The effect of conductive ventilation heterogeneity on diffusing capacity measurement

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1Respiratory Division, University Hospital (Universitair Ziekenhuis Brussel), Vrije Universiteit Brussel, Brussels, Belgium; 2Department of Allergy, Immunology and Respiratory Medicine, The Alfred Hospital, Melbourne, Victoria, Australia

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Verbanck S, Schuermans D, Van Malderen S, Vincken W, Thompson B. The effect of conductive ventilation heterogeneity on diffusing capacity measurement. J Appl Physiol 104: 1094–1100, 2008. First published February 14, 2008; doi:10.1152/japplphysiol.00917.2007.—It has long been assumed that the ventilation heterogeneity associated with lung disease could, in itself, affect the measurement of carbon monoxide transfer factor. The aim of this study was to investigate the potential estimation errors of carbon monoxide diffusing capacity (D\textsubscript{LCO}) measurement that are specifically due to conductive ventilation heterogeneity, i.e., due to a combination of ventilation heterogeneity and flow asynchrony between lung units larger than acini. We induced conductive airway ventilation heterogeneity in 35 never-smoker normal subjects by histamine provocation and related the resulting changes in conductive ventilation heterogeneity (derived from the multiple-breath washout test) to corresponding changes in diffusing capacity, alveolar volume, and inspired vital capacity (derived from the single-breath D\textsubscript{LCO} method). Average conductive ventilation heterogeneity doubled (P < 0.001), whereas D\textsubscript{LCO} decreased by 6% (P < 0.001), with no correlation between individual data (P > 0.1). Average inspired vital capacity and alveolar volume both decreased significantly by, respectively, 6 and 3%, and the individual changes in alveolar volume and in conductive ventilation heterogeneity were correlated (r = −0.46; P = 0.006). These findings can be brought in agreement with recent modeling work, where specific ventilation heterogeneity resulting from different distributions of either inspired volume or end-expiratory lung volume have been shown to affect D\textsubscript{LCO} estimation errors in opposite ways. Even in the presence of flow asynchrony, these errors appear to largely cancel out in our experimental situation of histamine-induced conductive ventilation heterogeneity. Finally, we also predicted that alternative combination of specific ventilation heterogeneity and flow asynchrony could affect D\textsubscript{LCO} estimate in a more substantial fashion in diseased lungs, irrespective of any diffusion-dependent effects.

histamine; ventilation maldistribution

IT HAS BEEN SUGGESTED THAT ventilatory heterogeneity associated with various pulmonary diseases could introduce artifacts in the carbon monoxide (CO) diffusing capacity (D\textsubscript{LCO}) measurement, such that its outcome does not reflect the true transfer factor (2, 3, 7, 15, 18, 19). It is generally assumed that this is particularly true when D\textsubscript{LCO} is measured with the most widely used method, i.e., the single-breath CO vital capacity maneuver (7). From inert-gas washout studies in microgravity (9), it was inferred that, in a vital capacity maneuver, conductive airway ventilation heterogeneity (i.e., ventilation heterogeneity originating between lung units larger than acini) increases the slope of phase III in the exhalation phase of an inert gas. If any type of ventilation heterogeneity also contributes to a steeper CO decline during exhalation than would be expected from CO disappearance into the bloodstream from a perfectly homogeneous lung, this would lead to an overestimation in the D\textsubscript{LCO}. This effect is partly canceled out by normalizing CO concentration by the inert-gas concentration for D\textsubscript{LCO} computation. However, model simulations with a two-compartment model indicate that, even with this normalization method, considerable D\textsubscript{LCO} estimation errors can occur with increased convection-dependent ventilation heterogeneity (19).

Modeling indeed revealed that the main mechanism for D\textsubscript{LCO} estimate errors in the presence of conductive ventilatory heterogeneity is related to a sampling error of exhaled CO, which cannot be corrected by the insoluble gas. The sampling error results from the inability to compute a complete volume-weighted average of the exhaled gas, which can only be done if the subject could exhale all lung gases, including those that remain confined to the residual lung volume. This affects the accurate estimation not only of the inert-gas concentration, but also that of the CO concentration. The predicted effect of conductive ventilation heterogeneity on transfer factor measurement is further complicated by the fact that it is not only related to the distribution of specific ventilation (i.e., concentration), but also to the absolute volumes of the different lung subunits during the CO uptake (19). For instance, in the case of equal distribution of end-expiratory lung compartments but unequal distribution of inspired gases, the transfer factor is underestimated with increasing heterogeneity, while the reverse is true in the case of unequal distribution of end-expiratory lung volumes with equal ventilation to both units. Hence, any distribution of specific ventilation may have a different effect on D\textsubscript{LCO} outcome, depending on which combination of inspired volume and end-expiratory lung volume distribution led to that particular distribution of specific ventilation. The alveolar volume (V\textsubscript{A}), estimated from the insoluble gas, which is also used in the D\textsubscript{LCO} computation, is expected to be affected by conductive ventilation heterogeneity in a more straightforward way. It is expected to be consistently underestimated by incomplete gas mixing in the lungs, which solely depends on specific ventilation distribution, irrespective of the underlying inspired and end-expiratory lung volume distributions.

In the present study, we assessed the net effect of the potential artifacts on transfer factor measurement in an experimental situation where different degrees of conductive ventilation heterogeneity are artificially introduced. For this purpose, we induced ventilation heterogeneity in normal subjects...
by histamine provocation, knowing that this will essentially
confine the induced ventilation distribution to the conductive
airways (24). In this case, the compartment model applies, and
no other artifacts from intra-acinar ventilation heterogeneity
are present to confound the DLCO estimation errors predicted
by the compartment model. Based on previous bronchoprovoc-
ation studies in normal subjects (23, 24), where conductive
ventilation heterogeneity was seen to increase twofold, on
average, with a wide intersubject scatter, we expected to cover
a wide range of ventilation heterogeneity, including that rep-
resentative of values encountered in patients with obstructive
lung disease. The chronic obstructive pulmonary disease pa-
tients in a recent study (21) had shown a conductive ventilation
heterogeneity of ~2.3-fold of that obtained in never-smokers.
This study is the first to quantitatively assess the effect of the
much postulated, but rarely substantiated, claim that diffusion
capacity would be in error in lung disease, owing to the
large-scale conductive ventilation heterogeneity occurring in
these patients.

METHODS

Subjects and protocols. Thirty five never-smokers were recruited
(27 men/8 women, age: 24 ± 7 yr) and provided written, informed
consent before starting the protocol, which was approved by the UZ
Brussel ethics committee (no. B14320071296). The test sequence
started with a series of baseline measurements, which included two
spirometry, two diffusing capacity, and three multiple-breath washout
(MBW) tests. Considering sufficient time intervals between O2
breathing (MBW) and DLCO measurements and the time evolution of
histamine effects, the exact test sequence before and after histamine
provocation was as follows: spirometry, DLCO test, three MBW tests,
DLCO test, spirometry. Using a dosimeter (model MB3; MEFAR,
Istanbul, Turkey), the histamine provocation procedure was discon-
tinued when forced expiratory volume in 1 s (FEV1) had reached a
>20% decrease vs. baseline FEV1 or a cumulative dose of 2 mg had
been inhaled. Heart rate was monitored as a safety measure through-
out the procedure.

Lung function and ventilation distribution testing. Spirometry and
diffusing capacity were measured using standard equipment (Vmax
Encore, Viavysy, Bilthoven, The Netherlands) and according to stan-
dardization procedures (11, 12). Prediction equations for the FEV1
were based on those provided by the European Community for Steel
and Coal. From the single-breath DLCO maneuver (using CH4 as the
inert tracer gas), we computed DLCO, inspired vital capacity (IVC),
and VA. We also applied a validated volume correction of DLCO
(DLCOcorr) proposed by Gonzalez Mangado et al. (8), by multiplying
DLCO by 

\[ \frac{1 + \frac{3}{2}(VA/TLCPRED)}{\frac{3}{2}(VA/TLCPRED)^2} \]

, where TLCPRED is the predicted total lung capacity for each subject. In a previous
study, the volume corrections via DLCOcorr were shown to be over-
correcting less for volume than the transfer coefficient (14). We also estimated slope of phase III from the CH4 trace. This CH4 phase III
slope is considered as an overall measure of ventilation heterogeneity,
dominated by units larger than acini, since the 10-s breath hold, which
is part of the DLCO maneuver, will have attenuated any intra-acinar
CH4 concentration differences.

Conductive and acinar components of ventilatory heterogeneity
were measured using the MBW test. Instrumentation, analysis,
and underlying theory of MBW tests have been extensively reported
elsewhere (20, 24). Briefly, tidal volume was targeted at 1 liter, and,
after a period of air breathing with stable end-expiratory lung volume
at functional residual capacity, inspired air was switched to the test
gas mixture. The test gas mixture consisted of pure O2, and 1-liter
tidal breathing continued for 20–25 breaths, depending on the sub-
ject’s lung volume (dilution). The test was discontinued when expired
N2 concentration was <2% of the initial alveolar N2 concentration.
From the MBW N2 tracings, indexes Scond and Saspin were derived to represent the conductive and acinar components of ventilation heter-
geneity, respectively, using an analysis that can be summarized as
follows. During each expiration, the N2 phase III slope is computed
between 0.6 and 1 liter of expired volume and normalized by the mean
expired N2 concentration. This leads to a normalized slope (Sn), which
increases as a function of lung turnover, where lung turnover is the
cumulative expired volume divided by the functional residual capacity
(as computed from the MBW test). On theoretical grounds (20), it can
be shown that 1) the rate of rise of the Sn curve is due to the convective
flow differences between lung units larger than acini, thus due to
heterogeneity originating in the conductive airways; and 2) the offset
of the Sn curve is mainly determined by diffusion-convection-depend-
ent heterogeneity generated in the acinar airways. Hence, Scond
is simply computed as the rate of Sn increase as a function of lung
turnover, between 1.5 and 6 lung turnovers (Fig. 1A). Then, Saspin
is computed as the Sn value of the first MBW expiration minus a
correction term to discard any conductive lung zone contribution; this
correction term equals the lung turnover corresponding to the first
breath, multiplied by Scond.

The theory of MBW phase III slope analysis leading to indexes
Scond and Saspin implies that ventilation heterogeneity can be attributed
to conductive and acinar lung zone, respectively, and that Scond and
Saspin are intrinsically independent (20, 24). Finally, since Scond and
Saspin are derived from phase III slopes, their value increases when
ventilation heterogeneity increases. In particular, Saspin will increase if
ventilation heterogeneity is increased in the acinar lung zone, due to
an alteration of the intra-acinar asymmetry (e.g., unequal narrowing of
affected respiratory bronchioles). Scond will increase when heteroge-
neous narrowing of conductive airways induces an alteration in the
specific ventilation and/or flow asynchrony between the lung units
subtended by these airways proximal to the terminal bronchioles.

Specific ventilation and flow asynchrony for VA and DLCO error estimation. The above-described analysis is specifically targeted to
distinguish between conductive and acinar ventilation heterogeneity
effects. The conductive component (represented by Scond) is, in turn,
a combination of specific ventilation heterogeneity (concentration
differences between lung units greater than acini) and flow asyn-
chrony between these units. Overall specific ventilation can be
roughly estimated from the curvilinearity of the N2 washout curve
(Curv), which is essentially unaffected by flow asynchrony. Curv is
computed as the ratio of the regression slope between three and six
lung turnovers and the regression slope between zero and three lung
 turnovers, in the semilog N2 concentration plot (as shown in Fig. 1B);
linearity corresponds to Curv = 1, and this value decreases with
increased curvilinearity. If Curv correlates inversely with Scond, it is
reasonable to assume that at least part of overall specific ventilation
heterogeneity reflected in Curv is also due to Scond. In that
case, it is possible to compute the inspired volume and end-expiratory
lung volume partitioning, compatible with the experimental Curv, and
the flow asynchrony compatible with the experimental Scond (13).

Expiratory flow asynchrony is applied between both lung compart-
ments, such that the best ventilated unit is the one to empty first (i.e.,
the relative flow of the best ventilated compartment decreases as a
function of expiratory time). Flow asynchrony is conveniently ex-
pressed as the deviation of flow at the onset of expiration with respect
to average flow to and from any compartment. For example, when
inspired volume and flow partitioning are 0.40±0.60, a flow deviation
of 0.01 signifies that the flow of the best ventilated unit is 0.61 at
the beginning and 0.59 at the end of expiration; for the worst ventilated
unit, these numbers correspond to 0.39 and 0.41, respectively.

The values for volume partitioning and flow asynchrony obtained
from the MBW indexes Curv and Scond can then be fed into a
two-compartment model that was previously developed for the study
of specific ventilation heterogeneity on VA and DLCO estimates (19).
This two compartment model consists of a common dead space and
two perfectly mixed alveolar compartments (with uniform diffusing capacity). With an initial volume of 2 liters, the single-breath DLCO maneuver is simulated by considering a 4-liter inhalation and a 10-s breath hold before exhalation. We applied the model in two modalities: 1) model A, whereby both model compartments have an equal volume at the onset of inspiration and specific ventilation heterogeneity is generated solely by a difference in inspired volume (or flow) to each compartment; 2) model B, whereby both lung compartments get the same inspired volume, and specific ventilation heterogeneity is generated solely by a difference in compartment volume at the onset of inspiration.

**RESULTS**

Four out of 35 subjects showed a >20% FEV₁ decrease after a cumulative histamine dose of <2 mg. On average, heart rate was 72.1 ± 8.7 (SD) beats/min prehistamine and 73.8 ± 8.5 (SD) beats/min posthistamine, with no significant difference between them (P > 0.1). We also verified that histamine did not affect air flow rates during crucial phases of the single-breath or multiple-breath maneuvers. Inspiratory times during the single-breath DLCO measurement maneuver were, respectively, 2.3 ± 0.5 (SD) s and 2.4 ± 0.6 (SD) s pre- and posthistamine (P > 0.1). The breathing frequency during the MBW test was, respectively, 11.6 ± 2.9 and 12.1 ± 3.2 (SD) breaths/min pre- and posthistamine, and, while the difference was significant (P = 0.01), the magnitude of this difference is negligible.

Table 1 shows lung function and ventilation heterogeneity data obtained in the 35 subjects under study, before and after histamine bronchoprovocation. The average FEV₁ decrease was only 8% predicted, while average ventilation heterogeneity in the conductive airway compartment almost doubled. By contrast, the acinar compartment did not respond to histamine provocation. On average, diffusing capacity was seen to decrease by 6% after histamine (P = 0.001), while VA and IVC obtained during the diffusing capacity test decreased by 3 and 6%, respectively (P < 0.001 for both). The correction of DLCO for volume showed a smaller reduction in DLCOcorr than in DLCO, yet the difference pre- vs. posthistamine remained significant.

Figure 2 illustrates the intersubject scatter in the response to histamine in terms of conductive ventilation heterogeneity (Scond; Fig. 2A) and the corresponding DLCO changes (Fig. 2B). In Figure 3, the histamine-induced changes in DLCO and VA are plotted vs. the corresponding changes in Scond. There was no association between change (Δ) in DLCO and VA and Scond (P > 0.1; Fig. 3A), whereas the correlation between ∆VA and ∆Scond was significant (r = −0.46; P = 0.006; Fig. 3B). There were no correlations between ∆DLCO or ∆VA and ∆FEV₁ following

![Fig. 1. Representative multiple-breath washout (MBW) curves obtained in one subject before and after 2-mg histamine. A: normalized slope (Sn) curve vs. lung turnover (TO). Solid and dotted lines illustrate, respectively, the pre- and posthistamine regression slope between 1.5 and 6 lung TOs for Scond computation (conductive ventilation heterogeneity). In this case, Scond = 0.020 liter⁻¹ (baseline) and Scond = 0.153 liter⁻¹ (posthistamine). B: semilog plot of the mean expired N₂ concentration washout curve vs. lung TO. Solid and dotted lines illustrate the pre- and posthistamine regression slopes, respectively, between 0 and 3 lung TOs, and between 3 and 6 lung TOs for Curv computation (curvilinearity of the washout curve). In this case, Curv = 0.77 (baseline) and Curv = 0.50 (posthistamine).](http://jap.physiology.org/)

**Table 1. Spirometry, diffusing capacity, and multiple-breath washout before and after histamine**

<table>
<thead>
<tr>
<th></th>
<th>Before Histamine</th>
<th>After Histamine</th>
<th>P</th>
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<tbody>
<tr>
<td>FEV₁, %predicted</td>
<td>106±10</td>
<td>98±13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLCO, ml·mmHg·min⁻¹·liter⁻¹</td>
<td>34.2±8.0</td>
<td>32.1±7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLCOcorr, ml·mmHg·min⁻¹·liter⁻¹</td>
<td>34.6±7.3</td>
<td>32.9±6.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VA, ml</td>
<td>6,637±1,467</td>
<td>6,421±1,458</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVC, ml</td>
<td>5,190±1,813</td>
<td>4,856±1,180</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CH₄ phase III slope, %/liter</td>
<td>0.0016±0.0007</td>
<td>0.0022±0.0014</td>
<td>0.004</td>
</tr>
<tr>
<td>Scond, liter⁻¹</td>
<td>0.029±0.008</td>
<td>0.058±0.030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Curv (–)</td>
<td>0.81±0.06</td>
<td>0.75±0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Sacin, liter⁻¹</td>
<td>0.078±0.030</td>
<td>0.079±0.031</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>FRC, ml</td>
<td>3,461±793</td>
<td>3,395±800</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

Values are averages ± SD. FEV₁, forced expired volume in 1 s; DLCO and DLCOcorr, diffusing capacity for carbon monoxide without and with volume correction, respectively (see text for details); VA, alveolar volume; IVC, inspired vital capacity; Sacin and Scond, multiple-breath washout-derived index of acinar and conductive ventilation heterogeneity, respectively; Curv, curvilinearity of the washout curve as depicted in Fig. 1; FRC, functional residual capacity measured by multiple-breath washout. P values indicate significance of difference between pre- and posthistamine values.
histamine bronchoprovocation (not represented in figure; \( P > 0.1 \) for both).

There was a significant correlation between posthistamine \( S_{\text{cond}} \) and Curv (\( r = -0.64; P < 0.001 \); Fig. 4A). The data point corresponding to the greatest posthistamine Curv and \( S_{\text{cond}} \) of 0.5 and 0.153 liter\(^{-1}\), respectively, relates to the subject with the pre- and posthistamine MBW curves illustrated in Fig. 1.

The correlation between histamine-induced changes in \( S_{\text{cond}} \) (derived from the MBW) and in \( CH_4 \) phase III slopes (derived from the single-breath \( DL_{\text{CO}} \) maneuver) is shown in Fig. 4B (\( r = 0.71; P < 0.001 \)). No significant correlations existed between \( S_{\text{acin}} \) and Curv, nor between corresponding \( S_{\text{acin}} \) and \( CH_4 \) phase III slope changes with histamine (\( P > 0.1 \) for all).

Table 2 summarizes the simulated \( DL_{\text{CO}} \) and VA estimates in the model A and model B modality when the model is considered with a distribution of compartment volume, inspired volume, and flow asynchrony corresponding to the experimental \( S_{\text{cond}} \) and Curv values. This was done for the two experimental situations obtained here (baseline and histamine), and any estimation error specifically due to histamine is considered with respect to its estimation error at baseline. For instance, the histamine-induced \( DL_{\text{CO}} \) estimation error in the case of model A is 11.0\% minus 7.8\%, or 3.2\%.

Summarizing all histamine simulations, the VA and \( DL_{\text{CO}} \) estimation errors relative to baseline amounted to 0.8 and 3.2\%, respectively.

Fig. 2. Measurements of \( S_{\text{cond}} \) (A) and diffusing capacity (\( DL_{\text{CO}} \); B), before and after bronchoprovocation with histamine. Corresponding average ± SD bars are also shown. x, Subjects who decreased their forced expiratory volume in 1 s (FEV\(_1\)) by 20\% baseline for a histamine dose <2 mg. *Excluding these subjects from the significance testing between baseline and histamine still led to \( P < 0.001 \) in both panels.

Fig. 3. Histamine induced changes (\( \Delta \)) in \( DL_{\text{CO}} \) (A) and alveolar volume (VA; B) vs. the corresponding changes in \( S_{\text{cond}} \). x, Subjects who decreased their FEV\(_1\) by 20\% baseline for a histamine dose <2 mg. Excluding these subjects from the correlation analyses in both panels did not affect the absence of significance in A; in B, this led to \( r = -0.44 \) with \( P = 0.012 \).
model B caps the $S_{\text{cond}}$ value by generating a horizontal $S_{\text{req}}$ asymptote well within the six lung turnover range, which is rarely encountered experimentally; Fig. 1A). Thus, in the hypothetical case of a lung disease where the increase in conductive ventilation heterogeneity is brought about entirely by an increased specific ventilation heterogeneity, VA and $DL_{\text{CO}}$ estimation errors with respect to baseline can amount to $-5.8$ and $-21.9\%$, respectively (model A).

DISCUSSION

The main aim of the present study was to specifically induce ventilation heterogeneity in the conductive airway compartment of normal subjects, such that the effects of large-scale ventilation differences (quantified by $S_{\text{cond}}$) on $DL_{\text{CO}}$ and VA measurement could be assessed. After histamine, $DL_{\text{CO}}$ and VA decreased significantly in the group as a whole (Table 1), yet these changes were relatively small (of the order of a few percent), given the amount of increase in ventilation heterogeneity that was being generated. Our data show that there was also an actual decrease in alveolar lung volume, as inferred from the concomitant change in IVC after histamine. Such a decrease in actual VA would automatically lead to a $DL_{\text{CO}}$ decrease. The correction of $DL_{\text{CO}}$ for lung volume via $DL_{\text{CO}}^{-\text{corr}}$ attenuated the actual diffusing capacity decrease after histamine challenge to an average of 4%.

The goal of the present study was to generate a considerable range in conductive ventilation heterogeneity, such that potential correlations between $\Delta S_{\text{cond}}$ and $\Delta DL_{\text{CO}}$ could be sought. In the range of experimental airway ventilation heterogeneity generated here, there was no significant correlation between conductive ventilation heterogeneity and $DL_{\text{CO}}$ measurement (Fig. 3A). Conductive ventilation heterogeneity did show a significant correlation with VA (Fig. 3B). The explanation for the correlations, or the absence thereof, between ventilation heterogeneity ($S_{\text{cond}}$) and $DL_{\text{CO}}$ or VA can be understood on the basis of model simulations in Thompson et al. (19). In that study, ventilation heterogeneity was simulated in a model with an unequal distribution of inspired volume (model A) and a model with an unequal distribution of end-expiratory volume (model B), thus simulating only conductive ventilation heterogeneity. We used the same model here to simulate the experimental conditions in terms of specific ventilation heterogeneity (as inferred from MBW index Curv) and incorporating flow asynchrony (as inferred from MBW index $S_{\text{cond}}$), to compute the corresponding VA and $DL_{\text{CO}}$ estimation errors (Table 2).

When considering average baseline and posthistamine values for Curv and $S_{\text{cond}}$, the predicted $DL_{\text{CO}}^{-\text{err}}$ due to histamine provocation is either $-3.2\%$ (model A) or $+2.1\%$ (model B), and the predicted VA error is either $-0.8\%$ (model A) or $-5.2\%$ (model B). Thus, depending on whether ventilation heterogeneities were brought about by unequal inspired volume (model A) or unequal end-expiratory lung volume (model B), or a mixture of both, the predicted $DL_{\text{CO}}^{-\text{err}}$ error ranges from negative to positive, while the predicted VA error is consistently negative. This probably explains why we found a correlation between histamine-induced VA changes and $S_{\text{cond}}$ changes, and not between corresponding $DL_{\text{CO}}^{-\text{err}}$ changes and $S_{\text{cond}}$ changes.

Unfortunately, it is impossible to determine which is the combination of end-expiratory lung volume and inspired vol-
LARGE-SCALE VENTILATION DISTRIBUTION AND TRANSFER FACTOR

Table 2. Simulated $V_A$ and $D_{LCO}$ error estimates for different model parameters

<table>
<thead>
<tr>
<th>MBW Indexes</th>
<th>Simulation Modality</th>
<th>Flow Asynchrony (deviation from average flow)</th>
<th>Estimated Error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Volume Partitioning</td>
<td>Flow Partitioning</td>
</tr>
<tr>
<td>Model A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.81</td>
<td>0.029</td>
<td>0.67:0.33</td>
</tr>
<tr>
<td>Posthistamine</td>
<td>0.75</td>
<td>0.058</td>
<td>0.70:0.30</td>
</tr>
<tr>
<td>Hypothetical (disease)</td>
<td>0.41</td>
<td>0.058</td>
<td>0.85:0.15</td>
</tr>
<tr>
<td>Model B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.81</td>
<td>0.029</td>
<td>0.70:0.30</td>
</tr>
<tr>
<td>Posthistamine</td>
<td>0.75</td>
<td>0.058</td>
<td>0.50:0.50</td>
</tr>
<tr>
<td>Hypothetical (disease)</td>
<td>0.41</td>
<td>0.058</td>
<td>N/A</td>
</tr>
</tbody>
</table>

For Model A, volume partitioning refers to inspired volume partitioning, and for Model B, volume partitioning refers to compartment volume partitioning. MBW, multiple-breath washout.

ume distribution that led to the specific ventilation heterogeneity induced here. However, it appears highly unlikely that, at the different length scales where conductive ventilation heterogeneity occurs in health and disease, ranging from the gravitational top-to-bottom, to the much smaller gravity-independent lung units (but still larger than acini), ventilation heterogeneity would show a uniform model A or model B behavior. As a consequence, it is not surprising that experimentally induced ventilation heterogeneity does not lead to a uniform overestimation or a uniform underestimation of $D_{LCO}$.

The MBW test was used to specifically assess the degree of conductive ventilation heterogeneity induced by histamine. This type of information cannot be retrieved from the phase III slope of the inert gas in the $D_{LCO}$ single-breath maneuver, since it intrinsically contains an unpredictable combination of diffusive and convective heterogeneities. Nevertheless, in the case of histamine provocation, where convective heterogeneities are shown to dominate, it could be expected that a correlation would exist between CH$_4$ phase III slope and $S_{cond}$ (Fig. 4B), and not between CH$_4$ phase III slope and $S_{acini}$. This also indicates that, despite being derived from a different test (the MBW test), the resulting parameters Curv and $S_{cond}$ were representative of the ventilation heterogeneity at the time of $D_{LCO}$ measurement.

Several authors have suggested significant $D_{LCO}$ and $V_A$ estimation errors due to ventilatory heterogeneity, with or without additional heterogeneity in diffusion properties of lung units across the blood-gas barrier. In a very early publication describing the single-breath method for measuring $D_{LCO}$, Forster et al. (7) stated that the test was only suitable for a homogeneous lung and that errors are likely to occur in patients with heterogeneity of ventilation and diffusion. Mathematical modeling by Piper and Sikand (15) predicted that increasing ventilation-to-diffusion ratio would decrease $D_{LCO}$ estimates. Later, Prisk et al. (16) showed reductions in $D_{LCO}$ estimates of up to 30% in the presence of ventilation-to-diffusion ratio heterogeneity compared with a perfectly homogeneous model. When considering estimation errors from any such model (including the one we used to interpret our data), some degree of ventilation heterogeneity exists, even in normal lungs. Hence, model-predicted estimation errors should not be related to a perfectly homogeneous lung model, but to a normal lung (with its intrinsic ventilation heterogeneity). This may be another reason why the $D_{LCO}$ estimation errors specifically due to ventilation heterogeneity generated by lung disease in a clinical setting may not be as important as is generally anticipated, given that reference $V_A$ and $D_{LCO}$ values are also obtained from normal subjects (corresponding to $V_A$ and $D_{LCO}$ estimation errors for the baseline condition in Table 2).

The histamine-induced conductive ventilation heterogeneity, as quantified by $S_{cond}$, was comparable to that previously obtained in patients with obstructive pulmonary disease. Considering those studies for which both $S_{cond}$ and $D_{LCO}$ data are available, the average $S_{cond}$ value obtained here after histamine (0.058 liter$^{-1}$) was comparable to that previously observed in asthmatic patients, with an average $S_{cond}$ of 0.076 liter$^{-1}$ (average $D_{LCO}$ = 97% predicted) (22) and in chronic obstructive pulmonary disease patients, with an average $S_{cond}$ of 0.064 liter$^{-1}$ in patients without emphysema (average $D_{LCO}$ = 82% predicted) and of 0.068 liter$^{-1}$ in patients with emphysema (average $D_{LCO}$ = 47% predicted) (21). In fact, the similarity of $S_{cond}$ Values in the face of $D_{LCO}$ differences across the different patient groups, and the normal $D_{LCO}$ values in the asthma study in particular, indicate that there is no straightforward link between $S_{cond}$ and $D_{LCO}$. It is possible that, depending on the disease state, $S_{cond}$ may predominantly arise from increased specific ventilation heterogeneity rather than an increase in flow asynchrony. Our model prediction is that, if the increase in $S_{cond}$ in any patient results essentially from an increased specific ventilation heterogeneity (hypothetical case simulation in Table 2), $D_{LCO}$ estimation errors may increase up to ~20% with respect to baseline [i.e., ($S_{cond}$) − (−7.8%) = −21.9%]. By considering $S_{cond}$ and Curv on a patient-per-patient basis, it may be possible to identify patient groups in whom ventilation heterogeneity affects $D_{LCO}$.

The reporting of Curv or any other washout curve-related indexes [such as the lung clearance index (LCI)] had been largely abandoned because these type of washout indexes only provide a global measure of ventilation distribution over the lung. However, these indexes have gained renewed interest over the past years, since it was shown that the LCI was a very sensitive index to signal early lung disease in children with cystic fibrosis (1). More recently, Downie et al. (5) also measured LCI values in asthma patients, reporting an average LCI of 8.2 (vs. 6.6 in normal subjects) with a corresponding average $S_{cond}$ value of 0.054 liter$^{-1}$ (vs. 0.023 liter$^{-1}$ in normal subjects). While LCI and Curv are different measures of specific ventilation, they are related, and when applied to our
baseline and hypothetical case of a greatly decreased Curv (from 0.81 to 0.41), we obtain a corresponding LCI increase from 5 to 7. Irrespective of the absolute LCI value (essentially due to dead space effects), this LCI increase is greater than that observed in the asthmatic subjects. Therefore, the predicted 22% DLCO estimation error in patients with a greatly increased specific ventilation heterogeneity can be considered an upper limit.

While our aim was to specifically induce ventilation heterogeneity and study its effect on DLCO estimation, we cannot a priori exclude the possibility that changes in overall perfusion and in the distribution of perfusion could have occurred. However, Echazarreta et al. (6) reported increases in the spread of perfusion and ventilation, but no change in overall perfusion, in mild asthmatic subjects following histamine challenge. These authors also showed that the changes in perfusion and ventilation did not lead to an increase in low ventilation-perfusion units, which would have otherwise resulted in a DLCO underestimation (19). Since the measurement of DLCO uses a diffusion-limited gas, the measurement is less sensitive to changes in blood flow than to changes in capillary blood volume. Prisk et al. (16) have shown that a change in body posture from standing to supine resulted in a capillary blood volume increase of 35% and a concomitant DLCO increase of only 15%. It is unlikely that the histamine challenge in our normal subjects, with a maximum FEV1 decrease of 20%, would have had a significant effect on capillary blood volume, and, if so, the effect on DLCO will have been only one-half of that. For all of these reasons, and given the stability of heart rate and respiratory rate throughout our study procedure, we doubt that any perfusion-related effects would have significantly contributed to the results obtained in this study.

In conclusion, we isolated the effect of large-scale ventilation heterogeneities (i.e., between units larger than acini) on the estimate of diffusing capacity, thereby avoiding the confounding effect from abnormal intra-acinar ventilation heterogeneity, which may also be present in patients with obstructive lung disease. It cannot be excluded that ventilation heterogeneity within the acinar air space contributes to artifacts in DLCO, but the study of an isolated acinar effect is almost impossible to achieve experimentally. The present experimental study shows that, in the case of histamine-induced conductive ventilation heterogeneity, there is an effect on DLCO measurement; however, it is only small. The experimental results are compatible with model simulations, which indicated 1) variable DLCO estimation errors because inspired volume and end-tidal lung volume distributions have opposing effects on DLCO estimation, and 2) a more consistent but small underestimation of Va due to ventilation heterogeneity. For very specific experimental conditions of ventilation heterogeneity that could be encountered in lung disease, the DLCO and Va measurement artifacts directly related to large-scale ventilation heterogeneity are expected not to exceed 20 and 5%, respectively. A methodology is proposed whereby indexes of conductive ventilation heterogeneity can be used to estimate DLCO error on a patient-per-patient basis.

REFERENCES


