Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans

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**Pietropaoli, Anthony P., Irene B. Perillo, Alfonso Torres, Peter T. Perkins, Lauren M. Frasier, Mark J. Utell, Mark W. Frampton, and Richard W. Hyde.** Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. J. Appl. Physiol. 87(4): 1532–1542, 1999.—Human airways produce nitric oxide (NO), and exhaled NO increases as expiratory flow rates fall. We show that mixing during exhalation between the NO produced by the lower, alveolar airways (V_{LNO}) and the upper conducting airways (V_{UNO}) explains this phenomenon and permits measurement of V_{LNO}, V_{UNO}, and the NO diffusing capacity of the conducting airways (D_{UNO}). After breath holding for 10–15 s the partial pressure of alveolar NO (P_A) becomes constant, and during a subsequent exhalation at a constant expiratory flow rate the alveoli will deliver a stable amount of NO to the conducting airways. The conducting airways secrete NO into the lumen (V_{UNO}), which mixes with P_A during exhalation, resulting in the observed expiratory concentration of NO (P_E). At fast exhalations, P_A makes a large contribution to P_E, and, at slow exhalations, NO from the conducting airways predominates. Simple equations describing this mixing, combined with measurements of P_E at several different expiratory flow rates, permit calculation of V_{UNO}, P_A, and D_{UNO}. In seven normal subjects, P_A = 1.6 ± 0.7 × 10^{-6} (SD) Torr, V_{LNO} = 0.19 ± 0.07 µl/min, V_{UNO} = 0.08 ± 0.05 µl/min, and D_{UNO} = 0.4 ± 0.4 ml·min⁻¹·Torr⁻¹. These quantitative measurements of V_{LNO} and V_{UNO} are suitable for exploring alterations in NO production at these sites by diseases and physiological stresses.

nitr|oxide diffusing capacity of airways; nitric oxide production by|airways; lung nitric oxide; breath holding

INCREASED EXHALED nitric oxide (NO) concentrations have attracted interest as a means for detecting inflammation of the airways in asthma (17). NO production by the lungs may be abnormal in diseases such as sepsis, cirrhosis, primary pulmonary hypertension, and interstitial lung diseases (18, 21, 26, 27). The exhaled concentration of NO (P_E) increases as expiratory flow rates (Q_E) fall (24), so Q_E must be kept constant to obtain reproducible measurements of P_E (Fig. 1). The reason for this flow dependence has recently been elucidated by Tsoukias and co-workers (28, 29). They show that during exhalation the mixing between NO from the lower alveolar airways perfused by the pulmonary circulation (V_{LNO}) with NO produced in the upper conducting airways (V_{UNO}) perfused by the bronchial circulation explains this phenomenon. Simple equations can describe this mixing. When combined with multiple measurements of P_E at different Q_E, these equations permit calculation of V_{UNO} and the partial pressure of NO in the lower alveolar airways (P_A). In this report, we describe an analysis of expired NO at different Q_E that also permits calculation of the diffusing capacity of the upper airways (D_{UNO}) and measurements of the pulmonary diffusing capacity of the lower airways (D_{LNO}) (12). Because diseases and physiological stress may cause changes in NO production and diffusing capacity by the alveoli different from those by the conducting airways, measurement of V_{LNO}, V_{UNO}, D_{UNO}, and D_{LNO} may provide new information about factors that alter NO production by the lungs.

**Glossary**

\[ \text{DL}_{NO} \] Diffusing capacity of the lower, alveolar airways recorded as milliliters of NO STPD moving from the air spaces into the tissues and blood per minute per Torr of NO in the air spaces

\[ \text{DU}_{NO} \] Diffusing capacity of the upper, conducting airways recorded as milliliters of NO STPD moving from the air spaces into the tissues and blood per minute per Torr of NO in the air spaces

\[ f \] Small fraction of \text{DU}_{NO}, \text{PU}, or \text{V}_{UNO}

\[ \text{FVC} \] Forced vital capacity

\[ \text{NO} \] Nitric oxide

\[ \text{PA} \] Partial pressure of NO in the alveoli

\[ \text{PB} \] Barometric pressure

\[ \text{PE} \] Partial pressure of NO in exhaled gas

\[ \text{PU} \] Partial pressure on NO in all or a segment of the upper conducting airway

\[ \text{Q_E} \] Expiratory flow rate

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METHODS

NO Exchange in the Alveolar Airways

The alveolar airways are defined as those tissues and air spaces well perfused by the pulmonary circulation, such as the alveoli, alveolar ducts, and respiratory bronchioles. In this zone, some of the NO produced by these lower airways diffuses into the air spaces. The fraction of the total NO produced in this alveolar compartment that enters the air spaces is called $V_{\text{LNO}}$. The NO in the alveoli can react with the blood and surrounding tissues (12) or diffuse rapidly through the alveolar capillary membrane into the perfusing blood. After elimination of ventilation by breath holding for 10–15 s, a steady state will develop, and the amount of NO entering the alveoli ($V_{\text{LNO}}$) equals the amount of NO diffusing into the perfusing blood and surrounding tissues (12) or

$$V_{\text{LNO}} = P_A \cdot D_{\text{LNO}} \tag{1}$$

where $D_{\text{LNO}}$ is the alveolar airway NO diffusing capacity, which is considered equivalent to the NO pulmonary diffusing capacity. Therefore, determination of $P_A$ multiplied by an independent measurement of $D_{\text{LNO}}$ permits calculation of $V_{\text{LNO}}$. $D_{\text{LNO}}$ was determined by a modification of the constant single exhalation method for measuring the pulmonary carbon monoxide diffusion capacity ($D_{\text{LCO}}$) described by Newth and co-workers (19) and Perillo and co-workers (20). Multiple values of $D_{\text{LNO}}$ are calculated during the exhalation and averaged.

NO Exchange in the Conducting Airways

The conducting airways are defined as those airways extending from the alveolar airways to the mouth. Strategies such as continuous positive pressure in the mouth (13, 24) or constant suction of gases from one nostril (9, 28, 29) can be used to avoid contamination of expired NO from the conducting airways (14). NO gas exchange in the conducting airways can be analyzed in the same manner as in the alveolar airways. Namely, a fraction of NO production by the conducting airways ($V_{\text{UNO}}$) enters the lumen. Some of this NO can diffuse back into the tissues of the conducting airways and enter the bronchial circulation in proportion to the partial pressure of NO in the lumen of the conducting airways ($P_U$). If the bronchial blood flow maintains the partial pressure of NO in the blood perfusing the tissues of the conducting airways at a negligible level, the amount of NO diffusing back into these tissues will equal $P_U \cdot D_{\text{UNO}}$. With exhalation at a constant flow rate, $P_U$ will reach a constant value, and during this steady state $V_{\text{UNO}}$ will equal the amount of NO diffusing back into the tissues or

$$V_{\text{UNO}} = P_U \cdot D_{\text{UNO}} \tag{2}$$

We describe two models of the conducting airways based on the above assumptions that allow the simultaneous calculation of $P_A$, $V_{\text{UNO}}$, and $D_{\text{UNO}}$ from multiple measurements of $P_E$ performed at different constant $Q_E$.

Model 1. Model 1 assumes a uniform concentration of NO throughout the conducting airways (Fig. 2), so $P_U = P_E$. After breath holding for 10–15 s, a constant $P_A$ is achieved (12), and subsequent exhalation at a steady flow rate ($Q_E$) delivers a constant amount of NO to the conducting airways equal to $Q_E (P_A - (P_B - 47))$, where $P_B$ is the barometric pressure, 47 is the partial pressure of water at body temperature in Torr, $Q_E$ is expressed in milliliters per minute STPD, and $P_A$ is expressed in Torr. This NO from the alveolar airways instantaneously mixes with NO in the conducting airways, resulting in a uniform partial pressure of NO in the conducting airways and the expired breath ($P_E$). The amount of NO exhaled at any instant (STPD) equals $Q_E (P_A - (P_B - 47))$. This equals the contribution from the alveolar airways $Q_E (P_A - (P_B - 47))$ plus $V_{\text{UNO}}$ less the NO diffusing from the lumen of the conducting airways back into the tissues and bronchial...
circulation of the conducting airways \( PE \cdot D_{UNO} \)

\[
\frac{\dot{P}E}{Q^e} = \frac{\dot{P}A}{Q^e} + \dot{V}_{UNO} - (PE \cdot D_{UNO})
\]  

(3)

Rearranging gives

\[
PE = \frac{1}{Q^e} \cdot (V_{UNO} - PE \cdot D_{UNO}) (PB - 47) + PA
\]  

(4)

Multiple sets of measurements of \( PE \) at different \( Q^e \) provide the data needed to determine \( PA \), \( V_{UNO} \), and \( D_{UNO} \) in Eqs. 3 and 4. First, \( PA \) is determined graphically by taking advantage of the following observation: At higher values of \( Q^e \) (i.e., >200 ml/s), \( PE \) is relatively small and results in the term \( PE \cdot D_{UNO} \) decreasing to <3% of \( V_{UNO} \). If \( PE \cdot D_{UNO} \) is considered insignificant at such flow rates, Eq. 4 becomes

\[
y = m x + b
\]

Equation 5 has the following form: \( y = mx + b \). A plot of \( PE \) vs. \( 1/Q^e \) resulted in the point where \( Q^e = \infty \) is the also the point where \( Q^e = \infty \). The slope equals \( V_{UNO}(PB - 47) \). We therefore calculated \( PA \) from the linear regression of \( PE \) plotted vs. \( 1/Q^e \) when \( Q^e > 200 \) ml/s (Fig. 3). If these data failed to result in a doubling of \( PE \), data at the next slower flow rate <200 ml/s were added until \( PE \) doubled its lowest value. This value of \( PA \) was combined with all the measurements of \( PE \) and \( Q^e \) collected at different constant \( Q^e \) to calculate the remaining two variables, \( V_{UNO} \) and \( D_{UNO} \), with use of Eq. 4 with the assistance of a curve-fitting program utilizing a quasi-Newton regression (8) (Fig. 4). The program forced the fit through the calculated value of \( PA \). To determine whether the quasi-Newton regression-fitting algorithm supplied a unique solution for \( V_{UNO} \) and \( D_{UNO} \), we also calculated their values using the Newton and the steepest descent-fitting algorithms for a representative subject. The three algorithms yielded the same values for \( V_{UNO} \) and \( D_{UNO} \). Therefore, the choice of curve-fitting algorithm does not influence identification of the unique solutions from these data. The curve-fitting program requires assumed starting values for \( V_{UNO} \) and \( D_{UNO} \). These were arbitrarily chosen to be 0.1 µl/min and 0.3 ml·min⁻¹·Torr⁻¹, respectively. In a representative subject, these starting values could be systematically varied 4· to 10-fold before deterioration of the fitted curve became apparent. If a poor fit is obtained, starting values would need to be changed to allow the program to identify a reasonable fit to the data.

To determine whether a reliable measurement of \( V_{UNO} \) was possible from just the faster values of \( Q^e \) used to calculate \( PA \), \( V_{UNO} \) was also calculated from these data with Eq. 5 and compared with \( V_{UNO} \) determined with all values of \( Q^e \) by use of Eq. 4.

This method for measuring \( PA \), \( V_{UNO} \), and \( D_{UNO} \) with model 1 assumes rapid arrival at a new steady state when the NO coming from the alveolar airways mixes with the NO in the conducting airways during exhalation. APPENDIX A describes an equation for calculating the changes in \( PE \) during mixing and shows the amount of gas needed to be exhaled to reach a steady state. The equation shows that once ~30% of the expiratory vital capacity has been exhaled after the initial breath-holding period, \( PE \) is within 99% of the constant equilibrated value, so Eqs. 4 and 5 are valid for measuring \( PA \), \( V_{UNO} \), and \( D_{UNO} \).

Model 2. Model 2 assumes stratification of the NO concentration in the conducting airways so the concentration of NO can gradually increase as the expired gas moves through the conducting airway (Fig. 5). In contrast to model 1, the conducting airway is considered to be a cylinder with a total volume \( K \) and an infinite number of uniform segments. Each segment has an equal fraction (f) of \( K \), \( V_{UNO} \), and \( D_{UNO} \), so that the dimensions of any segment are \( fK \), \( fV_{UNO} \), and \( fD_{UNO} \). At the start of exhalation at a constant \( Q^e \), \( PA \) enters the first segment, where \( V_{UNO} \) adds NO and \( D_{UNO} \) removes NO at a rate proportional to the partial pressure of NO in the segment. The bronchial blood flow in the wall of the upper airway is assumed to keep its partial pressure of NO at a negligible level. The resultant partial pressure of NO in the lumen of the segment equals \( Pu_1 \). \( Pu_1 \) then enters the next segment, and its fraction of \( V_{UNO} \) and \( D_{UNO} \) results in \( Pu_2 \), and so forth. At the proximal end of the conducting airway, \( Pu = PE \).

For any segment of the conducting airways, the amount of NO in the segment of volume \( fK \) equals \( fK[PU + (PB - 47)] \). It is changed by the NO production \( fV_{UNO} \) entering the seg-
ment less the amount diffusing out (Pu · fDuNO) or
\[
\frac{d}{dt} \left( \frac{P_u}{P_b - 47} \right) = fV_{UNO} - Pu \cdot fDuNO \tag{6}
\]
The solution of Eq. 6 given in detail in APPENDIX B is
\[
P_E = \frac{V_{UNO}}{DUNO} - P_A \left[ 1 - e^{-\frac{DUNO (P_b - 47)}{Q_E}} \right] + P_A \tag{7}
\]
\(P_A\) is obtained from data obtained at the faster \(Q_E\), as described above. With this value of \(P_A\) and all the measured pairs of \(P_E\) and \(Q_E\), \(V_{UNO}\) and \(DUNO\) are calculated using Eq. 7 with the assistance of a curve-fitting program utilizing a quasi-Newton regression (8).

Measurement of NO

Details of methods for measuring NO have been recently published (9). Briefly, a rapidly responding chemiluminescence NO analyzer (Sievers NOA, model 270B, Sievers, Boulder, CO) operating at a sample rate of 250 ml/min measured exhaled levels of NO at the mouthpiece with a 150-cm-long, 1.6-mm-ID, 3.2-mm-OD Tygon inlet tube. Response time of the analyzer was \(< 200\) ms for a signal 90% of full scale. The analyzer was adjusted to provide 40 measurements of the NO concentration per second that could be averaged over any time interval. The NO analyzer was calibrated daily by serial dilutions of a gas containing 229 parts per billion (ppb) of NO. To obtain gas samples free of NO, air from a gas cylinder containing \(< 2\) ppb of NO (Scott Specialty Gases, Plumsteadville, PA) was passed through a filter constructed from a 5.8-cm-ID, 19-cm-long cylinder (Gas Drying Unit, VWR Scientific, Rochester, NY) packed with potassium permanganate (Purafil, Thermoenvironmental Instruments, Franklin, MA) (4).

Because the air signal free of NO could drift as much as 2 ppb in 10 min, measurements of NO-free air were performed within 1 min before and after each NO measurement from expired gas samples, and these values were averaged to obtain the zero NO signal. The lag time between the volume signal obtained from a potentiometer attached to the spirometer and the change in the NO signal was determined daily and equaled 0.8 ± 0.1 (SD) s. Multiple repetitive measurements of gas mixtures of 2.8 and \(8.2 \times 10^{-6}\) Torr of NO showed a standard deviation of 0.09 \(\times 10^{-6}\) Torr. We assumed that the detection limit of our analyzer was two times the standard deviation of these multiple measurements or \(0.2 \times 10^{-6}\) Torr. During gas sampling the operator exhaled warm humidified gas from the mouth by the inlet of the NO analyzer approximately every 5–10 min, so the walls of the unheated inlet tubing were kept moist. This resulted in all gases being considered measured at ATPS. Measurements of NO in parts per billion (ppb) ATPS were converted to partial pressure of NO in Torr ATPS as follows: NO in Torr = (NO in ppb ATPS) \(\left( P_b - 47 \right) / \left( P_b - P_{H2O}\right)\)), where \(P_{H2O}\) is partial pressure of water at room temperature. For example, at \(P_b\) of 760 Torr and room temperature of 24°C where \(P_{H2O}\) = 22.4 Torr, 1 ppb NO = 0.735 \(\times 10^{-6}\) Torr of NO. The chart recorder (MacLab Recording Instrument, AD Instruments, Castle Hill, Australia) stored the volume signal and NO signal in a Macintosh LC computer (Apple Computer, Cupertino, CA). To obtain a stable constant value for the measurement of \(P_E\) after breath holding, we discarded an initial portion of the exhalate equal to four times the sum of the subject's estimated anatomic dead space and the instrument dead space of 100 ml, as well as the final 10% of the exhalate (Fig. 6). At flow rates < 45 ml/s, a constant value for \(P_E\) was obtained earlier during exhalation (APPENDIX A). At flow rates > 1,000 ml/s, a constant value for \(P_E\) was frequently not present until 40–50% of the breath had been expired. In these cases, the NO plateau level was determined by visual assessment of the NO signal displayed on the computer.

Maneuvers Used to Measure \(P_E\) and \(Q_E\)

Subjects exhaled to residual volume (RV) through the mouthpiece of the apparatus into the room and then rapidly Fig. 6. Record of NO concentration at mouth (PE) and lung gas volume signal during 20 s of breath holding followed by exhalation at 600 ml/s. PE is obtained from NO plateau that follows NO peak seen at start of exhalation. Peak is attributed to accumulation of NO in conducting airways during breath holding that is then flushed through mouthpiece at beginning of exhalation. Data for calculating PE was obtained after an initial expired volume equal to 4 times subject's and instrument's dead space (DS), as well as final 10% of forced vital capacity (FVC), was discarded. Qe was calculated from volume signal after initial and final 10% of FVC was discarded. NO signal was moved to left by 0.8 s to correct for lag between NO and volume signals.
and $Q^\dot{E}$ were averaged. $P_E$ was measured as described
inhalation of room air from a bag-in-box device to total lung
capacity (TLC) (Fig. 7). The subject then held this breath
for 10–20 s, valve is turned 90° into the spirometry circuit, and the subject
exhaled, maintaining a mouth pressure of $10–20$ Torr. $DLNO$ was obtained
from the spirometer's volume signals are displayed on a chart recorder and stored in
a computer.

**Measurement of $DLNO$**

$DLNO$ for each subject was calculated from the expired NO concentration measured after
inspiring 10 parts/million of NO in air placed in the bag in Fig. 7 from RV to TLC, breath
holding for 5 s, and then exhaling to RV at a constant flow rate of 500 ml/s with a
modification of the single-breath exhalation method for continuously measuring $DLNO$
during exhalation described by Newth and co-workers (19) and Perillo and co-workers (20).
Lung volume at any instant during exhalation was measured via side port on mouthpiece.
Changes in gas volume are measured with a spirometer attached to a potentiometer. NO
and volume signals are displayed on a chart recorder and stored in a computer.

The subject then held this breath for 10–20 s, so that $P_E$ reached a constant concentration
irrespective of the inhaled ambient NO concentration (12). At the end of the breath hold, the mouthpiece
valve was turned 90° into the spirometry circuit, and the subject exhaled, maintaining
mouth pressure at $5$ cm H$_2$O by watching a water manometer. Corks with various-sized holes
bored through their centers were placed in the expiratory tubing and resulted in
different expiratory resistances and $Q^\dot{E}$. Each subject performed measurements at seven
different flow rates that were as low as 6 ml/s and as high as 1,355 ml/s. Exhalations at each
flow rate were performed in triplicate, and the values for $P_E$ and $Q^\dot{E}$ were averaged. $P_E$ was measured as described
above, and $Q^\dot{E}$ was obtained from the spirometer's volume signal after the initial and final 10% of the expired volume
were discarded (Fig. 6). The entire experiment for each subject was completed within 4 h on the same day.

**Results**

$PA$, $V_{UNO}$, and $DLNO$ were measured in seven healthy, nonsmoking, 31- to 72-yr-old (mean 46 ± 18 yr) subjects. Five
were men and two were women. All subjects were free of cardiopulmonary disease. Spirometry showed values >90% of
predicted for the forced expiratory volume in 1 s, with a mean value of $104 ± 16$ (SD)% (2). This study was approved by the
University of Rochester's committee for investigations involving human subjects.

**Statistical Methods**

Values are means ± SD. In experiments where subjects served as their own control, results were compared using a
two-tailed paired t-test. Groups of subjects were compared with an unpaired t-test. $P < 0.05$ was required for statistical
significance. Regression lines and curves were fitted to the experimental data by the line of least mean squares referenced
to $P_E$.

**RESULTS**

$PA$, $V_{LNO}$, and $DLNO$

Figure 8 shows the values for $P_E$ and the reciprocal of $Q^\dot{E}$ ($1/Q^\dot{E}$) used to determine $PA$ from the faster exhalations
in the seven subjects. The linear regression of these points extrapolated to infinite flow, where $1/Q^\dot{E}$ = 0, equals $PA$. The regression line fitted the data closely, with $r^2$ = 0.965–0.999. $PA$ was $1.6 ± 0.7 × 10^{-6}$ (SD) Torr. $DLNO$ was $123 ± 19$ ml·min$^{-1}$·Torr$^{-1}$. $V_{LNO}$ (i.e., $PA·DLNO$) was $0.19 ± 0.07$ µl/min.

$V_{UNO}$ and $DLNO$

Figure 9 shows the paired values for $P_E$ and $1/Q^\dot{E}$ for all exhalations by the seven subjects used to determine
$V_{UNO}$ and $DLNO$. $Q^\dot{E}$ ranged from 6 to 1,355 ml/s. For model 1, $V_{UNO}$ was 0.077 ± 0.053 µl/min and $DLNO$ was
0.4 ± 0.4 ml·min$^{-1}$·Torr$^{-1}$; for model 2 the values were similar: 0.074 ± 0.052 µl/min and 0.5 ± 0.4 ml·min$^{-1}$·Torr$^{-1}$, respectively. The regression lines for both models fit the data closely, with $r^2 > 0.998$ in all subjects. The value of $r^2$ for the two models did not differ

![Figure 7. Apparatus for measuring NO production of conducting and alveolar airways.](http://jap.physiology.org/)
significantly: 0.9996 ± 0.0003 for model 1 and 0.9994 ± 0.0006 for model 2 (P = 0.30). \( V_{\text{UNO}} \) calculated with just the faster values of \( Q_{\text{E}} \) shown in Fig. 8 with use of Eq. 4 was 0.070 ± 0.048 µl/min. Although this value is slightly lower than 0.077 ± 0.053 µl/min with model 1 and 0.074 ± 0.052 µl/min with model 2, the difference was not significant (P = 0.2).

Comparison of \( V_{\text{LNO}} \) and \( V_{\text{UNO}} \)

Figure 10 shows that \( V_{\text{LNO}} \) of 0.19 ± 0.07 µl/min was consistently greater than \( V_{\text{UNO}} \) of 0.077 ± 0.053 µl/min with use of model 1 (P < 0.01). Calculating with model 2 gave similar results. \( V_{\text{LNO}} \) was 0.19 ± 0.07 µl/min compared with \( V_{\text{UNO}} \) of 0.074 ± 0.052 µl/min (P < 0.01).

Comparison of \( D_{\text{LNO}} \) and \( D_{\text{UNO}} \)

Table 1 shows that \( D_{\text{LNO}} \) is >100-fold greater than \( D_{\text{UNO}} \) calculated with model 1 or model 2.

**DISCUSSION**

These data show that a model of the human airways where exhaled NO from the alveoli mixes with the NO produced by the conducting airways precisely predicts the \( P_E \) observed at different \( Q_{\text{E}} \). Simple equations describing this mixing combined with values for \( P_E \) at different values of \( Q_{\text{E}} \) result in measurements of \( V_{\text{UNO}} \), \( D_{\text{UNO}} \), and \( P_A \). \( P_A \) multiplied by a separate measurement of \( D_{\text{LNO}} \) gives a measurement of \( V_{\text{LNO}} \).

**Common practice is to measure expired NO at a single relatively slow \( Q_{\text{E}} \) on the order of 100–250 ml/s** (13). The resultant observed values of \( P_E \) are three to five times \( P_A \) and, therefore, predominantly represent \( V_{\text{UNO}} \). Although these measurements at single relatively slow \( Q_{\text{E}} \) values provide a useful index of \( V_{\text{UNO}} \), they are at a disadvantage for detecting changes in \( P_A \) and \( V_{\text{LNO}} \).

A number of studies suggest that the mechanisms altering \( V_{\text{UNO}} \) and \( V_{\text{LNO}} \) may be different. The large increases in \( P_E \) seen in bronchial asthma likely come from upregulation of inducible NO synthase in the conducting airways (11, 30). Endothelial-derived NO synthase is reported to be located in the alveolar capillary membrane (10) and is upregulated in a rat model of the hepatopulmonary syndrome (7). This upregulation could explain the high levels of exhaled NO observed in some patients with cirrhosis and the hepatopulmonary syndrome (18). Downregulation of endothelial-derived NO synthase may account for the low levels of expired NO reported in primary pulmonary hypertension (3, 21). The technique described in this report for measuring \( V_{\text{UNO}} \) and \( V_{\text{LNO}} \) should provide a quantitative method to localize alteration in NO production to the alveoli or the conducting airways. Such measurements may result in more precise in the use of exhaled NO to assess lung injury or alterations in regulation of NO production by the lungs than that obtained with observations at a single \( Q_{\text{E}} \).

Table 1. \( D_{\text{LNO}} \) and \( D_{\text{UNO}} \) calculated with models 1 and 2 in healthy subjects

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<th>Mean ± SD</th>
<th>Range</th>
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<tr>
<td>( D_{\text{LNO}} )</td>
<td>123 ± 19</td>
<td>92–147</td>
</tr>
<tr>
<td>( D_{\text{UNO}} )</td>
<td>0.4 ± 0.4</td>
<td>0.04–1.1</td>
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<tr>
<td>Model 1</td>
<td>0.5 ± 0.4</td>
<td>0.08–1.2</td>
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<td>Model 2</td>
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Values are expressed in ml·min\(^{-1}\)·Torr\(^{-1}\); \( n = 7 \). \( D_{\text{LNO}} \), diffusing capacity of alveolar airways; \( D_{\text{UNO}} \), diffusing capacity of conducting airways.

**Choice of Lung Models to Explain the Change in \( P_E \) With Different Values of \( Q_{\text{E}} \)**

The simpler model (model 1) of the airways, where the conducting airways are considered one single uniform compartment, precisely described the observed data obtained at different values of \( Q_{\text{E}} \), with \( r^2 > 0.998 \) in all subjects. The multicompartment model of the conducting airways (model 2), with the more realistic
assumption that NO concentration in the conducting airways gradually approaches PE during exhalation, does not provide a better fit to the observed data. We also performed theoretical calculations to see if measurements of PE at Qₑ in humans as low as the practical limit of ~5 ml/s can be used to distinguish between the two models. These models generate different values for PE at Qₑ in human subjects, where Qₑ > 5 ml/s. Note similarity of shape of solid curves generated by models 1 and 2. This similarity makes it difficult to distinguish models 1 and 2 by use of measurements of exhaled NO and Qₑ.

Models 1 and 2 have limitations in their assumed dimensions, because the conducting airways must contain multiple compartments where the ratio of the surface area of the conducting airways that secretes NO into the gas volume in the lumen decreases as exhaled gas moves from the alveoli through the trachea (6, 23, 31). This anatomy results in uneven distribution between VₑNO, DₑNO, and conducting airway gas volume. Because the simple one-compartment model of the conducting airways so accurately predicts PE at different values of Qₑ, use of more realistic models of the conducting airways is not likely to result in a better measurable prediction of the experimental data.

Potential Errors in VₑNO Calculated With Eq. 2 With the Assumption That DₑNO Is Constant

If the decrease with lung volume observed for DₑCO is the same as that observed for DₑNO, VₑNO might be falsely high when values for DₑNO obtained at high lung volumes are used and falsely low when measurements of DₑNO obtained at low lung volumes are used. In the calculation of VₑNO with Eq. 2, we used a mean value of DₑNO obtained from DₑNO continuously calculated from the expired NO concentration recorded during expiration. The calculation started at a maximum volume equal to the subject’s TLC less four times the subject’s estimated anatomic dead space and ended when the subject reached a volume equal to the RV plus 15% of the forced vital capacity (19, 20). Newth and co-workers (19) reported that DₑCO measured with this method was unchanged as lung volume decreased. Preliminary measurements in nine subjects (20) showed that DₑNO decreased 9% over this volume interval, but this change did not reach statistical significance (P = 0.3). Therefore, the change in DₑNO with different lung volumes with use of the continuously calculated values during exhalation appears modest and would not be expected.
to result in large errors in \( V_{LNO} \). However, use of single-breath measurements of \( D_{UNO} \) obtained at TLC could result in overestimation of \( V_{LNO} \).

**Fraction of Total \( V_{LNO} \) and \( V_{UNO} \) Measured From Analyses of \( PE \)**

This method of measuring \( V_{LNO} \) and \( V_{UNO} \) assumes that NO produced in the tissues enters the air spaces and then diffuses into the surrounding tissues and perfusing blood. Some of the NO produced in the alveoli and the conducting airways will react with the tissues and blood and never enter the air spaces (16). This NO will not be measured by analyses of NO in the airways; therefore, \( V_{LNO} \) and \( V_{UNO} \) are likely underestimates of the true amount of NO produced by the alveoli and conducting airways. We are unaware of methods that can measure the fraction of NO that does not communicate with airways, and its size may be increased by diseases that impair diffusion of NO from the tissues into the air spaces.

**Comparison to Estimates of \( V_{LNO} \) and \( PA \) From Data of Others**

Because determination of \( PA \) requires breath holding or rebreathing for 10–15 s to achieve a constant value as well as rapid exhalations, most published values of \( PE \) do not permit calculations of \( PA \). However, Silkoff and co-workers (24) measured PE in 10 subjects at \( Q_{E} \) of 1,550 ml/s preceded by a 30-s breath hold and obtained a \( PE \) of 2.4 ± 1.0 × 10^{-6} Torr. With use of their mean data for \( PE \) at slower \( Q_{E} \), extrapolation of their data to an infinite value for \( Q_{E} \) gives \( PA \) of 1.9 ± 0.8 × 10^{-6} Torr, which is in close agreement with our value of 1.6 ± 0.7 × 10^{-6} Torr observed in our seven subjects.

Recently, Tsoukias and co-workers (28, 29) published a similar two-compartment model consisting of a nonexpansile compartment representing the conducting airways and an expansile compartment representing the alveolar region of the lungs. In their seven normal subjects, they determined \( PA \) from 8–12 measurements of \( PE \) and \( Q_{E} \) performed at constant values of \( Q_{E} \) that varied from 175 to 600 ml/s. With an equation equivalent to Eq. 3, they calculated \( PA \) and the flux of NO from the tissues of the conducting airways to the lumen. For model 1, flux equals \( V_{UNO} = (PE \cdot D_{UNO}) \). By plotting \( Q_{E} \) vs. \( PE \) on the vertical axis vs. \( Q_{E} \) on the horizontal axis, the intercept on the vertical axis equals flux and the slope equals \( PA \). Their values of \( PA \) of 4.1 ± 2.3 × 10^{-6} Torr were significantly greater than 1.6 ± 0.7 × 10^{-6} Torr obtained in our seven normal subjects (\( P = 0.025 \)). We have no explanation for the higher values of \( PA \) obtained by Tsoukias and co-workers. However, their flow rates ranged from only 175 to 600 ml/s, whereas \( Q_{E} \) for the subjects of Silkoff et al. (24) and our subjects varied from 4 ml/s to as high as 1,550 ml/s. This greater range in \( Q_{E} \) may provide more precision in determining \( PA \).

**Comparison to Estimates of \( V_{UNO} \) and \( D_{UNO} \) From Data of Others**

Only a few investigators have measured \( PE \) at a number of different constant \( Q_{E} \) that permit calculation of \( V_{UNO} \) or \( D_{UNO} \). Silkoff and co-workers (24) reported \( PE \) at nine different values of \( Q_{E} \) between 4.2 and 1,550 ml/s in 10 subjects. Their data shown in Fig. 13 permit calculation of \( V_{UNO} \) and \( D_{UNO} \) by use of Eq. 4 or 7. Note the similarity of their data to the findings in our subjects shown in Fig. 9. Model 1 closely fit the data of Silkoff and co-workers, with a mean \( r^{2} \) of 0.996 for their 10 subjects. \( V_{UNO} \) from their data was 0.061 ± 0.056 µl/min compared with 0.076 ± 0.053 µl/min in our subjects and did not differ significantly (\( P = 0.22 \)). \( D_{UNO} \) in their subjects was 0.4 ± 0.3 ml · min^{-1} · Torr^{-1} compared with 0.4 ± 0.4 ml · min^{-1} · Torr^{-1} in our subjects (\( P = 0.61 \)). Model 2 gave similar results with a close fit to the data (\( r^{2} = 0.995 \)). \( V_{UNO} \) was 0.053 ± 0.039 µl/min compared with 0.074 ± 0.052 µl/min in our subjects (\( P = 0.20 \)), and \( D_{UNO} \) was 0.5 ± 0.3 ml · min^{-1} · Torr^{-1} vs. 0.5 ± 0.4 ml · min^{-1} · Torr^{-1} in our subjects (\( P = 0.46 \)). The data of Silkoff and co-workers and our data show a wide scatter for the values of \( V_{UNO} \) and \( D_{UNO} \) in normal subjects, with coefficients of variation (CV) ranging from 60 to 90%. \( PA \) and \( V_{LNO} \) show less scatter, with a CV on the order of 40%.

Tsoukias and co-workers (28, 29) calculated flux from the data in their seven subjects, as described above. With use of representative values of \( PE \) in our subjects at \( Q_{E} \) of 175–600 ml/s used by Tsoukias and co-workers, their values of flux would only be 1–3% smaller than \( V_{UNO} \). Flux in their subjects was 0.043 ± 0.015 µl/min and did not significantly differ from the values of \( V_{UNO} \) of 0.070 ± 0.048 µl/min in our subjects with use of the faster \( Q_{E} \) shown in Fig. 8 (\( P = 0.20 \)) or 0.077 ± 0.053 µl/min with model 1 (\( P = 0.16 \)) or 0.074 ± 0.052 µl/min with model 2 (\( P = 0.18 \)) with use of faster and slower \( Q_{E} \).

**Evaluation of a Simplified Method to Measure \( V_{UNO} \) by Use of Only Faster \( Q_{E} \)**

Measurement of \( V_{UNO} \) with \( Q_{E} \) > 80–100 ml/s would have the advantage of fewer measurements of \( PE \) and elimination of the slow exhalations that are more difficult to perform because expiration must be continued for 25–150 s. The disadvantage is that \( D_{UNO} \) cannot...
be measured with any precision, because its accuracy
requires the higher concentrations of NO in the con-
ducting airways achieved with low values for QEI. In
our subjects, VUNO calculated with only the faster
QEI shown in Fig. 8 with use of Eq. 4 was 0.070 ± 0.048
µl/min compared with 0.077 ± 0.053 µl/min for model 1
and 0.074 ± 0.052 µl/min for model 2 by use of all the
values of PE and QEI shown in Fig. 9. The three values
did not differ significantly (P = 0.2) and have similar
CVs of ∼70%. Measuring VUNO with the useful ex-
pedient of using only faster QEI provides accept-
able values for VUNO but at the expense of measure-
ments of DUNO.

Choice of Analytic Method to Determine PA, VUNO, and
DUNO From Measurements of PE and QEI Performed
at Different Constant QEI

Tsoukias and co-workers (28, 29) measured PA and
flux by plotting the quantity of NO exhaled, which is
the product of QEI and PE = (PA − Pti) vs. QEI, so that
the slope of the graph equaled (PA − (PB − 47)) and the
intercept equaled flux (Eq. 3). We rearranged Eq. 3
to the form in Eqs. 4 and 5 and plotted PE vs. 1/QEI so that
QEI did not appear on both axes, thus eliminating
potential errors of mathematical coupling that can lead
to erroneous conclusions (1, 22). However, in our
normal subjects the two analytic techniques provide essen-
tially the same values for VUNO or flux and PE. For
example, the data using the higher values of QEI shown in
Fig. 8 with the analytic technique applied by Tsoukias
and co-workers (28, 29) using Eq. 3 resulted in flux of
0.065 ± 0.045 µl/min compared with VUNO of 0.070 ± 0.048 µl/min by use of Eq. 5 (P = 0.21). PA was 1.78 ± 0.77 × 10−6 Torr with the method of Tsoukias and
coworkers compared with 1.60 ± 0.72 × 10−6 Torr with
Eq. 6 (P = 0.14). Calculations with all seven sets of
values of QEI and PE resulted in flux of 0.067 ± 0.046
µl/min with the method of Tsoukias and coworkers
with use of Eq. 3 compared with VUNO of 0.077 ± 0.053
µl/min with Eq. 4. Therefore, in normal subjects the two
analytic methods result in identical data. Measurements
in less well-trained subjects are prone to greater varia-
tions in PE and QEI; therefore, it may be wise to analyze
data with both methods to determine whether math-
ematical coupling is influencing the results.

Alternate Models to Explain Expired NO Levels
at Different QEI

The models shown in Figs. 2 and 5 precisely predict expired NO concentrations in normal subjects. An alternate model of NO exchange in the upper conduct-
ing airways has been proposed that in preliminary
reports shows a similar close fit to the experimental
data (15, 25). These authors assume that the NO
production in the conducting airways results from a
constant partial pressure of NO in the tissue wall (Pti)
that can diffuse into the lumen at a rate proportional to
the concentration gradient. Then, for any small seg-
ment of the conducting airways of volume fV

\[
\frac{d}{dt} fV \cdot \frac{P_u}{P_B - 47} = fD_{UNO} (P_t - P_u)
\]  

where \( P_u \) is the partial pressure of NO in the segment.
and \( fD_{UNO} \) is the diffusing capacity for NO of the
segment. The solution of Eq. 8 is essentially the same
as described in APPENDIX B and results in

\[
PE = (P_t - P_A) \left[ 1 - e^{-\frac{D_{UNO} (P_B - 47)}{Q_E}} \right] + P_A \quad (9)
\]

The only difference from Eq. 7 describing the model in
Fig. 5 is that the term \( P_t \) replaces \( V_{UNO} \). Because all the other terms in Eqs. 7 and 9 are
identical, \( P_t \) in this model must equal \( V_{UNO} \) or \( D_{UNO} \) in
model 2 described above. Because the two models result
in identical solutions, the only difference in the models
is the terminology assigned to the measured constants.
For example, if the model with constant NO concen-
tration in the wall of the conducting airways (Pti)
is preferred, the value for Pti is readily determined by
dividing \( V_{UNO} \) by \( D_{UNO} \) obtained with model 1 or model 2.
In our subjects this value was 562 ± 798 and 313 ±
437 × 10−6 Torr for models 1 and 2, respectively. In the
10 subjects of Silkoff et al. (24) shown in Fig. 13, this
value was 573 ± 798 and 289 ± 531 × 10−6 Torr for
models 1 and 2, respectively.

In conclusion, these experiments show that NO pro-
duction into the lungs’ airways can be measured and
divided into contributions from the alveoli (VUNO)
and the conducting airways (VUNO). VUNO shows less scatter
in measurements in normal subjects and is two- to
fourfold greater than \( V_{UNO} \). DUNO is >100-fold greater
than \( D_{UNO} \). Because diffusion and control of NO produc-
tion in the alveoli and conducting airways are likely
governed by different mechanisms, this technique may
provide new information about processes that control
and alter NO production by the lungs.

APPENDIX A

Rate of Mixing of Alveolar Airway NO With Conducting
Airway NO in a Two-compartment Model

Model 1 assumes that, at the initiation of expiration at a
constant flow rate, NO in the conducting airways rapidly
arrives at a constant value that is maintained throughout
expiration. To determine the time required to reach this
constant value, we calculated the rate of change of NO in the
conducting airways (PE) as NO enters from the alveolar
airways. This instantaneous rate of change in the amount of
NO in the conducting airways equals \( \frac{d}{dt} \cdot \frac{P_t \cdot K}{(P_B - 47)} \),
where \( K \) is the volume of gas in the conducting airways and
PE is the partial pressure of NO in the conducting airways.
In this model, PE is determined by four variables: 1) NO from
the alveoli entering the conducting airways at a constant flow
rate \( QE \cdot PA \div (P_B - 47) \), 2) NO produced in the conducting
airway that enters its lumen \( (V_{UNO}) \), 3) NO diffusing out of
the lumen of the conducting airway into the surrounding
tissues \( (PE \cdot D_{UNO}) \), and 4) NO leaving the conducting
airway via exhalation \( (QE \cdot PE \div (P_B - 47)) \).

\[
\begin{align*}
\frac{d}{dt} \cdot \frac{P_t \cdot K}{(P_B - 47)} &= \frac{Q_E \cdot PA}{(P_B - 47)} + \frac{\dot{V}_{UNO}}{} \\quad (A1) \\
&= \frac{Q_E \cdot PE}{(P_B - 47)} - PE \cdot D_{UNO}
\end{align*}
\]
This can be rearranged
\[
\frac{dP_t}{dt} = \frac{1}{K} (Q_e \cdot P_A + V_{UNO}(P_B - 47)) - P_e \cdot \frac{1}{K} [Q_e + D_{UNO}(P_B - 47)]
\]
Equation A2 has the form \(dx/dt = a - bx\), the solution of which is \(x = (a/b)(1 - e^{-bt})\) or
\[
P_E = \frac{Q_e \cdot P_A + V_{UNO}(P_B - 47)}{Q_e + D_{UNO}(P_B - 47)} \left[1 - e^{-[Q_e + D_{UNO}(P_B - 47)]/K}\right] \tag{A3}
\]
When \(t\) is large, the exponent approaches zero and Eq. A3 becomes identical to Eq. 4. Because the expression \([Q_e \cdot P_A + V_{UNO}(P_B - 47)]/[Q_e + D_{UNO}(P_B - 47)]\) in Eq. A3 equals the value of \(P_E\) when mixing is complete \((P_E = \infty)\), Eq. A3 can be written as
\[
P_{E_t} = \frac{1 - e^{-[Q_e + D_{UNO}(P_B - 47)]/K}}{P_e} \tag{A4}
\]
where \(P_{E_t}\) is \(P_E\) at a selected time after initiation of exhalation. To estimate \(K\) in Eq. A4, we use the mean values obtained in our seven subjects for \(D_{UNO}\) of 0.5 ml·min\(^{-1}\)·Torr\(^{-1}\) and the measured half-time to reach a steady state during exhalation at \(Q_e = 250\) ml/min that equaled 0.25 min measured in one of the subjects. Then, according to Eq. A4
\[
1 + 2 = 1 - e^{-K/220} \quad \text{or} \quad K = 220\text{ ml}
\]
Then for any assumed flow rate \(Q_e\), the time to reach a specified ratio of \(P_E\) to \(P_{E_t}\) can be calculated. For example, if \(Q_e\) is 1,000 ml/s (60,000 ml/min), \(P_B = 760\) Torr, and the time to reach 99% equilibrium is desired, Eq. A4 becomes
\[
\frac{99}{100} = 1 - e^{-[60,000 \cdot 0.5]/220} \quad \text{or} \quad t = 1.01\text{ s}
\]
In these experiments, expired volume in our subjects was \(\sim 3,500\) ml STPD. At this \(Q_e\) of 1,000 ml/s, total time to exhale the breath is \(3,500 \div 1,000 = 3.5\) s. Therefore, in this subject, 99% equilibrium in \(P_E\) is reached when 1.01 + 3.5 or 29% of the breath has been exhaled. Figure 14 shows the required percentage of the breath exhaled to reach 99% and 99.9% equilibrium at different \(Q_e\) with use of the above representative values for \(D_{UNO}\) of 0.5 ml·min\(^{-1}\)·Torr\(^{-1}\) and \(K\) of 220 ml in our subjects. At \(Q_e \geq 80\) ml/s, 29% of the breath must be exhaled to achieve 99% mixing and 43% must be exhaled for 99.9% mixing. At slower flow rates, mixing is achieved at progressively smaller fractions of the exhaled breath, because \(P_A\) is a smaller fraction of the higher levels of NO present in the conducting airways with slow exhalations. In summary, this analysis shows that stable values for \(P_E\) can be expected once 30–40% of the expiratory vital capacity is exhaled.

**APPENDIX B**

**Determination of \(P_E\) in a Two-compartment Model of the Airways with Stratification of the Concentration of NO Along the Lumen of the Conducting Airways**

In any segment of the conducting airways illustrated in Fig. 5
\[
\frac{d}{dt} f_K \frac{P_{U_1}}{P_B - 47} = f_{V_{UNO}} - f_{D_{UNO}} \cdot P_{U_1} \tag{B1}
\]
}\[Fig. 14. Percentage of exhaled vital capacity required to reach a constant concentration of NO that is maintained during remainder of exhalation. At exhalations >80 ml/s, 99% mixing is achieved when 29% of breath has been expired and 99.9% mixing when 43% has been expired. At slower \(Q_e\), mixing is achieved more quickly, because \(P_A\) is a smaller fraction of higher levels of NO in conducting airways.\]

\[
\text{where } f_K \text{ is a small fraction of the total volume of gas in the conducting airway (K), } f_{V_{UNO}} \text{ is the same small fraction of } V_{UNO}, f_{D_{UNO}} \text{ is the same small fraction of } D_{UNO}, \text{ and } P_{U_1} \text{ is the partial pressure of NO in the segment. The ratio of } f_K, f_{V_{UNO}}, \text{ and } f_{D_{UNO}} \text{ is assumed constant throughout the conducting airways. As this volume of gas moves to the next segment, } f_{V_{UNO}} \text{ will add NO and } NO \text{ will be removed at a rate equal to } f_{D_{UNO}} \cdot P_{U_1}, \text{ where } P_{U_1} \text{ is the partial pressure of NO in the next segment that resulted from residence in the previous segment. In Eq. B1, the term } f \text{ cancels out, because each segment is defined as containing an equal fraction } f \text{ of } K, V_{UNO}, \text{ and } D_{UNO}. \text{ Rearranging gives}
\]
\[
\frac{d}{dt} P_U = \frac{V_{UNO}}{K} \left(\frac{P_B - 47}{K} - P_{U_1} \cdot D_{UNO} \frac{P_B - 47}{K}\right) \tag{B2}
\]
Equation B2 is of the form \(dx/dt = a - bx\). If the initial value of \(P_U\) is assumed to be zero, this type of equation has the solution
\[
x = (a + b) \left[1 - e^{-bt}\right]
\]
or
\[
P_U = \frac{V_{UNO}}{D_{UNO}} \left[1 - e^{-[D_{UNO}(P_B - 47)]/K}\right] \tag{B3}
\]
where \(P_U\) is the partial pressure of NO at the end of the conducting airways and equals \(P_E\), the expired partial pressure of NO. The term \(t\) is the time for the segment to traverse the conducting airways and equals the transit time for NO through the conducting airways. The volume of the conducting airways (K) divided by \(t\) equals \(Q_e\) or
\[
P_E = \frac{V_{UNO}}{D_{UNO}} \left[1 - e^{-[D_{UNO}(P_B - 47)]/K}\right] \tag{B4}
\]
Equation B4 states that as \(Q_e\) approaches zero, \(P_E\) reaches a maximum value of \(V_{UNO} \div D_{UNO}\). This value results because when there is no flow in the upper airways, the amount of NO
entering (V˙\textsubscript{UNO}) will equal the amount leaving, which equals PA. Rearranging, \( P\textsubscript{E} = V\dot{\text{UNO}} = \frac{V\dot{\text{UNO}}}{D\text{UNO}} \) when Q\dot{E} = 0. When Q\dot{E} is very fast, Eq. B4 states that PE approaches zero. However, unlike Eq. B3, a finite concentration of NO equal to PA is entering the conducting airways from the alveoli, so when Q\dot{E} is infinitely fast, P\dot{E} equals PA, not zero. Therefore, the correct limits for PE are as follows: PA when Q\dot{E} approaches infinity and \( \frac{V\dot{\text{UNO}}}{D\text{UNO}} \) when Q\dot{E} approaches zero. Equation B4 can therefore be expanded to

\[
PE = \left( \frac{V\dot{\text{UNO}}}{D\text{UNO}} - PA \right) \left( 1 - e^{-D\text{UNO}(PA - 47)/Q\dot{E}} \right) + PA
\]

**REFERENCES**


