No red cell resistance to NO? I Think Not!

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PULMONARY CAPILLARY BLOOD VOLUME and alveolar-membrane diffusing capacity are components to overall pulmonary diffusing capacity. The equation deriving pulmonary diffusing capacity into these components was first published in 1957 by Francis Roughton and Robert Forster (19) in which the total resistance to pulmonary diffusion is the addition of alveolar-membrane resistance and red blood cell resistance placed in series, such that

\[ \frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\Theta V_C} \]

where, \( D_L \) is the overall diffusing capacity of the lung, \( D_M \) is the true alveolar-membrane diffusing capacity separating the alveolar air from the blood, \( V_c \) is the total volume of blood in the pulmonary capillaries exposed to alveolar air, and \( \Theta \) is the number of milliliters of gas taken up by red blood cells in 1 ml of blood per 1 mmHg gradient of partial pressure of dissolved gas between the plasma and the interior of the red blood cell. While technically difficult to measure pulmonary diffusing capacity for oxygen, carbon monoxide (CO) is most commonly used as a surrogate of oxygen transfer. Therefore, \( D_L \) and \( D_M \) are reported as DMCO (alveolar-membrane diffusing capacity for CO) and DLCO (pulmonary diffusing capacity for CO) while \( \Theta \) is reported as \( \theta_{CO} \) (the blood transfer conductance for CO). According to the Roughton and Forster equation (19), the resistance of the red blood cell to the uptake of CO is about equal to the resistance of the alveolar-capillary membrane to the diffusion of gas across it.

To obtain DMCO and \( V_C \), DLCO has been traditionally measured at two different levels of alveolar PO2 (\( P_{A02} \)), e.g., at \( \sim 100\)–\(120 \) mmHg and \( \sim 600 \) mmHg. For each \( P_{A02} \) level, \( 1/DLCO \) is plotted on the \( y \)-axis and \( 1/\theta_{CO} \) is plotted on the \( x \)-axis. A line is drawn through the two points and the y-intercept (\( 1/DMCO \)) and slope (\( 1/V_C \)) can be solved. The formula for \( 1/\theta_{CO} \) varies across studies, but the most predominant formula used in studies today are from the original Roughton and Forster paper or a modification of this formula (12, 19).

However, there are at least three main technical issues with this traditional two-step method in obtaining DMCO and \( V_C \). First, at least four tests are needed (2 at low and 2 at high \( P_{A02} \)) to obtain a reliable measure of DMCO and \( V_C \). This places considerable time restraint in a clinical setting and increases patient effort. Second, with the traditional method, the CO gas distribution in the lungs may be different at two inspirations at different oxygen tensions, affecting DMCO and \( V_C \). Third, with the standard method, cardiac output may vary between measurements of DLCO at different oxygen tensions, which then have to be interpolated to obtain DLCO at the two oxygen tensions at the same cardiac output. This would also affect DMCO and \( V_C \).

In 1983, Colin Borland and his colleagues (3) examined the fate of inhaled NO, as it was an important component of cigarette smoke. They determined that nitric oxide (NO) uptake behaved similarly to CO uptake such that the ratio of pulmonary diffusing capacity for NO (DLNO) to DLCO was \( \sim 4.6 \), and concluded inhaled NO does not readily convert to nitrogen dioxide (NO2), a toxic gas (11, 21).1 Later, in 1987, Hervé Guénard et al. (13) made the assumption that since the reaction on NO with hemoglobin is effectively infinite, the blood transfer conductance for NO (\( \theta_{NO} \)) must also be infinite (13). Therefore, as the diffusivity of NO is about twice that of CO (\( \theta_{NO} = DMNO \approx 2 DMCO \)), Guénard assumed that DMCO and \( V_C \) could be calculated using a one-step maneuver.

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1 Other studies have determined that NO2 is formed at a rate of \( \sim 0.02 \) ppm/sec (\( \sim 1.2 \) ppm NO2/min) in a gas mixture containing 21% oxygen and 60 ppm NO. Therefore, the production of NO2 is negligible provided care is taken to prevent the mixture of NO with oxygen until immediately prior to inhalation.

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**Fig. 1.** Percentage increase in 3 variables: diffusing capacity for NO (DLNO), the ratio of DLNO-to-DLCO, and diffusing capacity for CO (DLCO) between baseline and after exchange transfusion (30 ml/kg) with Oxyglobin solution (cell-free hemoglobin-based oxygen carrier) in 3 foxhounds (28.3 (SD 1.0) kg). The percentage increase in DLNO and the ratio of DLNO-to-DLCO compared with baseline is statistically significant (\( P < 0.05 \)). \( P_{A02}, P_{ACO2}, \) and heart rate were maintained at \( \sim 140 \) mmHg, 35 mmHg, and 110 beats/min, respectively, while [Hb] and arterial oxygen content (\( CaO2 \)) decreased from 13.5 (1.5) to 11.4 (0.3) g/dl and 18 to 14 ml/dl, respectively, after exchange transfusion of Oxyglobin (Oxyglobin has a [Hb] content of 13 g/dl). The reduction in [Hb] and \( CaO2 \) with Oxyglobin is likely due to its known plasma expanding effect. By removing 850 ml of red blood cells from the foxhounds and then replacing 850 ml (\( \sim 40\)%) of total red blood cell volume with Oxyglobin solution, the red blood cell resistance to NO was greatly reduced. Therefore, the large percentage increase in both DLNO and the ratio of DLNO-to-DLCO between baseline and after exchange transfusion (30 ml/kg) is seen with no change in DLCO. This directly demonstrates a finite blood transfer conductance of NO (\( \theta_{NO} \)) in vivo. Data reproduced from Borland and colleagues (7).
in which both CO and NO are inhaled together. This was an ingenious idea. Hence, all the technical issues with the traditional Roughton and Forster two-step method are avoided with the new modified, one-step DLNO-DLCO technique. Since 1987, most studies that use this modified technique have assumed that ΘNO approaches infinity.

Nonetheless, a disagreement soon arose whether DLNO = DMNO (2, 6). Colin Borland and colleagues believed that ΘNO was less than finite, at ~4.5 ml NO·(ml blood-min·mmHg)^-1. This was derived from a value obtained in vitro with human red blood cells in 1958 in unphysiological conditions (8). In 2006, Borland and colleagues used a membrane oxygencator as a model for NO and CO transfer (5). Their membrane oxygencator tests various factors that affect NO and CO in physiological conditions that would be impossible to do in vivo or in an isolated lung preparation. The authors postulated that if DLNO = DMNO then hemolysis (which eliminates red blood cell resistance) would not alter DLNO. However, hemolysis actually increased DNO, providing evidence that there is significant red blood cell diffusive resistance to NO (i.e., DNO<DMNO). A limitation of this study was that the results were neither obtained in human blood nor were they obtained in vivo.

In this issue of the Journal of Applied Physiology, Colin Borland and colleagues (7) go one step further in that they use both in vitro (membrane oxygencator perfused with whole blood) and in vivo (foxhounds) data directly to examine whether ΘNO was infinite. They postulated that if there was significant red blood cell resistance to NO, both in vitro DLNO and in vivo DLNO would increase progressively as red blood cells were replaced with cell-free heme-based blood substitute (cell-free hemoglobin-based oxygen carrier). Both in vitro and in vivo replacement of whole blood with hemolyzed blood and a cell-free hemoglobin solution (see Fig. 1) caused DLNO to progressively increase, implying that DLNO<DMNO. The in vitro and in vivo data demonstrate a finite ΘNO of ~4.5 ml NO·(ml blood-min·mmHg)^-1, similar to data from 1958 (8). Borland and colleagues further demonstrate that the overall resistance to pulmonary NO uptake (1/DLNO) is 63% alveolar-capillary membrane resistance (1/DMNO) and 37% red blood cell resistance (1/ΘNO·Vc) (7).

What are the clinical implications of this study? First, although ΘNO is finite, DLNO does not need to be adjusted unless [Hb] is <8 g/dl (7). Therefore, in routine clinical practice and in research studies involving humans, ΘNO can still be assumed to be infinite and DLNO can be “clinically” equal to DMNO. In fact, altering [Hb] in humans does not alter DLNO (23). Second, to ensure internal consistency, it is recommended that future studies using combined DLNO and DLCO report the absolute values for alveolar oxygen pressure, hemoglobin concentration, and the formula for ΘCO so that, if need be, DM and Vc can be recalculated.

The use of DLNO may have possible health implications. Both DLCO and DLNO at rest are related to aerobic capacity (26), a strong independent predictor of death in women (14) and men (16). Thus a measurement of DLNO could be a prognostic marker for mortality in several patient populations.

2 Currently there are about eight different equations that describe the reaction rate of CO with hemoglobin (ΘCO) for humans, so reporting the equation for ΘCO in research studies would aid in between-study comparison.

References


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