ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

The effect of O_2 and CO_2 cross-sensitivity sensor error in the Eco Medics Exhalyzer D device on measures of conductive and acinar airway function

Jack Bozier, Edward Jeagal, Paul D. Robinson, G. Kim Prisk, David G. Chapman, Gregory G. King, Cindy Thamrin, Sandra Rutting

Please cite this article as: Bozier J, Jeagal E, Robinson PD, *et al*. The effect of O_2 and CO_2 cross-sensitivity sensor error in the Eco Medics Exhalyzer D device on measures of conductive and acinar airway function. *ERJ Open Res* 2022; in press (https://doi.org/10.1183/23120541.00614-2021).

The effect of O_2 and CO_2 cross-sensitivity sensor error in the Eco Medics Exhalyzer D device on measures of conductive and acinar airway function

Jack Bozier[1,2], Edward Jeagal[1,3], Paul D. Robinson[1,4], G. Kim Prisk[1,5], David G. Chapman[1-3], Gregory G. King[1,2,6], Cindy Thamrin[1,6] and Sandra Rutting[1,2]

[1] Woolcock Institute of Medical Research, University of Sydney, Glebe, NSW, Australia.

[2] Dept of Respiratory Medicine, Royal North Shore Hospital, St Leonards, NSW, Australia.

[3] School of Life Sciences, University of Technology Sydney, NSW, Australia

[4] Dept of Respiratory Medicine, The Children's Hospital at Westmead, Westmead, NSW, Australia.

[5] University of California, San Diego, California, USA.

[6] Faculty of Medicine and Health, The University of Sydney, NSW, Australia

jack.bozier@sydney.edu.au

edward.jeagal@sydney.edu.au

dr.pdrobinson@gmail.com

kprisk@health.ucsd.edu

david.chapman@woolcock.org.au

gregory.king@sydney.edu.au

cindy.thamrin@woolcock.org.au

sandra.rutting@sydney.edu.au

Corresponding author: Dr Sandra Rutting

Phone: 9463 2929

Address: Airway Physiology and Imaging Group,

Woolcock Institute of Medical Research,

431 Glebe Point Road, Glebe, NSW, 2037

Word count abstract: 250

Word count main text manuscript: 3267

Abstract

Introduction: Multiple-breath nitrogen washout (MBNW) test provides important clinical information in obstructive airways diseases. Recently, a significant cross-sensitivity error in the O_2 and CO_2 sensors of a widely-used commercial MBNW device (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) was detected, which leads to overestimation of N_2 concentrations. Significant errors in functional residual capacity (FRC) and lung clearance index (LCI) have been reported in infants and children. This study investigated the impact in adults, and on additional important indices reflecting conductive (Scond) and acinar (Sacin) ventilation heterogeneity, in health and disease.

Methods: Existing MBNW measurements of 27 healthy volunteers, 20 participants with asthma and 16 smokers were reanalysed using Spiroware software V3.3.1, which incorporates an error correction algorithm. Uncorrected and corrected indices were compared using paired t-tests and Bland-Altman plots.

Results: Correction of the sensor error significantly lowered FRC (mean difference 9%) and LCI (8%-10%) across all three groups. Scond was higher following correction (11%, 14%, and 36% in health, asthma and smokers, respectively) with significant proportional bias. Sacin was significantly lower following correction in the asthma and smoker groups, but the effect was small (2%-5%) and with no proportional bias.

Discussion: The O_2 and CO_2 cross-sensitivity sensor error significantly overestimated FRC and LCI in adults, consistent with data in infants and children. There was a high degree of underestimation of Scond but minimal impact on Sacin. The presence of significant proportional bias indicates that previous studies will require reanalysis to confirm previous findings and to allow comparability with future studies.

Key words: Multiple-breath nitrogen washout; ventilation heterogeneity; O_2 and CO_2 cross-sensitivity error; Exhalyzer D device; asthma.

Introduction

Multiple Breath Nitrogen (N_2) Washout (MBNW) test assesses ventilation heterogeneity, often increased in respiratory diseases such as asthma and COPD[1, 2]. The test involves measurement of the concentration of an inert tracer gas of interest (i.e. N_2) in expired breath, which is progressively washed out by inhalation of 100% oxygen over a series of tidal breaths. Analysis of the exhaled N_2 concentration versus exhaled volume of each breath allows calculation of a global measure of heterogeneity (lung clearance index - LCI), heterogeneity arising predominantly within the convection-dependent airways (Scond), heterogeneity arising in the more peripheral, diffusion-dependent acinar airways (Sacin), and functional residual capacity (FRC)[3].

MBNW has been extensively used as a research tool in various respiratory diseases, particularly in obstructive airway diseases. With the availability of commercially available devices and international guidelines, it has emerging utility in clinical care, especially in cystic fibrosis (CF). The LCI has shown to be a sensitive marker of early disease progression in children with CF and has also been included as a primary endpoint in several therapeutic trials[4, 5]. MBNW has yet to be a part of clinical management in other lung diseases, but studies have shown utility of Sacin and Scond in guiding up- vs down-titration of treatment[6, 7] and sensitivity to detect improvement in symptoms in response to treatment with high dose inhaled corticosteroid[8] or monoclonal antibody therapy in asthma[9]. These indices are also sensitive markers of small airway dysfunction and its reversibility in smokers with normal spirometry[10, 11].

Recently, the presence and impact of a critical sensor error in a commercial device used to perform MBNW (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) has been reported in infants and older children [12, 13]. This MBNW device relies on accurate measurements from O_2 and CO_2 sensors to calculate N_2 concentration indirectly. It was found that both sensors exhibit cross-sensitivities, i.e. the O_2 sensor estimation is dependent on CO_2 concentrations and vice versa, such that as the

washout progresses, O_2 and CO_2 concentrations are underestimated and N_2 concentrations increasingly overestimated, prolonging the washout. This has been shown to result in significant errors of up to 12 and 15-19% in the assessment of FRC and LCI, respectively[12-14]. A software update (v3.3.1) has now been released by the manufacturer with an implemented correction algorithm, which recalculates the N_2 concentration trace.

The magnitude of effect of this sensor error correction on these MBNW indices in adults is currently unknown, and to date there has been no description of the effects on additional important indices such as Scond and Sacin. This is essential to understand the validity of changes reported in previously published studies. Therefore, this study aimed to determine the effect of the CO_2 and O_2 sensor correction on MBNW parameters in both health and disease by examining three different adult cohorts: 1) healthy volunteers, 2) patients with asthma, and 3) long-term smokers. Secondly, we investigated whether correction of the sensor error affected the within- and between-session repeatability of MBNW parameters in health. Some of the data from the healthy and asthma participants have been previously published[15, 16].

Methods

Research participants

In this study we retrospectively reanalyzed MBNW measurements from healthy volunteers, participants with asthma and long-term smokers that were recruited from Royal North Shore Hospital and the Woolcock Institute of Medical Research. Healthy participants were current non-smokers with a smoking history of <10 pack-years and no respiratory disease. Patients with asthma had a physician-diagnosis of asthma and were current non-smokers with a smoking history of <10 pack-years. Long-term smokers were current smokers with at least 10 pack-year smoking exposure; these data were collected as part of a larger clinical trial (Australian Clinical Trials Registration Number (ACTRN): 12616001208493) in smokers with normal post-bronchodilator (BD) spirometry or GOLD Stage 1 (post-BD FEV1/FVC <0.7 but FEV1>80% predicted), with the additional inclusion criteria of abnormal Scond and/or Sacin as assessed by z-score <-1.64 using published predicted equations[11]. The original studies were approved by the local Human Research Ethics Committee (Northern Sydney Local Health District, LNR/16/HAWKE/11 and HREC/15/HAWKE/489).

Standard pulmonary function testing

After obtaining written informed consent, all participants underwent conventional lung function including spirometry, plethysmography and diffusing capacity for carbon monoxide (DLCO). These were performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria. All parameters were expressed as percent predicted using published predicted equations[17, 18].

MBNW testing

In the original studies, after a period of at least 10 min of rest, the healthy and asthmatic participants underwent MBNW testing by two commonly used breathing protocols: controlled and free-breathing protocols, in randomised order (assigned by a computer-based random number

generator); the group of smokers performed MBNW using the controlled breathing protocol only. A subset of healthy participants returned for testing within 3 months of their first visit, in which all measurements were repeated in the same order. Both controlled and free-breathing protocols were included as several published studies showed that indices of conductive and acinar ventilation heterogeneity were not comparable between breathing protocols[15, 16, 19].

MBNW was performed using the Exhalyzer D with Spiroware version 3.1.6 (Ecomedics, Duernten, Switzerland). Both the controlled breathing and free-breathing protocols were performed according to ERS consensus and are previously described in detail[15, 20]. In brief, after establishing a stable breathing pattern and end-expiratory lung volume (EELV), nitrogen washout during 100% O_2 inhalation was commenced. The controlled breathing protocol required participants to breathe at a RR between 8-12 breaths.min^{-1} and tidal volume (V_T) between 0.95-1.3 L following visual feedback until the N_2 concentration decreased to 1/40th of the starting end expiratory N_2 concentration. In the free breathing protocol, participants were encouraged to adopt relaxed tidal breathing but advised to adjust tidal volumes if insufficient expired N_2 phase III slope was observed; calculated S_{cond} and S_{acin} were adjusted for V_T, as per consensus guidelines[20]. At least 3 technically acceptable trials with FRC values <10% of the mean were obtained for each breathing protocol.

MBNW analysis

The effect of the sensor error was assessed by comparing the parameters of standard (uncorrected) analysis in Spiroware software version 3.1.6 with corrected parameters reanalyzed in new Spiroware software version 3.3.1, applying the sensor error correction algorithm. The correction algorithm has been described extensively before in Sandvik *et al.* [13] and Wyler *et al.* [12]. Briefly, the algorithm was derived using Exhalyzer D sensors and mass spectrometer to measure the O_2 and CO_2 concentrations of wide range of well-defined technical gas mixtures under various conditions, and used a polynomial function to correct for the errors observed. System settings, delay correction and

quality control remained unaltered (i.e., selection of breaths and any correction made to phase III slopes were consistent between both versions).

Statistical analysis

Statistical analysis was carried out with GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA). All data are expressed as mean ± SD, unless otherwise stated. Differences between uncorrected and corrected parameters were examined using paired Student's t-tests and Pearson's correlation. To investigate bias, we generated Bland-Altman plots as the difference (corrected minus uncorrected) versus the average, plotting the mean difference and 95% limits of agreement (95% limits of agreement, LA). We performed linear regression of the difference versus average to determine any proportional bias.

To make clear the consequence of the correction of the sensor error on prior studies, we present these results as the change in the outcome parameters of existing studies that result from this correction, i.e. with the uncorrected parameters as reference. For example, the sensor error results in expired N_2 being erroneously high towards the end of the washout. This in turn causes an overestimation in FRC. Our results are presented in the context of how FRC is altered when the sensor error is corrected, in this case a reduction in calculated FRC.

Within-session variability was expressed as the coefficient of variation (CoV) calculated as the ratio of the SD to the mean from three separate trials. To determine between-session variability, we calculated the difference (visit 2 minus visit 1) and 95% LoA separately for corrected and uncorrected parameters separately. We also report the between-session intra-class correlation coefficients (ICC), calculated using a two-way mixed effects ANOVA model based on absolute agreement, multiple measurements (k=3). A *p*-value smaller than 0.05 was considered statistically significant.

Results

Patient demographics

We reanalysed MBNW measurements from 27 healthy volunteers, 20 asthmatic patients and 16 long-term smokers. The patients' demographics and lung function are summarised in Table 1. The healthy volunteers were slightly younger than the asthmatic patients and smokers. The group of smokers had a mean±SD smoking history of 19.3 ± 8.6 pack years. Both plethysmography and MBNW-derived FRC were comparable across the groups, whereas MBNW indices of heterogeneity were significantly higher in the asthma and smoker groups compared to health, and higher in the smokers compared to asthma (in terms of Scond and Sacin).

Effects of sensor correction on MBNW parameters

Correction of CO_2 and O_2 sensor error had a significant effect on all MBNW parameters measured by the controlled breathing protocol (Table 2). Following correction, mean (95% CI) FRC and LCI decreased by 7.8 (7.0-8.4) % and 9.8 (8.8-10.8) %, respectively in health. Similar decreases in FRC and LCI were observed in asthma and long-term smokers. While uncorrected FRC measured by MBNW were comparable to FRC measured by body plethysmography, corrected FRC values were significantly lower compared to FRCpleth in all three groups (mean±SD differences of -0.26±0.47L (p=0.008), -0.26±0.37 (p=0.006), and -0.64±0.71 (p=0.003) in health, asthma, and smokers, respectively).

Notably, mean (95% CI) Scond significantly increased by 11.1 (-1.4-23.5) %, 14.0 (4.2-23.9) %, and 36 (19.8-52.2) % following sensor correction in health, asthma, and smokers, respectively. In contrast, Sacin was significantly lower following sensor correction in the asthma and smokers groups, with a trend to significance in the healthy group (p=0.07). The impact on Sacin, however was minimal with mean decreases (95% CI) of 1.8 (0.44-4.0), 2.9 (0.9-4.9), 4.8 (0.7-8.9) % observed in health, asthma,

and smokers, respectively. When using the free breathing protocol, similar effects for LCI, FRC, Scond and Sacin were observed in health and asthma (Online Supplement, Table S1).

There were strong correlations between all corrected and uncorrected MBNW values across the three groups (all r-values >0.85) (Figures 1-3, panels A-D) and for both breathing protocols (Figures S1 and S2). Bland-Altman plots showed that the effect of sensor correction on LCI and FRC demonstrated strong proportional bias in all three groups (greater difference with higher mean value) (Figures 1-3, panels E-H). The Bland-Altman plots also revealed large variance in Scond and significant proportional bias in health and smokers, but not in asthma. Less variance in differences was seen in Sacin and there was no evidence of proportional bias in all three groups.

Effects on within- and between session repeatability in health

Fifteen healthy volunteers underwent repeat testing. Within-session and between-session variability measurements are presented in table 3. There were no differences observed in within-session CoVs between corrected and uncorrected FRC (p=0.46) or LCI (p=0.84). Between-session variability was minimally affected by the sensor error. Corrected FRC and LCI showed narrower 95% LoAs, whereas Sacin and Scond showed slightly wider 95% LoAs. Between-session ICC values were numerically comparable between corrected and uncorrected values. Similar impact on within- and between session repeatability was observed with the free breathing protocol (Supplementary Table 2).

Discussion

In this study, we demonstrate that correction of the O_2 and CO_2 sensor error in the Exhalyzer D system results in significantly lower FRC and LCI, and higher Scond values in three different adult patient groups. The impact on Sacin, though statistically significant, was minimal. There were strong correlations between the corrected and uncorrected values for all MBNW parameters in all three groups. Importantly, the effect of the correction showed a significant proportional bias in FRC and LCI in all three groups, and significant proportional bias in Scond was also evident in health and smokers though not in asthma. The O_2 and CO_2 sensor error correction produced less variance in Sacin compared to other parameters and there was no evidence of proportional bias. Furthermore, sensor error correction had minimal impact on within-session and between-session variability, with a smaller 95% LoA for LCI between sessions.

Overestimation of FRC and LCI by the Exhalyzer system was first suggested when comparing the use of sulfur hexafluoride (SF_6) to N_2 as a tracer gases. Jensen *et al.* found in children with CF that N_2 resulted in larger estimates of FRC and LCI compared to SF_6 obtained using mass spectrometry[21]. In addition to differences in the diffusion front, the assumption was that back-secretion of N_2 from the tissues likely contributed to overestimation of FRC by MBNW. In fact, subsequent device comparison studies in adults tended to show FRC by the Exhalyzer D system to be larger than FRC_{pleth}[22, 23]. However, these findings are at odds with the idea that gas dilution techniques during tidal breathing can only access communicating lung units and not trapped gas compartments, such that the estimated FRC in disease should be lower than FRC obtained from plethysmography, which includes all compressible gas volume within the lungs. Prior to reanalysis, there were no differences between FRC_{Pleth} and FRC_{MBNW} in smokers, patients with asthma or in health but sensor error correction resulted in a significantly lower FRC_{mbnw} compared to FRC_{pleth} in all groups, more consistent with expectation. These results suggest that the sensor error explains most of the overestimation of FRC seen in the Exhalyzer device, just as Sandvik and colleagues found that sensor

error correction of MBNW removed the discrepancy in FRC between N_2 and SF_6[13]. It is unknown whether the error affects different commercially available MBW utilising O_2 and/or CO_2 sensors, a subject which warrants further investigation.

Our study is the first to demonstrate the impact of the O_2 and CO_2 sensor error correction on FRC and LCI in adults, and the first to investigate the impact on Scond and Sacin. The effect of the sensor error on LCI and FRC has been described previously in infants and children, and our data is consistent with their findings in both magnitude and presence of proportional bias [12, 13]. The alignment of these findings is important to understand consistency in the correction algorithm. The high correlations between uncorrected and corrected values suggest that previous findings involving correlations with MBNW indices may be preserved, but the presence of significant proportional bias indicates that previous studies examining interventional effects will require reanalysis, both to reconfirm previous findings and to allow comparability with future studies. Though a recent reanalysis of CF clinical trials was reassuring to a degree and showed that while treatment effects were reduced they were maintained following sensor correction[14].

Previous studies investigating the effect of sensor error correction were in infants and children[12-14] hence they did not include a comparison of phase III slope indices Scond and Sacin, which are not as commonly used in paediatric compared to adult age groups. Scond is calculated as the slope of the plot of normalised phase III slope (SnIII) versus lung turnover (TO), between TO 1.5-6, where SnIII is the slope of phase III in the N_2 expirogram normalised by mean or end-tidal N_2 concentration. Errors in Scond arise from two sources. First, the observed overestimation of FRC result in a lower TO, shortening the SnIII vs TO plot leftward and slightly elevating Scond. Second, as the washout progresses towards higher values of TO, the phase III slope is normalised by an increasingly overestimated N_2 concentration. The effect is a less steep SnIII vs TO plot, thus lowering calculated Scond. These effects are demonstrated in Figure 4, where corrected SnIII values for 3 different

patients are increased resulting in larger Scond as calculated between TO 1.5-6. In particular, the dominant effect of the effect on SnIII is clearly seen in panel 4C where uncorrected SnIII values deviate markedly from the corrected values at high TO. However, the change in SnIII in the first breath was minimal, both because the sensor error is smallest at high N_2 concentrations, and because the N_2 concentration used form normalization is large at this point in the washout. Much of the effect of sensor correction on Sacin likely comes from propagation of the Scond error into the correction applied to SnIII(1) to obtain Sacin[20].

Our comparison found Scond to be significantly increased by the sensor error correction, and furthermore with a significant proportional bias in both health and in smokers. However this distinction between groups is likely a manifestation of small numbers in each cohort, coupled with the inherent variability in the measurement of Scond. Indeed, when the three cohorts are combined into the single data set (Figure S3), it is clear that the sensor effort correction results in comparable effects on Scond regardless of the underlying pathophysiology.

Correction of the sensor error resulted in minimal impact on within-session and between-session variability in health. Within-session CoV remained small in FRC and LCI demonstrating that trial repeatability for MBNW was high even post reanalysis. Similarly, all parameters had minimal change in between-session difference, with a small change in the LoA for LCI which is likely attributed to the overall reduction in LCI caused by correction. Furthermore, we also reanalysed previously published data collected using both free breathing and controlled breathing[15, 16]. Sensor error correction did not affect the between-protocol differences in Scond and Sacin in health[15] or asthma[16], nor their dependences on the breathing pattern.

This study is limited by the selection criteria for the previous studies that we have included for reanalysis. Patients with asthma had relatively mild disease, and smokers were recruited for a larger

study based on having abnormal ventilation heterogeneity as described in the methods, and thus may not be representative of the population in general. Future reanalysis of MBNW data is required to understand the effect of sensor error correction in disease more broadly and the associated implications. Moreover, in our reanalysis, we chose to retain the same breath exclusions and other settings in the original analysis, to allow us to solely examine the effect of corrected N_2 concentrations on MBNW indices. There is a chance that the adjusted washout traces may result in e.g. changes in the shape of the expirogram, which may result in different quality control decisions by a manual operator. However, we attempted to maintain a consistent approach for quality control. The new software version also includes changes in the way in which delay between flow and gas concentration sampling is calculated, to include a dynamic delay correction[24], which was not implemented in our reanalysis, but which may be a factor affecting comparability between old and new studies in the literature involving the Exhalyzer D. This was intentionally done to focus on the effects of the cross-talk sensor error correction.

In conclusion, our study is the first to describe the effect of O_2 and CO_2 sensor error correction on the Exhalyzer D MBNW system in adults, and the first to investigate the effect on Scond and Sacin. Our results confirm the LCI and FRC effects seen in infants and children and demonstrate strong underestimation with proportional bias for Scond, with errors up to 50% observed in those with the greatest ventilation heterogeneity, but minimal effects on Sacin. While the discovery of the error is an important step towards improved accuracy of MBW devices, it also represents an important hurdle for ongoing efforts to support MBNW as a clinical tool or an endpoint for clinical studies. These findings provide important considerations for the interpretation of previously published adult MBNW studies, and those in younger age groups incorporating phase III slope analysis. The magnitude of effect supports reanalysis of that data to better understand the true findings.

	Health	Asthma	Smokers
Participants (n)	27	20	16
Males/females (n)	16/11	4/16	12/4
Age (years)	34 (19-65)	43 (24-78)[a]	43 (27-54)[b]
BMI (kg/m^2)	24.6 ± 3.4	25.5 ± 4.3	27.4 ± 6.4
Smoking history (pack years)	-	-	19.3 ± 8.6
Lung function			
FEV_1 (% predicted)	105 ± 14	89.2 ± 19[a]	98.8 ± 11[c]
FVC (% predicted)	105 ± 15	97.7 ± 20	105.5 ± 11
FEV_1/FVC (%)	83 ± 6	74 ± 8.4[a]	76 ± 6[b]
TLC (% predicted)	101 ± 23	103 ± 18	108 ± 11
FRC_{pleth} (% predicted)	97 ± 27	97 ± 17	109.1 ± 19
DLCO (% predicted)	102 ± 13	99 ± 15	90 ± 12[b]
FRC_{MBNW} (L)*	2.94 ± 0.89	2.62 ± 0.72	2.93 ± 0.84
LCI (TO)*	6.49 ± 0.47	7.23 ± 1.04[a]	7.05 ± 0.82[b]
Scond (L^{-1})*	0.019 ± 0.011	0.033 ± 0.018[a]	0.038 ± 0.019[c]
Sacin (L^{-1})*	0.056 ± 0.020	0.086 ± 0.049	0.095 ± 0.037[b,c]

Table 1 Participant demographics and lung function

Data are presented as mean±SD unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; FRC: functional residual capacity; DLCO: diffusing capacity of carbon monoxide. * Corrected, controlled breathing protocol values used. *a* = $p<0.05$ health vs asthma, *b* = $p<0.05$ health vs smokers, *c* = $p<0.05$ asthma vs smokers.

Table 2 Effects of sensor correction on main MBNW parameters

Parameter	Standard	Corrected	Mean difference (95% CI) absolute	Mean difference (95% CI) relative	*p*-value
Health					
FRC (L)	3.19±0.98	2.94±0.89	-0.25 (-0.29--0.21)	-7.7 (-8.4--7.0)	<0.0001
LCI (TO)	7.20±0.58	6.49±0.47	-0.71 (-0.80--0.63)	-9.8 (-10.8--8.8)	<0.0001
Scond L^{-1}	0.017±0.009	0.019±0.011	0.002 (0.0003-0.004)	11.1 (-1.4-23.5)	0.03
Sacin L^{-1}	0.057±0.020	0.056±0.020	-0.0009 (-0.002-0.0001)	-1.8 (-4.0-0.44)	0.08
Asthma					
FRC (L)	2.84±0.76	2.62±0.72	-0.22 (-0.26--0.18)	-8.0 (-9.2--6.8)	<0.0001
LCI (TO)	7.94±1.19	7.23±1.04	-0.71 (-0.84--0.58)	-8.8 (-10.0--7.6)	<0.0001
Scond L^{-1}	0.029±0.016	0.033±0.018	0.004 (0.001-0.006)	14.0 (4.2-23.9)	0.003
Sacin L^{-1}	0.088±0.049	0.086±0.049	-0.002 (-0.003--0.0008)	-2.9 (-4.9--0.9)	0.003
Smokers					
FRC (L)	3.19±0.91	2.93±0.84	-0.28 (-0.34--0.22)	-8.4 (-10.2--6.7)	<0.0001
LCI (TO)	7.69±0.96	7.05±0.82	-0.65 (-0.78--0.51)	-8.3 (-9.7--6.8)	<0.0001
Scond L^{-1}	0.029±0.015	0.038±0.019	0.009 (0.005-0.013)	36.0 (19.8-52.2)	0.0004
Sacin L^{-1}	0.10±0.040	0.095±0.037	-0.004 (-0.007—0.001)	-4.8 (-8.9--0.71)	0.008

Effects of sensor correction on main MBNW parameters. Data are presented as mean±SD unless otherwise stated. FRC; functional residual capacity; LCI: lung clearance index; Scond: conducting airways ventilation heterogeneity; Sacin: distal/intra-acinar airways ventilation heterogeneity. 95% CI: 95% confidence interval. The standard (uncorrected) value is the reference. Absolute difference is calculated as corrected-standard. Relative difference is calculated as corrected-standard/standard * 100.

Table 3. Effects on within- and between-session repeatability in health

	Within-session CoV	Between-session difference	Between-session 95% LOA	Between-session ICC
Uncorrected				
FRC (L)	3.3±2.9%	-0.03±0.52	-1.04-0.98	0.931
LCI (TO)	2.5±2.4%	0.06±0.47	-0.86-0.98	0.812
Scond (L^{-1})	-	-0.001±0.011	-0.017-0.015	0.836
Sacin (L^{-1})	-	-0.003±0.018	-0.037-0.031	0.835
Corrected				
FRC (L)	3.0±1.9%	-0.02±0.47	-0.94-0.91	0.927
LCI (TO)	2.4±1.7%	0.13±0.34	-0.53-0.79	0.849
Scond (L^{-1})	-	-0.003±0.009	-0.021-0.015	0.867
Sacin (L^{-1})	-	-0.002±0.02	-0.039-0.034	0.828

Data are presented as mean±SD unless otherwise stated. Mean differences are visit 2 minus visit 1. CoV: coefficient of variation; 95% LOA: 95% limits of agreement; ICC: intra-class correlation coefficient; FRC: functional residual capacity; LCI: lung clearance index; Scond: conducting airways ventilation heterogeneity; Sacin: distal/intra-acinar airways ventilation heterogeneity.

Figure legends

Figure 1. Comparison of standard (uncorrected) and corrected MBNW parameters in health. There were strong correlations between uncorrected and corrected functional residual capacity (FRC) (r=0.99 and p<0.0001) (**A**), lung clearance index (LCI) (r=0.94 and p<0.0001) (**B**), Scond (r=0.86, p<0.0001) (**C**) and Sacin (r=0.98, p<0.0001) (**D**). Bland-Altman plots shows that sensor correction results in a lower FRC (mean difference (95% limits of agreement) (-0.25 (-0.46, -0.04), p<0.0001)) (**E**) lower LCI (-0.71 (-1.14--0.28), p<0.0001) (**F**), higher Scond (0.002 (-0.007-0.012), p=0.027) (**G**), but no change in Sacin (-0.0009 (-0.006-0.004), p=0.08) (**H**). There also was significant proportional bias confirmed by linear regression for FRC (p<0.0001), LCI (p<0.0001), and Scond (p=0.0009).

Figure 2. Comparison of standard (uncorrected) and corrected MBNW parameters in asthma. There were strong correlations between uncorrected and corrected functional residual capacity (FRC) (r=0.99 and p<0.0001) (**A**), lung clearance index (LCI) (r=0.98 and p<0.0001) (**B**), Scond (r=0.94, p<0.0001) (**C**) and Sacin (r=0.98, p<0.0001) (**D**). Bland-Altman plots shows that sensor correction results in a lower FRC (mean difference (95% limits of agreement) (-0.22 (-0.38--0.06), p<0.0001)) (**E**) lower LCI (-0.71 (-1.24--0.18), p<0.0001) (**F**), higher Scond (0.004 (-0.006-0.014), p=0.003) (**G**) and lower Sacin (-0.002 (-0.007-0.003), p=0.003) (**H**). There also was significant proportional bias confirmed by linear regression for FRC (p=0.02) and LCI (p=0.01).

Figure 3. Comparison of standard (uncorrected) and corrected MBNW parameters in smokers. There were strong correlations between uncorrected and corrected functional residual capacity (FRC) (r=0.99 and p<0.0001) (**A**), lung clearance index (LCI) (r=0.97 and p<0.0001) (**B**), Scond (r=0.93, p<0.0001) (**C**) and Sacin (r=0.99, p<0.0001) (**D**). Bland-Altman plots shows that sensor correction results in a lower FRC (mean difference (95% limits of agreement) (-0.28 (-0.50--0.06), p<0.0001)) (**E**) lower LCI (-0.65 (-1.16--0.13), p<0.0001) (**F**), higher Scond (0.009 (-0.006-0.025), p=0.0004) (**G**) and lower Sacin (-0.004 (-0.015-0.006), p=0.008) (**H**). There also was significant proportional bias

confirmed by linear regression for FRC (p=0.04), LCI (p=0.03) and Scond (p=0.01). The uncorrected value is the reference. Absolute differences were calculated as corrected-uncorrected.

Figure 4. Normalised phase III slope (SNIII) vs lung turnovers (TO). Normalised phase III slope vs lung turnover (TO) graphs in health (**A**), asthma **(B**) and smokers **(C**). Open squares represent uncorrected values and solid squares corrected (with sensor correction) squares. Scond is calculated as the slope of the plot of SnIII versus lung TO, between TO 1.5-6. Corrected SnIII values are increased resulting in larger Scond as calculated between TO 1.5-6 in all three patient groups. The change in SnIII in the first breath was minimal, explaining the minimal effects seen in Sacin post correction.

Funding

SR was supported by the Berg Family Foundation. The smokers dataset was from a larger study funded by an investigator-initiated grant from GlaxoSmithKline, Australia.

Data sharing statement

The study protocol and raw data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

We would like to acknowledge the study participants for volunteering the time and effort required to conduct this study. We would also like to thank Blake Handley and Stephen Milne for assistance with the healthy and asthma datasets, and Prof. Christine Jenkins (ECOS Study) for the provision of the smokers data subset used for this study.

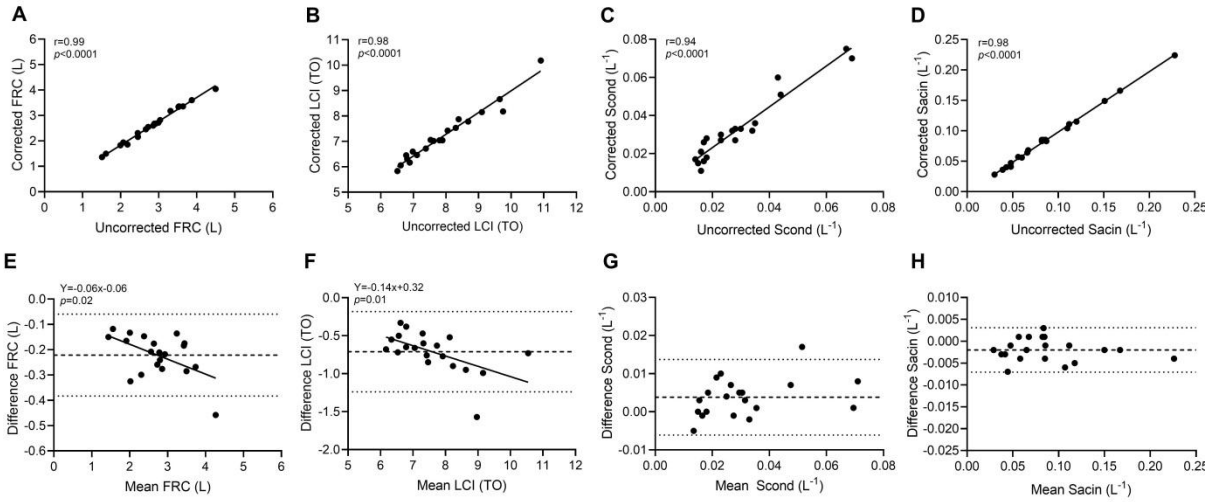
Disclosures

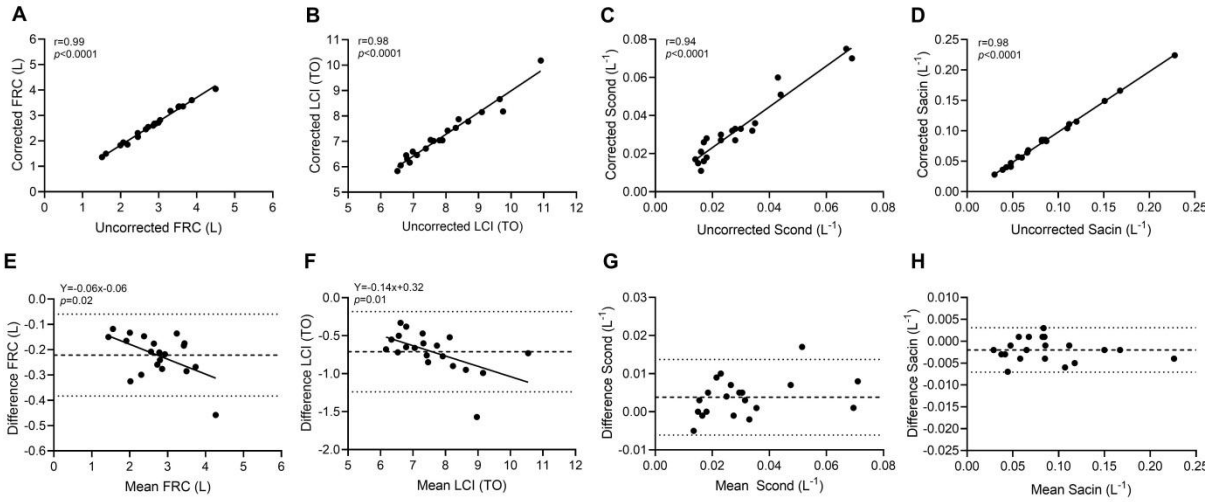
No conflicts of interest, financial or otherwise, relating to this study are declared by the authors.

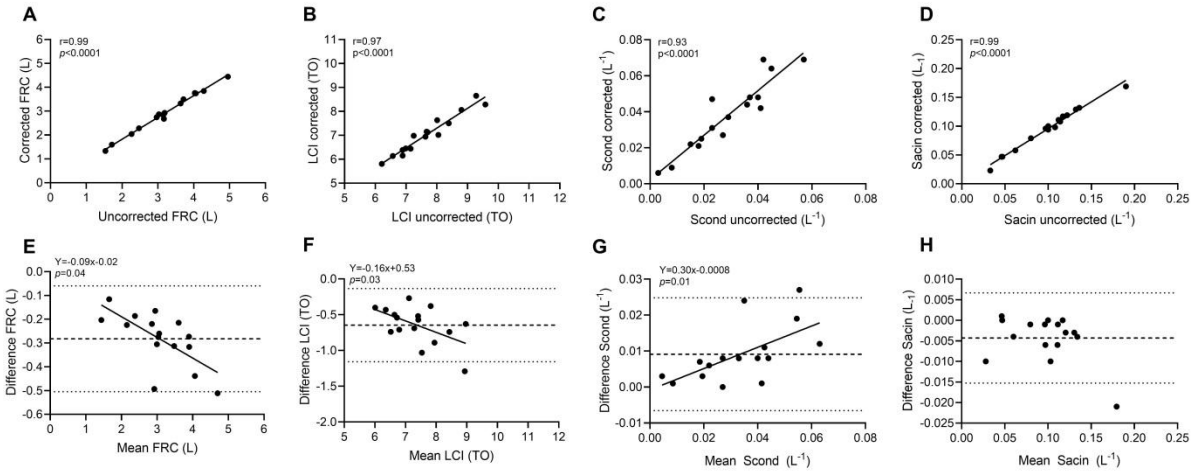
References

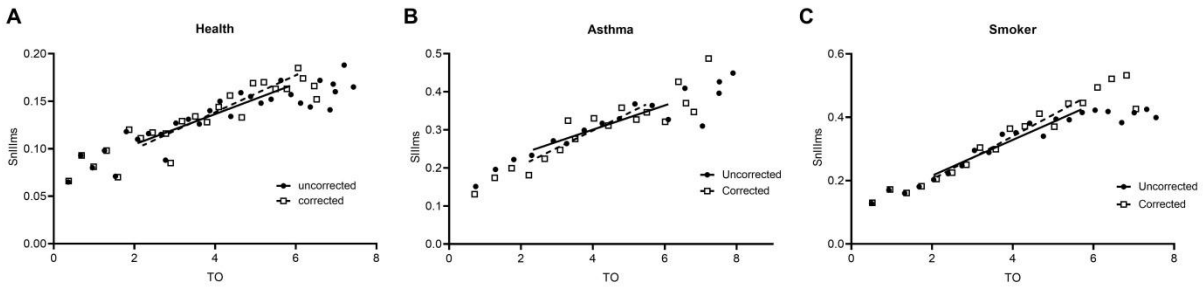
1. Verbanck S, Schuermans D, Vincken W. Inflammation and airway function in the lung periphery of patients with stable asthma. *The Journal of allergy and clinical immunology* 2010: 125(3): 611-616.
2. Verbanck S, Schuermans D, Van Muylem A, Melot C, Noppen M, Vincken W, Paiva M. Conductive and acinar lung-zone contributions to ventilation inhomogeneity in COPD. *American journal of respiratory and critical care medicine* 1998: 157(5 Pt 1): 1573-1577.
3. Zimmermann SC, Tonga KO, Thamrin C. Dismantling airway disease with the use of new pulmonary function indices. *European respiratory review : an official journal of the European Respiratory Society* 2019: 28(151).
4. Ratjen F, Davis SD, Stanojevic S, Kronmal RA, Hinckley Stukovsky KD, Jorgensen N, Rosenfeld M. Inhaled hypertonic saline in preschool children with cystic fibrosis (SHIP): a multicentre, randomised, double-blind, placebo-controlled trial. *The Lancet Respiratory medicine* 2019: 7(9): 802-809.
5. Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, Milla CE, Robinson PD, Waltz D, Davies JC. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *The Lancet Respiratory medicine* 2017: 5(7): 557-567.
6. Farah CS, King GG, Brown NJ, Peters MJ, Berend N, Salome CM. Ventilation heterogeneity predicts asthma control in adults following inhaled corticosteroid dose titration. *The Journal of allergy and clinical immunology* 2012: 130(1): 61-68.
7. Farah CS, King GG, Brown NJ, Downie SR, Kermode JA, Hardaker KM, Peters MJ, Berend N, Salome CM. The role of the small airways in the clinical expression of asthma in adults. *The Journal of allergy and clinical immunology* 2012: 129(2): 381-387, 387.e381.
8. Tang FSM, Rutting S, Farrow CE, Tonga KO, Watts J, Dame-Carrol JR, Bertolin A, King GG, Thamrin C, Chapman DG. Ventilation heterogeneity and oscillometry predict asthma control improvement following step-up inhaled therapy in uncontrolled asthma. *Respirology (Carlton, Vic)* 2020: 25(8): 827-835.
9. Farah CS, Badal T, Reed N, Rogers PG, King GG, Thamrin C, Peters MJ, Seccombe LM. Mepolizumab improves small airway function in severe eosinophilic asthma. *Respiratory medicine* 2019: 148: 49-53.
10. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, Salome CM, King GG. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. *Respirology (Carlton, Vic)* 2018: 23(5): 512-518.
11. Jetmalani K, Chapman DG, Thamrin C, Farah CS, Berend N, Salome CM, King GG. Bronchodilator responsiveness of peripheral airways in smokers with normal spirometry. *Respirology (Carlton, Vic)* 2016: 21(7): 1270-1276.
12. Wyler F, Oestreich MH, Frauchiger BS, Ramsey KA, Latzin PT. Correction of sensor crosstalk error in Exhalyzer D multiple-breath washout device significantly impacts outcomes in children with cystic fibrosis. *Journal of applied physiology (Bethesda, Md : 1985)* 2021.
13. Sandvik RM, Gustafsson PM, Lindblad A, Robinson PD, Nielsen KG. Improved agreement between N(2) and SF(6) multiple-breath washout in healthy infants and toddlers with improved EXHALYZER D sensor performance. *Journal of applied physiology (Bethesda, Md : 1985)* 2021: 131(1): 107-118.
14. Robinson PD, Jensen R, Seeto RA, Stanojevic S, Saunders C, Short C, Davies JC, Ratjen F. Impact of cross-sensitivity error correction on representative nitrogen-based multiple breath washout data from clinical trials. *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society* 2021.

15. Handley BM, Jeagal E, Schoeffel RE, Badal T, Chapman DG, Farrow CE, King GG, Robinson PD, Milne S, Thamrin C. Controlled versus free breathing for multiple breath nitrogen washout in healthy adults. *ERJ open research* 2021: 7(1).
16. Handley BM, Bozier J, Jeagal E, Rutting S, Schoeffel RE, Robinson PD, King GG, Milne S, Thamrin C. Controlled versus free breathing for multiple breath nitrogen washout in asthma. *ERJ open research* 2021: 00487-02021.
17. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012: 40(6): 1324-1343.
18. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. Lung volumes and forced ventilatory flows. *European Respiratory Journal* 1993: 6(Suppl 16): 5-40.
19. Verbanck S, Schuermans D, Paiva M, Robinson PD, Vanderhelst E. Mitigating increased variability of multiple breath washout indices due to tidal breathing. *The European respiratory journal* 2021: 57(2).
20. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *The European respiratory journal* 2013: 41(3): 507-522.
21. Jensen R, Stanojevic S, Gibney K, Salazar JG, Gustafsson P, Subbarao P, Ratjen F. Multiple breath nitrogen washout: a feasible alternative to mass spectrometry. *PloS one* 2013: 8(2): e56868.
22. Poncin W, Singer F, Aubriot AS, Lebecque P. Agreement between multiple-breath nitrogen washout systems in children and adults. *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society* 2017: 16(2): 258-266.
23. Tonga KO, Robinson PD, Farah CS, King GG, Thamrin C. In vitro and in vivo functional residual capacity comparisons between multiple-breath nitrogen washout devices. *ERJ open research* 2017: 3(4).
24. Gustafsson PM, Bengtsson L, Lindblad A, Robinson PD. The effect of inert gas choice on multiple breath washout in healthy infants: differences in lung function outcomes and breathing pattern. *Journal of applied physiology (Bethesda, Md : 1985)* 2017: 123(6): 1545-1554.


A
r=0.99
p<0.0001
Corrected FRC (L)
Uncorrected FRC (L)
B
r=0.98
p<0.0001
Corrected LCI (TO)
Uncorrected LCI (TO)
C
r=0.94
p<0.0001
Corrected Scond (L-1)
Uncorrected Scond (L-1)
D
r=0.98
p<0.0001
Corrected Sacin (L-1)
Uncorrected Sacin (L-1)
E
Y=-0.06x-0.06
p=0.02
Difference FRC (L)
Mean FRC (L)
F
Y=-0.14x+0.32
p=0.01
Difference LCI (TO)
Mean LCI (TO)
G
Difference Scond (L-1)
Mean Scond (L-1)
H
Difference Sacin (L-1)
Mean Sacin (L-1)


A
r=0.99
p<0.0001
Corrected FRC (L)
Uncorrected FRC (L)
B
r=0.98
p<0.0001
Corrected LCI (TO)
Uncorrected LCI (TO)
C
r=0.94
p<0.0001
Corrected Scond (L-1)
Uncorrected Scond (L-1)
D
r=0.98
p<0.0001
Corrected Sacin (L-1)
Uncorrected Sacin (L-1)
E
Y=-0.06x-0.06
p=0.02
Difference FRC (L)
Mean FRC (L)
F
Y=-0.14x+0.32
p=0.01
Difference LCI (TO)
Mean LCI (TO)
G
Difference Scond (L-1)
Mean Scond (L-1)
H
Difference Sacin (L-1)
Mean Sacin (L-1)


A
r=0.99
p<0.0001
Corrected FRC (L)
Uncorrected FRC (L)
B
r=0.97
p<0.0001
LCI corrected (TO)
LCI uncorrected (TO)
C
r=0.93
p<0.0001
Scond corrected (L-1)
Scond uncorrected (L-1)
D
r=0.99
p<0.0001
Sacin corrected (L-1)
Sacin uncorrected (L-1)
E
Y=-0.09x-0.02
p=0.04
Difference FRC (L)
Mean FRC (L)
F
Y=-0.16x+0.53
p=0.03
Difference LCI (TO)
Mean LCI (TO)
G
Y=0.30x-0.0008
p=0.01
Difference Scond (L-1)
Mean Scond (L-1)
H
Difference Sacin (L-1)
Mean Sacin (L-1)


A
Health
SnIIms
TO
uncorrected
corrected
B
Asthma
SIIms
TO
Uncorrected
Corrected
C
Smoker
SnIIms
TO
Uncorrected
Corrected

Online Supplement

The effect of O_2 and CO_2 cross-sensitivity sensor error in the Eco Medics Exhalyzer D device on measures of conductive and acinar airway function

Jack Bozier[1,2], Edward Jeagal[1,3], Paul D. Robinson[1,4], G. Kim Prisk[1,5], David G. Chapman[1-3], Gregory G. King[1,2,6], Cindy Thamrin[1,6] and Sandra Rutting[1,2]

[1] Woolcock Institute of Medical Research, University of Sydney, Glebe, NSW, Australia.

[2] Dept of Respiratory Medicine, Royal North Shore Hospital, St Leonards, NSW, Australia.

[3] School of Life Sciences, University of Technology Sydney, NSW, Australia

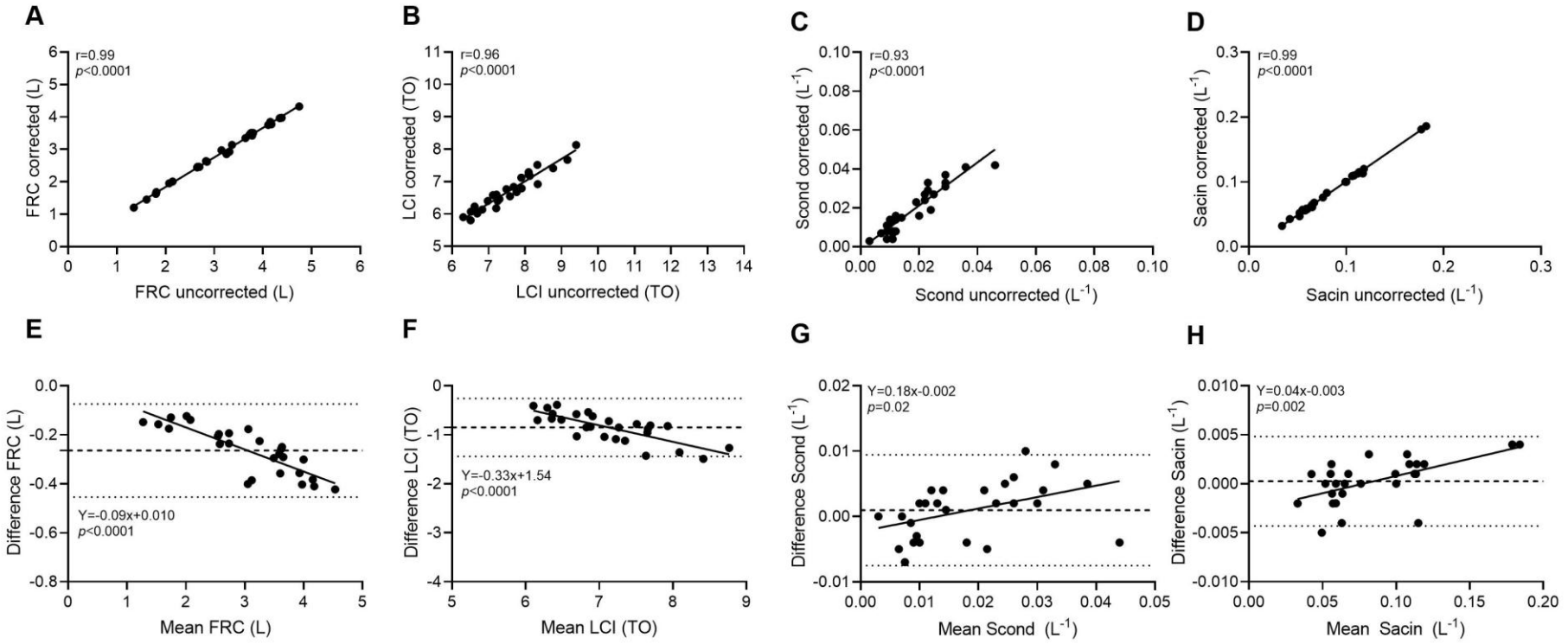
[4] Dept of Respiratory Medicine, The Children's Hospital at Westmead, Westmead, NSW, Australia.

[5] University of California, San Diego, California, USA.

[6] Faculty of Medicine and Health, The University of Sydney, NSW, Australia

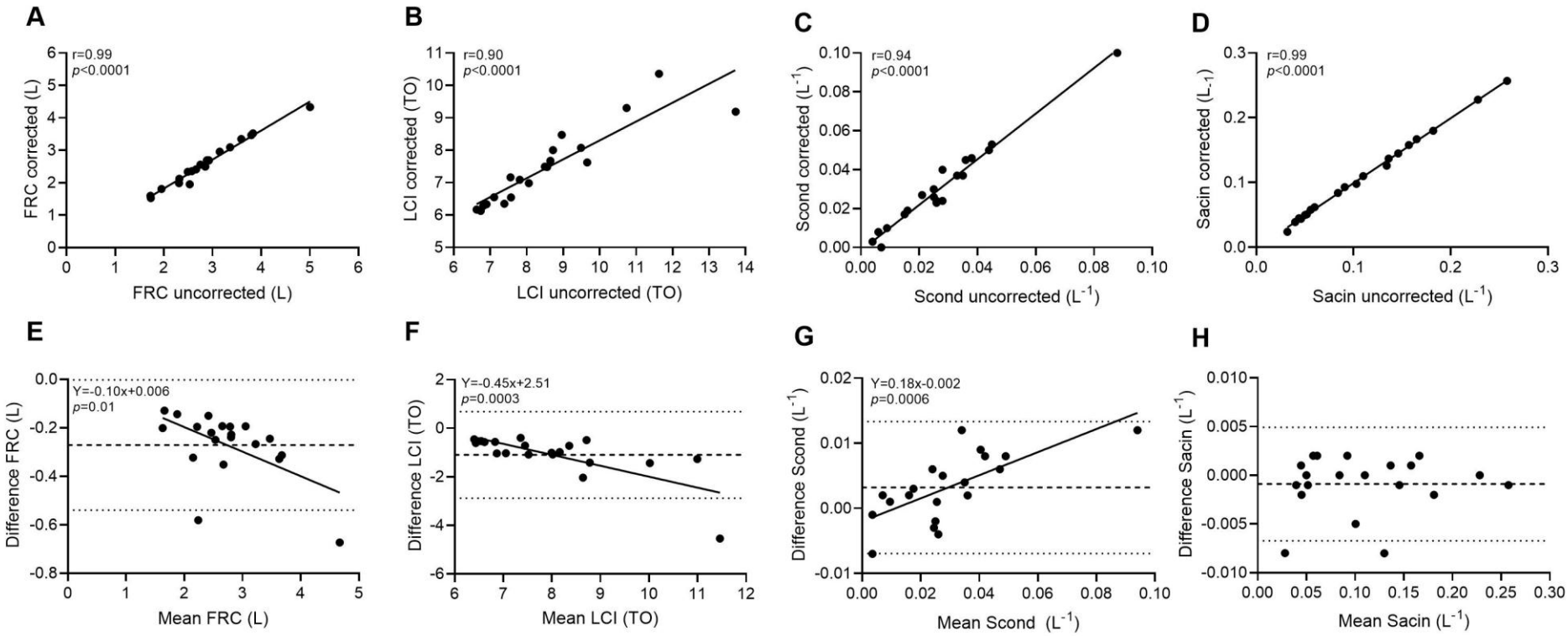
1. Figure S1 - Comparison of standard (uncorrected) and corrected MBNW parameters in health using free breathing protocol.
2. Figure S2 - Comparison of standard (uncorrected) and corrected MBNW parameters in asthma using free breathing protocol.
3. Table S1 - Effects of sensor correction on main MBNW parameters using free breathing protocol.
4. Table S2 - Effect of sensor correction on within-session and between-visit repeatability using free breathing protocol
5. Figure S3 - Comparison of standard (uncorrected) and corrected Scond for entire cohort using 1L breathing protocol.

Figure S1. Comparison of standard (uncorrected) and corrected MBNW parameters using free breathing protocol in health



There were strong correlations between uncorrected and corrected functional residual capacity (FRC) (r=0.99 and $p<0.0001$) (**A**), lung clearance index (LCI) (r=0.96 and $p<0.0001$) (**B**), Scond (r=0.93, p<0.0001) (**C**) and Sacin (r=0.99, p<0.0001). Bland-Altman plots shows that sensor correction results in a lower FRC (mean difference (95% limits of agreement) (-0.26 (-0.45--0.07), $p<0.0001$) (**E**) lower LCI (-0.85 (-1.44--0.26), $p<0.0001$) (**F**), but not change in Scond (0.001 (-0.008-0.009), $p=0.26$) (**G**) and Sacin (0.0003 (-0.004-0.005), $p=0.57$) (**H**). There also was significant proportional bias confirmed by linear regression for FRC (p<0.0001), LCI (p<0.0001), Scond ($p=0.02$) and Scond ($p=0.002$).

Figure S2. Comparison of standard (uncorrected) and corrected MBNW parameters using free breathing protocol in asthma



There were strong correlations between uncorrected and corrected functional residual capacity (FRC) (r=0.99 and *p*<0.0001) (**A**), lung clearance index (LCI) (r=0.90 and *p*<0.0001) (**B**), Scond (r=0.94, p<0.0001) (**C**) and Sacin (r=0.99, p<0.0001). Bland-Altman plots shows that sensor correction results in a lower FRC (mean difference (95% limits of agreement) (-0.27 (-0.54, -0.002), *p*<0.0001) (**E**) lower LCI (-1.1 (-2.9-0.68), *p*<0.0001) (**F**), higher Scond (0.003 (-0.007-0.013), *p*=0.012) (**G**), but no change in Sacin (-0.0009 (-0.007-0.005), *p*=0.19) (**H**). There also was significant proportional bias confirmed by linear regression for FRC (*p*=0.01), LCI (p=0.0003), and Scond (*p*=0.0006).

Table S1. Effects of sensor correction on main MBNW parameters using free breathing protocol

Parameter	Standard	Corrected	Mean difference (95% CI) absolute	Mean difference (95% CI) relative	*p*-value
Health					
FRC (L)	3.18±0.94	2.92±0.86	-0.26 (-0.30--0.23)	-8.3 (-8.9--7.6)	<0.0001
LCI (TO)	7.55±0.82	6.70±0.59	-0.85 (-0.97--0.73)	-11.0 (-12.2--9.9)	<0.0001
Scond L^{-1}	0.018±0.010	0.019±0.012	0.0010 (-0.0007-0.003)	2.0 (-8.9-13.0)	0.26
Sacin L^{-1}	0.085±0.04	0.085±0.04	0.0003 (-0.0007-0.0012)	-0.25 (-1.5-1.0)	0.57
Asthma					
FRC (L)	2.87±0.78	2.60±0.71	-0.27 (-0.34--0.21)	-9.45 (-11.3--7.6)	<0.0001
LCI (TO)	8.56±1.81	7.46±1.17	-1.10 (-1.53--0.67)	-11.9 (-14.9--9.0)	<0.0001
Scond L^{-1}	0.028±0.019	0.031±0.022	0.003 (0.0008-0.006)	6.2 (-7.9-20.1)	0.01
Sacin L^{-1}	0.109±0.066	0.108±0.066	-0.0009 (-0.0023-0.0005)	-1.6 (-4.5-1.2)	0.20

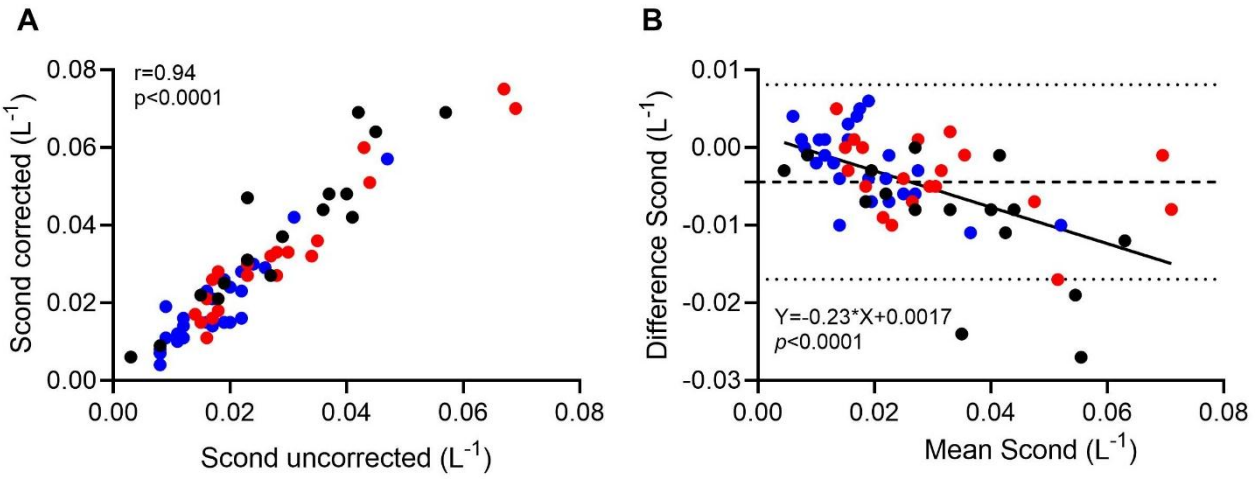
Effects of sensor correction on main MBNW parameters. Data are presented as mean±SD unless otherwise stated. FRC; functional residual capacity; LCI: lung clearance index; Scond: conducting airways ventilation heterogeneity; Sacin: distal/intra-acinar airways ventilation heterogeneity. 95% CI: 95% confidence interval. Absolute difference is calculated as corrected-standard. Relative difference is calculated as corrected-standard/standard * 100.

Table S2. Effect of sensor correction on within- and between-session repeatability using free breathing protocol

	Within-session CoV	Between-session difference	Between-session 95% LOA	Between-session ICC
Uncorrected				
FRC (L)	3.6 ± 2.3%	-0.05±0.29	-0.62-0.52	0.980
LCI (TO)	3.2 ± 1.5%	0.15±0.53	-0.89-1.19	0.850
Scond (L^{-1})		0.003±0.016	-0.027-0.033	0.158
Sacin (L^{-1})		0.004±0.052	-0.098-0.106	0.334
Corrected				
FRC (L)	3.9 ± 2.4%	-0.04±0.26	-0.54-0.47	0.979
LCI (TO)	3.1 ± 2.2%	0.15±0.51	-0.84-1.14	0.704
Scond (L^{-1})	-	0.006±0.016	-0.026-0.038	0.411
Sacin (L^{-1})	-	0.003±0.054	-0.103-0.109	0.278

Data are presented as mean±SD unless otherwise stated. Mean differences are visit 2 minus visit 1. CoV: coefficient of variation; 95% LOA: 95% limits of agreement; ICC: intra-class correlation coefficient; FRC: functional residual capacity; LCI: lung clearance index; Scond: conducting airways ventilation heterogeneity; Sacin: distal/intra-acinar airways ventilation heterogeneity.

Figure S3. Comparison of standard (uncorrected) and corrected Scond for entire cohort using 1L breathing protocol.



There was strong correlation between uncorrected and corrected Scond (r=0.94 and *p*<0.0001) for entire cohort (**A**). Bland-Altman plot shows that sensor correction results in a higher Scond (-0.004 (-0.017-0.008), *p*<0.0001) with significant proportional bias confirmed by linear regression (*p*<0.0001). Healthy volunteers are shown in blue, participants with asthma in red and smokers in black. These graphs demonstrate that these 3 cohorts are subsets of the same data set.