Alveolar and Bronchial Nitric Oxide Output in Healthy Children

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Summary. Exhaled nitric oxide (NO) concentration is a marker of pulmonary inflammation. It is usually measured at a single exhalation flow rate. However, measuring exhaled NO at multiple flow rates allows assessment of the flow-independent NO parameters: alveolar NO concentration, bronchial NO flux, bronchial wall NO concentration, and bronchial diffusing capacity of NO. Our aim was to determine the flow-independent NO parameters in healthy schoolchildren and to compare two different mathematical approaches. Exhaled NO was measured at four flow rates (10, 50, 100, and 200 ml/sec) in 253 schoolchildren (7–13 years old). Flow-independent NO parameters were calculated with linear method (flows ≥ 50 ml/sec) and non-linear method (all flows). Sixty-six children (32 boys and 34 girls) with normal spirometry and no history or present symptoms of asthma, allergy, atopy or other diseases were included in the analysis. Median bronchial NO flux was 0.4 nl/sec (mean SD: 0.5 ± 0.3 nl/sec) and median alveolar NO concentration was 1.9 ppb (2.0 ± 0.8 ppb) with the linear method. Bronchial NO flux correlated positively with height (r = 0.423; P < 0.001), FEV1 (r = 0.358; P = 0.003), and FVC (r = 0.359; P = 0.003). With the non-linear method, median bronchial wall NO concentration was 49.6 ppb (68.0 ± 53.3 ppb) and bronchial diffusing capacity of NO was 10.0 pl/sec/ppb (11.8 ± 7.5 pl/sec/ppb). The non-linear method gave lower alveolar NO concentration (1.4 [1.5 ± 0.7] ppb, P < 0.001) and higher bronchial NO flux (0.5 [0.6 ± 0.3] nl/sec, P < 0.001) than the linear method, but the results were highly correlated between the two methods (r = 0.854 and r = 0.971, P < 0.001). In conclusion, the multiple flow rate method is feasible in children but different mathematical methods give slightly different results. Reference values in healthy children are of value when applying bronchial and alveolar NO parameters in the diagnostics and follow-up of inflammatory lung diseases.


Key words: breath tests; bronchi; child; nitric oxide; pulmonary alveoli; reference values.

INTRODUCTION

Nitric oxide (NO) is a gaseous signaling molecule that regulates various physiological and pathophysiological functions in the lungs and in the whole human body.1,2 NO is synthesized in small amounts by constitutive nitric oxide synthases (cNOS) in physiological conditions. In inflammation, the inducible nitric oxide synthase (iNOS) produces higher amounts of nitric oxide for prolonged periods.3 NO concentration in exhaled air is elevated in inflammatory lung diseases such as asthma,4,5 COPD,6 and alveolitis.7,8 Exhaled NO measurement can be used for diagnostics and follow-up of inflammatory lung diseases in both adults and children.9,10

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According to the ERS and ATS recommendations, exhaled NO is usually measured at a single exhalation flow rate of 50 ml/sec.17 However, to attain a more precise interpretation of pulmonary NO dynamics and the underlying inflammatory process, applications of multiple exhalation flow rate method have been developed. The multiple flow methods are based on a mathematical model of pulmonary NO dynamics and allow assessment of alveolar and bronchial NO output separately.12–16 The multiple exhalation flow rate methods have been demonstrated to successfully differentiate between bronchial and alveolar inflammation in asthma, COPD, asbestosis, and allergic and fibrosing alveolitis.7,14–18

The two-compartment model introduced by Tsoukias and George12 divides lung in two parts: that is, the alveolar (peripheral) and the bronchial (central) compartments. In this model relative contributions of alveolar and bronchial levels to exhaled NO can be assessed by measuring exhaled NO at several exhalation flow rates. At higher flow rates (>50 ml/sec) there is a roughly linear relation between NO output and flow rate. The slope and intercept values of a linear regression line between NO output and exhalation flow rate correspond to alveolar NO concentration and bronchial flux, respectively. If lower flow rates (<50 ml/sec) are also included in measurements, bronchial NO flux can be divided into its two components: bronchial wall NO concentration and bronchial diffusing capacity of NO.14,15

Thus far there are only a few studies comparing results of different approaches of multiple flow rate method in children.19–24 In the present study we measured exhaled NO at multiple exhalation flow rates in 253 schoolchildren. The aim of the study was to assess flow-independent NO parameters in healthy schoolchildren and to compare the results obtained with two different mathematical approaches.

METHODS

Subjects

All children in a local comprehensive school were recruited for the present study (253 schoolchildren, 7–13 years old, 123–165 cm tall). Exhaled NO and spirometry were measured and ISAAC-questionnaire was filled in. The exclusion criteria were history or present symptoms of asthma, allergy or atopy according to the questionnaire, impaired lung function, or other chronic diseases. Lung function parameters were compared to normal values in Finnish children. Sixty-six children (32 boys and 34 girls) were eligible after the strict exclusion criteria, and they were included in the final analysis. The study was approved by the Ethics Committee of Tampere University Hospital. Both the children and their parents gave their written informed consent.

Symptom Questionnaire

Parents completed the International Study of Asthma and Allergies in Childhood (ISAAC) phase-one questionnaire.25 The questionnaire contained questions on atopy and allergy, and symptoms suggestive of eczema, rhinitis or asthma in past and present. Only airway symptoms appearing with acute respiratory infection were acceptable, and any other affirmative answers to the questions led to exclusion.

Exhaled NO

Exhaled NO was measured with a Sievers NOA 280 analyser (Sievers Instruments, Boulder, CO) at four exhalation flow rates (10, 50, 100, and 200 ml/sec). Flow rates were achieved with a servo-controlled variable flow resistor (NOFLA-device developed at the University of Tampere) that kept exhalation flow rate steady regardless of changes in expiratory effort.18,26

The exhalation flow channel of the NOFLA-device consists of a mass-flow meter, a pressure gauge, and a solenoid valve to adjust the flow resistance. Exhalation pressure measured by the pressure gauge was displayed on a computer screen, and the subjects were asked to maintain the pressure in the range of 5–20 cmH2O to avoid nasal contamination. The computer adjusted the flow resistance of the flow channel by increasing and decreasing the opening of the solenoid-valve in real time based on the flow rate measured by the mass-flow meter. If the subject’s expiratory effort increased during a measurement and the flow rate tended to increase above the desired level, the solenoid valve was closed a bit to increase the flow resistance and thereby to decrease the flow rate back to the desired level. If the subject’s expiratory effort diminished during a measurement, a reverse action of the valve was conducted. The cycle of measuring the flow and adjusting the solenoid valve was conducted ten times a second to reach a rapid and smooth control of exhalation flow rate. The accuracy of the flow control was ±0.5% or ±1 ml/sec of the set value.

Three acceptable measurements were performed at each flow rate. Repeatability of three consecutive exhaled NO measurements at each flow rate was calculated in a subset of 25 children. Mean (range) coefficient of variation at different flow rates were 5.7% (0.1–11.4%) at 10 ml/sec, 4.0% (0.1–9.6%) at 50 ml/sec, 4.1% (0.1–9.0%) at 100 ml/sec, and 5.3% (0.1–10.5%) at 200 ml/sec.

Flow-Independent NO Parameters

Exhaled NO concentration (FENO) can be described as an exponential function of exhalation flow rate:

\[
FENO = C_W \left(1 - e^{-\frac{\beta_{NO, Br}}{V}}\right) + C_{Alv} \times e^{-\frac{\beta_{NO, Br}}{V}}
\]  

(1)

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where $C_W$ is bronchial wall NO concentration, $D_{NO,Br}$ is bronchial diffusing capacity of NO, $C_{Alv}$ is alveolar NO concentration, and $V$ is exhalation flow rate.\textsuperscript{12,14,15}

In this non-linear method, exhaled NO values at all four exhalation flow rates (10, 50, 100, and 200 ml/ sec) were fit to the exponential equation above. Non-linear least squares regression was calculated with NLREG software (Version 4.1, Phillip H. Sherrod) (Fig. 1). Function convergence was attained in 54 out of 66 children.

At higher flow rates ($\geq 50$ ml/ sec) the exponential equation can be substituted with its linear approximate and rearranged to give:

$$V_{NO} = C_{Alv} \times V + J_{NO,Br} \quad (2)$$

where $V_{NO}$ is NO output (exhaled NO concentration $\times$ exhalation flow rate) and $J_{NO,Br}$ is bronchial NO flux.\textsuperscript{12,14}

By using this linear method, NO output was plotted against exhalation flow rate and a linear regression was set by least squares method (MS Excel 2003). The slope and the intercept of the regression line are approximates of alveolar NO concentration and bronchial NO flux, respectively. Only flow rates 50, 100, and 200 ml/ sec were used with the linear method (Fig. 2). Linearity was very good in all subjects with mean correlation coefficient of 0.98. Every subject had a correlation coefficient of at least 0.95.

**Statistics**

As exhaled NO concentrations and the flow-independent parameters were not normally distributed (Shapiro-Wilk’s test), non-parametric tests were used in the statistical analysis. Mann–Whitney U-test was used to compare differences between the sexes. Differences in the NO parameters calculated with linear and non-linear methods were tested by Wilcoxon test. Correlations were analyzed by Spearman’s rank correlation. Linear regression (stepwise method) was used to find the strongest explanatory variables for bronchial NO flux. SPSS 10.1 (SPSS, Inc., Chicago, IL) and InStat 3.05 (GraphPad Software, Inc., La Jolla, CA) softwares were used for statistical analysis. $P$-value $< 0.05$ was considered significant. Results are presented as medians and also as mean $\pm$ SD to allow easier comparison with the results of previous studies.

**RESULTS**

Girls were on average slightly older, taller and they had higher weight than the boys, but none of these differences were statistically significant. Girls had also higher exhaled NO concentrations at each single flow rate and also higher bronchial NO flux, but also these differences were non-significant (Table 1).

The linear method gave lower bronchial NO flux and higher alveolar NO concentration as compared with the non-linear method (Table 1). However, there was a strong correlation in bronchial NO flux ($r = 0.971$, $P < 0.001$) and in alveolar NO concentration ($r = 0.854$, $P < 0.001$) values between linear and non-linear methods.

Exhaled NO concentration at the flow rate of 50 ml/ sec correlated strongly with bronchial NO flux measured with both methods (linear: $r = 0.987$, $P < 0.001$; non-linear: $r = 0.970$, $P < 0.001$), but not with alveolar NO concentration (linear: $r = 0.075$, $P = 0.557$; non-linear: $r = -0.104$, $P = 0.452$).

In the whole group of children, height correlated positively with bronchial NO flux measured with the linear method ($r = 0.423$, $P < 0.001$) and with the non-linear
method \( r = 0.406, P = 0.003 \). Interestingly, correlation of height with bronchial NO flux calculated with the linear method was stronger in boys \( r = 0.529, P = 0.002 \) than in girls \( 0.334, P = 0.053 \), but there was no difference between the sexes in correlation between height and bronchial NO flux calculated with the non-linear method (boys: 0.437, \( P = 0.029 \); girls: 0.411, \( P = 0.041 \)). Bronchial NO flux and alveolar NO concentration calculated with the linear method are given by height-quartiles in Table 2. There was a statistically significant linear trend of increasing bronchial NO flux with increasing height.

Bronchial NO flux calculated with the linear method correlated positively also with FEV\(_1\) \( r = 0.358, P = 0.003 \) and FVC \( r = 0.359, P = 0.003 \). By using stepwise linear regression analysis, we found that the height was the strongest variable to explain variation in bronchial NO flux, and adding lung function parameters did not significantly improve the coefficient of determination (Fig. 3). Bronchial NO flux \( J_{\text{Br,NO}} \) can be represented as a function of the child’s height (in cm):

\[
J_{\text{Br,NO}} = -1.084 + 0.01124 \times \text{height}
\]

Alveolar NO concentration did not correlate with the child’s height or the measures of lung function. Age and sex had no significant effect on alveolar or bronchial NO parameters.

**DISCUSSION**

In the present study we measured exhaled NO concentration at multiple exhalation flow rates in healthy children. The multiple flow rate method is an extension and improvement of the exhaled NO measurement, as it allows separate assessment of NO output and inflammatory activity in alveolar and bronchial compartments. The current study shows, in accordance with a few studies published previously,\(^{19–22}\) that multiple flow method is

### TABLE 1—Basic Demographics, Exhaled NO Concentrations (ppb) at Different Exhalation Flow Rates, and Alveolar and Bronchial NO Parameters Calculated With the Linear and Non-Linear Methods

<table>
<thead>
<tr>
<th>Boys (n = 32)</th>
<th>Girls (n = 34)</th>
<th>P-value(^1)</th>
<th>All (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.0 (9.5 ± 1.7)</td>
<td>10.0 (9.9 ± 1.6)</td>
<td>0.344</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>136.0 (138.3 ± 10.9)</td>
<td>138.5 (140.1 ± 10.5)</td>
<td>0.426</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>31.5 (33.9 ± 8.4)</td>
<td>33.8 (34.9 ± 8.6)</td>
<td>0.568</td>
</tr>
<tr>
<td>FENO(_{0.01})</td>
<td>26.2 (34.2 ± 22.9)</td>
<td>33.8 (40.6 ± 18.3)</td>
<td>0.061</td>
</tr>
<tr>
<td>FENO(_{0.05})</td>
<td>9.5 (10.6 ± 5.1)</td>
<td>11.7 (12.8 ± 5.5)</td>
<td>0.089</td>
</tr>
<tr>
<td>FENO(_{0.1})</td>
<td>5.6 (6.2 ± 2.6)</td>
<td>6.8 (7.3 ± 2.9)</td>
<td>0.075</td>
</tr>
<tr>
<td>FENO(_{0.2})</td>
<td>3.7 (4.1 ± 1.4)</td>
<td>4.4 (4.7 ± 1.6)</td>
<td>0.086</td>
</tr>
<tr>
<td>Linear method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( J_{\text{Br,NO}} ) (nl/sec)</td>
<td>0.4 (0.4 ± 0.3)*</td>
<td>0.5 (0.5 ± 0.3)*</td>
<td>0.102</td>
</tr>
<tr>
<td>( C_{\text{Alv,NO}} ) (ppb)</td>
<td>2.0 (2.0 ± 0.8)*</td>
<td>1.9 (2.0 ± 0.8)*</td>
<td>0.873</td>
</tr>
<tr>
<td>Non-linear method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{W}} ) (ppb)</td>
<td>42.0 (53.1 ± 33.9)</td>
<td>71.6 (80.9 ± 63.5)</td>
<td>0.105</td>
</tr>
<tr>
<td>( D_{\text{Br,NO}} ) (pl/sec/ppb)</td>
<td>10.0 (12.4 ± 6.8)</td>
<td>10.0 (11.3 ± 8.1)</td>
<td>0.455</td>
</tr>
<tr>
<td>( J_{\text{Br,NO}} ) (nl/sec)</td>
<td>0.5 (0.5 ± 0.3)</td>
<td>0.6 (0.6 ± 0.3)</td>
<td>0.179</td>
</tr>
<tr>
<td>( C_{\text{Alv,NO}} ) (ppb)</td>
<td>1.4 (1.4 ± 0.6)</td>
<td>1.5 (1.6 ± 0.8)</td>
<td>0.633</td>
</tr>
</tbody>
</table>

Results are given as median \((\text{mean ± SD})\).

\(^1\)Mann–Whitney U-test between the sexes; \( J_{\text{Br,NO}} \), bronchial NO flux; \( C_{\text{Alv,NO}} \), alveolar NO concentration; \( C_{\text{W}} \), bronchial wall NO concentration; \( D_{\text{Br,NO}} \), bronchial diffusing capacity of NO.

\( *P < 0.001 \) as compared with non-linear method by Wilcoxon test.

### TABLE 2—Bronchial NO Flux, Alveolar NO Concentration and Age in the Healthy Children by Height-Quartiles

<table>
<thead>
<tr>
<th>Height (cm)</th>
<th>Age (years)</th>
<th>Bronchial NO flux (nl/sec)</th>
<th>Alveolar NO conc. (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile 1</td>
<td>127 (127 ± 2.6)</td>
<td>8.0 (8.0 ± 1.0)</td>
<td>0.3 (0.4 ± 0.3)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>135 (135 ± 2.0)</td>
<td>9.0 (9.1 ± 1.2)</td>
<td>0.4 (0.4 ± 0.2)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>140 (141 ± 2.4)</td>
<td>10.0 (10.0 ± 0.9)</td>
<td>0.4 (0.5 ± 0.3)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>151 (153 ± 6.2)</td>
<td>12.0 (11.4 ± 1.2)</td>
<td>0.6 (0.6 ± 0.3)</td>
</tr>
</tbody>
</table>

\( P\)-value*: \( <0.001 \) \( 0.011 \) \( 0.968 \)

Results are given as median \((\text{mean ± SD})\).

\( *P\)-values for a test of linear trend between the quartiles.
reliable in children as expected by the experience of using the same method in adults.7,14–17

The exclusion criteria for the present study were very strict. The aim was to attain healthy children without tendency to atopy, allergy or asthma, so any affirmative answer in the ISAAC-questionnaire led to exclusion. Therefore, the number of children was reduced and it is possible that also some healthy children were excluded.

Several studies have been published on exhaled NO levels at single flow rate of 50 ml/sec in healthy children and the mean results have ranged between 4.0 and 15.7 ppb10,27–29 which is in agreement with the present study with median and mean NO concentrations at the flow rate of 50 ml/sec being 10.3 and 11.7 ppb, respectively. Pedroletti et al. reported exhaled NO concentrations in schoolchildren at different exhalation flow rates in the range of 11–382 ml/sec. Their results were very similar with our exhaled NO concentration results at each flow rate.27

Exhaled NO concentration is highly flow-dependent, and NO measurements at a single flow rate are recommended to be conducted at the flow rate of 50 ml/sec. However, exhaled NO concentration at the flow rate of 50 ml/sec (FENO0.05) mainly reflects NO output in the large central airways and is not sensitive for possible changes in the lung periphery. This is reflected by the strong correlation of FENO0.05 with bronchial NO flux but not with alveolar NO concentration found also in the present study.

Approximates of alveolar NO concentration and bronchial NO flux calculated by the linear method introduced by Tsoukias and George12 may be affected by the range of the chosen exhalation flow rates. Inclusion of too low flow rates is likely to cause overestimation of the alveolar NO concentration and underestimation of the bronchial NO flux. At higher flow rates this problem diminishes. However, at higher flow rates the duration of a single exhalation is shorter due to the limited pulmonary capacity. The shorter duration of exhalation and low NO levels may cause inaccuracy in NO measurement due to the limited performance of current NO analyzers.

The suitable flow rates in linear method are also dependent on the child’s lung capacity and co-operation in the test situation. Due to the smaller lungs in children, total exhalation time at flow rates above 200 ml/sec is not always long enough to allow reaching a stable plateau in the NO concentration curve. This diminishes the reliability and the repeatability of the measurements. In addition, very low exhalation flow rates, like 10 ml/sec, are not invariably straightforward and comfortable for children. However, NO concentrations can usually be measured also at low flow rates depending on the child’s co-operation and the support from the measurement personnel. In our study we concluded to use flow rates of 50–200 ml/sec in the linear method, as these were the easiest flow rates for the children and linearity was very good.

As compared with the present results by the linear method, Suri et al.23 and Paraskakis et al.24 have reported similar levels of alveolar NO concentration and bronchial NO flux in healthy children. However, Mahut et al.21 found higher alveolar NO concentration (4.2 ± 2.0 ppb vs. 2.0 ± 0.8 ppb) and lower bronchial NO flux (0.32 ± 0.13 nl/sec vs. 0.5 ± 0.3 nl/sec) than in the present study. The difference between the results may be due to differences in the selection of the study subjects or due to differences in the measurements, for example, variability of the achieved exhalation flow rates. In our study, we used a servo-controlled variable flow-restrictor (NOFLA) to maintain accurate flow rate. This technique allows achievement of steady flow rates (±0.5% or ±1 ml/sec of the pre-set value) regardless of variations in exhalation effort during the measurement.

Solving the equation of the non-linear method provides a more precise analysis of airway status due to attainment of bronchial wall concentration ($C_W$) and diffusing capacity ($D_{NO, Br}$) of NO. In 12 cases the equation did not converge and results were not reached. Thus far there are only a few studies applying the non-linear method in children.19,20 Pedroletti et al.20 have published NO-parameters in asthmatic schoolchildren and healthy controls by using the non-linear method, and our results agree with those in the healthy controls. They found quite similar results of alveolar concentration (1.2 ± 0.1 ppb (mean ± SEM) vs. present study: 1.5 ± 0.7 ppb (mean ± SD)), $C_W$ (54.6 ± 8.4 ppb vs. present study: 68.0 ± 53.3 ppb), and $D_{NO, Br}$ (13.2 ± 1.7 pl/sec/ppb vs. present study: 11.8 ± 7.5 pl/sec/ppb). In results published by Shin et al.19 levels of $C_W$ and $D_{NO, Br}$ were somewhat different from ours ($C_W$: 198 ± 131 ppb and $D_{NO, Br}$: 4.82 ± 3.07 pl/sec/ppb).

In our study, there was a statistically significant difference in bronchial NO flux and alveolar NO concentration calculated with the linear and the non-linear
methods, but there was a strong correlation between the results obtained with the two different methods. One likely reason for the slight difference in bronchial NO flux between the linear and non-linear methods is that the non-linear method yields total maximal NO flux \( J_{DBr,NO} = CW \times DBr,NO \), whereas the linear method gives an approximate that is affected by alveolar NO concentration also (total NO flux, \( J_{DBr,NO} = (C_W-C_{Alv}) \times DBr,NO \)).

Our results showed that bronchial NO flux correlated positively with the child’s height as well as with FEV₁ and FVC; whereas age and sex had no effect on alveolar or bronchial NO parameters. The height explained about 18% of the variation in bronchial NO flux \( (r^2 = 0.179) \), and adding lung function parameters in the analysis did not significantly improve the coefficient of determination. Thus, the correlation between FEV₁ and bronchial NO flux is explained by the fact that both of these variables are related to height, and there was no independent relation between FEV₁ and bronchial NO flux. Interestingly, age was not correlated to bronchial NO flux, although age is related to height which in turn was correlated to bronchial NO flux. This suggests that the correlation between height and bronchial NO flux is explained by increasing bronchial tree and mucosal surface area available for NO production in taller children, and not by a mechanism related to age as such.

The girls had slightly higher exhaled NO concentrations at single flow rates and higher bronchial NO flux. Although the differences were not significant, they might be partially explained by the girls being slightly taller than the boys. However, the correlation between height and bronchial NO flux with the linear method was not quite significant in girls and was stronger in boys. There was no sex-difference in the correlation between height and bronchial NO flux using the non-linear method.

In conclusion, the multiple exhalation flow rate method can be considered as a reliable method to measure exhaled NO in schoolchildren. In healthy children, bronchial NO flux correlates with the child’s height and lung function, and the height was the strongest independent explanatory variable. Differences in chosen flow rates might cause variations in results, and the liner and non-linear methods give somewhat different results. There is an obvious need for standardization of the multiple exhalation flow rate method to obtain comparable results between laboratories. Knowledge of values of bronchial NO flux and alveolar NO concentration in healthy children enables their use in the diagnostics and follow-up of inflammatory lung diseases.

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REFERENCES