ORIGINAL ARTICLE
LUNG FUNCTION

The differing physiology of nitrogen and tracer gas multiple-breath washout techniques

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ABSTRACT

Background: Multiple-breath washout techniques are increasingly used to assess lung function. The principal statistic obtained is the lung clearance index (LCI), but values obtained for LCI using the nitrogen (N_2)-washout technique are higher than those obtained using an exogenous tracer gas such as sulfur hexafluoride. This study explored whether the pure oxygen (O_2) used for the N_2 washout could underlie these higher values.

Methods: A model of a homogenous, reciprocally ventilated acinus was constructed. Perfusion was kept constant, and ventilation adjusted by varying the swept volume during the breathing cycle. The blood supplying the acinus had a standard mixed-venous composition. Carbon dioxide and O_2 exchange between the blood and acinar gas proceeded to equilibrium. The model was initialised with either air or air plus tracer gas as the inspirate. Washouts were conducted with pure O_2 for the N_2 washout or with air for the tracer gas washout.

Results: At normal ventilation/perfusion (V'/Q') ratios, the rate of washout of N_2 and exogenous tracer gas was almost indistinguishable. At low V'/Q', the N_2 washout lagged the tracer gas washout. At very low V'/Q', N_2 became trapped in the acinus. Under low V'/Q' conditions, breathing pure O_2 introduced a marked asymmetry between the inspiratory and expiratory gas flow rates that was not present when breathing air.

Discussion: The use of pure O_2 to washout N_2 increases O_2 uptake in low V'/Q' units. This generates a background gas flow into the acinus that opposes flow out of the acinus during expiration, and so delays the washout of N_2.

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Introduction

Multiple-breath washout (MBW) techniques are increasingly being used to assess inhomogeneity of lung ventilation as a sensitive marker of early airways disease. The most commonly used index is the lung clearance index (LCI), which gives the number of turnovers of functional residual capacity (FRC) required to reduce the inert gas concentration to 1/40th of its starting value. However, in a consensus statement from the American Thoracic and European Respiratory Societies [1], it was recognised that there remained considerable variation between different techniques in relation to the values obtained for the LCI.

One common finding across studies is that values for the LCI are higher when measured using nitrogen (N_2) washout than when measured with an exogenous tracer gas, often sulfur hexafluoride (SF_6) [2–5]. These studies recognise that the divergence between N_2 and SF_6 techniques occurs later during the washout profiles, but concede that the underlying mechanism remains unclear. Possible causes suggested include methodological issues, physical differences between the test gases, differences in the pattern of breathing caused by pure oxygen (O_2) and the role of dissolved N_2 in the body.

The present study explores, using a model-based approach, whether the use of pure O_2 during a N_2 washout could delay the appearance of the N_2. Such a suggestion has been made previously in relation to infants with high metabolic rates and low lung volumes [6]. Here, we explore the role of pure O_2 with reference to acini with low specific ventilations and ventilation/perfusion (V'/Q') ratios. Such acini would be expected to influence the N_2 profile particularly during the later stages of the washout.

Methods

The model is illustrated in figure 1. The acinus has unit volume at FRC. The blood flow to the acinus (Q'_{ac}) is set at 2 units·min^{-1} to reflect that average pulmonary blood flow is approximately twice FRC every minute. For a specific, chosen value (K) for the V'/Q' ratio for the acinus, the minute ventilation to the acinus (V'_{ac}) may be calculated directly from the blood flow as K×Q'_{ac}. The acinus is ventilated by varying its volume with a saw-tooth waveform (figure 1). Inspiration lasts 1 s and expiration lasts 3 s. Thus, to achieve an overall minute ventilation of V'_{ac}, the acinus must increase its volume at a rate of 4×V'_{ac} during inspiration, and decrease its volume at a rate of (4/3)×V'_{ac} during expiration. The exchange of carbon dioxide (CO_2) and O_2 between the blood flowing through the acinus and the gas in the acinus is assumed to come to equilibrium.

The model was run in 10-ms steps, equivalent to a time interval (Δt) of (1/6000) min.

First, the volumes for CO_2 and O_2 (ΔV_{CO_2} and ΔV_{O_2}, respectively) that are released or taken up by the blood during the step are calculated as:

$$\Delta V_{CO_2} = Q'_{ac} \times (C_{vCO_2} - C_{cCO_2}) \times \Delta t, \text{ and}$$
$$\Delta V_{O2} = Q'_{ac} \times (C_{cO_2} - C_{vO_2}) \times \Delta t,$$

where C_{vCO_2} and C_{vO_2} are the mixed venous blood gas concentrations for CO_2 and O_2, respectively, and C_{cCO_2} and C_{cO_2} are the end-capillary blood gas concentrations for CO_2 and O_2, respectively. These

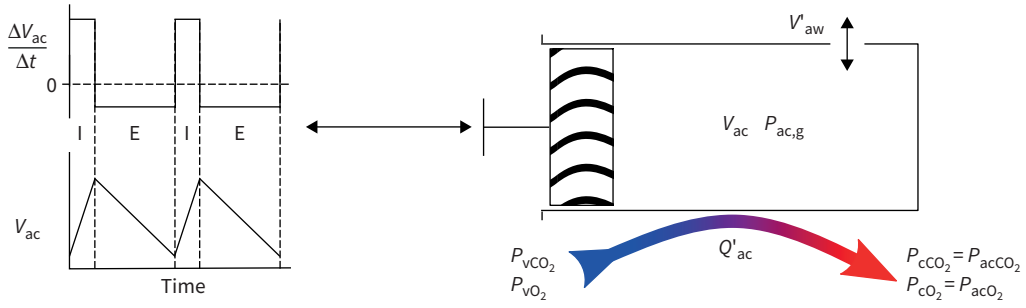

FIGURE 1 Diagram of model acinus. The volume of the acinus (V_{ac}) varies in a fixed saw-tooth pattern where the amplitude is set by the value chosen for acinar ventilation/perfusion ratio (V'_{ac}/Q'_{ac}). The end-capillary partial pressures for carbon dioxide (CO_2) and oxygen (O_2) (P_{cCO_2} and P_{cO_2}, respectively) are equilibrated with the partial pressures in the acinus (P_{acCO_2} and P_{acO_2}, respectively). The mixed venous partial pressures for CO_2 and O_2 (P_{vCO_2} and P_{vO_2}, respectively) are fixed at 46 mmHg (6.13 kPa) and 40 mmHg (5.33 kPa), respectively. The O_2 uptake and CO_2 output are calculated from the product of the arterio-venous difference and acinar blood flow (Q'_{ac}). These exchanges, together with the change in V_{ac}, are used to determine the overall flow of gas into or out of the airway (V'_{aw}). Δt: time interval; I: inspiration; E: expiration; $P_{ac,g}$: partial pressure of a gas in the acinus.

concentrations are calculated from the corresponding partial pressures (P_{CO_2} and P_{O_2}) *via* a model of the blood-gas dissociation curves [7]. For the mixed venous blood, standard values for P_{CO_2} and P_{O_2} were used (46 mmHg/6.13 kPa for CO_2 and 40 mmHg/5.33 kPa for O_2). For the end-capillary blood, as Δt is short and as complete equilibration is assumed, the P_{CO_2} and P_{O_2} values in the acinus at the start of the 10-ms step were used.

Second, the increment in volume of the acinus over the 10-ms step (ΔV_{ac}) is calculated, either as $4\times V'_{ac}\times\Delta t$ for inspiration or as $-(4/3)\times V'_{ac}\times\Delta t$ for expiration. From these volume changes, the total volume of gas entering from the airway over the 10-ms step (ΔV_{aw}) can then be calculated as:

$$\Delta V_{aw} = \Delta V_{ac} + \Delta V_{CO_2} - \Delta V_{O_2}$$

Finally, the amounts of CO_2, O_2, N_2 and exogenous tracer gas (if present) in the acinus can be updated from their amounts entering or leaving from the blood (CO_2 and O_2 only) and the airway. This completes the calculations for the current 10-ms step, and leaves the model with updated volume and gas concentrations in the acinus ready to undertake the next 10-ms step.

To simulate a N_2 washout, the model is first initialised using multiple breath cycles with air as the inspirate until the acinar concentrations are stable between breaths. The N_2 washout is then executed by substituting O_2 for air in the inspirate. To simulate an exogenous tracer gas washout, the initialisation period is undertaken with a gas that is similar to air, but where 1% tracer gas has been introduced by replacing an equivalent amount of N_2 so that the inspired O_2 concentration remains unaltered. The washout is then executed by switching the inspirate to air. Both N_2 and exogenous tracer gas are assumed insoluble in blood.

The primary outputs from the model are the acinar gas concentrations at end-expiration (end of each breath cycle), in particular the N_2 concentration for the N_2 washout and the exogenous tracer gas concentration for the tracer gas washout. The findings are illustrated as semi-log plots, with the x-axis representing the number of whole-lung turnovers, assuming V'/Q' is equal to 1 for the whole lung. Rather than displaying a large number of discrete points, curves were generated by fitting a spline through the values for the acinar gas concentrations. Secondary outputs included the average rate of gas flow into/out of the acinus, during inspiration and expiration, for each breath, and the relative rates of N_2 and tracer gas washout (normalised to their starting concentrations). Again, rather than display large numbers of data points, the results are shown as spline curves fitted through the datapoints.

Results

The results are shown in figure 2 for a standard V'/Q'=1, a low V'/Q'=0.1 and a very low V'/Q'=0.04.

For a V'/Q'=1, the concentration profiles for N_2 and exogenous tracer gas plotted against lung turnover were essentially indistinguishable (figure 2a). The inspiratory and expiratory gas flows (V'_{aw}) each relative to the rate of change of acinar volume ($\Delta V_{ac}/\Delta t$) were slightly above and below one, respectively, reflecting that the respiratory quotient was below unity (figure 2b). The relative rates of elimination of the two gases were always very similar (figure 2c).

For a V'/Q'=0.1, the concentration profile against lung turnover declined more steeply for the exogenous tracer gas than for N_2 (figure 2a). During the tracer gas washout, the inspiratory and expiratory gas flows remained constant throughout. This was not the case for the N_2 washout, where inspiratory flow increased and expiratory flow decreased (figure 2b). This behaviour was generated by the rise in acinar P_{O_2} during the washout increasing the uptake of O_2 into the blood by diffusion, and so generating a convective flow of gas from the airway into the acinus. The result was that the rate of elimination was lower for N_2 than for the tracer gas during the initial washout, and only reversed when the concentrations of the two gases in the acinus were sufficiently different (figure 2c).

For a V'/Q'=0.04, the results were qualitatively similar to those for a V'/Q'=0.1 except that the concentration profile for N_2 plotted against lung turnover plateaued, with almost all the N_2 left in the acinus (figure 2a). This occurred because expiratory flow fell below zero, and so gas flow was permanently into the acinus (figure 2b). Thus, most of the N_2 was trapped in the acinus and could never wash out through a purely convective process (figure 2c).

Discussion

The modelling provides a key reason why, physiologically, we should not expect MBW tests using N_2 and those using exogenous tracer gases to yield the same results, and why the N_2 washout will be slower. The essential difference is that exogenous tracer gases are washed out with air, while N_2 is washed out with

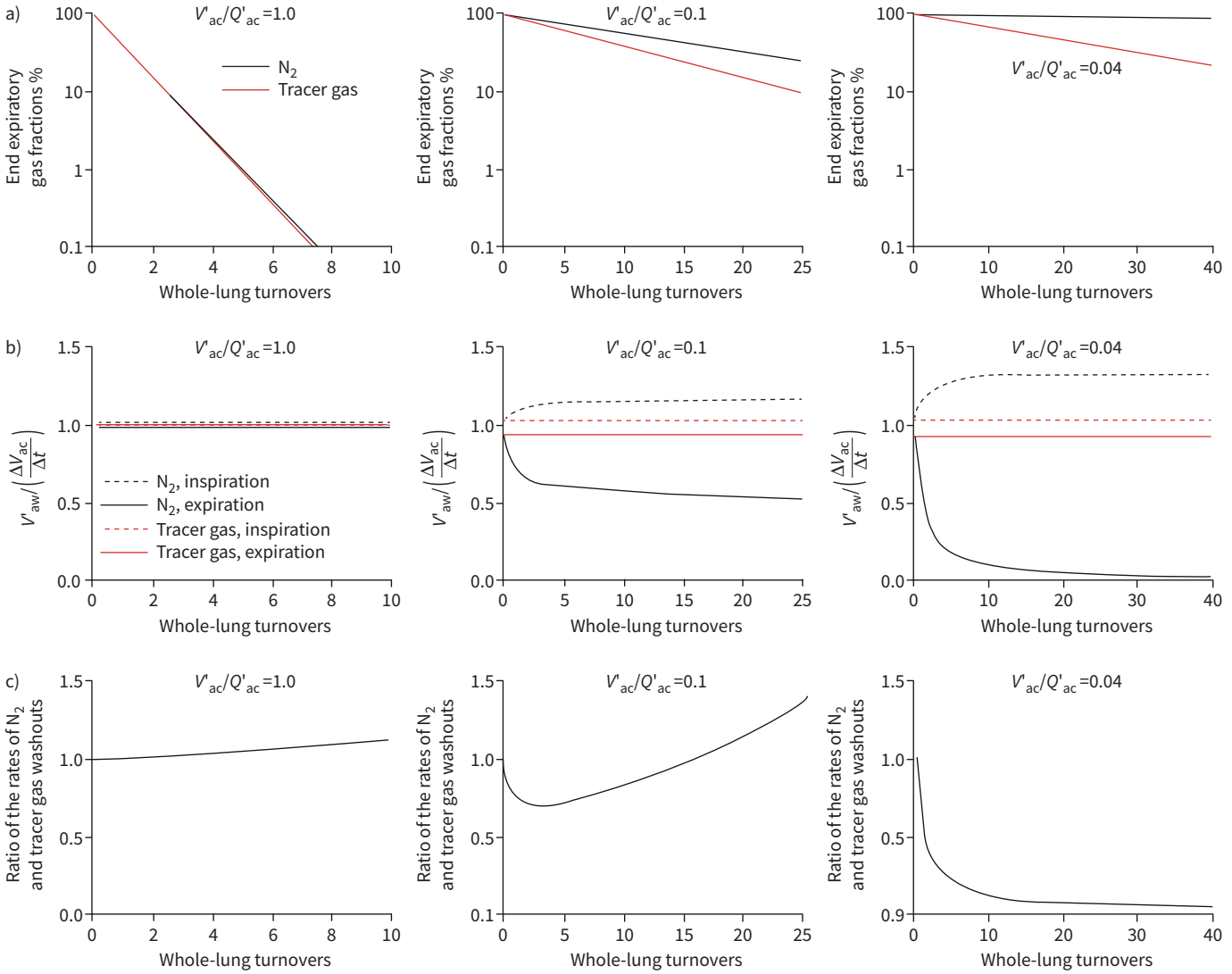

FIGURE 2 Comparison of the effects of low acinar ventilation/perfusion ratio (V'_{ac}/Q'_{ac}) on rates of nitrogen (N_2) and exogenous tracer gas washout. a) End-expiratory acinar fractions for N_2 and tracer gas, relative to their starting values at beginning of washout; b) ratio of the flow of gas into or out of the airway (V'_{aw}) relative to the rate of change of the volume of the acinus ($\Delta V_{ac}/\Delta t$) for inspiration and expiration; c) ratio for flow of N_2 relative to tracer gas out of the acinus during expiration. The flow for each gas species has been normalised to its concentration at start of the washout. Results are presented for values of V'_{ac}/Q'_{ac} equal to 1, 0.1 and 0.04. x-axes: number of turnovers of functional residual capacity for whole lung (occur every 30 s).

pure O_2, and the latter influences gas exchange in lung regions of low V'/Q'. In low V'/Q' units, there is little gas exchange when breathing air and this remains so when an exogenous tracer gas is washed out. However, when washing N_2 out with pure O_2, the rise in alveolar P_{O_2} generates an uptake of O_2 into the blood that then generates an underlying current of gas flow into the lung unit. This current of gas flow opposes the expiratory gas flow generated by breathing, and so reduces the rate at which N_2 is washed out of the lung unit.

In addition, the results illustrate that, in the case of very low V'/Q' units, physiological gas (N_2) trapping can occur in the lung without physical airways closure. This possibility has been recognised by others previously [8, 9]. In relation to this, residual N_2 in the lung can sometimes be detected at the end of a MBW by a period of forced breathing [10]. Such residual N_2 could be arising, in part, through this physiological mechanism as well as by physical gas trapping arising from airways closure. In our simulations, a completely regular breathing pattern was employed. However, variations in expiratory effort occur naturally, and these could result in N_2 release during some breaths, but not others. This may explain the difficulty that sometimes occurs identifying the cut off breath when calculating the LCI.

One question that arises naturally from this study is whether acini with low or very low V'/Q' exist within the lung. Early relatively gross measurements of regional V'/Q' distribution in healthy humans suggested that values for V'/Q' may vary between about 0.5 at the base of the lungs to 3 at the apex of the lungs [11]. Following the development of the multiple inert gas elimination technique to assess V'/Q' distribution, Wagner *et al.* [12] concluded that for healthy young people, >95% of both the blood flow and the ventilation was directed to units where V'/Q' was in the range of 0.3–2.1, but that for some older individuals V'/Q' values in the range 0.01–0.1 were observed. What could give rise to such low V'/Q' units? Pertinent to this question is a recent study by Verleden *et al.* [13], who used micro-computed tomography to study unused donor lungs and found that, once aged >30 years, there was a significant decline in the number of terminal bronchioles. If acini that are lacking terminal bronchioles are not to collapse, then they must be ventilated in some alternative, collateral way. One possible route for this ventilation to occur is *via* the pores of Kohn. These are considered a high-resistance pathway, potentially with an expiratory resistance that is substantially greater than the inspiratory resistance, and therefore such acini could form the basis of lung tissue with particularly low V'/Q' [14, 15]. Indeed, it should be noted that Verleden *et al.*'s study does not exclude the possibility that there may be acini without terminal bronchioles even in young, healthy lungs. Finally, in airways disease, terminal bronchioles may be lost [16] or simply closed by oedema/inflammation and/or plugged by mucus [17].

A further question that arises is whether very low V'/Q' units would remain stable when exposed to high O_2, or whether they would collapse. This depends on whether or not the low V'/Q' is associated with a very high inspiratory airways resistance [8]. In such a case, it would be possible for the gas uptake by the blood to exceed the gas flow into the acinus, and therefore collapse would follow. The speed of any such collapse would depend on the O_2 and N_2 content of the lung unit, and this would be increased if the N_2 content of mixed venous blood had been lowered by the period of high O_2 breathing so that there was an increased rate of diffusional uptake of N_2 into the blood. These matters have been considered in more detail elsewhere [9].

Apart from the influence of pure O_2 on the N_2 washout, there are other reasons why the washout of N_2 may be slower than for exogenous tracer gases. In particular, humans have ~1 L of dissolved N_2 in their tissues, and once the partial pressure of N_2 (P_{N_2}) is lowered in the lung, diffusion of N_2 from the blood into the lung will occur [4, 5]. Taking the solubility of N_2 in blood as 1.44×10^{-10} L(STPD)·L^{-1}·Pa^{-1}, an average value of P_{N_2} in the lung of 80 kPa and an average cardiac output of 4 L·min^{-1}, then the delivery of dissolved N_2 *via* the blood into the lung can be calculated as 46 mL·min^{-1}. If this were all to be eliminated, then for an alveolar ventilation of 4–5 L·min^{-1}, it would result in an inspired to end-tidal difference of ~1%. However, the full scale of this effect is unlikely to be sustained for long because, with a lowered P_{N_2} in the arterial blood, the dissolved N_2 will wash out of tissues with a high perfusion rate (*e.g.* kidney, heart) very quickly.

In addition to the physiological reasons for the difference between the rate of washout for N_2 and exogenous tracer gases, there are also issues relating to the N_2 measurement that could give rise to a falsely slow washout. Guglani *et al.* [4] identified cross-talk between the CO_2 and N_2 measurement within an N_2 MBW device (Exhalyzer; EcoMedics, Duernten, Switzerland) employed in their study. This resulted in an offset error for the N_2 measurement. In seeming contrast to those findings, Bayfield *et al.* [2] found similar LCI values were obtained from this device and from a respiratory mass spectrometer (AMIS 2000; Innovision, Odense, Denmark) when used in tandem during washout studies. However, in healthy volunteers, they also report that a plateau for N_2 washout was reached at ~1.3%. This appears high compared with measurements of N_2 exchange made using the highly precise technique of laser absorption

spectroscopy [18]. Finally, it is worth noting that considerable potential exists for cross-talk between CO_2 and N_2 when using mass spectrometry, as some of the CO_2 present may fragment to form carbon monoxide, which has the same molecular mass as N_2 at 28 u.

Apart from the routines relating to the blood gas dissociation curves, the model used in the present study was extremely simple. This is beneficial in terms of understanding why certain behaviours, such as N_2 trapping, can occur. However, it is also a significant limitation as the model does not have the detail necessary to predict with any accuracy the size of the effect. One very important limitation in relation to both N_2 and the exogenous tracer gas is that diffusion is constrained just to the acinus where full mixing is assumed. There is no scope for either gas to diffuse into the blood as both are considered insoluble. In addition, there is no scope for back-diffusion into the larger airways. This process is likely to become more important for the very low convective flow rates with which this study has been principally concerned causing the stationary front to migrate to a more proximal position locally within the airways. Therefore, these factors may limit the overall duration for which N_2 trapping can persist.

In conclusion, it is already well recognised that MBW tests conducted with N_2 as the tracer gas yield slower washouts than those conducted with exogenous tracer gases, and as a consequence that measurements made using the two different techniques are not simply interchangeable. The present, model-based study, illustrates how the use of pure O_2 to washout N_2 alters gas exchange, and hence slows N_2 washout in lung units with particularly low values for V'/Q'. In relation to interpreting parameters relating to the speed of a MBW test, such as the LCI, it is important to recognise that they are simply phenomenological parameterisations of a test result. The results from this study do not invalidate such measurements in the slightest, but by the same token they serve to illustrate further that the interpretation of such test results is complex. Indices such as the LCI are influenced by many factors, and they certainly should not be viewed simply as a measure of the ventilation inhomogeneity within the lung.

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