Assessment of exhaled nitric oxide kinetics in healthy infants

T. Martínez, A. Weist, T. Williams, C. Clem, P. Silkoff, and R. S. Tepper. Assessment of exhaled nitric oxide kinetics in healthy infants. J Appl Physiol 94: 2384–2390, 2003. First published January 31, 2003; 10.1152/japplphysiol.00758.2002.—Exhaled nitric oxide (FeNO) measurements provide a noninvasive approach to the evaluation of airway inflammation. Flow-independent NO exchange parameters [airway NO transfer factor (DNO) and airway wall NO concentration (CwNO)] can be estimated from FeNO measurements at low flows and may elucidate mechanisms of disturbances in NO exchange. We measured FeNO in sedated infants by using an adaptation of a raised lung volume rapid thoracic compression technique that creates forced expiration through a mass-flow controller that lasts 5–10 s, at a constant preset flow. We measured FeNO at expired flows of 50, 25, and 15 ml/s in five healthy infants (7–31 mo). Median FeNO increased [24, 40, and 60 parts per billion (ppb)] with decreasing expiratory flows (50, 25, and 15 ml/s). Group median (range) for DNO and CwNO were 12.7 (3.2–37) \( \times 10^{-3} \) nl·s\(^{-1} \)·ppb\(^{-1} \) and 108.9 (49–385) ppb, respectively, similar to values reported in healthy adults. Exhaled NO is flow dependent; flow-independent parameters of exhaled NO kinetics can be assessed in infants and are similar to values described in adults.

airway inflammation; lung function; nitric oxide measurement

AIRWAY INFLAMMATION CONTRIBUTES significantly to the pathophysiology of several respiratory diseases, including asthma. Airway inflammation in asthma is characterized by the influx of inflammatory cells and production of inflammatory mediators including cytokines, growth factors, and nitric oxide (NO) (7, 17). Invasive procedures (e.g., bronchoscopy, lavage, and biopsy) have been used to assess inflammation but are primarily research procedures and are unsuitable for repeated monitoring in the clinical setting (3, 6, 14).

Less invasive means of assessing airway inflammation include induced sputum analysis and, more recently, the measurement of exhaled gases, particularly exhaled NO (10, 12). The fractional concentration of exhaled NO (FeNO) is widely regarded as a noninvasive inflammatory marker of asthma, and use of FeNO has been advocated in the monitoring and diagnosis of other inflammatory lung diseases such as chronic obstructive pulmonary disease, cystic fibrosis, and the ciliary dyskinesia syndromes (15). Several factors may affect the reproducibility of FeNO measurements, and both the European Respiratory Society (ERS) and American Thoracic Society (ATS) have published recommendations aimed at standardizing the techniques used to measure FeNO in adults and children (1, 16). The factors that promote reproducibility of FeNO measurements include exclusion of the high concentrations of nasal NO, exhalation until the achievement of a NO concentration plateau, and, because NO is flow dependent, standardization of expiratory flow (19).

Recent studies have focused on mathematical models that calculate flow-independent parameters of exhaled NO kinetics from measurements of FeNO obtained at several expiratory flows (20, 23). In the model, alveolar gas, which contains very low concentrations of NO (<5 parts per billion (ppb)) owing to binding by hemoglobin, ascends the airway, where it is conditioned with NO diffusing from the airway wall to lumen under a concentration gradient. The flow-independent parameters include the NO transfer factor (DNO), the driving concentration for NO diffusion from airway wall to lumen (CwNO), and the alveolar NO concentration.

The techniques used to measure FeNO in older children and adults require subject cooperation and, therefore, have been difficult to reproduce in infants. Visual feedback mechanisms are used in older children and adults to help subjects achieve and maintain constant expiratory flow during FeNO measurements (8, 13, 19). Older subjects can voluntarily sustain expiratory effort for a prolonged period of time, which permits acquisition of clear NO concentration plateaux. In addition, elevation of the velum and exclusion of nasal NO during expiration have been demonstrated when adults actively expire through a high fixed resistance and target a constant airway pressure or flow (19).

Several studies have measured FeNO in infants during tidal breathing (2, 4). Although collection of FeNO from tidal breathing in young, uncooperative children is acceptable by both ERS and ATS standards, tidal.

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breathing is characterized by variable expiratory flow and may not exclude nasal NO. Stick and co-workers (24) recently described a method for measuring FENO utilizing a modification of their raised volume rapid thoracic compression technique for obtaining forced expiratory flows in infants. In their study, constant positive airway pressure and expiratory flow were maintained by manual increase of jacket compression pressure. Exclusion of nasal NO resulted from expiration against a resistance. However, Fig. 2 from their published report demonstrates an expiratory period of <2 s. Therefore, it is unclear whether true NO plateaus had been attained and whether NO measurements at lower flows, which require longer expiratory periods to achieve reproducible and accurate NO concentration plateaus, could be obtained by use of this methodology. In addition, flow-independent NO exchange parameters were not reported.

We describe an adaptation of our raised lung volume technique (5, 11) to measure FENO in normal infants. In addition, we report, for the first time, flow-independent NO diffusion parameters in healthy infants.

MATERIALS AND METHODS

Subjects. Five full-term normal healthy infants and toddlers (4 female) between 7 and 31 mo of age were evaluated. Subjects had a history negative for asthma, wheezing, lower respiratory tract infection, and congenital heart disease. In

Fig. 1. A: modified circuit used to measure exhaled nitric oxide in infants. B: 2-compartment mask. The mask is divided into a nasal and an oral compartment. Both compartments are attached to a 3-way sliding valve, which occludes the nasal compartment during forced exhalation.
addition, they were free of any acute respiratory symptoms for at least 3 wk before testing. Subjects received 50–75 mg/kg po of chloral hydrate and were evaluated while sleeping in the supine position. The study was reviewed and approved by the Institutional Review Board at Indiana University.

Equipment and technique. A modification of our previously described methodology for obtaining forced expiratory flows with the raised volume rapid thoracic compression technique was used (see Fig. 1A) (5, 11). Infants breathed through a two-compartment face mask into a circuit that had an adjustable bias flow from a Sechrist infant ventilator (model IV-100B) connected to NO-free compressed air. A two-compartment mask (dual port series, Hans Rudolph, Kansas City, MO) having a nasal and an oral compartment was fitted to each infant with a foam seal (Comfort Seal, Hans Rudolph). Each compartment had a port that was connected to a three-way sliding valve (Hans Rudolph model 8540) (Fig. 1B). A heated pneumotachometer, which is linear to 160 l/min (Hans Rudolph model 3700), measured inspiratory and expiratory flow. A differential pressure transducer (Validyne MP-45-871, Northridge, CA) measured airway pressure. Occlusion of the circuit’s expiratory segment by a balloon valve (Hans Rudolph ref. 200474) allowed a 20 l/min bias flow to augment inspiratory effort through both the nasal and oral compartments. Inflation of the respiratory system resulted in a lung volume at an airway pressure of 30 cmH2O, which was set by a pressure-relief valve (Sechrist, part IV-317). Opening of the expiratory segment of the circuit resulted in passive deflation of the respiratory system through the nasal and oral compartments. Several inflation-passive deflation cycles to lung volume at 30 cmH2O inhibited inspiratory effort, which produced a respiratory pause that often persisted for 5–10 s. After the last inflation, the sliding valve connected to the face mask occluded the port to the nasal chamber, and forced expiration via the oral compartment was initiated by rapid inflation of a jacket that encircled the infant’s thorax and abdomen. Jacket inflation was controlled by an electronic solenoid valve, which connected the jacket to a pressure reservoir. A three-way sliding valve (Hans Rudolph model 8540) redirected expiratory flow through a mass-flow controller (Kinetics Electronics, Yorba Linda, CA), which maintained a constant preset expiratory flow by continuously adjusting its resistance.

Exhaled NO was sampled at the oral compartment and measured continuously with a chemiluminescence NO analyzer (model 280; Sievers, Boulder, CO). The analyzer had a response time of 200 ms, repeatability of ±1 ppb, and a sampling rate of <5 ml/s. Calibration of the NO analyzer was performed with a zero-NO gas and a NO-standard gas of known NO concentration (45 ppm) before each session. Analog signals for flow, pressure, and NO were filtered, amplified, digitized, and displayed on a computer monitor in real time. Exhaled NO was measured at expiratory flows of 50, 25, and 15 ml/s, and data were stored for analysis.

Data analysis. Exhaled NO concentration was determined from NO plateau measurements obtained at each preset expiratory flow. The mouth pressure during the plateau was required to be above 15 cmH2O. NO output, the amount of NO exhaled per unit time (nl/s), was calculated as the product of the FENO concentration plateau (ppb) and flow (ml/s). DNO and CWNO were estimated by...
using a two-compartment mathematical model for NO kinetics described by Silkoff and co-workers (20). $D_{NO}$ and $C_{wNO}$ were calculated from the slope and $x$-intercept, respectively, derived from the linear regression of NO output and $F_{ENO}$ concentration.

**RESULTS**

The NO concentration and mouth pressure tracings obtained for subject 4 at three different forced expiratory flows (50, 25, and 15 ml/s) are illustrated in Fig. 2.
Table 1. Demographics, FE\textsubscript{ENO}, and NO kinetics data from 5 normal infants at 3 expiratory flows

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, mo</th>
<th>Weight, kg</th>
<th>Length, cm</th>
<th>Gender</th>
<th>Flow, ml/s</th>
<th>FE\textsubscript{ENO}, ppb</th>
<th>NO Output nl/s</th>
<th>D\textsubscript{NO}, nl·s\textsuperscript{-1}·ppb\textsuperscript{-1} ( \times 10^{-6} )</th>
<th>C\textsubscript{WNO}, ppb</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>11.7</td>
<td>81</td>
<td>F</td>
<td>13</td>
<td>36</td>
<td>480</td>
<td>37</td>
<td>49</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>8.2</td>
<td>69.8</td>
<td>F</td>
<td>17</td>
<td>61</td>
<td>1,036</td>
<td>385</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>8.7</td>
<td>66.4</td>
<td>F</td>
<td>11</td>
<td>60</td>
<td>639</td>
<td>14</td>
<td>109</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>12.5</td>
<td>88.6</td>
<td>F</td>
<td>16</td>
<td>36</td>
<td>566</td>
<td>12</td>
<td>87</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>12.5</td>
<td>92.9</td>
<td>M</td>
<td>13</td>
<td>237</td>
<td>3,086</td>
<td>32</td>
<td>326</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Median 14 11.7 81
Mean 11.7 10.7 79.7

\( \text{FE} \text{NO} \), fractional concentration of exhaled NO. \( \text{DNO} \), diffusion constant into airway lumen; \( \text{CWNO} \), airway wall concentration; F, female; M, male.

In Fig. 2A, a flow of 50 ml/s is established within 1 s and remains constant while airway pressure declines secondary to declining lung volume. In addition, NO concentration rises rapidly to a plateau level. A similar pattern in expiratory flow and airway pressure is demonstrated in the tracings obtained at expiratory flows of 25 and 15 ml/s; however, an early transient peak in \( \text{FENO} \) is seen followed by a fall to an NO plateau level. Mean duration of the NO concentration plateau for these three flows was 2 s, and the airway pressure remained above 15 cmH\textsubscript{2}O during the time the NO plateau level was observed.

The values for \( \text{FENO} \) concentration at each of the three flows for the five subjects are summarized in Table 1 and illustrated in Fig. 3. All infants demonstrated flow dependence of \( \text{FENO} \); \( \text{FENO} \) concentration decreased with increasing expiratory flow.

There was a linear relationship between NO output and \( \text{FENO} \) \( (R^2 > 0.91) \), for the 5 subjects (Fig. 4). Values for \( \text{DNO} \) and \( \text{CWNO} \) were calculated from the slope and \( x \)-intercept derived from these linear relationships, as previously described by Silkoff and co-workers (20) (Table 1). The median (range) for \( \text{DNO} \) and \( \text{CWNO} \) were 12.7 \( \times 10^{-5} \) nl·s\textsuperscript{-1}·ppb\textsuperscript{-1} (range 3.2–37) and 108.9 ppb (range 48.7–384.9), respectively.

**DISCUSSION**

Noninvasive measurement of \( \text{FENO} \) remains an innovative tool in the evaluation of inflammatory lung disease in older children and adults. In this report, we describe a method for measuring \( \text{FENO} \) in infants, which stems from a modification of a previously described raised-volume rapid thoracic compression technique used to measure infant pulmonary function in our laboratory. Flow dependence was established in all infants. By measuring \( \text{FENO} \) at several flows, we estimated flow-independent parameters of \( \text{FENO} \) (\( \text{DNO} \) and \( \text{CWNO} \)) for the first time in infants, which were similar in magnitude to those reported in adults (20).

In the present study, infants were allowed to inhale through both oral and nasal face mask compartments, which improved tolerance to the procedure and resulted in two distinct NO tracings. \( \text{FENO} \) either ascended from a baseline to a NO plateau level (Fig. 2A) or sharply ascended to an early peak with a subsequent fall to a plateau level (Fig. 2, B and C). The path taken during inspiration explains the observed variation in expiratory NO tracings. Figure 2A corresponds to a NO tracing expected from an oral inspiration, whereas Fig. 2, B and C, corresponds to the expected tracings after nasal inspiration. Prior studies in adults have demonstrated that \( \text{FENO} \) plateau levels are equivalent for both nasal and oral inspiratory maneuvers (19) once the nasal peak has been washed out. Therefore, nasal contamination of \( \text{FENO} \) during nasal inspiration is unlikely if plateau levels are achieved.

Nasal contamination of \( \text{FENO} \) measurements has been a limiting factor in the reproducibility of \( \text{FENO} \) levels. International guidelines recommend measuring \( \text{FENO} \) from an active oral exhalation against a high expiratory resistance to achieve vellum closure and thus isolation of the nasal cavity (1, 19). A previous study in infants has reported isolation of the nasal chamber by demonstrating low end-tidal CO\textsubscript{2} from the nasal face mask compartment during acquisition of \( \text{FENO} \) measurements, which we have also reproduced in several infants (24). Active oral exhalation is impossible to attain in sedated infants, and posterior isolation of the nasal compartment is difficult to evaluate noninvasively. It has been our observation that \( \text{FENO} \) rises in some subjects as mouth pressure decreases, which may indicate posterior nasal contamination from the nasal cavity at expiratory pressures that are higher than those used in adults. Therefore, in these subjects, we measured \( \text{FENO} \) at high positive pressures to minimize the contribution from nasal contamination.

\( \text{FENO} \) and \( \text{DNO} \) levels were markedly elevated in one of the infants (subject 5). A positive family history of...
asthma in this infant would make the presence of underlying airway inflammation in this subject plausible. Additionally, adult studies have also demonstrated a large variability for \( D_{NO} \) in healthy subjects (9, 18, 20). Whether the high levels in this infant stem from subclinical inflammation or reflect normal variability of \( F_{ENO} \) in infants requires further studies in a larger sample of subjects.

Studies in adults suggest that flow-independent parameters of \( F_{ENO} \) may help elucidate the mechanisms underlying perturbation of \( F_{ENO} \) levels (20, 22). \( D_{NO} \) and \( C_{WNO} \) values in the present report provide initial data for establishing normal values in infants, and our estimates for \( C_{WNO} \) are consistent with values in adults. Although the mean and median \( D_{NO} \) values fell within the values previously reported in adults, the range for \( D_{NO} \) was skewed to the right because two of our infants (subjects 1 and 5) had \( D_{NO} \) values that were higher than those reported in adults. The higher variability in flow-independent parameters observed in these infants could be explained by the mathematical model used for their calculation. The model assumes a homogeneous conducting airways compartment, where \( D_{NO} \) is largely proportional to the airway wall surface area. Infant airway radial distance and wall thickness are probably smaller than in adults, and airway wall surface area is large relative to airway volume, introducing variability, which could result in a wider range of normal values for flow-independent parameters in healthy infants. The relative importance of these factors remains unclear secondary to limited anatomical and physiological infant data at this time. In addition, our limited population of healthy infants may include subjects that are predisposed to develop asthma at an older age and thus increase intersubject variability.

Tsoukias and George (23) have previously described a two-compartment model of NO exchange dynamics, which calculates bronchial flux (the maximal quantity of NO diffusing into exhaled gas per unit time) from the linear relationship between NO output and expiratory flow. This measurement is equivalent in adults to the product of \( D_{NO} \) and \( C_{WNO} \) derived from the present model by Silkoff and co-workers (20). Bronchial flux values calculated for our subjects by both techniques differ significantly. The model described by Silkoff and co-workers derives bronchial NO flux from measurements obtained at low expiratory flow rates, whereas Tsoukias and co-workers derive flux from measurements obtained at high expiratory flow rates, and the discrepancy is difficult to explain from the small number of subjects evaluated. It is not clear which model may best describe the infant lung, which is not a scaled down version of the adult lung. The relative size of airway to surface area and lung volume, as well as the time constant for lung emptying, is greater in the infant than in the adult lung (21). However, the ability to separate bronchial flux components into \( D_{NO} \) and \( C_{WNO} \) is attractive because these measurements are themselves potentially useful in the evaluation of inflammatory airway disease in infants.

In conclusion, we report a modified version of our raised volume rapid thoracic compression technique to obtain \( F_{ENO} \) measurements in infants, which approximates ATS and ERS standards for adults. We were able to measure \( F_{ENO} \) at several expiratory flows, and calculate flow-independent parameters of NO exchange. The values for \( F_{ENO} \), \( D_{NO} \), and \( C_{WNO} \) paralleled those previously reported in adults. Future studies are required to establish normative data for \( F_{ENO} \) and flow-independent parameters of NO exchange in both healthy infants and infants with lung disease. This technique offers a new noninvasive tool with the potential to expand our understanding of infant lung disease.

This research was supported by National Institutes of Health Grant 54062.

REFERENCES


