Pulmonary Artery Occlusion Increases the Ratio of Diffusing Capacity for Nitric Oxide to Carbon Monoxide in Prone Sheep*

R. Scott Harris, MD, FCCP; Mehrnaz Hadian, MD; Dean R. Hess, RRT, PhD, FCCP; Yuchiao Chang, PhD; and José G. Venegas, PhD

Objective: To test the hypothesis that the ratio of diffusing capacity of the lung for nitric oxide (DLNO) to diffusing capacity of the lung for carbon monoxide (DLCO) would be affected by occlusion of a fraction of the pulmonary vascular bed.

Design: Interventional physiologic study.

Setting: Animal laboratory of a university hospital.

Subjects: Thirteen sheep.

Interventions: We simultaneously measured single-breath DLNO and DLCO in anesthetized and mechanically ventilated sheep (fraction of inspired oxygen [FIO2] of 1.0) before and after pulmonary artery occlusion by inflation of a balloon (n = 6), and by autologous clot embolism (n = 4). To see if the effect also occurred on FIO2 of 0.21, four animals were studied during ventilation with room air, one of which was also in the FIO2 of 1.0 group (14 total experiments with 13 sheep).

Results: On FIO2 of 1.0, the mean DLNO/DLCO ratio rose by 35% from 4.76 ± 0.41 in control to 6.42 ± 0.82 after balloon occlusion (p = 0.002), and by 54% from 7.55 ± 2.09 to 11.6 ± 2.61 (p = 0.005) after autologous clot embolism (± SD). An equivalent relative increase of 27% took place during ventilation with room air, but the DLNO/DLCO ratio was lower (3.14 ± 0.22 in control and 3.98 ± 0.38 after balloon occlusion). Independent of the method of obstruction or FIO2, the increase in DLNO/DLCO ratio was mostly due to a drop in DLCO. The DLNO/DLCO ratio reduced much of the intersubject variability of either DLNO or DLCO alone.

Conclusion: The DLNO/DLCO ratio increased after pulmonary artery occlusion regardless of the method of occlusion or FIO2. This increase may be a result of a greater sensitivity of DLCO than DLNO to a regional reduction in capillary blood flow. (CHEST 2004; 126:559–565)

Key words: carbon monoxide; diffusing capacity of the lung for CO; diffusing capacity of the lung for nitric oxide; nitric oxide; pulmonary embolism

Abbreviations: BO1.0 = balloon occlusion with fraction of inspired oxygen of 1.0; BO0.21 = balloon occlusion with fraction of inspired oxygen of 0.21; CV2 = squared coefficient of variation; DLCO = diffusing capacity of the lung for CO; DLNO = diffusing capacity of the lung for nitric oxide; FIO2 = fraction of inspired oxygen; NO = nitric oxide; PAO = pulmonary artery occlusion; PetCO2 = end-tidal carbon dioxide concentration; VA = alveolar mixing volume; Vd/Vt = Bohr dead space fraction

Chappell et al1 proposed that the diffusing capacity of the lung for carbon monoxide (DLCO) could be used to quantitatively monitor occlusive changes in the pulmonary vascular bed. By calculating the ratio of two serial measurements of DLCO conducted at two different concentrations of inhaled CO, they showed that the fraction of the vascular bed occluded could be reproducibly estimated. This

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Correspondence to: R. Scott Harris, MD, FCCP, Pulmonary and Critical Care Unit, Balfour 148, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114; e-mail: rharris@partners.org
approach makes use of the increase in “back pressure” of intracapillary CO generated in areas of stagnant capillary blood. The effect of that back pressure in the measurement of DLCO for a normal lung is negligible for four reasons: the high affinity of hemoglobin for CO, the low concentration of CO normally used (0.3%), the short duration of the measurement, and the continuous flow of blood with a low concentration of CO. However, in a lung with areas of stagnant blood flow, the local partial pressure of CO in the stagnant capillary blood increases during the measurement period, decreasing the calculated DLCO. Because this effect is magnified when the test gas contains higher concentrations of CO, the relative decrease in DLCO caused by increasing CO concentration is a function only of the fraction of the vascular bed with stagnant blood.

This approach for quantifying pulmonary vascular obstruction, however, has practical limitations. It requires two serial measurements of DLCO with a washout period in between of 100% O₂ breathing to obstruction, however, has practical limitations. It requires two serial measurements of DLCO with a washout period in between of 100% O₂ breathing to reduce the hemoglobin-bound CO accumulated during the first measurement. Physiologic changes in the pulmonary blood flow distribution, or subtle differences in the conduction of the test, can both affect the results. We reasoned that these problems could potentially be circumvented by measuring simultaneously diffusing capacities for two gases with different hemoglobin saturation kinetics. Given the much higher affinity for hemoglobin with nitric oxide (NO), and the much lower concentrations used clinically (parts per million), compared with CO, capillaries with stagnant blood flow could be expected to generate much lower back pressure with NO than CO. Thus, changes in the ratio of simultaneously measured DLNO to DLCO could potentially be affected by changes in the fraction of occluded capillary bed. This study was designed to test the hypothesis that the ratio of simultaneously measured DLNO and DLCO should systematically increase during obstruction of a fraction of the pulmonary vascular bed regardless of the method of pulmonary vascular bed obstruction or fraction of inspired oxygen (FiO₂).

Materials and Methods

Animal Preparation

The animal care committee of the Massachusetts General Hospital approved all protocols and procedures. Thirteen sheep weighing 21.2 ± 4.7 kg (± SD) [range, 16 to 35 kg] were anesthetized with thiopental sodium (30 mg/kg) and maintained under deep anesthesia with a continuous infusion. A tracheotomy was performed for insertion of a 7.0 endotracheal tube. The ventilator (Harvard Apparatus; Millis, MA) was set at a breathing frequency of 10 to 20 breaths/min, the inspiratory time was set to 30% of the breathing period, positive end-expiratory pressure was set to 5 cm H₂O, and FiO₂ was either 0.21 or 1.0, depending on the experiment (see below). Tidal volume was set to 7.7 to 13.1 mg/dL (mean, 9.9 ± 1.5 mg/dL). A 7.5F pulmonary thermocatheter was inserted in the left femoral vein for measurement of thermocatheter cardiac output, pulmonary arterial pressure, and pulmonary capillary wedge pressures. This catheter was also used to create pulmonary artery occlusion (PAO) by inflating the balloon with air and maintaining a wedge tracing on the strip chart recorder. For some animals, another pulmonary artery catheter was placed in the right internal jugular vein so that pulmonary artery pressure and cardiac output could be measured during balloon inflation of the other catheter. Pancuronium bromide was administered in 0.2 mg/kg IV doses as needed to prevent respiratory efforts after adequate sedation was achieved. All animals were administered full-dose heparin with a bolus dose (100 U/kg) and maintenance doses (50 U/kg q2h) throughout the experiment. Physiologic data collection included heart rate, arterial and pulmonary blood pressures, cardiac output, pulmonary capillary wedge pressure, and arterial and venous blood gases. Alveolar dead space was calculated using the modified Bohr equation: Vd/Vt = (PaCO₂ − PetCO₂)/PACO₂, where Vd/Vt is the Bohr dead space fraction, and PetCO₂ is the end-tidal CO₂ concentration. The PetCO₂ was obtained from a side-stream CO₂ monitor (Datex Instrumentalia; Oy, Finland) that was calibrated prior to beginning of each study day.

Measurement of DLNO and DLCO

Diffusing capacities for NO and CO were measured simultaneously in each condition using the single-breath method. A 3-L calibration syringe was flushed with standard 0.3% CO, 10% He, 21% O₂, and balance N₂ gas. Then the syringe was slowly filled with this gas while 800 ppm of NO gas was simultaneously injected through a three-way stopcock. The syringe was then sampled for CO and He concentration in percentage (PK Morgan Limited; Rainham Gillingham-Kent, UK), and NO concentration in parts per million (Sievers; Boulder, CO). All gas analyzers were calibrated prior to use according to the specifications of the manufacturer. The He analyzer was adjusted for the O₂ concentration of the sample gas (21% O₂). The resultant starting concentration ranges were 13 to 270 ppm for NO, 0.110 to 0.287% for CO, and 3.66 to 9.53% for He. The sheep was disconnected from the ventilator and allowed to passively exhale to relaxation lung volume. The calibration syringe was connected to the endotracheal tube; the lungs were inflated to total lung capacity (40 cm H₂O) and maintained at that pressure for 10 s. At that point, 200 mL were withdrawn back into the calibration syringe (anatomic dead space volume), a three-way stopcock was turned into a collection bag, and the sheep was allowed to passively exhale. The collection bag was then sampled for CO, He, and NO concentrations as before. For animals breathing 100% O₂, the He analyzer was adjusted for the new concentration of oxygen in the sample gas (nearly 100% O₂). If necessary, the NO analyzer scale was changed from parts per million to parts per billion for sampling the exhaled gas (range, 12 to 2,525 parts per billion). From the airway pressure tracing, the method of Ogilvie et al² was used to calculate the breath-hold time, taken as the time from onset of breath-hold to the beginning of the alveolar sample collection time. The diffusing capacities were calculated according to the following formula (equation 1):
where DLCO(NO) is in milliliters per minute per millimeters of mercury, Va is the alveolar volume, t is the breath-hold time, 160 is a constant representing the conversion from log10 to log10r seconds to minutes, and volumes percentage to partial pressure (at 760 mm Hg), CO(NO)inspired is the concentration of CO or NO inspired, CO(NO)expired is the concentration of CO or NO expired, Heexpired is the concentration of helium expired, and Heinspired is the concentration of helium inspired. Effective Va was calculated according to the following formula (equation 2):

\[
Va = \frac{Vinspired \times Heinspired}{Heexpired \times 0.95 \times BTPS}
\]

where Va is in liters, Vinspired is the volume delivered from the syringe in liters, Heinspired is the concentration of helium inspired, Heexpired is the concentration of helium expired, and BTPS is body temperature and pressure, saturated. Since approximately 5% CO2 is extracted from the expired samples in the calcium carbonate CO2 absorber prior to analysis, the 0.95 factor in equation 2 corrects for this concentrating effect.

**Protocols**

All sheep were studied in the prone position in two conditions: control and PAO. In each condition, four separate measurements of simultaneous single-breath DLCO and DLNO were made to ensure accuracy. During pulmonary artery balloon occlusion, a pulmonary capillary wedge tracing in conjunction with a drop in the PetCO2 confirmed occlusion. Given that the volume of the pulmonary artery catheter balloon is 1.5 mL, this likely resulted in a right- or left-main pulmonary artery obstruction. Six sheep were studied with an FIO2 of 1.0, and four sheep were studied with an FIO2 of 0.21. One sheep was studied before and after PAO with both O2 concentrations. Three of these animals had two pulmonary artery catheters in place to measure pulmonary artery pressure and cardiac output during balloon occlusion. For the autologous clot experiments, four sheep had 300 mL of blood removed with 1,000 mL of normal saline solution given as replacement volume. The blood was then used to form 5- to 7-mm cylindrical clots (equal height and diameter) by pouring the blood into a Plexiglas mold and allowing it to coagulate. Clots were then individually introduced through a large-bore internal jugular catheter until a stable mean pulmonary artery pressure between 30 mm Hg and 40 mm Hg was obtained. IV fluids were administered to maintain mean arterial pressure > 50 mm Hg. Animals were allowed to reach steady state in each condition as manifested by stability in heart rate, mean arterial BP, and arterial blood gases. Pulmonary vascular occlusion always followed the control condition. Steady state after PAO was achieved in approximately 15 min, and the four diffusing capacity measurements were completed within 30 min.

**Statistical Analysis**

Data are expressed as means ± SD unless otherwise noted. Random coefficient models were used to ascertain there was no time effect from the four repeated measures obtained for diffusing capacity data. Consequently, the average of the four measurements was used in further analysis. Paired t tests were used to determine significant changes before and after PAO in physiologic data and diffusing capacity data. The correlations between DLNO/DLCO and cardiac output data were summarized using Pearson correlation coefficients. Statistical significance was defined by p < 0.05. Statistical analysis was performed using SAS 8.2 (SAS Institute, Cary, NC).

**RESULTS**

**Global Physiologic Parameters**

Mean arterial pressure, heart rate, pulmonary capillary wedge pressure, and PaO2 did not change significantly during PAO compared to control (Table 1) in any of the experiments. Mean pulmonary artery pressure was higher during PAO with autologous clot embolism, since the goal was to give enough clots to increase the mean pulmonary artery pressure to approximately 35 mm Hg. Peak inspiratory pressure

<table>
<thead>
<tr>
<th>Table 1—Physiologic Variables*</th>
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</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
</tr>
<tr>
<td>SV, mL</td>
</tr>
<tr>
<td>MPAP, cm H2O</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
</tr>
<tr>
<td>PIP, cm H2O</td>
</tr>
<tr>
<td>eTCO2, torr</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>PCO2, torr</td>
</tr>
<tr>
<td>PaO2, torr</td>
</tr>
<tr>
<td>Vi/oVT</td>
</tr>
</tbody>
</table>

*Values are means ± SD. MAP = mean arterial pressure; HR = heart rate; SV = stroke volume; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PIP = peak inspiratory pressure; eTCO2 = end-tidal CO2.

‡n = 3 for these conditions.

1p < 0.05 compared to control for each experiment.
was also significantly higher in this experiment, but not during either of the balloon occlusion experiments. Since the pulmonary artery catheter balloon was used to create the balloon occlusion condition, the values for cardiac output, stroke volume, and mean pulmonary artery pressure were not recorded during the balloon occlusion with FIO2 of 1.0 (BO1.0). For balloon occlusion with FIO2 of 0.21 (BO0.21) and autologous clot embolism, cardiac output, stroke volume, and mean pulmonary artery pressure did not change during PAO compared to control. PetCO2 and arterial pH were significantly decreased, and PaoCO2 and Vd/VT were significantly increased during balloon occlusion with an FIO2 of 1.0 (BO1.0) and autologous clot embolism compared to control (Table 1). None of these changed significantly during BO0.21.

### DLNO, DLCO, and DLNO/DLCO Ratio

DLNO did not change after PAO for any of the experiments (Table 2). In contrast, DLCO decreased significantly for BO1.0 and autologous clot embolism. The DLNO/DLCO ratio increased significantly in all sheep (Table 2, Fig 1) after PAO. The DLNO/DLCO ratio varied substantially in the control condition (Fig 1), with the lowest values being for sheep studied on room air (BO0.21), and higher values for sheep studied with FIO2 of 1.0 (BO1.0 and autologous clot embolus). Despite this initial variability, all sheep demonstrated an increase in DLNO/DLCO ratio of similar magnitude (Fig 1). The mean DLNO/DLCO ratio rose after PAO (Table 2) in all experiments (3.14 to 3.98, p = 0.004; 4.76 to 6.42, p = 0.002; and 7.55 to 11.6, p = 0.005 for BO0.21, BO1.0, and autologous clot embolism, respectively). The effective VA did not change significantly after PAO in any of the experiments. The percentage change in DLCO was greater than the changes in either DLNO or VA (Fig 2), and the magnitude of the change progressively increased with 100% O2 and autologous clot. The relative change in DLNO was similar to that of VA (Fig 2).

### Cardiac Output and DLNO/DLCO Ratio

There was a strong correlation between DLNO/DLCO ratio and cardiac output before PAO (r = -0.903). That correlation was reduced after PAO (r = -0.765).

## Discussion

Chappell et al. demonstrated in a canine model that the ratio of DLCO from sequential measurements done at two concentrations of CO (0.3% and 3.3%) could be used to estimate the fraction of occluded pulmonary capillary bed. They theorized

### Table 2—DLNO, DLCO, DLNO/DLCO, and Va*

<table>
<thead>
<tr>
<th>Variables</th>
<th>BO0.21 (n = 4)</th>
<th>BO1.0 (n = 6)</th>
<th>AC, FIO2 of 1.0 (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PAO</td>
<td>Control</td>
</tr>
<tr>
<td>DLNO</td>
<td>47.4 ± 22.4</td>
<td>52.8 ± 28.2</td>
<td>69.5 ± 26.8</td>
</tr>
<tr>
<td>DLCO</td>
<td>15.2 ± 7.02</td>
<td>13.1 ± 6.38</td>
<td>14.6 ± 5.37</td>
</tr>
<tr>
<td>DLNO/DLCO ratio</td>
<td>3.14 ± 0.22†</td>
<td>3.98 ± 0.38†</td>
<td>4.76 ± 0.41†</td>
</tr>
<tr>
<td>Va</td>
<td>1.64 ± 0.74†</td>
<td>1.68 ± 0.67†</td>
<td>2.87 ± 0.18</td>
</tr>
</tbody>
</table>

*Values are means ± SD.
†p < 0.05 compared to control for each experiment.

![Figure 1: DLNO/DLCO ratio in control and PAO conditions for each experiment. The small open circles are for BO0.21, filled circles are for BO1.0, and large open circles are for autologous clot embolism. Each symbol represents an individual sheep with a line connecting its respective control and PAO values. The mean ratio for each experiment and condition is shown next to the individual symbols by its matching symbol and associated SE bar. As can be seen in this semi-log plot, each sheep had a rise in DLNO/DLCO ratio of similar magnitude after PAO. *p < 0.05 compared to control.](Image)
that, in capillaries with stagnant blood, the rate decline in alveolar CO concentration would be slower at higher inspired CO concentration due to saturation of hemoglobin, and thus DLCO would be lower. They found that occlusion of a pulmonary artery with a balloon catheter resulted in an increase in the ratio of DLCO, measured with 0.3% to that measured with 3.3%. In this study, we used a similar approach, but instead of sequential measurements of the same gas at different concentrations, we used simultaneous measurements of two gases with substantially different affinities for hemoglobin: NO and CO. There are several properties of NO that make its pulmonary uptake substantially faster than that of CO. First, the solubility of NO in water is twice as high as that of CO.7 Because the molecular weights of both molecules are similar, the Krogh diffusion constant of NO is about twice that of CO. Second, the affinity of hemoglobin for NO is several thousand times greater than that for CO.5–7 Third, the velocity constant of combination of NO with hemoglobin is about 280 times greater than that of CO.10 These factors should result in DLNO being substantially less affected by the presence of stagnant capillary blood than DLCO. Also, because the diffusing capacity of both gases is measured simultaneously, variability caused by temporal physiologic changes, or by subtle technique differences between two sequential measurements, is eliminated.

The purpose of this study was to test whether the ratio of simultaneously measured DLNO and DLCO would systematically increase following obstruction of a fraction of the pulmonary capillary bed. Although three different groups were studied, the purpose was not to compare the effect among the groups, but rather to compare before and after occlusion in each sheep. The main findings of the study are as follows: (1) the ratio of DLNO/DLCO in healthy prone sheep increased after obstruction of part of the pulmonary capillary bed by either balloon occlusion or autologous clot or when breathing 100% O2 or room air; (2) the rise in DLNO/DLCO ratio was almost exclusively caused by a fall in DLCO; and (3) the DLNO/DLCO ratio had substantially less intersubject variability than either DLNO or DLCO. An important corollary to finding 2 is that DLNO changes little with interruption in a substantial amount of blood flow. This means that, despite the fact that we were using a gas for the measurement of DLNO that is also a vasodilating drug, it is unlikely that any small change in the distribution of perfusion caused by the NO would have any effect on the measurement of DLNO. This is consistent with the findings of Tamhane et al,11 in which breathing 40 ppm of NO did not change the DLCO in nonsmoking healthy volunteers.

We used a volume of 200 mL for the Vd/Vt in order to be well above the expected anatomic dead space of 48 mL calculated for sheep of this size.12 It is unlikely that we included any anatomic dead space given that a tracheostomy was performed, thus shortening the trachea, and the valve for collecting the alveolar sample had a dead volume of <50 mL. In fact, the large amount of dead space gas discarded may have caused an overestimation of the diffusing capacity.13 Conveniently, even if the absolute values of DLCO or DLNO may have had systematic errors, their ratio should not be affected. We purposely chose not to correct the DLCO for hemoglobin since there is not an equivalent established hemoglobin correction for DLNO, and we thought we could introduce an artificial difference in DLNO and DLCO that would alter their ratio. There was little correlation between hemoglobin concentration and DLNO/DLCO ratio (r = 0.2488 before occlusion, r = 0.2484 after occlusion), making it unlikely that the large variability in hemoglobin concentration in these sheep contributed to the variability in diffusing capacities.

It is possible that ventilating the animals on 100% O2 while measuring DLCO and DLNO with a test gas mixture containing 21% O2 could have affected the measurement of the ratio. A single inspired breath of 21% O2 into a lung breathing 100% O2 can create an uneven distribution of alveolar O2 tension. This would be amplified by regional PAO that would rapidly decrease ventilation to that region. If the diffusing capacities measured with NO and CO change in different ways with changes in O2 tension, this could have affected the results. Indeed, Borland and Cox14 showed that the dependence of DLCO and DLNO were opposite with inspired O2 concentration.
(DLCO drops and DLNO rises), at least within the range they tested (15 to 25%). If all of the change in DLNO/DLCO were a result of a change in alveolar PO₂ after PAO, we should have seen both a decrease in DLCO and an increase in DLNO. DLNO actually decreased or remained the same in all animals. To further support these arguments, we tested four animals under room air conditions (Fig 1). As can be seen, the magnitude of DLNO/DLCO ratio is reduced in both conditions (control and PAO), but the magnitude of the change after PAO is similar. The average change in these four animals was 27%, very close to the 35% seen with 100% O₂.

The average DLNO/DLCO ratios were 3.14 and 4.76 in these prone sheep during normoxia and hyperoxia, respectively, in the balloon occlusion groups (BO₀.₂₁ and BO₁.₀), and 7.55 in the autologous clot embolism group (Table 2). These ratios are close to the values of 3.38 and 5.54 for normoxia and hyperoxia reported by Meyer et al¹⁵ in supine dogs using the rebreathing method. In our study, the ratio rose in a similar magnitude in all sheep after PAO regardless of the FiO₂ or the method of obstruction (Fig 1). The increase in ratio was 27%, 35%, and 54% for BO₀.₂₁, BO₁.₀, and autologous clot embolism, respectively. These increases were almost exclusively due to the 15%, 30%, and 41% drops in DLNO for the same conditions, respectively (Fig 2). Other investigators¹,²,¹⁶–¹⁸ have noted a drop in DLCO after PAO. In the PAO experiments by Chappell et al.,¹ the range of the drop in DLCO (6 to 20%) is similar to the range we saw for the balloon occlusion (15 to 30%) experiments, and corresponds to approximately a 35 to 40% vascular occlusion (right or left main pulmonary artery occlusion). The drop in DLCO was much higher during PAO with autologous clots, but this was a much more severe obstruction, resulting in an estimated 64% obstruction of the pulmonary vascular bed.⁹

Although this study was not designed to elucidate a mechanism for the increase in DLNO/DLCO ratio following occlusion of a fraction of the pulmonary capillary bed, a potential mechanism can be proposed. It is known that the DLCO increases with cardiac output during exercise,¹⁹ possibly by recruiting nonperfused pulmonary capillary bed. Conversely, one could expect that a decrease in cardiac output could reduce DLCO by a reduction in perfused capillary bed. Indeed, we observed a tight correlation between DLNO/DLCO and cardiac output in control conditions (r = -0.903). However, the correlation was reduced following PAO (r = -0.765). These observations could still be consistent with the hypothesis that DLNO/DLCO is a function of the fraction of recruited pulmonary capillary bed. In the normal lung, the amount of recruited capillary bed is likely directly related to the cardiac output. In the situation of PAO, a region of obstructed capillary bed would be formed, and blood flow that normally perfused this region would be redirected to other regions of the lung. In this situation, the effect on the DLNO/DLCO would depend on the balance of the amount of capillary occlusion to the amount of pulmonary capillaries recruited by the redirected blood flow. The net effect on pulmonary capillary recruitment would depend on the cardiac output response following PAO and the magnitude of redirected pulmonary blood flow. In a situation where the fraction of capillary bed occluded is large, this would be the dominant effect, causing a large increase in the CO back pressure, overwhelming the reduction in CO back pressure caused by capillary recruitment from redistributed blood to other regions in the lung. One could imagine, however, that with mild pulmonary capillary bed occlusions, the two effects (the loss of capillaries to occlusion and increase in capillary recruitment caused by redistributed blood flow) could balance each other, resulting in no change in DLNO/DLCO ratio. This would be especially likely in situations of reduced cardiac output prior to occlusion. This is a possible limitation of this method that requires further study.

We cannot say for certain that it is a difference in back pressure in the pulmonary capillary blood that is responsible for the increase in DLNO/DLCO ratio. However, being everything else equal during the simultaneous measurement of DLCO and DLNO, and given that DLNO was systematically affected much less than DLCO by vascular obstruction, we theorize that such effect has to be caused by differences in the diffusion and/or saturation kinetics between both gases. The flux of a gas depends on the membrane cross-sectional area, the gas diffusivity, and partial pressure gradient. Diffusivity, being a physical property, should not be affected by vascular obstruction, and any change in exposed cross-sectional area of the membrane should affect equally both gases. Since from the partial pressure gradient the alveolar concentration of the gases is known, this leaves the downstream concentration and thus the saturation kinetics of the gas as the only plausible explanation.

A potential advantage of DLNO/DLCO ratio over DLCO alone for monitoring pulmonary vascular obstruction is that any systematic errors in the measurement of each will be eliminated by their ratio since they are measured simultaneously. This is best demonstrated in the BO₁.₀ experiment (Table 2) by the much lower squared coefficient of variation (CV²) = (SD/mean)² = 0.01 in the control condition of DLNO/DLCO ratio among the six sheep vs the DLNO or DLCO individually (CV² = 0.15 and
CV² = 0.14, respectively). Regardless of experimental condition, the intersubject variability of DLNO/ DLCO (CV² = 0.15) was substantially higher than DLNO/ DLCO ratio (CV² = 0.03). Thus, although DLCO alone could be used as a follow changes in pulmonary vascular obstruction, only very large changes in DLCO can be confidently interpreted as actual changes in perfusion in the pulmonary vascular bed. In contrast, changes in the DLNO/DLCO ratio are less affected by systematic experimental errors, and thus should be more sensitive to changes in the pulmonary vascular bed than DLCO.

In summary, the ratio of single-breath DLNO/ DLCO ratio increased after PAO regardless of FIO₂ or mechanism of obstruction. The increase in DLNO/ DLCO ratio was due to a substantially greater fall in DLCO than DLNO after occlusion. The ratio of DLNO/DLCO greatly reduced the intersubject variability of DLCO measured alone. These results suggest that the DLNO/DLCO ratio is affected by changes in the fraction of perfused pulmonary capillary bed, but further study is needed to evaluate its sensitivity.

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