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The DL_{NO}/DL_{CO} ratio: Physiological significance and clinical implications

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ABSTRACT

DL_{NO}/DL_{CO} directly measures the ratio of the diffusing capacities of the lung for nitric oxide (NO) and carbon monoxide (CO). In terms of the Roughton and Forster (1957) equation, $1/DL = 1/Dm + 1/\theta Vc$, where Dm is the membrane (Dm) and θVc is the red cell component of the overall diffusing conductance (DL); DL_{NO} mostly reflects the Dm component and DL_{CO} the θVc red cell component.

The DL_{NO}/DL_{CO} ratio is positively related to the Dm_{CO}/Vc ratio and the CO red cell resistance ($1/\theta COVc$) as a percentage of the total resistance ($1/DL_{CO}$), independent of the absolute values of DL_{NO} or DL_{CO}.

In clinical studies, a raised DL_{NO}/DL_{CO} ratio (≥110% predicted versus a control group), plus a low DL_{NO} and DL_{CO} (<67% pred), predicts pulmonary vascular disease, while a low DL_{NO}/DL_{CO} ratio, with similarly reduced DL_{NO} and DL_{CO}, is associated with interstitial lung disease with fibrosis. More clinical studies are needed, and reference values need to be better defined.

1. Introduction

In the clinical setting, the diffusing capacity of the lung (DL) is usually measured with the single breath carbon monoxide (CO) uptake technique (DL_{CO}). The method has undergone little change since the original description (Ogilvie et al., 1957). In 1983–9, the measurement of diffusing capacity using a different haemoglobin (Hb)-reactive gas, nitric oxide (NO), was introduced (Guénard et al., 1987; Borland and Higenbottam, 1989), employing the same single breath technique. Both tracer gases (CO and NO) were inhaled from the same reservoir (together with an inert volume-marker gas, usually helium). The ratio of DL_{NO} to DL_{CO} in normal subjects was about 5.0 (Borland and Higenbottam, 1989). This fivefold ratio is caused by the different properties of NO versus CO, as they traverse the blood-gas barrier; the greatest difference lies in the more rapid (about 300 fold) uptake rate of NO versus CO by intracapillary haemoglobin.

It is too early to say whether the DL_{NO} will replace the DL_{CO} as the best test of the diffusing capacity of the lung. At the present time, measurement of the DL_{NO} is always accompanied by a simultaneous DL_{CO} measurement. In this article, we will review the physiological processes responsible for the fivefold difference in the NO-CO uptake rates, and consider the clinical implications of an abnormally high or low DL_{NO}/DL_{CO} ratio.

2. DL_{NO} versus DL_{CO}: differences and similarities

The conductance of CO from gas to blood is given by the classical equation (Roughton and Forster, 1957):

$$1/DL_{CO} = 1/Dm_{CO} + 1/\theta_{CO}Vc \quad (1)$$

where $1/DL_{CO}$ is the lung resistance to CO transfer (the reciprocal of the conductance), $1/Dm_{CO}$ (the molecular diffusion resistance of the lung membranes – from the surfactant lining layer to the red cell membrane – and $1/\theta Vc$ is the resistance to CO transfer within the red cell. The sum of the red cell and membrane resistances, which are in series, are the components of the overall resistance, $1/DL_{CO} \times \theta$ and Vc are defined under Nomenclature. The same equation applies to NO uptake:

$$1/DL_{NO} = 1/Dm_{NO} + 1/\theta_{NO}Vc \quad (2)$$

The molecular diffusive conductance for NO in lung tissue (~ tissue solubility/MW^{-2}) is 1.97 times that for CO; thus, the Dm_{NO}/Dm_{CO} ratio, designated α, is 1.97. The ratio of the red cell conductances for NO versus CO, θ_{NO}/θ_{CO}, on the best available evidence from in vitro and in vivo experiments (Guénard et al., 2016), at an alveolar PO_2 ~ 100 mmHg, is 4.5/0.56 = 8.01. θ_{CO} is O_2-sensitive, but θ_{NO} is essentially O_2-insensitive (Borland and Cox, 1991).

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Nomenclature

DL_{NO}	Diffusing capacity of the lung for inhaled nitric oxide (NO) in tracer quantities: the units are those of a conductance: mL min^{-1} mmHg^{-1} (traditional) and mmol min^{-1} kPa^{-1} (SI), also known as the transfer factor of the lung for nitric oxide TL_{NO}. $1/DL_{NO}$ is the transfer or diffusing capacity resistance
DL_{CO}	the same as for $DL_{NO,}$ but with carbon monoxide (CO) replacing NO
Dm_{CO} and Dm_{NO}	Membrane diffusing capacity for CO and NO
α	$Dm_{NO}/Dm_{CO} = 1.97$
θ_{CO} and θ_{NO}	Specific conductance for CO and NO transfer within the red cell: units are mL of CO or NO min^{-1} mmHg^{-1} per mL blood
$1/1/\theta_{CO}$ and $1/\theta_{NO}$	Specific resistances to transfer
Ψ	θ_{NO}/θ_{CO} ratio
Vc	The pulmonary capillary volume accessed by inhaled CO and NO
TLC	Total lung capacity
FRC	Functional residual capacity
MW	Molecular weight

Combining Eqs. (1) and (2) with $\alpha = Dm_{NO}/Dm_{CO} = 1.97$ and $\theta_{NO/\theta CO} = \Psi = 8.01$, and assuming [Hb] concentration is 100% predicted:

$$Vc = [(1/\theta_{CO})\cdot(1-\alpha/\Psi)]/(1/DL_{CO} - \alpha/DL_{NO}) \quad (3)$$

$$Dm_{CO} = (1/\alpha - 1/\Psi)/[(1/DL_{NO}) - 1/(\Psi\cdot DL_{CO})] \quad (4)$$

In Sections 4 and 5, we have used Eqs. (3) and (4) to explore the relationship between DL_{NO}/DL_{CO} and two ratios for CO diffusion derived from Eq. (1): a) the membrane conductance – pulmonary capillary volume ratio (Dm_{CO}/Vc) — θ_{CO} breathing air, is essentially constant—and b) the red cell resistance to CO diffusion ($1/\theta_{CO}Vc$) as a percentage of the total CO diffusion resistance ($1/DL_{CO}$), ~ Rrc/Rtot%, which is calculated as [1–(DL_{CO}/Dm_{CO})]%, calculating Dm_{CO} and Vc from given values of DL_{NO} and DL_{CO} (see Fig. 1, Tables 1, 2, and 3).

Eqs. (3) and (4) represent the consensus view at the present time. Nevertheless, some believe that estimates of θ_{NO} in vitro grossly underestimate the red cell conductance in vivo. According to this view, θ_{NO} in vivo approaches infinity, so that Eq. (2) can be rewritten:

$$1/DL_{NO} = 1/Dm_{NO} = 1/\alpha Dm_{CO} \quad (5)$$

2.1. A finite versus an infinite value for θ_{NO}

In oxygenated blood, NO reacts very rapidly with HbO_2 to form methaemoglobin and nitrate (NO_3). CO displaces O_2 from HbO_2 at a much slower rate (300 times slower than the NO reaction). But, when the reaction rates are measured in red cell suspensions (rather than cell-free Hb solutions) in vitro, the ratio θ_{NO}/θ_{CO} (Ψ) is 8 not 300. There are objections that the red cell θ may be an underestimate because of the presence of a layer of plasma (a "stagnant" pool) surrounding each cell. While everyone agrees on the basis of many studies in different species (Borland et al., RPNB this issue, 2017) that θ_{NO} *in vitro* is finite, confirmation that θ_{NO} *in vivo* is finite is harder to obtain. On the other hand, in anaesthetized dogs, an exchange transfusion of red cells for a cell-free blood substitute (bovine Hb-glutamer-200), giving NO more open access to the Hb molecule, increased DL_{NO} by 57 ± 16% and the DL_{NO}/DL_{CO} ratio from 4.5 to 7.1, DL_{CO} being unchanged (Borland et al., 2010).

2.2. θ_{NO} and θ_{CO} and absolute values of Dm_{CO} and Vc

Using Eq. (1) only (the classical method, measuring DL_{CO} at normal and hyperoxic alveolar PO_2), estimates of Dm_{CO} in normal subjects at rest are in the range 40–60 mL min^{-1} mmHg^{-1} (Hughes and Bates, 2003), rising to 130–180 with combined NO and CO inhalation using Eqs. (3) and (4) and a finite value for θ_{NO} (Zavorsky et al., 2017). Vc goes in the opposite sense, from 80 to 100 mL with the hyperoxic DL_{CO} to 65–85 mL with the combined DL_{NO}-DL_{CO} measurement. If an infinite θ_{NO} is taken, so that $Dm_{CO} = 0.5\ DL_{NO}$ (Eq. (5)), estimated Dm_{CO} falls and Vc rises towards the values found with the classical DL_{CO} multi-step O_2 technique. The higher values of Dm_{CO}, using the NO-CO analysis and a finite θ_{NO}, are more in line, particularly on exercise (Dm_{CO} ~ 260 mL min^{-1} mmHg^{-1} (Zavorsky et al., 2004)) with morphometric measurements (representing the theoretical maximum values) of 280 mL min^{-1} mmHg^{-1} (Gehr et al., 1978). This concordance of physiologic with morphometric values for Dm_{CO} is some justification, albeit indirect, for the use of the simultaneous NO-CO inhalation technique, coupled with a finite value for θ_{NO}. But, the issue of finite versus infinite θ_{NO} is still a matter for debate.

3. DL_{NO}/DL_{CO} ratio: physiological interpretation

With the classical analysis (Eq. (1)), the resistance split between $1/Dm_{CO}$ and $1/\theta_{CO}Vc$ was about 50:50 (Hsia et al., 1995). On clinical grounds, the DL_{CO} data from pulmonary vascular disease and anaemia seemed to favour a 25:75 partition between the membrane resistance, $1/Dm_{CO}$ (25%) and the red cell resistance, $1/\theta_{CO}Vc$ (75%), and this was supported by calculations of Dm_{CO} and Vc, using more recent estimates (Forster, 1987) of the $1/\theta_{CO}$ – PO_2 relationship (Hughes and Bates, 2003). Recent work using the NO-CO method with finite values for θ_{NO} (and, of course, θ_{CO}) suggest that the red cell resistance percentage for $1/DL_{NO}$ is much less (c.37%), and that $1/DL_{NO}$ is dominated by the membrane resistance (63%) (Borland et al., 2010). In simple terms, $1/DL_{NO}$ represents $1/(Dm_{CO}1.97)$, and $1/DL_{CO}$ reflects 1/Vc; thus, DL_{NO}/DL_{CO} is an expression of two related ratios, Dm_{CO}/Vc and the Rrc/Rtot% fraction for CO (Fig. 1, Table 1).

4. Relationship between DL_{NO}/DL_{CO} and Dm_{CO}/Vc

For this analysis, we have computed theoretical DL_{NO}, DL_{CO} and DL_{NO}/DL_{CO} values in terms of Eqs. (3) and (4) using the generally accepted value for θ_{NO} (Carlsen and Comroe, 1958) and a newly published equation for the $1/\theta_{CO}$-PO_2 relationship based on both in vitro and in vivo data (Guénard et al., 2016). Fig. 1 shows a curvilinear relationship between Dm_{CO}/Vc and DL_{NO}/DL_{CO}, which is independent of the absolute values of DL_{NO} and DL_{CO}, as shown in Table 1. Thus, on the basis of Dm_{NO}/Dm_{CO} of 1.97 (α) and θ_{NO}/θ_{CO} (Ψ) of 8.01, Dm_{CO}/Vc can be predicted from the DL_{NO}/DL_{CO} ratio. Rrc/Rtot% behaves similarly to Dm_{CO}/Vc.

5. DL_{NO}/DL_{CO} ratio in health and disease

5.1. Normal values

In the period 2007–8, three groups published reference values for DL_{NO} in European (van der Lee et al., 2007; Aguilaniu et al., 2008) and North American (Zavorsky et al., 2008a,b) adult populations. There were some important differences. The subjects in the North American study were younger and more athletic; two studies used the less sensitive electrochemical cell for NO analysis and a breath hold time (BHT) of 4 s (Aguilaniu et al., 2008) and 5.5 s (Zavorsky et al., 2008a,b), and one a high sensitivity chemilumines-

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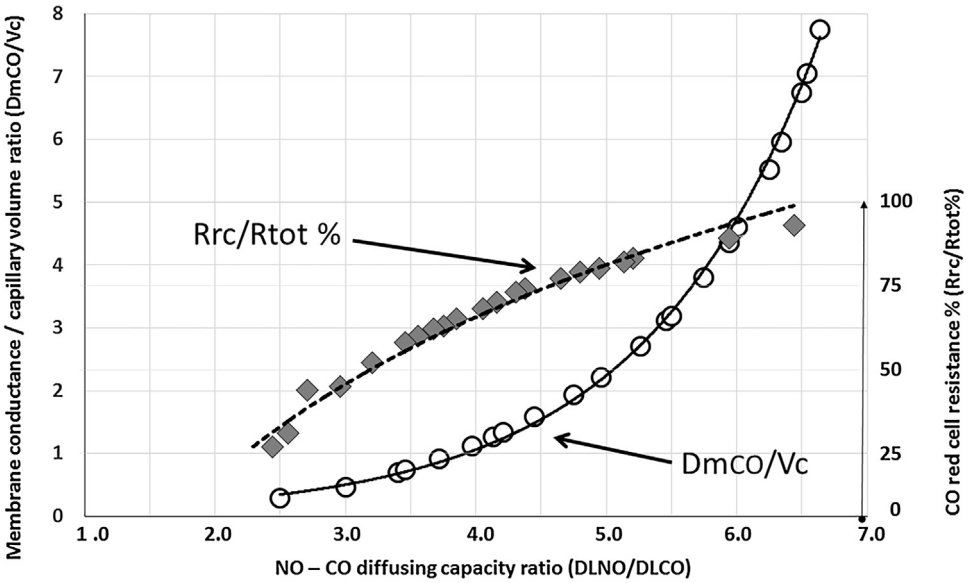

Fig. 1. Ratio of carbon monoxide (CO) membrane diffusing capacity (Dm_{CO}) to pulmonary capillary volume (Vc), Dm_{CO}/Vc, and red cell diffusion resistance (Rrc) for CO as a percentage of total (Rtot) CO diffusion resistance, Rrc/Rtot%, plotted against ratio of diffusing capacities for nitric oxide (NO) and CO (DL_{NO}/DL_{CO}) for normal male, aged 45 years, height 175 cm. Individual points calculated using Eqs. (3) and (4), and the computer programme in the on-line supplement of Zavorsky et al. (2017). The relationship is given by the equation $Dm_{CO}/Vc = 0.0536^{*}\exp^{0.7465^{*}DLNO/DLCO}$ and Rrc/Rtot% = 69.805 ln(DLNO/DLCO) – 31.963.

Table 1
Computations, using Eqs. (3) and (4) with θ_{NO} and θ_{CO} from Guénard et al. (2016), at constant DL_{NO}/DL_{CO} ratios (High. Normal and Low), showing that the Dm_{CO}/Vc ratio and red cell diffusion resistance for CO as percent total diffusion resistance (Rrc/Rtot%) do not depend on absolute values of DL_{NO} and DL_{CO}, only on the DL_{NO}/DL_{CO} ratio. All values except Dm_{CO}/Vc are as percent predicted normal for male aged 45 years, height 1.75 m (from on-line supplement, Zavorsky et al., 2017)[a].

DL_{NO}/DL_{CO}	DL_{NO} %	DL_{CO} %	Dm_{CO} %	Vc%	Dm_{CO}/Vc	Rrc/Rtot%
HIGH						
5.99	100	83	153	70	4.53	89
6.0	85	70	131	59	4.55	89
NORMAL						
4.85	110	113	108	108	2.07	79
4.85	70	72	69	69	2.08	79
LOW						
3.5	84	119	58	156	0.77	58
3.5	51	72	35	94	0.77	58

Dm_{CO}:. membrane diffusing capacity for carbon monoxide.
Vc: pulmonary capillary volume.
[a] 100% predicted = 166 (DLNO), 33.5 (DLCO), 166 (DmCO) 75 (Vc), 2.22 (DmCO/Vc), 80 (Rrc/Rtot%): units are mL min^{-1} mmHg^{-1}, except for Vc (mL) and DmCO/Vc and Rrc/RTot (dimensionless).

Table 2
Effects of acute and chronic exposure to the hypoxia of altitude in healthy lowlanders and highlanders, except for one study of highlanders with chronic mountain sickness (CMS). All results are as % of sea level controls for each study. Values <90% > 110% are in bold-italic. Dm_{CO} and Vc computed from DL_{NO} and DL_{CO} using Eqs. (3) and (4).

Authors	Subjects	n	Altitude exposure	DL_{NO}/DL_{CO}	DL_{NO} %	DL_{CO} %	Dm_{CO} %	Vc%
A.1 Martinot et al. (2013)	Lowlanders	25	2–3 days at 4300 m. Peru.	92	***121***	***131***	108	***138***
B.1 Faoro et al. (2014)	Lowlanders	13	2–4 days at 5,0050 m. Nepal.	93	103	***112****	94	***120***
C.1 de Bisschop et al. (2010)	Lowlanders	16	4 days at 4000 m. Bolivia.	***86***	***84***	97*	73	***111***
D.1 Groepenhoff et al. (2012)	Lowlanders	15	4 days at 4300 m. Peru.	***83***	***127***	***155***	102	***183***
A.2 Martinot et al. (2013)	Lowlanders	25	7–8 days. 4300 m	100	107	107	108	107
E. Taylor et al. (2016)	Lowlanders	7	40 days at 5150 m. Nepal.	106	***117***	110	***126***	104
C.2 de Bisschop et al. (2010)	Highlanders	8	4000 m. Bolivia.	***83***	110	***133*** (Hb 17.1)	***129***	***161*** ***(137**)***
B.2 Faoro et al. (2014)	Highlanders	28	5150 m. Nepal	***82***	***153***	***185*** (Hb 17.6)	***125***	***222*** ***(196**)***
D.2 Groepenhoff et al. (2012)	Highlanders	15	4300 m. Peru.	***79***	***132***	***167*** (Hb 18.0)	101	***208*** ***(169**)***
D.3 Groepenhoff et al. (2012)	Highlanders with CMS	13	4300 m. Peru.	***77***	***148***	***194*** (Hb 24.0)	110	***253*** ***(154**)***

* Corrected for polycythaemia to the standard haemoglobin level (13.4–14.6 g dL^{-1}).

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Table 3
Clinical studies of DL_{NO}/DL_{CO} ratios with related values and indices. All values are as percent of study controls. Dm_{CO}/Vc, Dm_{CO} and Vc were computed for patients and controls using Eqs. (3) and (4), and expressed as % of study controls. Bold italic ≤ two–thirds (67%) of control. Rows arranged in descending order of DL_{NO}/DL_{CO} ratios (% control) and in three sections (A > 110%, B ≤ 110% ≥ 95%, C < 95%).

Authors	Diagnosis	N[1]	DL_{NO}/DL_{CO}	DL_{NO} %	DL_{CO} %	Dm_{CO}/Vc	Dm_{CO} %	Vc%
GROUP A								
van der Lee et al. (2006)	PAH[2]	26	114	***58***	***65***	148	***69***	***40***
Borland et al. (1996)	PAH[3]	12	111	***65***	***62***	143	82	***57***
Degano et al. (2009)	HPS[4]	11	111	71	***66***	127	80	***63***
GROUP B								
van der Lee et al. (2009)	COPD: GOLD 1[5]	68	110	95	86	110	95	86
van der Lee et al. (2009)	Smokers: GOLD 0[6]	168	107	95	89	118	102	86
Magini et al. (2015)	CHF[7]	50	107	82	77	126	88	74
van der Lee et al., 2006	ILD[8]	41	105	***58***	***65***	117	***62***	***52***
Zavorsky et al. (2008a,b)	Morbid obesity	10	103	108	95	86	95	111
Farha et al. (2013)	PAH[3]	28	100	70	71	97	70	71
van der Lee et al. (2009)	COPD: GOLD 2[5]	26	97	88	86	91	80	89
GROUP C								
Moinard and Guénard (1990)	COPD: GOLD 3–4[5]	10	94	***52***	***56***	82	***48***	***59***
Barisione et al. (2016)	NSIP[9]	30	91	***52***	***58***	70	***45***	***64***
Barisione et al. (2016)	UIP–ILD[10]	30	89	***32***	***37***	***63***	***27***	***42***
Barisione et al. (2014)	BMT[11]	40	87	70	80	68	***62***	72
Dressel et al. (2009)	Cystic fibrosis	21	86	77	87	68	***67***	99
Phansalkar et al. (2004)	ILD[8] (sarcoidosis)	25	80	***35***	***43***	***52***	***28***	***54***

[1] number of patients in each study.
[2] pulmonary artery hypertension, 77% of whom had chronic thromboembolic disease.
[3] idiopathic peripheral pulmonary arterial hypertension.
[4] hepatopulmonary syndrome based on arterial hypoxaemia and positive contrast-enhanced echocardiography.
[5] GOLD staging of COPD severity ($FEV_1/FVC < 0.7$). GOLD 1 = FEV_1% predicted ≥ 80%: GOLD 2 = FEV_1% predicted < 80%: GOLD 3–4 = FEV_1% predicted < 50%.
[6] GOLD 0: asymptomatic smokers without airflow obstruction ($FEV_1/FVC > 0.7$).
[7] chronic heart failure.
[8] diffuse interstitial lung disease with fibrosis.
[9] non-specific interstitial pneumonia, associated with inflammation and fibrosis.
[10] usual interstitial pneumonia with lung fibrosis.
[11] patients selected for bone marrow transplantation for haematological malignancies.

cence analyser with the conventional 10 s BHT (van der Lee et al., 2007). Thus, differences exist in a "standard" value for DL_{NO}, DL_{CO} and DL_{NO}/DL_{CO} for these studies. For example, for a healthy male, aged 45 years, height 1.75 m, DL_{NO} varies from 143 (van der Lee et al., 2007) to 175 (Aguilaniu et al., 2008) mL min^{-1} $mmHg^{-1}$, and the DL_{NO}/DL_{CO} ratio varies from 4.4 (van der Lee et al., 2007) to 5.16 (Zavorsky et al., 2008a,b). In clinical studies (Tables 2 and 3), where the number of healthy controls is very much less, mean DL_{NO} for each control group varied from 89 to 178 mL min^{-1} $mmHg^{-1}$ and mean group DL_{NO}/DL_{CO} from 3.9 to 5.44.

Nevertheless, the ERS Task Force on DL_{NO} standardisation (Zavorsky et al., 2017) has analysed the individual results for 490 Caucasian subjects from the three studies mentioned (67 outliers including 22 non-Caucasians were excluded) and produced combined reference equations for DL_{NO}, DL_{CO}, VA, K_{NO} and K_{CO}. From the combined data, reference values for Dm_{CO}, Dm_{CO}/Vc and Vc were calculated using the best available values for θ_{NO} and θ_{CO} (Guénard et al., 2016); these reference values have been used in the percent predicted estimates in Table 1, and in the calculation of Dm_{CO} and Vc, Dm_{CO}/Vc in Figs. 1 and 2, and Tables 2 and 3. No predictions were possible for the DL_{NO}/DL_{CO} ratio, since age^2, height and sex contributed less than 5% to the variance. Thus, for clinical studies (Tables 2 and 3) the group mean DL_{NO}/DL_{CO} has been referenced to the group mean DL_{NO}/DL_{CO} of locally sourced healthy controls.

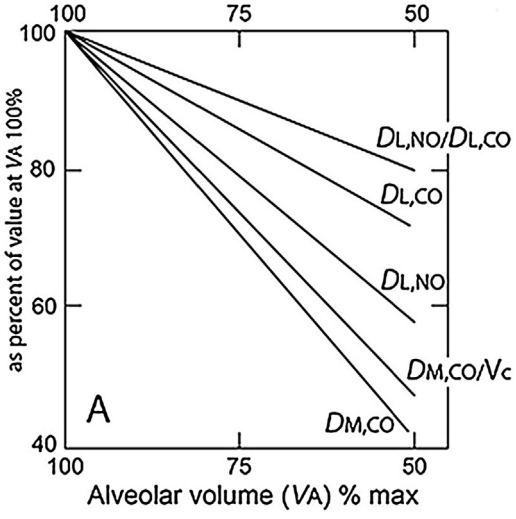

Fig. 2. Plot of diffusing capacities for nitric oxide (NO) and carbon monoxide (CO) and their ratio (DL_{NO}/DL_{CO}), membrane diffusing capacity for carbon monoxide (Dm_{CO}), and the Dm_{CO}–pulmonary capillary volume ratio (Dm_{CO}/Vc) (on the ordinate) versus alveolar volume (~lung volume minus anatomic dead space) as % maximum (~TLC minus dead space) while normal subjects voluntarily changed lung expansion (50% VA max is about FRC). Note DL_{NO}, Dm_{CO} and Dm_{CO}/Vc are more volume sensitive than DL_{CO} itself, implying that Vc is independent of lung expansion change, and that DL_{CO} is relatively independent, due to the influence of Vc. The Figure was reproduced from Zavorsky et al. (2017), and data were derived from van der Lee et al. (2007) and Hughes and van der Lee (2013).

5.2. Ageing

It is well known that DL_{NO} and DL_{CO} decline with increasing age. This is due to the K_{CO} (or K_{NO}) component of DL (=VA·K), not the VA component. Since VA is common to DL_{NO} and DL_{CO}, the effects of ageing on DL_{NO}/DL_{CO} should depend on the rate of change in the K_{NO}/K_{CO} ratio. Hughes and van der Lee (2013) found essentially no change in DL_{NO}/DL_{CO} which is in agreement with later analyses (Zavorsky et al., 2017). Age independence is a benefit when DL_{NO}/DL_{CO} ratios in the elderly are being considered.

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5.3. Exercise

The ERS–ATS Task Force (Zavorsky et al., 2017) has reviewed DL_{NO} exercise studies. DL_{NO} and DL_{CO} increase linearly as cardiac output and oxygen consumption increase, but with more scatter for the DL_{NO} relationship. With exercise, pulmonary vascular pressures increase and more alveolar surface is available for gas exchange from the opening up of closed capillary units in the alveolar septa (recruitment) and dilatation of already patent vessels. This recruitment and dilation increases Dm_{NO} and Dm_{CO} ("more" membrane now takes part in NO and CO transfer). In addition, DL_{CO} will increase as capillary volume (Vc) increases (this will be a smaller effect for DL_{NO}). As a result, the DL_{NO}/DL_{CO} ratio decreases linearly with increasing power output (Tamhane et al., 2001) by about 17–28% from rest to maximum exercise, or by 0.06 units for each 1.0 L min^{-1} increase in cardiac output (Zavorsky et al., 2007). In sarcoidosis with parenchymal fibrosis, the DL_{NO}/DL_{CO} ratio fell similarly from rest to exercise (Phansalkar et al., 2004).

5.4. Change in lung expansion (ΔVA)

In healthy subjects, DL_{NO} and DL_{CO} are measured at maximum inflation, i.e. at a breath hold at total lung capacity (TLC). In the single breath test, this breath hold lung volume is measured by inert gas dilution from gas expired after the breath hold. A subtraction of "non-gas exchanging" volume is made (instrumental plus anatomic dead space) and an "alveolar volume" (VA) calculated. This VA at TLC (~VAmax) is about 94% (SD 7%) of a separately measured TLC by multi–breath dilution (Roberts et al., 1990; van der Lee et al., 2007); this difference (from 100%) is 3–4% greater than expected from the anatomic dead space, and reflects incomplete alveolar mixing in the 10 s breath hold time.

Normal subjects can voluntarily stop the initial inspiration to TLC at a submaximal volume (VA50%max to VA90%max) and DL_{NO} and DL_{CO} and their components measured at different levels of alveolar expansion (van der Lee et al., 2007). In Fig. 2, as the alveoli reduce in size (down to 50% VAmax), the reduction in DL_{NO} exceeds that of DL_{CO} and the DL_{NO}/DL_{CO} ratio falls. This is because ΔDL_{NO} is more driven by ΔDm_{NO} than the more Vc-weighted DL_{CO}. Vc changes little as the lung becomes smaller; for example, the change in Dm_{CO}/Vc is almost the same as the change in Dm_{CO} itself. In the clinical setting, extrapulmonary restriction which reduces VA to 50% max should reduce the DL_{NO}/DL_{CO} ratio to 80% predicted.

5.5. Implication for DL_{NO}/DL_{CO} if VA is reduced other than by expansion loss

VA can be reduced by alveolar destruction or filling with fluid or inflammatory tissue. This may be local (e.g. pneumonectomy) or diffuse. A 50% loss of VA in pneumonectomy results in a DL_{CO} of 60% of predicted max, not 50%, because the blood flow and volume per unit alveolar volume in the remaining lung increases DL_{CO} by capillary dilatation and recruitment (Hughes and Pride, 2012). This "compensatory" effect (mediated by an increase in K_{CO} to 110–120% predicted) might be less for DL_{NO} (though DL_{NO} is also blood volume sensitive), so DL_{NO}/DL_{CO} might fall; there is no data one way or the other. A third cause of VA reduction is poor distribution of the inhaled marker gases; this occurs in airflow obstruction when the separately measured TLC exceeds the single breath VA. The effect on DL_{NO}/DL_{CO} is difficult to predict.

5.6. DL_{NO}/DL_{CO} ratios in altitude-induced hypoxia

There have been several studies of the effects of acute and chronic exposure to the hypoxia of altitude dwelling (summarised in Table 2). All values, including those for permanent residents (highlanders) are expressed as percent of that at sea level in the "lowlanders". Except for study A.2 (Table 2) the effect of acute (2–4 days) altitude exposure (rows A.1, B.1, C.1, D.1) is a fall in DL_{NO}/DL_{CO}, but the changes in the various components in Table 2 are more variable; the most consistent change is a rise in Vc, probably due to an increase in cardiac output and pulmonary capillary recruitment and dilatation. After 40 days exposure (study E), Vc appears to have returned to the sea level value, but this is only a single study. In a comparison of lowlanders and highlanders (acute versus chronic exposure) – B.1-2, C.1-2 and D.1-2 – highlanders have large increases in DL_{NO}, DL_{CO}, Dm_{CO} and Vc, with reductions in DL_{NO}/DL_{CO}. Highlanders have secondary polycythaemia, which is particularly marked in those with chronic mountain sickness (CMS) – D.3. The high haemoglobin explains a large part of the high DL_{CO} and Vc.

The main changes are probably a) an increase in pulmonary blood flow (~cardiac output) on acute exposure (high DL_{NO}, DL_{CO} and Vc) and b) secondary polycythaemia increasing DL_{CO} and Vc with chronic (lifelong) exposure through an expansion of the alveolar membrane surface (↑ Dm_{CO} in 2/3 studies); interestingly, Faoro et al. (2014) reported an increased VA in highlanders, as did Martinot et al. (2013) in 2/3 day exposure in lowlanders. Capillary recruitment (↑DL_{CO} and ↑Vc) must also occur.

5.7. DL_{NO}/DL_{CO} ratios in pulmonary and cardiac disease

Most of the clinical studies to date are listed in Table 3. Each study had their own controls (normal subjects) and all patient values are reported as "% control". This is important because, at this early stage in the development of the DL_{NO}, the "control" DL_{NO}/DL_{CO} varies widely from study to study (from 3.9 to 5.4).

In Table 3 (Group A), high DL_{NO}/DL_{CO} (>110% control) ratios plus DL_{CO} and/or DL_{NO} values <67% predicted normal are associated with pulmonary vascular disease, either pulmonary arterial hypertension or the pulmonary capillary remodelling and dilatation of the hepatopulmonary syndrome (HPS). In all three studies, DL_{NO} and DL_{CO} values are low, but the Dm_{CO}/Vc ratio is high. The reduction in Vc is greater than the reduction in the membrane conductance (Dm_{CO}).

In Group B (Table 3), DL_{NO}/DL_{CO} ratios are normal (110–97% control). Those studies with a reduced DL_{CO} (<80% control) are also associated with pulmonary hypertension, either arterial (Farha et al., 2013) or venous hypertension (chronic heart failure, Magini et al., 2015). The Magini et al. (2015) study (Dm_{CO} and Vc have been recalculated via the on-line supplement in Zavorsky et al., 2017) is interesting because the reduction in Vc is greater than the reduction in Dm_{CO}. In previous studies, using the normoxic–hyperoxic DL_{CO} analysis, the opposite has been found (Puri et al., 1995). The van der Lee et al. (2006) study in ILD will be discussed later.

In Group C, a low DL_{NO}/DL_{CO} (<95% control) was seen in destructive lung disease. Where the DL_{CO} and/or DL_{NO} values were <67% predicted normal (or, for Barisione et al., 2014; Dressel et al., 2009, where only the Dm_{CO} was ≤67% predicted), the pathological process was (in 4/6 examples) interstitial lung disease with diffuse fibrosis, or in one instance (Moinard and Guénard, 1990) destructive emphysema. The study of van der Lee (2006) in ILD (mostly sarcoidosis) with a *normal* DL_{NO}/DL_{CO} ratio (Group B), but reduced DL_{NO} and DL_{CO}, is an anomaly; the computed Dm_{CO}% was greater than the Vc%, whereas in the last four studies in Group C (with DL_{NO}/DL_{CO} < 95% controls) the opposite was the case. At present, there is no obvious explanation.

In summary, a high DL_{NO}/DL_{CO} is associated with pulmonary vascular disease and a low DL_{NO}/DL_{CO} with alveolar destruction (emphysema or fibrosis). The pattern of changes in Dm_{CO}/Vc (Table 3) and in Rrc/Rtot% (data not shown) mirror those of DL_{NO}/DL_{CO}, but Dm_{CO}/Vc and Rrc/Rtot% are computed variables, whereas DL_{NO}/DL_{CO} is directly measured.

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6. Conclusions

1. The ultra–rapid reaction of NO with red cell haemoglobin implies that NO conductance (DL_{NO}) from alveolar gas to pulmonary capillary blood mostly measures the alveolar–capillary membrane diffusing capacity (Dm), but because of the slower reaction of CO with oxygenated haemoglobin, DL_{CO} mainly reflects red cell conductance, and will be reduced when pulmonary capillary volume (Vc) is compromised.
2. The DL_{NO}/DL_{CO} ratio, according to the Roughton and Forster (1957) equation, is positively related to the Dm_{CO}/Vc ratio and to the CO red cell resistance fraction (Rrc/Rtot) in a curvilinear manner (Fig. 1).
3. The relationship between DL_{NO}/DL_{CO} and Dm_{CO}/Vc or Rrc/Rtot% is independent of the absolute values of DL_{NO} or DL_{CO} (Table 1).
4. The response of lowlanders to acute exposure to high altitude is variable, but there is a trend for a reduced DL_{NO}/DL_{CO} and a high Vc, probably due to capillary recruitment. With longer exposure, and in native highlanders, there is secondary polycythaemia, and a larger fall in DL_{NO}/DL_{CO} and rise in Vc, even after Hb correction (Table 2).
5. In clinical studies, three patterns emerged for the DL_{NO}/DL_{CO} ratio: A) high (≥110% predicted), associated with pulmonary vascular disease, B) normal (<110% >95%) in mild to moderate COPD, chronic heart failure and morbid obesity, C) low (≤95%) associated with moderate to severe COPD, cystic fibrosis, but predominantly with interstitial lung disease with fibrosis (Table 3).
6. Because of a wide spread of DL_{NO}/DL_{CO} values in healthy controls between studies (ranging from 3.9 to 5.4), we recommend each laboratory uses their own healthy controls..

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