



Controlled *versus* free breathing for multiple-breath nitrogen washout in asthma

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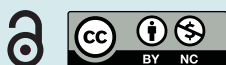
To the Editor:

Multiple-breath nitrogen washout (MBNW) is an emerging clinical test for assessing ventilation heterogeneity [1], often characteristically increased in asthma. MBNW indices both indicate and predict response to asthma treatment [2–4], and therefore may be an important tool for guiding treatment decisions [2]. Two established breathing protocols are currently in use: 1-L tidal volume (V_T) controlled breathing (CB) [5, 6] and unrestricted free breathing (FB) [7]. The CB protocol requires targeted V_T and respiratory rate, whereas the FB protocol encourages relaxed tidal breathing, making it more suitable for paediatrics [8]. Two recently published studies in healthy adults showed that indices of conductive and acinar ventilation heterogeneity (S_{cond} and S_{acin} , respectively) and, to a lesser extent, lung clearance index (LCI), were not comparable between breathing protocols [9, 10]. Importantly, differences between the protocols were dependent on the magnitude of ventilation heterogeneity. Thus, the assumption is that these effects would be amplified in disease, where ventilation heterogeneity is greater and clinical utility is most relevant. However, this has not been confirmed to date. We hypothesised that people with asthma, where ventilation heterogeneity is greater, would exhibit greater differences between the two protocols than the differences seen in healthy adults.

Therefore, this study aimed to determine in adults with asthma: 1) whether CB and FB MBNW protocols provide comparable functional residual capacity (FRC) and indices of ventilation heterogeneity (LCI, S_{cond} and S_{acin}), and 2) whether patient-related factors (anthropometrics and/or breathing pattern) influence any observable differences.

Written informed consent was obtained from participants with respiratory physician-diagnosed asthma recruited from the Woolcock Institute and Royal North Shore Hospital (ethics approval LNR/16/HAWKE/11). The study protocol has been previously published [9]. Briefly, spirometry and plethysmography were obtained according to ATS/ERS standards and current reference values [11, 12]. After a fixed period, participants then performed MBNW according to European Respiratory Society/American Thoracic Society consensus guidelines [8] with either the FB or CB protocol in successive triplicates (Exhalyzer D, collected in Spiroware v3.1.6 and reanalysed in v3.3.1; Eco Medics AG, Duernten, Switzerland), in randomised order. During each trial, once a stable breathing pattern and end-expiratory lung volume (EELV) was established, nitrogen washout during 100% oxygen inhalation was commenced. The CB protocol required participants to breathe at a respiratory rate between 8 and 12 breaths·min⁻¹, and V_T between 0.95 and 1.3 L following visual feedback. In the FB protocol, participants were encouraged to adopt relaxed tidal breathing but advised to adjust V_T upwards if insufficient expired nitrogen phase III slope was observed; calculated S_{cond} and S_{acin} were adjusted for V_T , as per consensus guidelines [8]. At least three technically acceptable trials with FRC values $\pm 10\%$ of the mean were obtained for each protocol, and quality control and *post hoc* analysis was performed by a single operator (B.M. Handley). For each MBNW parameter, the mean of three trials was compared between the FB and CB protocols using Pearson's correlation, paired t-tests and Bland–Altman plots. Associations between potential predictors (age, sex, height, body mass index (BMI), respiratory rate and V_T) and between-protocol differences (FB–CB) were examined using linear regression.

We studied 20 (16 female and four male) nonsmoking participants with a median (interquartile range) age of 43 (31.5) years and BMI of 25 (7.1) kg·m⁻². Study participants had mean \pm SD forced expiratory volume



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The lack of comparability in indices of ventilation heterogeneity between free- and controlled-breathing MBNW protocols is confirmed in asthma <https://bit.ly/3lmri4A>

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in 1 s (FEV_1) $89.2 \pm 19.2\%$ predicted and FEV_1 /forced vital capacity $74.4 \pm 8.35\%$. Compared to the CB protocol, the FB protocol had smaller mean V_T (mean \pm SD difference -0.36 ± 0.22 L, $p < 0.0001$) and a faster mean respiratory rate (mean \pm SD difference 3.16 ± 3.33 breaths \cdot min $^{-1}$, $p = 0.029$).

There was no significant difference in FRC measured between protocols (FRC_{CB} 2.62 ± 0.72 L versus FRC_{FB} 2.60 ± 0.71 L, $p = 0.64$), with strong correlation between the two ($r = 0.97$, $p < 0.0001$) and no evidence of proportional bias in the Bland–Altman plot ($p = 0.81$) (figure 1a). This is similar to observations in healthy adults [9], and supports the argument that FRC is not altered between MBNW protocols so long as care is taken to ensure stable breathing and EELV before and during washout. Both FRC_{CB} ($p = 0.006$) and FRC_{FB} ($p = 0.005$) were significantly reduced compared to plethysmographic FRC (2.87 ± 0.60 L), as may

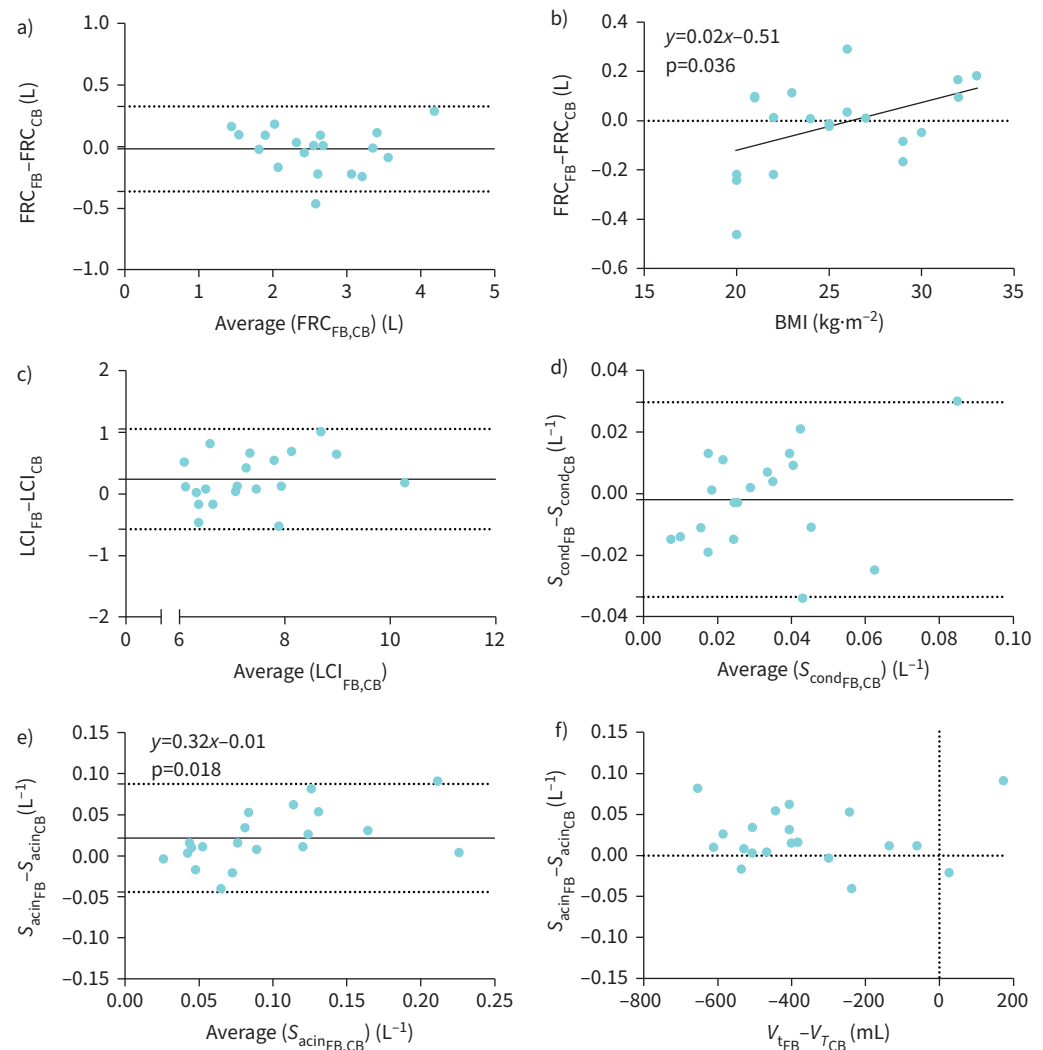


FIGURE 1 Differences between controlled (CB) and free breathing (FB) protocols, and associated factors. **a)** Functional residual capacity (FRC), showing no significant differences between the two protocols (mean difference (95% limits of agreement) -0.019 (-0.364 – -0.327) L, $p = 0.64$) and no proportional bias ($p = 0.81$), with **b)** between-protocol differences in FRC related to body mass index (BMI) ($p = 0.036$). **c)** Lung clearance index (LCI), showing significant differences (0.235 (-0.578 – -1.048), $p = 0.020$) but no proportional bias ($p = 0.179$) between protocols. **d)** Conductive ventilation heterogeneity (S_{cond}), showing no significant differences between protocols (-0.0020 (-0.034 – -0.030) L^{-1} , $p = 0.59$) and no proportional bias ($p = 0.203$). **e)** Acinar ventilation heterogeneity (S_{acin}) was significantly different between protocols (0.0215 (-0.044 – -0.087) L^{-1} , $p = 0.01$) with a significant proportional bias ($p = 0.018$), and **f)** between-protocol differences in S_{acin} were not predicted by between-protocol differences in tidal volume ($V_{t, FB} - V_{t, CB}$) ($p = 0.98$) or respiratory rate ($p = 0.38$, data not shown).

be expected in disease from a gas dilution method reliant on communicating lung volume. Interestingly, within-subject differences in FRC between protocols were associated with BMI ($y=0.02x-0.51$, $p=0.036$) (figure 1b) but not with age, sex, height, mean V_T or mean respiratory rate. We had previously found a trend towards a significance relationship between BMI and between-protocol differences in FRC [9]. The association we observed in this study could be attributed to a wider range for BMI, with more obese participants exhibiting higher FRC_{FB} values. The mechanisms for this are unknown but may have implications for testing in a clinical population.

Significant differences were seen in LCI between protocols, with higher values obtained using FB (LCI_{CB} 7.23 ± 1.04 versus LCI_{FB} 7.46 ± 1.17 , $p=0.020$), but the two protocols were strongly correlated ($r=0.94$, $p<0.0001$). This finding was consistent with our previous findings in health [9] except that now there was no proportional bias between protocols in asthma evident ($p=0.179$) (figure 1c). Previous studies suggested an effect of changing V_T on LCI [13], where shallow breathing may contribute to a higher LCI through an increased dead space to V_T ratio, and its effects on FRC and cumulative expired volume; though this effect was not statistically significant in our data. However, as we also previously demonstrated in health [9] and the lack of differences observed in other studies [14], the mean difference of 0.23 ± 0.41 seen here was relatively small and unlikely to be clinically significant. For comparison, the minimal clinically important difference for MBNW is yet to be established, however, a change of 1 unit is often used for LCI in interventional studies [15].

Results for S_{cond} and S_{acin} were also similar to those observed in health. S_{cond} was not significantly different between the CB and FB protocols ($S_{cond_{CB}}$ 0.033 ± 0.018 L⁻¹ versus $S_{cond_{FB}}$ 0.031 ± 0.022 L⁻¹, $p=0.59$), with significant correlation between the two ($r=0.70$, $p=0.0006$) and no evidence of proportional bias ($p=0.20$) (figure 1d). In contrast, S_{acin} was significantly different between the protocols ($S_{acin_{CB}}$ 0.086 ± 0.05 versus $S_{acin_{FB}}$ 0.108 ± 0.07 , $p=0.01$), with significant correlation between the two ($r=0.87$, $p<0.0001$), but evidence of proportional bias ($y=0.32x-0.01$, $p=0.018$) (figure 1e). These findings are consistent with the larger differences expected from the proportional bias observed in health [9, 10], particularly for S_{acin} . Neither between-protocol differences in S_{cond} nor in S_{acin} had any associations with age, sex, height, BMI, mean V_T or mean respiratory rate. The lack of dependence on breathing pattern in asthma is contrary to what we observed in health for S_{acin} and may suggest that the contribution of disease to between-protocol differences is larger than that of the breathing pattern. Alternatively, this could have been skewed by one individual whose V_T was greater during FB than CB (figure 1f).

It is interesting to note that the magnitudes of the between-protocol differences and limits of agreement seen in this study in asthma (-0.0020 (-0.034 – 0.030) L⁻¹ for S_{cond} and 0.0215 (-0.044 – 0.087) L⁻¹ for S_{acin}) were similar in range to those published in health (0.0002 (-0.030 – 0.030) L⁻¹ for S_{cond} and 0.029 (-0.045 – 0.103) L⁻¹ in S_{acin}) [9], despite the larger S_{cond} and S_{acin} values. A possible explanation may again be that the degree of abnormal ventilation distribution due to asthma is a stronger contributor to the measured S_{cond} and S_{acin} than variations in the breathing pattern. It is also possible that relative variability is lower in disease, unlike in health, where the small values of S_{cond} and S_{acin} close to zero render any variations proportionately larger.

We do not have data on between-session repeatability in these patients, although published studies exist for comparison [9, 16]. The between-protocol differences and limits of agreement seen here were similar or larger than previously reported between-session repeatability for the MBNW test in health (-0.003 (-0.021 – 0.015) L⁻¹ for S_{cond} and -0.002 (-0.039 – 0.034) L⁻¹ for S_{acin} over 2–10 weeks) [9] but less than the between-session repeatability in asthma (0.004 (-0.072 – 0.079) L⁻¹ for S_{cond} and -0.024 (-0.156 – 0.108) L⁻¹ for S_{acin} over 2 weeks) [16], reflecting contributions from protocol differences, test variability and disease.

The limitations of this study include the small sample size and the high proportion of participants who had undergone lung function testing before, although 15 out of 20 were naïve to MBNW. Nevertheless, these data confirm in disease that the two protocols should not be simply treated interchangeably in prospective studies, with implications for the interpretation of previously published data. It should also be noted that the data presented in this study were analysed using the updated software version for the Exhalys D device, which takes into account a recently documented sensor error [17, 18]; comparisons with health are also based on updated results, for which a correction has been issued. Further work is warranted to better understand the applicability of the V_T correction [10], dependence on phase III slope estimation [19] and other possible sources contributing to differences between these two established MBNW protocols.

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