A New Method for the Remote Collection of Nasal and Exhaled Nitric Oxide*

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Study objectives: The present study introduces a method that has been developed to improve the remote collection and transportation of gas samples from the nose and lungs.

Design: Assessment of agreement between two methods of clinical measurements.

Setting: Noninvasive exhaled gas measurement at a respiratory research laboratory.

Participants: Ten nonsmoking adult volunteers (median age, 44 years; age range, 33 to 53 years; men, 6; women, 4) were recruited.

Measurements and results: Exhaled nitric oxide (ENO) and nasal nitric oxide (NNO) outputs were measured directly (on-line) and remotely (off-line). With the velum closed, lung air was exhaled at fixed flows (ie, 6, 8, and 10 L/min) (ENO) or room-air was aspirated through the nose in series at one fixed flow (ie, 5 to 8 L/min) (NNO). The off-line nitric oxide (NO) measurements were achieved by a gas collection tube system, which consisted of a flow control unit, a tube reservoir with one-way valves at both ends, and an interrupter valve allowing the trapping of gas inside the tube and eliminating the inclusion of “dead space.” After clamping, the reservoir may store and transport the gas samples for delayed analysis. The coefficient of variation of three consecutive NO measurements was < 3% for both on-line and off-line ENO and NNO. The correlations between on-line and off-line measurements in both ENO and NNO outputs were high (r = 0.99; R^2 = 0.99), and, unlike previous studies using bag-collection, the ENO outputs for on-line and off-line measurements were in good agreement (Bland-Altman test) at all flows tested.

Conclusions: The tube gas collection system eliminates the dead space and contamination during the gas sampling and permits the cost-effective and reliable off-line collection of both nasal and exhaled gas samples.

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Key words: asthma; gas; lungs; nitric oxide; nose; off-line; rhinitis

Abbreviations: ENO = exhaled nitric oxide; NO = nitric oxide; NNO = nasal nitric oxide

Nitric oxide (NO) is generated by both the upper and lower airways. It is an important biological messenger that is involved in, among other things, vasoregulation, coagulation, neurotransmission, and immune responses.1,2 Physiologic levels of NO that are produced by constitutive NO synthase are produced continuously, whereas production may be elevated by the expression of inducible NO synthase in response to inflammation. The high baseline NO levels that are generated in the nasal cavities and paranasal sinuses may inhibit the multiplication of bacteria, viruses, fungi, and parasites, and may improve ciliary beat frequency.1,3 Autoinhalation of nasal NO (NNO) counteracts bronchoconstriction and enhances oxygen uptake in the lungs. However, in response to proinflammatory cytokines and oxidants, NO acts as a modulator and effector in several steps of the inflammatory process. The term “exhaled NO” (ENO) refers to the NO emitted below the palate. It increases significantly in inflammatory diseases such as asthma, bronchiectasis, and allergic rhinitis, whereas low levels are found in diseases such as cystic fibrosis, primary ciliary dysfunction, and acute sinusitis.1 Therefore, NO has been suggested as a simple noninvasive diagnostic tool and marker of airway inflammation and disease.

In the “on-line” method of measurement, air is drawn into the NO analyzer directly as it leaves the subject. This is not always practical or desirable. Several methods have been developed2,4–6 for the

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collection in bags of exhaled air for remote or delayed analysis of NO and other gases. The methods of collection, storage, and/or transportation have a number of shortcomings. These include elimination of the dead space, contamination from adjoining airways, and the properties and cost of the storage vessel. The objective of this study was to introduce and validate a new, improved, and cost-effective method that is suitable for the remote collection of either nasal or exhaled air for later analysis.

Materials and Methods

Ten healthy nonsmoking volunteers (age range, 33 to 53 years; median, 44 years; men, 6; women, 4) were recruited into this study. Three subjects had nonsymptomatic seasonal allergies, and one subject had an ongoing viral upper respiratory tract infection with moderate nasal obstruction. All subjects provided informed consent. The study was approved by the Human Ethics Committee of the University of Toronto.

NO Measurement and Sampling Techniques

NO was measured by a rapid-response chemiluminescent NO analyzer (model 280; Sievers; Boulder, CO). The sampling flow of the vacuum pump was 0.2 L/min. A daily 2-point calibration was performed, first with 100% nitrogen to zero, then with an analyzed standard gas (NO, 1.6 ppm; Praxair; Mississauga, ON, Canada) for the span. Zero and span measures were checked periodically throughout the testing. The NO analyzer signal output was fed to a computer data acquisition program (DaysLab for Windows; DaysTec Corp; Amherst, NH) with a real-time chart recorder-like display of NO vs time written directly to the hard disk of the computer as a data file. NO concentrations were measured during a data analysis program, written in-house (Visual Basic; Microsoft; Redmond, WA).

Comparison of On-line and Off-line NO Measurements

A prototype of the new device (patent pending) for the remote collection of gas from the nose or lungs was used. It consisted of a number of components the order of which can be rearranged (Fig 1). The principle underlying the pulmonary and nasal collections was identical, and the equipment differed in minor details only.

The on-line and off-line gas samples were taken from the same air stream during the same exhalation. The former was collected through a side-arm attached to the mouthpiece or the nozzle. This ensured the comparability of the gas taken for analysis.

Technique of ENO Measurement

In the off-line system (Fig 1, top, a) a mouthpiece (a) was connected to an adjustable resistor (c), followed by a three-way valve (d). A pressure gauge (b) with a measuring range of ±30 cm H2O was side-arm connected between the mouthpiece and the resistor. These detachable components were connected to a tube-shaped reservoir for gas collection (e). A one-way valve was placed in each end of the reservoir so that only air blown out by the subject could enter.

As soon as the air sample had been collected, the reservoir tube containing the exhaled air was sealed off at both ends by hemostatic clamps. Two small, manual, three-way valves, one near each end of the tube between the two one-way valves, permitted the gas to be sampled without releasing the clamps. The analyzer was attached to the more distal of these. The other was opened simultaneously to room air to ensure that the sample was at atmospheric pressure. The quantity of gas required by the analyzer was <10 mL. The reservoir tube held about 200 mL.

Technique of NNO Measurements

The mouthpiece was replaced by a nasal olive (Fig 1, bottom, b [a]), and an adjustable vacuum pump was introduced at the distal end of the system to suck air through the nostrils in series, while the velum was closed. Otherwise the components of the system were the same as those used for the ENO measurements above. The three-way escape valve (d) was relocated distal to the tube reservoir (e), and the order of the pressure gauge (c) and resistor (b) was switched to reflect the fact that the nasal air was aspirated rather than blown through the reservoir. Room air was aspirated through the nose, as it would be in the field. Therefore a correction had to be made for its NO content. A separate sample was collected for this. The aspiration flow rate was adjusted by the resistors, which had been calibrated and standardized against a rotameter (Matheson - FM 1050-serial). The highest flow that did not induce alar collapse or discomfort was applied to limit the time required to achieve maximal NO output. In two of the subjects with narrow noses, a flow of 5 L/min caused contralateral alar collapse. This was eliminated by the introduction of a nozzle. The aspiration flow rate ranged from 5 to 8 L/min.

Collection Procedure for ENO

On-line ENO measurements were performed according to American Thoracic Society recommendations. Each subject took a deep breath and exhaled through the mouthpiece, which was in line with the tubed reservoir and resistor (Fig 1, top, a) to a target pressure of 12 cm H2O. This pressure was sufficient to close the velum, thus preventing nasal contamination. The precalibration of three resistors ensured that flows of 6, 8, and 10 L/min would result when the subjects followed this procedure. After 10 s, the operator used the three-way valve switch to abruptly terminate the flow of air through the tube. The air thus trapped between the two one-way valves of the reservoir (Fig 1, top, a) was made secure with large hemostat clamps applied at either end.

Collection Procedure for NNO

For the collection of NNO, the subjects had to close the velopharyngeal aperture. Therefore, they were instructed to take a deep breath and perform an oral Valsalva maneuver against the closed velum. Meanwhile, a nozzle was positioned in one nostril, and room air was aspirated through the nasal cavities in series and the collection tube for 15 s at a constant flow. This action was terminated abruptly by the three-way valve, and the tube was clamped shut, as for the ENO collection described above.

On-line and Off-line NO Output Measurement

The NO concentrations, whether conducted directly to the NO analyzer (on-line) or stored briefly in the collection tube reservoir (off-line), were displayed on the computer screen. They were seen to plateau as the small amount of room air in the connection tubing was flushed out. Both the exhaled and the NNO concentrations were measured and analyzed independently three times. The NO output was calculated from the NO concentration and was multiplied by the flow rate.
The NNO measurements were combined with measurement of the NO concentration in room air and the amount was subtracted from the NO concentration before the output was calculated.

Properties of the Collection Tube Reservoir

The length and volume of the reservoir are adjustable, and it can be produced from a variety of materials with different sizes, lengths, and wall thicknesses to conform with requirements for stable storage and transportation of different gases. In this study, a polyethylene tubing (ordinary anesthesia tubing) with an internal diameter of 25 mm and a length of 40 cm had a volume of approximately 200 mL. Validation of the reservoir properties required to maintain a stable NO concentration (or those of other gases of interest) for a desired period of time was not the objective of this study and is under investigation.

Statistical Analysis and Power Calculations

Based on our previous repeated measurements of ENO and NNO output in healthy and allergic adults, we estimated that 10 subjects would give a power of at least 80%, which is sufficient to detect significant differences with a probability of \( p < 0.05 \).

Results

The mean ENO and NNO outputs for on-line and off-line measurements are shown in Table 1. As expected, the repeatability of the three consecutive NO measurements was high for both on-line measurements (coefficient of variation, < 2.6%) and off-line measurements (coefficient of variation, < 3.0%). The correlation between the 120 on-line measurements and the 120 off-line measurements (ENO and NNO) was extremely high \(( r = 0.99; R^2 = 0.99 \)) was shown in Figure 2. The largest difference for ENO was at a collection rate of 2 nL/min (10%), and for NNO the output was at 4 nL/min (2%). When analyzing the NNO and the ENO outputs at the different flows, the correlation coefficient remained at the same high level (Fig 2). Comparison between methods, by the Bland and Altman test, for ENO at 6 L/min and NNO is shown.
in Figure 3. The variance of ± 2 SD is 0.74 nL/min for ENO measurements and 0.88 nL/min for NNO measurements. These confidence intervals are much smaller than the changes that are considered to be of clinical importance. The differences in either NO concentration (at constant flow) or NO output should not exceed 10%.

There was no significant difference between the on-line and off-line measurements for NNO or ENO at any of the flows. When ENO outputs at different flows were compared, there was a significant increase in the ENO output from 6 to 8 L/min for on-line measurements and from 8 to 10 L/min for off-line measurements (Table 1; p < 0.01). The increase in the flow range appears to be fairly linear for this range of flows, with an increment of 7 nL/min NO for each 1 L/min increase in flow. The concentrations at different flows for the individual subjects and the mean ± SD are plotted in Figures 2 and 3.

**Table 1—ENO Output and NNO Output for On-line and Off-line NO Measurements**

<table>
<thead>
<tr>
<th>Output</th>
<th>On-Line NO Output, nL/min</th>
<th>Off-Line NO Output, nL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 L/min</td>
<td>133.2 ± 56.0</td>
<td>133.4 ± 56.1</td>
</tr>
<tr>
<td>8 L/min</td>
<td>147.5 ± 63.1</td>
<td>147.3 ± 63.0</td>
</tr>
<tr>
<td>10 L/min</td>
<td>156.3 ± 63.1</td>
<td>157.2 ± 63.4</td>
</tr>
<tr>
<td>NNO at 5–8 L/min</td>
<td>568.2 ± 127.5</td>
<td>564.4 ± 125.1</td>
</tr>
</tbody>
</table>

*Values given as mean ± SD.

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**Discussion**

If NO is to be measured outside the laboratory setting, the development of a reliable method for its collection and transportation is essential. Ideally, this method must be simple and easily learned. It should be nearly identical for nasal and pulmonary sampling, and it should use materials that are commonly available and inexpensive so that their loss or damage under field conditions is not disastrous to the investigation. No such system has been devised, but the tube reservoir method described and tested herein comes closer to fulfilling these requirements for both ENO and NNO than any other that has been proposed to date. The tube reservoir method provides NO values that are interchangeable with on-line measurements. This is the single most important consideration for any system involved in extralaboratory use. The agreement between on-line and off-line results for the open tube system developed herein was superior to those reported for NO collected in a bag or balloon.4–6

When collecting ENO in a balloon (Mylar balloon; Ellwood’s; Denver, CO) from a 15-s exhalation from total lung capacity at a flow of 45 mL/s (2.7 L/min), Silkoff et al6 found that off-line ENO levels were 30% lower than direct measurements. This large discrepancy was primarily due to the inclusion of the bag dead space in the early phase of exhalation. However, Jobsis et al4 who studied asthmatic children, found that the mean end-expiratory plateau concentration of NO during exhalation at an individually adjusted flow rate of
20% of the vital capacity (5.0%; range, 2.3 to 12.8%) was similar to values obtained when exhaling into a balloon at nonstandardized flows and pressures (4.8%; range, 2.2 to 17.2%). Although the mean values were similar with a ratio of 1.05, the correlation coefficient between the two methods was only 0.73. By comparison the tube reservoir method produced correlation coefficients of 0.99. In many of the subjects in the study by Jobsis et al,4 the difference in NO values between on-line and off-line measurements exceeded 30%, and in some cases, 50%.4 The mean difference with the tube reservoir method never exceeded 10%.

To evaluate the bag as a collection device for ENO, Paredi et al5 estimated that discarding the first 2 s of measurements was sufficient to exclude the dead space of the extrathoracic airways. This study reported a high correlation only for one of the four flows tested (10 L/min), whereas with the tube reservoir method excellent agreement was obtained for all flows for both ENO and NNO. Our data (Fig 4) showed that a washout period of up to 10 s may be required to obtain a stable plateau in exhaled NO measurements. Longer intervals are needed for lower flows. The early peak observed in on-line measurements is due to the contamination of exhaled air by NO originating from the nose before velar closure. In the off-line collection, this may be included in the reservoir if the washout time is < 10 s (Fig 4). This may explain the higher ENO values in the study by Paredi et al5 between off-line and on-line measurements, particularly at the flow rate of 6 L/min. However, at high flows (ie, 10 to 12 L/min), we observed that subjects with small total lung capacity had difficulty in maintaining a stable high flow at the end of exhalation. This may explain the lower off-line values at the highest flow rates (12 L/min) in that study.

On-line NNO measurement has been well-developed, and most studies employ the aspiration technique through the nasal airways in series.2 The tube reservoir system allows the same aspiration technique to be employed in off-line NNO sampling. This is not possible in bag collection.

Regardless of the collection technique used, the stability of the gas inside the reservoir is essential for the reliability of the measurements. Different gas containers have been tested in our study, and we found the least change in gas composition over time with the polyethylene anesthesia tube. In this study, the NO concentration remained stable for up to 72 h when the tube was kept in the freezer (−20°C). Tube reservoirs, closed off by quick and efficient clamping, allow the use of containers with thicker walls, as opposed to collection bags. Thus, tube reservoirs are easier to adapt to different gases and storage/transportation conditions than are bags. Clearly, tubes are much less expensive to manufacture and may be distributed in long coils. Furthermore, the tube method allows the subject to rehearse the exhalation procedure. Bags are also much more expensive and are intended primarily for single use, and the one-way valves of the bags make complete emptying of the bags time-consuming and virtually impossible without leaving a residual volume and condensate. The much smaller volume and thicker walls of the tubing reservoir, compared to the bag,
make handling and transport much easier and safer. If required, specialized reusable tube reservoirs in metal or other solid materials with permanent, built-in, one-way valves could be useful.

We conclude that the new method provides a simple, efficient, and inexpensive means for the reliable collection of gases originating from both the nasal and pulmonary airways. Samples of NO or other gases can be obtained easily, for example in the outpatient clinic, in field studies, or in the patient home, and can be transported to the laboratory for later analysis. The tube reservoir method yields NO values that are virtually identical, and thus interchangeable, with on-line measurements. The permitted transport and storage time will depend on the ambient temperature, concentration, solubility, and reactivity of the gas and on the material of which the tube reservoir is made. Thus, the properties of the reservoir chosen must be validated carefully for the gas concerned and the circumstances of its use.

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