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Chemiluminescent measurements of nitric oxide pulmonary diffusing capacity and alveolar production in humans

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Perillo, Irene B., Richard W. Hyde, Albert J. Olszowka, Anthony P. Pietropaoli, Lauren M. Frasier, Alfonso Torres, Peter T. Perkins, Robert E. Forster II, Mark J. Utell, and Mark W. Frampton. Chemiluminescent measurements of nitric oxide pulmonary diffusing capacity and alveolar production in humans. *J Appl Physiol* 91: 1931–1940, 2001.—Measurements of nitric oxide (NO) pulmonary diffusing capacity ($D_{L_{NO}}$) multiplied by alveolar NO partial pressure ($P_{A_{NO}}$) provide values for alveolar NO production ($\dot{V}_{A_{NO}}$). We evaluated applying a rapidly responding chemiluminescent NO analyzer to measure $D_{L_{NO}}$ during a single, constant exhalation (Dex_{NO}) or by rebreathing (Drb_{NO}). With the use of an initial inspiration of 5–10 parts/million of NO with a correction for the measured NO back pressure, Dex_{NO} in nine healthy subjects equaled 125 ± 29 (SD) $ml \cdot min^{-1} \cdot mmHg^{-1}$ and Drb_{NO} equaled 122 ± 26 $ml \cdot min^{-1} \cdot mmHg^{-1}$. These values were 4.7 ± 0.6 and 4.6 ± 0.6 times greater, respectively, than the subject's single-breath carbon monoxide diffusing capacity (Dsb_{CO}). Coefficients of variation were similar to previously reported breath-holding, single-breath measurements of Dsb_{CO}. $P_{A_{NO}}$ measured in seven of the subjects equaled 1.8 ± 0.7 mmHg × 10^{-6} and resulted in $\dot{V}_{A_{NO}}$ of 0.21 ± 0.06 μl/min using Dex_{NO} and 0.20 ± 0.6 μl/min with Drb_{NO}. Dex_{NO} remained constant at end-expiratory oxygen tensions varied from 42 to 682 Torr. Decreases in lung volume resulted in falls of Dex_{NO} and Drb_{NO} similar to the reported effect of volume changes on Dsb_{CO}. These data show that rapidly responding chemiluminescent NO analyzers provide reproducible measurements of $D_{L_{NO}}$ using single exhalations or rebreathing suitable for measuring $\dot{V}_{A_{NO}}$.

alveoli; rebreathing

THE RESPIRATORY TRACT PRODUCES nitric oxide (NO) in the nasal pharynx, the conducting airways, and the alveoli (10, 13, 24). Recently developed methods permit determination of the rate of NO production from these three locations (10, 24, 30). Representative values in normal subjects are 0.4 μl/min for the nose and nasal pharynx (10, 11), 0.08 μl/min from the conducting airways (24, 30), and 0.2 μl/min from the alveoli (24). The most popular method to estimate NO production by the conducting airways is based on measurement of expired NO concentration at constant expiratory flow rates from 50 to 200 ml/min while the subject exhales against a positive pressure of 5–20 cmH_2O (26). The positive pressure excludes contamination of the expired samples from NO in the nasal pharynx. At these relatively slow expiratory flow rates, the expired NO concentrations mainly reflect the NO production by the conducting airways, with little contribution from the alveoli. Airway inflammation from bronchial asthma can cause dramatic increases of conducting airway NO (26). Measurements of expired NO have attracted interest as a means to monitor airway inflammation and investigate the mechanisms controlling NO production by the conducting airways (26).

Determination of NO production by the alveoli ($\dot{V}_{A_{NO}}$) is more complex. It requires measurements of expired NO concentration collected while the subject expires against a positive pressure of 5–20 cmH_2O at a number of different constant expiratory flow rates (24, 29, 30). These measurements permit extrapolation to the NO concentration present at an infinitely fast expiratory flow rate that is free of any contribution from the conducting airways to the expired air. This extrapolated value represents the alveolar NO partial pressure ($P_{A_{NO}}$) present during a steady state without entry of NO into the alveoli from the conducting airways. $P_{A_{NO}}$ multiplied by the pulmonary NO diffusing capacity ($D_{L_{NO}}$) equals $\dot{V}_{A_{NO}}$ (15) or

$$\dot{V}_{A_{NO}} = P_{A_{NO}} \cdot D_{L_{NO}} \quad (1)$$

To date, $\dot{V}_{A_{NO}}$ has only been measured in normal human subjects (24), but it is suspected to be elevated

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in some diseases, such as cirrhosis of the liver (19), while decreased in other diseases, such as primary pulmonary hypertension (8).

Measurements of $\dot{V}_{ANO}$ require a measurement of D_{LNO} (*Eq. 1*). In humans, several groups have measured D_{LNO} with a modification of the carbon monoxide (CO) breath-holding, single-breath method (Dsb_{CO}) (2, 3, 12, 18). Short breath-holding times of 3–8 s were required because the rapid clearance of NO from the alveoli prevented accurate measurements of the small expired NO signal after 10 s of breath holding. The shorter breath-holding periods make it difficult to determine accurately the breath-holding time interval. In the past decade, rapidly responding, highly sensitive chemiluminescent NO analyzers have become available that permit continuous breath-by-breath measurements of NO concentration during the ventilatory cycle.

The purpose of this report is to see if these analyzers can be applied to make accurate measurements of D_{LNO} suitable for calculating $\dot{V}_{ANO}$. We describe a single exhalation method and rebreathing method that do not require a concurrent measurement of an inert, relatively insoluble gas, such as helium or methane. Compared with the breath-holding techniques, advantages include the use of lower concentrations of NO in the order of 5–10 ppm and the elimination of errors from estimating the breath-holding time interval. These methods are also compared with Dsb_{CO} obtained in the same subjects.

Glossary

CV	Coefficient of variation
Dex_{CO}	Pulmonary CO diffusing capacity measured during a single, constant maximum exhalation
Dex_{NO}	Pulmonary NO diffusing capacity measured during a single, constant maximum exhalation
D_L	Pulmonary diffusing capacity; test gas not specified
D_{LCO}	Pulmonary CO diffusing capacity; method of measurement not specified
D_{LNO}	Pulmonary NO diffusing capacity
Drb_{CO}	Pulmonary CO diffusing capacity measured during rebreathing CO
Drb_{NO}	Pulmonary NO diffusing capacity measured during rebreathing NO
Dsb_{CO}	Breath-holding, single-breath CO diffusing capacity
Dsb_{NO}	Breath-holding, single-breath NO diffusing capacity
P_{ANO}	Partial pressure of NO in alveoli
P_{NOex}	Expired concentration of NO
$P_{NOex\infty}$	Minimal partial pressure of NO that can be present during measurements of Dex_{NO}
$P_{NOrb\infty}$	Minimal partial pressure of NO that can be present during measurements of Drb_{NO}
V_A	Alveolar volume
$\dot{V}_{ANO}$	NO production by the alveoli

METHODS

Measurement of NO. Details of the method to measure NO have been previously described (11, 24). A rapidly responding chemiluminescent NO analyzer (model 270B, Sievers, Boulder, CO), operating at a sample rate of 250 ml/min, continuously measured exhaled levels of NO at the mouthpiece. Response time of the analyzer was <200 ms for a signal of 90% full scale with a lag time of 0.8 s. 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 dilution of a gas containing 229 parts per billion (ppb) of NO. To obtain reference 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 packed with potassium permanganate (Purafil, Thermoenvironmental Instruments, Franklin, MA).

Measurements of this NO-free air were performed within 2 min before and after each NO measurement of expired gas samples and averaged to obtain the zero NO signal. The lag time between the volume signal obtained from the potentiometer attached to the spirometer and change in the NO signal caused mainly by the transit time through the NO sampling tube was determined daily and equaled 0.8 ± 0.1 (SD) s. Multiple repetitive measurements of gas mixtures of 2.8 and 8.2 mmHg $\times 10^{-6}$ of NO showed a SD of 0.09 mmHg $\times 10^{-6}$. During gas sampling, the operator exhaled warm humidified gas from the mouth by the inlet of the NO analyzer every ~5–10 min; thus the walls of the unheated tygon inlet tubing (150 cm in length with an inside diameter of 1.6 mm and an outside diameter of 3.2 mm) were kept moist. This resulted in all gases being considered measured at ATPS. The chart recorder (MACLab Recording Instrument, AD, Castle Hill, NSW, Australia) stored the volume signal and NO signal in a Macintosh LC computer (Apple Computer, Cupertino, CA).

Single, rapid, maximal exhalation at constant flow rate for measuring D_{LNO}. After resting for 5 min in the sitting position, each subject first exhaled to residual volume (RV) through the mouthpiece of the apparatus attached to a four-way valve connected to room air (Fig. 1). The valve was turned 90°, and the subject rapidly inhaled 5–10 ppm of NO from the bag-in-box to total lung capacity, breath held for 2–3 s, and then exhaled into the spirometer at an expiratory flow rate of 460 ± 80 (SD) ml/s (range: 370–560 ml/s) to RV. For most of the measurements, the constant expiratory flow rate was facilitated by narrowing the expiratory line to the spirometer with a cork penetrated by an open tube with a cross-sectional area of 12 mm^2. The subject was instructed to maintain an expiratory pressure of +5 cmH_2O observed on the water manometer attached at the mouthpiece. The concentration of NO at the mouthpiece and changes in lung volume were recorded (Fig. 2). D_{LNO} measured during a single, constant maximum exhalation (Dex_{NO}) was calculated by using a modification of the method described by Cotton and coworkers (6). At any instant, the amount of NO leaving the alveolar volume (V_A) equals the amount of NO diffusing into the blood or

$$\frac{d}{dt}\left[\frac{P_{NOex}\cdot V_A}{(P_B - 47)}\right] = Dex_{NO}\,(P_{NOex} - P_{NOex\infty}) \qquad (2)$$

where Dex_{NO} is recorded in ml·min^{-1}·mmHg^{-1}, P_{NOex} is the partial pressure of NO at any instant in the expired gas measured at the mouth in mmHg, V_A is expressed in ml STPD, P_B is barometric pressure, and $P_{NOex\infty}$ is the minimal partial pressure of NO that can be present during the measurement

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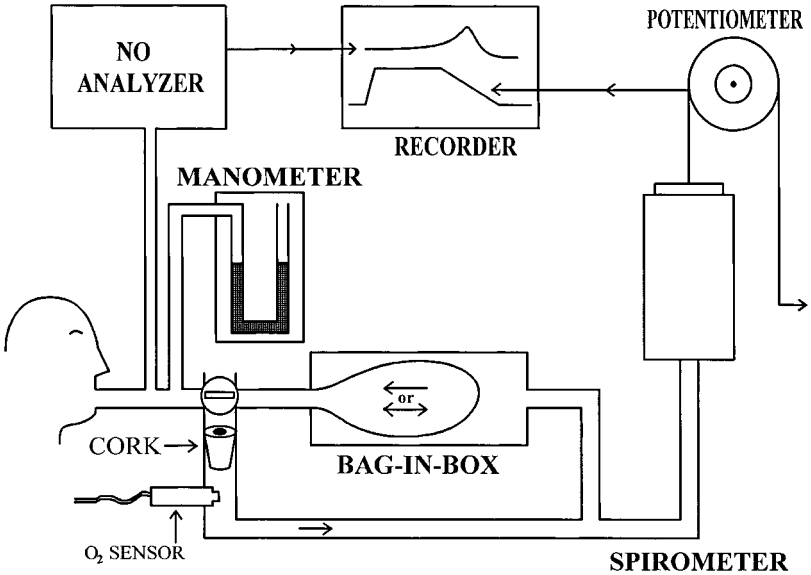

Fig. 1. Diagram of apparatus used to measure nitric oxide (NO) diffusing capacity with single, constant exhalations (Dex_{NO}) and rebreathing (Drb_{NO}). For measuring Dex_{NO}, the subject inhaled 5–10 parts/million (ppm) of NO from the bag-in-box, breath held for 2–3 s, and then exhaled through the orifice of the cork while maintaining +5 cm of pressure in the manometer. The cork and manometer result in a constant expiratory flow rate of ~0.5 l/s. To measure Drb_{NO}, the cork and manometer were removed, and subjects rebreathed 5–10 ppm of NO placed in the bag, emptying the bag at the end of each inspiration. For details see text.

whose subtraction corrects for NO production by the alveoli. Integration gives

$$Dex_{NO} = \frac{V_A \times 60}{(P_B - 47)(t_2 - t_1)} \ln\left(\frac{P_{NO_{ex1}} - P_{NO_{ex\infty}}}{P_{NO_{ex2}} - P_{NO_{ex\infty}}}\right) \quad (3)$$

where $P_{NO_{ex1}}$ and $P_{NO_{ex2}}$ are the initial and final values of $P_{NO_{ex}}$ during the time interval $t_2 - t_1$ recorded in seconds during the exhalation. The computer calculated Dex_{NO} every 0.025 s and then averaged this value with the previous 19 values, so that a value of Dex_{NO} was recorded as a 0.5-s moving average plotted every 0.025 s (Fig. 3). A single value for Dex_{NO} for each exhalation was then calculated from the mean of these values for Dex_{NO}, which was obtained after the initial data were discarded that was measured during exhaling of a volume equal to four times the total dead space, which was calculated as the sum of the subject's estimated anatomic dead space and the instrument's dead space of 100 ml. The subject's anatomic dead space was assumed to equal the subject's ideal body weight in pounds and was expressed in milliliters (4). The final 15% of the exhaled vital capacity was also discarded.

The V_A at any instant was calculated by the computer using the subject's RV plus the remaining fraction of the exhaled vital capacity recorded by the spirometer. RV was obtained from the subject's functional residual capacity measured with a body plethysmograph (9), less the expiratory reserve volume measured with a spirometer.

Estimation of the back pressure or $P_{NO_{ex\infty}}$. The subjects repeated the same maneuver used to measure Dex_{NO} with the bag-in-box filled with room air containing <15 ppb of NO. $P_{NO_{ex\infty}}$ was calculated from the mean value of the expired NO concentration that was recorded after the initial NO signal was discarded that was obtained while the subject exhaled a volume equal to four times the total dead space calculated as described above, as well as the final 10% of the expired

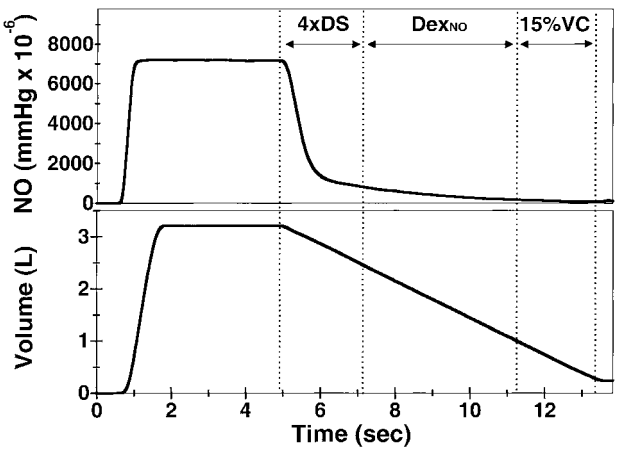

Fig. 2. Recording of NO signal and change in lung volume during the measurement of Dex_{NO}. The subject rapidly inhaled 3.3 liters of NO-enriched air, breath held for 3 s, and then exhaled at a constant flow rate of 370 ml/s. Dex_{NO} was calculated from the data collected after discarding an initial volume equal to 4 times the subject's and instrument's dead space (4 × DS) and the final 15% of the expired vital capacity (15% VC).

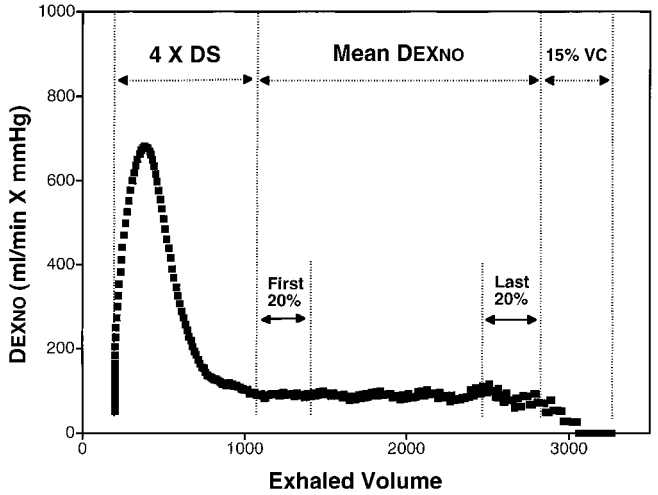

Fig. 3. Computer-generated values of Dex_{NO} vs. exhaled lung volume calculated from the data shown in Fig. 2. The mean value for Dex_{NO} was obtained from the average of recorded values after discarding an initial volume equal to the instrument's and subject's dead space (4 × DS) and the final 15% VC. To determine the effects of lung volume on Dex_{NO}, the value recorded during the first 20% of the measurement was compared with the last 20%.

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volume. For six of the subjects, measurements of $P_{NO_{ex\infty}}$ were made at several different constant expiratory flow rates above and below the flow rate used to measure Dex_{NO}. This allowed extrapolation to a value of $P_{NO_{ex\infty}}$ that matched the expiratory flow rate present during the measurement of Dex_{NO}.

Rebreathing method for measuring DL_{NO}. The rebreathing maneuver was performed by first filling the bag-in-box with 5–10 ppm of NO in air at a gas volume of ~1 liter less than the subject's vital capacity. The subject inserted the mouthpiece and exhaled into the room to RV. The valve at the mouthpiece was turned to the bag, and the subject inspired the contents of the bag and then rebreathed 1.6 ± 0.3 liters (STPD) at a constant rebreathing rate of 23 ± 4 breaths/min, emptying the bag with each breath for 20–30 s (Fig. 4, *top* and *middle*). The computer transformed the NO signal to a plot of the natural logarithm of the partial pressure of NO at the mouth less the minimal partial pressure of NO present during rebreathing ($P_{NO_{rb\infty}}$) vs. time (Fig. 4, *bottom*), where $P_{NO_{rb\infty}}$ is calculated as described below. After one to three breaths, there is a linear decrease in the logarithm of this NO signal for the subsequent six to eight breaths, and these data were used to calculate rebreathing DL_{NO} Drb_{NO}. Data collected after 20 s of rebreathing were usually discarded because of the low signal-to-noise ratio at NO concentrations <30 mmHg × 10^{-6} (Fig. 4, *bottom*). The operator measured the bag concentration (P_{bag}) recorded at the end of inspiration and the end-expiratory concentration ($P_{NO_{ex}}$) of these breaths. The computer calculated the slope of two parallel lines fitted to $P_{bag} - P_{NO_{rb\infty}}$ and $P_{NO_{ex}} - P_{NO_{rb\infty}}$ (k_{NO}) expressed as a fractional change in concentration per minute. From two parallel lines drawn through $P_{bag} - P_{NO_{rb\infty}}$ and $P_{NO_{ex}} - P_{NO_{rb\infty}}$, the ratio of $P_{bag} - P_{NO_{rb\infty}}$ to $P_{NO_{ex}} - P_{NO_{rb\infty}}$ at any instant (H) was calculated (Fig. 4). Drb_{NO} was then calculated with an equation similar to equations derived by Hook and Meyer (14) and Meyer and coworkers (21) that were used for calculating rebreathing measurements of oxygen, CO, and NO diffusing capacity (see APPENDIX for derivation)

$$Drb_{NO} = \frac{k_{NO}(V_{tot})}{P_B - 47} + \frac{\dot{V}_{eff}(H-1)^2}{(P_B - 47)H} \quad (4)$$

where V_{tot} is the total gas volume in ml (STPD) in the rebreathing circuit, which consists of the subject's RV, the gas volume of the initial inspiration, and the instrument's dead space of 100 ml. $\dot{V}_{eff}$ is effective ventilation in ml/min during rebreathing and was calculated as the mean volume of the rebreathing breaths less the instrument's dead space of 100 ml and the subject's dead space, which is estimated to be 25% of the rebreathing volume (23) multiplied by the rebreathing rate in breaths/min.

Estimation of the $P_{NO_{rb\infty}}$. $P_{NO_{rb\infty}}$ during rebreathing was measured by having the subject perform the same rebreathing maneuver for 15–20 s with the bag-in-box filled with room air. After 5–10 s of rebreathing, the NO concentration reaches a constant value equal to $P_{NO_{rb\infty}}$ (see Fig. 4 in Ref. 11). The value for $P_{NO_{rb\infty}}$ was measured from the mean NO concentration recorded at the mouth during the last 3 s of rebreathing after discarding the final exhalation during rebreathing (11). In seven of the subjects, the rebreathing maneuver to measure $P_{NO_{rb\infty}}$ was performed at ventilatory rates above and below the value present for measuring Drb_{NO}. These measurements permitted extrapolation to a value for $P_{NO_{rb\infty}}$ that matched the ventilatory rate present during the measurement of Drb_{NO}.

Effects of alveolar oxygen tension on Dex_{NO}. Five of the subjects performed measurements of Dex_{NO} at end-expiratory oxygen tensions varying between 42 and 570 Torr. End-expiratory O_2 was measured with an oxygen sensor (AG-17 O_2 sensor, Ceramatec, Salt Lake City, UT; and TED200-TX microprocessor, Teledyne, City of Industry, CA) installed in the expiratory line of the apparatus (Fig. 1). To vary the end-expiratory partial pressure of O_2, the bag-in-box was filled with O_2 concentrations varying from 1 to 99% before the NO was added just before the measurement of Dex_{NO}. To obtain end-expired O_2 tensions in excess of 500 Torr, the subject performed three slow vital capacity maneuvers while inhaling 100% oxygen before inhaling 5–10 ppm NO in 99% oxygen. Dex_{NO} was calculated as described above.

Effects of V_A on Dex_{NO} and Drb_{NO}. Effects of changes in V_A on Dex_{NO} were determined by comparing its value obtained during the first 20% of the expirate used to calculate Dex_{NO} to the final 20% (Fig. 3). Six of the subjects decreased their V_A during measurements of Drb_{NO} by starting the initial inspiration from RV, with the rebreathing bag containing a volume reduced to 2–3 liters less than the subject's vital capacity.

Measurement of Dsb_{CO}. Dsb_{CO} was measured by the technique of Jones and Meade (16) using automated equipment (P. K. Morgan, Haverhill, MA). Dsb_{CO} was multiplied by 5 to provide an estimated value of NO diffusing capacity (5 × Dsb_{CO}). The factor 5 was chosen on the basis of published reports showing a ratio of NO diffusing capacity to CO dif-

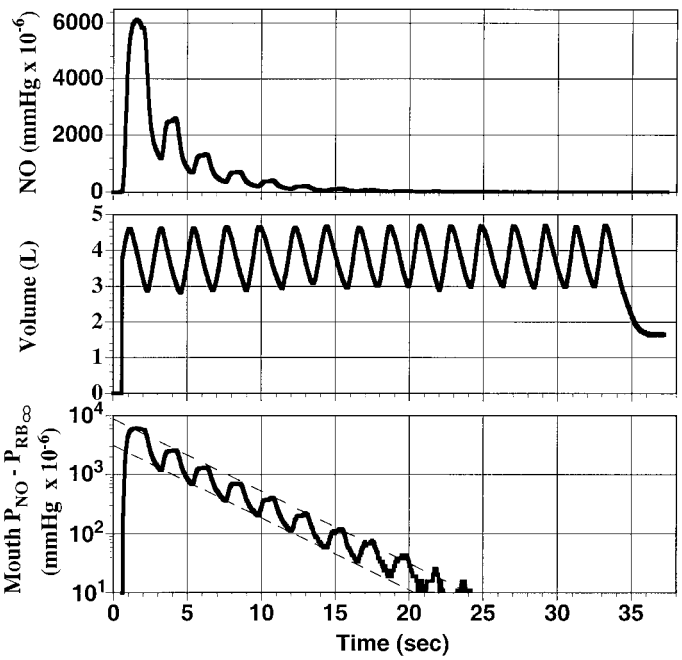

Fig. 4. Measurement of Drb_{NO}. *Top*: NO concentration at the mouthpiece during rebreathing of air enriched with 6,000 ppb of NO. *Middle*: changes in lung volume during rebreathing. The subject inspired 4.6 liters and then rebreathed 1.6 liters for 34 s. *Bottom*: the oscillating signal is the computer-processed NO concentration recorded as the difference between NO concentration at the mouth (P_{NO}) less the minimal partial pressure of NO present during rebreathing ($P_{NO_{rb\infty}}$). This signal is plotted as the logarithm of $P_{NO} - P_{NO_{rb}}$ vs. time. After the first 2 breaths were discarded, the computer fitted 2 straight, parallel, dashed lines to the inspired concentration from the breathing bag (*top* dashed line) and the end-expiratory concentration (*bottom* dashed line). The slope of these lines (k_{NO}) and the ratio of the concentration of the 2 dashed lines at any instant (H) were used to calculate Drb_{NO} with *Eq. 4*. In this subject, H can be calculated at *time 0* and equaled $9.0 \times 10^3 \div 3.2 \times 10^3$, or 2.8.

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fusing capacity breathing air ranging from 4.3 to 5.3 (2, 3, 12, 18, 28, 31).

Measurement of $\dot{V}A_{NO}$. $\dot{V}A_{NO}$ is the product of PA_{NO} and the diffusing capacity for NO (*Eq. 1*). PA_{NO} for seven of the subjects was measured with the technique described by Pietropaoli and coworkers (24), where subjects performed a series of exhalations at different constant expiratory flow rates after inspiring room air and breath holding for 10–15 s. The faster the exhalation, the less is the contribution of the NO produced by the conducting airways to the NO originating from the alveoli (PA_{NO}). The expired NO concentration in a series of expirations at different expiratory flow rates permits extrapolation to the concentration of NO at an infinite expiratory flow rate. This value was considered to be PA_{NO} free of contamination by NO from the conducting airways. $\dot{V}A_{NO}$ was then calculated with *Eq. 1*.

Subjects. Measurements of Dex_{NO}, Drb_{NO}, and Dsb_{CO} were made in nine healthy, nonsmoking subjects ranging in age from 31 to 72 yr (mean 46 ± 18 yr). Height was 173 ± 9 cm, and weight was 73 ± 11 kg. Six were men, and three were women. All subjects were free of cardiopulmonary disease and respiratory symptoms. Spirometry showed values of ≥90% of predicted (7) for the forced expiratory volume in 1 s with a mean value of 106 ± 14% of the predicted forced expiratory volume in 1 s. The study was approved by the University of Rochester's Research Subjects Review Board.

Statistical methods. Results are given as means ± SD. In experiments in which 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. A P value < 0.05 was required for statistical significance.

RESULTS

Values for Dex_{NO}, Drb_{NO}, and Dsb_{CO}. Figure 5 shows the individual values for Dex_{NO}, Drb_{NO}, and Dsb_{CO} multiplied by 5 in the nine subjects. Dex_{NO} equaled 125 ± 29 $ml \cdot min^{-1} \cdot mmHg^{-1}$, Drb_{NO} equaled 122 ± 26 $ml \cdot min^{-1} \cdot mmHg^{-1}$, and 5 × Dsb_{CO} equaled 135 ± 36 $ml \cdot min^{-1} \cdot mmHg^{-1}$. The 3% difference between Dex_{NO} and Drb_{NO} reached statistical significance ($P = 0.046$). Dex_{NO} was 4.7 ± 0.6 times greater than Dsb_{CO}, and Drb_{NO} was 4.6 ± 0.6 times greater than Dsb_{CO}.

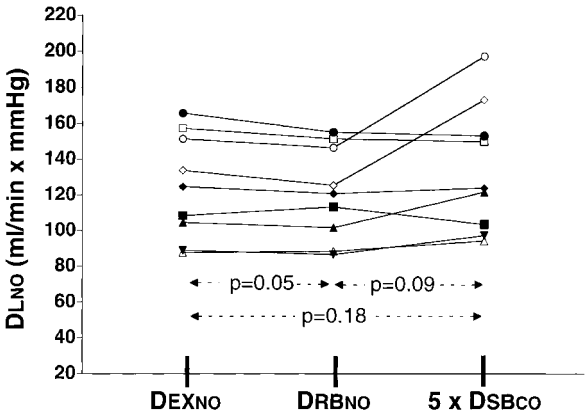

Fig. 5. Values for Dex_{NO}, Drb_{NO}, and 5 times the carbon monoxide breath-holding, single-breath diffusing capacity (5 × Dsb_{CO}) for 9 normal subjects. DL_{NO}, pulmonary lung diffusing capacity of NO. Symbols are as follows: ○, *subject MC* (31 yr, male); □, *subject AP* (33 yr, male); ●, *subject AT* (33 yr, male); ◇, *subject JB* (34 yr, male); ▲, *subject PP* (72 yr, male); ◆, *subject RH* (68 yr, male); ■, *subject CG* (32 yr, female); ▼, *subject IP* (32 yr, female); △, *subject SH* (65 yr, female).

Table 1. *Interday and intraday variability of Dex_{NO} and Drb_{NO} expressed as coefficients of variation*

	Intraday CV, %	Interday CV, %
Dex_{NO}	4.4 ± 3.9 (n = 9)	8.1 ± 4.9 (n = 7)
Drb_{NO}	3.4 ± 1.9 (n = 9)	13.6 ± 10.1 (n = 6)

Values are means ± SD; n, no. of subjects. CV, coefficient of variation; Dex_{NO}, NO diffusing capacity measured during a single exhalation; Drb_{NO}, NO diffusing capacity measured during rebreathing.

Intraday and interday variability of Dex_{NO} and Drb_{NO}. Table 1 shows interday and intraday variability of Dex_{NO} and Drb_{NO} expressed as the coefficient of variation (CV). Intraday CV in the nine subjects for Dex_{NO} was 4.4 ± 3.9% and for Drb_{NO} was 3.4 ± 1.9%. Interday CVs were slightly larger at 8.1 ± 4.9% for Dex_{NO} measured in seven of the subjects and 13.6 ± 10.1% for Drb_{NO} measured in six of the subjects.

Influence of alveolar oxygen tension on Dex_{NO}. Figure 6 shows values for Dex_{NO} at end-expiratory O_2 tensions that varied from 42 to 682 Torr in five of the subjects. Dex_{NO} showed no consistent change with different oxygen tensions. For example, the mean values for the five subjects obtained <100 Torr at 60 ± 10 Torr equaled 126 ± 30 $ml \cdot min^{-1} \cdot mmHg^{-1}$ compared with 128 ± 31 $ml \cdot min^{-1} \cdot mmHg^{-1}$ for all measurements at values >450 Torr obtained at 557 ± 68 Torr and did not differ significantly ($P = 0.14$).

Effects of changes in V_A on Dex_{NO} and Drb_{NO}. Figure 7*A* shows values for Dex_{NO} determined from the first 20% and the final 20% of the expirate used to determine PNO_{ex} in nine subjects. Dex_{NO} fell from 138 ± 35 to 100 ± 21 $ml \cdot min^{-1} \cdot mmHg^{-1}$, or 28% ($P = 0.001$), with a change in V_A from 6,578 ± 1,508 to 4,714 ± 1,112 ml BTPS. Figure 7*B* shows the changes for Drb_{NO} performed at V_A values of 6,186 ± 791 and 4,682 ± 373 ml BTPS in six of the subjects. Drb_{NO} fell from 127 ± 30 to 107 ± 21 $ml \cdot min^{-1} \cdot mmHg^{-1}$, or 19% ($P = 0.050$).

$\dot{V}A_{NO}$ in humans. In seven of the subjects, $\dot{V}A_{NO}$ was calculated from the product of PA_{NO} with Dex_{NO},

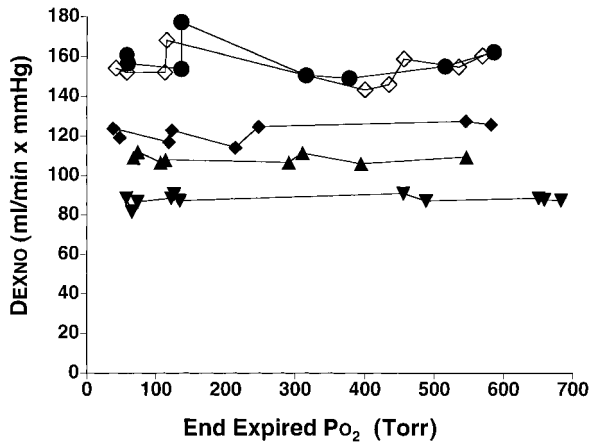

Fig. 6. Dex_{NO} at different end-expired P_{O_2} in 5 subjects. Dex_{NO} obtained at $P_{O_2} < 100$ Torr did not differ from values measured at $P_{O_2} > 450$ Torr. See Fig. 5 legend for definition of symbols.

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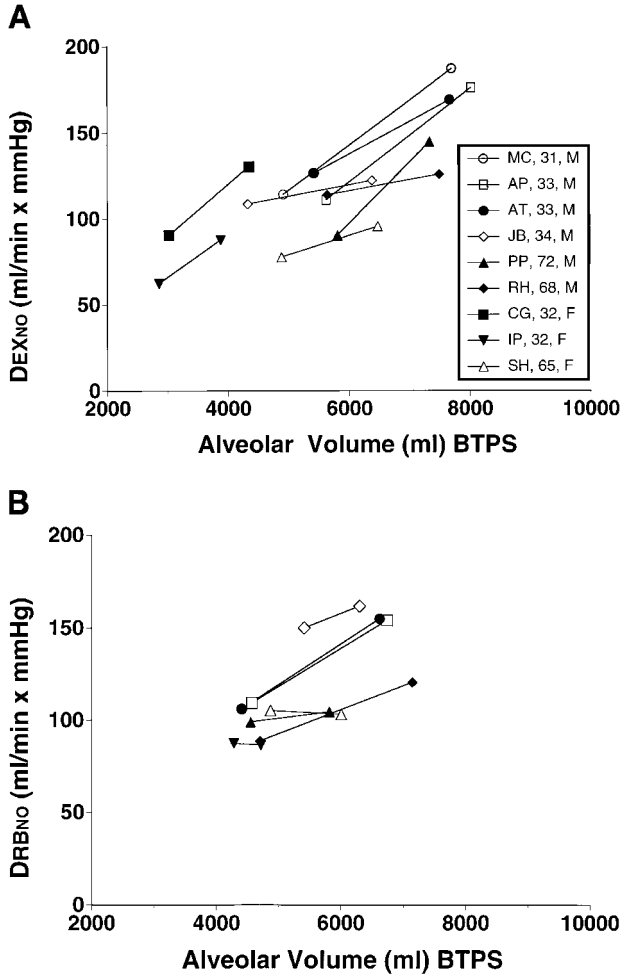

Fig. 7. *A*: changes in Dex_{NO} at different lung volumes in 9 subjects. *B*: changes in Drb_{NO} at different lung volumes in 6 of the subjects. M, male; F, female.

Drb_{NO}, and 5 × Dsb_{CO} (Fig. 8). P_{ANO} equaled 1.8 ± 0.7 mmHg × 10^{-6}. The respective values of $\dot{V}_{ANO}$ were 0.21 ± 0.06, 0.20 ± 0.06, and 0.22 ± 0.06 μl/min. The small difference between $\dot{V}_{ANO}$ calculated with Dex_{NO} and Drb_{NO} was significant (P = 0.009).

$P_{NOex\infty}$ and $P_{NOrb\infty}$ or back pressure. $P_{NOex\infty}$ equaled 3.8 ± 1.7 mmHg × 10^{-6} and $P_{NOrb\infty}$ equaled 2.6 ± 0.7 mmHg × 10^{-6}. If $P_{NOex\infty}$ and $P_{NOrb\infty}$ were assumed to equal zero, Dex_{NO} was underestimated by 2.3 ± 1.6% (P = 0.002) and Drb_{NO} by 1.7 ± 0.9% (P = 0.0002).

DISCUSSION

These data show that rapidly responding chemiluminescent NO analyzers, combined with simple pulmonary function equipment (Fig. 1), permit reproducible measurements of D_{LNO} in normal subjects using either single exhalations (Dex_{NO}) or rebreathing (Drb_{NO}). A separate measurement of the subject's RV with techniques such as body plethysmography or helium gas dilution is required. This measurement obviates the complexity of continuous analysis of an inert, insoluble gas such as helium with mass spectrometry, which was employed by previous investigators using single exhalations or rebreathing methods (6, 21, 28, 31). These are the first measurements of D_{LNO} that take into account the NO back pressure to diffusion resulting from NO production by the lungs.

Comparison of breath-holding, single-breath NO diffusing capacity and Dex_{NO} to measurements reported by others. Our values for Dex_{NO} of 125 ml·min^{-1}·mmHg^{-1} and Drb_{NO} of 122 ml·min^{-1}·mmHg^{-1} obtained in normal subjects are similar to measurements reported by others using breath holding [single-breath NO diffusing capacity (Dsb_{NO})] and constant exhalations (Dex_{NO}). Borland and Higenbottam (3) in one series obtained values for Dsb_{NO} of 147 ml·min^{-1}·mmHg^{-1} in 13 normal subjects and 126 ml·min^{-1}·mmHg^{-1} in another group of 10 normal subjects (2). Guenard and coworkers (12), using 3-s breath holdings in normal subjects, obtained values for Dsb_{NO} of 136 ml·min^{-1}·mmHg^{-1} in 14 subjects and 140 ml·min^{-1}·mmHg^{-1} in another series of 11 subjects (18). Tsoukias and coworkers (28, 31) recently reported values for Dex_{NO} of 130 ml·min^{-1}·mmHg^{-1} at a V_A of 5.4 liters (BTPS) in seven normal men aged 28 ± 4 (SD) yr. Values for Dsb_{NO} in the same subjects shown in a figure in their report (28) appear to be slightly lower at the same V_A.

The ratio of Dex_{NO} to Dsb_{CO} of 4.7 ± 0.6 and Drb_{NO} to Dsb_{CO} of 4.6 ± 0.6 in our subjects is similar to the values from the above breath-holding studies. Borland and coworkers (2, 3) reported ratios of Dsb_{NO} to Dsb_{CO} of 4.3 ± 0.3 and 4.5 ± 0.5. Guenard and coworkers (12, 18) obtained values of 5.3 ± 0.8 and 4.5 ± 0.6. Tsoukias and coworkers (28, 31) measured both Dex_{NO} and CO pulmonary diffusing capacity (D_L) (D_{LCO}) measured during single, constant, maximum exhalation (Dex_{CO}) and obtained a ratio of 5.3 ± 0.5.

Influence of NO back pressure on measurements of NO diffusing capacity. Production of NO by mammalian airways and its presence in exhaled air were not recognized until 1991 (13). Therefore, previous measurements of NO diffusing capacity by breath holding in humans and rebreathing in animals did not take this NO back pressure into account. Unlike for CO, NO

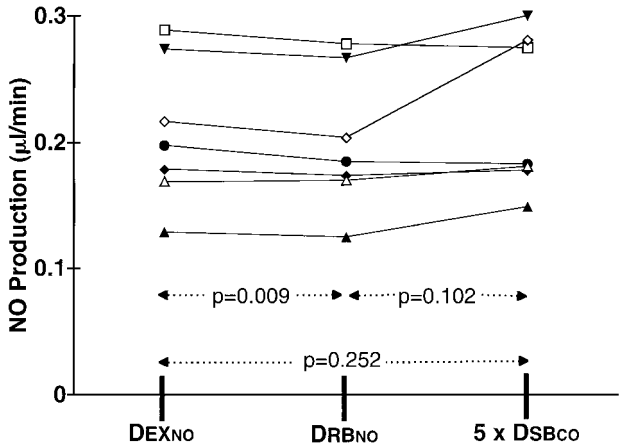

Fig. 8. Alveolar NO production calculated using Dex_{NO}, Drb_{NO}, and 5 × Dsb_{CO}. Symbols are as defined in Fig. 7*A*.

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back pressure has two components. First is the production of NO by the pulmonary capillary endothelium and the alveolar-capillary membrane that results in a $P_{A_{NO}}$ in the order of 2×10^{-6} mmHg (24, 30). Second, during exhalation this NO is added to the NO production by the conducting airways. This concentration from the conducting airways is highly dependent on expiratory flow rates (24, 26, 30), but typically at flow rates of 500 ml/s it equals $\sim 4 \times 10^{-6}$ mmHg (24). Alveolar NO during the measurement of Dex_{NO} or Drb_{NO} falls rapidly, with a half-time in the order of 2–3 s; thus the background pressure can make an appreciable contribution to the expired NO concentration later in the maneuver. Our measurements of Dex_{NO} and Drb_{NO} performed with 5–10 ppm of NO would be underestimated by ~2% if the back pressure were ignored. If the inspired NO concentration were decreased threefold to 2–3 ppm, the estimated error from ignoring NO back pressure would increase to 5% for Dex_{NO} and 3% for Drb_{NO}. Initially, inhaling a fourfold higher concentration of NO of 40 ppm reduces the estimated error to 0.6% for Dex_{NO} and 0.4% for Drb_{NO}. Inhaling 80 ppm of NO reduced the error at 40 ppm in half. Therefore, inhaling 40–80 ppm of NO instead of the 5–10 ppm employed in our normal subjects would make the error from ignoring NO back pressure trivial. However, subjects with elevated levels of exhaled NO, such as observed in asthmatic subjects, may require measurements of NO back pressure to avoid underestimation of NO diffusing capacity, even with the initial inhalation of 40–80 ppm of NO.

Effect of changes in lung volume on Dex_{NO}, Drb_{NO}, and Dsb_{NO}. Borland and Higenbottam (3) measured the effects of decreasing lung volume on Dsb_{NO} in five of their subjects. A 44 ± 9% decrease in V_A decreased Dsb_{NO} by 29 ± 11%, resulting in a slope of 0.63 ± 0.18 for the decrease in Dsb_{NO} vs. the decrease in V_A. This value is similar to the slope for Dex_{NO} vs. V_A of 0.95 ± 0.44 and Drb_{NO} vs. V_A of 0.45 ± 0.45 observed in our subjects. Recently, Tsoukias and coworkers (28, 31) completed a sophisticated analysis of the effects of V_A and sequential filling on Dex_{NO}. For the mean change of V_A from 6.6 to 4.7 liters shown in Fig. 8 of their report (28), seven normal subjects decreased Dex_{NO} from 132 to 116 $ml \cdot min^{-1} \cdot mmHg^{-1}$, resulting in a slope of 0.97. This value is in close agreement with the slope of 0.95 for V_A vs. Dex_{NO} found in our nine subjects.

Cause of lack of effect of changes in alveolar oxygen tension on Dsb_{NO} and Dex_{NO}. Borland and coworkers (3) observed no change in Dsb_{NO} in five of their subjects when they varied alveolar oxygen concentration from 19 to 69%, whereas Dsb_{CO} showed the expected fall from 34 to 20 $ml \cdot min^{-1} \cdot mmHg^{-1}$. We measured Dex_{NO} over a wider range with end-expired oxygen concentrations varying from 6 to 96% and also observed no significant change in Dex_{NO}. Lack of change in Dsb_{NO} and Dex_{NO} with the changes in oxygen tension has several explanations. First, unlike for CO, the very rapid rate of reaction of NO with oxyhemoglobin is similar to the reaction rate with reduced hemoglobin. The bimolecular rate constant for the combination of NO with oxyhemoglobin is reported to be 3.4×10^7 M/s vs. 2.2×10^7 M/s with reduced hemoglobin (17). Therefore, the rate of uptake of NO by red blood cells should not be markedly altered by changes in pulmonary capillary oxygen saturation and, if anything, should increase with higher oxygen saturations. Second, as pointed out by Morris and Gibson (22), the rate of reaction of NO with hemoglobin "is so high that effectively every molecule of NO which enters the reaction radius is captured by a heme group. The observed rate would then be a measure of diffusion to the site." Placing these concepts in the terminology developed by Roughton and Forster (25) for CO diffusion in the lungs, the uptake of NO by the pulmonary capillaries can be divided into its extra erythrocyte diffusion component and a reactive or intraerythrocyte component, $V_c \times \Theta_{NO}$, where V_c is the pulmonary capillary blood volume and Θ_{NO} is the rate of combination of NO with the hemoglobin in 1 ml of blood measured in vitro. According to Morris and Gibson (22), Θ_{NO} has a finite value because of the time required for the advancing front of NO to travel within the blood cell to the combining site on the hemoglobin molecule. Because the chemical reaction of NO with hemoglobin is limited by diffusion to the combining site within the red blood cells but not by the chemical reaction with hemoglobin, the modest differences in the rate of combination of NO with O_2 hemoglobin compared with reduced hemoglobin should not alter Θ_{NO}. As a result, $D_{L_{NO}}$ measured at different alveolar O_2 concentrations remains constant.

Effects of parallel heterogeneity of lung V_A, ventilation, and D_L on Drb_{NO} and Dex_{NO}. Meyer and coworkers (21) performed an elegant analysis of the effects of uneven distribution of V_A, ventilation ($\dot{V}_E$), and D_L on measured values of Drb_{NO} and rebreathing CO D_L (Drb_{CO}) in dogs. In their model containing two parallel compartments, uneven distribution of V_A between the compartments with the same D_L-to-$\dot{V}_E$ ratios caused overestimations of Drb_{NO} and Drb_{CO}, whereas uneven distribution of $\dot{V}_E$ and D_L resulted in underestimations. Most striking was their finding that all patterns of uneven distribution caused approximately twice as large an error in Drb_{NO} compared with Drb_{CO}.

Cotton and Graham (5) analyzed, in a similar two-compartment lung model, the effects of uneven distribution of $\dot{V}_E$, D_L, and other factors on Dex_{CO}. Dex_{CO} was altered by nonuniform distribution of $\dot{V}_E$ and D_L. The errors caused by nonuniform $\dot{V}_E$ could not be eliminated by recalculating the CO decay by reference to the simultaneously recorded helium concentration that was included in the inspired mixture. Models of effects of parallel heterogeneity of V_A, $\dot{V}_E$, and D_L on Dex_{NO} have not been published to our knowledge. However, because the disappearance rate of NO from the alveoli is three to four times more rapid than that observed for CO, for any given

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pattern of uneven distribution, the difference in NO concentrations between the two compartments will be larger for NO, and the resulting errors in Dex_{NO} will be greater than for Dex_{CO}. Therefore, all methods of measuring diffusing capacity with NO can be expected to have errors from heterogeneity of V_A, $\dot{V}_E$, and D_L considerably larger than observed with methods using CO, such as reported by Meyer and coworkers (21).

Measurements of $\dot{V}_{ANO}$ require measurements of NO diffusing capacity (*Eq. 1*). Errors in D_{LNO} from uneven distribution among V_A, $\dot{V}_E$, and D_L in the lungs will, therefore, result in similar errors in $\dot{V}_{ANO}$. D_{LCO} and D_{LNO} are not distorted to the same degree by uneven distribution. Therefore, errors from uneven distribution can be suspected if the ratio of D_{LNO} to D_{LCO} falls outside the usual range of 4.3–5.3 reported in healthy subjects. Measurements of D_{LNO} have attracted interest as a measurement that more fully represents the diffusing properties of the alveolar capillary membrane by lessening the importance of red cell kinetics (20). However, this theoretical advantage for D_{LNO} may be reduced by its greater distortion by maldistribution of V_A, $\dot{V}_E$, and D_L within the lungs (21).

Selection of method to measure D_{LNO} for calculating $\dot{V}_{ANO}$. Three practical methods are now available to measure D_{LNO}. They are single-breath measurement (Dsb_{NO}) with breath holdings usually between 3 and 8 s, the single, constant exhalation method (Dex_{NO}), and the rebreathing method (Drb_{NO}). Dsb_{NO} has the advantage of off-line measurements of NO with analyzers with slow response times but requires analysis of an inert gas such as helium in the inspired gas and the expired alveolar sample. Accurate measurements of the short breath-holding time are critical, and, with longer breath holds, the resulting lower exhaled NO concentration is difficult to measure accurately. We found that Drb_{NO} required considerable training even in normal subjects to obtain constant rebreathing rates and sufficient inspiratory effort with each breath to completely empty the rebreathing bag. For the measurement of Dex_{NO}, the constant exhalation rate obtained by maintaining a mouth pressure of +5 cmH_2O against a fixed resistance in the expiratory circuit was easily achieved by normal subjects. We avoided higher expiratory pressures because they reduce CO diffusing capacity and, by inference, Dex_{NO} (27). Dex_{NO} in our hands has proven to be a practical method for calculating $\dot{V}_{ANO}$ in ongoing studies evaluating the potential toxic effects of inhaled particulates on the lungs. Dex_{NO} has the added advantage of permitting measurements of NO diffusing capacity at different lung volumes (Fig. 3). This may contribute useful information for detecting uneven distribution of D_{LNO} within the lung. Whereas Dex_{NO} and Drb_{NO} do not require measurement of an inert insoluble gas during the maneuver, a separate measurement of RV is needed. All three methods have similar intraday and interday CV, except for Drb_{NO}, which had a greater interday CV than the other methods (Refs. 3, 12; Table 1).

APPENDIX

Derivation of Equations for Computing Drb_{NO}

Eq. 4 in METHODS is based on assumptions and equations similar to those published by Hook and Meyer (14) and Meyer and coworkers (21).

While the subjects rebreathed from a bag enriched with NO

$$\text{Amount of NO entering the bag per min} = \dot{V}_{eff}\, F_L \qquad (A1)$$

$$\text{Amount of NO leaving the bag per min} = \dot{V}_{eff}\, Fb \qquad (A2)$$

where F_L is the fractional concentration of NO in the lungs, Fb is the fractional concentration of NO in the rebreathing bag, and $\dot{V}_{eff}$ is in ml/min STPD and is calculated as the rebreathing frequency times the rebreathing tidal volume, less the instrument's dead space of 100 ml and the subject's dead space, which is estimated to equal 25% of the rebreathing tidal volume (23) (see Fig. 9).

The NO leaving the bag during rebreathing is assumed to enter the lungs, and conversely the NO leaving the lungs by ventilation is assumed to enter the bag. Thus for the lungs during rebreathing

$$\text{Amount of NO entering the lungs per min} = \dot{V}_{eff}\, Fb + \dot{V}_{L_{NO}} \qquad (A3)$$

$$\text{Amount of NO leaving the lungs per min} = \dot{V}_{eff}\, F_L + Drb_{NO}\, P_L \qquad (A4)$$

where $\dot{V}_{L_{NO}}$ is the rate of NO excretion by the lungs' tissues into the air spaces of the lungs in ml/min, Drb_{NO} is measured in ml NO STPD·min^{-1}·$mmHg^{-1}$, and P_L is partial pressure of NO in the lungs in Torr. Combining the above equations yields the following differential equations describing the changes in the NO concentration in the bag and the lungs during rebreathing.

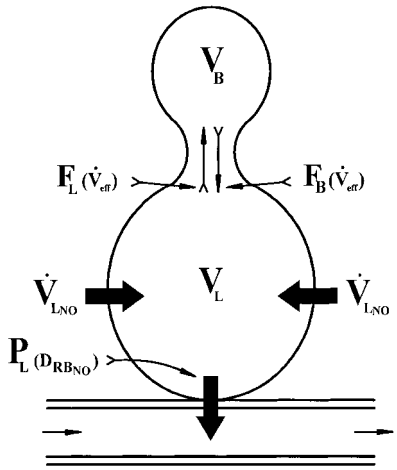

Fig. 9. Diagram of events taking place during the rebreathing of a gas enriched with NO initially placed in the rebreathing bag of volume Vb that exchanges with the gas in the lungs of volume V_L. Amount of NO entering the bag is F_L ($\dot{V}_{eff}$) and amount leaving is Fb($\dot{V}_{eff}$), where F_L and Fb are the fractional concentrations of NO in the lungs and the bag, respectively, and $\dot{V}_{eff}$ is the effective rate of ventilation between F_L and Fb, as defined in the text. The lungs produce NO that enters V_L ($\dot{V}_{L_{NO}}$). NO diffuses from V_L into the perfusing blood and surrounding tissues, and this amount of NO equals the product of partial pressure of NO in the lungs (P_L) and Drb_{NO} (*Eq. 1*).

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For the lungs of volume V_L in ml STPD

$$\frac{d}{dt} F_L V_L = -\dot{V}_{eff} F_L + \dot{V}_{eff} F_b - P_L Drb_{NO} + \dot{V}_{L_{NO}} \quad (A5)$$

Because $P_L = F_L (P_B - 47)$, the last two terms of *Eq. A5* can be rearranged giving

$$\frac{d}{dt} F_L(V_L) = -\dot{V}_{eff} F_L + \dot{V}_{eff} F_b - Drb_{NO}(P_B - 47)\left[F_L - \frac{\dot{V}_{L_{NO}}}{Drb_{NO}(P_B - 47)}\right] \quad (A6)$$

During an infinitely long period of rebreathing, F_L becomes constant at $F_{L\infty}$ (11), because a steady state is reached where the amount of NO entering the lungs ($\dot{V}_{L_{NO}}$) equals the amount leaving [$F_{L\infty}$ (Drb_{NO})(P_B − 47)] or

$$F_{L\infty} = \frac{\dot{V}_{L_{NO}}}{Drb_{NO}(P_B - 47)} \quad (A7)$$

Dividing through by V_L and substituting *Eq. A7* into *A6* gives

$$\frac{d}{dt} F_L = -\left(\frac{\dot{V}_{eff}}{V_L}\right)F_L + \left(\frac{\dot{V}_{eff}}{V_L}\right)F_b - \frac{Drb_{NO}(P_B - 47)(F_L - F_{L\infty})}{V_L} \quad (A8)$$

For the rebreathing bag of volume Vb in ml STPD

$$\frac{d}{dt} F_b(V_b) = \dot{V}_{eff} F_L - \dot{V}_{eff} F_b \quad (A9)$$

Dividing through by Vb

$$\frac{d}{dt} F_b = \left(\frac{\dot{V}_{eff}}{V_b}\right)F_L - \left(\frac{\dot{V}_{eff}}{V_b}\right)F_b \quad (A10)$$

Setting $\bar{F_L} = F_L - F_{L\infty}$, $\bar{F_b} = F_b - F_{L\infty}$, and because $d/dt\ F_{L\infty} = 0$, *Eqs. A8* and *A10* may be rewritten to give

$$\frac{d}{dt} \bar{F_L} = -\left(\frac{Drb_{NO}(P_B - 47)}{V_L} + \frac{\dot{V}_{eff}}{V_L}\right)\bar{F_L} + \frac{\dot{V}_{eff}}{V_L}\bar{F_b} \quad (A11)$$

$$\frac{d}{dt} \bar{F_b} = \frac{\dot{V}_{eff}}{V_b}\bar{F_L} - \frac{\dot{V}_{eff}}{V_b}\bar{F_b} \quad (A12)$$

Equations A11 and *A12* form a set of linear first-order differential equations that can be solved by vector analysis (1) if rebreathing ventilation is considered continuous, V_L constant, and $\dot{V}_{L_{NO}}$ the single source of NO excretion by the respiratory tract into the rebreathing system. The general solutions are

$$\bar{F_L} = F_L - F_{L\infty} = k_1 e^{\lambda_1 t} + k_2 e^{\lambda_2 t} \quad (A13)$$

$$\bar{F_b} = F_b - F_{L\infty} = H_1 k_1 e^{\lambda_1 t} + H_2 k_2 e^{\lambda_2 t} \quad (A14)$$

where

$$\lambda_i^2 + \left(\frac{Drb_{NO}(P_B - 47)}{V_L} + \frac{\dot{V}_{eff}}{V_b} + \frac{\dot{V}_{eff}}{V_L}\right)\lambda_i \times \frac{+Drb_{NO}(P_B - 47)\dot{V}_{eff}}{V_L V_b} = 0 \quad (A15)$$

$$H_i = \frac{\dot{V}_{eff}}{\dot{V}_{eff} + \lambda_i V_b} = \frac{Drb_{NO}(P_B - 47) + \dot{V}_{eff} + \lambda_i V_L}{\dot{V}_{eff}} \quad (A16)$$

$$H_2 = \frac{\dot{V}_{eff}}{\dot{V}_{eff} + \lambda_2 V_b} = \frac{Drb_{NO}(P_B - 47) + \dot{V}_{eff} + \lambda_2 V_L}{\dot{V}_{eff}} \quad (A17)$$

where t is time. After one or two breaths, the first exponential term in *Eqs. A13* and *A14* becomes very small, and the lung and P_{bag} data can be fitted to the equations

$$\bar{F_L} = F_L - F_{L\infty} = k_L e^{\lambda_2 t};\ \bar{F_b} = F_L - F_{L\infty} = k_b e^{\lambda_2 t} \quad (A18)$$

where $k_L = k_2$ and $H_2 = k_b/k_L$, where k_L and k_b are constants derived by the fitting process.

Equation A18 predicts that, after the first few breaths, the semilog plots against time of the lung and bag NO concentrations ($F_{L\infty}$) will fall along two parallel lines with slope λ_2, with the antilog of the distance between these two lines representing H_2. From the first and third parts of *Eq. A17*

$$Drb_{NO} = \frac{1}{P_B - 47}[\dot{V}_{eff}(H_2 - 1) - \lambda_2 V_L] \quad (A19)$$

Adding and subtracting λ_2 Vb yields

$$Drb_{NO} = \frac{1}{P_B - 47}[\dot{V}_{eff}(H_2 - 1) - \lambda_2 V_L - \lambda_2 V_b + \lambda_2 V_b] \quad (A20)$$

Gathering terms

$$Drb_{NO} = \frac{-\lambda_2(V_L + V_b)}{P_B - 47} + \frac{\dot{V}_{eff}(H_2 - 1) + \lambda_2 V_b}{P_B - 47} \quad (A21)$$

From the first two parts of *Eq. A17*

$$\lambda_2 V_b = \frac{\dot{V}_{eff}}{H_2} - \dot{V}_{eff} = \dot{V}_{eff}\frac{1 - H_2}{H_2} \quad (A22)$$

Substituting this expression into *Eq. A21* and replacing $-\lambda_2$ with k_{NO} and H_2 with H results in

$$Drb_{NO} = \frac{1}{P_B - 47}\left(k_{NO}(V_L + V_b) + \dot{V}_{eff} \times \left[\frac{1 - H}{H} + (H - 1)\right]\right) \quad (A23)$$

V_L + Vb equals the V_{tot}. Simplifying the term in brackets

$$Drb_{NO} = \frac{k_{NO}(V_{tot})}{P_B - 47} + \frac{\dot{V}_{eff}(H - 1)^2}{(P_B - 47)H} \quad (A24)$$

which is *Eq. 4* in METHODS.

Equation A19, from which *Eq. A24* is derived, can be shown to be equivalent to the formulas published by Hook and Meyer (14) and Meyer and coworkers (21) for measuring Drb_{NO} and Drb_{CO}. Combining *Eq. A19* with the first two parts of *Eq. A17*

$$Drb_{NO} = \frac{1}{P_B - 47}\left[\dot{V}_{eff}\left(\frac{\dot{V}_{eff}}{\dot{V}_{eff} + \lambda_2 V_b} - 1\right) - \lambda_2 V_L\right] = \frac{1}{P_B - 47}\left(\dot{V}_{eff}\frac{-\lambda_2 V_b}{\dot{V}_{eff} + \lambda_2 V_b} - \lambda_2 V_L\right) \quad (A25)$$

Replacing $-\lambda_2$ with k_{NO} and rearranging terms yields the equation published by the above group

$$Drb_{NO} = \frac{k_{NO} V_L}{P_B - 47}\left(1 + \frac{V_b/V_L}{1 - k_{NO} V_b/\dot{V}_{eff}}\right) \quad (A26)$$

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