Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions

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During the last year exhaled nitric oxide (NO) has been proposed as a marker of airway inflammation. More knowledge of the production and transfer of this molecule are needed in order for NO analysis to become a clinical tool. This was the aim of the study.

Exhaled NO values from multiple flow rates were used to model alveolar NO, transfer rate and tissue concentration of NO in the airways. Three flows rates, 0-005, 0-1 and 0-5 l sec^{-1} were found to be optimal. The NO transfer rate of the airways was 9±2 ml sec^{-1}, the tissue source was 75±28 ppb and the alveolar fraction of NO was 2±1 ppb in 10 healthy subjects (mean±CI95%).

In conclusion, we have shown that it is possible to get more information about the distribution of NO in the lungs and the airways than only a single value from one expiratory flow rate can give. Further studies will reveal if this airway modelling can be useful in disease of the respiratory system.

Key words: nitric oxide; human; respiratory system; asthma; smokers.


Introduction

During the past few years, the interest in monitoring exhaled nitric oxide (NO) has been increasing dramatically after it was proposed as a promising non-invasive tool in the diagnosis of asthma and other airway inflammatory disease. Early studies showed elevated fractions of NO (F_{E_{NO}}) in subjects with asthma (1,2). After more understanding had been achieved about the strong flow dependency of exhaled NO (3,4), as well as the techniques to separate the contributions from the nasal cavity and lower airways (3,5), it became evident that the results presented so far may not be comparable. The need to standardize the measurement procedures was realized and a recommendation of The European Respiratory Society Task Force on 'Measurements of nitric oxide in exhaled air' was published (6). The more recent research complying with the recommended measurement procedures has already been starting to show results that are contradictory (7). This seems to indicate that the link between NO output and lung or airway disease is much more complex than expected (8,9).

Exogenous inhaled NO selectively dilates pulmonary vessels in ventilated lung regions. In this way ventilation-perfusion matching can be modulated to improve oxygenation and lower pulmonary artery pressure in patients with hypoxaemia (10,11) and pulmonary hypertension (12,13). The NO contribution from the nose, and especially the sinus cavities, has been shown to be high (14). Therefore, not only exogenous NO but also endogenous NO may increase oxygenation of the blood. It has been reported that there was an increase in oxygen tension during nose breathing compared to mouth breathing (15). We therefore feel that appropriate attention should be given to the role of NO from the nose cavity during normal breathing when searching for the potential diagnostic value of the NO measurements. In this paper we want to present a novel approach for the measurement and the calculation of the NO derived in the nasal cavity utilizing simple procedures complementary to and in compliance with the ones recommended for the measurements of exhaled NO from the lower airways.

We also want to demonstrate that it is possible to utilize a simplified diffusion based modelling for the NO output to the airways during oral breathing during constant flow manoeuvres. For the basis of the experimental work we wanted to revisit the issue of the inhalation manoeuvre preceding the exhalation measurements. Inspiration to total lung capacity (TLC) is the recommended breathing manoeuvre, i.e. vital capacity (VC) manoeuvre, for attaining the plateau concentration of F_{E_{NO}} (6). This manoeuvre is difficult to achieve for subgroups of patients such as small children, patients with severe lung disease and in the elderly. In addition, repeated spirometry in asthmatics results in reduced F_{E_{NO}} (16). We therefore compared the F_{E_{NO}} from the VC manoeuvre with that of a deep inhalation, which is easier to perform.
Methods

EXPERIMENTS

Study population

Healthy volunteers (n = 52, 27 men and 25 women) were included in this study. They were non-smokers, and none had a history of respiratory infection during the previous month or any allergic symptoms. In addition, subjects with a diagnosis of asthma (n = 5, two men and three women) treated with inhaled steroids and smokers (n = 5, all women) with no history of respiratory infection during the previous month were investigated. All subjects were told not to eat or drink 2 h before the study occasion.

Analysing equipment

NO and end tidal CO2 concentrations, flow rate and airway pressure were measured with a computer-based single-breath NO system from Nitrograf AB, Hässleby, Sweden. Included in this system are a Sievers NOA 280 chemiluminescence analyser (Sievers, Boulder, CO, U.S.A.) and an infrared CO2 analyser. The sampling rate of the NO system was adjusted to 200 ml min⁻¹. The repeatability of the system was < 1 ppb with a response time of < 200 msec. The system was calibrated using a mixture of 460 ppb NO in nitrogen (AGA AB, Lidingö, Sweden) and the zero was set by feeding synthetic air (AGA AB) into a 2 l canister filled with Purafil II chemisorbant with purakol (Lindair AB, Ljusne, Sweden). Care was taken that the sampling system were done daily and the zero was controlled before each measurement. The temperature of the photomultiplier tube of the analyser differed less than 1°C from the temperature at which the calibration was performed. Since the NO signal is expiratory flow dependent (3,4) the NO system also includes measurements of expiratory flow rate. The flow sensor, D-liteTM (Datex-Ohmeda, Helsinki, Finland) was calibrated in the range of 0–0.51 sec⁻¹ (Dry Cal DC-2 flow calibrator, BIOS International, Pompton Plains, NJ, U.S.A.) Controls of calibration and flow rate of the sampling system were done daily and the zero was controlled before each measurement.

The subjects were instructed to perform a deep inhalation, except in protocol (a), and exhale with a constant flow. To facilitate for the volunteers to keep a constant expiratory flow, a resistance (Model #7100R 20, Hans Rudolph, Inc., Kansas City, MO, U.S.A.) was fitted onto the expiratory side of the two-way, non-rebreathing valve (Model 1410, Hans Rudolph). The expiratory pressure was 5 cm H₂O or above, but always less than 20 cm H₂O, to exclude NO from the nose cavity (3,5). Some volunteers needed increased resistance to be able to keep a constant expiratory flow and in these cases a higher resistance was used (Model #7100R 50, Hans Rudolph). The expiratory pressure has been shown not to influence the NO concentrations (3,4). The NO value was taken when there was a steady plateau longer than 3 sec. A mean value of three breaths was used for statistical analysis. The concentration of NO is expressed as FENO in ppb and the volume of exhaled NO is expressed as VENO in ml min⁻¹. VENO was calculated by multiplying the FENO plateau values with the expiratory flow rate.

Experimental procedure

(a) In six subjects the recommended procedure to measure exhaled NO (6), i.e. after a VC-manoeuvre, was compared to a deep inhalation. After each VC-manoeuvre the subject rested > 30 sec but < 1 min. The exhalation flow rate was 0.11 sec⁻¹. The time to achieve a plateau and the plateau value of exhaled NO was determined for the two different procedures.

(b) To establish the contribution of NO from the nasopharynx in relation to the exhaled NO from the tracheobronchial tree, FENO was investigated in 10 female and 10 male healthy subjects. FENO was measured at five different expiratory flow rates in the range of 0.05–0.321 sec⁻¹ during mouth breathing and nose breathing into mask covering the nose (CPAP mask S, Resperonic, Medela Medical AB, Täby, Sweden).

(c) In another eight healthy volunteers we tested whether it was possible to isolate the NO contribution from the nose. NO was measured in the pharynx at an inspiratory and expiratory flow rate of 0.115 l sec⁻¹ during nasal breathing. NO was sampled in the pharynx using an artificial airway no. 2 or 3 (Portex, Nerck-sur-Mer, France) where a channel had been made for the connection of the NO probe. To compare these values with NO levels during nasal exhalation and mouth exhalation we can utilize the requirement of the mass balance. The measured NO output from nasal exhalation needs to equal the sum of the NO output from the mouth and the net NO output from the nasal cavity. The output equals the flow multiplied by the concentration, and since the flow remaining the same on both sides of the equation it is cancelled out:

\[
F_{\text{NO-nose}} = F_{\text{NO-mouth}} + F_{\text{NO-nose}}
\]

By rearranging this for the net NO concentration that the nose adds to exhalation

\[
F_{\text{NO-nose}} = F_{\text{NO-nose}} - F_{\text{NO-nose}}
\]

The same procedure as applied to inhalation combines the NO content inhaled and the net output from the nasal cavity to the NO content measured at the pharynx as follows:

\[
F_{\text{NO-pharynx}} = F_{\text{NO-ambient air}}
\]

The range of ambient NO concentrations during these measurements were 0.2–2.4 ppb.
THEROY

Modelling of the lower airways

The entire tracheobronchial tree is assumed to be lumped into a single cylindrical tube with a constant inner radius \( r \) and length \( L \). A barrier layer of thickness \( d \) covers the inner wall of the airway lumen. The NO is generated in and temporarily stored by, the lumped single tissue layer and diffused through an aqueous barrier to the airway lumen. A portion of the generated NO will be diffused to the opposite direction and scavenged by the blood circulating in the tissue.

The gas enters the tube at constant volume flow \( \dot{Q} \) and has a NO concentration of \( F_{in NO} \). Let’s assume that the Fick’s law of diffusion controls the radial transfer rate of the NO from the source layer through the barrier layer:

\[
J_{NO} = \frac{kA_r}{d} (F_{in NO} - F_{NO}(x))
\]  

where \( J_{NO} \) is the volumetric transfer rate of NO, \( k \) the diffusion coefficient of NO in the barrier layer, \( d \) the thickness of the layer and \( A_r \) the area of the excreting surface element. The concentration equivalent to the partial pressure of NO in the wall source layer is

\[
F_{NO}(x) = F_{WNO} - (F_{WNO} - F_{INO}) e^{-\frac{\tau_{NO}}{Q^2}}
\]  

Then the exhaled NO concentration \( F_{out NO} \) at the exit of the tube is

\[
F_{out NO} = F_{INO}(L) = F_{WNO} - (F_{WNO} - F_{INO}) e^{-\frac{\tau_{NO}}{Q^2}}
\]  

The solution for the boundary condition \( F_{NO}(0) = F_{in NO} \) is

\[
F_{NO}(x) = F_{WNO} - (F_{WNO} - F_{INO}) e^{-\frac{\tau_{NO}}{Q^2}}
\]  

The rate of the NO volume released from a compartment \( (V_{NO}) \) is defined as NO output and is obtained by multiplying the concentration by flow:

\[
\dot{V}_{E NO} = F_{out NO} \cdot \dot{Q}
\]

\[
\dot{V}_{E NO} = (F_{INO} e^{-\frac{\tau_{NO}}{Q}} + F_{WNO} (1 - e^{-\frac{\tau_{NO}}{Q}})) \cdot \dot{Q}
\]

At high enough flows of \( \dot{Q} \) (when \( \dot{Q} >> D_{WNO} \), \( e^{-\dot{Q}_{NO}/\dot{Q}} = 1 - D_{WNO}/\dot{Q} \) and (9) can be written as:

\[
\dot{V}_{E NO} = (F_{INO} - F_{WNO}) D_{WNO} + F_{INO} \dot{Q}
\]

This equation represents a straight line with a slope of \( F_{INO} \) and offers an alternative way to determine the alveolar NO concentration if enough data points of \( \dot{V}_{E NO} \) vs. \( \dot{Q} \) are measured at high flows to find the slope of the line. This approach was also suggested by Tsoukias et al. (17).

STATISTICAL ANALYSIS

The t-test for paired samples and ANOVA for repeated measurements were used to compare data within the groups at different flow rates. The Tukey honest significant difference test was used for post hoc comparisons and probability values were calculated. For correlation the Spearman rank correlation coefficient was used. For all statistical calculations the Statistica/w 5.0 software package (StatSoft Inc., Tulsa, OK, U.S.A.) was used. Results are given as mean values and \( \pm 95\% \) confidence interval (CI95\%) in text, tables and figures.

Results

The subjects all performed well and the expiratory flow rates in the different protocols were less than \( \pm 10\% \) of the target flow.
Performing a VC manoeuvre, as in protocol (a), resulted in a $F_{ENO_{c_{tr}}}$ of 12.1 ± 4.7 ppb. The time to reach a plateau after a VC manoeuvre was 16.4 ± 3.7 sec. The corresponding values after a deep breath were 12.4 ± 4.7 ppb and 7.7 ± 2.7 sec. There was no statistical difference in $F_{ENO_{c_{tr}}}$ between the manoeuvres, but the time to reach a plateau was significantly longer ($P<0.01$) for the VC manoeuvre.

In protocol (b) the $F_{ENO_{n_{25}}}$ was 4.2 ± 1.2 ppb and $F_{ENO_{n_{1}, n_{1}}}$ was 8.3 ± 2.5 ppb. There was no difference between the exhaled NO in regards to gender and no correlation to age, height or body weight. During mouth breathing, the $F_{ENO_{c_{tr}}}$ was dependent of the expiratory flow ($r = -0.71$, $P<0.001$). To obtain the nasopharyngeal contribution to the expired plateau value the mouth values have to be subtracted according to equation (i). The calculated $F_{NO_{nose}}$ values from the nasopharynx were also flow dependent, $r = -0.71$, $P<0.001$. The $V_{ENO_{c_{tr}}}$-mouth and $V_{NO_{nose}}$-nose was significantly different for different flow rates ($P<0.001$). (Fig. 1). There was also a significant difference between the $V_{ENO_{c_{tr}}}$ and the $V_{NO_{nose}}$-nose ($P<0.001$). The $V_{NO_{c_{tr}}}$-nose was 4.4 ± 4.9 times higher than that from the tracheobronchial tree at the tested flow rates 0.05 ± 0.32 lsec$^{-1}$. There was no statistically significant difference ($P=0.75$) between the ratios measured at these flows.

In protocol (c) the measurements of NO obtained in the pharynx were used for calculations of the net NO from the nasal cavity. Applying the equations (i) and (ii) resulted in a $F_{ENO_{n_{25}}}$-nose of 36.6 ± 15.7 ppb and $F_{NO_{nose}}$-nose of 36.3 ± 16.0 ppb. These values were not significantly different and showed a good correlation, $r=0.98$ ($P<0.001$) (Fig. 2). Hence, there was no difference between exhaled and inhaled fractions of NO from the nose cavity at the measured flow rate. We also looked at the difference between the plateau values of the $F_{ENO_{c_{tr}}}$-mouth and the $F_{ENO_{c_{tr}}}$-pharynx since it has been proposed that the mouth cavity contributes to the exhaled NO concentration (18). We found no statistical difference between them, $F_{ENO_{c_{tr}}}$-mouth was 11.6 ± 8.1 ppb and the $F_{ENO_{c_{tr}}}$-pharynx was 10.2 ± 6.1 ppb (ns).

The 10 subjects used for modelling NO from the airways in protocol (d) had a $F_{ENO_{c_{tr}}}$ of 7.8 ± 2.4 ppb (Fig. 3). Using the equation (10) for calculation the $F_{A_{NO}}$, the $F_{wNO}$ and the $D_{wNO}$ showed no difference whether it was calculated with eleven flow rates or just three flow rates (Table 1). However, sample variance of the $F_{A_{NO}}$ determined by all 11 flow rates was 0.8 and the sample variance of the calculated $F_{A_{NO}}$...
TABLE 1. The computed values, from ten subjects, of the alveolar fraction of NO (FANO), the fraction of NO in the airway wall (FwNO) and the airway wall transfer rate of NO (DwNO).

<table>
<thead>
<tr>
<th>Expiratory flow rates</th>
<th>0–0.05–0.51 sec⁻¹</th>
<th>0–0.05, 0.1 and 0.5 sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated* FANO</td>
<td>Calculated† FANO</td>
</tr>
<tr>
<td>FANO ppb</td>
<td>1.5 ± 0.8</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>FwNO ppb</td>
<td>78.4 ± 26.7</td>
<td>76.5 ± 27.6</td>
</tr>
<tr>
<td>DwNO ml sec⁻¹</td>
<td>8.6 ± 2.7</td>
<td>8.7 ± 2.3</td>
</tr>
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Eleven different expiratory flow rates were compared with only three of these flow rates. Data are given in mean values and ±CI95%.∗

*The slope representing the FANO calculated by fitting from equation (10) after Tsoukias et al. (17).

†The slope representing the FANO calculated by subtracting the VENO from VEN₀ and dividing the sum by the difference in flow rate.

from the NO slope as described by Tsoukias et al. (17) was 0.3. When using only three flow rates the sample variance was decreased to 0.3. Using the flow rates 0.1 and 0.51 sec⁻¹ one can easily compute the slope by subtracting the VENO from VENO and dividing the sum by the difference in flow rate, as done in Table 1.

**Discussion**

We have shown in this study: (i) that extended information can be retrieved from an exhaled NO analysis when three different expiratory flows are used. (ii) There is no need for the use of a VC manoeuvre in order to obtain a reliable NO value. (iii) Inspired NO values from the nose can be estimated from expired values. (iv) The NO output from the nose during exhalation is about five times higher than the NO output from the mouth.

In trying to understand what the exhaled NO value means and the use of it in diagnosis and treatment of lung disease, we used the Fick’s law of diffusion to calculate the alveolar NO, the tissue source and the transfer rate of NO. Using the equations in the method section we were able to compute FANO, FwNO and DwNO as shown in Table 1. A step forward was that the numerous flow rates could be minimized to only three. We think that it is quite acceptable to let the patient exhale at three different flow rates in order to gather the information of FANO, FwNO and DwNO. A lot of information about the patient’s NO from the airways can be obtained if also the values from nose can be measured and calculated in a similar way. These measurements can easily be performed and the future will tell if they are of any use in lung function testing and monitoring of lung disease.

It is of considerable importance to use the appropriate expiratory flow rates to be able to show significant difference between different patient groups. An illustration of this can be seen in Fig. 3. The mean values for smokers (n = 5) and subjects with asthma (n = 5) are shown in comparison to the control group used for the diffusion measurements in protocol (d). The difference becomes greater the lower the expiratory flow. Laws of diffusion control the output of NO from the airways as explained in the theoretical part in the method. At high flows, when the contact time of the alveolar or peripheral gas with the airway surface is short, the peripheral NO concentration dominates. Then, to demonstrate the NO output from the airway wall, low flow rates may preferably be used to allow time for diffusion. The most likely explanation for the contradictory NO values obtained by Ho et al. in patients with bronchiectasis (7) is that a higher expiratory flow rate was used. Since it has been shown that patients with bronchiectasis have a strong expression of inducible NO synthase in the bronchial epithelium (19) one would expect to find increased levels of exhaled NO. Elevated levels of NO have also been found, when the NO measurements were done during a very slow vital capacity manoeuvre in this group of patients (20). This difference is most likely caused by the different expiratory flow rates. The same can be said for the studies of NO in patient with cystic fibrosis where both normal (21,22) and reduced values have been published (19). These patients have a very flat NO profile, i.e. at low expiratory flow rates the FENO is low (unpublished observation). Thus, using high flow rates no difference can be seen from control subjects but at very low flow rates a clear difference can be observed.

There was no difference in FENO whether an inspiration to TLC was performed before the exhalation or just a deep breath. The difference is in the longer exhalation time necessary to achieve a steady NO plateau during exhalation. Thus, it is important to wait until a plateau is achieved after a VC manoeuvre, and that no down sloping value should be accepted. A reliable NO value can be obtained even though the patient is unable to perform a VC manoeuvre. When repeated measurements in a research situation are needed it is of importance not to perform repeated VC manoeuvres since the FENO is known to decrease after lung function measurements (16).
It has been shown that an increase in oxygen tension occurs during nose breathing compared to mouth breathing (15). This points out the importance of measurements of the amount of endogenous inhaled NO. Our results indicate that it is possible to calculate the fraction of NO that is inhaled through the nose from the exhaled values. The optimal NO delivery to improve oxygen tension should produce a high alveolar fraction of NO. This is best achieved by a high delivery at the beginning of the inspiration in order to reach the best ventilated areas. During the respiratory pause NO is accumulated in the nose and a high fraction is inhaled in the beginning of the breath. If the inhaled NO fraction versus flow is known, it would be possible to calculate the amount of the NO per breath reaching the lung during any type of a flow pattern.

By measuring the exhaled NO by the same principle for mouth and nose values one can calculate the ratio between them. This ratio between the output from the nasal cavity and the lower airways did not depend on flow. On the other hand the nasal NO output as such was flow dependent in the flow range we covered. Previous studies performed by the nasal aspiration method have demonstrated that the nasal NO output varies with flow in the flow range of 0–7 to 2–7 l min\(^{-1}\) (23), but not in the range of 0·2 to 0·71 l min\(^{-1}\) (24). The role of the turbulences generated by nasal aerodynamics compared to the change of the diffusion properties at different flows requires further attention to understand the NO release mechanism in the nose. We measured NO during inhalation from the nose inside the pharynx only at one flow rate, so it is not excluded that different NO output mechanisms may be involved at essentially smaller or higher inhalation flows than our 0·11 l sec\(^{-1}\). A systematic comparison of the nasal NO concentration and output in the serial vs. parallel measurement configuration would be helpful in this context.

Inhalation of exogenous NO, as in smoking, causes the \(F_{\text{NO}}\) to decrease as shown in Fig. 3. Whether a compensatory increase in NO production occurs in the nose cavity and causes a shift in the ratio we do not currently know.

In conclusion, for patients that cannot co-operate to perform a VC-manoeuvre or in studies that need repeated measurements an exhalation after a deep inhalation can give an accurate NO value. Using a diffusion model we were able to compute alveolar NO fraction, airway transfer rate, and airway tissue source of NO. Clinical investigations are now needed to show the usefulness of these calculations in the diagnosis and treatment of respiratory diseases.

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References


