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# Comparison of Total-Breath and Single-Breath Diffusing Capacity in Healthy Volunteers and COPD Patients*

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***Background:*** **The measurement of single-breath diffusing capacity ($D_{LCO}^{SB}$) assumes that diffusing capacity per liter of alveolar volume ($D_{LCO}$/VA) determined in a 750-mL gas sample represents the diffusing capacity ($D_{LCO}$) of the entire lung. Fast-responding gas analyzers provide the opportunity to verify this assumption because of the possibility to measure CO and $CH_4$ fractions continuously throughout the entire expiration. Continuous gas sampling provides more information per measurement, but this information cannot be expressed in the traditional parameters. Our goals were to find new parameters to express the extra information of the continuous gas sampling, and to compare these new parameters with the traditional parameters.**

***Methods:*** **We compared a new method to determine $D_{LCO}$ with the traditional method in 62 healthy volunteers and 26 COPD patients. Traditionally, $D_{LCO}^{SB}$ is determined by multiplying $D_{LCO}$/VA with alveolar volume, both calculated from gas concentrations in a 750-mL gas sample. The new method calculates total-breath $D_{LCO}$ ($D_{LCO}^{TB}$) by integration of $D_{LCO}$/VA against exhaled volume.**

***Results:*** **In healthy volunteers, $D_{LCO}$/VA shows a slight upward slope during exhalation, while in COPD patients $D_{LCO}$/VA shows a horizontal line. Total-breath total lung capacity (TLC) is larger than single-breath TLC both in healthy volunteers and in COPD patients, leading to a $D_{LCO}^{TB}$ that is significantly larger than $D_{LCO}^{SB}$ in both groups ($p < 0.001$).**

***Conclusion:*** **The assumption that a 750-mL gas sample represents the entire lung seems to be correct for $D_{LCO}$/VA but not for the $CH_4$ fraction in case of ventilation inhomogeneity.**

***(CHEST 2007; 131:237–244)***

**Key words:** pulmonary diffusing capacity; pulmonary gas exchange; respiratory function tests

**Abbreviations:** $D_{LCO}$ = diffusing capacity; $D_{LCO}^{SB}$ = single-breath diffusing capacity; $D_{LCO}^{TB}$ = total-breath diffusing capacity; $D_{LCO}^{TB,VC}$ = CO transport in vital capacity; $D_{LCO}^{TB,RV}$ = CO transport in residual volume; $D_{LCO}$/VA = diffusing capacity per liter of alveolar volume; FRC = functional residual capacity; IVC = inspiratory vital capacity; RV = residual volume; $RV^{TB}$ = total-breath residual volume; TLC = total lung capacity; $TLC^{MB}$ = total lung capacity based on the multibreath functional residual capacity measurement; $TLC^{SB}$ = single-breath total lung capacity; $TLC^{TB}$ = total lung capacity determined with the total-breath method; VA = alveolar volume; $VA^{SB}$ = alveolar volume determined with the single-breath method; $VA^{TB}$ = alveolar volume determined with the total-breath method; VC = vital capacity; VCex = exhaled vital capacity

For many years, single-breath diffusing capacity ($D_{LCO}^{SB}$) and diffusing capacity per liter of alveolar volume ($D_{LCO}$/VA) have been used to evaluate gas transport across the alveolar-capillary membrane.[1,2] The patient first exhales to residual volume (RV), then inhales inspiratory vital capacity (IVC) of

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a gas mixture containing CO and an inert tracer gas. After 10 s of breath-holding at total lung capacity (TLC), the patient exhales again; 750 mL is discarded, and the next 750 mL of gas is used for analysis.[2] This sample is assumed to be representative of the entire lung. In healthy volunteers, this assumption seems to be acceptable.[3] However, in patients with uneven ventilation and/or uneven distribution of DLCO/VA, this assumption might not be correct and possibly leads to erroneous conclusions.[4,5]

Fast-responding gas analyzers allow continuous monitoring of gas fractions, obviating the need to rely on one gas sample only. Continuous gas sampling enables measurement of DLCO/VA during the entire exhalation and provides more information than can be expressed in the traditional parameters.[6–9] In the intrabreath method, diffusing capacity (DLCO) is determined during constant exhalation after a minimal breath-holding time. Disadvantages are the minimal breath-holding time, which may lead to a possible underestimation of alveolar volume (VA), and the constant exhalation flow that is required.[6] To maintain constant exhalation flow, a flow resistor is applied, possibly influencing the DLCO due to higher intrathoracic pressure. Therefore, we chose to use the single-breath maneuver and modified the analysis of the measurement only.

We intended to find new diffusion parameters that pertain to both well-ventilated and poorly ventilated lung areas. Another objective was to investigate whether the 750-mL gas sample used in the single-breath method is indeed representative of the entire lung both in normal subjects and in patients with uneven ventilation.

## Materials and Methods

The Erasmus University medical ethics committee approved the protocol. After verbal informed consent, measurements were performed in healthy volunteers and in COPD patients recruited from Rotterdam and its suburbs.

### *Healthy Volunteers*

A group of 62 healthy volunteers with no history of smoking or lung disease was studied. TLC based on multibreath functional residual capacity (FRC) measurement was determined to exclude restrictive pulmonary disease. $FEV_1$/IVC was used to exclude obstructive pulmonary disease. A range between + 1.64 SD or – 1.64 SD from predicted was assumed to be normal. Anthropometric and pulmonary function characteristics of the study population are presented in Tables 1, 2. Values are given as mean (SD). Volumes and their ratios are expressed as percentage of predicted values and Z scores.[10,11]

### *COPD Patients*

A group of 26 COPD patients was studied; 13 patients had moderate chronic airways obstruction, and 13 patients had severe obstruction.[12] Details are given in Tables 1, 2.

### *Spirometry*

Static lung volumes (TLC, IVC, FRC, and RV) were measured with a rolling-seal spirometer (Jaeger; Würzburg, Germany). FRC was determined with a closed-circuit multibreath helium dilution technique by tidal volume rebreathing. To distinguish between differently obtained TLCs, we named TLC based on the multibreath FRC measurement ($TLC^{MB}$). $FEV_1$ and $FEV_1$/IVC were determined with a Lilly-type flow transducer (Masterscreen PFT; Jaeger). Measurements were done according to European Respiratory Society and American Thoracic Society recommendations.[13–15]

### *Single-Breath Method*

The single-breath maneuver was performed with the ZAN 300 (ZAN Messgeräte; Oberthulba, Germany) according to European Respiratory Society and American Thoracic Society recommendations.[2] Inspiration gas contained 0.25% CO and 0.30% $CH_4$ balanced with air. After 10 s of breath-holding at TLC, the patient was asked to expire completely. Instead of collecting the second 750-mL gas for analysis, the expired gas was analyzed continuously during the entire exhalation.[6,16,17]

Fractions of $CH_4$ and CO in the exhaled gas were measured with a rapid-responding infrared analyzer (90% response time, 0.18 s; sample flow, 28 mL/s; dead space of the system, 80 mL; delay time, 0.75 s; cross sensitivity to water vapor and $CO_2$ negligible). The software took into account the time delay between volume and gas fraction signals.

Start of breath-holding time was assumed when 30% of the inspiration time was elapsed[18]; the end was set at each sample point. Measurements were performed in triplicate. Between consecutive measurements, we waited at least 4 min for washout of inert gas.[2]

### *Measurement Procedure and Expression of Test Results*

*Determination of TLC:* Measurements were interpreted according to two different methods: single-breath TLC ($TLC^{SB}$)

**Table 1—*Anthropometric Characteristics of the Study Participants**

| Characteristics | Healthy Volunteers (n = 62) | All COPD Patients (n = 26) | Moderate Obstruction (n = 13) | Severe Obstruction (n = 13) |
|---|---|---|---|---|
| Male/female gender, No. | 25/37 | 14/12 | 13/4 | 13/8 |
| Age, yr | 29 (11) | 56 (9) | 57 (11) | 54 (7) |
| Height, cm | 173 (10) | 172 (12) | 176 (12) | 167 (10) |
| Weight, kg | 68 (15) | 69 (16) | 76 (17) | 63 (12) |
| Body mass index, $kg/m^2$ | 23 (4) | 23 (4) | 24 (4) | 22 (4) |

*Data are presented as mean (SD) unless otherwise indicated.

**Table 2—*Pulmonary Function Characteristics of the Study Participants**

| Characteristics | Healthy Volunteers (n = 62) | | All COPD Patients (n = 26) | | Moderate Obstruction (n = 13) | | Severe Obstruction (n = 13) | |
|---|---|---|---|---|---|---|---|---|
| | % Predicted | Z score | % Predicted | Z score | % Predicted | Z score | % Predicted | Z score |
| IVC | 106 (14) | 0.5 (1.2) | 83 (16) | − 1.3 (1.2) | 88 (19) | − 1.0 (1.5) | 78 (10) | − 1.6 (0.8) |
| $FEV_1$ | 105 (12) | 0.4 (0.9) | 35 (18) | − 4.4 (1.2) | 49 (15) | − 3.5 (1.0) | 21 (4) | − 5.2 (0.7) |
| $FEV_1$/IVC | 101 (6) | 0.2 (0.7 | 42 (17) | − 6.6 (2.0) | 56 (12) | − 4.9 (1.4) | 29 (5) | − 8.3 (0.5) |
| TLC | 102 (12) | 0.2 (1.1) | 112 (14) | 1.1 (1.3) | 103 (12) | 0.3 (1.1) | 122 (10) | 1.9 (1.0) |
| RV | 94 (24) | − 0.2 (1.1) | 176 (50) | 4.1 (2.8) | 143 (42) | 2.5 (2.4) | 208 (34) | 5.8 (2.1) |
| RV/TLC | 89 (18) | − 0.6 (0.9) | 145 (31) | 2.9 (2.0) | 127 (31) | 1.7 (1.9) | 164 (18) | 4.1 (1.1) |

*Data are presented as mean (SD).

and total-breath TLC ($TLC^{TB}$). $TLC^{SB}$ is determined from the $CH_4$ fraction in the 750-mL gas sample after discarding 750 mL of gas for washout of dead space.[2,19,20] In this study, the 750-mL gas sample was not physically collected, but was derived from the gas fraction samples between 750 mL and 1,500 mL of expired volume.[21,22]

$TLC^{TB}$ is the sum of exhaled vital capacity (VCex) and RV obtained with the total-breath method ($RV^{TB}$). To determine $RV^{TB}$, a mass balance was used. The amount of inhaled $CH_4$ equals the amount of exhaled $CH_4$ obtained by integration of $CH_4$ against expired volume plus remaining $CH_4$ in $RV^{TB}$. $CH_4$ fractions in anatomic dead space and apparatus dead space were equal to inspired $CH_4$ fraction. The $CH_4$ fraction measured at 90% of exhaled volume was assumed to be representative of the $CH_4$ fraction in $RV^{TB}$.[21] Results obtained in the final 10% of the exhaled volume are possibly unreliable because at the end of the exhalation the sample flow might exceed the exhalation flow and then admixture of exhaled gas with room air leads to incorrect values for CO and $CH_4$ fractions.

*Determination of DLCO:* DLCO/VA, proportional to the rate constant of the CO disappearance,[23] was measured continuously during exhalation. Total CO transport is represented by DLCO and calculated according to two different methods: (1) $DLCO^{SB}$ is obtained by multiplying DLCO/VA with VA, both determined from CO and $CH_4$ fractions in the second 750-mL gas sample[2,19,20]; and (2) total-breath DLCO ($DLCO^{TB}$) is divided into two components: CO transport in vital capacity ($DLCO^{TB,VC}$) and CO transport in RV ($DLCO^{TB,RV}$):

$$DLCO^{TB} = DLCO^{TB,VC} + DLCO^{TB,RV}$$

Calculation of $DLCO^{TB,VC}$ is based on the integration of DLCO/VA against exhaled volume according to:

$$DLCO^{TB,VC} = \int^{90\%VCex} DLCO/VA \times dV$$

where dV = volume parts. Calculation of $DLCO^{TB,VC}$ was performed using 90% of the exhaled volume because of possible admixture of exhaled air with room air in the final 10% of exhalation as noted above. To determine $DLCO^{TB,RV}$, both $RV^{TB}$ and DLCO/VA in $RV^{TB}$ are needed. It is not possible to analyze the air that remains in $RV^{TB}$, so we assumed that DLCO/VA in $RV^{TB}$ was equal to DLCO/VA measured at 90% of the exhaled volume:

$$DLCO^{TB,RV} = DLCO^{90\%VCex}/VA \times RV^{TB}$$

DLCO and DLCO/VA of the COPD patients were corrected to a standard hemoglobin concentration. Mean hemoglobin concentrations in male and female COPD patients were 9.7 ± 1.2 mmol/L and 8.7 ± 1.0 mmol/L, respectively. Predicted hemoglobin concentrations were 9.2 ± 0.5 mmol/L and 8.2 ± 0.5 mmol/L, respectively, as determined in a group of 120 volunteers with the same demographic background in the Laboratory for Clinical Chemistry in our hospital (unpublished data).

### *Statistical Analysis*

Statistical software (SPSS for Windows 10.1.0; SPSS; Chicago, IL) was used for data analysis. The applied statistic for the comparison of means between the two different methods is the paired Student *t* test, in which differences are significant when $p < 0.05$.

## Results

### *Determination of TLC*

In healthy volunteers, the continuously measured exhaled $CH_4$, expressed as a fraction of inspired $CH_4$ and displayed as a function of exhaled volume, showed a nearly horizontal line with a minimal downward slope. In the COPD patients, the exhaled $CH_4$ fraction decreased rapidly during expiration. Examples are shown in Figures 1, 2, respectively. Figure 1, *top*, *1a*, and Figure 2, *top*, *2a* represent the single-breath method: the average $CH_4$ fraction in the second 750 mL (dashed area) is used to calculate single-breath VA ($VA^{SB}$) [shaded area]. Figure 1, *bottom*, *1b*, and Figure 2, *bottom*, *2b* represent the total-breath method: $CH_4$ fraction at 90% of the expiration is used to calculate $RV^{TB}$. The shaded area represents total-breath VA ($VA^{TB}$). The mean slope of the $CH_4$ fraction vs exhaled volume in percentage of VCex is $-0.022 \pm 0.012$ ($p < 0.001$) in healthy volunteers and $-0.23 \pm 0.11$ ($p < 0.001$) in COPD patients, $-0.15 \pm 0.08$ ($p < 0.001$) in patients with moderate COPD, and $-0.31 \pm 0.08$ ($p < 0.001$) in patients with severe COPD.

$TLC^{SB}$ and $TLC^{TB}$ are compared with each other and with $TLC^{MB}$; results are listed in Table 3. In healthy volunteers, $TLC^{TB}$ and $TLC^{MB}$ are not significantly different ($p = 0.07$). $TLC^{SB}$, however, is significantly lower than both $TLC^{TB}$ ($p < 0.001$) and

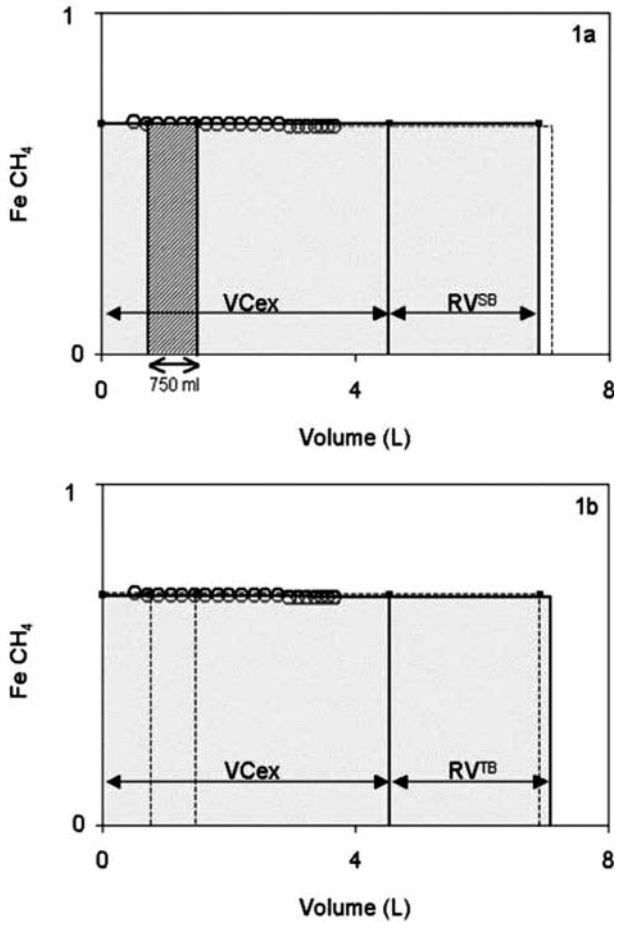


FIGURE 1. Typical example of the change in $CH_4$ fraction during exhalation in a healthy volunteer. *Top*, *1a*: Determination of $VA^{SB}$. The shaded area is a representation of $VA^{SB}$. The dashed area represents the second 750-mL gas sample. *Bottom*, *1b*: Determination of $VA^{TB}$. The shaded area is a representation of $VA^{TB}$. ○ Value of $CH_4$ fraction at each sample point.

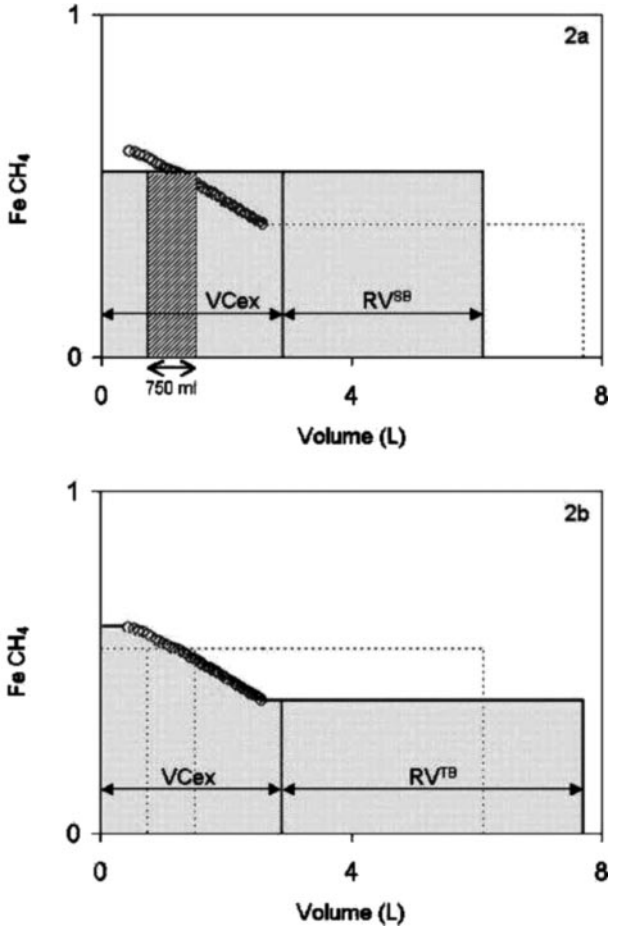


FIGURE 2. Typical example of the change in $CH_4$ fraction during exhalation in a patient with airways obstruction. *Top*, *2a*: Determination of $VA^{SB}$. The shaded area is a representation of $VA^{SB}$. The dashed area represents the second 750-mL gas sample. *Bottom*, *2b*: Determination of $VA^{TB}$. The shaded area is a representation of $VA^{TB}$. ○ Value of $CH_4$ fraction at each sample point.

$TLC^{MB}$ ($p < 0.001$). In all of the COPD patients, $TLC^{SB}$ is significantly lower than $TLC^{TB}$ ($p < 0.001$) and $TLC^{TB}$ is significantly lower than $TLC^{MB}$ ($p < 0.001$). In patients with moderate obstruction, the differences are smaller than in patients with severe obstruction.

Figure 3, *top*, *3a* shows a Bland-Altman plot of $TLC^{SB}$ and $TLC^{MB}$. Figure 3, *bottom*, *3b* shows a Bland-Altman plot of $TLC^{TB}$ and $TLC^{MB}$. The solid lines represent ± 2 SD difference of the healthy volunteers. The surface area between the solid lines represents the 95% confidence interval of the healthy volunteers. The dashed lines represent the mean difference of the patients. Table 4 lists the average indexes of gas mixing using the single-breath or the total-breath methods.

### *Determination of DLCO*

In healthy volunteers, continuously measured DLCO/VA showed a minimal upward slope. In COPD patients, continuously measured DLCO/VA showed a horizontal line. Examples are shown in Figures 4, 5, respectively. Figure 4, *top*, *4a*, and Figure 5, *top*, *5a* represent the single-breath method: the DLCO/VA in the second 750 mL (dashed area) is used to calculate $DLCO^{SB}$ (shaded area). Figure 4, *bottom*, *4b*, and Figure 5, *bottom*, *5b* represent the total-breath method: DLCO/VA value at 90% of the expiration is used to calculate $DLCO^{TB,RV}$. The shaded area represents $DLCO^{TB}$.

The mean slope of DLCO/VA vs exhaled volume in percentage of VCex is 0.017 ± 0.016 ($p < 0.05$) in healthy volunteers and − 0.008 ± 0.023 ($p = 0.11$) in COPD patients, − 0.003 ± 0.027 ($p = 0.71$) in patients with moderate COPD, and − 0.012 ± 0.019 ($p = 0.04$) in patients with severe COPD. Mean DLCO and DLCO/VA values are listed in Table 5. Total-breath DLCO/VA is calculated by dividing $DLCO^{TB}$ by $VA^{TB}$.

**Table 3—*Comparison of $TLC^{SB}$ and $TLC^{TB}$ with $TLC^{MB}$*****

| Variables | $TLC^{MB}$, L | $TLC^{SB}$, L | $TLC^{TB}$, L |
|---|---|---|---|
| Healthy volunteers (n = 62) | 6.21 (1.44) | 6.02 (1.43) | 6.14 (1.47) |
| All COPD patients (n = 26) | 6.93 (1.59) | 5.17 (1.36) | 6.02 (1.62) |
| Moderate COPD (n = 13) | 6.94 (1.77) | 5.63 (1.65) | 6.36 (1.88) |
| Severe COPD (n = 13) | 6.93 (1.46) | 4.71 (0.82) | 5.68 (1.30) |

*Data are presented as mean (SD).

## Discussion

One objective of this study was to investigate whether the assumption in the single-breath method is correct, that gas concentrations measured in the second 750 mL are representative of the entire lung. This assumption seems acceptable for $CH_4$ in healthy volunteers but not in COPD patients. TLC is best approximated with the multibreath helium dilution technique that lasts several minutes ($TLC^{MB}$). Theoretically, $TLC^{SB}$ cannot be larger than $TLC^{MB}$ because of the shorter time available for wash-in of inert gas. $TLC^{MB}$ was determined by helium dilution, while $TLC^{SB}$ and $TLC^{TB}$ were determined by $CH_4$ dilution. In some cases, we found that $TLC^{MB}$ was smaller than $TLC^{SB}$ or $TLC^{TB}$. This might be explained by the difference in solubility of helium and methane. Both in healthy volunteers and in patients with obstruction, average $TLC^{SB}$ is significantly smaller than $TLC^{MB}$. In healthy volunteers, this significant but small difference results from sequential filling and emptying and inhomogeneities caused by hydrostatic pressure differences due to gravitation, resulting in a small downward slope in the $CH_4$ vs exhaled volume relationship.[5,17,22,24–26] In all COPD patients, not only should these hydrostatic pressure differences be taken into account, but also uneven distribution of time constants resulting in asynchronous and inhomogeneous ventilation.[4] As a result, the downward slope in the $CH_4$ vs exhaled volume relationship is steeper in patients with obstruction than in healthy volunteers, causing a larger difference between $TLC^{SB}$ and $TLC^{TB}$. Differences are larger in the patients with severe obstruction than in patients with moderate obstruction. The single-breath method increasingly underestimates

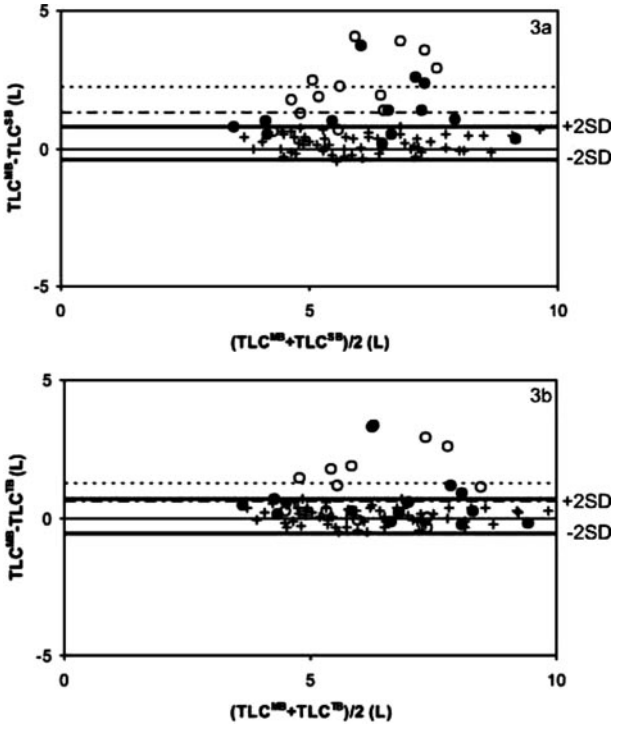


Figure 3. Bland-Altman plots. *Top*, *3a*: Single-breath method. The differences between $TLC^{MB}$ and $TLC^{SB}$ are plotted against the average values of these TLCs. The solid horizontal lines represent the 95% confidence interval of $TLC^{MB} - TLC^{SB}$ in healthy volunteers. The dotted line represents the mean value of $TLC^{MB} - TLC^{SB}$ of patients with severe obstruction. The dashed line represents the mean value of $TLC^{MB} - TLC^{SB}$ of patients with moderate obstruction. *Bottom*, *3b*: Total-breath method. The differences between $TLC^{MB}$ and $TLC^{TB}$ are plotted against the average values of these TLCs. The solid horizontal lines represent the 95% confidence interval of $TLC^{MB} - TLC^{TB}$ of healthy volunteers. The dotted line represents the mean value of $TLC^{MB} - TLC^{TB}$ of patients with severe obstruction. The dashed line represents the mean value of $TLC^{MB} - TLC^{TB}$ of patients with moderate obstruction. +Healthy volunteers. ○ Patients with severe obstruction. ● Patients with moderate obstruction.

**Table 4—*Average Indexes of Gas Mixing of Healthy Volunteers and COPD Patients***

| Methods | Index of Gas Mixing (SD) | 95% Confidence Interval | p Value |
|---|---|---|---|
| Healthy volunteers | | | |
| $TLC^{SB}/TLC^{MB}$ | 0.97 (0.05) | 0.96–0.98 | < 0.001 |
| $TLC^{TB}/TLC^{MB}$ | 0.99 (0.06) | 0.98–1.00 | 0.10 |
| All COPD patients | | | |
| $TLC^{SB}/TLC^{MB}$ | 0.75 (0.13) | 0.70–0.81 | < 0.001 |
| $TLC^{TB}/TLC^{MB}$ | 0.87 (0.14) | 0.82–0.93 | < 0.001 |
| Moderate COPD | | | |
| $TLC^{SB}/TLC^{MB}$ | 0.82 (0.12) | 0.74–0.89 | < 0.001 |
| $TLC^{TB}/TLC^{MB}$ | 0.92 (0.12) | 0.85–0.99 | 0.03 |
| Severe COPD | | | |
| $TLC^{SB}/TLC^{MB}$ | 0.69 (0.12) | 0.62–0.76 | < 0.001 |
| $TLC^{TB}/TLC^{MB}$ | 0.83 (0.15) | 0.74–0.92 | < 0.05 |

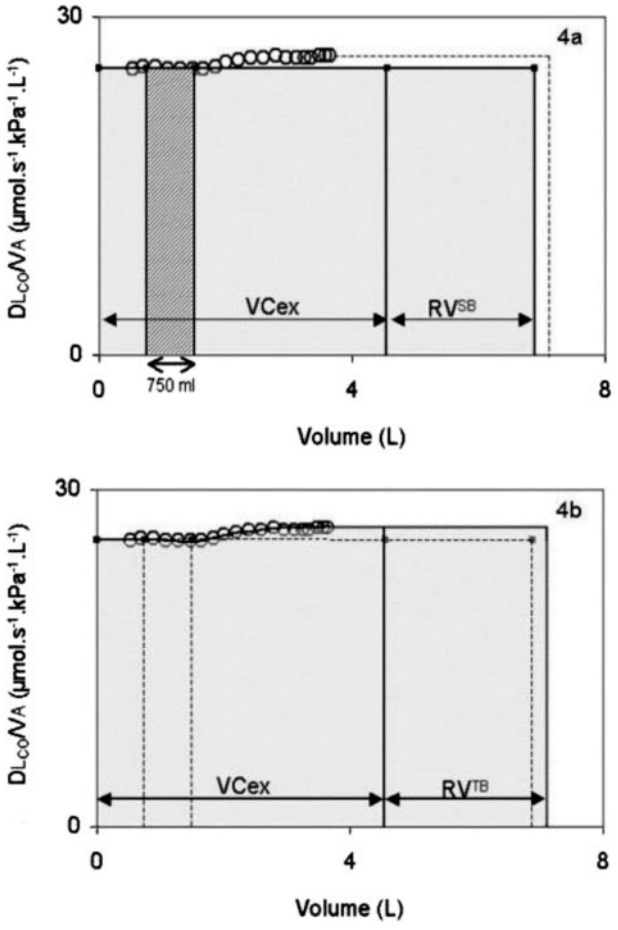


FIGURE 4. Typical example of the change in DLCO/VA during exhalation in a healthy volunteer. *Top*, *4a*: Determination of $DLCO^{SB}$. The shaded area is a representation of $DLCO^{SB}$. The dashed area represents the second 750-mL gas sample. *Bottom*, *4b*: Determination of $DLCO^{TB}$. The shaded area is a representation of $DLCO^{TB}$. ○ Value of DLCO/VA at each sample point.

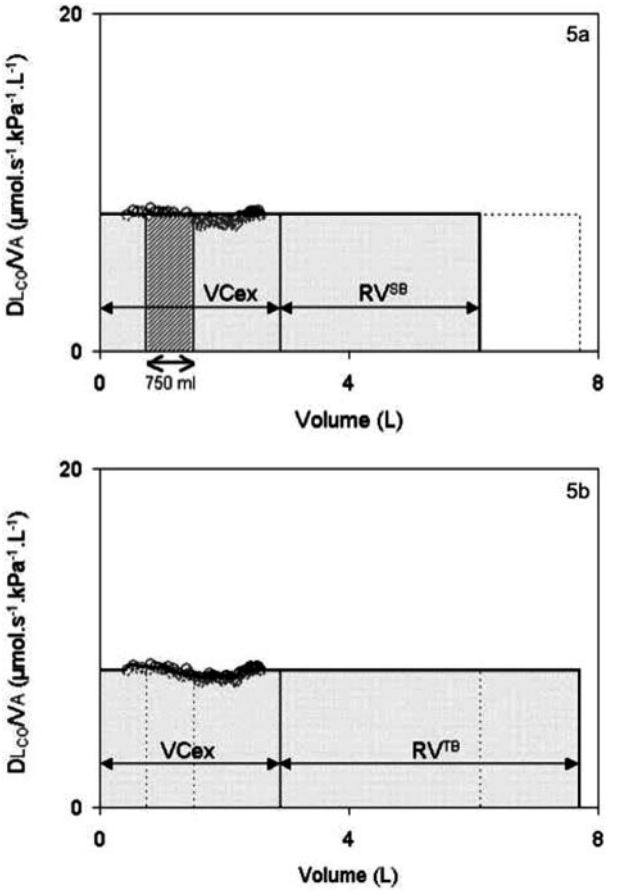


FIGURE 5. Typical example of the change in DLCO/VA during exhalation in a patient with airways obstruction. *Top*, *5a*: Determination of DLCO with the traditional method. The shaded area is a representation of $DLCO^{SB}$. The dashed area represents the second 750-mL gas sample. *Bottom*, *5b*: Determination of $DLCO^{TB}$. The shaded area is a representation of $DLCO^{TB}$. ○ Value of DLCO/VA at each sample point.

RV with increasing inhomogeneity of ventilation.[16,27–29] The Bland-Altman plots in Figure 3, *top*, *3a*, and *bottom*, *3b* show that the differences between $TLC^{MB}$ and $TLC^{SB}$ or $TLC^{TB}$ are larger in COPD patients than in healthy volunteers. In the total-breath method, the differences are smaller than in the single-breath method because in Figure 3, *bottom*, *3b*, the mean differences of patients (dashed lines) are closer to the 95% confidence interval of healthy volunteers (area between the solid lines). Therefore, we conclude that $TLC^{TB}$ is a better approximation of TLC than $TLC^{SB}$.

Results of DLCO measurements are less reliable if the gas-mixing index, $TLC^{SB}/TLC^{MB}$, is $< 0.85$. A ratio $< 0.85$ was assumed to be characteristic of uneven ventilation.[30] In healthy volunteers, $TLC^{SB}/TLC^{MB}$ was 0.97, indicating even distribution of the ventilation. In COPD patients, $TLC^{SB}/TLC^{MB}$ was $< 0.85$, indicating uneven ventilation and a less reliable determination of DLCO. $TLC^{TB}/TLC^{MB}$ of patients with severe obstruction was 0.83, still $< 0.85$ but significantly larger than $TLC^{SB}/TLC^{MB}$, which was 0.69. In the group of patients with moderate obstruction, however, $TLC^{TB}/TLC^{MB}$ was significantly $> 0.85$. This indicates a more reliable determination of the DLCO in patients with obstruction when using the total-breath method instead of the single-breath method.

In healthy volunteers, the DLCO/VA vs exhaled volume relationship went slightly upward (Fig 4), as was found by MacIntyre and Nadel.[3] Possible explanations might be the falling $PaO_2$ values during prolonged exhalation,[6] or the decreasing average volume during exhalation.[31] This needs further investigation.

A recent study by Thompson et al[32] showed that inhomogeneity of ventilation leads to unpredictable errors in measured DLCO because of a misrepresen-

**Table 5—*Comparison of Single-Breath and Total-Breath Methods**

| Variables | Single-Breath Method | Total-Breath Method | p Value |
|---|---|---|---|
| Healthy volunteers (n = 62) | | | |
| DLCO, μmol/s/kPa | 156 (40) | 166 (44) | <0.001 |
| DLCO/VA, μmol/s/kPa/L | 27 (5) | 28 (5) | <0.001 |
| COPD patients(n = 26) | | | |
| DLCO, μmol/s/kPa | 63 (36) | 73 (40) | <0.001 |
| DLCO/VA, μmol/s/kPa/L | 12 (6) | 13 (6) | 0.05 |
| Moderate COPD (n = 13) | | | |
| DLCO, μmol/s/kPa | 85 (37) | 98 (41) | <0.001 |
| DLCO/VA, μmol/s/kPa/L | 16 (6) | 16 (6) | 0.09 |
| Severe COPD (n = 13) | | | |
| DLCO, μmol/s/kPa | 40 (14) | 49 (18) | <0.001 |
| DLCO/VA, μmol/s/kPa/L | 9 (3) | 9 (3) | 0.32 |

*Data are presented as mean (SD).

tation of the true mean alveolar gas concentrations. In our group of COPD patients, this seems not to be the case because the slope of DLCO/VA against exhaled volume was not significantly different from 0. Possible explanations might be that DLCO/VA is evenly distributed over the lung despite the ventilation inhomogeneity, or that DLCO/VA is unevenly distributed but emptying of the different lung regions occurs in a constant ratio during the entire exhalation. However, if emptying of different lung regions would occur in a constant ratio, then $CH_4$ fractions should also have been constant. Therefore, we think it is more likely that DLCO/VA really is more or less constant over the VCex.

A clinically relevant finding is that the single-breath method determines the DLCO/VA fairly well, even in COPD patients with unequal ventilation, contrary to general opinion. DLCO, however, is underestimated with the traditional method because of the significant underestimation of TLC. The incorporation of the total-breath method into clinical practice is relatively simple: it requires fast CO and inert gas analyzers and software that determines DLCO/VA continuously during exhalation. An integration algorithm is needed to determine DLCO.

A weakness of the total-breath method is the assumed DLCO/VA in RV. $DLCO^{TB}$ assumes a CO disappearance in RV equal to $RV^{TB} \times$ DLCO/VA at 90% of VCex. This assumption for DLCO/VA in $RV^{TB}$ seems arbitrary, but the traditional method does more or less the same. The single-breath method assumes the DLCO/VA value of the second exhaled 750 mL to be representative of the rest of the vital capacity and RV. The extrapolation in the traditional method is therefore even bigger than in the total-breath method, and the error in the $DLCO^{SB}$ will be even larger than in the $DLCO^{TB}$. We conclude that the total-breath method needs further investigation but seems to be a valuable improvement in measuring DLCO.

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