Nitrogen back-diffusion during multiple-breath washout with 100% oxygen

To the Editor:

The lung clearance index (LCI) measured by multiple-breath washout (MBW) is defined as the number of lung volume turnovers needed to reduce the concentration of a blood-insoluble tracer gas by a factor of 40 during tidal breathing [1]. Over the past two decades, the MBW test has proven to be particularly useful in cystic fibrosis, and studies [2, 3] have demonstrated its superior sensitivity to that of forced expiratory volume in 1 s (FEV_1). The ideal tracer gas for the LCI test is so insoluble in blood that any gas exchange effects are insignificant, and it can be measured in concentrations low enough that the tracer itself does not affect the physical properties of the respired air or gas mix. Historically, SF_6 is the tracer gas that is most often used in the implementation of the MBW test [1]. However, it has been suggested that the LCI can instead be derived from the washout of nitrogen resident in the lungs using 100% oxygen, and this approach is being applied in a number of ongoing clinical trials.

Different LCI values with SF_6 and N_2 washout have been reported [4]. Two recent studies [5, 6] suggest that this difference is likely due to back-diffusion (diffusion of N_2 from blood and tissues during washout). Diffusion of N_2 from blood to tissues occurs along a partial pressure gradient, and has been described to affect N_2 washout in measurements of lung volume from as early as the 1940s [7]. The question of whether back-diffusion significantly affects the washout curve is important [5, 8], because if this is the case, the LCI based on N_2 depends on gas exchange factors that are usually not controlled or monitored in MBW protocols, such as pulmonary blood flow, regional tissue perfusion, etc. Back-diffusion would thus be a potential source of both false negative and false positive results in clinical trials using the N_2 LCI. However, the previous literature on this issue is based either on *indirect* comparisons of N_2 and SF_6 washout or on computer simulations. Here, we present the first study based on *simultaneous* measurement of SF_6 and N_2 washouts, analysed with the same software algorithms.

In order to perform the first direct measurement of N_2 back-diffusion, MBW tests were performed using a modified Innocor LCI device (PulmoTrace, Inc. Atlanta, GA, USA) simultaneously measuring N_2 (as 100% minus the sum of the measured O_2, CO_2 and SF_6 concentrations) and SF_6. The SF_6 and CO_2 were measured by a photoacoustic gas analyser, and O_2 was measured by a laser diode sensor (Oxigraf, Sunnyvale, CA, USA), both of which were integral to the Innocor device.

10 apparently healthy nonsmoking subjects aged 18–22 years (six males, mean body mass index 23 $kg \cdot m^{-2}$ (range 18–30 $kg \cdot m^{-2}$)) were recruited *via* advertisement, after approval by the institutional review board at the University of Pittsburgh, PA, USA (PRO16040202). Each subject performed two MBW tests. The SF_6 was washed in from a 120 L Douglas bag *via* a one-way valve, with a mixture of 0.2% SF_6 and 99.8% air. When the relative difference between inspired and expired SF_6 concentrations was <1%, the breathing valve switched to connect the patient to a second Douglas bag that was prefilled with 100% O_2. The subject continued breathing from this bag *via* another one-way valve until both SF_6 and N_2 concentrations were <1/40 of the starting concentration. During the first MBW test, the subjects were asked to hold their breath for 30 s at two different points (45 s and 120 s) after the start of washout. The second test was performed while the subject was exercising on a cycle ergometer at low power (25 W). During this test, the subjects were asked to hold their breath for 20 s after 25 and 75 s, respectively. The change in end-tidal N_2 and SF_6 occurring during the breath hold was measured as the difference between end-tidal concentrations before and after the breath hold. The difference between the increase in normalised N_2 concentration

Cite this article as: Sullivan L, Forno E, Pedersen K, *et al*. Nitrogen back-diffusion during multiple-breath washout with 100% oxygen. *Eur Respir J* 2017; 50: 1700679 [https://doi.org/10.1183/13993003.00679-2017].

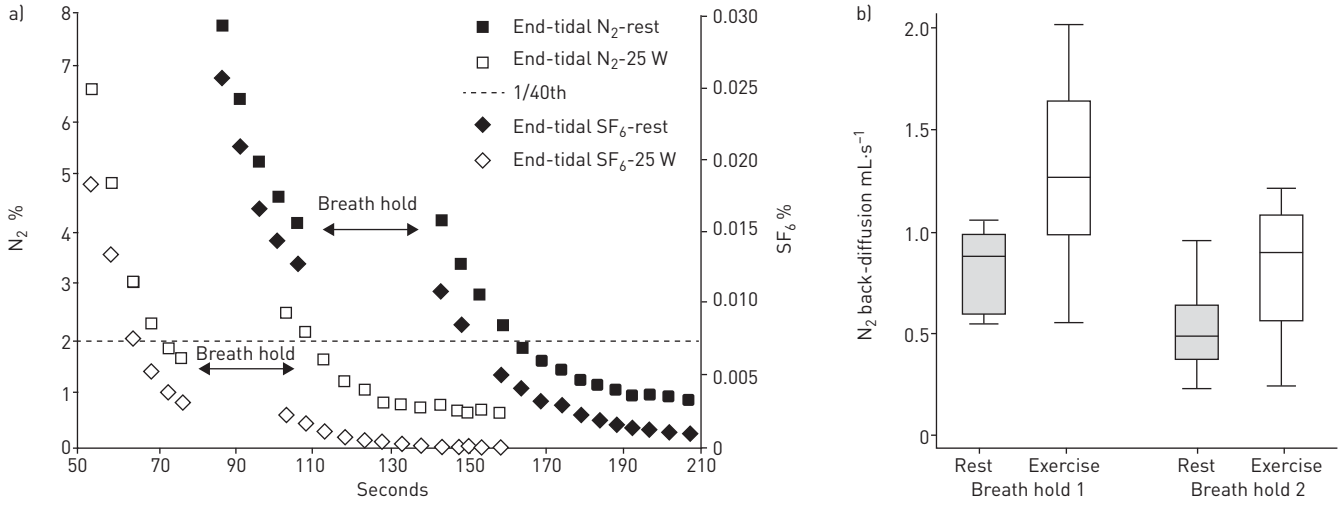

FIGURE 1 a) Multiple breath washout of both N_2 (left axis, squares) and SF_6 (right axis, diamonds) with two breath hold periods, conducted both at rest (filled symbols) and during low-level exercise (open symbols). b) N_2 back-diffusion during the breath holds at rest and during exercise.

during the breath hold and that of normalised SF_6 concentration was attributed to N_2 back-diffusion. Cardiac output was measured before each of the MBW tests using the inert gas rebreathing method [9].

Of the 10 subjects, two who had difficulty with the breath hold manoeuvres were excluded from the analysis. Cardiac output at rest was (mean±SD) 6.6±1.7 L·min^{-1} and was almost doubled during exercise to 10.4±1.6 L·min^{-1}. Representative tracings from one subject are shown in figure 1a. Figure 1b shows the boxplots of N_2 back-diffusion from both breath hold manoeuvres at rest and at exercise. During the first breath hold, N_2 back-diffusion rates were 0.83±0.21 mL·s^{-1} at rest and 1.29±0.49 mL·s^{-1} during exercise (p=0.048). During the second breath hold, N_2 back-diffusion was 0.53±0.25 mL·s^{-1} at rest and 0.82 ±0.34 mL·s^{-1} with exercise (p=0.033).

The second breath hold was timed later in the washout, near the LCI point. The alveolar N_2 concentration owing to back-diffusion at this point can be estimated from a one-compartment steady-state model of gas exchange [10], considering that the rate of back-diffusion (from the blood to the alveolus) is equal to the rate of N_2 excretion by ventilation. Using estimates of normal adult alveolar ventilation (5 L·min^{-1}), and our measured back-diffusion of 0.53 mL·s^{-1}, the estimated alveolar N_2 concentration is ~0.6%, which constitutes about 1/3 of the total concentration at the LCI point (by definition, one-40th of 79% or 1.95%). Thus, the N_2 LCI point represents the point at which the patient has reduced the concentration of resident N_2 in the lungs, not by a factor of 40 (as required by the definition of the LCI), but instead by a factor of about 60 (79/1.35). This error leads to overestimation of the LCI.

In a recent report, Yammine *et al.* [6] estimated the effect of back-diffusion on the LCI to be about 10% higher in a healthy subject, and 37% higher in a patient with cystic fibrosis. These two examples were based on indirect N_2 measurements and had no insoluble reference gas for comparison, as in our study. A theoretical study using published data on the kinetics of N_2 washout [5] showed that the fraction of N_2 stemming from back-diffusion at the LCI point is expected to be between 24% and 49%, similar to the result of 33% observed in the present study.

In conclusion, using simultaneous measurements of N_2 and SF_6, we found that the N_2 LCI point is significantly influenced by back-diffusion, which depends on cardiac output among other factors. Given that the effect is proportional to the cardiac output, complete rest might minimise the problem. Further studies are needed to investigate the importance of back-diffusion in patients with abnormal lung function.

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Received: March 31 2017 | Accepted after revision: June 05 2017

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

Acknowledgements: Author contributions: J.G. Nielsen and K. Pedersen initiated and designed the study. L. Sullivan, D.J. Weiner and K. Pedersen collected data and performed inert gas rebreathing and lung clearance index measurements. K. Pedersen and E. Forno analysed the data. All authors contributed to (participated in) the writing and reviewing of the manuscript.

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