The Relationship Between Single-Breath Diffusion Capacity of the Lung for Nitric Oxide and Carbon Monoxide During Various Exercise Intensities

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Study objectives: To determine the relationship between single-breath diffusion capacity of the lung for nitric oxide (DLNO) and single-breath diffusion capacity of the lung for carbon monoxide (DLCO), and to determine the single-breath DLNO/DLCO ratios during rest and at several exercise intensities using a commercial lung diffusion system that uses electrochemical cells to analyze gases.

Setting and participants: Eight healthy men (age, 27 ± 5 years; weight, 83.0 ± 11.8 kg; height, 180.4 ± 9.5 cm; maximal oxygen uptake [VO₂max], 47.6 ± 10.2 mL/kg/min [mean ± SD]) performed single-breath DLNO measurements (inspired nitric oxide concentration, 66.5 ± 10.6 ppm) and carbon monoxide (0.3%) randomized on 4 different days at rest and at various exercise intensities (40%, 75%, and 90% of VO₂max reserve [VO₂R]) on an electrically braked load simulator. The DLCO measured on day 1 was compared to the DLCO measured during the DLNO method from another day.

Results: The relationship between DLNO and DLCO was linear (DLNO = 4.47 × DLCO; r² = 0.91; standard error of the estimate = 0.04; p < 0.05). DLNO was 4.52 ± 0.24 times greater than DLCO, independent of exercise intensity. DLNO increased from 210.3 ± 18.2 mL/min/mm Hg at rest to 284.2 ± 38.6 mL/min/mm Hg at 90% VO₂R (oxygen uptake = 42.6 ± 9.8 mL/kg/min; 284.2 ± 31.6 W; p < 0.05). Steepwise regression demonstrated that DLNO is predicted by alveolar volume (VA) [in liters] and workload (watts) such that DLNO = 13.4 × VA + 0.23 × workload + 107.7 (r² = 0.90; SEE = 17.5; p < 0.05).

Conclusion: (1) Single-breath DLNO and DLCO increase linearly with increasing workload; (2) the single-breath DLNO/DLCO ratios are independent of exercise intensity, suggesting that using either nitric oxide or carbon monoxide as transfer gases are valid in the study of lung diffusion during any level of exercise; and (3) DLNO is mainly predicted by VA and workload.

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Key words: carbon monoxide; cycling; diffusion capacity; exercise; nitric oxide; oxygen uptake; single-breath technique; watts

Abbreviations: ANOVA = analysis of variance; ATS = American Thoracic Society; ΘCO = specific blood transfer conductance for carbon monoxide; DLCO = diffusion capacity of the lung for carbon monoxide; DLNO = diffusion capacity of the lung for nitric oxide; DMCO = membrane diffusing capacity for carbon monoxide; DMNO = membrane diffusing capacity for nitric oxide; HR = heart rate; ΘNO = specific blood transfer conductance for nitric oxide; SEE = standard error of estimate; VA = alveolar volume; VC = pulmonary capillary blood volume; VO₂ = oxygen uptake; VO₂max = maximal oxygen uptake; VO₂R = maximal oxygen uptake reserve

Diffusion capacity of the lung for carbon monoxide (DLCO) has been classically described by Roughton and Forster as the arrangement of membrane resistance and red cell resistance placed in series:

\[
DLCO = \frac{1}{DMCO} + \frac{1}{ΘCO × VC}
\]

where DMCO is pulmonary membrane diffusing capacity for carbon monoxide, ΘCO is the specific blood transfer conductance for carbon monoxide, and VC is pulmonary capillary blood volume. Membrane resistance (1/DMCO) and red cell resistance (1/ΘCO × VC) usually contribute equally to the overall diffusive resistance across the lung in healthy subjects. In order to obtain DMCO and VC, DLCO has been traditionally measured at two different levels of PaO₂, 120 mm Hg and approximately 600 mm Hg. For each level of PaO₂, 1/DLCO is then plotted on the y-axis and 1/ΘCO is plotted on the...
x-axis. Then the equation of the linear regression is obtained such that the x-intercept (1/DMCO) and slope (1/Vc) can be solved. This two-step method can be time consuming and uncomfortable for subjects to perform, especially during intense exercise. However, over the past 15 years, diffusion capacity of the lung for nitric oxide (DLNO) has been simultaneously used with DLCO to obtain DMCO and Vc in a single-breath maneuver, reducing the number of measurements and testing time by half. As well, the simultaneous measurement of nitric oxide and carbon monoxide transfer allows a similar distribution of the two gases so that no distortion occurs due to the breathing pattern used by the subject to perform the maneuver. The clever idea of using nitric oxide as the transfer gas is due to the fact that the reaction rate of nitric oxide binding to hemoglobin is 1,400 times faster than that of carbon monoxide, and the specific blood transfer conductance for nitric oxide (θNO) is so large that the red cell resistance to nitric oxide (1/θNO × Vc) approaches zero. Therefore, DLNO is equal to membrane diffusion capacity for nitric oxide (DMNO). Since the molecular weight of carbon monoxide and nitric oxide are 28 g and 30 g per mole, respectively, and solubilities (Bunsen coefficients) of carbon monoxide and nitric oxide in plasma at 37°C are 0.0215 and 0.0439, the diffusivity of nitric oxide (solubility/ square root of molecular weight) is approximately 1.97 times greater than that of carbon monoxide, and thus the theoretical relationship between membrane diffusing capacities for nitric oxide and carbon monoxide is as follows:

$$\text{DLNO} = \frac{\text{DMNO}}{\text{DMCO}} = \frac{\theta_{\text{NO}}}{\theta_{\text{CO}}} = 1.97$$

Several studies, therefore, have used 1.97 as the theoretical ratio of DLNO to DMCO such that Vc and DMCO can be calculated after DLCO and DLNO have been measured. Over the past 15 years, researchers have used nitric oxide chemiluminescence analyzers to obtain DLNO from single-breath or from rebreathing techniques along with the simultaneous measurement of DLCO to obtain DMCO and Vc rapidly and efficiently. Since brief exposure to nitric oxide does not interfere with physiologic function, it seems pertinent to use nitric oxide as a test gas to assess lung diffusion capacity. Data suggest that 16 s of rebreathing 40 ppm of nitric oxide can be used clinically to investigate pulmonary microvascular regulation at rest and during exercise. In that study, they determined that the DLNO/DLCO ratio averaged 3.98 ± 0.38 (mean ± SD) irrespective of exercise intensity, suggesting that using either nitric oxide or carbon monoxide as transfer gases is valid in the study of lung diffusion during any level of exercise. The clinical implication of using both nitric oxide and carbon monoxide concurrently in research and medical practice is that scientists and clinicians can immediately partition and quantify the components of lung diffusion capacity in a subject in a single 4-s breath-holding maneuver that requires minimal effort on the part of the patient, while simultaneously being able to pinpoint which component (membrane diffusion capacity, Vc, or both) is causing low (or high) lung diffusion capacities. This can be especially useful for helping with diagnosis of diseases that result in diffusion impairment (e.g., emphysema) or increased diffusion (e.g., Goodpasture syndrome).

To date, no study has compared the DLNO/DLCO ratio using a single-breath technique during various exercise intensities. The purpose of this study was to compare single-breath DLNO and DLCO during rest and at several exercise intensities (40%, 75%, 90%, of maximal oxygen uptake [VO2max]) reserve (VO2R) in healthy male subjects, and to calculate the ratio of single-breath DLNO/DLCO with the use of electrochemical cells to analyze nitric oxide and carbon monoxide.

**MATERIALS AND METHODS**

**Subjects**

Ten healthy male subjects were recruited, with 8 subjects completing the study (n = 8; age, 27 ± 5 years; weight, 83.0 ± 11.8 kg; height, 180.4 ± 9.5 cm; VO2max, 47.6 ± 10.2 mL/kg/min). All were nonsmokers with normal resting spirometry function and no history of cardiopulmonary disease. The study was approved by the Institutional Review Board of Concordia University. Subjects gave informed consent and completed a physical activity readiness questionnaire.

**Protocol**

Each subject was required to come into the laboratory on 3 separate days, with a minimum of 24 h between each testing session. The studies were performed at approximately the same
time of day. On day 1, subjects were screened for any signs of cardiopulmonary disease, performed resting spirometry, and then completed an incremental cycling protocol to determine Vo2\text{max}. Vo2\text{max} was assessed by having a racing bicycle placed on a computer-aided, electronically-braked load simulator (Computrainer PRO 8001; RacerMate; Seattle, WA), which commenced at 80 W and increased by 40 W every 2 min until volitional exhaustion. Metabolic variables were assessed with the Ergocard metabolic cart (Medisoft; Dimant, Belgium) using the breath-by-breath option. The software used was Exp'air (version 1.26; Medisoft). Heart rate (HR) was recorded using a Polar Vantage XL HR monitor (Polar Electro Oy, Kempele, Finland). The highest three consecutive oxygen uptake (Vo2) values (averaged over 20-s intervals) were defined as the Vo2\text{max}. On day 2 and day 3, subjects exercised for 5 min at each exercise intensity—light (40% Vo2\text{R}), moderate (75% Vo2\text{R}), and strenuous (90% Vo2\text{R})—followed by a 5- to 10-min break between each level of exercise such that HR would return close to baseline levels prior to the next measurement. The percentage of Vo2\text{R} was calculated from each subject's own linear relationship between Vo2 and workload (watts) from the Vo2\text{max} test, and then a set workload was calculated for each level of exercise intensity. Subjects were randomized to either perform the single-breath DLNO technique or the single-breath DLCO technique on day 2 followed by the other technique on day 3. Baseline measurements were obtained after 5 min of sitting in a chair. Each workload was sustained for 5 min to allow for a quasisteady state in HR and Vo2, after which subjects performed the single-breath maneuver. Volume and gas calibration of the Ergocard was performed just prior to each testing session.

**Single-Breath Apparatus and Technique**

Subjects exercised on a racing bicycle placed on computer-aided electronically braked load simulator (Computrainer PRO 8001; RacerMate). The subjects breathed through a three-way pneumatic valve developed by Medisoft. Dead space washout volume and expired sample volume were both adjusted for 900 mL. The instrument dead space was measured at 140 mL (including the mouthpiece, valve, and filter dead spaces). Anatomic dead space (milliliters) was estimated as bodyweight in kilograms × 2.2.\textsuperscript{14} The concentration of inspiratory gases were 0.30% carbon monoxide, 10% helium, 21% oxygen, and balance N2 for tank 1; and 232 ppm nitric oxide, balance N2 for tank 2. For the single-breath DLCO method, an inspiratory bag was filled with 5 to 8 L of gas mixture from tank 1, according to the subject's FVC. For the single-breath DLNO method, an inspiratory bag was filled with 5 to 8 L of gas mixtures from each tank, according to the subject's FVC. The same concentration of gases was present for the DLCO method except that nitric oxide was absent from the mixture. Inspired volume was measured and corrected for instrument and anatomic dead space, as well as being converted to standard temperature and pressure, dry conditions. Breath-holding time was calculated using the method of Jones and Meade.\textsuperscript{15}

The gas analyzers consisted of an nitric oxide electrochemical cell (Model MNO-1; City Technology Limited; Portsmouth, UK), detection range, 0 to 250 ppm, 0.1% accuracy, response time, < 10 s (10 to 90% full scale); a carbon monoxide electrochemical cell (Model A36/F; City Technology Limited), detection range, 0 to 400 ppm, 0.02% accuracy, response time, < 40 s (10 to 90% full scale); and a helium thermo conductivity analyzer (Medisoft), detection range, 0 to 15% helium, 0.02% accuracy, response time, < 20 s (10 to 90% full scale).

**Calculation of Diffusion Capacities and Vc**

DLNO and DLCO were calculated from the exponential disappearance rate of each gas with respect to helium using the method of Jones and Meade.\textsuperscript{15} The formulae for calculating DLCO are from the 1995 American Thoracic Society (ATS) guidelines.\textsuperscript{14} All results were standardized to a hemoglobin concentration of 14.6 g/dL and a PacO2 of 120 mm Hg. Breath-holding time of 4 s was chosen for both methods because it has been shown that DLCO values are not different at 3-s or 5-s breath-holding compared to 7-s and 10-s breath-hold.\textsuperscript{16} As well, a single-breath-holding time of approximately 9 s would result in a less than detectable amount of expired nitric oxide,\textsuperscript{3} so a compromise of 5-s breath-holding used. We did not account for nitric oxide backpressure in our calculations, as exhaled nitric oxide concentrations at rest are negligible, between 11 ppb and 66 ppb (0.010 to 0.026 ppm),\textsuperscript{17} and tend to decrease during exercise.\textsuperscript{18} The amount of nitric oxide backpressure then would minimally affect DLNO calculations as our measurements were carried out in the parts-per-million range, which is approximately 75 to 500 times larger than the exhaled nitric oxide concentration at rest or during exercise after a single-breath inspiration of 40 to 60 ppm of nitric oxide. Accounting for carbon monoxide back-pressure is also negligible, as it has been shown that 2 min between tests virtually eliminates all the carbon monoxide gas from the lungs in healthy subjects.\textsuperscript{19} As well, since the ATS\textsuperscript{14} recommends a minimum of 4 min between DLCO measurements, we had subjects perform the single-breath maneuver with a minimum of 5 min between tests.

**Data Analysis**

The research design was a randomized, single-blind, crossover design, with participants serving as their own control subjects. The different methods of diffusion capacity were the interventions. Repeated-measures analysis of variance (ANOVA) were used for measuring the primary dependent variables: DLNO, DLCO from the single-breath DLNO method, DLCO from the single-breath DLCO method, the DLNO/DLCO ratio, Vc, DMCO, HR, and Vo2. The experimental design was described as 8 × 3 × 2 × 1 × 3: subjects (S) crossed with method (M; DLNO, DLCO), crossed with intensities (I, rest, 40%, 75%, 90% Vo2\text{R}) for DLCO and HR. A one-way ANOVA was used to identify changes in Vc, DMCO, DLNO, DLNO/DLCO, HR, watts, alveolar volume (VA), and Vo2 with exercise intensity. An α level of 0.05 indicated statistical significance, and all data were reported as mean ± SD. A Newman-Kuels procedure was used to identify the significant differences in the dependent variables when main effects were present. Linear regressions and 95% confidence intervals were created for the relationship between the following: DLNO vs DLCO, DLNO vs Vo2, Vc, and Vo2, and DLNO/Vc vs DLCO/Vc. The variables Vo2, workload, VA, age, weight, height, and body surface area were examined by backward stepwise multiple-regression to predict DLNO. A Bland-Altman method comparison plot was used to compare the two methods of obtaining DLCO.\textsuperscript{20,21} The statistical program used was GB-Stat (version 7.0; Dynamic Microsystems; Silver Spring, MD).

**Results**

**Subject Characteristics**

Resting spirometry and Vo2\text{max} data completed during the preliminary session are shown in Table 1. Vo2\text{max} was within predicted norms based on peak

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**Table 1: Subject Characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
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<tr>
<td>BMI</td>
<td></td>
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<tr>
<td>Vo2\text{max}</td>
<td></td>
<td>Predicted norms based on peak</td>
</tr>
</tbody>
</table>
Table 1—Spirometry and $V_{O_2}\text{max}$ of Eight Healthy Men

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Percentage Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA, m²</td>
<td>2.10 ± 0.17</td>
<td>1.73-2.23</td>
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</tr>
<tr>
<td>Age, yr</td>
<td>27 ± 5</td>
<td>20-33</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>180.4 ± 9.5</td>
<td>161.0-191.0</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83.0 ± 11.8</td>
<td>66.3-99.2</td>
<td></td>
</tr>
<tr>
<td>FVC, L</td>
<td>5.7 ± 1.0</td>
<td>3.9-7.1</td>
<td>100.0 ± 8.01</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>4.7 ± 0.5</td>
<td>3.8-5.2</td>
<td>97.6 ± 11.39</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>80 ± 9</td>
<td>66-96</td>
<td>98.0 ± 11.02</td>
</tr>
<tr>
<td>PEF, L/s</td>
<td>9.2 ± 1.4</td>
<td>7.0-11.1</td>
<td>88.4 ± 10.81</td>
</tr>
<tr>
<td>$V_{O_2}\text{max}$, mL·kg⁻¹·min⁻¹</td>
<td>47.6 ± 10.2</td>
<td>37.8-68.4</td>
<td>100.9 ± 11.33</td>
</tr>
<tr>
<td>$V_{O_2}\text{max}$, L/min</td>
<td>3.91 ± 0.71</td>
<td>2.77-4.96</td>
<td>100.9 ± 11.33</td>
</tr>
<tr>
<td>RERmax</td>
<td>1.24 ± 0.09</td>
<td>1.14-1.41</td>
<td></td>
</tr>
<tr>
<td>Ve, L/min STPD</td>
<td>123.4 ± 14.5</td>
<td>99.9-146.3</td>
<td></td>
</tr>
<tr>
<td>HRmax, beats/min</td>
<td>186 ± 8</td>
<td>173-199</td>
<td></td>
</tr>
<tr>
<td>Peak wattage, W</td>
<td>295 ± 52</td>
<td>240-360</td>
<td></td>
</tr>
</tbody>
</table>

*BSA = body surface area; PEF = peak expiratory flow; RERmax = maximal respiratory exchange ratio; Ve = minute ventilation; HRmax = maximal HR; STPD = standard temperature and pressure, dry. Pulmonary function values in male subjects are presented as a percentage of normal values predicted for men of same height and age.

†Predicted value significantly different than measured value (p < 0.05).
‡Prediction equations are FEV₁, predicted = 0.05536 × 0.01305 × age − 0.000172 × age² + 0.0001408 × height²; FVC predicted = −0.1933 + 0.00064 × age − 0.000269 × age² + 0.0001864 × height²; peak expiratory flow rate = 1.0523 + 0.08272 × age − 0.001301 × age² + 0.00024962 × height²; from Hankinson et al.21
§Predicted $V_{O_2}\text{max}$ in male subjects = 10.51 × watts + 6.35 × weight − 10.49 × age + 510.3 ($r^2 = 0.88$; SEE = 212) from Storer et al.22

wattage, age, and weight.22 Except for peak expiratory flow rate, mean spirometry values were also within predicted norms.23 Resting DLCO values are presented in Table 2. The DLCO obtained from the DLNO method (46.2 ± 4.5 mL/min/mm Hg) were not significantly different compared to predicted values.24 However, paired t test results showed that the DLCO values at rest were significantly different by an average of 10% (4.2 mL/min/mm Hg) between the two methods performed on different days, (p < 0.05; Table 2). The breath-holding time for both DLCO and DLNO for the study was 5.0 ± 0.6 s, and the inspired nitric oxide concentration was 66.5 ± 10.6 ppm.

**Primary Results**

The single-breath DLNO/DLCO ratio averaged 4.52 ± 0.24 irrespective of exercise intensity (Table 3). This is significantly different to the ratio of 3.98 ± 0.38 found by others using a rebreathing technique (p < 0.05), but not significantly different from the ratio found by others in patients at rest who used the single-breath method (p > 0.05) [ratios ranged from 4.3 to 4.7; SD ranged from 0.3 to 0.6].3,7,10,12 As expected, DLNO increased linearly with $V_{O_2}$ ($r^2 = 0.54$) and workload ($r^2 = 0.67$) from rest throughout all levels of exercise with no tendency to plateau (Fig 1; p < 0.05). As well, VC ($r^2 = 0.42$) and DLCO ($r^2 = 0.50$) increased linearly with $V_{O_2}$ from rest throughout all levels of exercise with no tendency to plateau (Fig 1; p < 0.05). There was a very strong relationship between DLNO vs DLCO ($r^2 = 0.91$), and between DLNO/VA vs DLCO/VA ($r^2 = 0.83$) [Fig 2; p < 0.05]. HR values at rest and at all levels of exercise were not different between methods (p > 0.05), and there were no significant differences in DLCO between the two methods from the repeated-measures ANOVA.

Table 2—Baseline Lung Diffusion Capacity of Eight Healthy Men

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Percentage Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLCO from single-breath</td>
<td>46.2 ± 4.5</td>
<td>38.6-53.4</td>
<td>110.5 ± 12.8f</td>
</tr>
<tr>
<td>DLCO from single-breath</td>
<td>42.0 ± 2.6</td>
<td>36.7-47.0</td>
<td>100.4 ± 9.4f</td>
</tr>
<tr>
<td>Single-breath DLNO, mL/min/mm Hg</td>
<td>210.3 ± 18.2</td>
<td>183.1-235.3</td>
<td></td>
</tr>
<tr>
<td>VC, mL</td>
<td>116.3 ± 12.8</td>
<td>94.2-137.9</td>
<td></td>
</tr>
<tr>
<td>DMCO, mL/min/mm Hg</td>
<td>1067.7 ± 9.2</td>
<td>929.9-1194.4</td>
<td></td>
</tr>
</tbody>
</table>

*aDLCO significantly different from the DLCO obtained from DLNO method (p < 0.05).
†Predicted DLCO = 0.41 × height − 0.21 × age − 26.31 ($r^2 = 0.80$; SEE = 4.82) from Crapo and Morris.24 Pulmonary function values in male subjects are presented as a percentage of normal values predicted for men of the same height and age.

1022
Clinical Investigations
Table 3—VO2, HR, Wattage, and Diffusion Variables for Each Exercise Intensity*

<table>
<thead>
<tr>
<th>Variables (n = 8)</th>
<th>Rest</th>
<th>40% VO2R</th>
<th>75% VO2R</th>
<th>90% VO2R</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLNO/DLCO</td>
<td>4.56 ± 0.12</td>
<td>4.41 ± 0.26</td>
<td>4.63 ± 0.33</td>
<td>4.48 ± 0.25</td>
<td>4.52 ± 0.24</td>
</tr>
<tr>
<td>DLNO, mL/min/mm Hg</td>
<td>210.3 ± 18.2</td>
<td>232.1 ± 15.5</td>
<td>270.3 ± 31.9</td>
<td>284.2 ± 31.6</td>
<td></td>
</tr>
<tr>
<td>DLCO, mL/min/mm Hg</td>
<td>46.2 ± 4.5</td>
<td>52.8 ± 4.1</td>
<td>58.5 ± 7.1</td>
<td>63.6 ± 8.11</td>
<td></td>
</tr>
<tr>
<td>VC, mL</td>
<td>116.3 ± 12.8</td>
<td>137.7 ± 16.3</td>
<td>141.2 ± 16.1</td>
<td>162.8 ± 24.4</td>
<td></td>
</tr>
<tr>
<td>DMCO, mL/min/mm Hg</td>
<td>106.7 ± 9.2</td>
<td>117.8 ± 7.8</td>
<td>137.2 ± 16.2</td>
<td>144.2 ± 16.0</td>
<td></td>
</tr>
<tr>
<td>VA, L</td>
<td>7.5 ± 1.0</td>
<td>8.1 ± 1.1</td>
<td>8.6 ± 1.31</td>
<td>8.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>66 ± 10</td>
<td>130 ± 17</td>
<td>164 ± 16</td>
<td>179 ± 17</td>
<td></td>
</tr>
<tr>
<td>Watts</td>
<td>0 ± 0</td>
<td>77 ± 30</td>
<td>203 ± 37</td>
<td>253 ± 41</td>
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</tr>
<tr>
<td>VO2, L/min</td>
<td>0.29 ± 0.04</td>
<td>1.72 ± 0.33</td>
<td>2.96 ± 0.59</td>
<td>3.50 ± 0.71</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.

1Significantly different from rest and 40% VO2R only (p < 0.05).
2Significantly different from rest, 40%, and 75% VO2R (p < 0.05).

(p > 0.05). The DLCO values obtained between the two methods were highly correlated (r² = 0.98; standard error of the estimate [SEE] = 3.24; p < 0.05). However, using a Bland-Altman method comparison plot, there was mean bias of DLCO by 6%, or 3.1 mL/min/mm Hg from the single-breath DLNO method, compared to the single-breath DLCO method, when averaging all the results from rest up to 90% VO2R (Fig 3). Although a lot of scatter was present, exercise intensity did not magnify the differences in DLCO between methods.

The variables VO2, workload, VA, age, weight, height, and body surface area were examined by backward stepwise multiple regression to predict DLNO. The variables that appreciably affected DLNO were VO2 and workload. The regression formula was DLNO = 13.4 × VA + 0.23 × workload + 107.7 (r² = 0.90; SEE = 17.5; p < 0.05), where DLNO is expressed in milliliters per minute per millimeter of mercury, VA is in liters, and workload is in watts. Adding the other five variables to the equation only increased the coefficient of determination to 0.94, while reducing the SEE to 14.7. Therefore, DLNO was predicted essentially by VA and workload.

**DISCUSSION**

The main findings of the study are as follows: (1) single-breath DLNO and DLCO increase linearly with increasing workload, and the relationship between these two parameters is very strong; (2) the single-breath DLNO/DLCO ratios are independent of exercise intensity, suggesting that using either nitric oxide or carbon monoxide as transfer gases are valid in the study of lung diffusion during any level of exercise; and (3) DLNO is predicted by VA and workload.

**Relationship Between DLNO and DLCO**

The results of this study demonstrate that 91% of the variation in single-breath DLNO values is attributed to the variation in single-breath DLCO values. These results are very similar to those of Tamhane et al. as they found that 92% of the variation in DLNO was attributed to variances in DLCO values. However, their technique was different from the present study: they had their subjects rebreathe 40 ppm of nitric oxide for 16 s, while our subjects inspired 66.5 ppm of nitric oxide in a single—breath-holding maneuver lasting 4 to 5 s. Nevertheless, the slope of the regression line through the origin between DLNO and DLCO from rebreathing as shown by Tamhane et al. in which DLNO = 3.999 × DLCO resembles that of the single-breath method from the present study, in which we find that DLNO = 4.47 × DLCO.

**DLNO/DLCO Ratios**

We are also the first to demonstrate that single-breath DLNO/DLCO ratios are unaffected by exercise intensity. Tamhane et al. also showed that exercise did not affect the DLNO/DLCO ratios, but they used a rebreathing technique as opposed to our single-breath method. Nevertheless, the overall ratio we obtained was slightly higher to the DLNO/DLCO ratio from Tamhane et al. However, when our data (at rest) are compared to other studies, which have used the single-breath method (at rest), the DLNO/DLCO ratios were not significantly different from our data. This confirms that electrochemical nitric oxide and carbon monoxide cells can provide similar results to that obtained by chemiluminescence analyzers when using the single-breath technique. However, we acknowledge that the absolute values in DLNO at rest from this study are approximately 23 to 46% higher compared to other data. Indeed, it is possible that subjects from those studies had lower DLNO values at rest because their DLCO values were from 73 to 86% of predicted from age and height. Thus if DLCO values are lower than predicted, the DLNO values are concomitantly low as well.
**VA and Workload Affects DLNO**

Of all the variables that may have predicted DLNO (VO₂, workload, VA, age, weight, height, and body surface area), only VA and workload did so in a significant manner. These results are supported by Tsoukias et al.\(^{25}\) whose theoretical article suggests that unlike DLCO, DLNO is a strong function of VA. Their experimental data\(^{13}\) suggest that DLNO = 48 × VA\(^{0.67}\), but this function only holds true at rest and not during exercise. We also found that our data (rest and exercise) fit a power function such that DLNO = 57.7 × VA\(^{0.69}\) (\(r^2 = 0.45\); SEE = 26.5;
include both resting and exercise values, there was still an increase in DLCO measured from the DLNO method by 6% (3.1 mL/min/mm Hg). However, according to the ATS, an increase of 10% day-to-day variability in resting single-breath DLCO measurements is acceptable due to both day-to-day physiologic variability and technical variations. Inspired nitric oxide concentration was approximately 50 ppm higher than previous studies and thus the addition of the nitrogen/nitric oxide mixture to the initial diffusion gas mixture in the inspiratory bag may have reduced the oxygen concentration in the final mixture. According to our post hoc calculations, we diluted the initial oxygen/helium/carbon monoxide/N<sub>2</sub> diffusion gas mixture with a mixture of 232 ppm of nitric oxide, balance N<sub>2</sub>, as to obtain a final nitric oxide concentration of approximately 60 ppm. As our initial nitric oxide concentration in tank 2 was 232 ppm, the dilution factor is 66/232 (0.28), and thus we reduced the oxygen concentration by 28%. That leaves the oxygen concentration in the inspiratory bag of 15.1% instead of 21%. Given that the Pao<sub>2</sub> is approximately 90 mm Hg when inspiring from a gas mixture with 15.1% oxygen, the ΘCO is increased by approximately 14%, according to equation 5 developed by Roughton and Forster. Since there are fewer oxygen molecules competing with carbon monoxide molecules for the sites on the hemoglobin molecule, this can account for the overall 6% higher DLCO values from the single-breath DLNO method. Equation 1 shows that when DMCNO and Vc are kept the same, and ΘCO is changed by 14%, the change in DLCO is approximately 3.5 mL/min/mm Hg, which is very similar to the 3.1 mL/min/mm Hg mean difference we observed between the two methods. We suggest that future studies on DLNO ensure that inspiratory concentrations of helium, carbon monoxide, and oxygen should be similar for both the DLCO and DLNO tests. One can achieve this by either having a second initial diffusion gas mixture with higher concentrations of oxygen/helium/carbon monoxide for the DLNO test, or by using a higher concentration of nitric oxide so that there is less dilution of the initial diffusion gas mixture. Also, there may have been some errors in DLNO calculation leading to overestimation of DLNO. First is that nitric oxide undergoes spontaneous transformation to nitrogen dioxide in the presence of oxygen. This could lead to an overestimation of DLNO as the nitric oxide lost during conversion to nitrogen dioxide is measured as uptake by pulmonary capillary blood. Nitric oxide added to the diffusion mixture was used within 1 min of preparation. According to Fine, a 60 ppm concentration of nitric oxide left in an inspiratory bag with 21% oxygen for 1 min will result in a reduction of nitric oxide concentration by 1.5 to

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**Figure 2.** Top: A: The relationship between DLNO and DLCO. The equation is DLNO = 4.47 × DLCO (r<sup>2</sup> = 0.91; SEE = 0.04; p < 0.05). The 95% confidence intervals between DLNO and DLCO are provided (long dash). Bottom: B: The relationship between DLNO/VA and DLCO/VA. The equation is DLNO/VA = 4.50 × (DLCO/VA) (r<sup>2</sup> = 0.83; SEE = 0.04; p < 0.05). The 95% confidence intervals between DLNO/VA and DLCO/VA are also provided (long dash).

p < 0.05, but the relationship was not as strong as the linear regression incorporating both VA and workload in the prediction of DLNO. As the intensity of exercise increases, workload plays a much larger role in determination of DLNO. Others have also shown a 25% increase in DLNO between rest and 175 W. Our data corroborates theirs in that we found a 24% increase in DLNO per 175 W. It appears in this study that DLNO is best predicted by both VA and workload.

**Limitations**

Resting DLCO values as measured by DLCO and the DLNO method differed by 10%. When we
58.5 ppm, resulting in a overestimation of DLNO by only approximately 2.5 mL/min/mm Hg. Leaving the nitric oxide unused for another minute will only result in an overestimation of DLNO by approximately 5 mL/min/mm Hg. This is unlikely to significantly change the DLNO values, given that they were consistently > 200 mL/min/mm Hg. Another source of error in the DLNO is that the current method of Jones and Meade of calculating lung diffusion from the single-breath maneuver overestimates lung diffusion when the exhaled sample is collected late in the exhalation from alveolar regions that are not well mixed and, in part, filled sequentially. A sequential filling of the lung may increase the effective alveolar concentration gradient for diffusing gases such as nitric oxide in comparison with inert gases such as helium. The method of Jones and Meade ignores sequential filling of the lung, and thus an error may be introduced in the DLNO measurement. However, all of our subjects were healthy, and unlike those with prevalent lung diseases there is a reduced chance of sequential filling. Therefore, because of the very rapid reaction of nitric oxide with hemoglobin, this should not significantly impact on DLNO measurements. Furthermore, most of our data were collected during exercise, and exercise should improve gas mixing in all parts of the lung, thus reducing the supposed problem with the method of Jones and Meade of calculating lung diffusion from the single-breath maneuver.

One final concern was that one subject showed the presence of mild obstructive pulmonary disease (FEV1/FVC = 66%). It is well known that individuals with this problem have higher exhaled nitric oxide concentrations compared to normal subjects, and thus could alter our results. However, the increase in exhaled nitric oxide concentration in this type of patient is in the parts-per-billion range and not the parts-per-million range, which is too small to affect our results. Even so, when this subject was removed from the study and the statistical analyses were redone, there was no difference in the results. As such, he remained in the study.

CONCLUSION

The main findings of the study are as follows: (1) single-breath DLNO and DLCO increases linearly with increasing workload, and the relationship between these two parameters is very strong; (2) the single-breath DLNO/DLCO ratios are independent of exercise intensity, suggesting that using either nitric oxide or carbon monoxide as transfer gases are valid in the study of lung diffusion during any level of exercise; and (3) DLNO is mainly affected by VA and
workload. In conclusion, the results support the use of a single-breath inspiration of 66.5 ppm nitric oxide at rest and all levels of exercise as a measurement of alveolar-membrane diffusion capacity and Vc.

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