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 different expiratory flow rates, permit calculation of $V_{UNO}$ and $D_{UNO}$. $V_{LNO}$ is the product of $P_A$ and the alveolar airway diffusion capacity for NO. In seven normal subjects, $P_A = 1.6 \pm 0.7 \times 10^{-6}$ (SD) Torr, $V_{LNO} = 0.19 \pm 0.07 \mu l/min$, $V_{UNO} = 0.08 \pm 0.05 \mu l/min$, and $D_{UNO} = 0.4 \pm 0.4$ 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.

nitric 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 $V_{LNO}$. $V_{LNO}$ is determined from the product of $P_A$ 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

$D_{LNO}$ 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

$D_{UNO}$ 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

$P_A$ Partial pressure of NO in the alveoli

$P_B$ Barometric pressure

$P_E$ Partial pressure of NO in exhaled gas

$P_U$ Partial pressure on NO in all or a segment of the upper conducting airway

$Q_E$ 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_LNO. The NO in the alveoli can react with the produced in this alveolar compartment that enters the air

\[ V_{LNO} = P_A \cdot D_{LNO} \]  

where D_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_LNO permits calculation of V_LNO. D_LNO was determined by a modification of the constant single exhalation method for measuring the pulmonary carbon monoxide diffusing capacity (D_LCO) described by Newth and co-workers (19) and Perillo and co-workers (20). Multiple values of D_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 by the much higher concentration in the nasophar-

\[ V_{UNO} = P_U \cdot D_{UNO} \]  

We describe two models of the conducting airways based on the above assumptions that allow the simultaneous calculation of P_A, V_LNO, and D_LNO 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

\[ Q_E \left[ P_A - (P_B - 47) \right] \]

where P_B is the barometric pressure, 47 is the partial pressure of NO in the lumen of the conducting airways (P_U). If the bronchial 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 in the lumen that diffuses back into these tissues will equal P_U · D_{UNO}. With exhalation at a constant flow rate, P_U will reach a constant value, and during this steady state V_LNO will equal the amount of NO diffusing back into the tissues or

**Fig. 1.** Exhaled nitric oxide (NO) (P_E) vs. flow rate (Q_E) in normal subject AP after 15 s of breath holding. Note marked decrease in P_E as flow rates increase.
circulation of the conducting airways (PE · DUNO) or
\[
\frac{\cdot}{Q_e \cdot P_e} = \frac{\cdot}{P_a} + V_{UNO} - (PE \cdot DUNO) \tag{3}
\]
Rearranging gives
\[
PE = \frac{1}{Qe} (V_{UNO} - PE \cdot DUNO) (P_B - 47) + PA \tag{4}
\]

Multiple sets of measurements of PE at different Qe provide the data needed to determine Pa, VUNO, and DUNO in Eqs. 3 and 4. First, Pa is determined graphically by taking advantage of the following observation: At higher values of Qe (i.e., >200 ml/s), Pe is relatively small and results in the term PE · DUNO decreasing to <3% of VUNO. If PE · DUNO is considered insignificant at such flow rates, Eq. 4 becomes
\[
PE = \frac{1}{Qe} (V_{UNO} (PB - 47)) + PA \tag{5}
\]

Equation 5 has the following form: y = mx + b. A plot of PE vs. 1/Qe results in Pa at the y-intercept when 1/Qe = 0, which is also the point where Qe = ∞. The slope equals VUNO/(PB - 47). We therefore calculated Pa from the linear regression of PE plotted vs. 1/Qe when Qe > 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 Qe collected at different constant Qe to calculate the remaining two variables, VUNO and DUNO, with use of Eq. 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 VUNO and DUNO, 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 VUNO and DUNO. 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 VUNO and DUNO. 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 VUNO was possible from just the faster values of Qe used to calculate Pa, VUNO, and DUNO was also calculated from these data with Eq. 5 and compared with VUNO determined with all values of Qe by use of Eq. 4.

This method for measuring Pa, VUNO, and DUNO 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, VUNO, and DUNO.

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, VUNO, and DUNO, so that the dimensions of any segment are fK, fVUNO, and fDUNO. At the start of exhalation at a constant Qe, Pa enters the first segment, where VUNO adds NO and DUNO 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 + (PS - 47)). It is changed by the NO production (fVUNO) entering the seg-
ALVEOLAR AIRWAYS

Fig. 5. Model of lower alveolar airways and upper conducting airways that assumes a progressive change in partial pressure of NO in conducting airways (P<sub>U</sub>) during exhalation (model 2). In contrast to model 1 in Fig. 2, conducting airways are divided into an infinite number of segments, each with same fraction (f) of conducting airway volume, conducting airway NO production (P<sub>U</sub>), and conducting airway diffusing capacity (P<sub>DUNO</sub>). In 1st segment, P<sub>VU</sub> adds NO to lumen and P<sub>DUNO</sub> removes NO, resulting in a partial pressure of NO equal to P<sub>U1</sub>. P<sub>U1</sub> then moves to next segment, where P<sub>VU</sub> and P<sub>DUNO</sub> result in P<sub>U2</sub>. At end of conducting airway, final value for P<sub>U</sub> equals P<sub>E</sub>. Other symbols are identified in Fig. 2. See text and APPENDIX A for more details.

Measurement less the amount diffusing out (P<sub>U</sub> · P<sub>DUNO</sub>) or

\[
\frac{d}{dt} f_0 \frac{P_U}{P_B - 47} = f_0 V_{UNO} - P_U f_0 D_{UNO}
\]  

(6)

The solution of Eq. 6 given in detail in APPENDIX B is

\[
P_E = \left( \frac{V_{UNO}}{DU_{NO}} - P_A \right) \left[ 1 - e^{-DU_{NO} (P_B - 47) / Q_E} \right] + P_A
\]  

(7)

P<sub>A</sub> is obtained from data obtained at the faster Q<sub>E</sub>, as described above. With this value of P<sub>A</sub> and all the measured pairs of P<sub>E</sub> and Q<sub>E</sub>, V<sub>UNO</sub> and D<sub>UNO</sub> 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 Speciality 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 equalled 0.8 ± 0.1 (SD) s. Multiple repetitive measurements of gas mixtures of 2.8 and 8.2 × 10<sup>-6</sup> Torr of NO showed a standard deviation of 0.09 × 10<sup>-6</sup> Torr. We assumed that the detection limit of our analyzer was two times the standard deviation of these multiple measurements or 0.2 × 10<sup>-6</sup> 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 ATPS were converted to partial pressure of NO in Torr BTPS as follows: NO in Torr = (NO in ppb ATPS)(P<sub>B</sub> - 47) / (P<sub>B</sub> - P<sub>HO</sub>)(10<sup>9</sup>), where P<sub>HO</sub> is partial pressure of water at room temperature. For example, at P<sub>B</sub> of 760 Torr and room temperature of 24°C where P<sub>HO</sub> = 22.4 Torr, 1 ppb NO = 0.735 × 10<sup>-6</sup> 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<sub>E</sub> 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<sub>E</sub> was obtained earlier during exhalation (APPENDIX A). At flow rates >1,000 ml/s, a constant value for P<sub>E</sub> 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<sub>E</sub> and Q<sub>E</sub>

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 (P<sub>E</sub>) and lung gas volume signal during 20 s of breath holding followed by exhalation at 600 ml/s. P<sub>E</sub> 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 P<sub>E</sub> 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. Q<sub>E</sub> 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.](http://jap.physiology.org/)
and \( \dot{Q} \) were averaged. \( P_E \) was measured as described for 10–20 s, so that \( P_A \) reached a constant concentration capacity (TLC) (Fig. 7). The subject then held this breath and inhaled room air from a bag-in-box device to total lung capacity. After breath is held for 10–20 s, valve is turned 90° into spirometer circuit, and subject exhaled, maintaining a mouth pressure of +5 cmH\(_2\)O by watching water manometer. Corks with different-sized apertures were placed in expiratory tubing and provided variable resistance to exhalation, resulting in different constant \( Q_E \). NO concentration is continuously 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.

Measurement of \( D_{L_NO} \)

\( D_{L_NO} \) 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 \( D_{L_CO} \) during exhalation described by Newth and co-workers (19) and Perillo and co-workers (20). Lung volume at any instant during exhalation used in the calculation of the multiple values of \( D_{L_NO} \) was obtained by adding the amount of exhaled gas remaining above RV recorded by the spirometer (Fig. 7) to the subject’s RV. RV was obtained from the subject’s functional residual capacity (FRC) measured with body plethysmography (5) by subtracting the expiratory reserve volume obtained from a spirometer (P. K. Morgan, Haverhill, MA) from FRC. The multiple measurements of \( D_{L_NO} \) during the exhalation were averaged and performed in triplicate, and the mean value was recorded.

Subjects

\( P_A, V_{UNO}, \) and \( D_{UNO} \) 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 referred to \( P_E \).

RESULTS

\( P_A, V_{UNO}, \) and \( D_{UNO} \)

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

\( V_{UNO} \) and \( D_{UNO} \)

Figure 9 shows the paired values for \( P_E \) and 1/\( Q_E \) for all exhalations by the seven subjects used to determine \( V_{UNO} \) and \( D_{UNO} \). \( Q_E \) ranged from 6 to 1,355 ml/s. For model 1, \( V_{UNO} \) was 0.077 ± 0.053 µl/min and \( D_{UNO} \) 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.
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 $V_{\text{UNO}}$, $V_{\text{LNO}}$, and $P_A$. $P_A$ multiplied by a separate measurement of $D_{\text{LNO}}$ gives a measurement of $V_{\text{LNO}}$. Besides these separate quantitative measurements of $V_{\text{LNO}}$ and $V_{\text{UNO}}$, this model provides a reasonable physiological explanation for the rise in expired NO with slower $Q_{\text{E}}$ and helps define the physiological basis for observed values of expired NO reported by many investigators.

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 $V_{\text{LNO}}$ and $V_{\text{UNO}}$, 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.

### 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...
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 \( V_{UNO} \), \( D_{UNO} \), and conducting airway gas volume. Because the simple one-compartment model of the conducting airways so accurately predicts \( P_E \) at different values of \( Q_E \), use of more realistic models of the conducting airways is not likely to result in a better measurable prediction of the experimental data.

Lung Model Where NO Production Is Uniformly Distributed Throughout the Walls of the Conducting Airways

Tsoukias and George (28) reported what may be a more realistic model of the dynamics of pulmonary NO exchange in the conducting and alveolar airways. They define NO production as taking place uniformly throughout the walls of the lungs' tissues. From the differential mass balance of NO in the tissue, they derive a second-order partial differential equation (Eq. 1 in Ref. 28) that allows determination of the changes in \( P_E \) by interventions such as varying breath-holding time before exhalation, accelerating or slowing flow rates during exhalation, and varying the inspired NO concentration. Their experimental data obtained by measuring expired NO concentrations in seven normal subjects at different constant \( Q_E \) levels result in a fit close to their model, similar to that obtained using models 1 and 2 described above. Therefore, expired NO concentrations collected at different \( Q_E \) in normal subjects unfortunately do not provide a means to determine which of these various models most closely accounts for the observed profiles of expired NO concentration.

Potential Errors in \( V_{LNO} \) Calculated With Eq. 2 With the Assumption That \( D_{LNO} \) Is Constant

If the decrease with lung volume observed for \( D_{LCO} \) is the same as that observed for \( D_{LNO} \), \( V_{LNO} \) might be falsely high when values for \( D_{LNO} \) obtained at high lung volumes are used and falsely low when measurements of \( D_{LNO} \) measured at low lung volumes are used. In the calculation of \( V_{LNO} \) with Eq. 2, we used a mean value of \( D_{LNO} \) obtained from \( D_{LNO} \) 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 \( D_{LCO} \) measured with this method was unchanged as lung volume decreased. Preliminary measurements in nine subjects (20) showed that \( D_{LNO} \) decreased 9% over this volume interval, but this change did not reach statistical significance (\( P = 0.3 \)). Therefore, the change in \( D_{LNO} \) 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 $\dot{V}_{LNO}$. However, use of single-breath measurements of $D_{UNO}$ obtained at TLC could result in overestimation of $\dot{V}_{LNO}$.

Fraction of Total $\dot{V}_{LNO}$ and $\dot{V}_{UNO}$ Measured From Analyses of $P_E$

This method of measuring $\dot{V}_{LNO}$ and $\dot{V}_{UNO}$ 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, $\dot{V}_{LNO}$ and $\dot{V}_{UNO}$ 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 $\dot{V}_{LNO}$ and $P_A$ From Data of Others

Because determination of $P_A$ requires breath holding or rebreathing for 10–15 s to achieve a constant value as well as rapid exhalations, most published values of $P_E$ do not permit calculations of $P_A$. However, Silkoff and co-workers (24) measured $P_E$ in 10 subjects at $Q_E$ of 1,550 ml/s preceded by a 30-s breath hold and obtained a $P_E$ of $2.4 \pm 1.0 \times 10^{-6}$ Torr. With use of their mean data for $P_E$ at slower $Q_E$, extrapolation of their data to an infinite value for $Q_E$ gives $P_A$ of $1.9 \pm 0.8 \times 10^{-6}$ Torr, which is in close agreement with our value of $1.6 \pm 0.7 \times 10^{-6}$ 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 $P_A$ from 8–12 measurements of $P_E$ 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 $P_A$ and the flux of NO from the tissues of the conducting airways to the lumen. For model 1, flux equals $\dot{V}_{UNO} = (P_E \cdot D_{UNO})$. By plotting $Q_E[(P_E - D_{UNO})]$ on the vertical axis vs. $Q_E$ on the horizontal axis, the intercept on the vertical axis equals flux and the slope equals $P_A = (P_E - 47)$. Their values of $P_A$ of $4.1 \pm 2.3 \times 10^{-6}$ Torr were significantly greater than $1.6 \pm 0.7 \times 10^{-6}$ Torr obtained in our seven normal subjects ($P = 0.025$). We have no explanation for the higher values of $P_A$ 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 $P_A$.

Comparison to Estimates of $\dot{V}_{UNO}$ and $D_{UNO}$ From Data of Others

Only a few investigators have measured $P_E$ at a number of different constant $Q_E$ that permit calculation of $\dot{V}_{UNO}$ or $D_{UNO}$. Silkoff and co-workers (24) reported $P_E$ 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 $\dot{V}_{UNO}$ 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 fits the data of Silkoff and co-workers, with a mean $r^2$ of 0.996 for their 10 subjects. $\dot{V}_{UNO}$ from their data was $0.061 \pm 0.056 \mu l/min$ compared with $0.076 \pm 0.053 \mu l/min$ in our subjects and did not differ significantly ($P = 0.22$). $D_{UNO}$ in their subjects was $0.4 \pm 0.3 \mu l/min \cdot \text{Torr}^{-1}$ compared with $0.4 \pm 0.4 \mu l/min \cdot \text{Torr}^{-1}$ in our subjects ($P = 0.61$). Model 2 gave similar results with a close fit to the data ($r^2 = 0.995$). $\dot{V}_{UNO}$ was $0.053 \pm 0.039 \mu l/min$ compared with $0.074 \pm 0.052 \mu l/min$ in our subjects ($P = 0.20$), and $D_{UNO}$ was $0.5 \pm 0.3 \mu l/min \cdot \text{Torr}^{-1}$ vs. $0.5 \pm 0.4 \mu l/min \cdot \text{Torr}^{-1}$ in our subjects ($P = 0.46$). The data of Silkoff and co-workers and our data show a wide scatter for the values of $\dot{V}_{UNO}$ and $D_{UNO}$ in normal subjects, with coefficients of variation (CV) ranging from 60 to 90%. $P_A$ and $\dot{V}_{LNO}$ 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 $P_E$ 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 $\dot{V}_{UNO}$. Flux in their subjects was $0.043 \pm 0.015 \mu l/min$ and did not significantly differ from the values of $\dot{V}_{UNO}$ of $0.070 \pm 0.048 \mu l/min$ in our subjects with use of the faster $Q_E$ shown in Fig. 8 ($P = 0.20$) or $0.077 \pm 0.053 \mu l/min$ with model 1 ($P = 0.16$) or $0.074 \pm 0.052 \mu l/min$ with model 2 ($P = 0.18$) with use of faster and slower $Q_E$.

Evaluation of a Simplified Method to Measure $\dot{V}_{UNO}$ by Use of Only Faster $Q_E$

Measurement of $\dot{V}_{UNO}$ with $Q_E > 80$–100 ml/s would have the advantage of fewer measurements of $P_E$ 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

![Fig. 13. $P_E$ vs. $1/Q_E$ in 10 subjects reported by Silkoff and co-workers (24). $Q_E = 4.2$–1,550 ml/s. Note similarity to data in Fig. 9 for our 7 subjects. Values for $\dot{V}_{UNO}$ and $D_{UNO}$ determined from these data are given in text.](http://apjcp.oxfordjournals.org/content/37/7/1539.f13)
be measured with any precision, because its accuracy requires the higher concentrations of NO in the conducting airways achieved with low values for Q\text{E}. In our subjects, \( V_{UNO} \) calculated with only the faster Q\text{E} shown in Fig. 8 with use of Eq. 4 was \( 0.070 \pm 0.048 \) \( \mu l/\text{min} \) compared with \( 0.077 \pm 0.053 \) \( \mu l/\text{min} \) for model 1 and \( 0.074 \pm 0.052 \) \( \mu l/\text{min} \) for model 2 by use of all the values of PE and Q\text{E} shown in Fig. 9. The three values did not differ significantly (\( P = 0.2 \)) and have similar CVs of \( \approx 70\% \). Measuring \( V_{UNO} \) with the useful expediency of using only faster Q\text{E} provides acceptable values for \( V_{UNO} \) but at the expense of measurements of DUNO.

Choice of Analytic Method to Determine \( P_A, V_{UNO} \), and \( D_{UNO} \) From Measurements of \( PE \) and \( Q\text{E} \) Performed at Different Constant Q\text{E}

Tsoukias and co-workers (28, 29) measured \( P_A \) and flux by plotting the quantity of NO exhaled, which is the product of Q\text{E} and \( PE \), \( Q\text{E} \cdot PE \), vs. \( Q\text{E} \), so that the slope of the graph equaled \( P_A / (P_B - 47) \) and the intercept equaled flux (Eq. 3). We rearranged Eq. 3 to the form in Eqs. 4 and 5 and plotted PE vs. \( 1/Q\text{E} \) so that Q\text{E} 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 essentially the same values for \( V_{UNO} \) or flux and \( P_A \). For example, the data using the higher values of Q\text{E} shown in Fig. 8 with the analytic technique applied by Tsoukias and co-workers (28, 29) using Eq. 3 resulted in flux of \( 0.065 \pm 0.045 \) \( \mu l/\text{min} \) compared with \( V_{UNO} \) of \( 0.070 \pm 0.048 \) \( \mu l/\text{min} \) by use of Eq. 5 (\( P = 0.21 \)). \( P_A \) was \( 1.78 \pm 0.77 \times 10^{-6} \) Torr with the method of Tsoukias and co-workers compared with \( 1.60 \pm 0.72 \times 10^{-6} \) Torr with Eq. 3 (\( P = 0.14 \)). Calculations with all seven sets of values of Q\text{E} and PE resulted in flux of \( 0.067 \pm 0.046 \) \( \mu l/\text{min} \) with the method of Tsoukias and co-workers with use of Eq. 3 compared with \( V_{UNO} \) of \( 0.077 \pm 0.053 \) \( \mu l/\text{min} \) with Eq. 4. Therefore, in normal subjects the two analytic methods result in similar data. Measurements in less well-trained subjects are prone to greater variations in \( PE \) and Q\text{E}; therefore, it may be wise to analyze data with both methods to determine whether mathematical coupling is influencing the results.

Alternate Models to Explain Expired NO Levels at Different Q\text{E}

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 conducting 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 (P\text{ti}) that can diffuse into the lumen at a rate proportional to the concentration gradient. Then, for any small segment of the conducting airways of volume fV

\[
\frac{d}{dt} fV \cdot \frac{P_U}{P_B - 47} = fD_{UNO} (P_{ti} - P_U) \tag{8}
\]

where \( fD_{UNO} \) 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 A and results in

\[
PE = (P_{ti} - P_A) \left[ 1 - e^{-\frac{D_{UNO} (P_B - 47)}{Q\text{E}}} \right] + P_A \tag{9}
\]

The only difference from Eq. 7 describing the model in Fig. 5 is that the term \( P_{ti} \) replaces \( V_{UNO} \) 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 concentration in the wall of the conducting airways (\( P_{ti} \)) is preferred, the value for \( P_{ti} \) is readily determined by dividing \( V_{UNO} \) by \( D_{UNO} \) obtained with model 1 or model 2. In our subjects this value was \( 562 \pm 798 \) and \( 313 \pm 437 \times 10^{-6} \) Torr for models 1 and 2, respectively. In the 10 patients of Slikoff et al. (24) shown in Fig. 13, this value was \( 573 \pm 798 \) and \( 289 \pm 531 \times 10^{-6} \) Torr for models 1 and 2, respectively.

In conclusion, these experiments show that NO production into the lungs' airways can be measured and divided into contributions from the alveoli (\( V_{LNO} \)) and the conducting airways (\( V_{UNO} \)). \( V_{LNO} \) shows less scatter in measurements in normal subjects and is two- to fourfold greater than \( V_{UNO} \). \( D_{LNO} \) is \( >100\)-fold greater than \( D_{UNO} \). Because diffusion and control of NO production 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 \( \frac{d}{dt} (fP_{E} \cdot K)/(P_B - 47) \), 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 \( fQ\text{E} \cdot P_A / (P_B - 47) \), 2) NO produced in the conducting airways that enters its lumen \( V_{UNO} \), 3) NO diffusing out of the lumen of the conducting airway into the surrounding tissues \( fP_{E} \cdot D_{UNO} \), and 4) NO leaving the conducting airway via exhalation \( fQ\text{E} \cdot P_A / (P_B - 47) \). Therefore

\[
\frac{d}{dt} \frac{P_{E} \cdot K}{P_B - 47} = \frac{fQ\text{E} \cdot P_A}{P_B - 47} + V_{UNO} - fP_{E} \cdot D_{UNO} - \frac{fQ\text{E} \cdot P_{E}}{P_B - 47} \tag{A1}
\]
This can be rearranged
\[
\frac{dP_E}{dt} = \frac{1}{K} (Q_E \cdot P_A + V_U \cdot D_{UNO} (P_B - 47))
\]
\[\text{(A2)}\]
Equation A2 has the form \(dx/dt = a - bx\), the solution of which is \(x = (a/b)(1 - e^{-bt})\) or
\[
P_E = \frac{Q_E \cdot P_A + V_U \cdot D_{UNO} (P_B - 47)}{Q_E + D_{UNO} (P_B - 47)} \left[ 1 - \frac{[Q_E \cdot D_{UNO} (P_B - 47)]}{K} \right] ^{\frac{1}{2}}
\]
\[\text{(A3)}\]
When \(t\) is large, the exponent approaches zero and Eq. A3 becomes identical to Eq. 4. Because the expression \([Q_E \cdot P_A + V_U \cdot D_{UNO} (P_B - 47)]/[Q_E + D_{UNO} (P_B - 47)]\) in Eq. A3 equals the value of \(P_E\) when mixing is complete \((P_E = \infty)\). Eq. A3 can be written as
\[
P_E = 1 - e^{\frac{[Q_E \cdot D_{UNO} (P_B - 47)]}{K}}
\]
\[\text{(A4)}\]
where \(P_E\) 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 0.50 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
\[
1/2 = 1 - e^{\frac{[Q_E \cdot D_{UNO} (P_B - 47)]}{K}}
\]
or \(K = 220\) mL
Then for any assumed flow rate \(Q_E\), the time to reach a specified ratio of \(P_E\) to \(P_E\) can be calculated. For example, if \(Q_E = 1,000\) mL/s (60,000 mL/min), \(P_B = 760\) Torr, and the time to reach 99% equilibrium is desired, Eq. A4 becomes
\[
\frac{99}{100} = 1 - e^{\frac{[60,000 \cdot 0.5 \cdot 728]}{220}}
\]
or \(t = 1.01\) 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 \(3,500 = 1,000\) or 3.5 s. Therefore, in this subject, 99% equilibrium in \(P_E\) is reached when \(1.01 \div 3.5\) 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 0.50 mL·min\(^{-1}\)·Torr\(^{-1}\) and \(K \approx 220\) 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
\[
\frac{d}{dt} f_k \frac{P_{U_1}}{P_B - 47} = f_{V_{UNO}} - f_{D_{UNO}} \cdot P_{U_1}
\]
\[\text{(B1)}\]
entering ($V_{UNO}$) will equal the amount leaving, which equals $P_E = V_{UNO} \cdot D_{UNO}$ when $Q_E = 0$. When $Q_E$ is very fast, Eq. B4 states that $P_E$ approaches zero. However, unlike Eq. B3, a finite concentration of NO equal to $P_A$ is entering the conducting airways from the alveoli, so when $Q_E$ is infinitely fast, $P_E$ equals $P_A$, not zero. Therefore, the correct limits for $P_E$ are as follows: $P_A$ when $Q_E$ approaches infinity and $V_{UNO} \cdot D_{UNO}$ when $Q_E$ approaches zero. Equation B4 can therefore be expanded to

$$P_E = \frac{V_{UNO}}{D_{UNO}} - PA \left(1 - e^{-D_{UNO}(P_A - 4)} \right) + PA \quad (B5)$$

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