Attempt to Normalize Simulated Exhaled Nitric Oxide According to Ventilatory Settings

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Summary. Background: Simulated exhaled nitric oxide (eNO) depends on ventilatory settings used in different experimental conditions. Objectives: To normalize the simulated minute exhaled nitric oxide according to different ventilatory settings. Working Hypothesis: Different ventilatory settings influence the concentrations of exhaled nitric oxide and these results can be normalized. Methodology and Study Design: We used a rubber lung model (50 ml) with an orifice through which a 3 mm endotracheal tube was introduced. The NO, which simulated that of endogenous production, was delivered through the base of the lung using a unidirectional rotameter and obtaining a concentration of around 25 ppb. The sample of gas was recorded through a 6 F arterial catheter introduced into the endotracheal tube to its tip. The ventilator used was a Babylog 8000. Air delivered was compressed and filtered and had an NO content of under 0.3 ppb. The NO level assessed was the plateau value given by the software of the Sievers NOA apparatus. Each experiment involved sampling during 1 min, three times. Normalization was done using a multiple cubic regression formula. Results: An increase in respiratory frequency or in peak of inspiratory pressure were accompanied by a decrease in eNO (ppb). Minute volume was adjusted for the percentage of leakage given by the ventilator. Normalization was obtained analyzing 518 respirations with different ventilatory settings. The coefficient of variation fell from 15.5% to 0.27%. Validation of the normalization formula was performed in other three groups (320, 372, and 372 respirations) with different simulated NO concentrations (25, 16, and 50 ppb), resulting in reduction of the coefficient of variation from 42.7% to 9.3%, from 42.3% to 10.6% and from 45.2% to 9.6%, respectively. Conclusions: Normalization of simulated minute eNO according to ventilatory settings is possible using the equipment and experimental set-up reported. Extrapolation to patients is not possible without constraints. Pediatr Pulmonol. 2008; 43:1167–1174.

Key words: exhaled nitric oxide; premature infants; newborn; mechanical ventilation; bronchopulmonary dysplasia.

INTRODUCTION

Improved survival among the more immature preterm infants has led to a rise in bronchopulmonary dysplasia (BPD), a chronic lung disease1 that increases length of hospital stay and causes greater morbidity and mortality of respiratory origin. The physiopathological changes involved in BPD include epithelial and endothelial damage, increased vascular permeability, surfactant inactivation, and activation of alveolar neutrophiles and macrophages. The cellular composition of tracheal aspirate is the gold standard for defining lung inflammation.2 Due to the activation of alveolar macrophages, greater expression of inducible NO-synthase is to be expected, with elevation of nitrites and nitrates in endotracheal aspirate and of minute exhaled nitric oxide (NO). Exhaled NO (eNO) is raised in adults3 and asthmatic children.4 It has also been detected among the gases exhaled by

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neonates receiving mechanical ventilation. If BPD appears in the evolution of a preterm survivor, it can be expected that eNO will already be raised in the first days of life, when these preterm infants are usually intubated and receive mechanical ventilation. Recently Roiha et al. and Williams et al. have reported different results in eNO in BPD: a lower NO output in infants breathing spontaneously and higher exhaled NO peak levels in ventilated infants, respectively. These differences may be also due to different ways of measuring eNO. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide should be followed, but no clear standard on analysis of eNO in ventilated preterm infants are reported.

Williams et al. analyzed the influence of ventilatory settings on the measurement of eNO levels and they noticed that a rise of inspiratory pressure, of ventilator rate or of FiO₂ implied a reduction in the NO peaks. They suggested the need to standardize these values, but without indicating how this should be carried out. These authors stated that the ideal point for sampling was at the tip of the endotracheal tube. Their experiment dealt with NO levels of over 2,000 ppb, whereas in neonatal pathology levels do not usually exceed 20 ppb.

The present experimental work is an attempt to duplicate the work by Williams et al. but adapted to clinical reality, by studying the eNO obtained at the end of the plateau which occurs during expiration, and working with NO levels below 20 ppb. We also attempt to normalize the eNO figures according to different ventilatory settings, and expressing the results as minute eNO.

**MATERIALS AND METHODS**

The experimental design is shown schematically in Figure 1.

**Lung Model**

The lung used has a baseline capacity of 50 ml, which rises when it is subjected to pressure (inspiration), returning to its baseline on account of its elasticity. A 3 mm endotracheal tube is introduced into the lung through a narrow orifice.

**Simulated Exhaled Nitric Oxide**

The NO which simulates that of endogenous production is delivered through the base of the lung using a unidirectional rotameter, 0.1 L/min of NO at 100 ppb from a gas cylinder (Abelló Linde NO 100 ppb). This flow produces a concentration inside the lung of around 25 ppb of NO, similar to the highest levels found in ventilated preterm infants in a clinical context.

**Ventilator: Equipment and Ventilatory Settings**

The ventilator used was a Babylog 8000 (Dräger Medical Hispania, S.A., Barcelona, Spain), specifically designed for premature infants. The IPPV mode was employed, that is, totally controlled intermittent positive pressure. This mode of operation has a continuous flow of gas (6 L/min) and preset limit of inspiration pressure, so that the volume delivered per minute varies depending on the pressure limit set and the distensibility of the lung (fixed in the experiment). In the experimental model,
airway resistance is not taken into account. Oxygen used is NO-free. Air delivered is compressed and filtered (with active carbon), and has an NO content of under 0.3 ppb. Ventilatory settings used in different experiments included two constant parameters (Positive end expiratory pressure = PEEP = 5 cm water; Inspiratory time/Expiratory time = I/E = 1/2) and three parameters that changed within a range habitual in clinical practice (Fraction of inspiratory oxygen = FiO2 = 0.21–1; Peak of inspiratory pressure = PIP, 10–30 cm water; Respiratory frequency = RF, 30–50 respirations/minute).

**NO Sampling: Equipment Specifications**

The sample of gas in which NO is analyzed is obtained through a “6 French” arterial catheter introduced into the endotracheal tube to its tip. This catheter is the narrowest that permits adequate aspiration of the gas sample without generating an alarm in the Sievers NOA apparatus. Furthermore it occupies very little of the endotracheal cross-sectional area, and hence does not affect ventilation of the lung. Sievers NOA 280i is able to detect from 0.5 ppb to 500 ppm of NO, with a sensitivity of 0.5 ppb, aspirating a gas sample of 10–200 ml/min (100 ml/min in our experiments) and with a lag time of 1 sec and an electronics response time of 0.067 sec (sampling rate of 15 Hz) to 90% full scale. This high speed means the apparatus can differentiate between inspired gas (contaminated with compressed air) and expired gas, which adopts the form of a plateau.

**Calculation of Plateau NO and Minute Exhaled NO**

The NO level assessed is not the highest peak, but rather the plateau value given by the software of the Sievers NOA apparatus, and which is the most reliable equivalent to the NO exhaled during expiration (corresponds to the average NO detected between 1/2 and 7/8 of the duration of expiration). Minute exhaled NO or Minute excretion of NO was calculated using the formula:

\[
V'_{NO} (\text{nl/min}) = \text{Plateau NO (ppb = nl/L)} \times V_E \text{ (minute ventilation in L/min)}
\]

\[V_E (\text{L/min}) = \frac{\text{Volume per minute (L/min)}}{(100 - \text{leakage})/100}
\]

Each experiment, with its three or four subgroups according to different ventilatory settings, involved sampling during 1 min, repeated 3 times, for each subgroup.

**Experiment 1**

The baseline ventilation parameters were: FiO2 = 0.21, PEEP = 5 cm water, I/E = 1/2, PIP = 20 cm water, RF = 50 respirations/min. These ventilatory settings were changed while eNO was determined.

**Experiment 2**

We analyzed the influence of sample aspiration of NOA Sievers apparatus on ventilatory parameters. Aspiration was done for obtaining the sample in which NO was determined. We recorded the volume per minute (VM) in L/min, the tidal volume (TV) in ml/respiration, and leakage (%), to calculate the minute exhaled NO.

An attempt to normalize results for “minute exhaled NO” was also done. The objective of the normalization was to apply a formula which allows calculating the “normalized minute exhaled NO” based on an exhaled NO obtained with particular values of VM, PIP and RF. The following premises were considered: (1) The values of minute eNO would be relativized by the maximum eNO obtained in each experiment, which obviously would never be falsely high. (2) The normalization would obtain a formula that reduces the differences in minute eNO between the groups with different ventilatory settings. (3) The normalized experimental value of minute exhaled NO would be obtained under ventilation with the basic settings for a ventilated preterm infant (FiO2 = 0.21, PEEP = 5 cm water, I/E = 1/2, PIP = 20 cm water, RF = 50 respirations/min). The normalization formula was obtained calculating all possible multiple linear and nonlinear regressions and choosing the variables with \( P < 0.05 \). To check if the results obtained applying normalization were more uniform, the formula was used in 518 respirations.

**Experiment 3**

We tried to validate the normalization formula. It was similar to Experiment 2, but using different ventilatory settings in three groups with distinct lung simulated exhaled NO concentrations (25, 16, and 50 ppb). Three hundred twenty, 372, and 372 respirations were analyzed, respectively.

**Statistical Analysis**

Continuous variables are presented as mean ± standard deviation (SD) if normally distributed or as median and interquartile range (IQR: 25th–75th percentiles) if not normally distributed. All continuous variables were normally distributed with the exception of some normalized minute exhaled NO in experiments 2 and 3. The influence of NO in compressed air on eNO was analyzed by means of Pearson linear correlation. One-way analysis of variance and the Scheffe test were performed to compare plateau NO, volume per minute and minute eNO.
among the different groups. Kruskal–Wallis and Mann–Whitney U-tests were used in case of not normally distributed variables. Different types of multiple regressions between deviation of minute eNO, VM, PIP, plateau NO and RF were considered: linear, quadratic and cubic. The normalization formula was obtained from the analysis of 518 respirations calculating all possible multiple linear and nonlinear regressions and choosing the variables with \( P < 0.05 \). Validation of the normalization formula was performed in three groups with different lung simulated exhaled NO concentrations, including 320, 372, and 372 respirations, respectively. Validation was accepted as valid if the coefficient of variation (Standard deviation \( \times 100/Mean \)) was below 10%.

RESULTS

Influence of NO in Compressed Air on Simulated Exhaled NO (Experiment 1)

Compressed air was almost NO-free (concentrations of NO between 0.1 and 0.4 ppb). Exhaled NO (eNO) concentrations (ppb) were not correlated with NO in compressed air (\( n = 1,004; r = 0.011; P = 0.719 \)). Therefore, eNO values were not influenced by NO in compressed air.

Influence of \( \text{FiO}_{2} \), RF, and PIP on Simulated eNO (Experiment 1)

An increase in \( \text{FiO}_{2} \) was accompanied by an increase in eNO, especially for very high oxygen concentrations. An increase in RF or PIP was accompanied by a decrease in eNO. Moreover, the variation with \( \text{FiO}_{2} \), although statistically significant, had little clinical relevance (10.7 ppb with \( \text{FiO}_{2} = 0.21 \) and 11.3 ppb with \( \text{FiO}_{2} = 1 \)) (Table 1).

Influence of Sample Aspiration of NOA Sievers Apparatus on Ventilatory Parameters (Experiment 2)

The results (Table 2) were as expected: aspiration of the volume of the sample decreases the volume recovered by the ventilator, this being interpreted as an increase in leakage. However, if the mathematical calculations to normalize the above data are done assuming leakage was 0%, the results obtained are also shown in Table 2. The figures are practically the same, indicating that they should always be adjusted for the percentage of leakage, normalizing for non-existent leakage (i.e., 0%).

Influence of Different Ventilatory Settings on Simulated Minute Exhaled NO (Experiment 2)

The results (Table 3) confirmed what had been expected: lower PIP corresponded to lower volume per minute and higher eNO; lower RF corresponded to lower volume per minute and higher eNO. However, the figures for minute exhaled NO were not similar under all four conditions, even though the amount of NO delivered to the lung during the experiment was always constant. For this reason results for minute exhaled NO in ventilated neonates should always be normalized according to ventilatory settings employed.

Attempt to Normalize Simulated Minute Exhaled NO According to Ventilatory Settings (Experiment 2)

The normalization aimed at obtaining a formula that reduced the differences in minute eNO among the groups with different ventilatory settings. The first group is the basic one for a ventilated preterm infant: \( \text{FiO}_{2} = 0.21 \), PEEP: 5, I/E: 1/2, PIP: 20, RF: 50. The normalization took

| TABLE 1— Influence of \( \text{FiO}_{2} \), RF and PIP on Exhaled NO in Experiment 1 |
|---------------------------------|-----------------|-----------------|
| Influence of \( \text{FiO}_{2} \) on exhaled NO (ppb) |
| 1. \( \text{FiO}_{2} = 0.21 \) | 141 | 10.73 | 0.64 |
| 2. \( \text{FiO}_{2} = 0.4 \) | 142 | 10.74 | 0.81 |
| 3. \( \text{FiO}_{2} = 1 \) | 145 | 11.29 | 0.74 |
| One-way/Scheffé (\( P < 0.05 \)) | \( P < 0.001; 1–3, 2–3 \) |
| Influence of RF on exhaled NO (ppb) |
| 1. RF of 30 respirations/min | 86 | 16.88 | 0.94 |
| 2. RF of 50 respirations/min | 141 | 13.43 | 0.73 |
| One-way | \( P < 0.001 \) |
| Influence of PIP on exhaled NO (ppb) |
| 1. PIP of 10 cm water | 83 | 20.82 | 0.84 |
| 2. PIP of 20 cm water | 120 | 12.25 | 0.93 |
| 3. PIP of 30 cm water | 146 | 9.20 | 0.75 |
| One-way/Scheffé (\( P < 0.05 \)) | \( P < 0.001; 1–2, 1–3, 2–3 \) |

RF, respiratory frequency; PIP, peak of inspiratory pressure (cm water).

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into account the following formulas to calculate “minute eNO deviation” and to obtain the “normalized minute eNO”:

\[
\text{Minute eNO without leakage} = \frac{\text{plateau NO} \times \text{Volume per minute}}{100 - \text{leakage}} \times 100
\]

\[
\text{Minute eNO deviation} = \text{Minute eNO without leakage} - \text{maximum minute eNO from experiment}
\]

Calculation of the multiple linear and nonlinear regressions for minute eNO deviation. Initially we analyzed four cubic regressions simultaneously: Minute eNO deviation versus eNO, VM, PIP, and RF. Global R² was 0.998. The multiple cubic regression formula that included all the variables with \( P < 0.05 \) was:

\[
\text{Minute eNO deviation} = -173.668 \times \left( \frac{\text{eNO}}{100} \right)^3 + 49.513 \\
- 12.486 \times \left( \frac{\text{VM}}{100} \right)^3 + 24.075 \times \left( \frac{\text{PIP}}{100} \right) \\
+ 12.371 \times \left( \frac{\text{RF}}{100} \right)^3 - 8.126 - \text{maximum minute eNO}
\]

Normalized minute eNO = minute eNO without leakage – minute eNO deviation

Applying this formula to the 518 respirations analyzed, we obtained the results shown in Table 4. The large differences in minute eNO among the four groups have practically disappeared. After normalization the coefficient of variation has fallen from 15.5% to the virtually insignificant figure of 0.27%.

**Validation of the Normalization Formula (Experiment 3)**

Applying the formula for normalization to the other three groups with different lung simulated exhaled NO concentrations (320, 372, and 372 respirations analyzed, respectively) yielded the results shown in Table 5, in comparison to the initial data for each group. The large differences among the three subgroups in each group have been notably reduced. The coefficient of variation has fallen from 42.7% to 9.3%, 42.3% to 10.6%, and 45.2% to 9.6% in each group, respectively. It may be assumed that the proposed formula would also be useful for other sets of ventilatory parameters.

**DISCUSSION**

Exhaled nitric oxide output depends on ventilatory settings used. It is possible to normalize these values using the formula reported in the present study. BPD in former premature infants is associated with an inflammatory response. Nitrate concentration in bronchoalveolar lavage fluid was found to be higher in infants 14 days old who developed BPD. Roiha et al. have recently described a lower nitric oxide output in premature infants with chronic lung disease and breathing spontaneously at 44 weeks postconceptional age. Williams et al. did not find in these mechanically ventilated infants higher exhaled NO peak levels in the first week after birth. These different results may be due to the different postnatal age of the infants and to the different ways of evaluating the exhaled NO (breathing spontaneously or mechanically ventilated, output or minute ventilation obtained using plateau NO values or peak or maximum values of exhaled NO). Olsen et al. reported that minute excretion

### Table 2—Influence of Aspiration of Apparatus on Ventilatory Parameters in Experiment 2

<table>
<thead>
<tr>
<th></th>
<th>No aspiration</th>
<th>During aspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without correction for losses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume per minute (L/min)</td>
<td>0.35</td>
<td>0.30</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td>7.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Losses (leakage) (%)</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>With correction for losses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume per minute (L/min)</td>
<td>0.40</td>
<td>0.39</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td>8.33</td>
<td>8.27</td>
</tr>
<tr>
<td>Losses (leakage) (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3—Influence of Different Ventilatory Settings on Minute Exhaled NO in Experiment 2

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>eNO Plateau</th>
<th>Vol/minute</th>
<th>Minute eNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PIP = 20, RF = 50</td>
<td>144</td>
<td>15.87 ± 0.81</td>
<td>0.30 ± 0.00</td>
<td>4.76 ± 0.24</td>
</tr>
<tr>
<td>2. PIP = 15, RF = 50</td>
<td>143</td>
<td>20.18 ± 0.93</td>
<td>0.19 ± 0.01</td>
<td>3.83 ± 0.11</td>
</tr>
<tr>
<td>3. PIP = 20, RF = 40</td>
<td>115</td>
<td>16.71 ± 0.83</td>
<td>0.24 ± 0.00</td>
<td>4.01 ± 0.20</td>
</tr>
<tr>
<td>4. PIP = 15, RF = 40</td>
<td>116</td>
<td>20.76 ± 0.81</td>
<td>0.15 ± 0.00</td>
<td>3.11 ± 0.12</td>
</tr>
</tbody>
</table>

One-way/Scheffé (\( P < 0.05 \))

- \( P < 0.001 \); 1–2, 1–3, 1–4, 2–3, 2–4, 3–4
- \( P < 0.001 \); 1–2, 1–3, 1–4, 2–3, 2–4, 3–4
- \( P < 0.001 \); 1–2, 1–3, 1–4, 2–3, 2–4, 3–4

Mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Vol/minute</th>
<th>Minute eNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PIP = 20, RF = 50</td>
<td>144</td>
<td>0.30 ± 0.00</td>
<td>4.76 ± 0.24</td>
</tr>
<tr>
<td>2. PIP = 15, RF = 50</td>
<td>143</td>
<td>0.19 ± 0.01</td>
<td>3.83 ± 0.11</td>
</tr>
<tr>
<td>3. PIP = 20, RF = 40</td>
<td>115</td>
<td>0.24 ± 0.00</td>
<td>4.01 ± 0.20</td>
</tr>
<tr>
<td>4. PIP = 15, RF = 40</td>
<td>116</td>
<td>0.15 ± 0.00</td>
<td>3.11 ± 0.12</td>
</tr>
</tbody>
</table>

Mean ± SD.

PIP, peak of inspiratory pressure (cm water); RF, respiratory frequency; eNO, exhaled NO (ppb); Vol/minute, Volume per minute (L/min); minute eNO, minute exhaled NO (ppb/minute).

Not corrected by % of leakage.
of NO (divided by the weight) in premature infants with respiratory distress syndrome and mechanically ventilated at 24 hr of life was higher than in controls (0.405 nl/min/kg vs. 0.099 nl/min/kg; \( P < 0.001 \)). In our experimental work we have chosen to show the results as “minute exhaled NO” (in nl/min), a simulation of endogenous NO production of bronchoalveolar origin.

We have followed from the outset the recommendations of Williams et al.\(^{14} \) to position the catheter used for obtaining samples with its end at the tip of the endotracheal tube, in order to avoid the dilution effect of the ventilator gases. In Experiment 1, the changes in the peak of inspiration pressure and in the frequency of respiration have repeated the findings already published by Williams et al.,\(^{14} \) because a rise in either of them causes an increase in the volume per minute and a decrease in the eNO due to a dilution effect. However, in our study the changes related to FiO\(_2\) were minimal. Williams et al.\(^{14} \) explain their finding of a fall in eNO when FiO\(_2\) increases as probably due to a higher conversion of NO to NO\(_2\).

In their work the supply of NO to the experimental bag yielded 2,857 ppb, whereas our design yielded around

### Table 4—Normalization of the Results of Experiment 2

<table>
<thead>
<tr>
<th>Lung NO: 25 ppb</th>
<th>n</th>
<th>Minute eNO</th>
<th>Minute eNO corrected by leakage</th>
<th>Normalized minute eNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PIP = 20, RF = 50</td>
<td>144</td>
<td>3.09 ± 0.55</td>
<td>3.96 ± 0.70</td>
<td>5.26 (4.95–5.51)</td>
</tr>
<tr>
<td>2. PIP = 10, RF = 50</td>
<td>111</td>
<td>1.18 ± 0.29</td>
<td>2.35 ± 0.58</td>
<td>4.80 (4.08–5.01)</td>
</tr>
<tr>
<td>3. PIP = 20, RF = 30</td>
<td>85</td>
<td>2.01 ± 0.16</td>
<td>2.61 ± 0.21</td>
<td>5.18 (4.91–5.31)</td>
</tr>
<tr>
<td>All</td>
<td>320</td>
<td>2.14 ± 0.91</td>
<td>3.04 ± 0.93</td>
<td>5.08 (4.80–5.30)</td>
</tr>
</tbody>
</table>

One-way/Scheffé (\( P < 0.05 \))

Coefficient of variation 42.7%

Mean ± SD; Median (IQR).

PIP, peak of inspiratory pressure (cm water); RF, respiratory frequency; eNO, exhaled NO (ppb); minute eNO, minute exhaled NO (ppb/minute); NS, not significant.

*Kruskal–Wallis/Mann–Whitney U-test (\( P < 0.05 \)).

### Table 5—Validation of the Proposed Normalization in Experiment 3

<table>
<thead>
<tr>
<th>Lung NO: 16 ppb</th>
<th>n</th>
<th>Minute eNO</th>
<th>Minute eNO corrected by leakage</th>
<th>Normalized minute eNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PIP = 20, RF = 50</td>
<td>144</td>
<td>2.60 ± 0.29</td>
<td>3.45 ± 0.37</td>
<td>4.57 ± 0.06</td>
</tr>
<tr>
<td>2. PIP = 10, RF = 50</td>
<td>144</td>
<td>0.93 ± 0.07</td>
<td>2.01 ± 0.14</td>
<td>5.20 ± 0.21</td>
</tr>
<tr>
<td>3. PIP = 20, RF = 30</td>
<td>84</td>
<td>2.11 ± 0.15</td>
<td>2.76 ± 0.21</td>
<td>5.96 ± 0.03</td>
</tr>
<tr>
<td>All</td>
<td>372</td>
<td>1.84 ± 0.77</td>
<td>2.74 ± 0.68</td>
<td>5.13 ± 0.54</td>
</tr>
</tbody>
</table>

One-way/Scheffé (\( P < 0.05 \))

Coefficient of variation 42.3%

Mean ± SD; Median (IQR).

PIP, peak of inspiratory pressure (cm water); RF, respiratory frequency; eNO, exhaled NO (ppb); minute eNO, minute exhaled NO (ppb/minute).

*Kruskal–Wallis/Mann–Whitney U (\( P < 0.05 \)).
25 ppb (100 times less). Perhaps this explains why no substantial fall in eNO was found, when increasing FiO2, suggesting that it would have little importance in the clinical setting.

One difficulty when attempting to analyze exhaled NO of bronchoalveolar origin is the risk that the determination could be invalidated due to NO contained in the gas inhaled. This is not the case when using the “breath program” software which accompanies the Sievers Nitric Oxide Analyzer (NOA 280i) in the mode designated “use pressure for breath detection mode,” and with “on-line sampling.” It is considered that the gas in the region between 1/2 and 7/8 of the duration of expiration is equivalent to the third and fourth quartiles of the expiration time, when the gas obtained is exclusively bronchoalveolar,16 with no possibility of external contamination. The third quartile of expiration shows the lowest breath-to-breath variability of the flow.16 However, the mean flow during the third quartile is higher than during the entire expiration, and this should be taken into account when results are compared with the existing literature. We do not use this way of calculation reported by Hall et al.16 in newborn infants breathing spontaneously. The manufacturers of the NOA 280i only recommend the use of medicinal air (NO-free) when environmental NO levels exceed 40–50 ppb. Our compressed air NO levels never reached these values. In Experiment 2 it was verified that the variable Volume per Minute was highly influenced by the percentage of leakage given by the ventilator, although this can be corrected mathematically, and indeed this should always be done. In our work, minute exhaled NO was obtained multiplying the plateau NO value by the volume per minute, as Olsen et al.8 reported, but volume per minute was previously corrected by the percentage of leakage.

The proposed normalization formula is capable of eliminating differences in minute eNO figures obtained with different ventilatory settings, both in Experiment 2 as in Experiment 3, which constitutes a validation of the technique (coefficients of variation of 9.3%, 10.6%, and 9.6%).

The main feature of our study is the possibility to normalize “simulated minute exhaled NO” by means of a formula which includes the ventilatory parameters used. The volume per minute is corrected by taking into account the percentage of leakage. Although in our experiment the leakage of gas around the endotracheal tube is almost zero, the sample of gas aspirated by the analyzer is considered as a leak by the ventilator. Another important point is the similarity between the ventilatory parameters analyzed in the experiments and those used in clinical practice when a preterm infant has to be mechanically ventilated. However, we have not corroborated our findings by duplicating the experiments using different equipment for the management of exhaled NO.

Our results lead us to suggest the following points when collecting gas samples from ventilated premature infants to obtain minute exhaled NO: (1) It would suffice to collect exhaled NO during 1 min, although it is recommended to repeat collection 3 times.8 (2) For each sample collection, the parameters to record should be: FiO2, PIP, PEEP, RF, I/E, Volume per minute and percentage of leakage (the two latter ones, every 15 sec during collection). (3) The results should be expressed as “exhaled NO” (ppb plateau value), “minute exhaled NO after correction for leakage” (exhaled NO × Volume per minute = nl or ppb/min) and “normalized minute exhaled NO after correction for leakage.” However, in real life, the association between ventilatory setting and washing NO out of the lungs is dependent on other factors, such as, for example, body size and underlying disease. Therefore extrapolation of the results of the model used in this study to patients is not possible without constraints.

We conclude that normalization of simulated minute exhaled NO according to ventilatory settings is possible using the particular equipment and experimental set-up reported in the present study. Extrapolation of the results of the model used in this study to patients is not possible without constraints.

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