Impact of Analysis Interval on the Multiple Exhalation Flow Technique to Partition Exhaled Nitric Oxide

James L. Puckett, PhD, Richard W.E. Taylor, BS, Stanley P. Galant, MD, and Steven C. George, MD, PhD

Summary. Exhaled nitric oxide (eNO) is elevated in asthmatics and is a purported marker of airway inflammation. By measuring eNO at multiple flows and applying models of eNO exchange dynamics, the signal can be partitioned into its proximal airway \([J_{awNO}\) (nl/sec)] and distal airway/alveolar contributions \([CANO\) (ppb)]. Several studies have demonstrated the potential significance of such an approach in children with asthma. However, techniques to partition eNO are variable, limiting comparisons among studies. The objective of this study is to examine the impact of the analysis interval (time or volume) on eNO plateau concentrations and the estimation of \(J_{awNO}\) and \(CANO\). In 30 children with mild to moderate asthma, spirometry and eNO at multiple flows (50, 100, and 200 ml/sec) were measured. The plateau concentration of eNO at each flow was determined using two different methods of analysis: (1) constant time interval and (2) constant volume interval. For both methods of analysis, a two-compartment model with axial diffusion was used to characterize \(J_{awNO}\) and \(CANO\). At a flow of 200 ml/sec, the time interval analysis predicts values for eNO that are smaller than the volume interval analysis. As a result, there are significant differences in \(CANO\) between the methods of analysis (volume > time). When using the multiple flow technique to partition eNO, the method of analysis (constant time vs. constant volume interval) significantly affects the estimation of \(CANO\), and thus potentially the assessment and interpretation of distal lung inflammation. Pediatr Pulmonol. 2010; 45:182–191. © 2010 Wiley-Liss, Inc.

Key words: asthma; NO; inflammation; children.

INTRODUCTION

Nitric oxide (NO) was first measured in the exhaled breath of humans in 1991. Since its discovery there have been significant efforts to develop methods to accurately and reliably characterize the concentration of NO in the exhaled breath. Research has shown that the shape and magnitude of the NO exhalation profile depends strongly on the exhalation flow, presence of inflammation, and lung volume. These unique features are a consequence of the significant proximal airway source relative to the small concentration in the distal airway/alveolar region, and creates new challenges to develop methodologies that effectively characterize the exhaled NO signal.

Guidelines for the online measurement of exhaled NO were initially presented in 1997, and later updated in 2005 by the American Thoracic Society (ATS) and the European Respiratory Society (ERS). Three major features of the current guidelines include: (1) exclusion of the nasal sinuses by exhaling at a pressure >5 cmH₂O and subsequently closing the velopharyngeal aperture, (2) a constant exhalation flow of 50 ml/sec, and (3) prior to analysis, exhalation should occur for at least 4 sec in subjects <12 years old or 6 sec in subjects ≥12 years old. If these conditions are satisfied, the guidelines suggest that a plateau concentration of NO can be recorded. The plateau concentration is defined as a time-averaged value over a 3 sec window (signal does not vary by >10%), which is denoted as the fractional concentration of exhaled NO in exhaled breath (FE₅₀). The current guidelines have only been established for a single exhalation flow of 50 ml/sec (FE₅₀). At an exhalation flow of 50 ml/sec, FE₅₀ is predominately of proximal airway origin and the much smaller concentration...
tration from the distal airways cannot be ascertained. However, our group, as well as others, have presented numerous techniques in which the exhalation flow is varied (either within or between consecutive single exhalations) from 50 ml/sec to as high as 300 ml/sec in an effort to partition the exhaled NO signal into its proximal airway \( \left[ J_{\text{awNO}} \right] \) (ml/sec), maximum airway flow and distal airway/alveolar contributions \( \left[ C_{\text{ANO}} \right] \) (ppb), alveolar NO concentration). \(^4,7,15–17\) Several studies have demonstrated the potential significance of such an approach, \(^18–24\) particularly in children with asthma. \(^14,25–27\) However, if the interval of analysis to determine \( F_{\text{ENo}} \) is a fixed time (i.e., 3 sec) then incommensurate volumes of exhaled breath will be analyzed at different flows (e.g., 150 ml at 50 ml/sec and 900 ml at 300 ml/sec) and at different lung volumes. This could affect the calculated value of \( F_{\text{ENo}} \) at each flow and hence the estimation of \( J_{\text{awNO}} \) and \( C_{\text{ANO}} \), since the slope of the exhaled NO profile is statistically negative in healthy adults, decreasing by approximately 6% per second of exhalation. \(^7\) The objective of our study was to examine the impact of the analysis interval (time or volume) on NO plateau concentrations and partitioning the exhaled NO signal into proximal airway and distal airway/alveolar contributions in children with asthma.

**MATERIALS AND METHODS**

**Subjects**

Thirty pediatric patients between the ages of 6–17 years with mild to moderate asthma who presented to the Children’s Hospital of Orange County (CHOC) Breathmobile \(^8\) for a routine asthma evaluation participated in the study. Criteria for the diagnosis of asthma included a previous history of recurrent coughing, wheezing, shortness of breath (at rest or following exercise), and symptomatic improvement following short-acting bronchodilator. \(^28\) Patients were excluded from the study if they had any other heart or lung disease or any smoking within the past 5 years. Short and long acting \( \beta_2 \) agonists were withheld for 12 hr prior to the study. Each subject and their guardian began their visit by reading and completing the requirements stated in the informed consent documents; the consent form had been approved by the University of California, Irvine and CHOC Institutional Review Boards.

**Measurements**

Due to the potential confounding effect of lung function tests on the exhaled NO signal, \(^29\) the exhaled NO measurements at multiple flows (NIOX Flex, Aerocrine Ltd, Stockholm, Sweden) were performed prior to spirometry. Briefly, the patients inhaled through a NO-scrubbing filter (inspired NO-free air) via a mouthpiece to total lung capacity. This was followed immediately by full exhalation at a constant flow (50, 100, or 200 ml/sec) and pressure (>5 cm H\(_2\)O) through the mouthpiece into the NO measuring device. We chose 200 ml/sec as the fastest flow since the total exhalation time at flows >200 ml/sec is not always long enough in children to achieve a stable plateau concentration. We chose 50 ml/sec as the lowest flow as this should be high enough to maintain a constant airway NO flux during a single exhalation in children, \(^2\) and it is the recommended flow for a single breath exhaled NO measurement. The exhaled NO measurements at multiple flows (50, 100, and 200 ml/sec) were randomized and performed in triplicate for a total of nine single exhalations. To ensure an adequate plateau region for analysis in the NO exhalation profile, and account for progressively larger lung volumes as children’s age, the exhalation times for the 50, 100, and 200 ml/sec maneuvers were set to 10, 8, and 6 or 15, 10, and 8 or 20, 15, and 10 sec for ages 6–9, 10–13, and 14–17 years, respectively. The subjects were allowed to rest for at least 30 sec between attempts. Standard spirometry was performed (WinDx Spirometer, Creative Biomedics International, CA) in accordance with ATS criteria. \(^30\) The best spirometric measure of at least three maneuvers was recorded for analysis.

**Data Analysis**

The plateau concentrations of NO at multiple flows (50, 100, and 200 ml/sec) were determined using two different methods: (1) time interval analysis and (2) volume interval analysis. First, in accordance with current guidelines, the exhaled NO plateau concentration was determined as a time-averaged value over a 3 sec window. We analyzed the same time interval for all three flows within each subject, and chose the time interval to be the final 3 sec from the highest flow, since the lower two flows have longer exhalation times. Hence, the time interval for analysis was 4–6, 6–8, and 8–10 sec for ages 6–9, 10–13, and 14–17, respectively. A profile was removed from the analysis if the variation of NO concentration in the window varied by more than 10% (consistent with ATS/ERS guidelines). This is consistent with ATS and ERS guidelines, and allowed for a progressively longer exhalation time for older (and hence larger) children.

Second, the exhaled NO signal was analyzed based on the volume of exhaled breath. In addition, we also sought to determine the exhaled NO concentration at equivalent lung volumes across all subjects. Hence, we normalized the exhaled volume by an estimate of conducting airway volume, or exhaled airway volume turnovers \( \left( V_{\text{ex}}/V_{\text{aw}} \right) \), where \( V_{\text{ex}} \) is the exhaled volume and \( V_{\text{aw}} \) is an estimate of the subject’s conducting airway volume). The subject’s conducting airway volume was estimated in milliliters using the following previously reported relationship:

\[
V_{\text{aw}} = 1.018 \times \text{Height (cm)} - 76.2.
\]

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For each exhalation, we examined both the NO and flow tracings over one to 10 airflow volume turnovers in 15 sequentially increasing increments: 1–3, 1.5–3.5, 2.5–4, 2.5–4.5, 3–5, 3.5–4.5, 4–6, 4.5–6.5, 5–7, 5.5–7.5, 6–8, 6.5–8.5, 7–9, 7.5–9.5, and 8–10. Over each airflow volume turnover increment, with respect to both flow and exhaled NO, we measured the mean, the standard deviation, and the slope by linear regression. We normalized the slope and standard deviation by the mean. The criteria to determine the ideal airway volume turnover were consistent with current ATS and ERS guidelines: (1) the normalized slope of the flow and exhaled NO profile was approximately equal to zero (i.e., a plateau had been achieved), (2) the coefficient of variation of the flow was ≤5%, and (3) the coefficient of variation of exhaled NO was ≤10%.

**Calculation of J’awNO and CANO**

At flows ≥50 ml/sec in children, the elimination rate, VawNO (pl/sec, product of mean exhaled NO concentration and flow), of NO can be approximated by the following linear equation: 

\[ V_{awNO} = C_{A_{NO}} \times V_E + J'_{awNO} \]

where CANO is the distal airway/alveolar NO concentration (ppb), J’awNO is the maximum airway NO flux (pl/sec) and V_E is the exhalation flow (ml/sec).2 Thus, to estimate CANO and J’awNO, one can plot VawNO against V_E and apply a linear least squares analysis to determine the slope, S, and the intercept, I. The two-compartment model with axial diffusion can then be applied to estimate CANO and J’awNO using the following simple relationships: 

\[ C_{ANO} = S - I/a \]  
\[ J'_{awNO} = I \times b \]

where “a” and “b” are constants determined from the mathematical model which account for axial (or “back”) diffusion of NO.15 These relationships, to estimate J’awNO and CANO, do not consider variability in airway volume, which impacts the size and shape of the trumpet, and thus the cross-sectional area for axial diffusion. If both children (>4 years) and adults are considered, Vaw can range broadly from 25 to 300 ml. Details of the mathematical model have been presented previously,15 thus, we will present only the salient features here to account for a variable Vaw.

Briefly, to account for changes in Vaw, we scaled the size of the trumpet (length and cross-sectional area) based on the bifurcating structure of the Weibel lung model A,2 which has a volume of 217 ml through generation 17, the end of the airway compartment, and beginning of the alveolar compartment. In other words, the lengths and diameters of the symmetric bifurcating Weibel model were each scaled by \((V_{aw}/217)^{1/3}\), then the resulting dimensions were fit to the previously described trumpet shape: 

\[ A = A_0(z/z_0)^{2/3} \]

where A is the cross-sectional area of the trumpet, z is the axial position along the trumpet, and the subscript “0” refers to the axial position at generation 17.15 The constants “a” and “b” are then determined using the governing equations for the model as previously described15 for discrete values of Vaw ranging from 25 to 300 ml. The resulting relationship fits a power law extremely well \((R^2 = 0.998)\) resulting in the following equations to estimate J’awNO and CANO:

\[ J'_{awNO} = I(1.2(V_{aw}^{0.087})) \]  
\[ CANO(ppb) = S - \left(\frac{I}{840V_{aw}^{-0.012}}\right) \]

where Vaw is expressed in milliliters. Equations (1) and (2) were used in both methods of analysis to determine the region specific J’awNO and CANO.

**Statistical Analysis**

Statistical analysis was performed using Sigma Stat (Systat Software, San Jose, CA). Kolmogorov–Smirnov test was used to determine normality. Differences between time interval and volume interval analysis were determined using a paired Student’s t-test if the data set passed the Kolmogorov–Smirnov test, otherwise the Wilcoxon Signed Rank Test was used. Differences between endpoints evaluated over different time or exhaled volume intervals were assessed with analysis of variance (ANOVA). A value \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Patient Characteristics**

Thirty asthmatic subjects between the ages of 6–17 years were enrolled into the study. All of the enrolled subjects were able to perform the exhaled NO (none of the subjects had previous experience using the NIOX Flex) and spirometric maneuvers. The general patient characteristics are shown in Table 1.

**TABLE 1—Demographics of Subjects and Pre-Bronchodilator Spirometry**

<table>
<thead>
<tr>
<th>Subjects, n</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Male/female gender, n</td>
<td>14 (47)/16 (53)</td>
</tr>
<tr>
<td>Atopy, n (%)</td>
<td>85%</td>
</tr>
<tr>
<td>ICS treated/ICS naïve, n (%)</td>
<td>15 (50)/15 (50)</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>108 ± 15</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>105 ± 16</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>FEF25–75, % predicted</td>
<td>102 ± 30</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Inhaled corticosteroid (ICS) naïve was defined as no oral or ICS within the last 8 weeks and ICS treated was defined as prescribed ICS treatment for at least 8 weeks.
Determining the Ideal Airway Volume Turnover Interval

At all three flows, the subjects initially exhaled at a rate greater than the target; however, the flow quickly reached a steady value (Figs. 1A, 2A, and 3A). At a flow of 50 ml/sec, the normalized slope of the flow was approximately equal to zero and the coefficient of variation of the flow was $\leq 5\%$ at 2.5–4.5 airway volume turnovers (Fig. 1B,C). There is an inverse relationship between exhalation flow and exhaled NO concentration (Figs. 1D, 2D, and 3D). The normalized slope of exhaled NO was approximately equal to zero and the coefficient of variation of the exhaled NO signal was $\leq 10\%$ at 4.5–6.5 airway volume turnovers (Fig. 1E,F).

At a flow of 100 ml/sec, the normalized slope of the flow was approximately equal to zero and the coefficient of variation of the flow was $\leq 5\%$ at 3–5 airway volume turnovers (Fig. 2B,C). The normalized slope of exhaled NO was approximately equal to zero and the coefficient of variation of the exhaled NO signal was $\leq 10\%$ at 5–7 airway volume turnovers (Fig. 2E,F).

At a flow of 200 ml/sec, the normalized slope of the flow was approximately equal to zero and the coefficient of variation of the flow was $\leq 5\%$ at 4–6 airway volume turnovers (Fig. 3B,C). The normalized slope of exhaled NO was approximately equal to zero and the coefficient of variation of the exhaled NO signal was $\leq 10\%$ at 5–7 airway volume turnovers (Fig. 3E,F). At each flow, the criteria of having normalized slopes of the flow and normalized slopes of exhaled NO were met at 4.5–6.5 airway volume turnovers. Data presented as mean, upper and lower 95th confidence intervals.
exhaled NO profile equal to zero and coefficients of variation of the flow and slope ≤5% and ≤10%, respectively were satisfied for the remaining airway volume turnovers (i.e., $7 < V_{ex}/V_{aw} < 10$).

The first airway volume turnover which fulfilled our ideal analysis criteria for all three flows was 5–7 airway volume turnovers. Therefore, we determined $J_{aw}^{NO}$ and $C_{ANO}$ for each of seven sequentially increasing volume turnover increments of analysis: 5–7, 5.5–7.5, 6–8, 6.5–8.5, 7–9, 7.5–9.5, and 8–10. There were no differences in the estimation of $J_{aw}^{NO} (P = 1.0)$ or $C_{ANO} (P = 0.4)$ across these airway volume intervals. An interval of 5–10 airway volume turnovers also allows for analysis of approximately a minimum of 3 sec, even at the highest flow (i.e., 200 ml/sec). Hence, we chose $5 < V_{ex}/V_{aw} < 10$ as the ideal volume interval window to analyze and apply the model of NO exchange dynamics.

**Comparison of Time Interval and Volume Interval Analysis**

Next, we compared the effect of time interval analysis (i.e., 3 sec) and volume interval analysis (i.e., 5–10 airway volume turnovers) on the measurement of the flow dependent $F_{NO,50}$, $F_{NO,100}$, and $F_{NO,200}$ and calculation of the flow independent $J_{aw}^{NO}$ and $C_{ANO}$ (Fig. 4).

All data sets passed the Kolmogorov–Smirnov test with the exception of $F_{NO,50}$; however, the $P$-value for several of the data sets was close to the cut point of $P = 0.05$; hence, Table presents the results of both the paired $t$-test and the Wilcoxon rank sum test. Regardless of the statistical test employed, there were no differences between the plateau concentrations obtained from time interval and volume interval analyses for the flows of 50 ml/sec ($F_{NO,50}$) and 100 ml/sec ($F_{NO,100}$). However,
there was a statistical difference between the plateau concentrations obtained from the different methods of analyses with respect to the highest flow of 200 ml/sec (FENO,200). The mean differences (limits of agreement) [FENO based on volume interval – FENO based on time interval] were −0.2 ppb (−3.7 to 3.2 ppb), 0.2 ppb (−1.8 to 2.2 ppb) and 1.0 ppb (−1.4 to 3.4 ppb) for 50, 100, and 200 ml/sec, respectively.

The region specific NO parameters, J_{awNO} and CA_{NO}, were determined using the FENO plateau concentrations calculated from both time interval analyses and volume interval analyses. With respect to J_{awNO}, there was no statistical difference (regardless of statistical test employed) between time interval and volume interval methods of analyses (Table 2). The mean difference (volume − time) was 1.4 ppb with limits of agreement ranging from −1.7 ppb below to 4.4 ppb above and discrepancies of up to approximately 6 ppb.

DISCUSSION

This study has examined the impact of the analysis interval (constant time or exhaled volume interval) on FENO measurements at multiple exhalation flows, and the partitioning of the exhaled NO signal into its proximal airway (J_{awNO}) and distal airway/alveolar (CA_{NO}) contributions in children with asthma. We have presented a method to analyze the exhaled NO signal based on
equivalent exhaled airway volumes, and suggest an ideal volume interval (5–10 airway volume turnovers) to analyze and apply the model of NO exchange dynamics. The constant time interval predicts values for FE\textsubscript{NO,200} that are smaller than the constant exhaled volume interval. The result is a systematic bias for the constant time interval analysis that results in a significant underestimation of CANO. This finding is particularly relevant given the numerous observations demonstrating the potential clinical utility of CANO in asthma.\textsuperscript{33}

There is mounting evidence supporting FE\textsubscript{NO,50} as a reproducible,\textsuperscript{34} non-invasive measure of inflammation in the asthmatic lung.\textsuperscript{35–38} Several studies suggest that FE\textsubscript{NO,50} can be used to diagnose asthma,\textsuperscript{39–41} especially in combination with the results of more traditional measures of lung function such as spirometry.\textsuperscript{42} The constant time interval and constant volume interval predict values for FE\textsubscript{NO,50} that are highly correlated, and not different from each other. This finding is dependent on the choice of the time interval relative to the volume interval. For

TABLE 2—Exhaled NO Measurements Evaluated by Time and Volume Intervals

<table>
<thead>
<tr>
<th></th>
<th>Time interval</th>
<th>Volume interval</th>
<th>Kolmogorov–Smirnov</th>
<th>Wilcoxon rank P-value</th>
<th>Paired t-test P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE\textsubscript{NO,50} (ppb)</td>
<td>46.0 ± 38.7</td>
<td>46.0 ± 38.4</td>
<td>Failed (P &lt; 0.05)</td>
<td>0.45</td>
<td>0.49</td>
</tr>
<tr>
<td>FE\textsubscript{NO,100} (ppb)</td>
<td>27.0 ± 22.5</td>
<td>27.2 ± 23.0</td>
<td>Passed (P = 0.086)</td>
<td>0.36</td>
<td>0.76</td>
</tr>
<tr>
<td>FE\textsubscript{NO,200} (ppb)</td>
<td>14.5 ± 11.8</td>
<td>15.5 ± 12.4</td>
<td>Passed (P = 0.10)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CA\textsubscript{NO} (nl/sec)</td>
<td>3.9 ± 3.4</td>
<td>3.8 ± 3.3</td>
<td>Passed (P = 0.19)</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>CA\textsubscript{NO} (ppb)</td>
<td>1.1 ± 1.0</td>
<td>2.6 ± 2.1</td>
<td>Passed (P = 0.05)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.
example, we chose a time interval consistent with the last 3 sec of the highest flow (e.g., 4–6 sec in 6–9 years old). This corresponds to approximately 4–6 airway volume turnovers (see Fig. 4), which, although slightly earlier in the exhalation profile relative to the constant volume interval method, overlaps with the constant volume interval (5–10 airway volume turnovers), and hence there is no statistical difference.

When examining the impact of a constant time interval versus a constant exhaled volume interval at higher flows, the difference between the regions of analysis depends on the flow (Fig. 4). For an exhalation flow of 100 ml/sec, the time interval analysis (e.g., 4–6 sec for 6–9 years old) corresponds to a larger volume interval (approximately 7 < V_ex/V_aw < 11, Fig. 4), but, again, there is significant overlap with the constant volume interval analysis (5 < V_ex/V_aw < 10); hence, the difference in our analysis is negligible. At the highest flow, the region of analysis for the constant time interval is at a much larger V_ex/V_aw (15–20, Fig. 4), and the result is a smaller estimate in FENO at 200 ml/hr due the slightly negative slope of the exhalation profile at larger exhaled volumes (V_ex/V_aw > 8, Fig. 3E). Therefore, V_NO at 200 ml/sec is significantly larger which creates a steeper slope and larger CA_NO for the constant volume interval analysis (Fig. 4).

Within a given study, the multiple exhalation flow method to partition exhaled NO has been shown to be a reproducible43 and reliable method to estimate CA_NO in healthy14,24 and asthmatic14,26,27 children. In fact, numerous potentially clinically significant findings have been reported, highlighting the biological relevance of CA_NO in the context of asthma. For example, increased levels of CA_NO have been reported in asthmatics with nocturnal symptoms,44 asthmatics with poor control14 and in asthmatics refractory to ICS treatment.18 Furthermore, oral steroids18,43 and leukotriene receptor antagonists,45 but not inhaled corticosteroids,23,46 have been shown to reduce CA_NO. These findings suggest that partitioning exhaled NO to determine CA_NO may improve the clinical relevance of the exhaled NO signal, and thus accurate methods to estimate CA_NO are needed.

Despite these important findings, there are no specific guidelines on the collection technique or method to determine CA_NO, and variations in published CA_NO concentrations, in healthy and asthmatic children have been reported. Some of the discrepancy in the asthmatic children may be attributed to heterogeneity in disease severity or symptoms between the studies, or neglecting axial diffusion of NO; however, the reported differences in healthy children are most likely attributable to variable techniques of measurement. For example, in healthy children, Mahut et al.26 reported an average CA_NO of 4.2 ± 2.0 ppb, while Sepponen et al.24 reported 2.0 ± 0.8 ppb. In our study, the mean concentrations of CA_NO based on time and volume interval analysis were 1.1 and 2.6 ppb, respectively, and were significantly different from each other (P < 0.001; Table 2). Our results suggest that differences between studies may stem from ATS and ERS guidelines which do not require a constant exhaled volume interval analysis, and the requirements for a constant exhalation time window are flexible enough to result in significant differences in estimated CA_NO.

Two key features of the current ATS guidelines with respect to measuring the FE_NO plateau concentration are: (1) prior to analysis, exhalation should occur for at least 4 sec in subjects younger than 12 years old or 6 sec in subjects older than 12 years old and (2) the FE_NO concentration is defined as a time-averaged value over a 3 sec window in which the guideline criteria for a stable plateau are met (i.e., the FE_NO concentration does not vary by more than 10%). These guidelines are not adequate to address features of FE_NO measured at multiple exhalation flows. For example, if the interval of analysis to determine FE_NO is a fixed time (i.e., 3 sec), then an exhaled volume of 150 ml is analyzed at a flow of 50 ml/sec, but 600 ml is analyzed at a flow of 200 ml/sec. Furthermore, if the analysis window begins after 6 sec of exhalation in adults and children >12 years, this corresponds to an exhaled volume of 300 ml at a flow of 50 ml/sec and 1,200 ml at a flow of 200 ml/sec. Hence, when using current guidelines to estimate J_0 NO and CA_NO by measuring FE_NO at multiple flows, the model is applied over different exhaled volumes of air and at different lung volumes. Additionally, the guidelines do not consider variation in the size of the subjects, despite the positive correlation between FE_NO and height.24,47 An algorithm to analyze FE_NO based on airway volume turnover intervals is physiologically more accurate than the time interval analysis because the volume interval method considers the height of the individual and permits application of the quantitative model of NO exchange across equivalent exhaled volumes of air and lung volumes.

In conclusion, we have contrasted methods to analyze the exhaled NO profile based on constant exhalation time intervals and volume intervals to determine the effect on both FE_NO and the multiple exhalation flow technique to partition exhaled nitric oxide into its proximal airway (J_0 aw_NO) and distal airway/alveolar contributions (CA_NO). The volume interval analysis method is based on an estimate of the subject’s airway volume, which considers the height of the individual and facilitates application of the two-compartment model across equivalent exhaled breath volumes and lung volumes. Analysis of a constant time interval results in a significantly reduced FE_NO at higher flows and thus a systematic bias leading to an underestimation of CA_NO. The magnitude of the bias will depend on the choice of the time interval. This result is particularly relevant given the recent clinical studies demonstrating the potential of CA_NO to characterize distal lung inflammation.14,25–27,33 An ideal volume interval to
analyze the exhaled NO signal based on achieving relatively steady flows and exhaled NO concentrations is 5–10 airway turnovers. Future studies must address the underlying mechanisms of the negative slope in the exhaled NO profile, and optimal flow ranges for children and adults as we move towards standardizing the methods to assess proximal and distal NO levels in the lungs.

ACKNOWLEDGMENTS
This work was supported by a grant from the National Institutes of Health (R01 HL070645) and the Children’s Hospital of Orange County.

REFERENCES