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Invited Review

Modeling pulmonary nitric oxide exchange

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George, Steven C., Marieann Hogman, Solbert Permutt, and Philip E. Silkoff. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 96: 831–839, 2004; 10.1152/japplphysiol.00950.2003.—Nitric oxide (NO) was first detected in the exhaled breath more than a decade ago and has since been investigated as a noninvasive means of assessing lung inflammation. Exhaled NO arises from the airway and alveolar compartments, and new analytical methods have been developed to characterize these sources. A simple two-compartment model can adequately represent many of the observed experimental observations of exhaled concentration, including the marked dependence on exhalation flow rate. The model characterizes NO exchange by using three flow-independent exchange parameters. Two of the parameters describe the airway compartment (airway NO diffusing capacity and either the maximum airway wall NO flux or the airway wall NO concentration), and the third parameter describes the alveolar region (steady-state alveolar NO concentration). A potential advantage of the two-compartment model is the ability to partition exhaled NO into an airway and alveolar source and thus improve the specificity of detecting altered NO exchange dynamics that differentially impact these regions of the lungs. Several analytical techniques have been developed to estimate the flow-independent parameters in both health and disease. Future studies will focus on improving our fundamental understanding of NO exchange dynamics, the analytical techniques used to characterize NO exchange dynamics, as well as the physiological interpretation and the clinical relevance of the flow-independent parameters.

NO; model; airways; alveoli; inflammation

NITRIC OXIDE (NO) was discovered in exhaled breath in humans and other vertebrates in 1991 (14). This was followed by a series of reports that the concentration of NO in the exhaled breath was substantially elevated in subjects with asthma who were not treated with corticosteroids, suggesting that exhaled NO was a potential noninvasive index of airway inflammation (1, 23, 30, 56). In 1997, it was discovered that the concentration of NO in the exhaled breath was highly dependent on the exhalation flow rate (20, 43), differing significantly from other endogenous gases evolved in the exhaled air such as carbon dioxide and nitrogen. Additional key observations were made in parallel that further distinguished the gas exchange dynamics of NO as unique and included the nasal epithelium (28), the airway epithelium (2), the alveolar epithelium (2), the vascular endothelium (22), and the blood as potential sources of exhaled NO (31, 46). This series of important findings spurred an era of intense investigation focused on improving our understanding of the unique gas exchange mechanisms of NO in the lungs in both health and disease.

Because NO exchange dynamics are significantly different from other well-studied gases such as carbon dioxide, new models and analytical methods have been developed to understand the underlying physiology and gas exchange mechanisms. A simple two-compartment model of the lungs has been described by several research groups (17, 34, 44, 48) that adequately explains many of the unique features of NO exchange dynamics, in particular the dependence on exhalation flow rate. The two-compartment model describes exhaled NO arising from two compartments, the airways and the alveolar region, using three flow-independent exchange parameters: one describing the alveolar region (the steady-state NO alveolar concentration), and two describing the airway region (airway NO diffusing capacity and either the maximum airway wall NO flux or the airway wall NO concentration). With the use of these three parameters, the two-compartment model can then predict the exhaled concentration at any desired exhalation flow rate. It is important to note that the two-compartment model does not consider the nasal compartment and the significant NO production in the paranasal sinuses (28). Thus special precautions to close the soft palate during exhalation must be made to avoid nasal contamination when model predictions are compared to experimental data.

Since the original description of the two-compartment model, research has focused on three areas: *1*) the development of experimental breathing and analytical techniques to accurately and reproducibly estimate the flow-independent NO exchange parameters, *2*) the estimation of the flow-independent NO parameters in health and disease, and *3*) the further development and testing of the underlying assumptions in the simple two-compartment model. These research thrusts have enhanced our understanding of NO exchange mechanisms, as

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well as the pathophysiological interpretation of the flow-independent NO exchange parameters.

Glossary

$C_{NO}(V)$ or $F_{NO}(V)$ Fractional concentration of NO in the gas phase within the airway compartment [parts/billion (ppb)]

$C_{A_{NO}}(t)$ or $F_{A_{NO}}(t)$ Mixed or average fractional concentration of NO in the gas phase of the alveolar region (ppb). This concentration may depend on time during a single breath or during the tidal breathing cycle. A steady-state concentration is achieved for breath hold or exhalation times of >10 s

$Calv_{NO}$ or $Falv_{NO}$ Mean alveolar tissue concentration of NO equivalent to the steady-state concentration in the gas phase of the alveolar compartment during a breath hold or exhalation of >10 s

$C_{E_{NO}}$ or $F_{E_{NO}}$ Fractional exhaled concentration of NO, which may be a function of time, exhaled volume, and exhalation flow rate (ppb)

$C_{E_{NO,i}}$ or $F_{E_{NO,i}}$ Fractional exhaled concentration of NO in phase III of the exhalation profile at constant exhalation flow rate *i* (ppb)

Caw_{NO} or Faw_{NO} Mean (by radial position) airway tissue concentration of NO (wall concentration) equivalent to the steady-state concentration in the gas phase of the airway compartment during a prolonged breath hold (ppb)

Daw_{NO} Global or total airway compartment diffusing capacity, transfer factor, or conductance for radial mass transfer of NO from the airway wall to the gas stream ($pl \cdot ppb^{-1} \cdot s^{-1}$)

$Dalv_{NO}$ Global or total alveolar compartment diffusing capacity, transfer factor, or conductance for mass transfer of NO across the alveolar membrane ($pl \cdot ppb^{-1} \cdot s^{-1}$)

$J'aw_{NO}$ Global or total maximum flux (rate of radial transport) of NO in the airway compartment (pl/s), which occurs as the gas phase concentration in the airway compartment approaches zero, and is equal to the product $Daw_{NO} \times Caw_{NO}$

Jaw_{NO} Global or total flux of NO in the airway compartment (pl/s). This flux may depend on time during a single breath or tidal breathing cycle and approaches $J'aw_{NO}$ for exhalation flow rates of >50 ml/s in healthy adults

$J'alv_{NO}$ Global or total maximum flux of NO in the alveolar compartment (pl/s)

Vaw Volume of the airway compartment exchanging NO, approximately equivalent to the anatomic dead space (ml)

V_A Volume of the alveolar compartment that depends on exhalation time and volume (ml)

$\dot{V}_{NO}$ Elimination rate of NO from the breath during exhalation (ml/s)

$\dot{V}_E$ Exhalation flow rate (ml/s)

TWO-COMPARTMENT MODEL

The two-compartment model has been described in detail in prior publications (17, 34, 44, 48), and only the salient features will be described herein. Figure 1 depicts the basic features of the model. The alveolar NO concentration, which probably changes in a cyclical manner with respiration, reflects the balance between NO produced locally or inhaled and NO destroyed or diffusing away. During an exhalation or breath hold of more than ~8–10 s (3, 13, 21, 47–49, 52), the concentration in the alveolar region, $C_{A_{NO}}$, reaches a steady-state concentration. As alveolar air is convected through the airways toward the mouth during exhalation, the gas stream is conditioned with NO diffusing from the airway wall. Briefly, the amount of NO absorbed by the airstream from the airways per unit time is referred to as the flux of NO from the airways, Jaw_{NO} (pl/s), and is expressed as a linear function of the airway gas phase NO concentration, C_{NO}, by the following (48)

$$Jaw_{NO} = J'aw_{NO} - Daw_{NO}C_{NO} \quad (1)$$

or

$$Jaw_{NO} = Daw_{NO}(Caw_{NO} - C_{NO}) \quad (2)$$

$J'aw_{NO}$ is the maximum flux of NO from the airway tissue, which is approximately equal to the airway compartment flux if C_{NO} were zero, or, alternatively, simply the product $Daw_{NO} \times Caw_{NO}$ (see DETERMINING FLOW-INDEPENDENT NO PA-

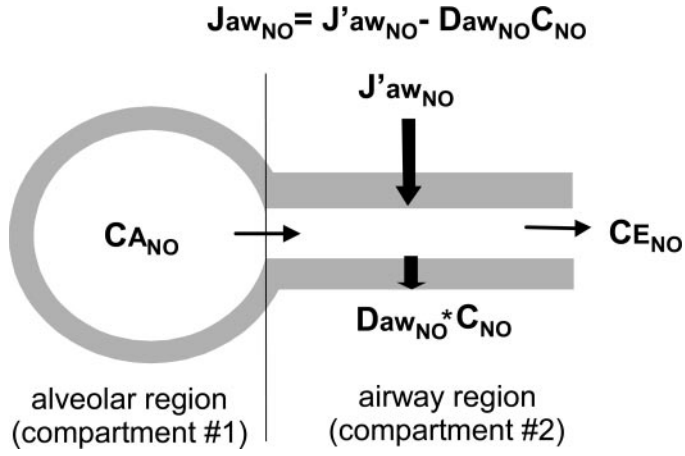

Fig. 1. Schematic of 2-compartment model used to describe nitric oxide (NO) exchange dynamics. Exhaled NO concentration ($C_{E_{NO}}$) is the sum of 2 contributions, the alveolar region and the airway region, which depends on 3 flow-independent parameters: maximum total volumetric flux of NO from the airway wall ($J'aw_{NO}$, pl/s), diffusing capacity of NO in the airways (Daw_{NO}, $pl \cdot s^{-1} \cdot ppb^{-1}$), and steady-state alveolar concentration ($C_{A_{NO}}$, ppb). Jaw_{NO}, total flux (pl/s) of NO between the tissue and gas phase in the airway and is an inverse function of the exhalation flow rate ($\dot{V}_E$); C_{NO}, concentration of NO in the gas phase within the airway compartment.

RAMETERS). $D\text{aw}_{NO}$ is the airway NO diffusing capacity (conductance for mass transfer or transfer factor) for radial transport of NO between the airway tissue and the gas phase.

Once the flow-independent parameters are known, the two-compartment model can be used to predict the exhaled concentration of NO, $C_{E_{NO}}$ (ppb) (or $F_{E_{NO}}$) at any constant exhalation flow ($\dot{V}_E$) by using the relatively simple exponential expression (17, 34, 44, 48)

$$C_{E_{NO}} = \text{Caw}_{NO} + (C_{A_{NO}} - \text{Caw}_{NO}) \cdot \exp(-D\text{aw}_{NO}/\dot{V}_E) \quad (3)$$

Our laboratory has previously demonstrated that $C_{E_{NO}}$ predicted by *Eq. 3* is no different than the experimentally measured $C_{E_{NO}}$ in phase III of the exhalation profile in healthy adults (37–39), with the advantage that intersubject and interpopulation variations in flow rate can be accounted for by calculating $C_{E_{NO}}$ at a precise desired flow rate (e.g., 50 ml/s).

DETERMINING FLOW-INDEPENDENT NO PARAMETERS

The potential of the flow-independent NO parameters lies in their ability to partition exhaled NO into two important anatomic subdivisions of the lungs, the airways and the alveolar region, and also provide both structural and metabolic information about the airways relevant to the NO pathways. The challenge has been to develop robust breathing techniques and analytical methods to accurately and reliably estimate their values in healthy adults and children and in those with lung disease. To uniquely determine the airway and alveolar contribution to exhaled NO, multiple exhalation flow rates must be sampled (17, 34, 44, 48). Two general approaches have been described in the literature: multiple constant exhalation flows and a dynamically changing flow within a single exhalation.

Multiple constant exhalation flows. Several groups have manipulated the governing equations of the two-compartment model to arrive at analytical expressions that can be used in conjunction with multiple constant exhalation flows to estimate one or more of the flow-independent NO exchange parameters. When $\dot{V}_E$ is large compared with $D\text{aw}_{NO}$, the exponential function of *Eq. 3* approaches the first-order linear approximation [i.e., $\exp(-D\text{aw}_{NO}/\dot{V}_E) = 1 - D\text{aw}_{NO}/\dot{V}_E$]. This approximation occurs for $\dot{V}_E > \sim 5 \times D\text{aw}_{NO}$ ml/s or ~50 ml/s in healthy adults (see mean values of $D\text{aw}_{NO}$ in healthy adults in Table 1). Under this condition, *Eq. 3* reduces to the following simple expression

$$C_{E_{NO,\dot{V}}} = C_{A_{NO}} + (\text{Caw}_{NO} - C_{A_{NO}}) \cdot D\text{aw}_{NO} \cdot \frac{1}{\dot{V}} \quad (4)$$

Under most circumstances (see Tables 1 and 2), $C_{A_{NO}}$ is normally <2% of Caw_{NO} and can thus be neglected from the second term on the right-hand side of *Eq. 4*. Making this simplification and recognizing that $J'\text{aw}_{NO} = D\text{aw}_{NO} \times \text{Caw}_{NO}$, the following simple expression for $C_{E_{NO}}$ is derived

$$C_{E_{NO}} = C_{A_{NO}} + J'\text{aw}_{NO} \cdot \frac{1}{\dot{V}} \quad (5)$$

Then, if both sides of *Eq. 5* are multiplied by $\dot{V}_E$, the following expression for the elimination rate of NO, $\dot{V}_{NO}$ ($C_{E_{NO}} \cdot \dot{V}_E$, ml NO/s), is derived

$$\dot{V}_{NO} = C_{A_{NO}} \cdot \dot{V}_E + J'\text{aw}_{NO} \quad (6)$$

Tsoukias et al. ("Tsoukias" technique) (51) used *Eq. 6* to estimate $C_{A_{NO}}$ and $J'\text{aw}_{NO}$ by measuring $\dot{V}_{NO}$ at multiple constant exhalation flow rates. As shown in Fig. 2*A*, the slope and intercept of the resulting linear relationship provides an estimate of $C_{A_{NO}}$ and $J'\text{aw}_{NO}$, respectively. Note that in some disease states (e.g., cystic fibrosis, Table 2), $C_{A_{NO}}$ is larger than 2% of Caw_{NO} and thus the intercept should be rigorously interpreted as $D\text{aw}_{NO} \times (\text{Caw}_{NO} - C_{A_{NO}})$, as described earlier.

Pietropaoli et al. ("Pietropaoli" technique) (34) followed with a similar technique to estimate $J'\text{aw}_{NO}$ and $C_{A_{NO}}$ but also included the estimate of $D\text{aw}_{NO}$. They used *Eq. 5* (rather than *Eq. 6*) and plotted $C_{E_{NO}}$ vs. $1/\dot{V}_E$ for $\dot{V}_E > 200$ ml/s and used the intercept as an estimate of $C_{A_{NO}}$. Then, using this value for $C_{A_{NO}}$, combined with additional flow rates as low as 6 ml/s, they used nonlinear regression techniques (steepest descent and Newton's algorithm) and *Eq. 3* to estimate $J'\text{aw}_{NO}$ and $D\text{aw}_{NO}$. Figure 2*B* demonstrates a plot of $C_{E_{NO}}$ vs. $1/\dot{V}_E$ where the intercept is $C_{A_{NO}}$ and the slope is $J'\text{aw}_{NO}$ (*Eq. 5*).

Silkoff et al. ("Silkoff" 9-flow technique) (44) utilized a nonlinear regression technique using *Eq. 3* and nine different flows (range 4–1,550 ml/s) to estimate all three flow-independent parameters. Then they demonstrated that $D\text{aw}_{NO}$ and

Table 1. *Analytical techniques to estimate flow-independent NO parameters*

Technique	Determined Parameters				Flow Rate Requirements	Reference
	$J'\text{aw}_{NO}$	$D\text{aw}_{NO}$	$C_{A_{NO}}$	Caw_{NO}		
Tsoukias	X		X		Two constant-exhalation vital capacity maneuvers. Flow rate range 100–500 ml/s	51
Pietropaoli	X		X		Two constant-exhalation vital capacity maneuvers. Flow rate range 100–500 ml/s	34
Silkoff 2-flow	X	X		X	Two constant-exhalation vital capacity maneuvers. Flow rate range 15–50 ml/s	44
Silkoff 9-flow	X	X	X	X	Nine constant-exhalation vital capacity maneuvers. Flow rate range 4.2–1,550 ml/s	44
Hogman	X	X	X	X	Three constant-exhalation vital capacity maneuvers. Flow rate range 5–500 ml/s	17, 19
Tsoukias SB	X	X	X	X	One vital capacity maneuver with exhalation flow rate dynamically changing from 6 to 1% of vital capacity per second.	50

See glossary for definitions of parameters and DETERMINING FLOW-INDEPENDENT NO PARAMETERS for descriptions of techniques.

Table 2. *Flow-independent NO parameters in healthy adults and children*

$J'aw_{NO}$, pl/s	Daw_{NO}, pl·s^{-1}·ppb^{-1}	$C_{A_{NO}}$, ppb	Caw_{NO}, ppb	Year	*n*	Technique	Reference
			Adults				
710±310		5.6±3.1		1998	7	Tsoukias	51
1,280±350	5.7±2.8	2.1±0.36	224±171	1999	7	Pietropaoli	34
1,020±500	6.8±3.8	5.0±1.0	150±101	2000	10	Silkoff (9-flow)	44
680±390	9.2±1.9	1.6±0.3	75±28	2000	10	Hogman	17
600±320		1.3±0.6		2000	10	Tsoukias	27
640±42	4.2±0.67	2.5±0.23	208±46	2001	10	Tsoukias SB	39
700±400		1.0±0.8		2001	16	Tsoukias	26
700±750		1.1±0.75		2001	57	Tsoukias	25
1,200±900		1.8±0.9		2002	20	Tsoukias	12
752±395	7.7±2.5	1.9±0.9	97.6±44.5	2002	40	Hogman	19
418±237	2.27±0.74	1.81±1.58	225±211	2003	10	Tsoukias SB	37
530±234	3.13±1.57	3.08±2.39	220±177	2003	24	Tsoukias SB	38
			Children (age 8–18 yr)				
784±465	4.82±3.07	4.63±3.59	198±131	2002	9	Tsoukias SB	40
719±464	13.2±6.69	1.16±0.39	54.6±32.6	2003	15	Hogman	32
			Infants (age <36 mo)				
3,150±4,120	19±14		191±153	2003	5	Silkoff (2-flow)	29

Values are means ± SD; *n*, no. of subjects.

$J'aw_{NO}$ (and hence Caw_{NO}) could be estimated from two low flow rates ($\dot{V}_E < 50$ ml/s, Silkoff 2-flow technique) by using the slope ($-Daw_{NO}$) and intercept ($J'aw_{NO}/Daw_{NO}$) of a plot of $\dot{V}_{NO}$ vs. $C_{E_{NO}}$ (Fig. 2*C*).

Hogman and colleagues ("Hogman" technique) (17, 19) followed Silkoff with a slightly modified technique that combined the technique of Tsoukias and also the concepts of Pietropaoli and Silkoff. In this technique, only three exhalation

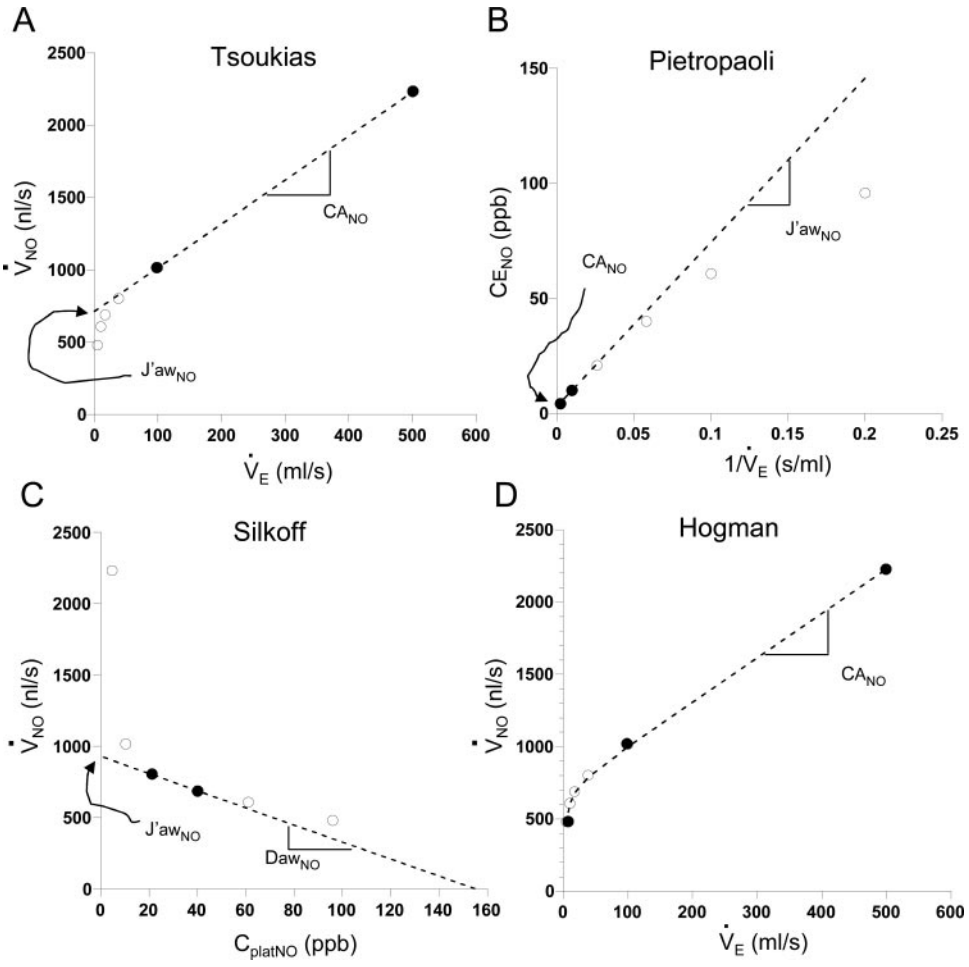

Fig. 2. Schematics depicting 4 different analytical techniques that have been described in the literature to estimate the flow-independent NO parameters by using a minimum of 2 constant exhalation flow rates and a maximum of 3. *A*: Tsoukias technique. *B*: Pietropaoli technique. *C*: Silkoff technique. *D*: Hogman technique. Circles have been produced by the analytical solution of the 2-compartment model (*Eq. 3*) by using representative values for the flow-independent parameters (Daw_{NO} = 5 pl·s^{-1}·ppb^{-1}; $J'aw_{NO}$ = 750 pl/s; $C_{A_{NO}}$ = 3 ppb) at 5 different exhalation flow rates: 5, 17.2, 38.2, 100, and 500 ml/s. ●, Flow rates used by each of the techniques to estimate 2 or more of the flow-independent parameters only in linear regression; ○, flow rate of the 2-compartment model. Dashed lines, main feature of each technique (described in greater detail in the text).

flow rates are needed, one very low (5 ml/s), one medium flow around 100 ml/s, and a high flow that needs to be 300 ml/s or above but not over 500 ml/s. A line is regressed through the medium and high flow rates to determine $C_{A_{NO}}$ as described in the Tsoukias technique; then $D_{aw_{NO}}$ and $J'_{aw_{NO}}$ are determined from all three flows by use of an iterative technique (Fig. 2*D*) that uses the second-order term to minimize the error induced from the first-order linear approximation of *Eq. 4*. Thus three unknown parameters are determined from the minimum number of data points (i.e., three).

The advantage of the Tsoukias and Pietropaoli techniques is the use of only two exhalation flow rates, which are easily performed by most subjects, and the very simple analytical method of analysis. The primary disadvantage is that $D_{aw_{NO}}$ cannot be determined. The Silkoff 2-flow technique also utilizes only two exhalation flow rates, albeit lower flow rates (<50 ml/s). It may be difficult for some subjects with compromised lung function to continue exhaling long enough at these low flows. To exhale the initial airway volume of ~200 ml and achieve a 3-s plateau, a subject needs to exhale for a minimum of 15 s at an exhalation flow rate of 17.2 ml/s. In addition, the Silkoff 2-flow technique does not provide data to determine $C_{A_{NO}}$, but the significant advantage of the Silkoff 2-flow technique is the estimation of $D_{aw_{NO}}$, which has been shown to be elevated in asthma and independent of steroid therapy (38, 44). The technique of Hogman has the obvious advantage that all of the flow-independent NO exchange parameters can be determined, at the cost of one additional low exhalation flow rate (which may be difficult for some subjects to complete as described above) and a nonlinear regression technique that is numerically more complex.

Dynamically changing flow rate. To avoid performing multiple constant exhalation flow rates, the exhalation flow rate during a single exhalation maneuver can be dynamically altered to sample essentially an infinite (depends on the sampling frequency) number of exhalation flow rates. This strategy was employed in a second technique by Tsoukias et al. ("Tsoukias SB" technique, where SB stands for "single breath") (50). To employ this strategy, the residence time, τ_{res}, of each exiting differential bolus of air must be known. Then, from *Eq. 3*, the exhaled concentration, $C_{E_{NO}}$, is simply

$$C_{E_{NO}} = C_{aw_{NO}} + (C_{A_{NO}} - C_{aw_{NO}}) \cdot \exp(-D_{aw_{NO}} \cdot \tau_{res}/Vaw) \quad (7)$$

where Vaw is the airway compartment volume. Note the similarity between *Eq. 7* and *Eq. 3*, with the notable exception that $\dot{V}_E$ has been replaced with Vaw/τ_{res}; hence, this technique not only requires τ_{res}, but also requires one to estimate Vaw. Tsoukias et al. (50) used backward integration of the exhalation flow rate signal to determine τ_{res} at each exhalation time point and the subjects' body weight in pounds plus age in years to estimate Vaw in units of milliliters.

As described earlier, both low (<50 ml/s) and high (>100 ml/s) exhalation flow rates must be sampled to estimate all of the flow-independent exhalation NO parameters. In a single exhalation maneuver, Tsoukias et al. (50) used a preexpiratory breath hold of 20 s followed by a decreasing exhalation maneuver (~300 to 50 ml/s for adults). The result is an exhalation profile that has a large peak early in the exhalation due to the breath hold and accumulation of NO in the airway compartment followed by a progressively increasing NO concentration in phase III. Phases I and II are sensitive to $D_{aw_{NO}}$ (low flow, actually a zero flow from the breath hold) and $J'_{aw_{NO}}$, whereas phase III is sensitive to $C_{A_{NO}}$ and $J'_{aw_{NO}}$. Hence, all three parameters can be uniquely determined (Fig. 3) from a nonlinear least squares algorithm. The primary advantage of this technique is that only a single breathing maneuver is needed and a precise exhalation flow rate is unnecessary (one only needs to record the exhalation flow rate). The primary disadvantages are that some subject populations (young children or those with compromised lung function) cannot perform the 20-s breath hold, the parameter estimation algorithm is numerically complex and thus less portable, and Vaw must be estimated for the analysis. A summary of the analytical techniques to estimate the flow-independent NO exchange parameters is provided in Table 1.

FLOW-INDEPENDENT NO PARAMETERS IN HEALTH AND DISEASE

The techniques described above have been used to estimate values for the flow-independent NO exchange parameters in healthy adults, children, and infants, as well as several diseases including asthma (19, 25–27, 32, 38, 44), allergic alveolitis (25, 27), cystic fibrosis (40), scleroderma (12), allergic rhinitis (19), chronic obstructive pulmonary disease (COPD) (19), Sjogrens syndrome (16), and smoking (18, 19). Tables 2 and 3 summarize these values including the size of the sample and the standard deviation of the estimated mean. Because the techniques utilized have unique sources of error, absolute values for the flow-independent NO exchange parameters may vary between studies for the same group of subjects. Hence, Tables 2 and 3 also include the technique utilized in each study.

In healthy adults, a range of 1.0–5.6 ppb for $C_{A_{NO}}$ has been reported, which is consistent with the direct bronchoscopic measurements of the lower airways (8, 9). Although direct measurement of the alveolar maximum flux, $J'_{alv_{NO}}$, is not possible, an estimate can be made by using reports of the alveolar diffusing capacity, $D_{alv_{NO}}$ (also reported as $D_{L_{NO}}$), and the simple relationship at steady state, $J'_{alv_{NO}} = D_{alv_{NO}} \times C_{A_{NO}}$. Reported ranges for $D_{L_{NO}}$ in healthy adults at total lung capacity (alveolar volumes of 5–7 liters) are 1,580–2,100 $pl \cdot s^{-1} \cdot ppb^{-1}$ using several different techniques (4, 13, 33, 47, 52). This provides a range of 1,580–11,760 pl/s for $J'_{alv_{NO}}$. For $J'_{aw_{NO}}$, $D_{aw_{NO}}$, and $C_{aw_{NO}}$, the ranges in healthy adults are 420–1,280 pl/s, 3.1–9.2 $pl \cdot s^{-1} \cdot ppb^{-1}$, and 75–225 ppb, respectively. Note that $D_{alv_{NO}}$ and $J'_{alv_{NO}}$ are significantly larger than $D_{aw_{NO}}$ and $J'_{aw_{NO}}$, respectively. This is likely due to the enormous surface area in the alveolar region; however, note that the tissue concentration is much larger in the airways (ratio of maximum flux and diffusing capacity).

In disease states, there are several interesting trends, many of which are consistent with our present understanding of regional inflammation in these diseases. Although asthma is a heterogeneous disease, it appears that steroid-treated subjects with asthma have an elevated $D_{aw_{NO}}$, whereas subjects with asthma who are not treated with steroids have elevated $D_{aw_{NO}}$, $J'_{aw_{NO}}$, and possibly $C_{A_{NO}}$. Subjects with allergic alveolitis and Sjogrens have elevated $C_{A_{NO}}$, whereas subjects with

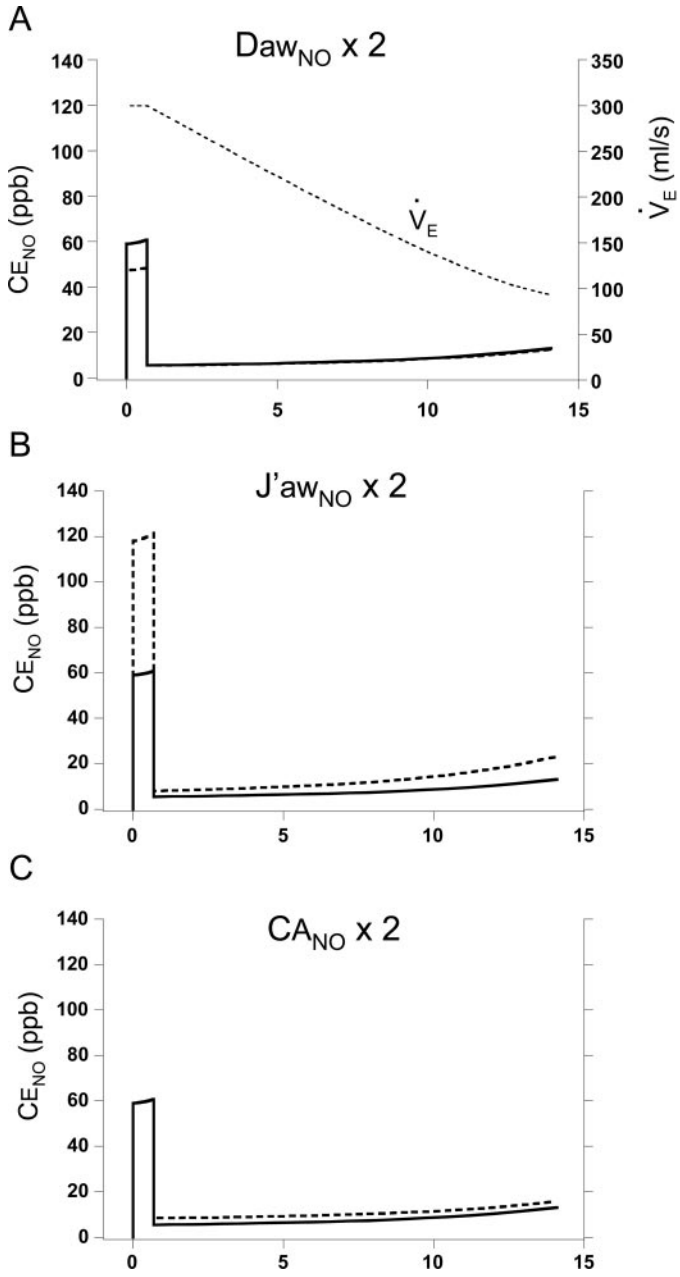

Fig. 3. The 2-compartment model prediction of the exhaled NO profile is shown for a single exhalation maneuver with a 20-s preexpiratory breath hold and a decreasing exhalation flow rate. Representative values for lung volumes of a healthy adult have been used, and the "control" values for the flow-independent parameters are $D\mathrm{aw}_{NO} = 5$ pl·s^{-1}·ppb^{-1} (*A*); $J'\mathrm{aw}_{NO} = 750$ pl/s (*B*); $\mathrm{CA}_{NO} = 3$ ppb (*C*). In each panel, the control profile (solid line) is shown together with the exhaled profile when 1 of the flow-independent parameters is doubled (dashed line). In *A*, the decreasing exhalation flow rate is also shown on the second *y*-axis. This informal sensitivity analysis demonstrates graphically which part of the profile is impacted by each parameter. It can be seen that each parameter uniquely impacts the exhaled profile and can thus be uniquely determined. Note that $D\mathrm{aw}_{NO}$ primarily impacts phases I and II, CA_{NO} impacts primarily phase III, whereas $J'\mathrm{aw}_{NO}$ impacts all 3 phases.

scleroderma have an elevated CA_{NO} but a reduced $J'\mathrm{aw}_{NO}$. Subjects with cystic fibrosis have an elevated $D\mathrm{aw}_{NO}$ but a reduced Caw_{NO}. Allergic rhinitis appears to increase $D\mathrm{aw}_{NO}$. For subjects with COPD, CA_{NO} is elevated, whereas $D\mathrm{aw}_{NO}$ is elevated in one group and decreased in another group relative to healthy controls, suggesting distinct subpopulations in NO gas exchange dynamics. In smoking, Caw_{NO} and $J'\mathrm{aw}_{NO}$ are both reduced.

FUTURE DIRECTIONS

Future progress in our understanding of NO exchange dynamics and potential clinical applicability will depend on our ability to improve our basic understanding of the chemical and physical factors that impact NO gas exchange, understanding limitations of the two-compartment model, improving analytical and experimental models, and completing additional cross-sectional and longitudinal clinical studies. Additionally, practical measurement methods that are applicable to a wide range of ages and disease processes will be necessary.

The strength of the two-compartment model is its relative simplicity in characterizing NO exchange dynamics with only three flow-independent parameters. However, this simplicity comes at the cost of several major simplifying assumptions that make the governing equations tractable. Characterizing the lungs with only two compartments is obviously a gross simplification. The transition region between the bronchioles and the alveoli (terminal and respiratory bronchioles) is not described in the two-compartment model. This is evident by the abrupt change in concentration between the emptying of the airway and the alveolar compartment in the model predictions shown in Fig. 3 for the single-breath maneuver with a preexpiratory breath hold. The experimental exhaled NO tracings for a similar maneuver produce a smooth rise and fall as the airway compartment is emptied during exhalation. The analytical technique to estimate the flow-independent NO parameters compensates for this model limitation by predicting the total volume of NO exhaled in phases I and II rather than predicting the precise shape of this portion of the tracing.

Another model simplification is that axial diffusion of NO in the gas phase as a mechanism of transport of NO is neglected. It has recently been demonstrated theoretically that if a substantial source of NO arises in the small airways, neglecting axial diffusion may cause the two-compartment model to significantly underestimate the flux of NO from the airways due to back-diffusion into the alveolar region (36, 53). Future work must focus on experimental validation of such predictions and also on the progressive development and validation of the two-compartment simplifications.

One area in which the analytical methods could be substantially improved is in the analysis of exhaled NO tracings during normal tidal breathing. The expired NO concentration profile during tidal breathing has been investigated in infants (15), but partitioning the exhaled NO into airway and alveolar compartments was not accomplished. In adults, all of the techniques to date utilize a single-breath maneuver, which can be cumbersome for some subject populations such as infants or those with compromised lung function. Several challenges must be overcome with tidal breathing. Changes in lung volume for tidal breathing (400–800 ml) are smaller than for single-breath maneuvers (1,500–3,000 ml). A single cycle (inhalation and exhalation) occurs over a relatively short time frame (4–8 s), and exhalation profiles are observed in a narrow window (2–4 s). Over the short exhalation, there is insufficient time to accumulate appreciable amounts of NO in the airway and alveolar compartments. Hence, tidal breathing profiles are

Table 3. *Flow-independent NO parameters in lung disease*

$J'aw_{NO}$, pl/s	Daw_{NO}, $pl \cdot s^{-1} \cdot ppb^{-1}$	$C_{A_{NO}}$, ppb	Caw_{NO}, ppb	Year	*n*	Technique	Reference
			Asthma (steroid treated)				
Adults							
2,416±1,042	22.3±13.5		108±70	2000	25	Silkoff (2-flow)	44
700±400		1.2±0.4		2001	16	Tsoukias	26
1,711±900	11.9±3.7	1.7±0.6	144±80	2002	15	Hogman	19
1,200±837	11.8±11.7	3.30±2.27	143±66	2003	12	Tsoukias SB	38
Children (age 8–18 yr)							
3,780±2,060	28.3±13.3	1.54±0.65	134±57.4	2003	10	Hogman	32
			Asthma (steroid free)				
Adults							
6,512±4,330	25.5±19		255±232	2000	25	Silkoff (2-flow)	44
2,600±1,900		1.5±0.9		2000	10	Tsoukias	27
2,500±1,900		1.1±1.26		2001	40	Tsoukias	25
3,600±1,600		1.2±2.0		2001	16	Tsoukias	26
2,690±1,690	8.71±5.74	5.68±3.22	438±312	2003	8	Tsoukias SB	38
Children (age 8–18 yr)							
4,350±1,920	22.3±10.6	1.51±0.95	195±134	2003	5	Hogman	32
			Allergic Alveolitis				
600±220		4.1±1.3		2000	5	Tsoukias	27
700±410		4.1±1.2		2001	17	Tsoukias	25
			Scleroderma				
600±450		4.7±2.2		2002	15	Tsoukias	12
			Cystic fibrosis (age 10–14 yr)				
607±648	17.6±12.1	1.96±1.18	38.0±25.0	2002	9	Tsoukias SB	40
			Allergic rhinitis				
1,161±770	11.8±4.9	1.8±0.6	98±40	2002	15	Hogman	19
			COPD				
1,137±1,420	10.5±8.3	3.9±2.0	108±63	2002	20	Hogman	19
			Sjogrens syndrome				
811±815	6.70±2.92	3.98±1.27	121±76	2002	5	Hogman	16
			Smokers				
438±360	7.8±4.0	2.1±0.7	56±36	2002	20	Hogman	19

Values are means ± SD.

flatter and lack the easily recognizable characteristics of single-breath maneuvers. Furthermore, expired NO levels for tidal breathing are roughly fourfold lower (5–10 ppb) than those observed for single-breath maneuvers. Some of these obstacles can be overcome by observing a series of sequential tidal breaths, whereas others will need to be addressed with improved analytical instrumentation and data-filtering strategies to improve the signal-to-noise ratio.

New experimental methods, techniques, and protocols will also be necessary to complete our understanding of NO exchange dynamics in the lungs. Our present understanding of NO metabolism and transport within lung tissue remains in its infancy. It is clear that exhaled NO is derived from several isoforms of NO synthase in the lungs (54, 55); however, NO also undergoes many chemical reactions that both consume and secondarily produce NO. The substrates for these reactions are numerous and include and *S*-nitrosothiols, proteins, superoxide, and oxygen (10, 41). The models to describe NO exchange utilize a lumped first-order rate constant and distribute production and consumption uniformly throughout the airway tree. However, there is evidence that NO production is heterogeneous in the airways with a relatively larger production in the upper airways (7, 42). These simplifications will need to be addressed with more advanced experiments such as the use of inhaled agents that can potentially map NO production and consumption within the lungs (5, 6, 24, 35, 45, 56, 57).

Although there have been numerous studies that have addressed changes in the flow-independent NO parameters in disease, applications in the clinical assessment and treatment of patients will require numerous additional studies. The focus of these studies should include repeatability with different analytical techniques, controlled treatment interventions, and longitudinal analysis of the parameters. For example, it has been proposed that Daw_{NO}, which depends on physical and anatomical determinants in the airways such as gas solubility and airway surface area, may be impacted by the well-documented structural and physiological changes termed "airway remodeling" in asthma that occur over the time frame of years (38, 44). In contrast, although $J'aw_{NO}$ and Caw_{NO} also depend on structural and physical determinants in the airways, they also depend on the production rate of NO in the airway tissue (48). Hence, they may be sensitive to more acute interventions in

asthma such as corticosteroid therapy, which impacts the pathophysiology of asthma over the time frame of days to weeks (26, 44). More advanced clinical studies are needed to address these possible applications of NO exchange models.

SUMMARY

Exhaled NO has multiple sources in the lungs, and thus new analytical techniques have been developed to capture this rich feature. To date, a two-compartment (airways and alveoli) model of the lungs has proven to be a simple and robust means of describing NO exchange dynamics. The two compartments can be described by three flow-independent NO parameters, two of which characterize the airway compartment ($D\mathrm{aw}_{NO}$ and $J'\mathrm{aw}_{NO}$ or Caw_{NO}) and one which characterizes the alveolar compartment (CA_{NO}). Several analytical techniques have been employed to estimate these parameters in both health and disease. The techniques utilize either multiple vital capacity maneuvers at two or more constant exhalation flow rates or a single vital capacity maneuver in which the flow rate is dynamically altered during the exhalation. Although future studies must address several important model simplifications, early reports suggest the flow-independent NO parameters are uniquely altered in several disease states such as asthma, cystic fibrosis, scleroderma, alveolitis, COPD, and allergic rhinitis and thus may provide pathophysiological insight or assist in the clinical management of inflammatory lung diseases.

GRANTS

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