Measurement of transfer factor during constant exhalation

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Abstract

Background – Transfer factor of the lung for carbon monoxide (TLCO) was measured by a new method based on analysis of the ratio of the concentrations of carbon monoxide to an inert gas (methane) relative to lung volume during a constant exhalation. Since this new technique is based solely upon exhalation, anomalies associated with inspiration and breath holding do not affect results. Additionally, because prolonged breath holding is not required, measurements can readily be made in dyspnoeic patients.

Methods – Exhalation TLCO (TLCO,ex) was compared with the standard (Jones and Meade) 10 second breath holding TLCO (TLCO,bh) in 100 consecutive patients. Patients did not practise the exhalation manoeuvre prior to testing.

Results – The comparative results were very close; mean difference (bias) $\pm$ standard deviation (precision) was 0.05 (0.84) mmol/min/kPa. The relation was equally strong in patients with severe pulmonary disease; for patients with FEV₁ <1.5 l the mean difference was 0.21 (0.80) mmol/min/kPa.

Conclusions – Since the results were essentially identical between the techniques, it seems that comparable pathophysiological factors affect TLCO during breath holding and constant exhalation. Constant exhalation may therefore be a useful alternative to the breath holding technique for clinical measurement of TLCO.

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Transfer factor of the lung for carbon monoxide (TLCO) is usually measured by a "standard" 10 second breath holding technique originally developed by Ogilvie and colleagues¹ and modified by Jones and Meade.² Calculation of breath holding TLCO (TLCO,bh) is based on the Bohr equation which states that for any TLCO, at constant lung volume, gas absorption is proportional to time of breath holding (see below). The breath holding method is particularly prone to problems because, during both inspiratory and expiratory flow, gas absorption follows patterns which are different from breath holding^{3,4}; an "equivalent" breath holding time during these phases of respiration is estimated to account for the differing gas absorption patterns.²

With the introduction of rapid gas analysers an alternative approach to TLCO,bh has become

available. Gas diffusion during constant exhalation has been analysed theoretically.^{3,4} Such an approach does not depend upon length of time or other conditions during either inspiration or breath holding. Although mathematical analysis of gas absorption during constant exhalation suggests that the absorption of carbon monoxide is proportional to the relative rate of change of lung volume,^{3,4} several studies have calculated TLCO by using breath holding equations over discrete decrements of lung volume – for example, 2% or 10% vital capacity – with or without smoothing of data.⁵⁻⁹

We describe here a method which uses constant exhalation following minimal breath holding developed from a theoretical analysis of gas diffusion and absorption during constant exhalation.^{3,4} Comparison TLCO data for 100 patients between this new exhalation technique (TLCO,ex) and TLCO,bh are also presented.

Methods

THEORY

The breath holding method is based on an idealised breathing manoeuvre during which inspiratory and expiratory phases are accounted for as "equivalent" breath holding time. To approximate this ideal manoeuvre, long breath holding and relatively short inspiration and expiration times are desirable.¹ Jones and Meade analysed gas absorption during inhalation and exhalation as well as during breath holding.^{2,3} They suggested that errors associated with non-instantaneous inspiratory and expiratory times could be reduced by calculating breath holding time as the interval between 30% of the inspiratory time and the midpoint of collection of the exhaled sample.² With this standard method of measurement TLCO,bh is given by:

$$TLCO, sb = \frac{V_{A_0}}{t} \ln \frac{F_A}{F_{A_0}} \frac{60 \times 1000}{0.938 \times 101.3 \times 22.4} \quad (1)$$

where TLCO = the transfer factor of the lung for carbon monoxide, V_A = alveolar compartment volume in litres, V_{A0} = alveolar compartment volume at end of inspiratory flow in litres, F_A = fractional pressure of CO in alveolar compartment, F_{A0} = fractional pressure of CO at end of inspiratory flow, and t = breath holding time in seconds. The constant terms are: 60 s/min, 1000 ml/l, 0.938 fraction of dry gas, 101.3 kPa/atmosphere, 22.4 l/mol.

The analyses of both Jones and Meade³ and Martonen and Wilson⁴ indicated that, during

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Figure 1 Change of alveolar volume (lower lines) and fraction of carbon monoxide (upper lines) during measurement of TLCO by constant exhalation (continuous lines) and breath holding (dashed lines) techniques in the same person.

constant exhalation, the relation between carbon monoxide concentration and lung volume declines at a rate proportional to TLCO and the constant expiratory flow rate, $\dot{V}_E$ (in l/s). Using this approach TLCO_{ex} can be calculated as shown in equation 2:

$$TLCO_{ex} = \dot{V}_E \frac{\ln FA/FA_0}{\ln VA/VA_0} \frac{60 \times 1000}{0.938 \times 101.3 \times 22.4} \quad (2)$$

Figure 2 Concentrations of carbon monoxide and methane which would be measured at the mouth in the example illustrated in fig 1 for both constant exhalation and breath holding techniques. Closing volume is 0.5 l above residual volume. As in fig 1, continuous lines represent TLCO_{ex} and dashed lines represent TLCO_{bh} methods; data utilised in calculation of TLCO are between the vertical lines.

Since this method of measurement is based only upon analysis of gas absorption during constant exhalation, an extended breath holding time is not necessary. The equation is similar to that previously used to measure pulmonary capillary blood flow except that carbon monoxide replaces acetylene and the effect of pulmonary tissue volume does not have to be considered.^{10 11}

In fig 1 the two techniques are compared with breathing pattern (lower display: volume v time) and alveolar concentration of methane and carbon monoxide (upper displays); the scale on the ordinate refers only to the gas concentrations (as % inspired). The shorter breath holding time and longer exhalation time of the TLCO_{ex} (continuous lines) compared with the TLCO_{bh} (dotted lines) method are depicted for a normal individual with residual volume of 2 litres, inspired volume of 4 litres, dead space volume of 0.3 litres, and TLCO of 10 mmol/min/kPa. For both methods time of inspiration is two seconds; breath holding time is 1.5 seconds for the constant exhalation technique and eight seconds for the breath holding technique; exhalation time is six seconds for the constant exhalation method and three seconds for the breath holding method. Alveolar concentrations of carbon monoxide were calculated from equations derived by Martonen and Wilson⁴ (see Appendix). Alveolar carbon monoxide concentrations are lower after the longer breath holding time; with both techniques FACO declines more rapidly as the lung volume becomes smaller.

In fig 2 the concentrations of carbon monoxide and methane which would be measured near the mouth are illustrated for the two techniques in the same individual as in fig 1. The same conventions and physiological values used in fig 1 are used with the exception that the effect of closing volume 0.5 l above residual volume is also shown. At the mouth, inspired concentrations of carbon monoxide and methane will be recorded until dead space gas is expired. Two sets of vertical lines are present in this figure. These lines represent the gas volumes and gas concentrations which will subsequently be used for calculation of TLCO_{bh} and TLCO_{ex}. With the breath holding technique (dashed line) a small sample of gas following dead space clearance is used to determine the rate of carbon monoxide absorption. With the constant exhalation method (continuous line) exhaled gas is constantly monitored between dead space clearance and closing volume for calculation of TLCO_{ex}.

In fig 3 $\ln FA/FA_0$, the calculated variable used to calculate TLCO in both equations 1 and 2, is displayed against either time or $\ln VA/VA_0$ as appropriate. Only the information between the first set of dotted lines in fig 2 is shown for TLCO_{ex} (continuous line, plotted against $\ln VA/VA_0$). For calculation of TLCO_{bh}, values of $\ln FA/FA_0$ are plotted against time (dashed line) from the beginning of inspiration to closing volume. The lower filled circle represents the data between the second set of vertical dotted lines in fig 2 delayed for transport through the dead space. The upper circle

Figure 3 Calculated data used in computation of $TLCO_{ex}$ and $TLCO_{bh}$ displayed as they are used in the measurements. $TLCO_{ex}$ is proportional to the slope of $\ln FA/FA_0$ versus $\ln VA/VA_0$ during exhalation between dead space washout and closing volume (continuous line) so it is shown only for that portion of exhalation. In contrast, since $TLCO_{bh}$ is proportional to $\ln FA/FA_0$ versus time during breath holding but uses data from the beginning of inspiration through early exhalation, all data from beginning of inspiration to closing volume are shown (dotted line). The two large filled circles are values used in the Jones and Meade² technique for calculation of $TLCO_{bh}$ (see text).

is the time value chosen by Jones and Meade² to represent the virtual beginning of gas absorption. Note that the continuous line connecting these points very closely follows the calculated alveolar values (dashed line).

PATIENTS

One hundred consecutive patients referred to the pulmonary function laboratory of the University of California Irvine Medical Center for evaluation were studied. The range of age, height, and pulmonary function is shown in the table. Using previously published criteria,¹² patients were classified as probably normal (44), obstructive defect (25), restrictive defect (18), or mixed (13). For analysis, patients were divided into two groups by the ratio FEV_1/FVC ; 77.5% was chosen as the dividing value

Mean (SD) selected anthropometric and pulmonary function values

	FEV_1/FVC >77.5%	FEV_1/FVC <77.5%
M:F	23:27	31:19
Age (years)	48.3 (17.3)	56.7 (13.8)
Height (m)	1.64 (0.13)	1.69 (0.09)
Weight (kg)	71.4 (20.8)	77.8 (25.6)
FEV_1 (% predicted)	93.4 (25.1)	62.9 (22.1)
FVC (% predicted)	82.6 (19.9)	73.7 (15.3)
TLC (% predicted) ¹⁴	97.1 (21.5)	105.9 (223.5)
$TLCO_{bh}$ (% predicted) ¹³	67.6 (20.3)	55.8 (25.8)
FEV_1/FVC (%)	85.3 (4.2)	61.2 (12.7)
Single breath N_2 (%/l)	2.7 (2.5)	5.3 (3.7)
Pao_2 (kPa)	11.2 (1.6)	10.2 (1.7)
$Paco_2$ (kPa)	5.2 (0.5)	5.2 (0.7)

FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; $TLCO_{bh}$ = transfer factor of the lung for carbon monoxide by the breath holding method; Pao_2 , $Paco_2$ = arterial oxygen and carbon dioxide tension.

because it separated patients into two equal groups of 50. Group details are given in the table.^{13,14} Those in the lower FEV_1/FVC group were, as expected, more obstructed, had lower $TLCO$ and Pao_2 values, as well as greater single breath nitrogen values. In all 100 patients single determinations of $TLCO$ by both the breath holding and expiratory techniques were performed in random order; in 59 patients $TLCO_{bh}$ was also measured after administration of a bronchodilator.

PROCEDURES

For measurement of $TLCO_{bh}$ a pulmonary function laboratory (SensorMedics 2450, Yorba Linda, California, USA) was used. With this system the expired gas sample was collected in a bag for automatic analysis; gases used were 0.3% carbon monoxide, 10% helium (as the inert marker), and 21% oxygen; the remainder was nitrogen. Carbon monoxide was measured by an infrared analyser; carbon dioxide was absorbed by soda lime; helium was measured with a catharometer. Volume was measured by the bag in box technique.¹³ Breath holding time was calculated using the approach of Jones and Meade.² In a few patients $TLCO_{bh}$ was measured with the SensorMedics 2200 (see below). With both instruments the criteria established by the ATS were met both in performance of the breath holding manoeuvre and for computation.¹⁵

For constant exhalation measurements a pulmonary function cart (SensorMedics 2200, Yorba Linda, California, USA) was used. This device records airflow continuously with a mass flow sensor; volume is obtained by computer integration. Gases used were 0.3% carbon monoxide and 0.3% methane (as the inert marker), and 21% oxygen; nitrogen made up the bulk of the remainder except for 0.3% acetylene which is not used in the measurement of $TLCO_{ex}$. Both gas concentrations were continuously monitored with an infrared sensor. Data were digitised at the rate of 31 samples/second and recorded for subsequent calculation of $TLCO_{ex}$ and pulmonary function variables on the fixed drive of a built-in personal computer (IBM 50Z series 2). Other characteristics of the instrument have been described elsewhere.^{11,16} Relative lung volume was calculated by integration of the flow signal. Absolute lung volume was calculated from volume inspired minus dead space multiplied by the ratio of inspired to alveolar methane concentrations. For both techniques anatomical dead space was estimated to be equal to the age in years plus weight in pounds¹⁷; the maximum value used was 0.3 l. The slope of the relation $\ln FA/FA_0$ v $\ln VA/VA_0$ (equation 2) was determined by least squares regression analysis. All data manipulation and calculations were made utilising software which is an integral part of the SensorMedics 2200. To assist with the measurement of $TLCO_{ex}$ two displays were used: (1) volume and gas concentrations measured at the mouth v time (fig 2), and (2) $\ln FA/FA_0$ v $\ln VA/VA_0$ (lower continuous line in fig 3); these displays aided in selection of those

portions of exhaled data to be used for analysis. Computation was restricted to data which demonstrated constant expiratory flow and were situated between the end of dead space washout and beginning of closing volume; these physiological events are readily identified on the expired methane curve (fig 2, upper part). Even in patients with severe maldistribution of ventilation the end of dead space washout can be clearly distinguished but the closing volume may not be easily recognised; in these cases the end of constant flow was used as the last data point. In several patients TLCO_{ex} measurements were repeated because of technically unacceptable breathing manoeuvres; this was usually lack of constant exhalation for at least one second.

Constant expiratory flow was achieved by requesting patients to exhale continuously with constant but moderate effort against a fixed resistance. Several types of fixed expiratory resistances were evaluated; eventually, we settled on an orifice of 5 mm diameter and 13 mm length (provided by the manufacturer Sensormedics) for patients with vital capacities above 2.5 l; a variable orifice of 2 mm diameter and 8 mm length (Discofix four-way stopcock, Burron Medical, Bethlehem, Pennsylvania, USA) was used for patients with smaller vital capacities. The larger orifice raised proximal airway pressure to 2.6 cm H₂O at the average expiratory flow rate of 0.5 l/s (actual patient expiratory flow rates varied between 0.23 and 0.97 with mean of 0.55 and standard deviation of 0.17 l/s). The smaller orifice raised pressure to 8 cm H₂O at the same flow rate when fully

open (the usual operating condition). Additionally, visual feedback information on flow rate was provided on the initial monitor display (not shown in fig 2). Patients did not practise the constant exhalation manoeuvre before measurement but every patient was able to complete the test.

Every patient had technically acceptable tests, including those repeated as described above, except for one which was not recognised early enough to be repeated; this patient was replaced by another. Data collected were evaluated by standard techniques; mean, standard deviation, and analysis of variance were calculated as appropriate for the analysis (Systat, Evanston, Illinois, USA). Bias, precision, and 95% confidence intervals were calculated and plotted by the methods of Bland and Altman.¹⁸

Results

As expected, mean (SD) breath holding time was substantially less with the constant exhalation technique (3.48 (0.89) s) than with the standard method (11.1 (0.78) s). Despite expiration times of between 1.4 and 14 s with the expiratory method (mean (SD) 5.8 (2.2) s), expiration flow rates were less scattered because of varied vital capacities and ranged from 0.25 to 0.91 l/s.

There was very little difference between TLCO_{bh} and TLCO_{ex} in the 100 patients studied. In fig 4 the difference between exhalation and breath holding methods is compared with the mean TLCO (average of both methods) for all patients. The differences between the two methods were evenly arrayed around zero difference; this relation was similar at all values of mean TLCO. Mean difference (bias) was 0.05 with a standard deviation (precision) of 0.84 mmol/min/kPa. Comparison of the two equal groups divided by an FEV₁/FVC of 77.5% revealed similar results: -0.15 (0.80) above 77.5% and 0.26 (0.83) below 77.5%; individual patients are shown in fig 3 as either filled (FEV₁/FVC >77.5%) or open circles (FEV₁/FVC <77.5%). The association remained equally strong in patients with severe pulmonary disease: bias 0.23 and precision 0.75 mmol/min/kPa for the 21 patients with FEV₁/FVC <60%.

The slope of the single breath nitrogen exhalation curve was recorded in 56 patients. There was no correlation between single breath nitrogen and the difference between TLCO_{bh} and TLCO_{ex} ($r=0.004$, $p=0.978$).

As a test of reproducibility TLCO_{bh} and TLCO_{ex} were tested 3 times in 6 normal subjects. The results were similar for both techniques (coefficient of variation 6.3 (1.6)% for TLCO_{bh} and 6.9 (2.1)% for TLCO_{ex}). It was also possible to compare TLCO_{bh} before and after bronchodilator (bias 0.12, precision 0.60 mmol/min/kPa) with the TLCO_{bh} versus TLCO_{ex} difference (bias 0.05, precision 0.84) in 59 patients; the two mean differences and standard deviations were similar, suggesting that precision with either technique is also similar.

Alveolar volume was slightly (and significantly) smaller with the exhalation tech-

Figure 4 Comparison of results of measurement of TLCO by breath holding and constant exhalation techniques in 100 consecutive patients. The difference between the techniques is plotted against the mean of the two techniques; 95% confidence limits are indicated by dotted lines. The 50 values obtained from patients with FEV₁/FVC >77.5% (●) are distributed similarly to the 50 patients with FEV₁/FVC values <77.5% (○).

nique with a mean difference of 0.27 (0.50) l. Because of this difference $TLCO_{ex}/VA$ was slightly larger than $TLCO_{bh}/VA$ (mean difference 0.18 (0.52) mmol/min/kPa/l. Alveolar volume was smaller than TLC with both techniques (mean difference 0.89 (0.97) l with the breath holding method and 1.18 (1.13) l with the exhalation method). As expected, the difference between TLC and VA was greater in obstructed patients (mean difference 1.24 (0.99) in patients with $FEV_1/FVC < 77.5\%$ v 0.57 (0.83) in patients with $FEV_1/FVC > 77.5\%$); this disparity was highly significant.

Discussion

In this study we have compared breath holding and exhalation methods of $TLCO$ measurement across a large patient sample. Although the patients in this study varied greatly in age, weight, height, and pulmonary disease (table), $TLCO$ determinations obtained by both methods were consistently close (fig 4).

Since the breath holding technique has potential theoretical problems not shared with the constant exhalation technique, it was reassuring to see that the two techniques produced results which are virtually identical. This close similarity of results between the methods supports the conclusion that the Jones and Meade² modification of the breath holding $TLCO$ technique is remarkably accurate.

The long breath holding time required for the standard method can be difficult for some patients to obtain. Dyspnoeic or exercising patients, and even normal subjects who are near or at maximal exercise capacity, may not be able to hold their breath for 10 seconds.¹⁹ Expiratory time was longer during the constant exhalation than the breath holding method in many patients. Although it is easier for dyspnoeic individuals to prolong exhalation than to hold their breath,^{20,21} exhalation time can be shortened to only a few seconds if necessary (see below). We have found that normal subjects can successfully perform the exhalation method even at maximal exercise.

The exhalation method requires a period of constant flow during exhalation; in practice, one second of exhalation data is sufficient since this interval will provide 31 data points, an amount adequate for calculation of the relation $\ln FA/FA_0$ v $\ln VA/VA_0$ (equation 2, fig 3). In the 100 patients studied expiratory time varied between 1.4 and 14.0 seconds. As expected, there was a strong positive correlation between forced vital capacity and expiratory time but the size of the fixed expiratory resistance also played an important part. Individual patients with severe obstruction experienced difficulty in reproducing flow patterns or following instructions and often generated only short segments in which expiratory flow was constant. Nevertheless, even in these patients $TLCO_{ex}$ values were very comparable to $TLCO_{bh}$.

Back pressure from the expiratory orifice might reduce $TLCO_{ex}$ by compression of pulmonary capillaries.²² However, even with a maximal Valsalva manoeuvre a fall of only about 15% in $TLCO_{bh}$ has been reported.²² The pres-

sures generated by the orifice (2.4 cm H_2O for the standard orifice and 8 cm H_2O for the small orifice used with small vital capacities under normal operating conditions) were more than one order of magnitude less than those created in a maximal Valsalva manoeuvre and did not appear to alter values of $TLCO_{ex}$ in comparison with $TLCO_{bh}$.

One potential drawback to a method in which breath holding is minimised is the possibility of uneven distribution of inhaled gas, particularly in patients with long time constants. Since we found essentially no correlation between the slope of the single breath nitrogen curve and the difference between the two techniques, it seems likely that the effect of uneven distribution of gas upon $TLCO$ did not differ between a short and longer period of breath holding, or was diminished by slow exhalation, or both.

Transfer factor of the lung for carbon monoxide has also been measured during exhalation by a method which estimates $TLCO_{ex}$ over small intervals by using the breath holding relation (equation 1).⁵⁻⁹ While this approach yields values similar to the constant exhalation method, it is an approximation^{3,4} and produces results which may vary considerably during exhalation, possibly because of pulmonary circulatory pulsatility.²³ With the small interval technique we observed, as Newth and colleagues had earlier,⁵ widely changing values of $TLCO$ during exhalation. These fluctuations of $TLCO$ are probably caused by regional differences in gas absorption and emptying. Despite fluctuating $TLCO$ values in these patients, the slope of $\ln FA/FA_0$ v $\ln VA/VA_0$ was relatively smooth, suggesting that the concept of overall lung $TLCO$ is probably valid.

Calculated VA was smaller with the constant exhalation technique than the breath holding technique, suggesting that a long period of breath holding, particularly when combined with continued inspiratory effort, allows better distribution of gas to very poorly ventilated lung; in the two groups separated by FEV_1/FVC values the mean difference was 0.165 l in the $FEV_1/FVC > 77.5\%$ group and 0.385 l in the obstructed group. Since $TLCO_{bh}$ and $TLCO_{ex}$ values were so close, even in patients with maldistribution of ventilation, presumably the additional volume penetrated by the inert marker methane during prolonged breath holding did not participate significantly in gas exchange.

Since oxygen competes with carbon monoxide for combination with haemoglobin, the effects of length of breath holding and exhalation times on alveolar PO_2 and $TLCO$ need to be considered. Frey and associates reported that $TLCO_{bh}$ fell at the rate of 2.573%/kPa PAO_2 .²⁴ In five normal subjects we found that expired PO_2 (immediately following dead space washout) was 17.68 (0.89) kPa after a two second breath hold and 16.57 (0.71) kPa after a 10 second breath hold. Expired PO_2 dropped at the rate of 0.25 kPa/s during a constant 0.54 (0.06) l/s flow rate following the two second breath hold, and fell at the rate of 0.23 (0.05) kPa/s during a constant

0.55 (0.05) l/s flow rate after the 10 second breath hold. Because of falling PAO_2 values during prolonged exhalation $TLCO_{ex}$ should slowly rise, but we found that the mean difference between $TLCO$ techniques was virtually unaffected by differing breath holding and expiratory times; the mean difference between techniques was 0.037 mmol/min/kPa per second of expiratory time ($r=0.38$). Further, assuming that the midpoint of exhalation represents the average PAO_2 for the exhalation method and a value one second after dead space washout represents the average PAO_2 for the breath holding method, mean PAO_2 during the breath holding method would be only 0.44 (0.39) kPa lower than the exhalation method and consequently $TLCO_{bh}$ would be expected to be only 1.13 (1.00)% higher than $TLCO_{ex}$ – amounts which are statistically and clinically trivial and insignificant.

In summary, we have shown that a method based upon the theoretical relation of gas absorption to lung volume during constant exhalation compares favourably with the standard breath holding methods in 100 patients who ranged from near normal to severe lung disease. All patients were able adequately to complete the test with this new technique. Potential uses of this new method include the evaluation of dyspnoeic patients and the measurement of $TLCO$ during exercise.

Appendix

EQUATIONS DESCRIBING CARBON MONOXIDE ABSORPTION⁴

During inspiration:

$$F_{IF} = \frac{F_I \dot{V}_{IIF}}{V_I + TL_{IIF}} \left[1 - \frac{(\dot{V}_{IDS}) + (V_{A_0} - V_I)_{IIF}}{V_{It} + (\dot{V}_{A_0} - \dot{V}_I)_{IIF}} \right]^{1 + \frac{TL}{V_I}}$$

During breath holding:

$$FAI = FA_{IF} e^{\left(\frac{TL(\pi F - t)}{V_{A_0}} \right)}$$

During constant exhalation:

$$FAE = FAI \left(1 + \frac{\dot{V}_E(\pi - t)}{V_{A_0}} \right)^{\frac{TL}{V_E}}$$

where F = fraction of gas, V = volume of gas, $\dot{V}$ = gas flow rate, t = time, TL = transfer factor of lung, A = alveolar, A_0 = at full inspiration, I = inspiratory flow, I = inspiratory, E = expiratory, and DS = dead space.

Equations for methane are identical except

that they simplify by setting $TLCO$ as equal to zero.

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