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The single breath transfer factor ($T_{L,CO}$) and the transfer coefficient (K_{CO}): a window onto the pulmonary microcirculation

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Summary

The transfer factor, $T_{L,CO}$ (with the transfer coefficient, K_{CO}, also known as the transfer factor per unit alveolar volume, [T_L/V_A]), is one of the most useful clinical tests of pulmonary function, the only one which specifically focuses on pulmonary microcirculation. It was originally devised in 1909 as a physiological tool to assess the diffusive capacity of the lung as a gas exchanger. It was subsequently developed as a clinical tool, but cumbersome analytical techniques delayed its introduction into clinical medicine until 1950s. The physiology of the carbon monoxide transfer factor (also called the diffusing capacity $D_{L,CO}$) is based on the Roughton–Forster equation which partitions $D_{L,CO}$, a conductance, into membrane (D_M) and red cell (θV_C) diffusion conductances. Recent work (1987–2001) suggests that 70–80% of the resistance to CO (and O_2) diffusion may reside in the red cell fraction. The clinical implication is that $T_{L,CO}$ and K_{CO} are 'windows' onto the pulmonary microcirculation. As regards reference values for clinical use, $T_{L,CO}$ depends on age, height and gender. K_{CO}, which is actually a rate constant, is independent of gender, and is affected principally by age. A schema is presented for the clinical interpretation of $T_{L,CO}$. As $T_{L,CO}$ is derived from the product of K_{CO} and the accessible alveolar volume (V_A), examination of these two components (K_{CO} and V_A) will usually suggest a specific pathophysiological mechanism as the explanation for a reduction in $T_{L,CO}$.

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Key words

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Introduction

The transfer factor of the lung for carbon monoxide ($T_{L,CO}$), also known as the diffusing capacity ($D_{L,CO}$), has become one of the key tests of pulmonary function. $T_{L,CO}$ measures the potential of the lung for gas exchange. For example, a patient with interstitial lung disease might have a low $T_{L,CO}$ ($D_{L,CO}$), (say <50% predicted normal), but could still have a normal arterial PO_2 (PaO_2) at rest; but on exercise, PaO_2 will fall, often severely, because the lung has insufficient gas exchanging surface area to meet the additional oxygen demand. As will be seen later, the particular surface area which is crucial is that of the microvascular bed, in particular the number of capillaries.

History of the $T_{L,CO}$ ($D_{L,CO}$) measurement

Most of our current pulmonary function tests were introduced in the 1950s. $D_{L,CO}$, as it was called then, has a much longer history. It was devised originally by Krogh & Krogh (1909), in Denmark, by August Krogh and Marie (his wife), as a physiological tool to test the notion, long since abandoned, that the lung, like the swim bladder of deep-sea fish, could secrete oxygen against the normal pressure gradient exerted by the inspired air. Subsequently, $D_{L,CO}$ was introduced as a clinical test by Krogh (1915), but the measurement never caught on because methods of measuring carbon monoxide (CO) – by combusting the gas with oxygen to produce CO_2 – were cumbersome. It was not until after the Second World War (1939–45), following the invention in Germany of the infra-red technique for CO detection that Marie Krogh's original measurements were repeated and refined for clinical use; the principle modification, suggested by W.S. Fowler (Forster *et al.*, 1954a), was the addition of an inert gas (helium) to the CO–air mixture, so that CO_0 could be calculated (see Fig. 1) rather than measured. In M. Krogh's (1915) original technique, CO_0 was obtained from an initial expiration from total lung capacity (TLC) to mid-lung volume; after a breath hold of 6–8 s, a second expiration was made and CO_t sampled.

The diffusing capacity for oxygen

Lilienthal *et al.* (1946) published a landmark paper describing a method for measuring the oxygen diffusing capacity (D_{L,O_2}),

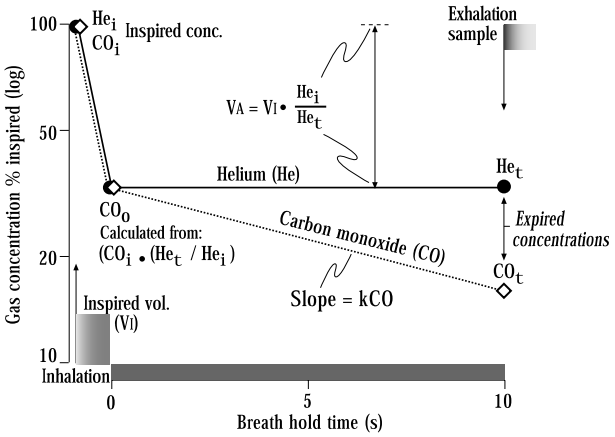

Figure 1 Concentrations of the test gases (carbon monoxide and helium) plotted against breath hold time for the single breath $T_{L,CO}$ manoeuvre illustrating the origin and calculation of the two components (K_{CO} and V_A) from which the $T_{L,CO}$ is derived. V_A = alveolar volume at which the K_{CO} was measured.

for which $D_{L,CO}$ was after all only a substitute. It could be argued that this paper ushered in a new era in gas exchange research. The ingenuity of Lilienthal's method lay in the differentiation of end-capillary ($Pc'O_2$) from arterial (PaO_2) oxygen tension; this was achieved by measuring alveolar and arterial PO_2 on exercise breathing air, followed by a 10–12% O_2 hypoxic mixture. They were delighted when the steady state D_LO_2 values were similar to Krogh's (1915) single breath $D_{L,CO}$ measurements, also made on exercise.

Steady state $D_{L,CO}$

With the revival of interest in diffusion measurements, several groups (Bates, 1952; Filley *et al.*, 1954; Bates *et al.*, 1955) thought it more appropriate to measure DL,CO by a steady state technique ($D_{L,CO}$ ss), similar to that for D_LO_2. The measurement of $D_{L,CO}$ ss was a much easier proposition than D_LO_2. There was no need to repeat the measurement under hypoxic conditions, $P\bar{c}$ (the *mean* capillary tension) for CO could be ignored (haemoglobin being considered an infinite sink for CO), and $D_{L,CO}$ ss computed as $\dot{V}_{CO}$/PACO, where $\dot{V}_{CO}$ is CO uptake in ml min^{-1}, and PA is the alveolar tension. [note: $D_LO_2 = (\dot{V}O_2 / (PAO_2 - P\bar{c}O_2)$]. The measurement of $D_{L,CO}$ ss was straightforward, but the assessment of PACO by direct sampling was problematic in the presence of a sloping alveolar plateau of CO concentration on expiration, as was frequently the case in respiratory disease. The solution of Filley *et al.* (1954) was to calculate an 'ideal' PACO, as for the alveolar–arterial gradient for oxygen, although this involved taking an arterial sample for $PaCO_2$. A few simultaneous comparisons, mostly in normal subjects, were made on exercise of $D_{L,CO}$ ss and D_LO_2 and there was reasonable concordance (Forster *et al.*, 1955; Shepard *et al.*, 1958). $D_{L,CO}$ sb exceeded $D_{L,CO}$ ss at rest by about 33% (Marshall, 1958), but the two measurements converged on exercise.

Steady state or single breath $D_{L,CO}$?

In the late 1950s, Ogilvie *et al.* (1957) published their 'standardized technique' for the single breath $D_{L,CO}$, called $D_{L,CO}$ sb, incorporating Fowler's helium modification (Forster *et al.* (1954a); see earlier]. In the 1960s, $D_{L,CO}$ ss or $D_{L,CO}$ sb were being introduced in the pulmonary function laboratories of most University Departments. By the 1970s, the concept and clinical usefulness of $D_{L,CO}$ (now called $T_{L,CO}$ throughout Europe) was widely accepted and it was the single breath technique which became the method of choice. The reasons were pragmatic. $D_{L,CO}$ sb did not require an arterial sample, nor meticulous timing of alveolar samples. It was more acceptable to patients and staff. In 1965, an automated apparatus for measuring $D_{L,CO}$ sb came onto the market (Meade *et al.*, 1965). It was a success and other manufacturers followed suit. Once $D_{L,CO}$ became a 'black box' test, any inhibitions clinicians might have had about setting it up were quickly dispelled!

Physiology of $D_{L,CO}$ ($T_{L,CO}$)

The Kroghs believed that CO uptake from alveolar gas occurred by passive diffusion across the alveolar-capillary membranes, driven by the PA–$P\bar{c}$ gradient for CO. Krogh & Krogh (1909) converted $D_{L,CO}$ to D_{L,O_2} by multiplying by 1.23 which is the ratio of the tissue diffusivities of O_2 and CO. Following Christian Bohr (1909) who was August Krogh's teacher, they reasoned that $P\bar{c}CO$ would be negligible because of the high affinity of CO for Hb. As we have seen, this assumption greatly simplifies the measurement. Like many assumptions, it proved to be only partially correct (see later)! Nevertheless, until the 1940s, resistance to gas exchange was considered to be 'diffusive', i.e. proportional to the thickness/ surface area ratio of the intervening membranes and tissues. For pulmonary gas exchange, the final step for oxygen, the combination with haemoglobin to form HbO_2, was thought to be practically instantaneous (just a few milliseconds). Roughton (1932), on the other hand, [from his work with Hartridge (Hartridge & Roughton, 1923)], noted that the reaction velocity for O_2 or CO was seven to 10 times slower in intact red cells than in haemoglobin solutions, and he concluded that diffusion resistance of the red cell membrane and the interior of the cell was responsible.

In the 1950s, R.E. Forster was asked by Julius Comroe (Head of Physiology at the University of Pennsylvania) to repeat Krogh's (1915) measurements with the aim of developing a clinically useful test (Comroe, 1975). Forster became (fortunately for us!) sidetracked by his desire to understand exactly what was being measured by $D_{L,CO}$ (Forster *et al.*, 1954b). His research, with several collaborators including Roughton (a frequent visitor to Philadelphia from his laboratory in Cambridge), culminated in the publication of a famous article (Roughton & Forster, 1957) containing the equation:

$$\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta V_C} \quad (1)$$

where D_M is the diffusing capacity of the membranes separating the alveolar gas from the red cell itself; θ is the rate of reaction of CO with red cells (sometimes called the diffusing capacity per ml of blood), and V_C is the microvascular (capillary) blood volume in contact with the inhaled CO. Kruhoffer (1954), who worked in the same Department in Copenhagen as did the Kroghs, had published a similar equation 3 years earlier, but without giving a formal proof, and with erroneous values for θ.

The Roughton–Forster equation is the key to understanding what the $D_{L,CO}$ is measuring. $D_{L,CO}$ is a conductance (ml min^{-1} mmHg^{-1}), and the reciprocals in equation (1) are resistances. $1/\theta V_C$ is the oxygen-dependent part of the resistance, located in the pulmonary capillaries, and $1/D_M$ is what remains when $1/D_L$ is back-extrapolated to zero PO_2. $[1/D_L - 1/D_M]/[1/D_L]$ is the 'red cell' fraction of the total diffusion resistance. Roughton & Forster (1957) also showed, from measurements of $D_{L,CO}$ at two or more alveolar PO_2 levels (ideally 150–200 and 500–600 mmHg) and knowing θCO at the appropriate PAO_2, that D_M and V_C could be calculated by a simple graphical method.

The membrane diffusing capacity

The D_M is determined by the tissue diffusivity [solubility/(mol. wt)$^{-2}$] of O_2 or CO in lung tissue and the surface area/thickness ratio of the epithelial, interstitial, endothelial and plasma barriers. In physiological terms, D_M is a function of the expansion of the lung. With a doubling of the gas volume of the lung (from 50 to 100% TLC) D_M increases by about 75%, but $D_{L,CO}$ by only 25% (V_C does not change) (Stam *et al.*, 1991). Increases of D_M as the lung expands are caused by a mixture of airspace spherical expansion and unfolding of the surface. Unfortunately, the measurement of D_M is not independent of V_C, because intracapillary Hb must be present for D_M to be detected. Also, changes in the dimensions of the V_C component will alter D_M.

The diffusing capacity of blood

In vivo, θCO is inversely proportional to PO_2 . The original *in vitro* measurements of Roughton & Forster (1957) were carried out at pH 7.8–8.0. More recent estimates (Forster, 1987) at physiological pH have resulted in significantly lower $1/\theta$ values at high PO_2s. If, in the Roughton–Forster equation, the 1987 $1/\theta$ are substituted for the 1957 ones, D_M increases 2.5–3.5 times, and V_C decreases by 33% . Nearly all published D_M and V_C values have used the 1957 θCO values, a notable exception being Borland & Cox (1991) and Borland *et al.* (2001).

Pulmonary capillary volume

As measured by the Roughton–Forster technique, capillary volumes (with 1957 $1/\theta$) are in the range 80–100 ml (females) and 100–120 ml (males) at rest, and 125–210 ml (males and females) on exercise (Hsia *et al.*, 1995). These values would be about 33% lower if the 1987 $1/\theta$ were to be used. Morphometric values for V_C at rest, from post-mortem lungs, are about 200 ml (ranging from 120–280 ml depending on body weight) (Gehr *et al.*, 1978). Because of the Fåhreus–Lindqvist effect, whereby red cells accelerate relative to mean plasma flow in their passage through the capillary bed, capillary haematocrit (Hct) is less than that in larger vessels. In fact, pulmonary capillary Hct is about 67% of large vessel Hct (Brudin *et al.*, 1986). This effect does not change V_C estimates because there is an equal and opposite reduction in θCO.

Diffusing capacity for nitric oxide

In the last 15 years, the alveolar uptake of nitric oxide (NO) has been studied (Guenard *et al.*, 1987; Borland & Higenbottam, 1989). The theory and technique for estimating $D_{L,NO}$ is identical to that for $D_{L,CO}$. $D_{L,NO}$ is four to five times greater than $D_{L,CO}$. The reason is that θ_{NO} is nearly seven times larger than θ_{CO} (Carlsen & Comroe, 1958). From simultaneous inhalation of NO and CO, $D_{L,NO}$ and $D_{L,CO}$ can be calculated, and the Roughton–Forster equation solved on the basis of two values of $1/\theta$ ($1/\theta_{NO}$ and $1/\theta_{CO}$), instead of two values of $1/\theta_{CO}$ at different PAO_2 s (Borland & Cox, 1991; Borland *et al.*, 2001). This has the effect of increasing $D_{M,CO}$ by three to four times, and reducing the V_C/D_M ratio from about 2 to 0.35.

Red cell resistance fraction

As mentioned earlier, the general view, before the Roughton & Forster (1957) paper, was that $D_{L,CO} \sim D_M$ with no resistance to diffusion attributable to the reaction of CO with haemoglobin. When $D_{L,CO}$ was partitioned into D_M and V_C using the original 1957 θ_{CO} values, the red cell resistance fraction varied from 32–56% (Forster, 1957). With the morphometric analysis, the red cell resistance fraction is in the range 50–80% (Gehr *et al.*, 1978). Using the 1987 θ_{CO} values and Borland & Cox (1991) and Borland *et al.*'s (2001) θ_{NO}–θ_{CO} technique, this fraction has risen to 80%, i.e. most of the diffusion resistance is intracapillary. Thus, $D_{L,CO}$ measurements may be heavily weighted towards the numbers of red cells and/ or the number of capillary vessels. This new perception supports the views of clinicians who have maintained that $D_{L,CO}$ is a 'window on the pulmonary microcirculation'. Striking changes in $D_{L,CO}$ in severe anaemia (↓) [Rankin *et al.*, 1961], on exercise (↑) [Hsia *et al.*, 1995], in intrapulmonary haemorrhage (↑) [Ewan *et al.*, 1976], and in pulmonary vasculitis (↓) emphasize the pre-eminent role of the capillary bed.

The ratio $D_L,O_2/D_{L,CO}$ in hypoxia is about 1.2 (Meyer *et al.*, 1981), the same as predicted (but for normoxia) by Krogh and Krogh in 1909. If the $D_{L,CO}$ is measured in normoxia, the D_L,O_2 / $D_{L,CO}$ ratio is 1.7, reflecting a combination of O_2 /CO ratios for D_M (1.23) and θ [c. 2.0; Forster, 1987).

Perhaps surprisingly, partitioning the $D_{L,CO}$ into its D_M and V_C components (not difficult to do even in patients) has not proved useful clinically. There are two reasons: first, $D_{L,CO}$ is dominated by its θV_C component, and secondly, D_M and V_C measurements are coupled in the sense that V_C must exist for D_M to be measurable. The only examples of 'uncoupling' are congestive heart failure (Puri *et al.*, 1995) (D_M is reduced when V_C is normal or high) and intrapulmonary haemorrhage (Ewan *et al.*, 1976) (V_C high, D_M normal or reduced).

Clinical interpretation of $T_{L,CO}$ and K_{CO}

Marie Krogh (1915) pointed out that the single breath $T_{L,CO}$ was the product of two separate measurements – the rate constant for CO removal from alveolar gas (which she called k_{CO}) and the alveolar volume (V_A). This simple concept is the key, in our opinion (Hughes & Pride, 2001), to its clinical interpretation. k_{CO} is measured as the exponential decay in fractional concentration of CO over a period of breath-holding (BHT) — see Fig. 1:

$$k_{CO} = [\log_e(CO_0/CO_t)]/BHT \quad (2)$$

where CO_0 and CO_t are the alveolar CO concentrations at the start and finish of BHT. The units of k_{CO} are s^{-1} or min^{-1}.

The total CO transfer of the lungs is calculated as:

$$T_{L,CO} = [k_{CO} \times V_{A\ STPD}]/[P_B - P_{H_2O}] \quad (3)$$

where P_B and P_{H_2O} are the barometric pressure and the water vapour pressure (at 37°C) which standardize for the driving pressure for CO uptake, i.e. the pressure of CO in the alveoli (P_{ACO}). The units of $T_{L,CO}$ are mmol min^{-1} kPa^{-1} (SI) and ml min^{-1} $mmHg^{-1}$ (traditional).

In the original clinical description (Ogilvie *et al.*, 1957), and until 1965 when automated apparatus (and calculations) were introduced (Meade *et al.*, 1965), TLC was calculated independently from closed-circuit inert gas dilution (or body plethysmography) and used as 'V_A'. In the absence of airflow obstruction, V_A and TLC are approximately the same, but single breath V_A may be considerably less than TLC when gas mixing is slow as in airflow obstruction (see later). Nowadays, the simultaneously measured single breath V_A has replaced a separate measurement of TLC for logistic reasons; clinicians request $T_{L,CO}$ more frequently than TLC.

It is our contention that the logical way to interpret $T_{L,CO}$, in the clinical context, is in terms of its components (V_A and k_{CO}) from which it is derived. Unfortunately, the simplicity of this approach has been obscured by modern nomenclature. Today, Krogh's k_{CO} is deployed in different units (although it is the same rate constant) as the *carbon monoxide transfer coefficient* (K_{CO}), whose units of mmol min^{-1} kPa^{-1} L^{-1} BTPS (in SI units) give misleadingly the appearance of being a ratio, an impression enhanced by its alternative terminology (T_L/V_A or D_L/V_A). In SI units, k_{CO} [min^{-1}] converts to K_{CO} (T_L/V_A) by dividing by 2.56, and in traditional units by dividing by 0.853.

Nomenclature

The Kroghs term for the $D_{L,CO}$ was 'diffusion constant', but 'diffusing capacity' replaced it in the 1950s. John Cotes proposed (Cotes & Meade, 1963 'transfer factor' ($T_{L,CO}$) in recognition of the θ V_C term in the Roughton–Forster equation. $T_{L,CO}$ is in general use throughout Europe, although $D_{L,CO}$ remains in use in North America. Krogh (1915) referred to k_{CO} as the 'permeability' factor. In modern parlance, the T_L/V_A or D_L/V_A is referred to as the 'transfer factor per unit lung volume', although 'transfer coefficient' is now the preferred term in Europe. Reflecting the original Krogh concept, the term K_{CO} is replacing T_L/V_A or D_L/V_A.

Reference values

M. Krogh (1915) found that $D_{L,CO}$ was greater in men than in women, and Ogilvie *et al.* (1957) described the dependence of $D_{L,CO}$ on body surface area. Modern reference equations for $T_{L,CO}$ have height and age as coefficients, with separate regressions for men and women. The regression on height is determined by the V_A component of $T_{L,CO}$ (V_A in normal subjects being a surrogate for TLC). The regression on age is largely determined by the k_{CO} component.

Cotes & Hall (1970) pointed out that in young adults K_{CO} was the same in both sexes, but declined with age at a faster rate in men than in women. For a man and a woman who started with the same K_{CO} at age 25 years (say 1.8 in SI units), the male K_{CO} at age 65 years would be 16% less than the woman's (from Cotes & Hall, 1970). Population studies of K_{CO}, which have included both men and women, show a dependence on age with the coefficient being significantly slightly greater in men (–0.023 $years^{-1}$ in males versus –0.016 $years^{-1}$ in females) (Hughes & Pride, in preparation). Most of the studies showed, in addition, a dependence on *height*, although there is no logical reason why a rate constant, which is what K_{CO} ($\sim T_L/V_A$) actually represents, should be dependent on stature or gender. The dependence on height is intriguing. Gulsvik *et al.* (1992) have made the interesting suggestion that, in the seated position, and in taller people, the apices of the lungs may be more poorly perfused relative to the mid and lower zones for gravitational reasons; the resulting inhomogeneity in blood flow and blood volume would reduce the measured K_{CO} for taller people.

In a review of the literature, we have found no significant gender difference for K_{CO} at age 45 years, although a small (non-significant) difference emerges at age 65 years. The current EEC recommendations (Cotes *et al.*, 1993) for reference values for K_{CO} ($\sim T_L/V_A$) are based on [$T_{L,CO}$(predicted) / TLC (predicted)]. The use of separate predictors, each with individual gender, height and (plus age for the $T_{L,CO}$) coefficients, for the K_{CO}, which is actually a rate constant, seems illogical. Further investigation seems to be required.

Interpretation of $T_{L,CO}$ and K_{CO} in lung disease

From eqn (3), $T_{L,CO} = k_{CO} \times V_A$. Therefore, a low $T_{L,CO}$ must be caused by a low K_{CO} or a low V_A or a combination of the two. It is also possible for K_{CO} to be high (as a percentage of that expected at the predicted TLC). As a mechanical analogy, note that $FEV_1 = FEV_1/VC \times VC$, and the explanation for a low FEV_1 must be either a low FEV_1/VC or a low VC or a combination (like K_{CO}, FEV_1/VC may be high). Note that the single breath $T_{L,CO}$ is performed at full inflation, close to TLC, in the seated position, and at rest. In the absence of airflow obstruction with impaired gas mixing, the single breath estimate of V_A should approach that of TLC minus the anatomic dead space (about 200 ml). In practice, V_A is about 94 ± 7% of TLC, 0.1–0.6 L less in absolute terms (Roberts *et al.*, 1990). In airflow obstruction the single breath V_A may be considerably less than the true TLC measured by multi-breath gas dilution or body plethysmography (Roberts *et al.*, 1990).

Causes of a low K_{CO}

The common causes of a low K_{CO} are well known – particularly emphysema and diffuse alveolar–capillary damage associated with connective tissue/ autoimmune disease (see Table 1). In the earlier section 'Physiology of $D_{L,CO}$', we emphasized that most of the resistance to CO uptake lay within the microvasculature. Consequently, in lung disease, loss or destruction of the pulmonary capillary bed is a much more important mechanism for reducing K_{CO} than thickening or inflammatory change in the extravascular tissues. In the Churg–Strauss syndrome and in bronchiolitis (Table 1) a low K_{CO} suggests vasculitis (the pulmonary arterioles and bronchioles share a common connective tissue sheath). In addition, a *physiological* cause of a low K_{CO} is a reduced haemoglobin level, and it is customary to make a correction for this (American Thoracic Society, 1995; Cotes *et al.*, 1972, 1993).

Causes of a high K_{CO}

This is a more difficult concept to grasp. There are physiological reasons for a high K_{CO} (in terms of percentage predicted for a normal TLC): first, incomplete alveolar expansion (but without alveolar disruption) in which the lungs are not inflated to the level of the predicted TLC, secondly, an increase in pulmonary blood flow per unit lung volume. K_{CO}, in terms of the Roughton–Forster equation, consists of two conductances, D_M/V_A and $\theta V_C/V_A$ [θ may be ignored if PAO_2 and (Hb) are normal].

In the case of incomplete alveolar expansion in an otherwise normal lung, T_L/V_A or K_{CO} increases linearly so that at FRC (~50% of V_A at full inflation, i.e. TLC) K_{CO} is > 150% of K_{CO} at TLC (Stam *et al.*, 1994; Hughes & Pride, 2001. This K_{CO} rise is caused mostly by an increase in the V_C/V_A term (V_C does not change and V_A falls), while the DM/V_A ratio remains constant or falls slightly (Stam *et al.*, 1991). Clinically, a rise in K_{CO} (up to 150% of that predicted for a normal TLC) will occur in neuromuscular, pleural or chest wall disease if TLC is reduced. Secondary factors, such as atelectasis or parenchymal disease, may limit the expected rise of K_{CO}.

Table 1 Some of the commoner causes of a K_{CO} which is lower or higher than the reference value (adapted from Hughes & Pride, 2001).

Low K_{CO}	High K_{CO}
Diffuse alveolar–capillary damage	Loss of units (discrete)
Pulmonary fibrosis	Pneumonectomy
Connective tissue/ autoimmune diseases	Local destruction / infiltrates
Sarcoidosis, asbestosis, bleomycin	
Pulmonary hypertension associated	Incomplete alveolar expansion:
Vasculitis	Pleural disease
Thromboembolic	Neuromuscular
Congestive heart failure / mitral stenosis	Chest wall deformity
Pulmonary oedema	Poor technique
Intrapulmonary shunting	Alveolar haemorrhage
Pulmonary arteriovenous malformations (PAVMs)	Anti-GBM disease
Hepatopulmonary syndrome (HPS)	Pulmonary vasculitis
	Wegener's granulomatosis
	SLE
	Idiopathic haemosiderosis
Airflow obstruction	Increased pulmonary blood flow
Emphysema	ASD
Churg–Strauss syndrome	Asthma
Bronchiolitis	
Low $\theta CO/V_A$[a]	High $\theta CO/V_A$[a]
Anaemia	Polycythaemia rubra vera
	Secondary polycythaemia

GBM, glomerular basement membrane; SLE, systemic lupus erythematosus; ASD, atrial septal defect.

[a] Corrections can be made for an abnormal haemoglobin level.

Table 2 Different mechanisms reducing single breath V_A in respiratory disease (adapted from Hughes & Pride, 2001).

Restrictive Disease with a small TLC and normal V_A/TLC ratio			*Obstructive Disease with normal or increased TLC*
I	**II**	**III**	**IV**
Lack of lung expansion: lung structure normal	Loss of units: remaining lung structure normal	Diffuse alveolar damage	Sampled V_A < TLC due to incomplete mixing during breath-holding
Examples			
Acute inspiratory muscle weakness. Chest wall disease and pleural disease.	Pneumonectomy. Local alveolar infiltrate, collapse, consolidation or local destruction	Fibrosing alveolitis. Pulmonary oedema, congestive heart failure, mitral stenosis, bleomycin lung, Wegener's granulomatosis.	Incomplete mixing may be associated with alveolar destruction (emphysema), space-occupying lesions (bullae) or normal alveolar structure (asthma)

An increase in pulmonary blood volume also increases K_{CO}, because the V_C/V_A ratio rises (as does the D_M/V_A ratio). Apart from an increase in pulmonary venous pressure (as in mitral stenosis or congestive heart failure) (Puri *et al.*, 1995), the cause of the increase in V_C is an increase in cardiac output. On exercise K_{CO} (and $T_{L,CO}$) increase by about 20% per 5 l min^{-1} increase in cardiac output from its resting value (Hsia *et al.*, 1995). At rest, pulmonary blood flow may increase as a result of left to right shunts, or blood flow may become more 'homogeneous', as in asthma (Collard *et al.*, 1994), representing an 'effective' blood flow increase. A much commoner situation in lung disease is where blood flow is diverted from diseased lung to normal lung, whose blood flow per unit volume increases. A clear-cut example is pneumonectomy, where blood flow per unit volume at rest doubles in the remaining lung, and K_{CO} increases. Corris *et al.* (1987), in a study of 28 patients before and after pneumonectomy, found that the post-pneumonectomy K_{CO} was 110–131% predicted. Hughes & Pride (2001) have referred to this situation as loss of alveolar units (discrete) where discrete means that some normal (unaffected) lung units are present. There may be many causes (see Table 1 and Table 2 [II]).

A low K_{CO} will usually be associated with a low $T_{L,CO}$, as it is unusual for V_A to exceed its predicted normal value. A high K_{CO} may be associated with a high, normal or a low $T_{L,CO}$, depending (a) on the level of K_{CO}, and (b) whether V_A is normal or reduced. In alveolar haemorrhage, K_{CO} is only elevated when active bleeding is taking place (Ewan *et al.*, 1976), but the Hb-corrected K_{CO} may be sufficiently high to raise $T_{L,CO}$, although V_A may be somewhat reduced.

Causes of a low V_A

There are two causes of a low V_A; restrictive lung disease when absolute lung volumes are small (Table 2; I–III), but the VA/TLC ratio is normal, and obstructive lung disease (Table 2, IV) where TLC is usually normal or increased, but the sampled V_A is <TLC due to incomplete mixing during breath holding. Restrictive lung disease can be subdivided further (see Table 2) into (I) lack of alveolar expansion, (II) loss of alveolar units, discrete, and (III) diffuse alveolar–capillary damage.

Causes of a low $T_{L,CO}$

The same reduction in $T_{L,CO}$ (say to 60% predicted) can be produced by several combinations of K_{CO} and V_A. Hughes & Pride (2001) have shown that, in restrictive lung disease, a $T_{L,CO}$ of 60% predicted could be associated with (a) acute neuromuscular disease, (b) alveolar haemorrhage, (c) lung resection or collapse, (d) diffuse alveolar damage (connective tissue disease) or (e) pulmonary vascular pathology, depending on the precise combination of K_{CO} and V_A. Therefore, inspection of the components of $T_{L,CO}$ (K_{CO} and V_A) is essential if a reasonable interpretation of the $T_{L,CO}$ test is to be made.

Clinical examples

Table 3 lists examples of a low $T_{L,CO}$ in some pulmonary diseases. In inspiratory muscle weakness (Hart *et al.*, 2002), K_{CO} is 130% predicted (for a normal TLC), but at this low V_A (50% predicted) the K_{CO} for a normal subject would be >150% predicted (Hughes & Pride, 2001). As previously mentioned, parenchymal lung damage or atelectasis limit the rise in K_{CO} in 'extrapulmonary' restriction.

The K_{CO} for a 50% reduction in V_A due to loss of lung units is about 115% predicted (Hughes & Pride, 2001), and this is what occurs post-pneumonectomy (Corris *et al.*, 1987). The situations in Table 3 where K_{CO} is normal (sarcoidosis, bronchiectasis) but V_A is reduced (and there is no airflow obstruction as a cause) are compatible with loss of lung units due to underlying disease with sparing of the remainder of the lung.

Where K_{CO} is moderately impaired (84–85%) in CHF and fibrosing alveolitis, there is presumably a spectrum of lung damage – normal units with a K_{CO} in the 100–110% range and diseased units with a low K_{CO} (<80%). In Table 3, note four situations (post-pneumonectomy, fibrosing alveolitis, primary PHT and emphysema) where $T_{L,CO}$ is essentially the same (54–58% predicted) but inspection of the V_A and K_{CO} patterns (and, in the case of emphysema, the FEV_1/VC ratio) reveals different

Table 3 Typical values of a low $T_{L,CO}$ and its components (V_A and K_{CO}) in some common pulmonary conditions. % is percent of predicted normal value.

Diagnosis	Source	FEV_1/VC (actual)	Vc %	V_A %	K_{CO}%	$T_{L,CO}$%	Comment
High/normal K_{CO} with low V_A							
Inspiratory muscle weakness	Hart (2002)	0·79	50	55	130	66	Lack of alveolar expansion (see text)
Pneumonectomy	Corris (1987)	NA	82*	77*	98*	78*	*Pre **Post
		NA	53**	51**	111**	58**†	Loss of lung units, discrete
Sarcoidosis with infiltrates	Hughes (1999)	0·8	83	75	105	76	Loss of lung units predominantly
Bronchiectasis§	Perez (1998)	0·75#	96	82#	99	82	Loss of lung units predominantly
Low K_{CO} with low/normal V_A							
CHF (NYTHA III)	Puri (1995)	0·71	76	75	85	72	Loss of units; some microvascular damage
Fibrosing alveolitis	Hughes (1999)	0·78	73	66	84	54†	Loss of units; some alveolar–capillary damage
Primary PHT	Hughes (1999)	0·9	91	100	58	58†	Diffuse microvascular damage
Emphysema	Gevenois (1996)	0·55	84	74	49	54†	Diffuse alveolar–capillary damage

#, estimated value; §, on CT scan evidence (rheumatoid arthritis patients); NA, not available.
PHT, pulmonary hypertension; CHF, congestive heart failure; NYHA III, New York Heart Association, grade III.
†Note similar $T_{L,CO}$ in these four instances, but different combinations of V_A and K_{CO}.

pathophysiological mechanisms in each case see Hughes (1999) for more examples.

$T_{L,CO}$, exercise and arterial hypoxaemia

The $T_{L,CO}$ is rarely measured on exercise except in research protocols. In normal subjects, $T_{L,CO}$ increases in proportion to the workload; there is no evidence of a plateau (Hsia *et al.*, 1995). At near maximal exercise, $T_{L,CO}$ is 50% higher than resting values in men (25% higher in women) (Hsia *et al.*, 1995). In patients with lung disease, the fractional (*percentage*) increase in $T_{L,CO}$ for a given level of exercise is similar to that in normal subjects (reviewed in Hughes, 1991), although exercise levels are quite low ($\dot{V}O_2 \leq 1.0$ L min^{-1}). When resting $T_{L,CO}$ is <60% predicted, worsening of arterial hypoxaemia on exercise is seen almost invariably. Patients with interstitial lung disease and diffuse alveolar–capillary damage have been the subjects of most studies. It is not easy to differentiate diffusion limitation from $\dot{V}_A/\dot{Q}$ mismatch as causes of the exercise-induced hypoxaemia – a problem first studied in the 1940s (Baldwin *et al.*, 1949).

In interstitial lung disease patients with a low $T_{L,CO}$, diffusion limitation, in terms of the contribution of the PA–Pc′ (alveolar to end-capillary) gradient to the overall PA–Pa (alveolar to arterial) oxygen gradient, is about 10% at rest, 20–30% on light exercise and more than 50% at a $\dot{V}O_2$ of 1.0 L min^{-1} (Hughes, 1991). The reason for the widening PA–Pc′ gradient (failure of capillary PO_2 to equilibrate with alveolar PO_2 before red cells leave the alveolus) is a low diffusion–perfusion ratio ($\sim T_{L,CO}/\dot{Q}$) within gas exchanging units, or for the lung overall if the disease process is diffuse. In interstitial lung disease, the $T_L/\dot{Q}$ ratio at rest is generally above the critical threshold for diffusion limitation, but on exercise, the increase in $T_{L,CO}$, starting from a low base, is insufficient to match the increase in blood flow, so the $T_L/\dot{Q}$ ratio falls and a PA–Pc′ gradient emerges. On the other hand, in patients with airflow obstruction (emphysema, for example), any change in PaO_2 on exercise will reflect ventilatory limitation and local $\dot{V}_A/\dot{Q}$ mismatching more than any decline in $T_L/\dot{Q}$ ratios.

Conclusion

Recent physiological evidence, using the Roughton–Forster analysis, and morphometric measurements on lungs post-mortem, suggest that most of the resistance to CO transfer from alveolar gas to pulmonary capillary blood may lie in the red cell itself. The clinical implication is that $T_{L,CO}$ and K_{CO} are focused primarily on the pulmonary capillary bed. Although *non-specific* diffuse alveolar damage, as in interstitial fibrosis, or alveolar destruction, as in emphysema, will compromise the microvasculature and reduce $T_{L,CO}$ and K_{CO}, pathology *specific* to the pulmonary circulation, without diffuse alveolar damage, such as vasculitis, raised pulmonary venous pressure or microvascular dilatation (as in PAVMs and HPS) also reduces $T_{L,CO}$ and K_{CO}.

As simple monitors of the integrity of the pulmonary microcirculation, $T_{L,CO}$ and K_{CO} are uniquely valuable and important clinical tests.

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