Eur Respir J 2013; 41: 453–461
DOI: 10.1183/09031936.00082112

SERIES "PHYSIOLOGY IN RESPIRATORY MEDICINE"
Edited by R. Naeije, D. Chemla, A. Vonk Noordegraaf and A.T. Dinh-Xuan
Number 2 in this Series

The $T_{L,NO}/T_{L,CO}$ ratio in pulmonary function test interpretation

J. Michael B. Hughes* and Ivo van der Lee#

ABSTRACT: The transfer factor of the lung for nitric oxide ($T_{L,NO}$) is a new test for pulmonary gas exchange. The procedure is similar to the already well-established transfer factor of the lung for carbon monoxide ($T_{L,CO}$). Physiologically, $T_{L,NO}$ predominantly measures the diffusion pathway from the alveoli to capillary plasma. In the Roughton–Forster equation, $T_{L,NO}$ acts as a surrogate for the membrane diffusing capacity (D_M). The red blood cell resistance to carbon monoxide uptake accounts for ~50% of the total resistance from gas to blood, but it is much less for nitric oxide.

$T_{L,NO}$ and $T_{L,CO}$ can be measured simultaneously with the single breath technique, and D_M and pulmonary capillary blood volume (V_c) can be estimated. $T_{L,NO}$, unlike $T_{L,CO}$, is independent of oxygen tension and haematocrit. The $T_{L,NO}/T_{L,CO}$ ratio is weighted towards the D_M/V_c ratio and to α; where α is the ratio of physical diffusivities of NO to CO (α=1.97). The $T_{L,NO}/T_{L,CO}$ ratio is increased in heavy smokers, with and without computed tomography evidence of emphysema, and reduced in the voluntary restriction of lung expansion; it is expected to be reduced in chronic heart failure. The $T_{L,NO}/T_{L,CO}$ ratio is a new index of gas exchange that may, more than derivations from them of D_M and V_c with their in-built assumptions, give additional insights into pulmonary pathology.

KEYWORDS: Carbon monoxide, diffusing capacity, lung function in disease, nitric oxide, transfer factor

AFFILIATIONS
*National Heart and Lung Institute, Imperial College School of Medicine, Hammersmith Hospital, London, UK.
#Dept of Pulmonary Diseases, Spaarne Hospital, Hoofddorp, The Netherlands.

CORRESPONDENCE
J.M.B Hughes
4 Cedars Road
London
SW13 0HP
UK
E-mail: mike.hughes@imperial.ac.uk

Received:
May 25 2012
Accepted after revision:
Aug 06 2012
First published online:
Aug 30 2012

The classical technique for measuring gas transfer from the alveolus to the pulmonary capillary blood is the single breath transfer factor of the lung for carbon monoxide ($T_{L,CO}$), but known in North America as the diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$). In the last two decades, the single breath measurement of diffusing capacity of the lung for nitric oxide ($T_{L,NO}$ or $D_{L,NO}$) has been introduced [1, 2]. Since the work of ROUGHTON and FORSTER [3], the model for gas transfer from alveolus to blood consists of two resistances in series:

$$1/T_L\,(1/D_L) = 1/D_M + 1/\Theta_{bl}\cdot V_c \qquad (1)$$

where $1/T_L$ is the total resistance to gas transfer ($mmol^{-1}\cdot min\cdot kPa$ in SI units or $mL^{-1}\cdot min\cdot mmHg$ in traditional units), $1/D_M$ is the resistance to passive diffusion across the alveolar–capillary membrane and intracapillary plasma (D_M is the membrane diffusing capacity), and $1/\Theta_{bl}\cdot V_c$ is the resistance to gas transfer of the red blood cell, which includes, for reactive gases such as carbon monoxide (CO) and nitric oxide (NO), chemical combination with the red blood cell haemoglobin (Hb) ($1/\Theta_{bl}$ is the resistance of red blood cells to gas transfer, *e.g.* CO or NO, per mL of blood and V_c is the pulmonary capillary blood volume measured in mL). Θ_{bl} is the specific transfer conductance of blood (measured *in vitro*) for a specified gas.

For CO these two resistances ($1/D_M$ and $1/\Theta_{bl}\cdot V_c$) are approximately equal. For NO, the total resistance to alveolar–capillary transfer ($1/T_L$) is much less, ~20–25% of that for CO, thus $T_{L,NO}$ is four to five times greater than $T_{L,CO}$, and the resistance

Previous articles in this series: No. 1: Naeije R, Vachiery J-L, Yerly P, *et al.* The transpulmonary pressure gradient for the diagnosis of pulmonary vascular diseases. *Eur Respir J* 2013; 41: 217–223.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

resides mostly in the $1/D_M$ component. This occurs for two reasons: 1) the physical diffusivity of NO is approximately twice that of CO and its resistance ($1/D_M$) is half; and 2) the rate of combination of NO with blood *in vitro* is considerably faster than for CO [4]. Because the blood cell resistance for NO is low compared to the membrane resistance, the measurement of $T_{L,NO}$ has been regarded as a surrogate for D_M. In essence, $T_{L,NO}$ measures D_M and $T_{L,CO}$ measures D_M and $\Theta_{bl}\cdot V_c$; thus, the $T_{L,NO}/T_{L,CO}$ ratio will be weighted towards the $D_M/\Theta_{bl}\cdot V_c$ ratio times the ratio of diffusivities for NO and CO.

In this issue of the series we review measurements of the $T_{L,NO}/T_{L,CO}$ ratio that have been reported in normal subjects and in various respiratory and pulmonary vascular conditions. It should be noted that the $T_{L,NO}/T_{L,CO}$ ratio is equivalent to the ratio of the transfer coefficients for NO (K_{NO}) *versus* CO (K_{CO}) because $T_L = K \times$ alveolar volume (V_A), where K is the rate of uptake per min·mmHg^{-1} for NO tension (P_{NO}) or CO tension (P_{CO}), and V_A is common to $T_{L,NO}$ and $T_{L,CO}$.

PHYSIOLOGICAL DETERMINANTS OF $T_{L,NO}$ AND K_{NO}

There are important differences in the way NO and CO are handled by tissues and blood namely: 1) the diffusivity (solubility/MW^2) of NO in plasma is 1.97 times that of CO, and 2) the rate of NO uptake per mmHg of NO tension per mL of blood, *i.e.* its specific conductance (Θ) [4], is 5.75 times faster than the uptake of CO at a P_{O_2} of 100 mmHg [3]. The chemical reactions of NO and CO with blood are also different. For example, NO reacts directly with the oxygen of oxyhaemoglobin to form a nitrate plus a deoxygenated form of Hb called methaemoglobin (metHb) in which the iron atoms of the haem ring are oxidised from the ferrous (Fe^{++}) to the ferric (Fe^{+++}) form [5]:

$$NO + Hb(Fe^{++})O_2 \rightarrow metHb(Fe^{+++}) + NO_3^- \quad (2)$$

CO does not react with O_2 but competes with oxygen for the Fe^{++} site on the haem ring:

$$CO + Hb(Fe^{++})O_2 \rightarrow Hb\ (Fe^{++})CO + Hb(Fe^{++})O_2 \quad (3)$$

The increased affinity of CO for Hb (~220 times that for O_2) is due to the different angles of attachment of CO and O_2 to the haem ring [6]. NO and CO are tightly bound to Hb through their extremely slow dissociation constants. Unlike NO, the rate of reaction of CO with oxyhaemoglobin is P_{O_2} dependent; once Hb is saturated with oxygen, the specific resistance reaction rate ($1/\Theta$) is linearly related to P_{O_2}. This is the basis of the Roughton–Forster formulation (equation 1). $T_{L,NO}$, on the other hand, is independent of the level of alveolar P_{O_2} (P_{A,O_2}) [7] because NO reacts directly with haemoglobin (equation 2) rather than competing with oxygen for Hb binding sites (equation 3).

IS THERE SIGNIFICANT BLOOD RESISTANCE TO NO UPTAKE?

Investigators have cited the rapid reaction of NO with Hb (250 times faster than CO) as a reason for considering $T_{L,NO}$ to be a surrogate for D_M [1]. The assumption that $\Theta_{bl,NO}$ for red blood cells is infinite cannot, in theory, be correct because of the advancing front phenomenon, *i.e.* the reaction rate of NO with Hb is so high that, according to MORRIS and GIBSON [8], ''effectively every molecule of NO which enters the reaction radius is captured [instantaneously] by a heme group. The observed rate [Θ_{bl}] would then be a measure of the rate of diffusion to the site.'' This means that a diffusion pathway, either across the red blood cell membrane or within the substance of the cell, or both, is an essential component of $\Theta_{bl,NO}$.

Experimentally, red blood cell lysis (by the addition of water to blood in a membrane oxygenator model of NO and CO transfer [9]), or red blood cell substitution, in anaesthetised dogs, with cell-free haem based oxyglobin [10] increased $T_{L,NO}$ substantially, but hardly altered $T_{L,CO}$. This suggested, for NO uptake, that there was significant resistance in the red blood cell membrane, or its interior, or in a stagnant layer of plasma immediately surrounding the cell, and separate from any resistance stemming from the chemical combination with haemoglobin; conversely, most of the red blood cell resistance to CO uptake appeared to be associated with the haemoglobin molecule itself. Unlike $T_{L,CO}$, $T_{L,NO}$ is unaffected by changes in P_{A,O_2} [7]; as already mentioned, this is not surprising considering the chemistry involved (equation 2), but it supports the notion that the red blood cell resistance to NO uptake is independent of the haemoglobin molecule. In addition, $T_{L,NO}$ but not $T_{L,CO}$ seems to be relatively independent of the haemoglobin concentration in blood [11]. BORLAND *et al.* [10] estimated that 37% of the resistance to NO uptake lies in the $1/\Theta_{bl}\cdot V_c$ component (~50–60% for CO uptake), but this figure must be treated with caution as it involved exchange transfusion in dogs, substituting bovine Hb-glutamer-200 (a cell-free blood substitute) for whole blood. To conclude, significant blood resistance to NO uptake exists, both for theoretical reasons and from experimental data, but in absolute terms $1/\Theta_{bl,NO}$ is a small fraction (<16%) of $1/\Theta_{bl,CO}$. Thus, it is not inappropriate to regard the $T_{L,NO}$, much more than the $T_{L,CO}$, as weighted towards D_M.

PHYSIOLOGICAL DETERMINANTS OF THE $T_{L,NO}/T_{L,CO}$ RATIO

If, as a simplifying assumption, $T_{L,NO}$ ''operationally'' equals D_M, the Roughton–Forster equation can be rewritten for NO and CO as follows:

$$1/T_{L,NO} = 1/D_{M,NO} \quad (4)$$

$$D_{M,NO}/D_{M,CO} = \alpha \quad (5)$$

$$1/T_{L,NO} = 1/\alpha\cdot D_{M,CO} \quad (6)$$

where α (=1.97) is the ratio of membrane diffusivities of NO to CO in plasma. Assuming that $1/\Theta_{bl,NO}$ was negligible, GUENARD *et al.* [1] showed that D_M and V_c could be calculated from a single breath manoeuvre with CO and NO as test gases, using a value for Θ_{CO} appropriate for the single breath P_{A,O_2}:

$$1/V_c = \Theta_{CO}\ (1/T_{L,CO} - \alpha/T_{L,NO}) \quad (7)$$

where V_c is calculated in mL and $D_{M,CO}$ is calculated separately from equations 4 and 6. This was a more convenient solution than the ROUGHTON and FORSTER [3] two-step approach at two different P_{A,O_2} values. Reasonable values were found in normal subjects for $D_{M,CO}$ and V_c [1], but the estimates for V_c are dependent on the values chosen for $\Theta_{bl,CO}$ as explained in the Appendix.

Equation 7 can be rearranged (equation 1, adapted for CO uptake, and divided by equation 6) as follows:

$$T_{L,NO}/T_{L,CO} = \alpha(1 + D_{M,CO}/\Theta_{CO}\cdot V_c) \quad (8)$$

This illustrates the dependence of the ratio on $D_{M,CO}/V_c$ since α and $\Theta_{bl,CO}$ (at a given P_{O_2}) are fixed quantities.

Alternatively, if there is finite resistance to red blood cell NO uptake [10], $1/D_{M,NO}$ must decrease, for a fixed value of $1/T_{L,NO}$, when $1/\Theta_{bl,NO}\cdot V_c$ increases from zero, as equation 4 reverts to equation 1. Thus, $D_{M,NO}$ will now exceed $T_{L,NO}$. This increase in $D_{M,NO}$ ($T_{L,NO}$, $T_{L,CO}$ and $D_{M,CO}$ being unchanged) ''forces'' α (in equations 5 and 8) to increase, even though it is a physical constant. Nevertheless, the dependence on the $D_{M,CO}/V_c$ ratio in equation 8 will remain.

GLÉNET *et al*. [12] have presented a diffusion model (in two dimensions) for the $T_{L,NO}/T_{L,CO}$ ratio, which is a rectangular box whose height and width define the thickness of the alveolar–capillary membranes and the thickness of the blood sheet; they show that the $T_{L,NO}/T_{L,CO}$ ratio is related to the tissue diffusivity (for NO) and inversely to the product (approximately the area of the box) of the thickness of the blood and tissue sheets, and to $\Theta_{bl,CO}$. The sheet is thicker at functional residual capacity, mainly due to increased blood thickness (V_c/V_A), and thinner with continuous positive pressure breathing or haemodilution; in all cases the $T_{L,NO}/T_{L,CO}$ ratio changed appropriately. Thus, one would predict that in extrapulmonary restriction the $T_{L,NO}/T_{L,CO}$ ratio ($\sim K_{NO}/K_{CO}$) would fall and that this might be clinically useful, and this prediction is supported by measurements in normal subjects at different levels of lung expansion (fig. 1b).

$D_{M,CO}$ AND V_c FROM SIMULTANEOUS SINGLE BREATH $T_{L,NO}$ AND $T_{L,CO}$

Using equation 6, $D_{M,CO}$ can be calculated if $T_{L,NO}$ and α are known, on the assumption that the blood resistance to NO uptake ($1/\Theta_{bl,NO}\cdot V_c$) is 0, V_c can then be derived from the Roughton–Forster equations if Θ_{CO} at a P_{O_2} of 100 mmHg is known (equation 7). Nevertheless, there are several uncertainties in this calculation of V_c. There are seven separate equations [15], differing in slope and intercept, for the expression $1/\Theta_{CO} = \alpha\cdot P_{O_2} + \beta$, all measured *in vitro* under different experimental conditions, with α being a temperature and pH-dependent coefficient linked to the reaction of CO with Hb. β is related to λ, the ratio of the permeability of the red blood cell membrane to the interior of the cell, but may also depend on stagnant layers of plasma adjacent to the cell [16]. Thus, $1/\Theta_{CO}$ a P_{O_2} 100 mmHg (13.3 kPa) may vary from 0.82 to 1.71 $min^{-1}\cdot mmHg^{-1}$. Another variable is the $D_{M,NO}/D_{M,CO}$ ratio (α) which, on physical principles, should be in the range 1.93–1.97. Investigators have ''forced'' α to 2.42 [17] or 2.08–2.26 [18] to give a ''best fit'' with the $D_{M,CO}$ and V_c calculated from the oxygen two-step Roughton–Forster $T_{L,CO}$ method. Since α is defined as the physical diffusivity ratio of NO/CO, this approach cannot be correct physiologically. A third uncertainty is the $D_{M,NO}/T_{L,NO}$ ratio, generally assumed on the basis of the zero blood cell resistance to NO uptake to be 1.0 [1], although values of 1.57 have been measured experimentally [10], albeit under rather artificial conditions of red cell substitution with cell-free haem oxyglobin. The dependence of estimates of pulmonary capillary volume (V_c), on Θ_{CO} and the

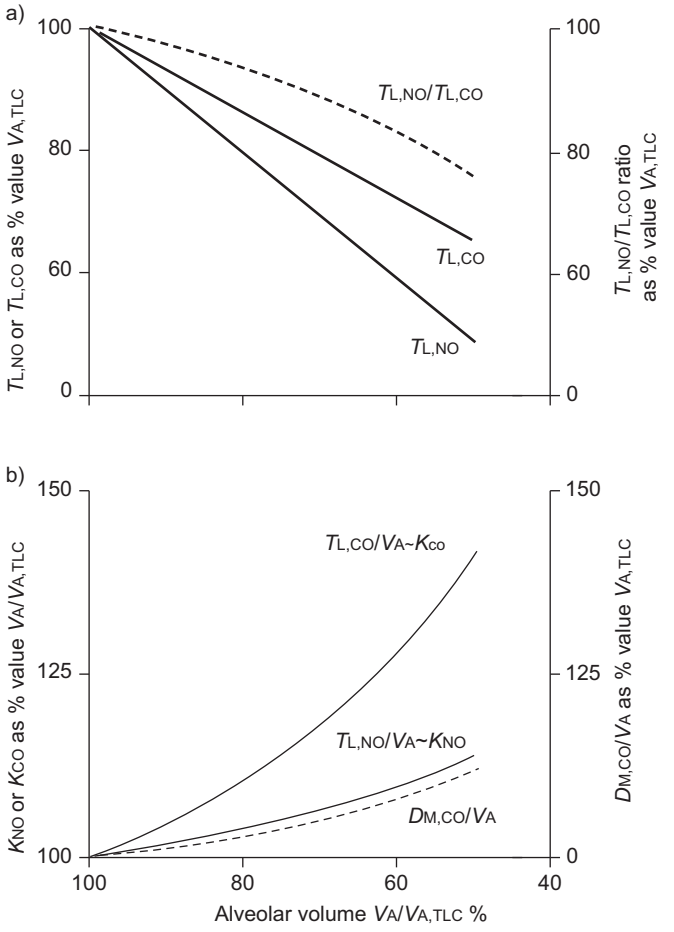

FIGURE 1. Effect of voluntary reduction of lung volume from total lung capacity (TLC) in normal subjects on a) transfer factor of the lung for nitric oxide ($T_{L,NO}$) and carbon monoxide ($T_{L,CO}$) and $T_{L,NO}/T_{L,CO}$ ratio, and b) transfer coefficient of the lung (K) for nitric oxide (K_{NO}; $\sim T_{L,NO}$/alveolar volume (V_A)) or carbon monoxide (K_{CO}; $\sim T_{L,CO}/V_A$), and for membrane diffusing capacity per unit volume for CO ($D_{M,CO}$)/V_A. Lung expansion expressed as single breath V_A as per cent V_A at maximal inflation ($V_{A,TLC}$). Note larger rise in K_{CO} (*versus* K_{NO}) with diminished expansion in (b), which buffers decline of $T_{L,CO}$ *versus* $T_{L,NO}$ in (a), causing a fall in $T_{L,NO}/T_{L,CO}$ ratio, as would occur in extrapulmonary restriction. Data taken from a) [13] and b) [14].

NO red blood cell resistance proportion, for fixed values of $T_{L,NO}$ and the $T_{L,NO}/T_{L,CO}$ ratio, is shown in the Appendix where $T_{L,NO}$ at rest (144 $mL\cdot min^{-1}\cdot mmHg^{-1}$) is taken from ZAVORSKY *et al*. [19] and the $T_{L,NO}/T_{L,CO}$ ratio (4.5) from the average of eight studies.

In the Appendix we show that calculations of V_c, from simultaneous $T_{L,NO}$ and $T_{L,CO}$ measurements, using equation 7, are very dependent on the choice of $1/\Theta_{bl,CO}$ and that $D_{M,CO}$ is dependent on the value chosen for the blood resistance fraction of NO uptake ($(1/\Theta_{bl,NO}\cdot V_c)/(1/T_{L,NO})$). We propose, therefore, that calculations of $D_{M,CO}$ and V_c from simultaneous measurement of $T_{L,NO}$ and $T_{L,CO}$ be set aside until there is more consensus concerning the $1/\Theta_{CO}$–P_{O_2} relationship and the $D_{M,NO}/T_{L,NO}$ ratio. The $T_{L,NO}/T_{L,CO}$ ratio avoids these uncertainties and assumptions; it also has the advantage that it represents the K_{NO}/K_{CO} ratio (V_A being

TABLE 1 Literature review of values in normal subjects for transfer factor of the lung for nitric oxide ($T_{L,NO}$), transfer factor of the lung for carbon monoxide ($T_{L,CO}$) and $T_{L,NO}/T_{L,CO}$ ratio

First authors [ref.]	Male/female	$T_{L,NO}$ mmol min^{-1} kPa^{-1}			$T_{L,CO}$ mmol·min^{-1} kPa^{-1}			$T_{L,NO}/T_{L,CO}$ ratio		
		Total	Male	Female	Total	Male	Female	Total	Male	Female
VAN DER LEE [13]	65/59		54±8.7	39±6.3		12±2.2	9.2±1.6		4.6±0.5	4.3±0.4
AGUILANIU [20]#	161/142		70	61		14.4	12.8		4.85+	4.8+
ZAVORSKY [21]¶	66/64		56	45		10.8	8.8		5.19+	5.13+
GUENARD [1]	7/7		52±6.7	39±2		10±0.5	7.3±0.37		5.2+	5.3+
ZAVORSKY [19]	10	46±8.9			8.5±1.5			5.4±0.3		
GLÉNET [12]	20/7	64±13			13.2±2.8			4.9±0.3		
ZAVORSKY [22]	8/0	70±6.1			15.4±1.5			4.6±0.1		
DE BISSCHOP [23]	8/8	57±12			13±2.3			4.4±0.3		
VAN DER LEE [24]	35/36	48±11			10.9±2.4			4.36±0.6		
DEGANO [25]	27/8	40±6.7			9.0±1.3			4.34±0.33		
DRESSEL [26]	13/8	35±12			9.1±2.7			3.8±0.4		

Data are presented as n or mean±SD . #: calculated from regression equations (table 2 [20]) for height 1.75 m and age ⩽59 yrs. ¶: calculated from regression equations (see appendix [19]) for height 1.75 m and age 40 yrs. +: calculated as mean $T_{L,NO}$/mean $T_{L,CO}$.

common to both measurements), which, as rate constants, have a direct bearing on gas exchange efficiency.

$T_{L,NO}$ AND $T_{L,NO}/T_{L,CO}$: NORMAL VALUES AND EFFECTS OF AGEING, LUNG VOLUME AND EXERCISE

We present a literature review of simultaneous measurements of $T_{L,NO}$ and $T_{L,CO}$ in normal subjects in table 1. Although there is a wide spectrum in the mean values between studies (for example, the subjects in ZAVORSKY *et al.* [19] were probably more athletic), it is more pertinent to relate reference values for $T_{L,NO}$ to $T_{L,CO}$ values measured at the same time, as $T_{L,NO}/T_{L,CO}$ ratios. In two large European series [13, 20] (table 1), the $T_{L,NO}/T_{L,CO}$ ratio averaged 4.45 and 4.8, respectively, and in a North American study [21] averaged 5.16. The average value of eight smaller studies [1, 12, 19, 22–26], weighted for numbers, was 4.5. At the present time, each laboratory should establish its own standard for the $T_{L,NO}/T_{L,CO}$ ratio in healthy subjects, although the current consensus is that the ratio is in the range of 4.3–4.9.

Ageing

In the age range 25–55 yrs, VAN DER LEE *et al.* [13] found the $T_{L,NO}/T_{L,CO}$ ratio increased by 0.33% per year, but three other studies [12, 20, 21] found no change in the ratio with ageing. Thus, $T_{L,NO}$ and $T_{L,CO}$ seem to decline with ageing at essentially the same rate.

Lung volume

$T_{L,NO}$ is more sensitive to V_A deflation than $T_{L,CO}$. For example, from $V_{A,max}$ to $V_{A,50\%max}$ the $T_{L,NO}$ declines by 43% *versus* 29% for $T_{L,CO}$ (fig. 1a) [13]. The explanation is that the fall in $T_{L,CO}$ is buffered by a greater increase in K_{CO} (+42%) than K_{NO} (+14%) (fig. 1b) [14]. This is due to a greater decrease in D_M than V_c as lung volume decreases. In other words, a rise in V_c/V_A is the principal reason for the increase of K_{CO} [14]. If K_{NO} ($\sim T_{L,NO}/V_A$) reflects D_M/V_A, the effects of volume change on K_{NO} (fig. 1b) should be similar to $D_{M,CO}/V_A$, as calculated from the Roughton–Forster $D_{L,CO}$ analysis [14]. In fact, at $V_{A,50\%max}$ (as a fraction of the value at $V_{A,max}$), the K_{NO} ratio (1.14) from VAN DER LEE *et al.* [13] is almost the same as the $D_{M,CO}/V_A$ ratio (1.12) from the data of STAM *et al.* [14], although there was considerable inter-subject variability. Figure 1b shows that volume change affects $T_{L,NO}/V_A$ ($\sim K_{NO}$) and $D_{M,CO}/V_A$ in a very similar way, quite differently from $T_{L,CO}/V_A$ ($\sim K_{CO}$), lending further support to the notion that $T_{L,NO}$ is ''effectively'' measuring D_M.

The rise in K_{CO} as lung volume and expansion diminishes is the reason for the fall in the $T_{L,NO}/T_{L,CO}$ ratio (fig. 1a) when lung volume is lowered, and this fall may be a useful marker of extrapulmonary restriction *versus* other pathologies (fig. 2).

Exercise

ZAVORSKY *et al.* [19] have summarised the data from seven studies on the effect of moderate-to-heavy (maximum oxygen uptake 46.5 mL·min^{-1}·kg^{-1}) exercise. There was a linear increase in $T_{L,NO}$ and $T_{L,CO}$, which were highly correlated. The $T_{L,NO}/T_{L,CO}$ ratio decreased by an average of 9% (range -2 to -16%). D_M and V_c both increased on exercise [17], but $T_{L,NO}$ will not share the increase in V_c caused by capillary recruitment and distension, so the $T_{L,NO}/T_{L,CO}$ ratio will fall.

Breath holding time

DRESSEL *et al.* [28] found slightly higher $T_{L,NO}$ values at very low breath holding times of 4 s; this effect has not been reproduced by other researchers. No significant differences were seen between 6- and 8-s breath-holding times [28]. Although there are advantages in sticking to the usual 10 s, for the sake of comparison with previous single breath $T_{L,CO}$ estimations, the sensitivity and response time of some NO analysers (table 2) will force some researchers into accepting a 6- or 8-s breath holding time.

MEASUREMENT OF $T_{L,NO}$ AND K_{NO}: TECHNICAL MATTERS

Most investigators use the single breath technique with breath holding as described for the $T_{L,CO}$ ($D_{L,CO}$) by OGILVIE *et al.* [30],

TABLE 2 Methodological aspects of the transfer factor of the lung for nitric oxide ($T_{L,NO}$) measurement in five reference studies

	VAN DER LEE [13]	AGUILANIU [20]	ZAVORSKY [21]	PHANSALKAR [29]	DRESSEL [26]
Technique	Single breath	Single breath	Single breath	Rebreathing	Single breath
Commercial system	MasterLab Pro (Erich Jaeger[¶])	HypAir (Medisoft[+])	HypAir (Medisoft)		Masterscreen PFT (Viasys[§])
NO analyser					
Make	Chemiluminescence CLD 77AM (Eco Physics[f])	Electrochemical cell (Medisoft)	Electrochemical cell (Medisoft)	Sievers nitric oxide analyzer 280 (Sievers Instruments, Inc.[##])	Electrochemical cell (Viasys)
Specification	Lower limit 0.02 ppb Upper limit 10 ppm Response time 0.1 s	Lower limit 0.1 ppm Upper limit 450 ppm Response time <10 s	Lower limit 0.1 ppm Upper limit 450 ppm Response time <10 s	Response time <0.5 s	Unknown
NO source	750 ppm in N_2	450 ppm in N_2	450 ppm in N_2		448 ppm in N_2
$F_{I,NO}$ ppm	8	40	40	c. 40	45
Other gases %					
Carbon monoxide	0.25	0.28	0.28	0.3	0.28
Helium	9.17	9.47 or 14	9.47 or 14		9.5
Oxygen		19 or 21	19 or 21	30	
Methane				0.3	
Acetylene[#]				0.4–0.8	
Balance gas	Air	N_2	N_2	N_2	Air
Breath hold time s	10	4	5.5	16 (rebreathe)	8
Discard volume mL	750	800	900	NA	750
Sample volume mL	750	600	900	NA	750

$F_{I,NO}$: inspiratory nitric oxide fraction; NA: not available; [#]: used for measuring total pulmonary blood flow; [¶]: Erich Jaeger, Friedberg, Germany; [+]: Medisoft, Dinant, Belgium; [§]: Viasys, Hoechberg, Germany; [f]: Eco Physics, Zurich, Switzerland; [##]: Sievers Instruments, Inc., Boulder, CO, USA.

with the breath-hold time estimated according to JONES and MEADE [31] or GRAHAM *et al.* [32]. Table 2 summarises the technical aspects from the principal reference studies. NO oxidises to NO_2 when in contact with air, so it is stored in a nitrogen tank and dispensed just before use. This reaction is rather slow; therefore, mixing the NO with air in the inspiratory bag does not immediately lead to significant NO_2 formation. NO reacts with certain plastics and connections to and from the dispensing bag, and these connections should be made of polytetrafluoroethylene (*e.g.* Teflon™; DuPont, Wilmington, DE, USA) or stainless steel. BORLAND and HIGENBOTTAM [2] showed that there is no interaction between NO and CO. The commercially available combined $T_{L,NO}$ and $T_{L,CO}$ apparatus has similar values for $T_{L,CO}$ as the traditional $T_{L,CO}$ apparatus when the same subjects are tested on both [32].

Because the rate of uptake from alveolar gas (~K_{NO}) is four to five times faster than for CO (~K_{CO}), breath holding times have, in general, been shorter than the 10 s that is the usual for $T_{L,CO}$. Nevertheless, note that the very sensitive chemiluminescence NO analyser used by VAN DER LEE *et al.* [13] allows them to extend the breath hold time to the usual 10 s, and this increases the accuracy of both the $T_{L,NO}$ and the $T_{L,CO}$ measurements. Endogenous levels of NO and CO are usually ignored. For normal populations a $T_{L,CO}$ and K_{CO} correction for Hb is waived; for clinical studies, a Hb correction to a standard [Hb] is recommended but it is not required for $T_{L,NO}$ and K_{NO} [11]. Smoking is generally forbidden for 24 h before testing because of its effects in raising plasma CO tension (''back-pressure'' effect) and increasing HbCO (''anaemia'' effect), but smoking and CO do not affect the $T_{L,NO}$.

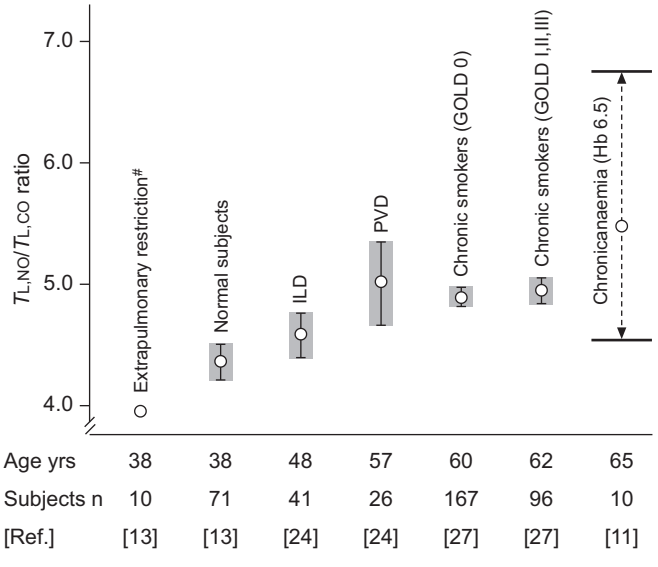

FIGURE 2. Ratio of transfer factor of the lung for nitric oxide ($T_{L,NO}$) to transfer factor of the lung for carbon monoxide ($T_{L,CO}$) in normal subjects at full inflation and with voluntary reduction of lung volume (mimicking ''extrapulmonary restriction'') and in different clinical situations. ILD: interstitial lung disease; PVD: pulmonary vascular disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; Hb: haemoglobin. Data are presented with SEM±2 error bars. Dashed line represents range. [#]: alveolar volume/alveolar volume total lung capacity 0.7.

THE $T_{L,NO}/T_{L,CO}$ RATIO ($\sim K_{NO}/K_{CO}$) IN DISEASE

The $T_{L,NO}/T_{L,CO}$ ratio can be normal, increased or decreased. A normal $T_{L,NO}/T_{L,CO}$ ratio does not exclude a pathophysiological state, because both the $T_{L,NO}$ and $T_{L,CO}$ can be lowered equally, but it is unlikely that a pathological process will affect both components proportionately. According to equation 8, the $T_{L,NO}/T_{L,CO}$ ratio is mainly influenced by the $D_{M,CO}/\Theta_{CO}\cdot V_c$ ratio, or the ratio of the membrane to red blood cell conductance for CO. Figure 2 illustrates the clinical situations in which the $T_{L,NO}/T_{L,CO}$ ratio is increased or decreased, and table 3 lists situations where the ratio is high or low with an explanation in terms of alterations in the pulmonary microcirculation *versus* changes in alveolar surface area.

Increase in the $T_{L,NO}/T_{L,CO}$ ratio

$T_{L,NO}$ is independent of Hb level, but the $T_{L,CO}$ falls in anaemia; therefore, the $T_{L,NO}/T_{L,CO}$ ratio, uncorrected for the Hb concentration, increases (fig. 2) [11]. Similarly, $T_{L,NO}$ is independent of P_{A,O_2}, but the $T_{L,CO}$ falls as P_{A,O_2} increases; therefore, the $T_{L,NO}/T_{L,CO}$ ratio increases. In 26 patients with pulmonary vascular disease [24] (77% had a diagnosis of chronic thromboembolic pulmonary hypertension), the $T_{L,NO}/T_{L,CO}$ ratio was slightly increased (112%), but this was no more sensitive than the reduction in $T_{L,NO}$, $T_{L,CO}$, K_{NO} or K_{CO}. In a subgroup (n=36) of heavy smokers (n=236) with computed tomography (CT)-proven emphysema [27], 92% had a low K_{NO} compared to 78% who had a low forced expiratory volume at 1 s/forced vital capacity (FVC) ratio (<0.7 being considered abnormal). The area under the receiver operating characteristic curve (ROC) (most right and least wrong: maximum=1.0) for the detection of CT-based emphysema was 0.894 for K_{NO} and 0.822 for K_{CO}. The negative predictive value of K_{NO} was much greater than its positive predictive value. The $T_{L,NO}/T_{L,CO}$ ratio was raised in this cohort of heavy smokers (4.9 *versus* 4.36), but the ratio did not differentiate between those with CT-diagnosed emphysema and those without.

Decrease in the $T_{L,NO}/T_{L,CO}$ ratio

The small (~10%) fall in the $T_{L,NO}/T_{L,CO}$ ratio with exercise is consistent with an increase in pulmonary capillary diameters (increase in V_c versus D_M, and fall in the $D_M/\Theta_{CO}\cdot V_c$ ratio). Pulmonary capillary recruitment, which also occurs, increases surface area (D_M) as well as V_c, and this limits the fall in the $T_{L,NO}/T_{L,CO}$ ratio. With deflation of the lung in normal subjects the $T_{L,NO}/T_{L,CO}$ ratio falls [13, 24], so a $T_{L,NO}/T_{L,CO}$ ratio decrease should be a marker for extrapulmonary restriction.

In 25 nonsmoking patients with stage II–III sarcoidosis [29] the $T_{L,NO}/T_{L,CO}$ ratio ($\sim K_{NO}/K_{CO}$ ratio) determined by a rebreathing technique was reduced (85% predicted) in keeping with the low D_M/V_c ratio (79% normal). $T_{L,NO}$ was more reduced than K_{NO} (34% pred normal *versus* 60%), a similar pattern to $T_{L,CO}$ and K_{CO}, which suggests that loss of alveolar membrane surface area (loss of alveolar units) exceeded membrane thickening. If all ventilated units were equally involved in the membrane thickening, we would expect K_{NO} and $T_{L,NO}$, as % pred, to be equally reduced. On exercise [29], recruitment of diffusing capacity (as % of resting values) was similar for normal subjects and patients with sarcoidosis, with a decrease (-15%) in the $T_{L,NO}/T_{L,CO}$ ratio, consistent with capillary dilatation on exercise, which would not be ''seen'' by NO diffusion. In another study of 41 patients with diffuse interstitial lung disease (66% had sarcoidosis) the $T_{L,NO}/T_{L,CO}$ ratio increased [24]; we speculate that these patients may have had more end-stage disease and fibrosis.

SUGGESTIONS FOR FUTURE RESEARCH

Chronic heart failure

A reduction in $D_{M,CO}$ with normal or elevated V_c is a characteristic finding in chronic heart failure, at least in the early stages [35, 36]. Therefore, a decreased $T_{L,NO}/T_{L,CO}$ ratio would be expected in the New York Heart Association (NYHA) grades I and II. As

TABLE 3 The $T_{L,NO}/T_{L,CO}$ ratio in different situations and conditions

$T_{L,NO}/T_{L,CO}$	Situation/diagnosis	Explanation
Increased	High P_{O_2}	Less binding sites available for CO which lowers $T_{L,CO}$
	anaemia (uncorrected) [11]	but not $T_{L,NO}$
Increased	Heavy smokers [27]	Greater involvement of microvascular compartment
	Diffuse parenchymal disease# [24]	reduces $T_{L,CO}$ more than $T_{L,NO}$
	Chronic thromboembolic pulmonary hypertension [24]	
	Hepatopulmonary syndrome [25]	
	Pulmonary artery occlusion in sheep [34]	
Decreased	Rest to exercise (normals) [22]	Expansion of capillary volume (per unit V_A) increases
	Voluntary restriction of lung expansion [13] mimicking	$T_{L,CO}$ more than $T_{L,NO}$
	''extrapulmonary restriction''	
Decreased	Sarcoidosis¶ [29]	Alveolar surface area reduction exceeds microvascular
	Lifelong altitude exposure+ [23]	damage, and affects $T_{L,NO}$ more than $T_{L,CO}$
	Cystic fibrosis [26]	
	Morbid obesity [19]	
	Chronic heart failure [35] (unconfirmed for $T_{L,NO}/T_{L,CO}$ ratio)	

$T_{L,NO}$: transfer factor of the lung for nitric oxide; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; P_{O_2}: oxygen tension; CO: carbon monoxide; V_A: alveolar volume. #: weighted towards sarcoidosis with end-stage disease; ¶: sarcoidosis in stages II–III and younger than those in #; +: ''highlanders'', corrected for polycythaemia.

pulmonary hypertension intervenes in NYHA grades III and IV, the $T_{L,NO}/T_{L,CO}$ ratio might return to normal or increase.

Extrapulmonary restriction

The interpretation of the $T_{L,CO}$ in extrapulmonary restriction is complicated by the rise in K_{CO} ($\sim T_{L,CO}/V_A$) to >120% pred when alveolar expansion diminishes (fig. 1b). The $T_{L,NO}/V_A$ ($\sim K_{NO}$) is relatively independent of volume expansion, and this would make the interpretation of the $T_{L,NO}$ in extrapulmonary restriction more straightforward. In addition, the expected fall in the $T_{L,NO}/T_{L,CO}$ ratio would add diagnostic usefulness to the finding of a raised K_{CO} *per se*.

Interstitial lung disease

Conventionally, $D_{M,CO}$ and V_c are reduced equally in interstitial lung disease. Table 3 shows that sarcoidosis with end-stage disease and fibrosis [24] had a raised $T_{L,NO}/T_{L,CO}$ ratio, but sarcoidosis without fibrosis [29] had a reduced ratio. An increased ratio suggests that V_c is more compromised than the alveolar–capillary membranes, whereas greater membrane involvement would lead to a reduced $T_{L,NO}/T_{L,CO}$ ratio. Thus, replacement of inflammation by fibrosis might be associated with a $T_{L,NO}/T_{L,CO}$ ratio, which rises from normal or less than normal to a value >100% pred. Similarly, the development of vascular remodelling with pulmonary hypertension in scleroderma (systemic sclerosis), for example, might also see the $T_{L,NO}/T_{L,CO}$ ratio rise above normal.

Chronic obstructive pulmonary disease

Further studies in chronic obstructive pulmonary disease, in relation to high-resolution CT quantitation of emphysema would be welcome. Studies of the ratio in bronchiectasis and obliterative bronchiolitis (*e.g.* post bone-marrow transplant) would be of interest.

CONCLUSION

The $T_{L,NO}$ is a relatively new pulmonary function test, similar in many ways to the more established $T_{L,CO}$. It differs from the $T_{L,CO}$ in being independent of P_{O_2} and haematocrit. Physiologically, the $T_{L,NO}$ behaves as if most of its transfer resistance lies in the thickness of the pulmonary membranes and blood, with red blood cell access including the binding of NO to Hb to form metHb being relatively unimportant. The $T_{L,NO}/T_{L,CO}$ ratio is weighted towards the D_M/V_c ratio and α, the ratio of diffusivities in plasma of NO to CO (α=1.97). The normal ratio lies between 4.3 and 4.9. The $T_{L,NO}/T_{L,CO}$ ratio is reduced in extrapulmonary restriction and is predicted to be reduced in chronic heart failure. The $T_{L,NO}/T_{L,CO}$ ratio is increased in interstitial and pulmonary vascular disease, and in heavy smokers, but it is not yet known if it will predict the onset of emphysema. The $T_{L,NO}/T_{L,CO}$ ratio provides

TABLE 4 Calculations of membrane diffusing capacity for nitric oxide ($D_{M,NO}$), membrane diffusing capacity for carbon monoxide ($D_{M,CO}$) and pulmonary capillary volume (V_c); see Appendix for explanation and commentary

	Data set	$D_{M,NO}$ mL·min^{-1}·mmHg^{-1}	$D_{M,CO}$[#] mL·min^{-1}·mmHg^{-1}	1/$D_{M,CO}$ mL^{-1}·min·mmHg	1/$\Theta_{bl,CO}$·V_c mL^{-1}·min·mmHg	1/$\Theta_{bl,CO}$ mL^{-1}·min·mmHg·mL^{-1}	V_c mL	1/$\Theta_{bl,CO}$·V_c/(1/$T_{L,CO}$) ~ $T_{L,CO}$ red blood cell resistance %	Comment
NO blood resistance = 0 1/$\Theta_{bl,NO}$ = 0									
	A	144	73	0.0137	0.0173	1.31	76	55	Most used Θ_{bl} value but at pH 8.0 [3]
	B	144	73	0.0137	0.0173	1.71	99	55	Θ_{bl} value at pH 7.4 [15]
	C	144	73	0.0137	0.0173	0.82	47	55	Θ_{bl} thin film exps [16]
Finite NO blood resistance (1/$\Theta_{bl,NO}$·V_c)/(1/$T_{L,NO}$) = 37%									
	D	230	117	0.0086	0.0224	1.31	58	72	$D_{M,NO}$ from [10]:
	E	230	117	0.0086	0.0224	1.71	76	72	$D_{M,NO}$ from [10]:
	F	230	117	0.0086	0.0224	0.82	37	72	$D_{M,NO}$ from [10]:
	G	230			0.0026[¶]	0.22[+]	88	36[§]	From Roughton–Forster equation and 1/$D_{M,NO}$ and 1/$\Theta_{bl,NO}$

The calculations for $D_{M,CO}$ and V_c were derived from ''normal, resting'' transfer factor of the lung for NO ($T_{L,NO}$; 144 mL·min^{-1}·mmHg^{-1}) and $T_{L,NO}/T_{L,CO}$ ratio (4.5) [1, 12, 19, 22–26] ($T_{L,CO}$ = 32 mL min^{-1} mmHg^{-1}); for situations where NO uptake blood resistance is zero (1/$D_{M,NO}$ = 1/$T_{L,NO}$) or finite (1/$D_{M,NO}$<1/$T_{L,NO}$). $D_{M,CO}$ derived from $D_{M,NO}$ for α = 1.97 (NO/CO diffusivity ratio), and V_c derived *via* Roughton–Forster equation 1 for different experimental values of the blood resistance to carbon monoxide transfer (1/$\Theta_{bl,CO}$; at P_{O_2} 100 mmHg). Multiply by three to convert to SI units (mmol·min^{-1}·kPa^{-1}1) from mL·min^{-1}·mmHg^{-1}. [#]: α =1.97; [¶]: 1/$\Theta_{bl,NO}$·V_c was used not 1/$\Theta_{bl,CO}$·V_c; [+]: 1/$\Theta_{bl,NO}$ was used not 1/$\Theta_{bl,CO}$; [§]: ~NO red blood cell resistance % was used not $T_{L,CO}$ red blood cell resistance %.

an alternative way of investigating the blood gas barrier and alveolar–capillary pathology.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We would like to thank N. Pride (National Heart and Lung Institute, Imperial College, London, UK) for his review of a draft of the manuscript and D. Simmonds (Medical Artist, London) for figures.

APPENDIX: SEE TABLE 4

Calculations were made using the Roughton–Forster equation ($1/T_L = 1/D_M + 1/\Theta_{bl}\cdot V_c$) with fixed values for $1/T_{L,NO}$ (1/144) and $1/T_{L,CO}$ (1/32). $1/D_{M,NO}$ was calculated from $1/T_{L,NO}$ on the assumption of: 1) zero red blood cell resistance ($1/D_{M,NO} = 1/T_{L,NO}$) (table 4 data sets A–C); or 2) with a red blood cell resistance equal to 37% of the total resistance ($1/D_{M,NO} = 1/T_{L,NO} \times 0.63$) (table 4 data sets D–G) [10]. $D_{M,CO}$ was calculated from $D_{M,NO}$ using α, the NO/CO physical diffusivity ratio (1.97). $1/T_{L,CO}$ (given) - $1/D_{M,CO}$ (derived) = $1/\Theta_{bl,CO}\cdot V_c$, from which V_c was estimated from various equations for the $1/\Theta_{bl,CO}$ *versus* P_{O_2} relationship (at P_{O_2} 100 mmHg). The red blood cell resistance proportion for CO uptake ($(1/\Theta_{bl,CO}\cdot V_c)/(1/T_{L,CO})$) was calculated. Finally, V_c was derived from $1/\Theta_{bl,NO}\cdot V_c$ ($= 1/T_{L,NO} - 1/D_{M,NO}$) using the value by CARLSEN and COMROE [4] for $\Theta_{bl,NO}$ (4.5 $mL\cdot min^{-1}\cdot mmHg^{-1}\cdot mL^{-1}$), (table 4 data set G).

Comment

The effect (table 4, C to B) of an increase in $1/\Theta_{CO}$ of one unit is to increase estimates of V_c from 47 to 99 mL (+106%) or (table 4, F to E) from 37 to 76 mL (+105%). Θ_{NO} becoming finite (table 4, D to F) increases $D_{M,CO}$, but decreases V_c by 20% (table 4, A *versus* D and B *versus* E). Even the highest values of $D_{M,CO}$ and V_c (117 and 99, respectively) fall short of morphometric estimates [37] at rest of V_c (180 mL) and $D_{M,CO}$ (463 mL min^{-1} $mmHg^{-1}$, but corrected down to 272 mL min^{-1} $mmHg^{-1}$ [38]). These calculations highlight the uncertainties in deriving $D_{M,CO}$ and V_c from simultaneous measurements of $T_{L,NO}$ and $T_{L,CO}$.

REFERENCES

1 Guenard H, Varenne N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity by the measurements of NO and CO transfer. *Respir Physiol* 1987; 70: 113–120.
2 Borland CD, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur Respir J* 1989; 2: 56–63.
3 Roughton FJW, Forster RE. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. *J Appl Physiol* 1957; 11: 290–302.
4 Carlsen E, Comroe JH. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. *J Gen Physiol* 1958; 42: 83–107.
5 Umbreit J. Methemoglobin – it's not just blue: a concise review. *Am J Hematol* 2007; 82: 134–144.
6 Haab P, Dyrand-Arczynska W. Carbon monoxide effects on oxygen transport. *In*: RG Crystal, ed. The Lung: Scientific Foundations. 2nd Edn. Philadelphia, Lippincott Williams & Wilkins, 1997; pp. 1673–1680.
7 Borland CD, Cox Y. Effect of varying alveolar oxygen partial pressure on diffusing capacity for nitric oxide and carbon monoxide, membrane diffusing capacity and lung capillary blood volume. *Clin Sci* 1991; 81: 759–765.
8 Morris RJ, Gibson QH. The role of diffusion in limiting the rate of ligand binding to hemoglobin. *J Biol Chem* 1980; 255: 8050–8053.
9 Borland CD, Dunningham H, Bottril F, *et al.* Can a membrane oxygenator be a model for NO and CO transfer? *J Appl Physiol* 2006; 100: 1527–1538.
10 Borland CD, Dunningham H, Bottril F, *et al.* Significant blood resistance to nitric oxide transfer in the lung. *J Appl Physiol* 2010; 108: 1052–1060.
11 van der Lee I, Zanen P, Blesma DH, *et al.* The effect of red cell transfusion on nitric oxide diffusing capacity. *Respiration* 2005; 72: 512–516.
12 Glénet SN, Bisschop CD, Vargas F, *et al.* Deciphering the nitric oxide to carbon monoxide lung transfer ratio: physiological implications. *J Physiol* 2007; 582: 767–775.
13 van der Lee I, Zanen P, Stigter N, *et al.* Diffusing capacity for nitric oxide: Reference values and dependence on alveolar volume. *Respir Med* 2007; 101: 1579–1584.
14 Stam H, Kreuzer FJA, Versprille A. Effect of lung volume and positional changes on pulmonary diffusing capacity and its components. *J Appl Physiol* 1991; 71: 1477–1488.
15 Forster RE. Diffusion of gases across the alveolar membrane. *In*: Fishman AP, Farhi LE, Tenney SM, eds. Handbook of Physiology: Section 3: The Respiratory System: Vol. IV. Gas Exchange. Bethseda, American Physiological Society, 1987; pp. 71–88.
16 Reeves RB, Park HK. CO uptake kinetics of red cells and CO diffusing capacity. *Respir Physiol* 1992; 88: 1–21.
17 Tamhane RM, Johnson RL, Hsia CC. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. *Chest* 2001; 120: 1850–186.
18 Ceridon ML, Beck KC, Olson TP, *et al.* Calculating alveolar capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. *J Appl Physiol* 2010; 109: 643–653.
19 Zavorsky GS, Kim DJ, McGregor ER, *et al.* Pulmonary diffusing capacity for nitric oxide during exercise in morbid obesity. *Obesity* 2008; 16: 2431–2438.
20 Aguilaniu B, Maitre J, Glénet S, *et al.* European reference equations for CO and NO lung transfer. *Eur Respir J* 2008; 31: 1091–1097.
21 Zavorsky GS, Cao J, Murias JM. Reference values of pulmonary diffusing capacity for nitric oxide in an adult population. *Nitric oxide* 2008; 18: 70–79.
22 Zavorsky GS, Quiron KB, Massarelli PS, *et al.* The relationship between single-breath diffusion capacity of the lung for nitric oxide and carbon monoxide during various exercise intensities. *Chest* 2004; 125: 1019–1027.
23 de Bisschop C, Kiger L, Marden MC, *et al.* Pulmonary capillary blood volume and membrane conductance in Andeans and lowlanders at high altitude: a cross sectional study. *Nitric oxide* 2010; 23: 187–193.
24 van der Lee I, Zanen P, Grutters JC, *et al.* Diffusing capacity for nitric oxide and carbon monoxide in patients with diffuse parenchymal disease and pulmonary arterial hypertension. *Chest* 2006; 129: 378–383.
25 Degano B, Mittaine M, Guénard H, *et al.* Nitric oxide and carbon monoxide lung transfer in patient with advanced liver cirrhosis. *J Appl Physiol* 2009; 107: 139–143.
26 Dressel H, Filser L, Fischer R, *et al.* Lung diffusing capacity for nitric oxide and carbon monoxide in relation to morphological changes as assessed by computed tomography in patients with cystic fibrosis. *BMC Pulm Med* 2009; 9: 30.

27 van der Lee I, Gietema HA, Zanen P, *et al.* Nitric oxide diffusing capacity *versus* spirometry in the early diagnosis of emphysema in smokers. *Respir Med* 2009; 103: 1892–1897.

28 Dressel H, Filser L, Fischer R, *et al.* Lung diffusing capacity for nitric oxide and carbon monoxide: dependence on breath-hold time. *Chest* 2008; 133: 1149–1154.

29 Phansalkar AR, Hanson CM, Shakir AR, *et al.* Nitric oxide diffusing capacity and microvascular recruitment in sarcoidosis. *Am J Respir Crit Care Med* 2004; 169: 1034–1040.

30 Ogilvie CM, Forster RE, Blakemore WS, *et al.* A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 1957; 36: 1–17.

31 Jones RS, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q J Exp Physiol Cogn Med Sci* 1961; 46: 131–143.

32 Graham BL, Mink IT, Cotton DJ. Improved accuracy and precision of single–breath CO diffusing capacity measurements. *J Appl Physiol* 1981; 51: 1306–1313.

33 Zavorsky GS, Murias JM. A small amount of inhaled nitric oxide does not increase lung diffusing capacity. *Eur Respir J* 2006; 27: 1251–1257.

34 Harris RS, Hadian M, Hess DR, *et al.* Pulmonary artery occlusion increases the ratio of diffusing capacity for nitric oxide to carbon monoxide in prone sheep. *Chest* 2004; 126: 559–565.

35 Puri S, Baker BL, Dutka DP, *et al.* Reduced alveolar-capillary membrane diffusing capacity in chronic heart failure. Its pathophysiological relevance and relationship to exercise performance. *Circulation* 1995; 91: 2769–2774.

36 Guazzi M. Alveolar–capillary membrane dysfunction in heart failure: evidence of a pathophysiological role. *Chest* 2003; 124: 1090–1102.

37 Gehr P, Bachofen M, Weibel ER. The normal human lung: ultrastructure and morphometric estimation of diffusion capacity. *Respir Physiol* 1978; 32: 121–140.

38 Hsia CCW, McBrayer DG, Ramanathan M. Reference values of pulmonary diffusing capacity during exercise by a rebreathing technique. *Am J Respir Crit Care Med* 1995; 152: 658–665.