Pulmonary Membrane Diffusing Capacity and Capillary Blood Volume Measured During Exercise From Nitric Oxide Uptake*

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**Study objectives:** To validate lung diffusing capacity for nitric oxide (DLNO) as an index of conductance of the alveolar-capillary membrane during exercise, we compared DLNO to lung diffusing capacity for carbon monoxide (DLCO) and pulmonary membrane diffusing capacity for carbon monoxide (DMCO), and compared pulmonary capillary blood volume (Vc) calculated by two methods.

**Setting and participants:** The study was performed at a university medical center involving 12 nonsmoking healthy volunteers (age range, 23 to 79 years). DLCO, DLNO, cardiac output (Qc), and lung volume were measured simultaneously at rest and during graded ergometer exercise by a rebreathing technique. Pulmonary membrane diffusing capacity and Vc were compared by (1) the classic technique of Roughton and Forster from DLCO measured at two alveolar oxygen tension (PAO2) levels, and (2) from DLNO and DLCO assuming negligible erythrocyte resistance to nitric oxide (NO) uptake, ie, DLNO approximately equal to pulmonary membrane diffusing capacity for nitric oxide.

**Results:** In all subjects, DLNO increased linearly from rest to exercise; age, Qc, and lung volume were the major determinants of DLNO by stepwise regression analysis. The DLNO/DLCO ratio averaged 3.98 ± 0.38 (± SD) and the DLNO/DMCO ratio averaged 2.49 ± 0.28 irrespective of exercise intensity. Changing PAO2 did not alter DLNO. Brief exposure to 40 ppm of inhaled NO during 16 s of rebreathing did not alter either DLCO or Qc. Estimates of pulmonary membrane diffusing capacity and Vc by the two methods showed a strong correlation.

**Conclusion:** Results support DLNO as a direct measure of pulmonary membrane diffusing capacity, allowing the estimation of Vc in a single rebreathing maneuver during exercise. The DLNO-DLCO rebreathing technique can be applied clinically in the investigation of pulmonary microvascular regulation.

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**Key words:** carbon monoxide; cardiac output; lung diffusing capacity; pulmonary capillary blood volume; rebreathing technique

**Abbreviations:** DLCO = lung diffusing capacity for carbon monoxide; DLNO = lung diffusing capacity estimated using nitric oxide as the tracer gas; DMCO = pulmonary membrane diffusing capacity for carbon monoxide; DMNO = pulmonary membrane diffusing capacity for nitric oxide; Hb = hemoglobin; MW = molecular weight; NO = nitric oxide; PAO2 = alveolar oxygen tension; Qc = cardiac output; Vc = pulmonary capillary blood volume; θCO = rate of carbon monoxide uptake

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According to the classic model of pulmonary diffusion proposed by Roughton and Forster, lung diffusing capacity for carbon monoxide (DLCO) is composed of two resistances arranged in series:

\[
\frac{1}{DLCO} = \frac{1}{DMCO} + \frac{1}{\theta CO \cdot Vc}
\]

where pulmonary membrane diffusing capacity for carbon monoxide (DMCO) is the carbon monoxide conductance across the alveolar-capillary tissue membrane and plasma barrier; \( \theta CO \) is the rate of carbon monoxide uptake by whole blood and combination with hemoglobin (Hb) measured in vitro; and Vc is the pulmonary capillary blood volume. In normal subjects, resistances of the membrane (1/DMCO) and erythrocyte (1/\([\theta CO \cdot Vc]\)) contribute almost equally to the overall diffusive resistance across the lung. In order to estimate DMCO and Vc from carbon monoxide uptake,
DLCO must be measured at two alveolar oxygen tension (PAO₂) levels, with the assumption that PAO₂ does not alter DMCO.

Lung diffusing capacity estimated using nitric oxide (NO) as the tracer gas (DLNO) has been proposed as a direct measure of the conductance of alveolar membrane (pulmonary membrane diffusing capacity for NO [DMNO]). Because the reaction rate of NO binding to Hb is some 280 times faster than that of carbon monoxide, the rate of NO uptake by blood (DNO) is extremely large and 1/(DNO · Vc) becomes negligible, ie, DLNO is approximately equal to DMNO.

Based on the molecular weights (MWs) of carbon monoxide (MW = 28) and NO (MW = 30) and the solubility factors (α) of carbon monoxide (0.0183 mL⁻¹·atm⁻¹) and NO (0.0364 mL⁻¹·atm⁻¹) in water at 37.5°C, the theoretical relationship between membrane diffusing capacities for NO and carbon monoxide (Dmco) is as follows:

\[ \frac{DLNO}{DMCO} = \frac{Dmno}{DMCO} \times \alpha_{NO} \times \sqrt{\frac{MWCO}{MWNO}} = 1.93 \]

Previous studies have measured DLNO and DLCO simultaneously at rest in humans²,⁴ and in animals using a single-breath⁵ or rebreathing³ technique. However, DLNO and DLCO have not been compared during exercise. There has been no direct comparison of DLNO and DMCO at rest or during exercise, and the relationship between DLNO and cardiac output (Qc) has not been established.

Our objective is to validate the measurement of DLNO during exercise as an index of pulmonary membrane diffusing capacity by determining (1) the relationships between DLNO and Qc and between DLNO and DMCO, and (2) the effect of a brief exposure to inhaled NO during testing on Qc and DLCO. We hypothesized that there is a close correlation between DLNO and DMCO from rest to exercise, and that the DLNO/DMCO ratio should be approximately 2 irrespective of exercise intensity.

We utilized a rebreathing technique to measure DLCO, DLNO, and Qc in normal subjects at rest and during exercise at two levels of inspired oxygen tension. In addition, we compared Vc estimated from DLCO measured at two inspired oxygen tensions with that estimated from simultaneous measurements of DLNO and DLCO. If our hypothesis is true, it should be possible to simultaneously estimate DLNO, DMCO, Vc, and Qc at rest or exercise without having to repeat the measurement at more than one level of inspired oxygen tension.

**Materials and Methods**

**Subjects**

Twelve healthy subjects (9 men and 3 women; age range, 23 to 79 years) were studied. All were nonsmokers with normal resting spirometry measurements and no history of cardiopulmonary disease. Written informed consent was obtained, and the experimental protocol was approved by the Institutional Review Board for Human Research.

**Rebreathing Apparatus and Technique**

The experimental apparatus has been described. Subjects exercised on a bicycle ergometer, breathing through two three-way pneumatically controlled balloon valves (model 8500; Hans Rudolph; Kansas City, MO). Airflow was measured by a turbine flowmeter (VMM 2; Interface Associates; Aliso Viejo, CA). Oxygen uptake, carbon dioxide production, ventilation, and heart rate were measured continuously by a metabolic cart (Vmax 229; Sensormedics; Yorba Linda, CA). At the end of a selected expiration, the pneumatic valves switched to allow the subject to inspire to total lung capacity one breath of test gas mixture from a reservoir bag containing 0.3% carbon monoxide, 0.3% methane, 0.8% acetylene, and either 30% oxygen in a balance of nitrogen or 99% oxygen. When needed, medical-grade NO at a concentration of 40 ppm was added to the test gas mixture just prior to each measurement. After inspiring the test gas to total lung capacity, a second valve switch allowed the subject to rebreathe and out of an anesthetic bag for 16 s while gas concentrations were continuously monitored at the mouth. At rest, the rebreathing rate was set at 30 breaths/min synchronized by a metronome. During exercise, subjects were allowed to breathe at their spontaneously chosen rate.

Concentrations of carbon monoxide, methane, and acetylene were measured by rapid-response infrared gas analyzers (Sensors; Saline MI) via a recirculating sampling system. The concentration of NO was measured by a chemiluminescence analyzer (model NOA280; Sievers Instruments; Boulder, CO); linearity and reproducibility of the analyzer were verified in the range from 0 to 400 ppm. The sampling flow rate was 200 mL/min. The response time of the analyzer was < 500 ms. The NO analyzer was calibrated daily using a zero NO filter and a test gas containing 26 ppm NO. Analog output of the NO analyzers was passed through a medium-gain, direct-current amplifier and digitized by a computer along with signals from the infrared analyzer, flowmeter, and the metabolic cart.

**Calculation of Diffusing Capacities and Qc**

Diffusing capacities for carbon monoxide or NO were calculated from the slope of the exponential disappearance rate of each gas with respect to methane during rebreathing. The first three end-tidal points and points after approximately 12 s were routinely discarded to avoid incomplete mixing and recirculation, respectively. The rate of carbon monoxide uptake by erythrocytes (Dmco) was calculated from the mean PAO₂ during rebreathing and Hb concentration

\[ \frac{1}{\Theta_{CO}} = (0.73 + 0.0058 \times PAO₂) \times \frac{14.6}{Hb} \]
DMCO and Vc were calculated from DLCO measured at the two levels of PAO2; results were used to calculate DLCO at a standard Hb concentration of 14.6 g/dL and a PAO2 of 120 mm Hg. All results of DLCO are expressed under these standard conditions. Vc was also calculated from DLCO and DLNO measured during a single rebreathing maneuver. Qc was measured from the slope of the end-tidal exponential disappearance of acetylene with respect to methane.

Protocol

Measurements were conducted on two separate days. On day 1, spirometry was measured. Maximal oxygen uptake was determined by an incremental exercise protocol; workload was increased by 30 W every 2 min until volitional termination or until a plateau in oxygen uptake with respect to workload was obtained. On day 2, the subject exercised at workloads equivalent to 25%, 50%, and 80% of their predetermined maximal oxygen uptake. Each workload was sustained for 3 min to reach a quasisteady state in heart rate, respiratory rate, and oxygen uptake; the rebreathing maneuver was then performed. Baseline measurements were obtained at rest sitting on the bicycle. Between measurements, the subject rested for 10 to 20 min or until heart rate and respiratory rate returned to baseline. Measurements were repeated with 30% or 99% oxygen in the test gas mixture corresponding to a mean PAO2 during rebreathing of about 150 mm Hg and 600 mm Hg, respectively. Prior to rebreathing the test mixture containing 99% oxygen, the subject prebreathed 100% oxygen for 4 min at rest and 1 min during heavy exercise. All measurements were performed first without NO in the test gas mixture, and then repeated with 40 ppm of NO added to the reservoir bag. A venous blood sample was drawn to measure Hb concentration.

Data Analysis

Diffusing capacities were normalized by body surface area and analyzed with respect to cardiac index. The slope and correlation coefficient of the DLNO vs cardiac index plot were calculated for each subject and averaged for all subjects. The dependence of DLNO on cardiac index, lung volume, age, body surface area, height, and weight was examined by stepwise linear regression analysis. The correlation between DLNO and DMCO, between DLNO and DLNO, and between DLNO measured at two levels of PAO2 was determined. The DLNO/DMCO and DLNO/DMCO standard ratios at different workloads were compared by repeated-measures analysis of variance. Statistical analysis was performed using commercial software (Statview, version 4.5; SAS Institute; Cary, NC).

RESULTS

DLNO

In all subjects, DLNO increased linearly from rest to exercise with cardiac index (Fig 1, top); individual slopes of the relationship averaged 3.36 ± 0.19 (± SE) while the correlation coefficient \( r^2 \) averaged 0.95 ± 0.01. Resting value of DLNO is variable across subjects, consistent with that reported by other investigators.\(^2,4\) We found that the variability reflects mainly differences in lung volume. When DLNO is normalized with respect to end-inspiratory

lung volume at full inspiration, the intersubject variability is reduced (Fig 1, middle). Results of stepwise linear regression analysis of DLNO with

\[
DLNO = (4.235 \times Qc) + (19.705 \\
\times EIIV) - (0.706 \times age) - 7.133,
\]

where EIIV = end-inspiratory lung volume at full inspiration in liters, and age is in years. Solid line = identity; dashed line = regression through data points.

Figure 1. Top: In each subject, DLNO increased linearly with respect to cardiac index from rest to exercise. The slope of this relationship averaged 3.36 ± 0.19, and \( r^2 \) averaged 0.95 ± 0.01 (± SE). Middle: Normalizing DLNO by end-expiratory lung volume reduces intersubject variability in the elevation of the relationship. Bottom: There is a close relationship between observed DLNO and that fitted to the multivariate regression equation:

\[
y = x - 0.0085
\]

\[R^2 = 0.95\]
respect to independent variables are presented in Table 1. The significant determinants of DLNO are Qc, end-inspiratory lung volume, and age. Using age as an independent variable accounts for more of the variance than height, weight, or body surface area. The relationship between observed DLNO and DLNO fitted to the multivariate regression equation is shown in Figure 1, bottom.

**DLCO and DMCO**

Both DLCO and DMCO increased linearly with respect to Qc (Fig 2, top and bottom), closely matching previously published reference values from our laboratory based on Qc, age, and body surface area.6

**Comparisons of Diffusing Capacities for Carbon Monoxide and NO**

Figure 3, top, shows the relationship between DLNO and DMCO. The slope of the regression line through the origin (DLNO/DMCO) is 2.49, about 30% above the expected DMNO/DMCO ratio of 1.93. Figure 3, bottom, shows the relationship between DLNO and DLCO; the slope of this relationship is 4.0. The average DLNO/DMCO and DLNO/DLCO standard ratios at different exercise intensities are shown in Table 2; there are no significant differences among workloads. Thus, the DLNO/DMCO and DLNO/DLCO ratios are independent of exercise intensity.

**Effect of PAO2 on DLNO**

There is an excellent correlation between DLNO measured at two levels of mean PAO2 at a given workload. DLNO (breathing 100% oxygen) = 0.993 × DLNO (breathing 30% oxygen) \([ r^2 = 0.97 \])]. Hence, altering PAO2 has no significant effect on the measurement of DLNO.

**Effect of NO on DLCO and Qc**

The brief exposure to 40 ppm NO during 16 s of rebreathing does not significantly affect the measurement of DLCO or Qc. Pooled data from all subjects at all workloads performed in the presence or absence of NO in the rebreathing mixture show the following correlations: DLCO (with NO) = 0.968 × DLCO (without NO) \([ r^2 = 0.93 \]), Qc (with NO) = 0.977 × Qc (without NO) \([ r^2 = 0.92 \]).

**VC**

Similarly, there is a close correlation between VC (in milliliters) estimated using the technique of Roughton and Forster1 and that estimated from simultaneous measurements of DLNO and DLCO: VC (DLNO-DLCO technique) = 1.04 × VC (Roughton-Forster technique) \([ r^2 = 0.74 \]).

**DISCUSSION**

**Summary of Results**

From rest to exercise, DLNO increases linearly with increasing Qc; the slope of this relationship is

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**Table 1—Stepwise Linear Regression Analysis**

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>DLNO Intercept mL/min/mm Hg</th>
<th>Qc L/min</th>
<th>Alveolar Volume L</th>
<th>Age yr</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>intercept</td>
<td>50.901</td>
<td>7.711</td>
<td>18.402</td>
<td>18.625</td>
<td>0.546</td>
</tr>
<tr>
<td>age</td>
<td>-18.402</td>
<td>7.133</td>
<td>19.705</td>
<td>0.706</td>
<td>0.943</td>
</tr>
</tbody>
</table>

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**Figure 1.** Cardiac Output (L/min) vs. DLNO (mL/min/mm Hg) and DMCO (mL/min/mm Hg).

**Figure 2.** Top: DLCO increased linearly with Qc from rest to exercise in all subjects (solid line; DLCO = 1.98 × Qc + 12.09; \( r^2 = 0.665 \)); previously published reference values from our laboratory6 (dashed line; DLCO = 1.67 × Qc + 13.18; \( r^2 = 0.71 \)). Bottom: DMCO also increased linearly with Qc in all subjects (solid line; DMCO = 3.00 × Qc + 19.33; \( r^2 = 0.658 \)); previously published reference values (dashed line; DMCO = 2.52 × Qc + 24.12; \( r^2 = 0.49 \)).
erythrocyte resistance to NO uptake. Changing PA wrong direction to be explained by a significant carbon monoxide alone, the difference is in the average D
lno
. Although these data support the use of D
lno
/DMCO
, rang-
ing from 8 ppm in humans to 600 ppm in animals. Long-term exposure to high concentrations of NO, such as for the treatment of respiratory failure, can potentially reduce oxygen-carrying capacity of the blood through the formation of methemoglobin, and the oxidation products of NO may cause lung injury. Also, NO-mediated pulmonary vasodilatation during physiologic increases in blood flow may alter the physiologic parameters under investigation. Our data show that brief exposures to NO (16 s) at an initial concentration of 40 ppm have no toxic effects, do not alter Qc or DLCO, and allow an adequate number of end-tidal points to be sampled for calculating the slope of NO disappearance.

Comparison With Previous Measurements of DLNO

Previous measurements of DLNO in human subjects have been conducted using the single-breath technique at rest. Guénard et al
4
reported a resting DLNO of 136 ± 32 mL/min/mm Hg (mean ± SD) with a breath-hold of 3 s and an inspired NO concentration of 8 ppm. Borland and Higenbottam
2
reported a resting DLNO of 147 ± 31 mL/min/mm Hg (mean ± SD) with a breath-hold of 10 s and an inspired NO concentration of 40 ppm. Moinard and Guénard
18
reported a resting DLNO of 124 ± 13 mL/min/mm Hg (mean ± SE) with a breath-hold of 3 s and an inspired NO concentration of 8 ppm. Because of the faster NO-uptake kinetics, the breath-hold time had to be reduced. In comparison, our measurement of DLNO at rest by the rebreathing technique (117 ± 7 mL/min/mm Hg; mean ± SE; range, 69 to 178 mL/min/mm Hg) is somewhat lower. The DLNO/DLCO ratio of 4.0 in this study is similar to the DLNO/DLCO ratio of 4.3 reported by others. However, in previous studies, DLCO was not expressed at a standard PAO2 or Hb concentration.

Table 2—Ratios of DLNO/DMCO and DLNO/DLCO*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Rest</th>
<th>25%</th>
<th>50%</th>
<th>80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLNO/DMCO</td>
<td>2.42 ± 0.10</td>
<td>2.49 ± 0.09</td>
<td>2.52 ± 0.06</td>
<td>2.54 ± 0.07</td>
</tr>
<tr>
<td>DLNO/DLCO</td>
<td>4.09 ± 0.13</td>
<td>3.94 ± 0.15</td>
<td>3.94 ± 0.08</td>
<td>3.94 ± 0.09</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM. DLCO is expressed at standard conditions (mean PAO2 of 120 mm Hg and Hb concentration of 14.6 g/dL). No significant differences by repeated-measures analysis of variance.
Correlation Between DLNO and DMNO

If there was a significant erythrocyte resistance to NO uptake, then the measured DLNO would underestimate true DMNO, ie, the observed DLNO/DMCO ratio would be lower than the expected ratio of 1.93. We observed a DLNO/DMCO ratio of 2.49, 29% higher than expected. A number of factors that could potentially contribute to this difference are discussed below:

1. NO undergoes spontaneous transformation to nitrogen dioxide in the presence of oxygen. This chemical reaction could lead to an overestimation of DLNO, as the NO lost during conversion to nitrogen dioxide is measured as uptake by the pulmonary capillary blood. The reaction rate of NO to nitrogen dioxide is measured as uptake by the pulmonary capillary blood. The reaction rate of NO to nitrogen dioxide is directly proportional to the square of NO concentration and to the concentration of oxygen.3 However, the rate of gas-phase oxidation of NO is slower by several magnitudes compared to the kinetics of NO uptake in the lungs.3,11 We added NO to the test gas mixture just before each measurement; thus, it seems unlikely that NO oxidation should be a significant factor affecting the measurement of DLNO.

2. Chemical transformation of NO can occur in the superficial lining fluid, bronchial mucosa, and other tissues of the respiratory system. This reaction is difficult to quantify, but both in vivo and in vitro studies using radiolabeled NO have shown that NO passes rapidly into the blood before reacting significantly with lung tissue.19,20 Spiestersbach et al,21 using isolated rabbit lungs perfused with buffer equilibrated with NO, also showed that diffusive uptake of NO is not affected by its reaction with lung tissue; the rate of reaction is much slower than the rate of diffusion across the alveolar-capillary membrane and hence should not affect estimates of DLNO.

3. Reversible gas exchange of NO occurs in the conducting airways.22–24 The NO diffusing capacity of the conducting airways calculated by Pietropaoli et al24 is in the range of 0.4 to 1.2 mL/min/mm Hg; these values are too small in magnitude (< 1%) to cause a major error in our measured DLNO.

4. NO is produced within the respiratory tract.22–24 Endogenous NO from the lung, airway, and nasal tissue could potentially cause an underestimation of DLNO, as it is not accounted for in the inspired NO concentration. The rate of endogenous NO production measured in spontaneously exhaled air is in the parts per billion (10^-9 L/min) range.22–24 It is unlikely that such minute concentrations of NO would significantly affect our measurements, which were carried out in the (parts per million) range.

5. The expected DMNO/DMCO ratio of 1.93 is based on the solubility of NO in water with the implicit assumption that NO solubility in alveolar tissue and plasma is similar. The accuracy of this assumption is untested; thus, the expected ratio is only a rough approximation.

6. The accuracy of DMCO calculated using the Roughton-Forster technique depends on the accuracy of the relationship of 1/θCO to intraerythrocyte oxygen tension. This relationship varies among the studies in which it has been measured,25 and could easily account for a 25% underestimation of true DMNO. In addition, the calculation of 1/θCO assumes that mean PAO2 within capillary erythrocytes is the same as that in alveolar air; this assumption may also be in error. Thus, errors in the assumed value of θCO may well explain the observed difference in DLNO/DMCO ratios by the two techniques. Since the observed difference between experimental and theoretical DLNO/DMCO ratios is in the wrong direction to be caused by a significant erythrocyte resistance to NO uptake, these data do not contradict the major tenet underlying the combined DLNO-DLNO method, ie, DLNO approximates the true diffusing capacity of the alveolar-capillary membrane.

Conclusion

We conclude that inhaled NO can be used as a test gas in conjunction with CO to more directly estimate alveolar-capillary membrane diffusing capacity and pulmonary capillary blood volume during a single rebreathing measurement at rest or during exercise. The combined DLNO-DLNO method represents a significant improvement over the classic Roughton-Forster technique in two ways: (1) there is no need to measure diffusing capacity at two PAO2 levels, reducing the number of necessary measurements and testing time by half; (2) potential errors described above arising from inherent assumptions in the Roughton-Forster technique could be avoided. Brief exposure to NO during rebreathing does not interfere with physiologic measurements. The present method provides more direct estimates of both membrane diffusing capacity and Vc, and could be utilized clinically to examine the regulation of alveolar-capillary membrane and pulmonary microvascular function at rest and during exercise.

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