A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model

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Condorelli P, Shin H-W, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. J Appl Physiol 102: 417–425, 2007. The most common technique employed to describe pulmonary gas exchange of nitric oxide (NO) combines multiple constant flow exhalations with a two-compartment model (2CM) that neglects the trumpet shape (increasing surface area per unit volume) of the airway tree and 2) gas phase axial diffusion of NO. However, recent evidence suggests that these features of the lungs are important determinants of NO exchange. The goal of this study is to test an algorithm that characterizes NO exchange using multiple constant flow exhalations and a model that considers the trumpet shape of the airway tree and axial diffusion (model TMAD). Solution of the diffusion equation for the TMAD for exhalation flows >100 ml/s can be reduced to the same linear relationship between the NO elimination rate and the flow; however, the interpretation of the slope and the intercept depend on the model. We tested the TMAD in healthy subjects (n = 8) using commonly used and easily performed exhalation flows (100, 150, 200, and 250 ml/s). Compared with the 2CM, estimates (mean ± SD) from the TMAD for the maximum airway flux are statistically higher (J′awNO = 770 ± 470 compared with 440 ± 270 pl/s), whereas estimates for the steady-state alveolar concentration are statistically lower (CNO = 0.66 ± 0.98 compared with 1.2 ± 0.8 parts/billion). Furthermore, CNO from the TMAD is not different from zero. We conclude that proximal (airways) NO production is larger than previously predicted with the 2CM and that peripheral (respiratory bronchioles and alveoli) NO is near zero in healthy subjects.

Nitric oxide (NO) was first detected in the exhaled breath of healthy and asthmatic humans in the 1990s (1, 11). Because NO modulates many functions in the lungs (e.g., smooth muscle tone, neurotransmission, and inflammation), there has been considerable interest in understanding NO as a potentially useful noninvasive biological marker (3, 4, 7, 19, 31). Early work established a strong inverse relationship between the concentration and the exhalation flow (15, 29, 35), yet a positive relationship between the elimination rate (product of concentration and flow) and exhalation flow (29, 35). To explain these observations, two-compartment models (2CMs) were developed in which both the airways (rigid tubes) and the alveolar (flexible balloon) regions were sources of exhaled NO (13, 18, 30, 32). The 2CM was attractive because the analytical solution could easily be adapted to create algorithms that analyzed breathing maneuvers with mathematical techniques in which exhaled NO could be partitioned into alveolar (peripheral) and airway (proximal) contributions. This led to the rapid application of these techniques to characterize proximal and peripheral NO in a range of normal and pathological conditions including exercise (25), asthma (8, 14, 16, 17, 26, 30), chronic obstructive pulmonary disease (14), cystic fibrosis (28), and scleroderma (10).

The simplicity of the 2CM is both its strength and weakness. Although the initial description of the model (32) considered the increasing cross-sectional area with distance into the airway tree (i.e., the “trumpet” shape), the subsequent early descriptions neglected this feature (13, 18, 30), and all of the early models neglected axial (as opposed to radial) diffusion of NO in the gas phase. More recently, advanced theoretical and experimental studies (with heliox) by our group (21–24) and others (36) have now established that both the trumpet geometry and gas-phase axial diffusion of NO are critical features of NO exchange that should be considered in the analytical methods.

Although several techniques have been presented that employ a breathhold (single or multiple; Refs. 22, 33) or tidal breathing (6) maneuver, the most common method by far is a series of single exhalation maneuvers from maximal inspiratory to expiratory Society (2), usually performed at a single exhalation flow. Although our most recent work has incorporated axial diffusion and the trumpet shape of the airway tree into the governing material balance equations of the 2CM, the solutions have required cumbersome numerical techniques or considered a transient no-flow (breathhold) condition (21–24), which may be difficult for some patients. The goals of the current study are threefold: 1) develop a steady-state model of NO exchange that considers axial diffusion and the trumpet shape of the airway tree, 2) use the model to develop an algorithm that analyzes a series of steady-state constant flow exhalations and partitions exhaled NO into proximal and peripheral components, and 3) compare the performance of the new model with the earlier simpler model that neglects axial diffusion and the trumpet shape of the airway tree.

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**METHODS**

Experimental exhaled NO. We collected exhaled NO concentration and exhalation flow from healthy nonsmoking, nonasthmatic adults with no history of respiratory disease. The protocol focused on an exhalation flow range that is practical and easy to perform in terms of the magnitude of the flow itself, as well as the number of flows and breathing maneuvers. In addition, we sought high enough flows to ensure that the wall flux of NO from the airway tree (JawNO, pl/s) is constant (independent of flow) and approaches the maximum airflow wall flux of NO (JawNO, pl/s; Ref. 9). This condition is safely met for flows >100 ml/s in healthy adults (9, 32) and greatly simplifies the solution of the governing equation (see Model and APPENDIX). Thus the target flows were 100, 150, 200, and 250 ml/s. These flows have been commonly employed by other research groups, and can be performed by nearly all subjects. Furthermore, by performing each maneuver in triplicate, we limited the number of exhalation flows to 12 for each subject. The group consisted of eight subjects (5 female) with (mean ± SD) age, height, weight, and forced expiratory volume in 1 s (FEV1) (Vmax229; Sensormedics, Yorba Linda, CA) of 31 ± 5 yr, 165 ± 10.1 cm, 62.3 ± 12.3 kg, and 3.5 ± 0.6 liters (102 ± 4.5% predicted), respectively (see Table 1 for details). Each exhalation flow was achieved using flow restrictors. Flow, pressure, and NO concentration (model 280B, Ionics, Boulder, CO) for each maneuver were recorded simultaneously. The protocol was approved by the Institutional Review Board at the University of California, Irvine, and written informed consent was obtained from each subject.

We previously demonstrated that the slope of the exhalation NO profile in phase III is statistically negative (between 4 and 12% of the concentration per liter exhaled) at a constant exhalation flow (35). Thus, when determining exhaled NO concentration (CENO) for multiple exhalation flows, it is important to analyze the exhaled concentration over a similar lung volume. We also wanted to ensure that the wall flux of NO from the airway wall (JawNO) can be considered constant and equal to the maximum airflow flux JawNO (9). Because the flow is larger than 100 ml/s, JawNO can be considered constant and equal to the maximum airflow flux JawNO (9). Thus the concentration increases with z-position and the concentration at the mouth (the exhaled concentration, CENO) is described by Eq. 2.

Axial diffusion of NO is described by Fick’s 1st law of diffusion and transports NO from high to low concentration; thus NO is simultaneously transported by diffusion in the axial direction back toward the alveolar region. Airway volume is defined as the volume between positions z1 and z2 and is estimated in ml as the sum of the subjects age in years plus ideal body weight in pounds (5, 33). The cross-sectional area of the trumpet decreases with increasing z-position and is determined by the relationship in Eq. 1 by mapping the airway dimensions to that of the Weibel symmetric bifurcating airway tree (37).

![Fig. 1. Schematic of the trumpet model. Alveolar air with nitric oxide (NO) steady-state alveolar concentration (CANO) exits the alveolar region at position z1 (generation 17) and is transported toward the mouth (position z2) by convection at a steady volumetric flow rate (V)]. NO is added to the airstream at a rate equal to the wall flux of NO from the airway wall (JawNO pl/s). Because the flow is larger than 100 ml/s, JawNO can be considered constant and equal to the maximum airflow flux JawNO (9). Thus the concentration increases with z-position and the concentration at the mouth (the exhaled concentration, CENO) is described by Eq. 2. Axial diffusion of NO is described by Fick’s 1st law of diffusion and transports NO from high to low concentration; thus NO is simultaneously transported by diffusion in the axial direction back toward the alveolar region. Airway volume is defined as the volume between positions z1 and z2 and is estimated in ml as the sum of the subjects age in years plus ideal body weight in pounds (5, 33). The cross-sectional area of the trumpet decreases with increasing z-position and is determined by the relationship in Eq. 1 by mapping the airway dimensions to that of the Weibel symmetric bifurcating airway tree (37).](fig1)

**Table 1. Physical characteristics of subjects**

<table>
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<th>Sex</th>
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<th>Ht, cm</th>
<th>Wt, kg</th>
<th>Iwt, kg</th>
<th>Vaw, ml</th>
<th>FEV1, liters</th>
<th>%predicted</th>
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Ht, height; Wt, body weight; Iwt, ideal body weight; Vaw, volume of the airway compartment estimated in ml as the sum of the subjects ideal body weight (lbs) plus age (yrs Ref. 34); FEV1, forced expiratory volumes in 1 s (liters and %predicted).
to the axial position at the end of generation 17; see Fig. 1), and $m = 2$ provides an excellent match to the data of Weibel (22, 37). The **APPENDIX** provides details of a solution to the steady-state diffusion equation generating the following solution for the exhaled concentration of NO at the mouth,

$$C_{\text{ENO}} = C_{\text{ANO}} + \frac{J_{\text{awNO}}}{V} \cdot f(V, D_{\text{NO,air}}, A_1)$$  \hspace{1cm} (2)

where $f$ is a function (see **APPENDIX**) of the exhalation flow, the molecular diffusivity of NO in the insufflating gas (i.e., $D_{\text{NO,air}}$ or axial diffusion), and the cross-sectional area of the airway tree at the airway-alveolar junction (i.e., the shape of the trumpet). The key assumption in the solution to the governing equation is that the flux of NO from the airway tree is a constant (i.e., does not depend on flow), and thus our solution is valid for approximately exhalation flows $>100$ ml/s in healthy adults. Note that if $f$ approaches unity, the simple solution of the 2CM is attained (9, 32, 35).

**Parameter estimation.** Equation 2 can be simplified more by limiting the flow to the range to 100 $<$ $V$ $<$ 250 ml/s, which is the range commonly employed in experimental studies including the current study. In this range, $f$ is nearly a linear function of $V$ ($r^2 = 0.98$, see **APPENDIX**, Fig. 5) and can be approximated by $f = (0.00078 s/ml) V + 0.57$. If this relationship is inserted into Equation 2, and both sides of the equation are multiplied by $V$, the following linear relationship for the elimination rate ($V_{\text{NO}}$, pl/s) of NO as a function of flow is attained,

$$V_{\text{NO}} = (C_{\text{ANO}} + J_{\text{awNO}} \cdot 0.00078) V + \frac{J_{\text{awNO}}}{1.7}$$ \hspace{1cm} (3)

where the factor 1.7 is the inverse of 0.57. Thus the model predicts that a plot of $V_{\text{NO}}$ vs. $V$ produces a linear relationship in which the slope, $S$, is equal to $C_{\text{ANO}} + J_{\text{awNO}} \cdot 0.00078$ and the intercept, $I$, is equal to $J_{\text{awNO}}/1.7$. Hence, $C_{\text{ANO}}$ and $J_{\text{awNO}}$ can be estimated from a plot of $V_{\text{NO}}$ vs. $V$ using the TMAD and the following simple relationships,

$$C_{\text{ANO}} = S - \frac{I}{0.00078 s/ml}$$

$$J_{\text{awNO}} = \frac{1.7 \cdot I}{740 \text{ ml/s}}$$ \hspace{1cm} (4)

where $S$ is the slope and $I$ is the y-intercept using simple linear regression. This can be contrasted with the 2CM in which $C_{\text{ANO}}$ and $J_{\text{awNO}}$ can be approximated as simply $S$ and $I$, respectively (9, 32, 35). Values for $J_{\text{awNO}}$ and $C_{\text{ANO}}$ were thus determined by applying linear least squares to a plot of $V_{\text{NO}}$ vs. $V$ for each subject using both the 2CM and the TMAD (Eqs. 4 and 5). The slope was constrained to be greater than or equal to zero.

We previously described in a response to a letter to the editor of the *Journal of Applied Physiology* (20) the advantages of using $V_{\text{NO}}$ vs. $V$ instead of alternate forms that use $C_{\text{ENO}}$ as the dependent variable. In brief, using $V_{\text{NO}}$ as the dependent variable effectively places more weight on the data obtained at higher flow. This can be justified because the assumption of a constant wall flux becomes more accurate as the flow increases (and thus the model is more accurate); thus this technique provides a more accurate estimate of $J_{\text{awNO}}$ and $C_{\text{ANO}}$.

**Statistics.** Confidence intervals (95%) for the determined parameters were calculated assuming a normally distributed error using the $t$-statistic for the slope and intercept of $V_{\text{NO}}$ vs. $V$ for each subject. Differences between the determined parameters using the TMAD and 2CM models as well as comparing the determined parameters to a mean value of zero were calculated using a paired $t$-test or single population $t$-test, respectively. Statistical significance was assumed for $P < 0.05$.

**RESULTS**

In each subject, one or more maneuvers were eliminated by not meeting the criteria for a constant exhalation flow. Figure 2 shows representative exhalation profiles from subject 1 for a typical maneuver that was included (Fig. 2A) and excluded (Fig. 2B). Of the 96 breathing maneuvers (12 maneuvers/subject $\times$ 8 subjects), 65 (68%) were included for further analysis, the remaining 31 having been eliminated by exceeding the maximum variation in flow during the analysis window (standard deviation $>5\%$). Figure 2C demonstrates $C_{\text{ENO}}$ of the profiles that met the inclusion criteria as a function of exhalation flow for all eight subjects. The data demonstrate the inverse relationship between $C_{\text{ENO}}$ and $V$ for all eight subjects that has been previously reported (15, 29, 35).

Figure 3 demonstrates the relationship between $V_{\text{NO}}$ and $V$ for all eight subjects, including the best fit line. $V_{\text{NO}}$ has been calculated using the individual NO concentrations and flows shown in Fig. 2C. Note that a positive relationship between $V_{\text{NO}}$ and $V$ exists for seven of the eight subjects. For subject 5, the best fit line (constraining the slope to be $\geq 0$) has a zero slope. Figure 4 compares the estimated values for $C_{\text{ANO}}$ and $J_{\text{awNO}}$ using the 2CM and TMAD (Eqs. 4 and 5) models. The mean ($\pm$ SD) value of $C_{\text{ANO}}$ for the TMAD model is 0.66 $\pm$ 0.98 parts/billion (ppb), which is not statistically different from zero ($P > 0.05$) and statistically smaller than the mean value determined with the 2CM model (1.2 $\pm$ 0.80 ppb, which is statistically larger than zero). The mean ($\pm$ SD) value of $J_{\text{awNO}}$ for the TMAD model is 770 $\pm$ 470 pl/s, which is statistically larger (1.7 times, see Eq. 4) than the mean value determined from the 2CM model (440 $\pm$ 270 pl/s).

An important consideration in a method to determine unknown parameters is the uncertainty in the estimate. Table 2 presents the uncertainty (95% confidence interval) in the estimates for $C_{\text{ANO}}$ and $J_{\text{awNO}}$ for each of the subjects and for each of the models. The 95% confidence interval for $C_{\text{ANO}}$ spans zero for six and seven subjects, respectively, for the 2CM and TMAD models. For $J_{\text{awNO}}$, the 95% confidence interval spans zero for only three of the eight subjects for both models. The mean maximum deviation from the central value for $J_{\text{awNO}}$ (for both models) is 154 $\pm$ 225%; however, this value is significantly skewed by subject 8, in whom the central value is small (94 pl/s) and the uncertainty high. If this subject is removed, the mean maximum deviation is 74 $\pm$ 41%.

**DISCUSSION**

We have described a new technique to partition proximal and peripheral NO exchange in the lungs that incorporates previously neglected, yet relevant, physical features of the airway tree and gas exchange while maintaining mathematical and computational simplicity. By limiting the flow range to 100–250 ml/s, we are able to incorporate both the trumpet shape of the airway tree and axial diffusion of NO yet still produce a solution of the governing equation and computational technique that uses the slope and intercept of NO elimination vs. flow. The result is a 1.7-fold larger flux of NO from the airway tree and a near zero alveolar (peripheral) NO concentration. These results are consistent with previous, yet more complicated, numerical models, which included the trumpet shape and axial diffusion (21–24, 36). Thus our technique provides a more accurate description of NO exchange dynamics than the previously described and commonly employed 2CM for constant flow exhalations, but maintains simplicity,
Fig. 2. Determination of plateau exhaled concentrations. Representative exhalation flow profiles from subject 1 at the targeted exhalation flow of 150 ml/s for an exhalation maneuver included in the final analysis (A) and a maneuver excluded from analysis (B). The ‘+’ are NO concentration from an unfiltered signal and the solid line is exhalation flow. The space between the vertical dashed lines represents the window of analysis (between 5 and 10 exhaled airway volumes). Note that in maneuver marked for inclusion, several airway volumes are needed to be exhaled before a steady flow is achieved; then, during the analysis window, the standard deviation of the flow is 1.2% (number in parenthesis). Note also that despite a constant flow, a negative slope in the NO concentration is evident highlighting the need to analyze the profiles over a constant exhaled volume window that is scaled to the subjects lung volume. In the maneuver marked for exclusion, the exhalation flow is not constant (standard deviation 11.4%) until after the analysis window. The last panel (C) summarizes the plateau NO concentrations from all 8 subjects of the profiles marked for inclusion, including the mean value (•) at each of the targeted exhalation flows (100, 150, 200, and 250 ml/s).

and thus may be broadly useful to describe proximal and peripheral NO exchange in lung disease.

We previously showed that adding axial diffusion of NO alone (i.e., maintaining the cylindrical geometry) does not significantly impact NO exchange (21, 22). However, the combination of axial diffusion with the trumpet shape dramatically increases the loss of NO to the alveolar region. This is due to the fact that the rate of axial diffusion is proportional to the product of the concentration gradient (change in concentration with axial position) and the cross-sectional area (see Appendix). The dramatic increase in the cross-sectional area in the peripheral regions of the lungs effectively reduces the resistance of NO diffusion in the axial direction, making this a significant physical force that cannot be neglected. Because the observed concentration of NO at the mouth is unchanged, the predicted flux of NO from the airway tree must increase to account for the loss of NO due to back diffusion into the alveolar region. Previous estimates by our group and others (21–24, 36) using breathhold techniques has estimated this increase to be between two- and fivefold, which is consistent with our current prediction using constant flow exhalations (1.7-fold increase).

The large pool of blood in the alveolar region provides a near infinite sink (primarily hemoglobin) to scavenge NO. Thus any additional NO that diffuses from the airway tree toward the alveolar region is immediately bound and does not impact the steady alveolar concentration. We previously demonstrated that the relative impact of axial diffusion decreases as exhalation flow increases (23). This is due to the shift in the balance between convection (movement of NO from the bulk flow of air) of NO and diffusion (Brownian motion of NO molecules) of NO. The rate of convective transport of NO increases in proportion to the exhalation flow, but does not impact the rate of axial diffusive transport. Thus, as flow increases, the loss of NO to the alveolar region by diffusion decreases. This phenomenon by itself can produce a positive slope in the plot of NO elimination vs. exhalation flow of ~1 ppb per ml/s (ppb) over a flow range of 100–250 ml/s in healthy subjects (23). The relative impact should depend on the flux of NO from the airway tree. The larger the airway flux, the larger the gradient of NO in the airway tree, and thus the larger the impact of axial diffusion. This trend is exactly what our model predicts. The alveolar concentration is equal to the value of slope of the NO elimination vs. flow minus a term that is proportional to the airway flux (Eq. 5). For example, for our predicted mean airway flux of 770 ml/s, the impact of axial diffusion, and the trumpet shape of the airway tree can produce a slope of 0.60 ppb [1/740 s/ml = J′awNO/(1.7*740 s/ml), Eqs. 4 and 5], which must be subtracted from the slope to reveal the true alveolar concentration. Because the mean slope of NO elimination vs. flow in our subjects was only 1.2 ppb, the predicted alveolar concentration by the TMAD is near zero (0.66 ppb) and consistent with our previous predictions using more complex numerical solutions and breathhold techniques (21, 23, 24).

The result in Fig. 4 and Table 2 that CNO is negative using the TMAD model in two subjects should not be interpreted as a true negative concentration as this has no physical meaning. The 95% confidence interval presented in Table 2 for CNO is the true range of possible values (with 95% confidence), and this range includes positives values for all subjects. The fact that the 95% confidence interval includes negative values simply reflects the noise and error in the experimental measurement and mathematical model. It is important to note that
the mean value for all subjects is greater than zero, albeit not statistically different from zero.

An important limitation in the current technique is the inability to characterize the airway diffusing capacity of NO, or $D_{aw, NO}$ (9). We and others previously showed that to estimate $D_{aw, NO}$, the exhalation flow must be low enough such that the concentration of NO in the airway tree, $C_{NO}$, can reach a high enough level to decrease the airway flux (9, 13, 18, 30, 33). In other words, the airway flux is no longer a constant and equal to the maximum airway flux. This phenomenon occurs for exhalation flows less than $\sim 50$ ml/s ($5*D_{aw, NO}$) in healthy subjects (9). However, even when such low flows are used, the uncertainty in determining $D_{aw, NO}$ remains large, and obtaining a reliable plateau exhaled concentration is difficult for many subjects due to the necessary long exhalation time. For example, if one were to examine the same exhaled volume...
may be due to different subject populations. There is significant variation in NO elimination among the healthy population, and our current study included only eight subjects. In the current study, the estimated \( J^{awNO} \) using the 2CM (440 pl/s) is smaller than most other estimates using constant flow exhalations (range 700–1,280 pl/s; Ref. 9). In addition, the difference in the magnitude of \( J^{awNO} \) may reflect differences in the techniques. For example, the breathhold technique is a transient technique, and the estimated value of \( J^{awNO} \) is proportional to the estimated value of \( V_{aw} \). In contrast, the current technique depends on steady-state measurements of exhaled concentration and flow and is independent of \( V_{aw} \) (with the minor exception that the window of analysis to determine \( C_E^{NO} \) is based on \( V_{aw} \)). In any event, although the relative impact of axial diffusion and the trumpet shape is consistent between the techniques, caution must be exercised in comparing absolute values of the determined parameters between techniques.

The uncertainty in estimating \( J^{awNO} \) in the current technique is significant in healthy subjects. In three of the subjects, the 95% confidence interval spanned zero, suggesting not that \( J^{awNO} \) was necessarily zero (the NO is coming from somewhere), but rather that the technique could not determine a positive value with 95% confidence. The uncertainty is due to the noise in the experimental data of plotting \( \dot{V}_{NO} \) vs. \( V \) and using only 5–10 data points (depending on the subject). Additional breathing maneuvers will improve the accuracy of the estimated value at the expense of additional effort on the part of the subject. Single breath techniques with a prescribed decrease in the exhalation flow during the maneuver (26, 27, 33) may provide a more accurate estimate of airway and alveolar NO contributions with much fewer breathing maneuvers, but they require more sophisticated mathematical tools and have not yet been tested with axial diffusion and the trumpet shape of the airway tree.

Finally, the flow range used in the current study was chosen based on the relative ease at which subjects can perform them and the need to keep the airway flux constant (i.e., \( J^{awNO} \gg D_{awNO}C_{NO}^{aw} \)). However, several research groups have presented constant flow exhalations using flows larger than 250 ml/s (13, 16), and in healthy subjects the airway flux may be constant for flows as low as 50 ml/s (9). Furthermore, the

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**Table 2. Airway and alveolar NO exchange parameters and confidence intervals**

<table>
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<tr>
<th>Subject</th>
<th>( C_{NO} )</th>
<th>( J^{awNO} )</th>
<th>( C_{NO} )</th>
<th>( J^{awNO} )</th>
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2CM, 2-compartment model; TMAD, trumpet model with axial diffusion; \( C_{NO} \), alveolar concentration of NO; \( J^{awNO} \), maximum airway flux of NO; “lower” and “upper” refer to the limits of the 95% confidence interval for the central determined value. *Not statistically different from zero (\( P > 0.05 \)); †statistically different from the 2CM model.
approximation for f and relationships for CANO and r
impacts the approximate linear relationship for f and subsequent
gators in characterizing proximal and peripheral NO in lung
Thus the technique may be useful to a broad range of investi-
gation as a function of position, 

\[
\frac{d^2C_{NO}}{dz^2} + \left( \frac{1}{A} \frac{V}{D_{NO,air}} \right) \frac{dC_{NO}}{dz} + \frac{J_{aw.NO}}{D_{NO,air}V_{aw}} = 0 \tag{A1}
\]
with the following two boundary conditions,

\[
C_{NO}(z = z_1) = C_{ANO} \tag{A2}
\]
\[
\frac{dC_{NO}}{dz}(z = z_2) = 0 \tag{A3}
\]

The first boundary condition (Eq. A2) simply states that the concentration of NO entering the trumpet at position \( z_1 \) (generation 17) is equal to the alveolar concentration, CANO. The second boundary condition states that convective flow is large enough near mouth (position \( z_2 \)) that the concentration gradient in the \( z \)-position is negligible or approaches zero (21–24).

The values for \( z_1 \) and \( z_2 \) are determined using the data from Weibel (37) for generations 0–23 and from Hanna and Scherer (12) for the dimensions of the oropharynx and oral cavities. Thus \( z_1 = 0.468 \) cm (end of generation 17) and \( z_2 = 40.4 \) cm. Note that the airway volume can be easily estimated by integrating \( A(z)dz \) over the length of the trumpet,

\[
V_{aw} = \int_{z_2}^{z_1} A(z)dz = A_1z_1 - \int_{z_1}^{z_2} x^{-3}dx = A_1z_1(1 - x_2^{-3}) \tag{A4}
\]

where \( x = z/z_1 \) (and thus \( x_2 = z_2/z_1 = 84.6 \)) and \( A_1 \) is the cross-sectional area at position \( z_1 \) (300 cm², Fig. 1). Equation A4 produces a value for Vaw of 142 ml that is in close agreement with our population mean estimate of 166 ml using the sum of the subject’s ideal body weight in pounds plus age in years.

The solution to the governing equation (Eq. A1) is most readily attained by defining the following nondimensional parameters: \( \phi = C_{NO}/C_{ANO} \), \( x = z/z_1 \), \( Pe_1 = z_1V_{aw}/D_{NO,air} \) where \( Pe_1 \) is the Peclet number at \( z \)-position \( z_1 \) representing the ratio of the rate of bulk convection of NO to rate of axial diffusion, and \( \alpha \) is proportional to

\[
\text{APPENDIX}
\]

Model development. The development of the governing equation for the model begins with a differential mass balance over a thickness \( \Delta z \) in the airway tube. The salient features of the model are 1) a cross-sectional area, A, that depends on \( z \)-position (trumpet shape, Eq. 1); 2) a constant average flow per unit volume from the airway wall (radial diffusion) equal to the total maximum airway wall flux from the entire airway tree, \( J_{aw.NO} \) (pl/s) divided by the airway volume, Vaw; 3) axial diffusion in the \( z \)-direction of NO in the gas phase is governing by Fick’s 1st law of diffusion \( (A^*D_{NO,air})*dC_{NO}/dz) \), pl NO/s, where \( D_{NO,air} \) is the molecular diffusivity of NO in air; 4) convection of NO in the \( z \)-direction is characterized by the bulk exhalation flow, \( V \) (ml/s); and 5) steady-state conditions. The result is the following form of the convective-diffusion equation describing the concentration of NO and CANO (ppb or pl NO/cm³) in the airway tree as a function of position,

\[
\frac{d^2C_{NO}}{dz^2} + \left( \frac{1}{A} \frac{V}{D_{NO,air}} \right) \frac{dC_{NO}}{dz} + \frac{J_{aw.NO}}{D_{NO,air}V_{aw}} = 0 \tag{A1}
\]

Table 3. Approximation for f and subsequent relationships for CANO and J_{aw.NO}

<table>
<thead>
<tr>
<th>Flow Range (ml/s)</th>
<th>f = a * V + b</th>
<th>CANO = S - I/c</th>
<th>J_{aw.NO} = I * d</th>
</tr>
</thead>
<tbody>
<tr>
<td>50–250</td>
<td>0.00100</td>
<td>0.53</td>
<td>0.94</td>
</tr>
<tr>
<td>50–500</td>
<td>0.00056</td>
<td>0.59</td>
<td>0.89</td>
</tr>
<tr>
<td>100–250</td>
<td>0.00078</td>
<td>0.57</td>
<td>0.98</td>
</tr>
<tr>
<td>100–300</td>
<td>0.00068</td>
<td>0.59</td>
<td>0.97</td>
</tr>
<tr>
<td>100–400</td>
<td>0.00055</td>
<td>0.61</td>
<td>0.95</td>
</tr>
<tr>
<td>100–500</td>
<td>0.00045</td>
<td>0.63</td>
<td>0.94</td>
</tr>
</tbody>
</table>

S, slope of \( V_{NO} \) (pl/s) vs. \( V \) (ml/s); I, intercept of \( V_{NO} \) (pl/s) vs. \( V \) (ml/s); \( r^2 \), coefficient of determination for the linear approximation of the complex function f (19 data points with evenly distributed values for exhalation flow were used in the linear regression).
the ratio of the rate of radial diffusion of NO to rate of axial diffusion of NO. Inserting these relationships into Eqs. A1-A3 results in the following simpler set of equations,

\[ \phi'' + \frac{[Pe_1 x^2 + 2x^{-1}] \phi'}{\phi} + \alpha = 0 \quad (A5) \]

with boundary conditions,

\[ \phi(x = 1) = 1 \quad (A6) \]
\[ \phi'(x = x_2) = 0 \quad (A7) \]

where \( \phi' \) and \( \phi'' \) are the first and second derivatives with respect to \( x \). The solution to Eq. A5 (second-order inhomogeneous ordinary differential equation with variable coefficients) can be solved analytically for the concentration of NO exiting the mouth (C_{ENO}, equivalent to C_{NO} at position \( z_2 \) or \( \phi \) at position \( x_2 \)) by using an integrating factor and integrating by parts. The result is,

\[ \phi(x_2) = 1 + \frac{\alpha}{Pe_1} \left( \frac{Pe_3}{3} e^{\frac{Pe_3}{3} x_2^3} - \Gamma \left( \frac{Pe_3}{3} \right) \right) - \frac{e^{-x_2^3}}{3} \quad (A8) \]

where \( Pe_3 \) is the Peclet number at position \( z_2 \) (\( z_2 V/D_{NO,air} A_3 \)), and \( \Gamma(u,n) \) is the lower incomplete gamma function defined by,

\[ \Gamma(u,n) = \int_0^n t^{u-1} e^{-t} dt \quad (A9) \]

One can rewrite Eq. A8 by reintroducing the dimensional parameters to arrive at Eq. 2 in the main body of the text with the form,

\[ f = \frac{\left( \frac{Pe_1}{3} e^{\frac{Pe_1}{3} x_2^3} - \Gamma \left( \frac{Pe_1}{3} \right) \right) - \frac{e^{-x_2^3}}{3}}{1 - \frac{x_2^3}{3}} \quad (A10) \]

In Eq. A10, \( \Gamma(Pe_2/3) \) has been replaced by the constant value of 1.354, which is valid for exhalation flows \( > 10 \mu L/s \). Figure 5 shows the dependence of \( f \) with exhalation flow over the flow range 100 < \( V < 250 \) mL/s. Note the near linear relationship in which \( f \) can be approximated \( (r^2 = 0.98) \) by the much simpler form,

\[ f = 0.00078(V) + 0.57 \quad (A11) \]

which can then be inserted into Eq. 2 as shown in the main text to arrive at Eqs. 3–5.

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GRANTS

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