UC Irvine
UC Irvine Previously Published Works

Title
Measurement of transfer factor during constant exhalation.

Permalink
https://escholarship.org/uc/item/0b2310wx

Journal
Thorax, 49(11)

ISSN
0040-6376

Authors
Wilson, AF
Hearne, J
Brenner, M
et al.

Publication Date
1994-11-01

DOI
10.1136/thx.49.11.1121

Peer reviewed

Measurement of transfer factor during constant exhalation

Archie F Wilson, Jennifer Hearne, Matthew Brenner, Ray Alfonso

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 FEV_1 <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.

(*Thorax* 1994;49:1121–1126)

Department of Medicine, University of California Irvine Medical Center, 101 City Dr. So., Orange, California 92668, USA
A F Wilson
J Hearne
M Brenner
R Alfonso

Reprint requests to:
Dr A F Wilson.

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 colleagues[1] 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:

$$TLCO,sb = \frac{V_{A_0}}{t} \ln \frac{F_A}{F_{A_0}} \frac{60 \times 1000}{0\cdot938 \times 101\cdot3 \times 22\cdot4}$$

(1)

where TLCO = the transfer factor of the lung for carbon monoxide, V_A = alveolar compartment volume in litres, V_{A_0} = alveolar compartment volume at end of inspiratory flow in litres, F_A = fractional pressure of CO in alveolar compartment, F_{A_0} = 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 Meade[3] and Martonen and Wilson[4] indicated that, during
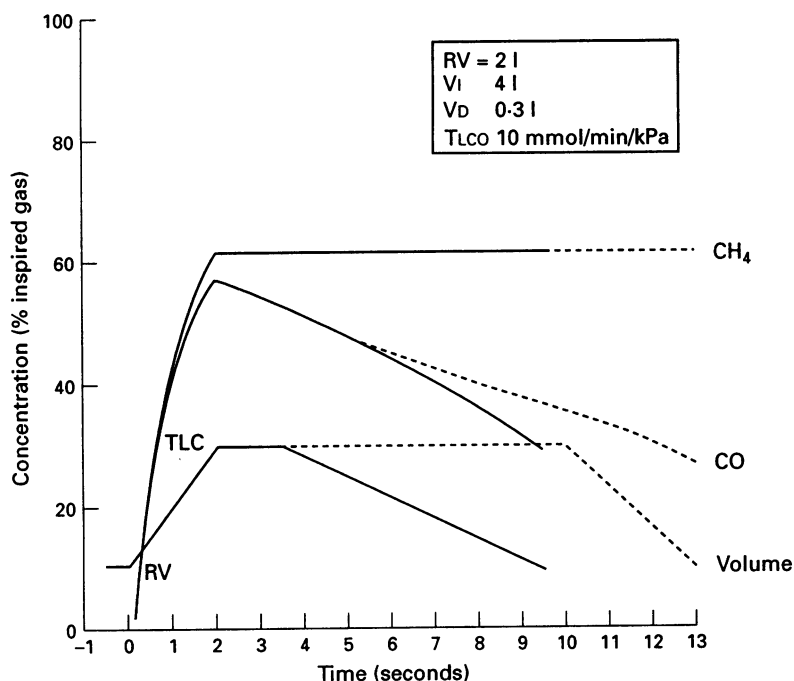
Figure 1 Change of alveolar volume (lower lines) and fraction of carbon monoxide (upper lines) during measurement of TLCO by constant exhalation (continuous lines) and breath holding (dashed lines) techniques in the same person.

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:

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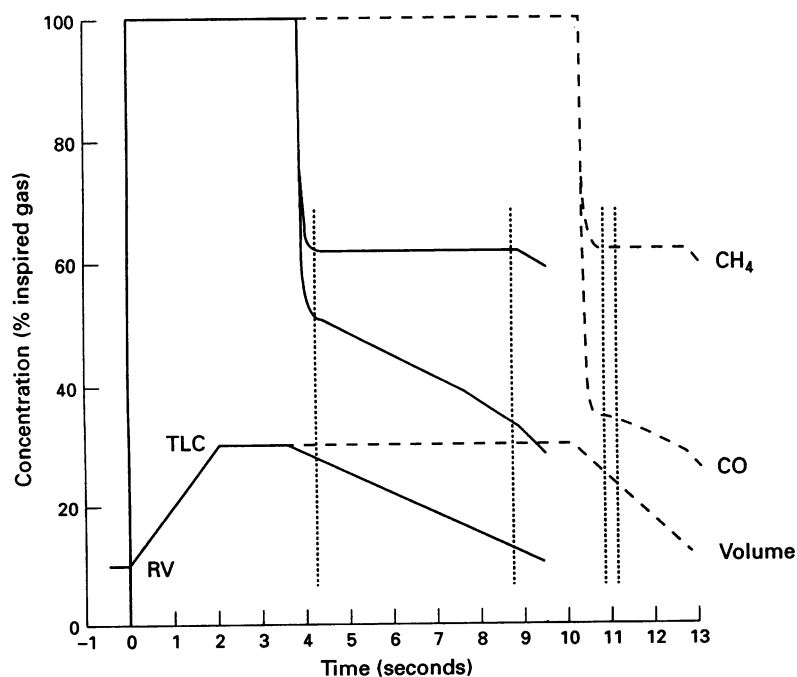
(2)

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 Wilson[4] (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 0.51 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
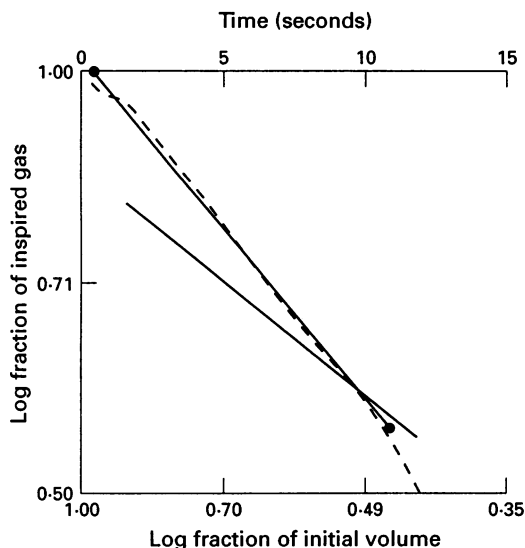
Time (seconds)

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/FA₀ versus ln VA/VA₀ 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/FA₀ 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 Meade[2] 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 N₂ (%/l)	2·7 (2·5)	5·3 (3·7)
Pao₂ (kPa)	11·2 (1·6)	10·2 (1·7)
Paco₂ (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; Pao₂, Paco₂ = 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 Pao₂ 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 pounds[17]; the maximum value used was 0·31. The slope of the relation ln FA/FA₀ v ln VA/VA₀ (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/FA₀ v ln VA/VA₀ (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 H_2O 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 H_2O 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 FEV_1/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 (FEV_1/FVC >77·5%) or open circles (FEV_1/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 FEV_1/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.

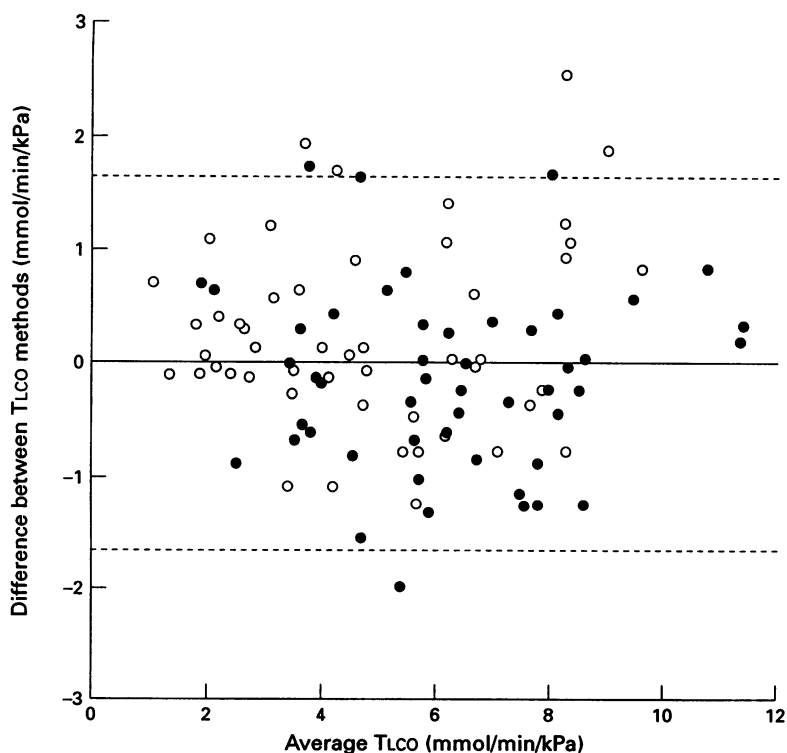Alveolar volume was slightly (and significantly) smaller with the exhalation tech-

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 T_{LCO},ex/VA was slightly larger than T_{LCO},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 T_{LCO} measurement across a large patient sample. Although the patients in this study varied greatly in age, weight, height, and pulmonary disease (table), T_{LCO} 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 Meade[2] modification of the breath holding T_{LCO} 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 T_{LCO},ex values were very comparable to T_{LCO},bh.

Back pressure from the expiratory orifice might reduce T_{LCO},ex by compression of pulmonary capillaries.[22] However, even with a maximal Valsalva manoeuvre a fall of only about 15% in T_{LCO},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 T_{LCO},ex in comparison with T_{LCO},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 T_{LCO} 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 T_{LCO},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[34] 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 T_{LCO} during exhalation. These fluctuations of T_{LCO} are probably caused by regional differences in gas absorption and emptying. Despite fluctuating T_{LCO} 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 T_{LCO} 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 T_{LCO},bh and T_{LCO},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 P_{O_2} and T_{LCO} need to be considered. Frey and associates reported that T_{LCO},bh fell at the rate of 2·573%/kPa P_{AO_2}.[24] In five normal subjects we found that expired P_{O_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 P_{O_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 ABSORPTION[4]

During inspiration:

$$FIF = \frac{FI\dot{V}I\pi F}{VI + TL\pi F}\left[1 - \frac{(\dot{V}I tDS) + (VA_0 - VI)\pi F}{VI t + (\dot{V}A_0 - \dot{V}I)\pi F}\right]^{\left(1+\frac{TL}{VI}\right)}$$

During breath holding:

$$FAI = FAIF\ e^{\left(\frac{TL(\pi F - t)}{VA_0}\right)}$$

During constant exhalation:

$$FAE = FAI\left(1 + \frac{\dot{V}E(\pi - t)}{VA_0}\right)^{\frac{TL}{\dot{V}E}}$$

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, IF = 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.

1 Ogilvie CM, Forster RE, Blakemore WS, Morton J. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 1957;**36**:1–17.
2 Jones RJ, Meade F. Pulmonary diffusing capacity: an improved single-breath method. *Lancet* 1960;**i**:94–5.
3 Jones RJ, Meade F. A theoretical and experimental analysis of anomalies in the estimation of pulmonary diffusing capacity by the single breath method. *Q J Exp Physiol* 1961;**56**:131–43.
4 Martonen TB, Wilson AF. Theoretical basis for single breath gas absorption tests. *J Math Biol* 1982;**14**:203–20.
5 Newth CJL, Cotton DJ, Nadel JA. Pulmonary diffusing capacity measured at multiple intervals during a single exhalation in man. *J Appl Physiol* 1977;**43**:617–25.
6 Hallenborg C, Holden W, Menzel T, Dozor R, Nadel JA. The clinical usefulness of a screening test to detect static pulmonary blood using a multiple-breath analysis of diffusing capacity. *Am Rev Respir Dis* 1979;**119**:349–56.
7 Graham BK, Mink JT, Cotton DJ. Dynamic measurements of CO diffusing capacity during discrete samples of alveolar gas. *J Appl Physiol* 1983;**54**:73–9.
8 Saumon G, Loiseau A, Loiseau P, Georges R. Lung transfer factor for carbon monoxide measured during a slow single breath without breath-holding and during slow exhalation. *Bull Eur Physiopathol Respir* 1984;**20**:353–60.
9 Ramage JE Jr, Coleman RE, MacIntyre NR. Rest and exercise cardiac output and diffusing capacity assessed by a single slow exhalation of methane, acetylene and carbon monoxide. *Chest* 1992;**92**:44–50.
10 Elkayam U, Wilson AF, Morrison J, Meltzer P, Davis J, Klosterman P, *et al.* Non-invasive measurement of cardiac output by single breath constant exhalation technique. *Thorax* 1984;**39**:107–13.
11 Zenger M, Brenner M, Haruno M, Mahon D, Wilson AF. Measurement of cardiac output by automated single-breath technique, and comparison with thermodilution and Fick methods in patients with cardiac disease. *Am J Cardiol* 1993;**71**:105–9.
12 Wilson AF. Patterns of disturbance. In: Wilson AF, ed. *Pulmonary function testing: indications and interpretations.* Orlando: Grune and Stratton, 1985:243–54.
13 Cotes JE. Lung function throughout life; determinants and reference values. In: *Lung function: assessment and application in medicine.* 4th edn. Oxford: Blackwell, 1979: 329–89.
14 Grimby G, Soderholm B. Spirometric studies in normal subjects: III. Static lung volumes and maximum breathing ventilation in adults with a note on physical fitness. *Acta Med Scand* 1963;**173**:199–206.
15 Statement of the American Thoracic Society. Single breath carbon monoxide diffusing capacity (transfer factor): recommendations for a standard technique. *Am Rev Respir Dis* 1987;**136**:1299–307.
16 Chang YCT, MacIntyre NR. Real-time gas analysis improves the measurement of single-breath diffusing capacity. *Am Rev Respir Dis* 1992;**146**:946–50.
17 Cotes JE. Measurement of the transfer factor (diffusing capacity) for the lung and its subdivisions. In: *Lung function: assessment and application in medicine.* 4th edn. Oxford: Blackwell, 1979:230–50.
18 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**i**:307–10.
19 Van Kessel AL. Pulmonary diffusing capacity for carbon monoxide. In: Clausen JL, ed. *Pulmonary function testing: guidelines and controversies.* New York: Academic Press, 1982:166–85.
20 Fowler WS. Breaking point of breath-holding. *J Appl Physiol* 1954;**6**:539–45.
21 Mithoefer JC. Lung volume restriction as a ventilatory stimulus to breathing. *J Appl Physiol* 1959;**14**:701–5.
22 Smith TC, Rankin J. Pulmonary diffusing capacity and the capillary bed during Valsalva and Muller maneuvers. *Am J Physiol* 1969;**27**:826–33.
23 Sackner MA. Measurement of cardiac output by alveolar gas exchange. In: Fishman AP, Fahri LE, Tennet SM, Geiger SR, eds. *Handbook of physiology: respiratory system.* Vol 4. Bethesda, Maryland: American Physiological Society, 1987:233–55.
24 Frey TM, Crapo RO, Jensen RL, Kanner RE, Kass JE, Castriotta RJ, *et al.* Adjustment of DLco for varying COHb and alveolar PO_2 using a theoretical equation. *Respir Physiol* 1990;**81**:303–12.