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Nitrogen offset in N_2 multiple washout method

To the Editor:

In a recent study of the nitrogen multiple breath washout (MBW) method to measure lung clearance index (LCI) using the Exhalyzer device (Eco Medics AG, Dürnten, Switzerland), Bayfield *et al.* [1] reported an N_2 offset signal of ~1.4%, slightly higher than reported in several previous studies. There was no similar offset using sulfur hexafluoride as the tracer gas measured with the Innocor device (Innovision ApS, Glamsbjerg, Denmark), a finding that is in line with previous reports. The results of this and other studies are extremely important as the Exhalyzer is the device that is currently used in ⩾100 cystic fibrosis centres in the European Cystic Fibrosis Society Clinical Trial Network and the Cystic Fibrosis Foundation Therapeutics Development Network in various drug trials [2].

There is no plausible explanation for a persisting offset within the constraints of the washout model underlying the definition of LCI.

At a first glance, an offset error of 1.4% might seem harmless but in the context of LCI measurements, it is devastating because the LCI point is, by definition, at a concentration of 2.5% of the value at the beginning of washout or 1.95%. So, more than two-thirds of the measured concentration at the LCI point is not related to washout of N_2 from the lungs but to something else. Mathematically, the offset error affects the calculated LCI more in lungs with a high degree ventilation heterogeneity because the washout curve is not as steep as in healthy lungs. The error therefore exaggerates ventilation heterogeneity or, in other words, makes the patient appear sicker than they are.

The authors mention several possible explanations for the offset but seem reluctant to draw a conclusion, although this has important implications for current and future use of the N_2 MBW method.

I agree with authors that the explanation is most likely not the difference in physical properties of SF_6 and N_2 for the following reasons: 1) a study [3] has shown no difference between washout of helium and SF_6, two gases with much larger differences in diffusivity than SF_6 and N_2; 2) another study [4] has shown a slightly larger Fowler dead space for SF_6 than He, an effect that would tend to slow SF_6 washout compared to N_2, opposite to the experimental data; and 3) model calculations [5] have failed to explain the observed difference between N_2 and SF_6.

The two most likely explanations are an offset error in indirect N_2 measurement, and back diffusion of N_2 from blood and tissue.

Indirect N_2 measurement at low N_2 concentrations is problematic for two main reasons: 1) mathematically, small relative errors in measured oxygen and carbon dioxide concentrations propagate into a ~50 times larger error in the calculated N_2 concentration [6]; and 2) the laser diode system used to measure O_2 in the Exhalyzer is affected by CO_2.

In a previous study [7], we demonstrated that ~50% of the offset stems from an offset error caused by interference from CO_2. It is due to a well-known mechanism where absorption of laser light depends on which other gases are present (molecular collision). The authors mention our data but appear to require confirmation by the manufacturer of the Exhalyzer device. If the authors had any doubts on the validity of our data, it would have been easy to perform a test in their own laboratory. The test takes <5 min, and all it requires is a gas mixture with 95% O_2 and 5% CO_2.

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Cite this article as: Nielsen JG. Nitrogen offset in N_2 multiple washout method. *ERJ Open Res* 2020; 6: 00335-2019 [https://doi.org/10.1183/23120541.00335-2019].

So, how about back diffusion? A simple one-compartment lung model predicts that back diffusion will generate as much as a 1% alveolar N_2 concentration at resting cardiac output and back diffusion is known to be slow (hours rather than minutes). Therefore, N_2 back diffusion tops the list of suspected causes of the offset error. In a recent study [8], we were able to measure N_2 back diffusion in the first couple of minutes after start of washout, confirming the magnitude of the problem predicted by the one-compartment model. We were also able to demonstrate direct proportionality between back diffusion and cardiac output in a setup that was insensitive to errors from indirect measurement of N_2.

The authors mention one study [9] that was unable to explain the overreading of functional residual capacity (FRC) of the Exhalyzer by N_2 back diffusion, a study that offered no other explanation. However, the study: 1) did not use back diffusion data from the time interval that is relevant in the MBW test; 2) applied an erroneous equation to correct for back diffusion (see our comment to [9]); and 3) it did not account for the technical offset error.

In summary, the theoretical considerations estimating the two most likely causes of the N_2 offset agree with experimental data from several independent groups. Proof does not get much stronger than that in physiology.

Several previous studies have demonstrated accurate measurements of FRC in physical lung models with the Exhalyzer. The explanation is simple: the physical model has no CO_2 and no N_2 back diffusion, and thus the data confirm rather than contradict the offered explanation for the *in vivo* N_2 offset.

Does it matter? The reported overestimation of LCI due to the N_2 offset error was 55% in CF patients. However, the offset also overestimates FRC, which blunts the LCI error. Using a common FRC in the simultaneous measurements (which is the right thing to do) increases the error in LCI to 100%. In our study [7] we found an error of 70% in a comparable situation. We have estimated that about half of the error is due to a technical offset and the other half is due to back diffusion, which is therefore responsible for an error of ~50% in the CF patients. This error depends primarily on the balance between cardiac output and alveolar ventilation, which are therefore important confounders in longitudinal studies. The technical offset error depends on expired CO_2 concentration, which varies both between subjects and within the same subject.

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Received: 02 Dec 2019 | Accepted after revision: 04 Dec 2019

Conflict of interest: J.G. Nielsen reports that he is the previous owner of Innovision, which develops and markets the Innocor device mentioned in the study, and to which his comments relate, but he has no financial interests in the company.

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