Normative multiple-breath washout data in school-aged children corrected for sensor error

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To the Editor:

The nitrogen (N_2) multiple-breath washout (MBW) technique is a lung function test used to assess ventilation distribution and lung volumes. The lung clearance index (LCI) is a marker of global ventilation inhomogeneity from the technique. It is calculated by the number of functional residual capacity (FRC) turnovers required to wash out resident N_2 from the lungs by breathing 100% oxygen (O_2) [1]. We have previously published normative data for MBW outcomes in healthy, white, school-aged children measured using the commercially available Exhalyzer D MBW device (Eco Medics, Duernten, Switzerland) in the *European Respiratory Journal* [2]. Recently, Wyler *et al.* [3] identified an error in the cross-sensitivity correction for the O_2 and carbon dioxide gas sensors within the Exhalyzer D device. This error results in an overestimation of N_2 concentration and MBW outcomes, including LCI and FRC. Correction of this error and reanalysis of MBW data is possible and has now been implemented into an updated software version by the manufacturer (Spiroware version 3.3.1; Eco Medics). The aim of this study was to provide updated N_2MBW normative data in healthy children collected on the Exhalyzer D device and analysed using Spiroware 3.3.1, and to report the differences in outcomes from the previous version Spiroware 3.2.1 [2].

We reanalysed all 485 N_2MBW trials from 180 children reported previously and determined the mean±SD values for FRC, LCI and the moment ratios (M_1/M_0 and M_2/M_0), and upper limits of normal (ULN) corresponding to the 97.5th percentiles (mean+1.96SD). Within-test variability was calculated as relative difference (($trial_1-trial_2$)/mean(%)) for participants with two trials and as the coefficient of variation (SD/mean (%)) for participants with three or more trials. Absolute and relative differences in primary outcomes and within-test variability between Spiroware 3.3.1 and Spiroware 3.2.1 were calculated, and Bland–Altman plots and paired t-tests were used for comparisons between the software versions. In addition, we provide updated prediction and z-score equations for FRC. For the statistical analysis we used R version 3.4.3 (R Project for Statistical Computing, www.r-project.org) and Stata statistical software (release 14; StataCorp, College Station, TX, USA).

Reanalysis using the updated crosstalk correction led to significant decreases in MBW outcomes compared with our previously published outcomes. Mean±SD $LCI_{2.5\%}$ was 6.3±0.4 in Spiroware 3.3.1 compared with 7.0±0.5 in Spiroware 3.2.1. The new ULN is 7.1 compared with the previously reported 7.9 (figure 1a). The mean (95% CI) absolute difference (Spiroware 3.3.1 – Spiroware 3.2.1) was −0.8 (−0.8 to −0.7), which corresponds to a relative difference of −10.4% (−11.2% to −9.6%). The Bland–Altman plot revealed a bias whereby the difference in $LCI_{2.5\%}$ values between software versions increased as mean LCI values increased (limits of agreement −1.4 to −0.2) (figure 1b).

The results were similar for $LCI_{5\%}$ and the moment ratios. For $LCI_{5\%}$, the mean±SD value was 4.9±0.3 and ULN was 5.5 in Spiroware 3.3.1, compared with a mean of 5.1±0.3 and ULN of 5.7 in Spiroware 3.2.1 (absolute difference −0.2 (−0.2 to −0.2), relative difference −3.4% (−3.7% to −3.0%); p<0.001). For M1/M0, the mean±SD value was 1.5±0.1 and ULN was 1.7 in Spiroware 3.3.1, compared with 1.6±0.1 and 1.8 in Spiroware 3.2.1 (absolute difference −0.1 (−0.1 to −0.1), relative difference −6.4% (−6.9% to −6.0%); p<0.001). For M2/M0, the mean±SD value was 4.2±0.5 and the ULN was 5.2 in Spiroware 3.3.1, compared with 5.0±0.6 and 6.2 in Spiroware 3.2.1 (absolute difference −0.8 (−0.8 to −0.7), relative difference −15% (−16.3% to −13.8%); p<0.001).

Shareable abstract (@ERSpublications)
This study provides updated reference values for nitrogen multiple-breath washout outcomes in healthy, white, school-aged children in a commercially available device corrected for a known sensor error. https://bit.ly/3mzBoyR

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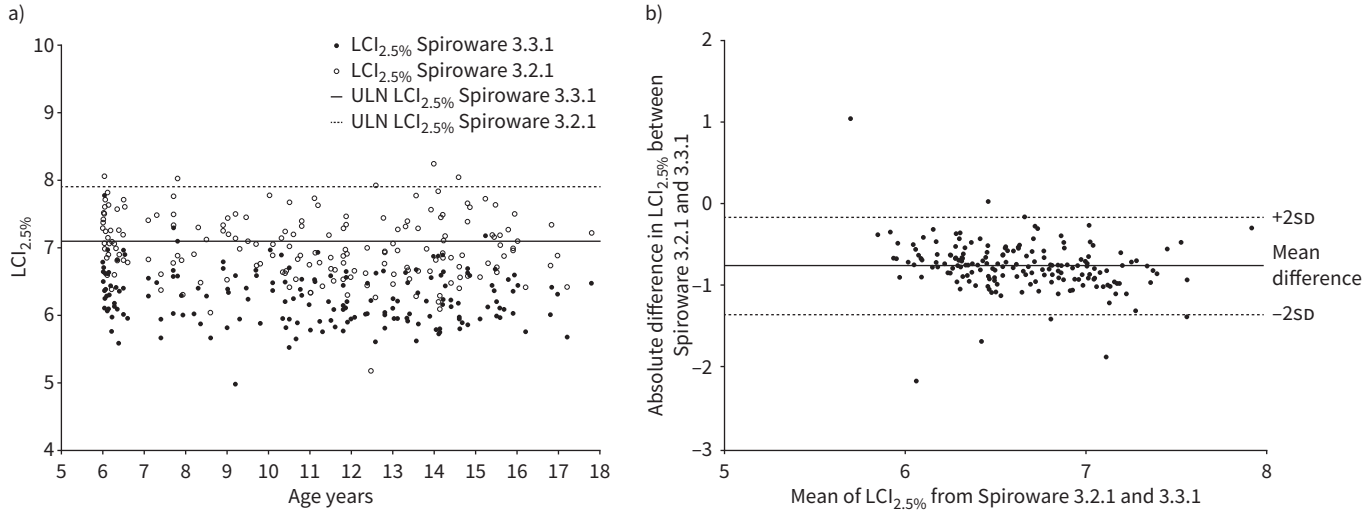

FIGURE 1 Impact of Spiroware 3.3.1 software on the lung clearance index (LCI) in healthy children. a) Relationship between $LCI_{2.5\%}$ and age. The revised upper limit of normal (ULN) for $LCI_{2.5\%}$ is 7.1 for Spiroware 3.3.1 compared with 7.9 for Spiroware 3.2.1. b) Bland–Altman plot of the agreement in $LCI_{2.5\%}$ between Spiroware 3.3.1 and Spiroware 3.2.1. The solid horizontal line represents the mean difference, and the dashed lines represent the limits of agreement (mean difference±2SD). The mean absolute difference between software versions (Spiroware 3.3.1 – Spiroware 3.2.1) was −0.8 (95% CI −0.8 to −0.7), and the limits of agreement −1.4 to −0.2. The difference between software versions increased as mean LCI values increased.

Bland–Altman plots also revealed a bias for $LCI_{5\%}$, M1/M0 and M2/M0, whereby the difference between software versions increased as mean values increased (data not shown).

The number of breaths needed to reach the end of test criteria (2.5% of starting tracer gas concentration) significantly decreased by 5±2.4 breaths, while the within-test variability of MBW outcomes did not change. Associations between MBW outcomes and demographic (age, height, weight) and physiological factors (tidal volume, equipment dead space) were unchanged following reanalysis (data not shown). Natural log (ln) transformed FRC values remained independently associated with ln(height) and ln(weight), with only minor adjustments to the coefficients. The updated FRC prediction equation for Spiroware 3.3.1 is presented as equations 1 and 2 (where FRC is expressed in L, height in cm and weight in kg):

$$\text{predicted FRC} = e^{-18.83} \times \text{height}^{4.13} \times \text{weight}^{-0.36} \quad (1)$$

$$\text{z-score FRC} = \frac{\ln(\text{measured FRC} - \text{predicted FRC})}{0.1706} \quad (2)$$

As FRC values from the Eco Medics device have been shown previously to exceed those from plethysmography [4], we compared our new predicted FRC values to reference equations from plethysmography in healthy school-aged children [5]. The mean±SD predicted FRC for our study population was 1.6±0.7 L using our Spiroware 3.3.1 equations compared with 1.9±0.7 L using the plethysmography reference equations (Spiroware 3.3.1 – plethysmography: absolute difference −0.2 (−0.3 to −0.2); paired t-test $p<0.001$), which suggest that updated FRC values will not exceed those from plethysmography in healthy children.

We found that reanalysis of N_2MBW data using the updated software version significantly decreased MBW outcomes in healthy children. Relationships between MBW outcomes and demographic or physiological variables were unchanged; therefore, static ULN for LCI and moment ratios are still appropriate within this age range. The new ULN for MBW outcomes are lower than published previously, and care is needed to ensure that appropriate reference values are used for the interpretation of MBW data collected or analysed using software versions that incorporate the new crosstalk correction (*i.e.* Spiroware 3.3.1 and higher).

We report a similar impact of the crosstalk correction on outcomes to those reported in children with cystic fibrosis and healthy children [3] and healthy infants and toddlers [6]. Our results support previous findings that the impact of the crosstalk correction is dependent on the magnitude of the outcomes. Larger reductions in LCI were seen in children with cystic fibrosis compared with healthy controls, and particularly those with more severe disease and longer washout times [3]. These data suggest that the magnitude of the difference between groups (*e.g.* healthy *versus* cystic fibrosis) may be smaller than previously reported on this device, but is likely to be similar to the differences observed in MBW measurements using sulfur hexafluoride (SF_6) as the tracer gas [7].

The impact of the crosstalk correction was examined following reanalysis of data from clinical observational and interventional trials in children with cystic fibrosis [8]. Reassuringly, despite small changes in coefficients, the correction did not change observed treatment effects or interpretation of longitudinal tracking data. The within- and between-subject variability in MBW outcomes was reduced with the correction, which means we may have greater precision to distinguish between groups. The reduced number of breaths needed to complete the washout using the updated software also suggests that MBW test duration will be shorter and potentially more feasible in young children and in clinical practice [3]. These data are encouraging for the interpretation of previously published data using this device and use of MBW as a surveillance method in the future.

The sensor error in the Eco Medics device may partially explain differences in outcomes reported between MBW systems [7]. One study has reported improved agreement following correction between N_2MBW and SF_6MBW outcomes in healthy infants and toddlers [6]. However, additional methodological differences are likely to remain between N_2MBW devices (sensor types, signal processing, software algorithms) and systems using different tracer gases (gas distributions of resident *versus* exogenous gas, tissue nitrogen diffusion, impact of tracer gases on breathing pattern).

The sensor error correction may influence the comparability of FRC values between the MBW technique and other lung volume methods, such as lung plethysmography. Collation of data from multiple centres, methods and devices will help to improve our understanding of the remaining differences between systems.

In summary, we report updated normative data in school-aged, white children from four international centres with extensive experience in MBW testing. Further work is needed to assess the impact of the crosstalk correction on MBW outcomes across a wider age range and to understand remaining differences in outcomes between devices.

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