

Implementing the three-equation method of measuring single breath carbon monoxide diffusing capacity

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Conventional methods of measuring the single breath diffusing capacity of the lung for carbon monoxide (DL_{CO}^{SB}) are based on the Krogh equation, which is valid only during breath holding. Rigid standardization is used to approximate a pure breath hold manoeuvre, but variations in performing the manoeuvre cause errors in the measurement of DL_{CO}^{SB} . The authors previously described a method of measuring DL_{CO}^{SB} using separate equations describing carbon monoxide uptake during each phase of the manoeuvre: inhalation, breath holding and exhalation. The method is manoeuvre-independent, uses all of the exhaled alveolar gas to improve estimates of mean DL_{CO}^{SB} and lung volume, and is more accurate and precise than conventional methods. A slow, submaximal, more physiological single breath manoeuvre can be used to measure DL_{CO}^{SB} in patients who cannot achieve the flow rates and breath hold times necessary for the standardized manoeuvre. The method was initially implemented using prototype equipment but commercial systems are now available that are capable of implementing this method. The authors describe how to implement the method and discuss considerations to be made in its use.

Key Words: *Diffusion, Diffusing capacity, Gas exchange, Lung volume, Transfer factor*

Utilisation de la méthode à trois équations pour mesurer la capacité de diffusion du monoxyde de carbone en apnée

RÉSUMÉ : Les méthodes traditionnelles pour mesurer la capacité de diffusion du monoxyde de carbone (DL_{CO}) en apnée sont basées sur la formule de Krogh, qui est valide uniquement pendant l'apnée. On procède alors à une normalisation arbitraire pour se rapprocher de la manoeuvre en apnée pure, mais les variations qui se produisent en pratiquant la manoeuvre sont une source d'erreur dans la mesure de la DL_{CO} en apnée. Les auteurs ont précédemment décrit une méthode de mesure de la DL_{CO} en apnée où l'on utilisait des équations séparées décrivant la capture du monoxyde de carbone pendant chaque phase de la manoeuvre : inspiration, apnée et expiration. La méthode est indépendante de la manoeuvre, utilise la totalité du gaz alvéolaire expiré pour améliorer les estimations de la DL_{CO} moyenne en apnée et du volume pulmonaire, et est plus fiable et précise que les méthodes traditionnelles. Une manoeuvre en apnée lente, sous-maximale et plus physiologique peut être utilisée pour mesurer la DL_{CO} en apnée chez les patients qui ne peuvent atteindre les débits et rester en apnée pendant le temps nécessaire à la manoeuvre normalisée. La méthode a été initialement développée en utilisant un matériel prototype, cependant des appareils commerciaux sont maintenant disponibles pour permettre la mise en application de cette méthode. Les auteurs décrivent comment procéder et discutent des considérations relatives à son utilisation.

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The single breath diffusing capacity of the lung for carbon monoxide (DL_{CO}^{SB}) was first measured by Krogh (1), who developed an equation to describe the uptake of carbon monoxide during breath holding. Conventional methods of measuring DL_{CO}^{SB} still use the Krogh equation (1) and are therefore constrained by the requirement of a rigidly standardized single breath manoeuvre, which approximates a pure breath hold but obviously includes inhalation and exhalation. Although the uptake of carbon monoxide by the lungs measured in this manner is more appropriately called the transfer factor (2), we have used the American Thoracic Society (ATS) convention of calling it diffusing capacity (3).

Most current clinical measurements of DL_{CO}^{SB} use some variation of the technique described by Ogilvie et al (4). The Jones and Meade method (5) applies corrections to the timing of the breath hold period and recommends the collection of a very small alveolar gas sample immediately following dead space washout. The method recommended by Ferris (6), referred to as Epidemiology Standardization Project method, appears to have been developed for ease of computation and standardization without a theoretical or clinical rationale. We have previously shown that all of these methods overestimate DL_{CO}^{SB} measured from a lung model (7) and normal subjects (8), particularly when the exhaled flow rates are reduced.

DL_{CO}^{SB} is overestimated by conventional methods because they are based on a single equation that is valid only during breath holding. The methods rely on the use of a standardized manoeuvre consisting of rapid inhalation, 10 s of breath holding and rapid exhalation to approximate a pure breath hold manoeuvre (2,3). However, the measurement of DL_{CO}^{SB} depends on how well the subject can perform the manoeuvre. If the inspiratory or expiratory flow rates are reduced, if the breath hold time is changed, if the alveolar sample is collected later in exhalation, or if the volume of the alveolar sample is changed, then the measured DL_{CO}^{SB} will be spuriously affected (7-9).

For normal subjects who can inhale and exhale with high flow rates and hold their breath for 10 s, measurement of DL_{CO}^{SB} is not a problem because the differences between the various methods are small (9). For patients with airflow obstruction, or any other subject who cannot adequately perform the standardized single breath manoeuvre, DL_{CO}^{SB} is usually overestimated (8).

We developed a method of measuring DL_{CO}^{SB} based on three equations – one each for inhalation, breath holding and exhalation (7). These equations analytically account for the differences in the rate of carbon monoxide uptake during inhalation, breath holding and exhalation. Diffusion of carbon monoxide during all three phases of the manoeuvre – inhalation, breath holding and exhalation – can be determined, eliminating the need to assume that all carbon monoxide transfer occurs during breath hold. This makes the measurement of DL_{CO}^{SB} independent of the manoeuvre, and increases precision and accuracy of DL_{CO}^{SB} measurements (9). The subject is not required to inhale and exhale with maximum flow rates and breath hold for 10 s to approximate a pure breath hold manoeuvre. Theoretically, variations in

the flow rates and breath hold times do not cause errors in the measurement of DL_{CO}^{SB} .

With the three-equation method, gas concentrations and lung volume are monitored continuously throughout the single breath manoeuvre. These data are used to calculate the carbon monoxide concentrations in the alveolar space and to predict the uptake of carbon monoxide. By comparing the observed with the predicted carbon monoxide uptake, DL_{CO}^{SB} can be calculated. Because the inspiratory flow and gas concentrations are monitored and the carbon monoxide uptake is computed during inhalation, the assumptions for start of breath holding time and carbon monoxide concentration at 'time zero' used by conventional methods are not required.

The three-equation method permits the measurement of DL_{CO}^{SB} at different lung volumes (10), different breath hold times (11) and different flow rates (9). The method also permits all of the exhaled alveolar gas to be used in the calculation of DL_{CO}^{SB} . This yields a measurement of DL_{CO}^{SB} that more accurately reflects the mean carbon monoxide uptake of the entire lung. In contrast, conventional methods use smaller alveolar gas samples collected immediately after dead space washout to reduce timing errors, but, unfortunately, the measurements of both the DL_{CO}^{SB} and the absolute lung volume are more influenced by ventilation inhomogeneities in the lung when measured using small gas samples.

We have focused the implementation of this method on the use of a manoeuvre with relaxed flow rates with submaximal lung volumes that can be done equally well by subjects or patients and that more closely approximates normal breathing. The manoeuvre can be done with or without breath holding. The method is by no means restricted to this or any other particular manoeuvre.

The three-equation DL_{CO}^{SB} method was initially developed using a mass spectrometer, a modified infrared absorption type carbon monoxide analyzer and other prototype equipment (8,9). With the continuing refinement of computerized pulmonary function equipment and the improvement of gas analyzers, it is now feasible to use commercial pulmonary function systems to implement this method. We present considerations for the implementation of the method and an efficient computer algorithm for rapid calculation of DL_{CO}^{SB} .

EQUIPMENT

Gas analyzers: While, in theory, the three-equation method could be implemented by using the mean carbon monoxide concentration measured from any size of gas sample collected during exhalation (7), in practice, problems of dead space washout, sample timing and sample volume prevent the advantages of the three-equation method from being fully realized when applied to data collected by conventional DL_{CO}^{SB} equipment (12). The availability of continuously measured concentrations of carbon monoxide and a tracer gas not only facilitates implementation of the three-equation algorithm but also permits simultaneous measurements of ventilation and gas mixing from the same single breath manoeuvre (10).

TABLE 1
Error in DLco^{SB} due to carbon monoxide analyzer response time

$t_{0-90\%}$ (ms)	τ (ms)	Shift (points)	Error (%)
0	0	0	0
67	29	1	-0.01
133	58	2	-0.22
200	87	3	-0.68
267	116	4	-0.91
333	145	5	-1.13
400	174	6	-1.41
467	203	7	-1.63
533	232	8	-1.87
600	261	9	-2.15
667	290	10	-2.47

Data from a simulated normal adult lung model using the single breath manoeuvre shown in Figure 5. Response times of carbon monoxide analyzer were simulated to give optimal shifts of 0 to 10 sample points. The error was reduced by an average of 66% when the breath hold time was increased to 10 s. There was little change in the error when the single breath diffusing capacity of the lung for carbon monoxide (DLco^{SB}) was changed from 10 through 40 mL/min/torr. $t_{0-90\%}$ is the 0% to 90% response time of carbon monoxide analyzer (ms); τ is the analyzer time constant (ms); shift is the optimal shift in sample points (20 ms); error is the percentage error in the measured DLco^{SB} compared with the simulated DLco^{SB}

Infrared absorption type analyzers are commonly used for measuring carbon monoxide concentration. Care must be taken to ensure that background carbon dioxide and water vapour do not interfere with the carbon monoxide concentration signal, particularly in view of the very low carbon monoxide levels to be measured. An additional compensation strategy that is incorporated in the algorithm is discussed below.

The response time of the analyzer is also of concern. A slowly responding analyzer will produce inaccurate DLco^{SB} measurements (Table 1). The response time is generally a function of the size of the sample chamber, the sample aspiration rate, the gas pressure in the sample cell and the geometry of the system. Increasing the aspiration flow rate, which usually decreases the chamber pressure (and hence decreases the density of the test gas in the chamber), will improve the response time, but will decrease the signal to noise ratio. Similarly, using a smaller sample chamber will increase response time but will also diminish the signal to noise ratio. A balance between response time and noise must be found. In the test system described here, a commercially available analyzer was modified to provide a 0% to 90% response time of 110 ms at an aspiration rate of 125 mL/s with a root-mean-square noise level of 1% full scale.

The signal to noise ratio of sampled data increases proportionally with the square root of the number of samples considered. For example, averaging 100 samples to obtain zero level readings improves the signal to noise ratio by a factor of 10 to 0.1% full scale. There is a similar improvement in determining the mean carbon monoxide concentration in the exhaled gas.

Digital techniques for compensating for the response time

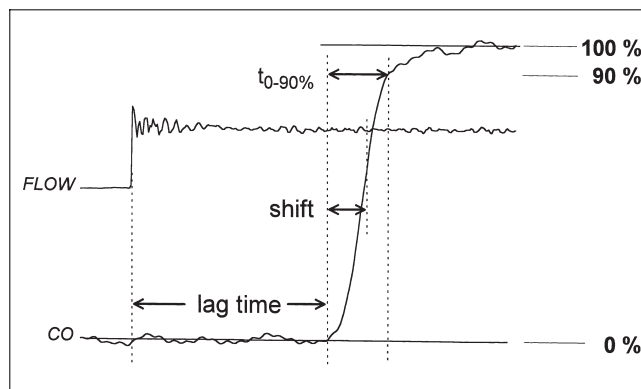


Figure 1 Lag and response times for carbon monoxide: the response time of the analyzer was estimated by rapidly switching the gas being sampled from zero to full scale carbon monoxide. The change in the flow signal shows the time at which the switch was made from medical air to test gas. The lag time, the 0% to 90% response time ($t_{0-90\%}$) and the optimal shift are calculated from the resulting response curve. A similar analysis was done for the helium concentration signal

of the gas analyzers are available, but the noise levels typical of infrared analyzers prevent these methods from being very useful. A more suitable compensation technique, given the noise levels and sampling rates used, is an optimal shift of the signal, which is the natural logarithm of twice the time constant (13).

The lag time of the analyzer, which is the transport time of the aspirated gas through the tubing to the sample chamber, is generally not a problem because it can be measured and simply added to the response time shift (Figure 1). The only concern with the increasing lag time is gas mixing within the sampling tube, which blunts the response of the instrument. A similar lag and response time correction is applied to the measurement of the tracer gas concentration.

Helium was used as the tracer gas. Helium concentration was measured by a mass spectrometer. The advantages of the mass spectrometer are that very low aspiration rates are used (typically 1 mL/s), the response is rapid (typically 0% to 90% response of 80 ms) and the signal to noise ratio is high (noise less than 0.1% full scale). The mass spectrometer cannot be used to measure carbon monoxide concentration because both carbon monoxide and nitrogen have a molecular mass of about 28. A stable isotope with a higher molecular mass, C¹⁸O, has been used, but it is expensive and potentially noisy because the tiny signal for C¹⁸O falls directly between the very large peaks in the mass spectrum for nitrogen and oxygen. Dry test gas was used containing 0.3% carbon monoxide, 10% helium, 21% oxygen, balance nitrogen.

Methane (0.3%) has also been used as a tracer gas (14) and measured by an infrared analyzer with similar problems as the measurement of carbon monoxide. Unfortunately, using methane in very low concentrations makes noise more of a problem. The increased solubility of methane can lead to an overestimation of lung volume, but the predicted effect is small (14).

The linearity of gas analyzers presents an additional prob-

TABLE 2
Error in DL_{CO}^{SB} due to carbon monoxide analyzer nonlinearity

	Nonlinearity (%)				
	0	0.5	1.0	1.5	2.0
%error (0 s bht)	0	1.77	3.23	4.93	6.61
%error (10 s bht)	0	0.69	1.46	2.18	2.92
	DL_{CO}^{SB} (mL/min/torr)				
	10	20	30	40	
%error (0 s bht)	8.74	4.92	3.23	2.67	
%error (10 s bht)	3.74	2.04	1.46	1.17	

A nonlinearity of the form $f(x) = ax^2 + bx$ was simulated for the carbon monoxide data from a normal adult lung model with a single breath diffusing capacity of the lung for carbon monoxide (DL_{CO}^{SB}) of 30 mL/min/torr. In the upper panel, the nonlinearity specifies the maximum deviation as a percentage of full scale. The error gives the percentage error in the measured DL_{CO}^{SB} from simulated manoeuvres with 0 s and 10 s breath hold time (bht). The lower panel shows the interaction between the nonlinearity of the carbon monoxide analyzer and the DL_{CO}^{SB} in the lung model. DL_{CO}^{SB} was varied from 10 to 40 mL/min/torr. The nonlinearity in the carbon monoxide analyzer for these simulations was 1% full scale. The percentage error in the measured DL_{CO}^{SB} for 0 s and 10 s breath hold manoeuvres is shown

lem. Typically, gas analyzers are checked at zero and at full scale. Table 2 shows the effects of nonlinearity of the carbon monoxide analyzer on DL_{CO}^{SB} measurements. Note that the error in DL_{CO}^{SB} due to analyzer nonlinearity is dependent upon the carbon monoxide concentration, and hence varies with both breath hold time and DL_{CO}^{SB} . The linearity of the analyzers should be checked regularly (see calibration section).

Gas flow: A pneumotach mounted in the wall of a bags-in-box system was used to measure flow (8,9,11) and the signal was integrated to obtain volume. A #3 Fleisch pneumotach with a ± 2 cmH₂O differential pressure transducer was used. Ambient room air always flows through the pneumotach. Linearizing tubes that are six times longer than the diameter of the pneumotach are used to maintain a uniform flow profile. Other systems use in-line pneumotachs or mass flow sensors that must be compensated for gases of different densities and temperatures. Nonlinearities may be compensated by digitally processing the flow signal rather than by using linearizing cones or screens. If slow flow rates are used for the single breath manoeuvre, recommended for the three-equation method, fewer problems are encountered in measuring flow. An alternative strategy is to use a spirometer to measure volume.

Breathing circuit: It is important to keep equipment dead space to a minimum because increasing the dead space of the breathing circuit will increase the response time of the system. The effectiveness of rapidly responding gas analyzers will be lost if the sampling ports are located in a part of the circuit distant from the mouth or where mixing is slow. When using continuous monitoring of gas concentration signals, the functional equipment dead space is the volume of the tubing between the gas sample ports and the mouth (Figure 2). The gas sample ports should, therefore, be located as close to the mouth as possible. A low dead space two-way valve, the

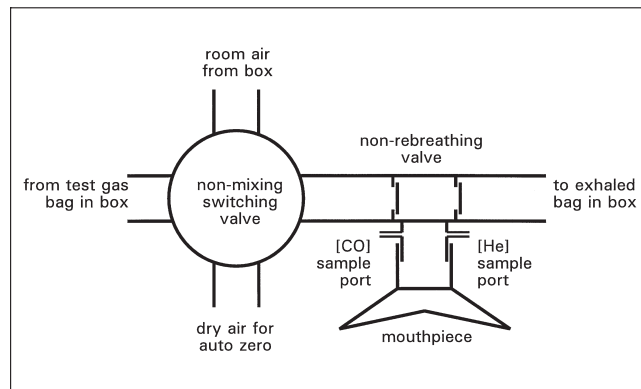


Figure 2) The breathing circuit for a bags-in-box system

inspiratory limb of which was connected to a three-way switching valve, was used with the gas sampling ports located just distal to the mouthpiece. Using an in-line flow sensor will increase the equipment dead space, but such devices are now available in small sizes with low resistance. These systems may use demand valves to deliver test gas, which require some negative pressure during inhalation, but it is unlikely that pressures will be in the range to influence diffusing capacity (15). The use of disposable filters will increase both dead space and response time.

It is very important to ensure that the system is free from leaks. With low gas concentrations and high aspiration rates, a small leak can make a significant difference in the signal. A method should be in place to check routinely for leaks. The method the authors use is to flush the system with medical air (21% oxygen, 79% nitrogen), close off the mouthpiece and blow test gas around the valves and tubing. If there is a leak then the meters will register an increase in carbon monoxide or helium concentration.

CALIBRATION

Flow meter: Because forced inhaled and exhaled flow rates are not used, the flow meter does not have to meet ATS requirements (3). The flow meter was checked for linearity by using a rotameter system. The flow meter was linear to within 1% of full scale over the range of 0.05 to 3 L/s for both inhalation and exhalation. If a nonlinearity had been detected it would have been corrected with digital signal processing. On a daily basis, the flow signal is calibrated using a 3 L syringe. The first calibration check is done with the gas analyzers off so that they are not aspirating any gas. The calibration is accepted when the inspired and expired volumes measured from the syringe are within 1% of the syringe volume at flow rates of 0.1, 0.5 and 2 L/s.

The flow calibration is then repeated with the gas analyzers aspirating to compensate for the combined aspiration flow rate of the gas analyzers. A significant day to day change in the analyzer aspiration rate usually indicates a blockage, malfunction or leak in the system. The analyzer aspiration flow rate determined from the calibration is used to offset the flow signal during actual manoeuvres. The analyzer aspiration flow rate is also used to determine the

point near the end of exhalation when the subject's exhaled flow rate falls below the analyzer aspiration rate. Since beyond this point the carbon monoxide and helium concentration data are meaningless, this becomes the end-of-test point for gas analysis.

Gas analyzers: For measurements of DL_{CO}^{SB} the absolute accuracy of the analyzers is unimportant. Because ratios of gas concentrations are being compared, it is the relative accuracy, and hence the linearity of the meters, that is the main concern. The preferred method of determining the linearity of a gas analyzer is to deliver known dilutions of a given test gas to the analyzer and to compare the observed with the expected measurement. A Wöststoff Pump (Bochum, Germany) was used for this purpose. The linearities of the helium and the carbon monoxide concentration analyzers were measured separately. As part of the daily calibration routine, test gas is diluted with dry medical air and the output of the mass spectrometer is compared with the output of the carbon monoxide concentration analyzer (Figure 3). This procedure requires only a few minutes to perform because the output of the analyzers is sampled while the flow rates of the test gas and medical air are changed. This procedure would also be valid for dual gas analyzers (eg, infrared carbon monoxide and methane), which use different sensors for each gas.

Algorithm calibration: The authors have no device to calibrate the entire system. The hardware components are calibrated as described above. The software is verified by using data from a computer simulation of the single breath manoeuvre. Raw data from the analogue to digital converter are simulated and subsequently processed by the same software used to analyze real data. If the measured variables such as DL_{CO}^{SB} and lung volume are not the same as the simulated values for those variables over a range of expected operating conditions then the error in the software must be corrected.

DATA ACQUISITION

The analogue signals for flow, carbon monoxide concentration and helium concentration must be digitized for subsequent processing by the computer. The signals are filtered with a second-order 10 Hz low-pass filter and sampled at a rate of 50 Hz per channel. A 12 bit analogue to digital converter is used with the signal amplitude matched to the range of the converter. The effect of sampling rate was examined in lung models and healthy subjects. It was found that, for manoeuvres using flow rates less than 2 L/s, a rate of 50 Hz was adequate.

To guide the subject through the manoeuvre, a template of volume versus time was displayed for the prescribed manoeuvre, including the volume history control, on a computer screen. The subject's vital capacity (VC) and inspiratory capacity (IC) were measured in a practice manoeuvre without test gas. A real time tracing of the subject's volume versus time was displayed over the template. Most subjects and patients can easily adjust their flow rates to match the template with little practice. When the subject reached total lung capacity (TLC), the volume tracing was adjusted, if neces-

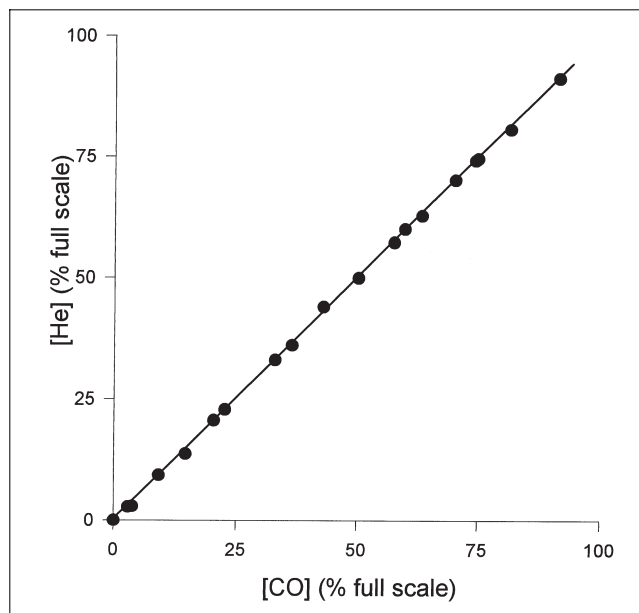


Figure 3) A typical check of analyzer nonlinearity comparing the carbon monoxide analyzer with the helium analyzer. Different dilutions of test gas are sampled simultaneously by the two analyzers. If either analyzer becomes nonlinear, the data points deviate from the line of identity

sary, to superimpose the template at TLC during the volume history control breath. The subject then exhaled to the pre-measured functional residual capacity (FRC) to start inhalation of test gas using the template as a guide.

Washout between tests: When the subject first breathed through the mouthpiece, helium concentration was measured to ensure that adequate washout had occurred since the previous test. If the helium concentration rose above 0.1% within the first three tidal breaths then the test was aborted.

Zero levels: Before and after each single breath manoeuvre the average zero levels of the analyzers and flow meter over a 2 s period were determined with the flow meter tube occluded and the gas analyzers aspirating dry medical air. This gives a 'dry' zero for the carbon monoxide concentration analyzer with no carbon dioxide or water vapour. To control volume history each single breath manoeuvre was preceded by a standardized breath of room air with slow inhalation from FRC to TLC, a 5 s breath hold at TLC and slow exhalation back to FRC. During this deep breath, the ambient carbon monoxide concentration was measured during inspiration, and the premanoeuvre background carbon monoxide concentration in alveolar gas was measured during exhalation. The latter measurement was used as the 'wet' zero for the analyzer, incorporating any residual interference effects of water vapour and carbon dioxide in addition to background carbon monoxide, which includes carbon monoxide back pressure from smoking, environmental exposure or previous DL_{CO}^{SB} tests. The 'wet' zero was applied to the carbon monoxide signal during the expiratory phase of the single breath manoeuvre, while the 'dry' zero was applied during the inspiratory phase. This strategy compensates for the back pressure of carbon monoxide, which is otherwise

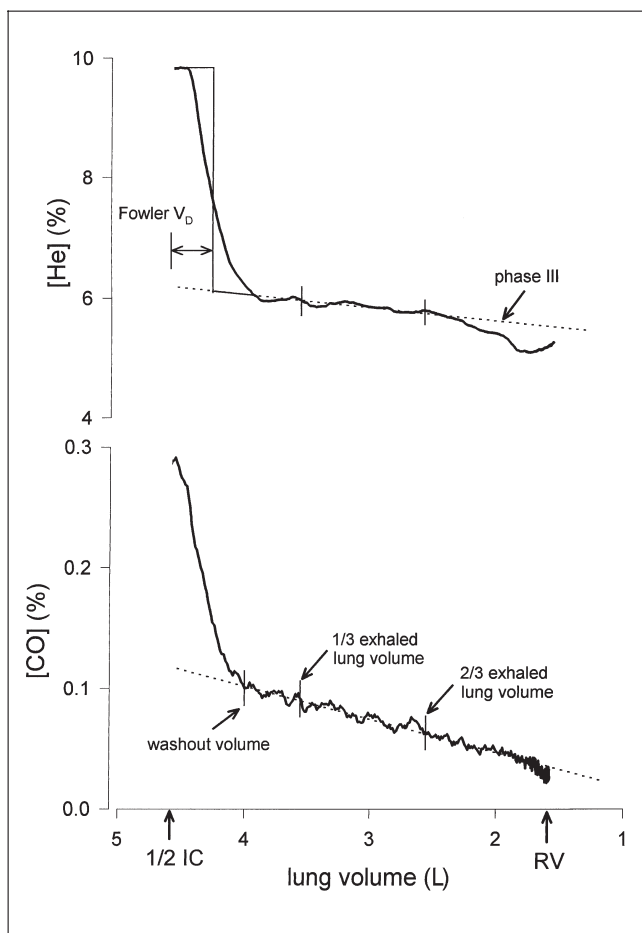


Figure 4 The Fowler dead space (V_D) and the phase III slope are measured from the helium concentration washout curve (upper panel). The point of dead space washout is measured from the carbon monoxide concentration washout curve (lower panel). IC Inspiratory capacity; RV Residual volume

assumed to be zero and which can be a significant factor when analyzing serial single breath manoeuvres and especially when studying smokers.

The 'dry' zero baseline levels before and after the manoeuvre were compared. If the carbon monoxide concentration zero level drifted by more than 20 parts/million, the helium concentration zero level drifted by more than 300 parts/million or the flow zero level drifted by more than 10 mL/s, then the test was rejected.

Water vapour was removed from the gas sampled by the carbon monoxide analyzer using tubing that is selectively permeable to water vapour. The tubing is inside a sheath through which dry medical air is continuously flushed. While this eliminates most of the water vapour, the zero level procedure compensates for the small, residual interference.

Some commercial systems simply equilibrate water vapour to the ambient level in room air by aspirating the sample through water vapour permeable tubing exposed to room air. The above procedure of obtaining a 'wet' zero level should still be used to compensate for the back pressure of carbon monoxide and any residual interference of carbon dioxide and water vapour.

LUNG VOLUMES AND DISTRIBUTION OF VENTILATION

Volume: The measurement of $DLCO^{SB}$ requires a measurement of absolute lung volume (V_A). In conventional $DLCO^{SB}$ methods, V_A is estimated as inspired tracer gas concentration ($[He]_{INSP}$) times inspired volume (V_{INSP}) divided by tracer gas concentration in the exhaled gas sample ($[He]_{SAMP}$). For example, for helium as tracer gas:

$$V_A = \frac{[He]_{INSP} \cdot V_{INSP}}{[He]_{SAMP}}$$

The inherent assumption is that $[He]_{SAMP}$ is equal to the mean gas concentration in the lung at end-inspiration. Whereas this approximation may be reasonable in subjects with little ventilation inhomogeneity, it usually underestimates lung volume in the patients with lung disease (11). The lung volume measurement is also influenced by the timing of the exhaled gas sample because $[He]_{SAMP}$ will be smaller if the sample is collected later in exhalation.

If a continuous recording of the tracer gas is available, more information can be used to calculate V_A . The algorithm the authors use (11) measures all of the tracer gas inhaled and all of the tracer gas exhaled to determine how much of the tracer gas remains in the lung at residual volume (RV). V_A is calculated by assuming that the tracer gas concentration measured at end-expiration is equal to the mean concentration of tracer gas in the lung at RV.

t_0 was defined as the start of inhalation of test gas, t_1 as the end of inspiration – the point of maximum lung volume – and t_2 as the end of exhalation – the point when the expiratory flow falls to the level of the combined analyzer aspiration rate. V_A at end-exhalation, $V_A(t_2)$, is found as:

$$V_A(t_2) = \int_{t_0}^{t_2} \frac{He(t) \, dV(t)}{He(t_2)}$$

where $V(t)$ is the lung volume at time t and $He(t)$ is the fractional helium concentration at time t . To reduce noise, the mean of the last 10 sample points is used for $He(t_2)$. In contrast to conventional methods, this algorithm yields a robust calculation of V_A that does not change with breath hold time (11). This calculation of lung volume includes the equipment dead space proximal to the gas sample ports, the anatomic dead space and the alveolar volume. The measured flow signal is converted to conditions of body temperature saturated with water vapour (BTPS) and, hence, V_A is also BTPS.

Dead space: Anatomic dead space is determined by a computerized algorithm based on the Fowler method (16) with the obvious changes to accommodate the wash-in of a tracer gas rather than the washout of nitrogen. It was found that dead space changed with lung volume (10) in a similar manner to that previously reported (17). Dead space also varies with breath hold time (10,11). This measurement of dead space includes the equipment dead space between the gas analyzer sample ports and the mouth. In the three-equation algorithm, dead space is subtracted from V_A to give alveolar lung volume.

Washout volume: The point of dead space washout was determined from the carbon monoxide concentration washout curve. A linear regression line through the middle third (by volume) of exhaled carbon monoxide versus lung volume was found. The point at which the observed carbon monoxide concentration first crossed this line was considered to be the point of dead space washout (Figure 4). The washout volume was the volume exhaled from maximum lung volume to this point. The washout volume varied with flow rate; exhaling at 0.5 L/s resulted in a smaller washout volume than exhaling at 2 L/s (10).

Phase III slope: The measurement of the slope of the alveolar plateau (phase III) of the tracer gas washout curve ($\Delta\text{He}/L$) was based on the single breath nitrogen washout method (18). A linear regression line through the middle third (by volume) of exhaled helium versus lung volume was found. The slope of this line was $\Delta\text{He}/L$. Because $\Delta\text{He}/L$ depends on the concentration of helium in the lung, which in turn depends on the specific ventilation (10), the slope is normalized to account for differences in specific ventilation. The predicted mean end-inspiratory alveolar helium concentration ($[\text{He}]_{\text{APRED}}$), assuming perfect mixing, is calculated by the equation:

$$[\text{He}]_{\text{APRED}} = \int_{t_0}^{t_D} \frac{\text{He}(t)dV(t)}{V_A(t)}$$

where t_D is the time when $V(t) = V(t_1) - V_D$, V_D is the Fowler dead space, and $V_A(t_1)$ is the alveolar volume at end-inhalation. The normalized phase III slope (S_n) is:

$$S_n = \frac{\Delta\text{He}/L}{[\text{He}]_{\text{APRED}}}$$

S_n was found to be independent of gas concentration, whereas $\Delta\text{He}/L$ increased with increasing helium concentration (10). The reproducibility of S_n was much better than that of $\Delta\text{He}/L$.

Mixing efficiency: The calculated mixing efficiency was similar to that of Cumming and Guyatt (19), but the authors used the wash-in of helium rather than washout of nitrogen (20). The mean exhaled helium concentration for all of the exhaled gas was found ($[\text{He}]_{\text{MEAS}}$). The mixing efficiency (E_{MIX}) was the ratio of the predicted to the observed mean helium concentration, ie:

$$E_{\text{MIX}} = \frac{[\text{He}]_{\text{APRED}}}{[\text{He}]_{\text{MEAS}}}$$

E_{MIX} was found to be very reproducible, with a coefficient of variation of less than 0.5% (10,20,21).

THREE-EQUATIONS ALGORITHM

The equations for the uptake of carbon monoxide are all based on the general mass balance equation, which states that the change in the mass of carbon monoxide in the lung is equal to the mass of carbon monoxide diffusing out of the lung plus the mass of carbon monoxide coming into or out of the mouth, ie, change in carbon monoxide in lung = - carbon monoxide diffused + carbon monoxide flow at mouth:

$$\frac{dV(t)\text{CO}(t)}{dt} = -D_{\text{LCO}} \cdot P_{\text{Aco}}(t) + \text{CO}_m(t) \frac{dV(t)}{dt} \quad [1]$$

where $V(t)$ is the absolute alveolar lung volume at time t , $\text{CO}(t)$ is the fractional concentration of carbon monoxide in the dry gas in the lung, $\text{CO}_m(t)$ is the fractional concentration of carbon monoxide in the dry gas at the mouth and $P_{\text{Aco}}(t)$ is the partial pressure of carbon monoxide in the alveolar gas. If P_B is the barometric pressure in torr then the partial pressure of the dry gas is $P_D = P_B - 47$ and $P_{\text{Aco}}(t) = P_D \cdot \text{CO}(t)$. For inhalation equation [1] becomes:

$$V(t) \frac{d\text{CO}(t)}{dt} + \text{CO}(t) \frac{dV(t)}{dt} = -D_{\text{LCO}} \cdot P_b \cdot \text{CO}(t) + \text{CO}_m(t) \frac{dV(t)}{dt} \quad [2]$$

During breath holding at some constant volume V_{BH} equation 1 becomes

$$V_{\text{BH}} \frac{d\text{CO}(t)}{dt} = -D_{\text{LCO}} \cdot P_b \cdot \text{CO}(t) \quad [3]$$

which is equivalent to the familiar Krogh equation (1). During exhalation, carbon monoxide concentration in the lung is assumed to be equal to the carbon monoxide concentration at the mouth. In the computer algorithm a time delay equal to the dead space transit time is used to eliminate this assumption. During exhalation, equation [1] becomes:

$$V(t) \frac{d\text{CO}(t)}{dt} = -D_{\text{LCO}} \cdot P_b \cdot \text{CO}(t) \quad [4]$$

If inhalation occurs from time t_0 to time t_1 , $\text{CO}(t_0) = 0$, $f(t) = dV(t)/dt$ and $\text{CO}_m(t)$ is monitored, then equation [2] can be solved to give the carbon monoxide concentration in the lung at end-inhalation (equation [5], below). If exhalation occurs from time t_1 to time t_2 , then equation [2] can be solved to give the carbon monoxide concentration during exhalation, $\text{CO}_E(t)$:

$$\text{CO}_E(t) = \text{CO}(t_1) \cdot \exp \left[-D_{\text{LCO}} \cdot P_b \int_{t_1}^t \frac{dt}{V(t)} \right] \quad [6]$$

where $t_1 \leq t \leq t_2$. If a period of breath holding were to occur

$$\text{CO}(t_1) = \exp \left[- \int_{t_0}^{t_1} \frac{f(t) + D_{\text{LCO}} P_b}{V(t)} dt \right] \cdot \int_{t_0}^{t_1} \frac{\text{CO}_m(t) \cdot f(t)}{V(t)} dt \cdot \exp \left[\int_{t_0}^{t_1} \frac{f(t) + D_{\text{LCO}} \cdot P_b}{V(t)} dt \right] \quad [5]$$

from time t_x to time t_y , then the carbon monoxide at the end of breath holding would be:

$$CO_{E(t_y)} = CO_{E(t_x)} \cdot \exp\left(-\frac{DLCO \cdot P_o \cdot (t_y - t_x)}{V(t_x)}\right)$$

However, during breath holding, both the equations 2 and 4 reduce to equation 3. Therefore, in the implementation, only two equations, 5 and 6, are used but the name 'three-equation method' has been retained to avoid confusion with previous reports. Any breath hold period occurring before or after the point of maximum volume will be adequately handled by equations 5 and 6, respectively. This eliminates the need to define the start and end of breath holding for the calculation of $DLCO^{SB}$.

Equation 6 can be used to predict the mean carbon monoxide concentration in the exhaled gas over a selected period t_a to t_b , where $t_1 < t_a < t_b < t_2$:

$$[CO]_{PRED} = \frac{\int_{t_a}^{t_b} CO_{E(t)} dV(t)}{V(t_b) - V(t_a)}$$

The mean carbon monoxide concentration over this same interval can be measured ($[CO]_{MEAS}$). An iteration technique can now be used to solve the equations for $DLCO$. A simple bisection method was chosen for stability. Although this method is slower than other iteration techniques, current computer processor speeds make the extra time for more iterations negligible.

The bisection method finds an upper and lower bound for $DLCO$ and uses the midpoint for successive iterations. One starts by arbitrarily assuming $DLCO = 30$ mL/min/torr, STPD. If the initial value for $DLCO$ is too high then $[CO]_{PRED}$ will be lower than $[CO]_{MEAS}$. Conversely, if the initial value for $DLCO$ is too low then $[CO]_{PRED}$ will be higher than $[CO]_{MEAS}$. If 30 mL/min/torr is too high then it becomes the upper bound and 0 becomes the lower bound. If 30 mL/min/torr is too low, then it becomes the lower bound. The $DLCO$ is then doubled until an upper bound is established.

Once the upper and lower bounds are found the new value for $DLCO$ is:

$$DLCO_{new} = \frac{DLCO_{upper} + DLCO_{lower}}{2}$$

$DLCO_{new}$ is then used to find $[CO]_{PRED}$, which is compared with $[CO]_{MEAS}$ to determine whether $DLCO_{new}$ is too high or too low. If it is too high, then it becomes the upper bound for the next iteration. If it is too low then it becomes the lower bound.

This process is repeated until $[CO]_{PRED}$ is within a specified tolerance of $[CO]_{MEAS}$. The authors use 0.1%. The $DLCO$ value thus found is then converted to mL/min/torr STPD. In most instances, 12 or fewer iterations are required to converge to the solution for $DLCO$.

$DLCO$ is not permitted to be negative. If $DLCO = 0$ returns a value of $[CO]_{PRED}$ that is too low, then a value of zero is returned for $DLCO$.

The algorithm was written in QUICKBASIC (Microsoft

Corporation, Washington). For a typical run, the lung volumes and diffusing capacity were calculated within 5 s using a PC type computer with a 486 central processor.

Sample size: A physical sample of alveolar gas is not collected in a bag for analysis. Rather, any specific sample can be constructed from the flow and gas concentration data. The largest exhaled gas sample for analysis begins at the point of dead space washout and continues to end-exhalation, which is defined as the point when the exhaled flow rate decreases to the level of the aspiration rate of the analyzers. The main advantages of using the largest possible exhaled gas sample are, first, the estimate of $DLCO^{SB}$ will be more representative of the entire lung and less influenced by ventilation and/or diffusion inhomogeneities in the lung; and second, the measurement of VA will similarly be more accurate. Additionally, the effect of carbon monoxide analyzer noise will be diminished as the signal to noise ratio improves proportionally to the square root of the number of samples.

Index of diffusion inhomogeneity: The effects of diffusion inhomogeneity on measurements of $DLCO^{SB}$ were recently described (21). The overall diffusing capacity was found by using the maximum size sample as described above. The large sample of alveolar gas was then divided into four equal volume samples and the data were reanalyzed four times, with a separate $DLCO^{SB}$ calculated for each of the four smaller samples. The VA found from the maximum size sample was used for the subsequent analysis of the smaller samples. For submaximal manoeuvres with no breath holding, it was found that $DLCO^{SB}$ measured from the samples collected early in exhalation were reduced while $DLCO^{SB}$ from later samples was increased compared with the $DLCO^{SB}$ from the total sample (21). With the addition of 6 s of breath holding the $DLCO^{SB}$ from the small exhaled alveolar gas samples were all the same as the $DLCO^{SB}$ from the entire exhaled alveolar gas sample. There was no difference in the $DLCO^{SB}$ from the entire exhaled sample for the 0 and 6 s breath hold manoeuvres.

The authors have since developed an index to quantify diffusion nonuniformity, (D_I). The $DLCO^{SB}$ for each small sample ($DLCO^{SB}_i$) is normalized by expressing it as a percentage of the $DLCO^{SB}$ for the large sample. D_I is calculated as:

$$D_I = \left[\frac{1}{4} \sum_{i=1}^4 (DLCO^{SB}_i - 100)^2 \right]^{1/2}$$

Manoeuvre parameters: While this method can be applied to any single breath manoeuvre, the focus here is on a slow, submaximal manoeuvre. The single breath manoeuvre is done seated, immediately preceded by deep breath to control volume history. The control breath consists of inhaling room air from FRC to TLC, breath holding at TLC for 5 s and exhaling back to FRC. The single breath manoeuvre is then an inhalation of test gas from FRC to one-half of the IC and exhalation without breath holding to RV (Figure 5). All flow rates should be at a rate corresponding to 10% of the VC/s.

Position: $DLCO^{SB}$ measured in supine (22) or 15° head down (23) position is increased compared with sitting. Addition-

ally, gravitationally dependent ventilation inhomogeneity is decreased in the supine position (23). However, the DL_{CO}^{SB} in the upright seated position may be more reflective of ambient gas exchange in ambulatory persons. If further investigations are warranted, supine tests can be done to determine the amount of recruitment of pulmonary vasculature or increase in blood volume.

Volume history: An inhalation to TLC before the inhalation of test gas significantly increases the subsequent measurement of DL_{CO}^{SB} compared with DL_{CO}^{SB} measurements made after a 10 min control period of tidal breathing (20,24,25). To control volume history, the choices are to precede the inhalation of test gas by either a deep breath or a controlled period of tidal breathing. Using a deep breath as the control has many advantages. It is easier to perform, easier to control and easier to standardize than tidal breathing. Because the effect of a deep breath can last for 3 mins (25), a long tidal breathing control period would be required. Patients instructed to breathe normally on a mouthpiece typically hyperventilate. A deep breath also provides a TLC reference for subsequent submaximal manoeuvres and permits carbon monoxide back pressure measurements.

Inspired volume: The traditional manoeuvre starts at RV with inspiration to TLC. Starting at FRC and inhaling one-half IC is closer to normal breathing than going from RV to TLC. In normal subjects, the DL_{CO}^{SB} measured from this manoeuvre is about 10% lower than that measured from an RV-TLC manoeuvre (10,24). While a VC inhalation minimizes ventilation nonuniformities in the lung and would be easier to standardize in the clinical setting, a submaximal breath from FRC is more sensitive in the detection of peripheral inhomogeneities in the lung, which are usually worse in disease (10). A possible problem with inhaling a small volume of test gas is a greater dilution of test gas and consequently reduced signal to noise ratios. This is partially offset by the reduction in breath hold time. Further enhancement may be possible by using higher concentrations of carbon monoxide and tracer gas in the test gas.

Breath hold time: Eliminating breath holding makes the manoeuvre more natural and easier to perform. It also makes the measurement of DL_{CO}^{SB} and lung mechanics sensitive to gas mixing inhomogeneities, which are not observed with increased breath hold times (10,20,21). If warranted for a given patient with maldistribution of ventilation, a second measurement can be made at 6 to 10 s of breath holding to determine whether increased gas mixing alters DL_{CO}^{SB} . For purposes of describing the manoeuvre, the breath hold time is defined as the interval from 100 mL below TLC on the inspiratory limb to 100 mL below TLC on the expiratory limb.

Exhaled volume: One of the strengths of the three-equation method is the ability to include all of the exhaled gas data in the calculation of DL_{CO}^{SB} . The largest possible exhaled volume gives the best mean estimate of DL_{CO}^{SB} . For this reason exhalation should be done to RV. Exhaling to RV also provides measurements of VC and RV from the same manoeuvre.

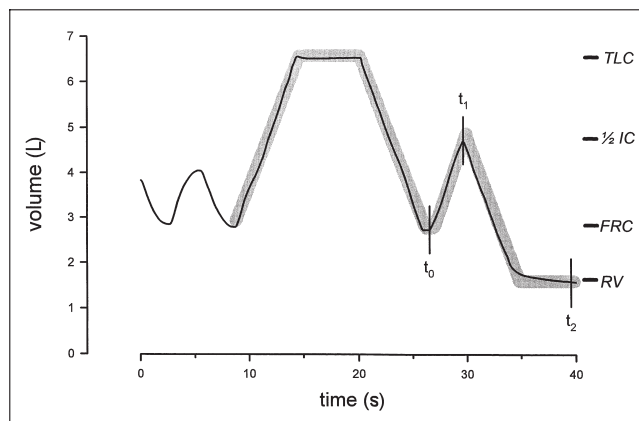


Figure 5 Functional residual capacity (FRC) is determined from tidal breathing before the manoeuvre. A standard deep breath of room air to total lung capacity (TLC) with breath holding for 5 s and exhalation back to the premeasured FRC is done for volume history control. The subject then inhales test gas from FRC to half inspiratory capacity ($1/2$ IC) and exhales to residual volume (RV). All flow rates are slow, 10% of the vital capacity per second. The subject matches a real-time display of volume-time to a template of the manoeuvre (shaded area) on the computer screen to attain the desired volumes and flow rates

Flow rate: A slow flow rate is not only more physiological but permits the single breath manoeuvre to be done equally well by normal subjects and patients with obstructive lung disease. Much of the work done by the authors has used a flow rate of 0.5 L/s, which was arbitrarily determined. For children and subjects with low volumes, a flow rate of 10% of the VC per second was used. Standardizing the flow rate to a fraction of the VC rather than a fixed flow rate will make the manoeuvre more comparable between subjects with different lung volumes.

A disadvantage of using manoeuvres with high flow rates is that far more rapidly responding gas analyzers are required because a significant volume may be exhaled during the transient response time of the analyzer. This may have been one of the reasons for the increased washout volumes reported by Huang and MacIntyre (14), who studied standardized (rapid) single breath manoeuvres with a 300 ms response time analyzer.

'Intrabreath' measurements of DL_{CO} : The three-equation method is not to be confused with measurements of DL_{CO} during exhalation, referred to as ' DL_{CO} (exhaled)', 'intra-breath DL_{CO} ' or 'within-breath DL_{CO} '. Newth et al (26) described a technique of measuring DL_{CO} during exhalation based on an approximation of the Krogh equation. The authors showed that this method was influenced by ventilation inhomogeneity, causing the measured DL_{CO} to vary with exhaled flow rate (27). Additionally, such measurements are very sensitive to noise in the monitored carbon monoxide signal. The authors proposed using a discrete sample method of measuring DL_{CO} (exhaled) (28), one variation of which used all of the exhaled data to determine a single value of DL_{CO} (exhaled). Huang et al (29) reported measurements of DL_{CO} (exhaled) using the analysis of Martonen and Wilson (30). This method also has the advantage of using all of the

exhaled gas to calculate a single value of $DL_{CO}(\text{exhaled})$, but relies on the assumption of a linear decline in $\ln[CO]$ with lung volume and a constant exhaled flow rate. It was found that intrabreath methods of measuring DL_{CO} , which essentially measure the slope of the exhaled carbon monoxide concentration curve, are spuriously affected by ventilation inhomogeneity, cannot reliably measure DL_{CO} and cannot distinguish between nonuniform ventilation and nonuniform diffusion in the lung (31).

DISCUSSION

While the measurement of DL_{CO}^{SB} has been used extensively for clinical and research purposes, the full potential of its value has never been realized. The limitations of conventional DL_{CO}^{SB} measurements have led to spurious variations in the results due to variations in flow rates, breath hold times, breath hold volume, and the size and timing of the alveolar gas sample. Using separate, analytic equations to describe the total manoeuvre eliminates these errors and removes the constraint of performing an unnatural, standardized manoeuvre that is very difficult for some patients to perform adequately.

The concept in our method that has been challenged most frequently is the assumption that, within a given single breath manoeuvre, DL_{CO}^{SB} is constant and does not vary with time or lung volume. Conventional methods are based on the assumption that all the carbon monoxide uptake occurs at peak lung volume with a constant DL_{CO}^{SB} . While we could have assumed DL_{CO}^{SB} to be some function of volume or time, results from normal subjects suggest that this is not the case (8,9,11). Assuming DL_{CO}^{SB} to be a constant yields the most useful results.

The effect of this assumption is that the DL_{CO}^{SB} that is calculated by our method gives the mean diffusing capacity over the entire manoeuvre that would explain the observed uptake of carbon monoxide in a uniform, homogeneous lung. Each manoeuvre is analyzed separately so that DL_{CO}^{SB} is measured independently in each single breath manoeuvre. Hence, the assumption that DL_{CO}^{SB} does not vary with lung volume within a given single breath manoeuvre does not prevent the study of how DL_{CO}^{SB} changes with lung volume from manoeuvre to manoeuvre. If there were a decrease in DL_{CO}^{SB} with lung volume, then a lower rate of carbon monoxide uptake would be observed for a manoeuvre done at a lower lung volume, and a lower DL_{CO}^{SB} would be calculated.

In normal subjects, DL_{CO}^{SB} increases slightly as peak lung volume increases, but is not affected by changes in the time-weighted mean lung volume. At short breath hold times mean lung volume can be much less than peak lung volume, while if inhalation and exhalation are done very rapidly and the breath hold is long then mean lung volume approaches peak lung volume. We showed previously that DL_{CO}^{SB} is not affected by breath hold time in normal subjects, and is thus independent of mean lung volume (11). The effect of peak lung volume on DL_{CO}^{SB} is nonlinear and is dependent on volume history (24). DL_{CO}^{SB}/VA varies with lung volume (24).

In equations 1 to 4 we could have assumed that DL_{CO}^{SB} was some function of time and/or lung volume. Saidel et al (32) used the assumption that diffusing capacity was directly proportional to surface area, which, assuming a spherical compartment, would be proportional to volume to the two-thirds power. In our lung models, we obtained the best fit of simulated to observed data from normal subjects when DL_{CO} was constant (7). In patients with lung disease, this may not be the case. Saidel and Modarreszadeh (33) found that DL_{CO}^{SB} was relatively independent of lung volume in normal subjects, but it became more volume-dependent in patients with chronic obstructive pulmonary disease. We found that the observed pattern of carbon monoxide uptake and tracer gas washout in patients with emphysema could be modelled with constant DL_{CO}^{SB} but with the introduction of incomplete mixing of gas in the lungs.

We have shown that DL_{CO}^{SB} measured by the Jones and Meade method (5) in normal subjects who can perform the standardized manoeuvre very well is very similar to the three-equation DL_{CO}^{SB} (9). Beck et al (12) also found that, in patients who adequately performed the standardized single-breath manoeuvre, the three-equation analysis applied to an ATS-type sample of exhaled gas yielded similar results to a Jones and Meade analysis of the same sample. This implies that, until such time as a normal population-based study can be done to derive normal regression formulas, current normal regressions developed using conventional methods may be applied as a reasonable approximation. As recommended by the ATS, each laboratory should do a study of normal persons to verify the applicability of any set of regression equations (3).

An important improvement to the measurement of DL_{CO}^{SB} that is afforded by the technology of continuous monitoring of exhaled concentrations is the determination of the point of dead space washout. This eliminates the necessity of using an arbitrary volume to be discarded before the accumulation of an alveolar sample. This alone improves the precision of conventional methods (14) because there can be considerable variation in the gas required to wash out dead space (9). Continuous monitoring also allows the calculation of the Fowler dead space, which can be subtracted from the measured total lung volume to estimate alveolar lung volume. The Fowler dead space is not constant for any individual but varies with lung volume, flow rate and breath hold time (17,20). The Fowler dead space provides an estimate of the portion of the measured lung volume that is not participating in gas exchange. This is a measure of functional rather than 'anatomic' dead space.

While breath hold time has little effect on DL_{CO}^{SB} in normal subjects, DL_{CO}^{SB} increases with increasing breath hold time in patients with emphysema and in patients with asthma (11). The index of diffusion uniformity, D_I , changes much more with breath hold time in patients and in smokers than in normal subjects. Smokers with normal DL_{CO}^{SB} have abnormal D_I . In the assessment of some patients it may be useful to measure DL_{CO}^{SB} at both 0 s and 6 s of breath hold to determine the degree of diffusion inhomogeneity.

In summary, measurements of DLco^{SB} using the three-equation method can potentially improve both the precision and the accuracy of DLco^{SB} while permitting the measurement to be made from a single breath manoeuvre, which can be done equally well by normal subjects and by patients with lung disease, and which far more closely resembles normal respiration. These improvements may help to achieve more of the potential value for DLco^{SB} as an important index of lung function.

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