Single-exhalation profiles of NO and CO$_2$ in humans: effect of dynamically changing flow rate

NIKOLAOS M. TSOUKIAS,1 ZIAD TANNOUS,2 ARCHIE F. WILSON,2 AND STEVEN C. GEORGE1
1Department of Chemical and Biochemical Engineering and Materials Science and 2Division of Pulmonary and Critical Care, Department of Medicine, University of California at Irvine, Irvine, California 92697-2575

Tsoukias, Nikolaos M., Ziad Tannous, Archie F. Wilson, and Steven C. George. Single-exhalation profiles of NO and CO$_2$ in humans: effect of dynamically changing flow rate. J. Appl. Physiol. 85(2): 642–652, 1998.—Endogenous production of nitric oxide (NO) in the human lungs has many important pathophysiological roles and can be detected in the exhaled breath. An understanding of the factors that dictate the shape of the NO exhalation profile is fundamental to our understanding of normal and diseased lung function. We collected single-exhalation profiles of NO and CO$_2$ from normal human subjects after inhalation of ambient air (15 parts/billion) and examined the effect of a 15-s breath hold and exhalation flow rate ($V_E$) on the following features of the NO profile: 1) series dead space, 2) average concentration in phase III with respect to time and volume, 3) normalized slope of phase III with respect to time and volume, and 4) elimination rate at end exhalation. The dead space is ~50% smaller for NO than for CO$_2$ and is substantially reduced after a breath hold. The concentration of exhaled NO is inversely related to $V_E$, but the average NO concentration with respect to time has a stronger inverse relationship than that with respect to volume. The normalized slope of phase III NO with respect to time and that with respect to volume are negative at a constant $V_E$ but can be made to change signs if the flow rate continuously decreases during the exhalation. In addition, NO elimination at end exhalation vs. $V_E$ produces a nonzero intercept and slope that are subject dependent and can be used to quantitate the relative contribution of the airways and the alveoli to exhaled NO. We conclude that exhaled NO has an airway and an alveolar source.

endogenous; exhalation flow rate; phase III slope; elimination rate

NITRIC OXIDE (NO) is a highly reactive and abundant molecule in the body that has many important physiological roles, including neurotransmission, host defense response, and smooth muscle relaxation (5). Recently, NO has been detected in the exhaled breath of humans (2, 21). The concentration of NO in the exhaled breath increases in inflammatory lung diseases such as bronchial asthma (15, 22). This finding generated excitement about a possible use of NO as a biomarker of pulmonary inflammation (14). In addition, exogenous NO has been shown to selectively vasodilate the pulmonary vasculature and is capable of modulating bronchial smooth muscle tone in animals and humans (8–10). The above findings suggest the possible use of NO as a therapeutic intervention for a number of pulmonary diseases. However, there is only limited information on the fundamental gas exchange dynamics of NO in the lungs.

Early studies of exhaled NO demonstrated an increase in NO elimination from the lungs with hyperventilation and exercise, providing evidence that alveolar gas contained a nonzero concentration (21). Subsequent studies demonstrated NO production from the conducting airway space below the larynx on the basis of direct measurement (24) as well as after breath hold (16, 21). The most recent studies have reported significant levels of NO in the nasal cavity (3, 16, 24). In fact, the production is approximately an order of magnitude larger in the nasal cavity than in the lower respiratory tract. These studies have documented that endogenous NO production in the airways and lungs is heterogeneous and likely plays a variety of roles in normal lung function.

The heterogeneity in the source of exhaled NO complicates the interpretation of the exhalation profile. Historically, the exhalation profile of the respiratory gases, CO$_2$ and O$_2$, and other inert gases, such as He and N$_2$, has provided great insight into the gas exchange dynamics of the specific gas and the lungs, in general. The shape of the NO exhalation profile has not been fully characterized. Recently, NO concentration was shown to be inversely related to exhalation flow rate ($V_E$) (28). This result was also consistent with conducting airway production of NO and highlighted the importance of further study into the dynamics of NO gas exchange.

The objective of our present study is threefold: 1) to quantitatively characterize the features of the NO oral exhalation profile, 2) to further explore the dependence of the NO exhalation profile on $V_E$, and 3) to develop an experimental technique that can provide an estimate of the relative contribution of the airways and the alveoli to exhaled NO.

METHODS

Subjects. We collected oral exhalation profiles of NO and CO$_2$ in seven normal men [28.4 ± 3.8 (SD) yr] with no history of smoking or lung diseases. The protocol was approved by the Institutional Review Board at the University of California, Irvine. Subjects were categorized as normal on the basis of standard spirometry that included forced vital capacity, forced expiratory volume in 1 s, and forced expiratory flow between 25 and 75% of the exhaled volume. All subjects included in the study had spirometry values >75% of predicted on the basis of their race, age, weight, and height (Table 1).

Isolation of nasal cavity. Several researchers have reported the nasal cavity as a significant source of NO in the respiratory tract (3, 16, 24) that is capable of contaminating the oral exhalation profile. Hence, to collect a true oral exhalation profile to describe the exchange dynamics of NO in the lower respiratory tract, the nasal cavity must be isolated from the lower respiratory tract. To achieve this, we applied a small negative pressure (~20 cmH$_2$O) to the nasal cavity. Each
subject was then instructed to swallow, which elevated the soft palate and sealed the nasal cavity. Closure of the soft palate was confirmed by monitoring the nasal cavity pressure and maintaining a nasal concentration of CO2 of <0.5% during expiration. Any maneuvers where movement of the soft palate was detected (change in nasal cavity pressure) were discarded and repeated. All exhalation profiles had a constant negative nasal pressure between −10 and −20 cmH2O. Under these conditions, all subjects maintained a nasal CO2 concentration <0.5% during the course of the exhalation maneuver.

The negative nasal cavity pressure provides several advantages over other reported methods. 1) Elevation of the soft palate requires no special effort from the subject. In contrast, balloon occlusion is invasive, and voluntary closure of the soft palate requires subject training. 2) Minor openings of the soft palate will result in a convective flow into the nasal cavity and will not contaminate the oral exhalation. 3) By recording the pressure in the nasal cavity, even minor openings of the soft palate can be detected immediately. 4) The nasal cavity is isolated during inspiration and expiration.

Protocol. The design of the experimental protocol focuses on the effect of \( V_E \) on the NO exhalation profile. After isolation of the nasal cavity, each subject was asked to breathe comfortably (3–5 tidal breaths) before performing a single-exhalation maneuver. In our single exhalation a subject inspires from functional residual capacity to total lung capacity, then slowly exhales to approximately functional residual capacity. The exhalate passed through a mouthpiece connected to a “j” valve (two 1-way valves), allowing the subjects to inspire ambient air [10–15 parts/billion (ppb) NO] through one port and exhale through the second port. Gas (NO and CO2) was measured from a sampling port at the side of the mouthpiece, ~1 cm from the mouth.

Each subject performed four different \( V_E \) patterns: 1) constant slow \( V_E \) of −250 ml/s (control maneuver), 2) constant faster \( V_E \) of −500 ml/s, 3) linearly (in time) increasing \( V_E \), and 4) linearly (in time) decreasing \( V_E \). Each subject performed the maneuvers in triplicate, with or without a 15-s breath hold before the exhalation. Hence, each subject performed 24 technically acceptable exhalation maneuvers (4 \( V_E \) × 3 replicates × 2 presence or absence of breath hold). Exhalation with constant flow rate was facilitated by use of a Starling resistor. The resistance to flow through a Starling resistor increases with increasing flow; thus the resistor serves to maintain a constant flow rate, independent of expiratory effort of the subject. On-line representation of the flow signal on the screen of the computer assisted the subjects during performance of the increasing and decreasing \( V_E \) maneuvers.

Airstream analysis. NO concentration was measured using a chemiluminescence NO analyzer (model NOA280, Sievers Instruments, Boulder, CO). The instrument is capable of providing highly accurate ( repeatability ±1 ppb vol) gas phase measurements with a very small detection threshold [−5 ppb vol − 500 parts/million (ppm) vol] and a fast response time (0–90% response time of 200 ms). We used a needle valve to restrict flow and maintain an operating reaction cell pressure of 7.5 mmHg and a sampling rate of 250 ml/min. The factory calibration values of the instrument were tested periodically using certified NO gas (27.8 ppm in N2).

CO2 concentration was measured by using a fast-response (0–90% response time of 100 ms) mass spectrometer (Perkin-Elmer Medical Instruments, Pomona, CA). The convective transport times for NO and CO2 from the mouth to the analyzers were 350 and 450 ms, respectively. These times were subtracted from the profiles before analysis.

Flow rate was measured with a pneumotachometer (model 50-MC2-M2, Marom). Analog signals from the pneumotachometer, the mass spectrometer, and the chemiluminescent analyzer were digitized through a 12-bit analog-to-digital converter. The analog-to-digital card was programmed to scan three channels simultaneously every 50 ms. The resulting measurements (20 Hz) were stored in a computer for later analysis. Figure 1 is a schematic of the experimental setup.

Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FVC, liters</th>
<th>FEV1, %pred</th>
<th>FEF25–75, %pred</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>173</td>
<td>63</td>
<td>4.19</td>
<td>84</td>
<td>75</td>
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<tr>
<td>2</td>
<td>167</td>
<td>55</td>
<td>4.25</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>191</td>
<td>70</td>
<td>5.42</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>175</td>
<td>77</td>
<td>4.85</td>
<td>101</td>
<td>112</td>
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<tr>
<td>5</td>
<td>167</td>
<td>61</td>
<td>3.81</td>
<td>83</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>173</td>
<td>66</td>
<td>4.58</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>172</td>
<td>72</td>
<td>4.00</td>
<td>85</td>
<td>84</td>
</tr>
<tr>
<td>Mean</td>
<td>172.5</td>
<td>66.3</td>
<td>4.44</td>
<td>91.4</td>
<td>89.6</td>
</tr>
</tbody>
</table>

FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; FEF25–75, forced expiratory flow at 25–75% of exhaled volume.
Data analysis. We used three parameters to characterize the exhalation profile: 1) serial dead space, 2) average concentration of phase III, and 3) slope of phase III. In addition, we determined the elimination rate of each gas from the lungs.

The dead space for CO₂ (VdCO₂) was calculated as described by Meyer et al. (18) using backextrapolation of the phase III slope with respect to exhaled volume [SIII,CO₂(V)] (18, 19, 25, 26). Because the shape of the exhalation profile of NO is quite different from that of CO₂ (see RESULTS), we defined the dead space of NO (VDNO) in a different manner. The exhalation profile of NO is characterized by a rapid rise to an initial peak in phase I. The slope of phase III can be negative or positive depending on Vₑ (see RESULTS). The size of the initial peak depends on the length of breath hold. We defined VdNO as the volume corresponding to the midpoint NO concentration between the ambient level and the initial peak value in the breath-hold maneuver.

An average value for concentration and flow rate that characterizes the exhalation profile was calculated using the data in phase III (alveolar gas) of the exhale. Average concentrations were calculated with respect to time and exhaled volume. The average concentrations and flow rates with respect to time (Cₑ(NO), Cₑ(CO₂), and Vₑ(t)) were calculated over a fixed time interval of 6 s beginning 2 s after the start of exhalation and ending 8 s after the start of exhalation.

\[ \bar{C}(t) = \frac{\int_2^6 C \, dt}{\int_2^8 dt} \]  

The average concentrations and flow rates with respect to volume (Cₑ(NO)(V), Cₑ(CO₂)(V), and Vₑ(V)) were calculated using the exhale between 20% (Vₑ20) and 60% (Vₑ60) of each subject’s vital capacity (VC).

\[ \bar{C}(V) = \frac{\int_{V₂₀}^{V₆₀} C \, dV}{\int_{V₂₀}^{V₆₀} dV} \]  

Fig. 2. Schematic detailing analysis of exhalation profiles for concentration and flow rate. Average concentrations and flow rates were estimated for exhaled volume and exhaled time by use of interval of 20–60% of vital capacity (VC) or 2–8 s, respectively. Phase III slope was calculated using linear least squares over this same interval and then normalized by average value over interval. Vₑ, exhalation flow rate; Cₑ(NO), Cₑ(CO₂) and NO concentration, respectively; Vₑ(CO₂), Vₑ(NO), and Vₑ(NO); average Vₑ, Cₑ(CO₂), and Cₑ(NO); respectively; Sₑ(NO), Sₑ(CO₂), and Sₑ(NO); phase III slopes for NO, CO₂, and NO, respectively.
enough through the airway tree

\[ E_{\text{NO}} = \dot{V}_{\text{EE}}C_{\text{alvee}} + \int_{g,\text{air}} J_{\text{t:g,air}} A_{\text{s,air}} \]  

(10)

where \( C_{\text{alvee}} \) is alveolar concentration at end exhalation, \( \int_{g,\text{air}} J_{\text{t:g,air}} A_{\text{s,air}} \) is the average (over axial position) flux (\( \text{nl} \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \)) of NO from the airway tissue into the gas phase, and \( A_{\text{s,air}} \) is the total surface area of the airways (\( \text{cm}^2 \)). Recently, Silkoff et al. (28) reported a linear relationship between \( E_{\text{NO}} \) and \( V_{\dot{E}} \) over a wide range of flow rates, which suggests that 1) \( C_{\text{alvee}} \) approaches a steady-state value \( (C_{\text{alvee},\text{ss}}) \) and 2) \( \int_{g,\text{air}} J_{\text{t:g,air}} A_{\text{s,air}} \) is essentially constant over the examined flow rate range. The above observations were verified with the theoretical model (30). In this fashion the slope of \( E_{\text{NO}} \) vs. \( V_{\dot{E}} \) provides an estimate of \( C_{\text{alvee},\text{ss}} \), and the intercept provides an estimate of the contribution from the conducting airway space to total lung elimination.

Statistics. A two-tailed paired \( t \)-test was used to detect differences in the dead space between gases and between different maneuvers. The same statistical method was used to examine the effect of breath hold on the average exhaled concentrations and phase III slope. The flow rate dependence of the parameters was examined with linear least squares regression. The null hypothesis of no dependence (i.e., zero slope) was tested with a single-sample two-tailed \( t \)-test with use of the average slope from all seven subjects. \( P < 0.05 \) was considered statistically significant in all analyses.

RESULTS

Figure 3A represents a typical exhalation profile of NO and CO\(_2\) under the control conditions. The \( V_{\dot{E}} \) signal is also shown. The CO\(_2\) profile demonstrates the well-documented dead space (phase I) followed by a rapid rise (phase II) to a sloping alveolar plateau (phase III). The exhalation profile of NO is substantially different. The initial concentration is equal to the ambient concentration (\( \sim 14 \) ppb). Phase I is present and is visually smaller than that of CO\(_2\) (see Series dead space). Phase II is characterized by an initial peak and then a slow decrease during exhalation of the alveolar gas (phase III). Hence, under the control conditions of our experiment, \( S_{\text{III},\text{NO}} \) is negative (see Phase III slope).

Figure 3B represents the exhalation profiles in the same subject after a 15-s breath hold. NO and CO\(_2\) scales have been reduced to allow depiction of the initial NO peak. The results are typical of those presented by previous investigators. For CO\(_2\), phase I is reduced, phase II rises to a larger concentration before the start of phase III, and phase III has a flatter slope. For NO the initial peak in phase I is much larger in magnitude (70 ppb vs. 17 ppb in this particular subject), and phase III is similar in shape.

Figure 3C depicts the effect of a dynamically changing flow rate on the exhalation profiles (in this case a linearly decreasing \( V_{\dot{E}} \), again, in the same subject). The CO\(_2\) profile does not change appreciably. However, the NO profile demonstrates marked changes. After the initial peak the concentration decreases gradually to a minimum and then begins to steadily increase. This phenomenon generates a positive phase III slope (see Phase III slope).

Series dead space. Mean values of \( V_{\text{DCO}_2} \) and \( V_{\text{DNO}} \) for all seven subjects, under different exhalation maneuvers, are summarized in Table 2. \( V_{\text{DNO}} \) is \( \sim 50–60\% \) smaller than \( V_{\text{DCO}_2} \) under all conditions. \( V_{\text{DCO}_2} \) and \( V_{\text{DNO}} \) decrease significantly (\( \sim 40–60\% \), \( P < 0.05 \)) after a 15-s breath hold but are independent of a twofold increase in the flow rate.

Average concentrations. Table 3 summarizes the average concentrations for NO and CO\(_2\) for the control maneuver. After a 15-s breath hold, \( C_{E,\text{NO}}(t) \) and \( C_{E,\text{NO}}(V) \) were slightly decreased for most of the subjects; however, this decrease was not statistically signifi-
Table 2. Series dead spaces

<table>
<thead>
<tr>
<th></th>
<th>Slow</th>
<th>Fast</th>
<th>15-s Breath Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>180 ± 24†</td>
<td>182 ± 35</td>
<td>77 ± 24  91 ± 14</td>
</tr>
<tr>
<td>NO</td>
<td>87 ± 25*</td>
<td>75 ± 40</td>
<td>34 ± 10  47 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SD in ml for 7 subjects. *Significantly different from breath hold (P < 0.05). †Significantly different from nitric oxide (NO) (P < 0.05).

Table 3. Average concentrations and flow rates

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>15-s Breath Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE(NO)(t), ppb</td>
<td>9.35 ± 3.49</td>
<td>8.86 ± 3.32</td>
</tr>
<tr>
<td>CE(NO)(t), %</td>
<td>4.67 ± 0.23</td>
<td>5.68 ± 0.23*</td>
</tr>
<tr>
<td>VE(t), ml/s</td>
<td>262 ± 42</td>
<td>255 ± 35</td>
</tr>
<tr>
<td>CE(NO)(V), ppb</td>
<td>8.98 ± 3.29</td>
<td>8.61 ± 3.18</td>
</tr>
<tr>
<td>CE(NO)(V), %</td>
<td>4.98 ± 0.26</td>
<td>5.83 ± 0.25*</td>
</tr>
<tr>
<td>VE(V), ml/s</td>
<td>264 ± 39</td>
<td>264 ± 25</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 subjects. CE(NO)(t) and CE(NO)(V), average NO concentration with respect to time, respectively; CE(NO)(t) and CE(NO)(V), average NO concentration with respect to volume, respectively; VE(t) and VE(V), average exhalation flow rate with respect to time and volume, respectively; ppb, parts/billion. *Significantly different from control (P < 0.05).

positive for CO₂ and negative for NO. In addition, a 15-s breath hold reduces the absolute value of the intercept for CO₂ and NO; i.e., the phase III slopes become flatter (Fig. 3B).

Elimination rate. ENO for the constant exhalation maneuvers is displayed as a function of VEE, in Fig. 7. A 15-s breath hold had no statistical effect on the elimination rates at end exhalation; hence, all the data have been lumped together. The mean slope (an index of C_{a\infty}) and intercept (an index of airflow flux or production) for each subject are presented in Table 4. ENO is generally a positive function of VEE; however, the standard deviation of ENO is large. In fact, the slope of NO for subject 1 was zero. The slope for all subjects, and hence the average steady-state alveolar concentration, was 5.6 ± 3.1 (SD) ppb. The intercept, and hence the average net total airflow flux of NO (I_{T\text{gf},A_{\text{air}}}), for the seven subjects was 0.71 ± 0.31 × 10⁻⁶ ml/s and is statistically different from zero.

**DISCUSSION**

Our experimental data suggest two different but not contradictory hypotheses: 1) NO may originate from the airway region of the lung, which is fairly nonexpansible; 2) NO may originate from the expansible alveolar region of the lung. The combination of hypotheses 1 and 2 can provide a possible explanation for the unique behavior of the NO exhalation profile and the differences with CO₂ that have been observed by us and others. However, the existence of alternative explanations cannot be excluded. Some are discussed below. The relative importance or contribution of each of these mechanisms is examined in greater detail with the development of a simplified two-compartment mathematical model (30).

Series dead space. There is a significant difference between VDCO₂ and VDNNO. Although VDCO₂ (180 ± 24 ml) is probably underestimated [a phenomenon previously reported (18) and attributed to an artifact arising from backextrapolation of the nonlinear CO₂ profile], VDNNO (87 ± 25 ml) is significantly smaller. Hence, even with the nasal passages isolated, NO appears in the exhaled breath before CO₂. This result is consistent with considerable production of NO in an area of the lung before the alveolar region, the source of CO₂ (hypothesis 1).

The considerable decrease in the dead space volume for both gases after the short breath-hold time has been previously reported (4, 18) and is attributed primarily to cardiogenically induced convective mixing during the breath hold. Although the techniques used to determine VDNNO and VDCO₂ were different, they are quite similar and cannot account for the large difference between VDNNO and VDCO₂.

Average phase III concentrations. The decrease in the average exhaled concentration over the specified volume interval (20–60% of VC) in the constant flow rate maneuvers with higher V̇E (Fig. 4) cannot be attributed to the same mechanism for both gases. For CO₂ this decrease can be explained through the mechanism of continuous gas exchange in the alveolar region of the lung (6, 18). Over a constant-volume interval, a slower
\( \dot{V}_E \) will result in an increased expiration time (similar to breath holding); hence, the result is an increased \( \text{CO}_2 \) concentration due to continuous production and evolution into the alveolar gas. In contrast, when the average \( \text{CO}_2 \) concentration is calculated over a specified time interval (Fig. 4D), the effective time for gas exchange is the same under the different flow rate conditions. As a result, there is no statistical flow rate dependence on \( \text{CCO}_2(\dot{V}) \). Small changes in \( \text{CCO}_2(\dot{V}) \) with flow rate can be attributed to other mechanisms such as stratified or parallel inhomogeneities of the lung during exhalation (19).

In contrast, \( \text{CNO}(\dot{V}) \) and \( \text{CNO}(\text{V}) \) are inverse functions of flow, suggesting inherently different gas exchange dynamics. The exact mechanism behind this dependence is not known. However, a probable explanation, which is in agreement with our findings for the series dead space, is that a significant part of the exhaled NO is derived from the relatively nonexpansile airway region of the lung proximal to the alveolar region (hypothesis 1). As \( V_E \) increases, the rate of washout in the airways (fixed volume) increases (or mean residence time decreases), thus decreasing the concentration. This phenomenon does not occur for \( \text{CO}_2 \), the source of which is the expansile alveoli. As the alveoli shrink during exhalation, \( \text{CO}_2 \) concentration in the alveolar gas is maintained relatively constant (in the absence of production or consumption) and is independent of flow rate. This concept is developed more fully in the form of a mathematical model (30). In addition, consistent with the theory of airway NO production is the fact that several cell types present in the airways, including the bronchial epithelium, fibroblast, smooth muscle cell, macrophage, and neutrophil, have been shown in vitro to produce NO and/or to contain the enzyme necessary for NO production, NO synthase (12, 13, 17, 23, 27).

Interestingly, the slope of \( \text{CNO}(\dot{V}) \) vs. \( V_E \) is statistically steeper than the slope of \( \text{CNO}(\text{V}) \) vs. \( V_E \). If one assumes a net consumption during exhalation of NO in the alveolar region because of fast reaction with the hemoglobin of the blood in the pulmonary capillary bed, then the relationship of NO concentration to flow rate can be viewed as a result of two different antagonistic mechanisms: 1) concentration is an inverse function of the flow due to NO production in the nonexpansile part of the lungs, and 2) concentration is a positive function of flow as a result of a decrease in the expiration time and hence less NO consumption by the pulmonary blood. Mechanism 2 presumes a nonzero alveolar concentration (hypothesis 2), which is consistent with experimentally observed in vitro production of NO by the alveolar epithelium (7) as well as theoretically by Hyde et al.
These two mechanisms are consistent with a steeper slope of $C_{NO}(t)$ vs. $V_E$ (same exhalation time and hence only mechanism 1) compared with $C_{NO}(V)$ vs. $V_E$ (different exhalation times and hence mechanism 2 contributes).

Mechanisms other than continuing gas exchange, such as parallel or stratified inhomogeneity, may also impact the observed results. For example, parallel inhomogeneities in the lungs exist because of different alveolar ventilation-to-volume ratios. During exhalation, regions of higher ventilation will empty first from the lung and will have a lower (or higher) concentration of gas because of alveolar dilution (or enrichment) during inspiration (19). Thus, over a constant time interval, a higher $V_E$ will increase the contribution of alveolar air in poorly ventilated regions, which have a higher (or lower) concentration. Whether the poorly ventilated regions have a higher or lower NO concentration will depend on whether there is a net positive or negative flux of NO in the specific alveolar region, respectively. A positive or negative flux depends on several factors, including the alveolar diffusing capacity, the inhaled concentration, and the volume of the specific alveolar region (11, 30). This mechanism would predict a positive or negative relationship of $C_{NO}(t)$ to $V_E$. Because $C_{NO}(t)$ is a stronger inverse function of $V_E$ than $C_{NO}(V)$ is, parallel inhomogeneities would be an important mechanism underlying the phase III slope if a net consumption of NO occurs in poorly ventilated regions.

Recently, Silkoff et al. (28) performed an extensive study on the effect of $V_E$ on the exhaled NO concentration during oral exhalation (nasal cavity isolated during expiration). Although they applied significant positive end-expiratory pressure, which has been reported by others to affect exhaled NO concentration (20), they also report an inverse relationship of concentration to $V_E$.

Phase III slope. Phase III slopes have been defined in several ways by previous investigators (18, 19, 25, 26). When phase III slope data are reported, several factors
must be considered: 1) absolute vs. relative values, 2) slope with respect to time or exhaled volume, 3) range of the exhalation profile over which to calculate slope, and 4) method of normalization.

We showed earlier that $V_E$ can affect the average CO$_2$ concentration by changing the expiration time (continuous alveolar production) or the exhaled volume (parallel, stratified inhomogeneities). The positive dependence of $S_{III,CO_2}(t)$ and the small negative relationship of $S_{III,CO_2}(V)$ to $V_E$ can be explained using the same rationale. The change in CO$_2$ concentration per unit time [$S_{III,CO_2}(t)$] will increase in the constant flow rate maneuvers with a higher $V_E$ due to parallel inhomogeneity, since the exhaled volume per unit time will increase. This change is small compared with the effect of continuing gas exchange (18). The change in CO$_2$ concentration per unit volume [$S_{III,CO_2}(V)$] will slightly decrease with higher flow because of continuing alveolar gas exchange (continuous production), since the expiration time per unit volume will decrease.

If it is assumed that the average flow during the examined part of phase III does not change significantly between maneuvers, $S_{III,NO}(t)$ is not expected to change with the dynamically changing flow rate maneuvers (Fig. 6, C and D). On the other hand, $S_{III,NO}$ behaves completely differently. $S_{III,NO}(t)$ and $S_{III,NO}(V)$ are essentially independent of the magnitude of a constant $V_E$ but depend significantly on a changing $V_E$ throughout the course of the single exhalation (Fig. 5, A and B, and Fig. 6, A and B).

Figure 6 displays this unique feature of the NO exhalation profile. Depending on the slope of the exhalation flow, the alveolar plateau can range from positive to negative values. The mechanisms underlying the direct relationship of the exhaled NO concentration to the flow rate presented earlier can also be invoked to
explain this phenomenon. At any given exhaled volume or time after the beginning of exhalation, an increase or decrease in $V_E$ causes a decrease or increase in NO concentration, respectively, because of dilutional effects in the nonexpansile airways (hypothesis 1). For example, if $V_E$ is continuously decreasing [i.e., negative $S_{III,V_E}(t)$], NO concentration will continuously increase [i.e., positive $S_{III,NO}(t)$].

An interesting finding related to the dynamically changing flow rate maneuvers is the nonzero intercept for NO in Fig. 6, A and B. The existence of a negative $S_{III,NO}$ when $V_E$ is constant indicates that, although the phase III slope is predominantly influenced by flow rate changes, other mechanisms also contribute. We have already suggested a nonzero alveolar concentration (hypothesis 2) coupled with continuing gas exchange (in the case of continuing NO consumption) as a mechanism for this negative slope. However, for continuous consumption of NO to occur in the alveolar region, alveolar concentration at end inspiration would have to be larger than the steady-state value. A steady-state alveolar NO concentration is expected when the net rate of production from the cells of the alveolar membrane is balanced by diffusion into the pulmonary blood (11, 30). Hence, it is possible that inspired conditions such as concentration and flow rate may affect the alveolar concentration at end inspiration. For example, if the inspired NO concentration were zero, then the alveolar concentration at end inspiration may be less than the steady-state value. In this case, continuing gas exchange would cause an increase in the alveolar concentration during exhalation and a positive phase III slope. This finding would be modulated by inspiration flow rate, which impacts the time of inspiration as well as absorption of NO from the airways. Our finding of a negative phase III slope may be attributed to inhaling ambient concentrations of NO that were on the order of 15 ppb. The effect of inspired conditions on the NO exhalation profile is explored further elsewhere (30) with use of a mathematical model and should be explored further experimentally.

Breath hold. The effect of breath hold on the NO and CO$_2$ exhalation profiles has been reported previously and can be explained in a manner consistent with the mechanisms related to average concentration and the phase III slope. During breath hold, NO and CO$_2$ concentrations in the airway and alveolar regions approach their steady-state values. NO accumulates in the airway space [but could also accumulate in alveoli (11)], and CO$_2$ accumulates in the alveolar region. Hence, during exhalation, a large initial peak in NO concentration is observed in phase I and a larger concentration of CO$_2$ is observed at the start of phase III.

The dynamics of NO exchange in the alveolar region are more complicated because of the somewhat unexpected result of the effect of breath hold on the average NO concentration in phase III. If continuing NO exchange in the alveolar region is the predominant mechanism underlying the negative phase III slope under control conditions (constant flow rate), then the average NO concentration in phase III should decrease after the 15-s breath hold (e.g., the alveolar concentration is expected to decrease because of consumption by the pulmonary blood). CO$_2$ and NO have similar relative rates of increase or decrease, respectively, during a single exhalation with constant flow rate (~5%/s over the 2- to 8-s time period without breath hold and 1.7%/s after the breath hold). However, only CO$_2$ concentrations were statistically affected by a breath hold. Although $C_{E_{NO}}(t)$ and $C_{E_{NO}}(V)$ were slightly decreased (consistent with continuous uptake by the pulmonary blood) during breath hold, this was not statistically significant. There are several possible explanations: 1) NO consumption by the pulmonary blood may be limited and the negative $S_{III,NO}$, in contrast to $S_{III,CO_2}$, is generated predominantly by parallel or stratified inhomogeneities and not as a result of continuous gas exchange in the alveoli. 2) NO concentrations in the airway space become quite elevated during the breath hold (Fig. 3B). The subsequent steep axial gradients in NO concentration may lead to substantial axial diffusion from NO in the airways to the alveolar region, leading to an alveolar concentration, at the end of breath hold, above its normal steady-state value. The distance of diffusion ($L_D$) over a 15-s breath hold can be

![Fig. 7. Elimination rates for NO ($E_{NO}$) as a function of $V_E$ at end exhalation ($V_{E,ee}$). Only constant $V_E$ maneuvers are shown, with and without breath hold. Lines represent least squares linear regression through all data points for each subject.](image)

Table 4. Slope and intercept of $E_{NO}$ vs. $V_E$

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Slope, ppb</th>
<th>Intercept, ml·s$^{-1}$·10$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>1.12</td>
</tr>
<tr>
<td>3</td>
<td>7.9</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>8.1</td>
<td>0.59</td>
</tr>
<tr>
<td>7</td>
<td>7.7</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$E_{NO}$, NO elimination rate; $V_E$, exhalation flow rate.

$E_{NO}$, NO elimination rate; $V_{E,ee}$, exhalation flow rate.
eliminated. The alveolar concentration is independent of $V_{\dot{E}}$ (in the constant and in the dynamically changing maneuvers), thus this decrease was not sufficient to preserve a constant $E_{NO}$ (Eq. 9). There are at least three explanations. 1) As discussed earlier (hypothesis 2), there may be a nonzero alveolar concentration of NO. Because of the expansile nature of the alveoli, the alveolar concentration is independent of $V_{\dot{E}}$ (in the absence of production or consumption should alveolar concentration remain constant during exhalation). Hence, the elimination rate of a gas is proportional to $V_{\dot{E}}$ (Eq. 10). This explanation is quite probable in light of the evidence that the alveolar epithelium is capable of producing NO (7, 29). 2) Equation 10 suggests that a positive relationship between $V_{\dot{E}}$ and $E_{NO}$ can be generated by a flow-dependent flux of NO from the airway wall ($J_{\text{air}}$). If transport of NO into the airstream within the airways is limited by diffusion in the gas phase, then increasing $V_{\dot{E}}$ may increase the net uptake of NO within the airways (28). As $V_{\dot{E}}$ increases, the concentration of NO decreases by dilution. Hence, the driving force for radial diffusion (difference between tissue concentration and airstream concentration or radial concentration gradient) is increased. The very small solubility of NO in water and tissue creates a very small relative gas-phase resistance to diffusion, making this explanation unlikely (30). The consumption of NO by chemical reaction, at least first order in NO concentration, within the airway tissue may also generate a flow-dependent flux. Increasing $V_{\dot{E}}$ and thus decreasing NO concentration in the airstream and within the adjacent tissue may decrease the rate of consumption by chemical reaction while the rate of endogenous production remains unaffected. It is difficult to speculate on the importance of this mechanism; however, the mathematical model provides valuable insight and suggests that reduced consumption in the airway tissue with increasing $V_{\dot{E}}$ is not important in explaining the observed flow rate dependence of the NO elimination.

There is a quite significant variability in the exhaled levels of NO compared with CO$_2$, even among normal human subjects. The large standard deviations of the intercept and slope in Fig. 6 suggest that such variability originates from the airway as well as the alveolar region of the lung. This variability can be attributed to a number of parameters, including the endogenous production of NO in the airway or alveolar tissue as well as the diffusing capacity of NO, and they are examined in detail elsewhere (30).

Conclusions. We have utilized a technique to establish satisfactory isolation of the nasal cavity while collecting the experimental oral exhalation profile of NO and CO$_2$ from normal human subjects. An experimental protocol was designed that focuses on the effect of $V_{\dot{E}}$ on the exhaled concentration of the endogenously produced gases. There are five major findings of the study related to NO exchange dynamics: 1) the average phase III exhaled concentration of NO is an inverse function of $V_{\dot{E}}$; 2) $S_{III,NO}$ is highly dependent on the dynamic changes of flow rate during exhalation and can vary from positive to negative values; 3) for constant $V_{\dot{E}}, S_{III,NO}$ is slightly negative when ambient levels of NO are inhaled (~15 ppb); 4) the elimination rate of NO is a positive linear function of $V_{\dot{E}}$ with a nonzero intercept and can be used to quantitate the relative contribution of the alveolar and airway regions to exhaled NO; and 5) there is a large intersubject variability in exhaled NO concentration compared with CO$_2$. All these findings are consistent with the alveoli and the airways as sources of exhaled endogenous NO. These findings can provide directions for collecting reproducible exhalation NO profiles. For example, one must consider the following factors when assessing NO concentration in the exhaled breath: $V_{\dot{E}}$, exhalation volume, and region of the exhalation profile where the measurement is recorded. In addition, inspired conditions such as volume, flow rate, and concentration may impact the exhalation profile. These factors are considered by a mathematical model (30) but must also be addressed in future experimental designs.

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