Early View

Research letter

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Controlled versus free breathing for multiple breath nitrogen washout in asthma

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Take home: The lack of comparability in indices of ventilation heterogeneity between free- and controlled-breathing MBNW protocols is confirmed in asthma.
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Conflicts of Interest: E Jeagal has nothing to disclose
Conflicts of Interest: S Rutting has nothing to disclose
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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-litre tidal volume-controlled breathing (CB)(5, 6) and unrestricted free breathing (FB)(7). The CB protocol requires targeted tidal volume ($V_T$) and respiratory rate (RR), 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 ERS/ATS 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% O₂ inhalation was commenced. The CB protocol required participants to breathe at a RR between 8-12 breaths.min⁻¹ and Vₜ between 0.95-1.3 L following visual feedback. In the FB protocol, participants were encouraged to adopt relaxed tidal breathing but advised to adjust tidal volumes upwards if insufficient expired N₂ phase III slope was observed; calculated S-cond and S-acin were adjusted for Vₜ, as per consensus guidelines(8). At least 3 technically acceptable trials with FRC values ±10% of the mean were obtained for each protocol, and quality control and post hoc analysis was performed by a single operator (BMH). For each MBNW parameter, the mean of 3 trials was compared between the FB vs CB protocols using Pearson’s correlation, paired t-tests, and Bland-Altman plots. Associations between potential predictors (age, sex, height, BMI, RR and Vₜ) and between-protocol differences (FB-CB) were examined using linear regression.

We studied 20 (16 female, 4 male) non-smoking participants with a median(IQR) age of 43(31.5) and BMI of 25(7.1). Study participants had mean±SD %predicted FEV₁ 89.2±19.2%predicted and FEV₁/FVC 74.4±8.35 %. Compared to the CB protocol, the FB protocol had smaller mean Vₜ (mean difference±SD -0.36±0.22 L, p<0.0001), and a faster mean RR (mean difference±SD 3.16±3.33 breaths.min⁻¹, p=0.029).

There was no significant difference in FRC measured between protocols (FRC_CB 2.62±0.72 L vs 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) (Fig 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 FRC_pleth (2.87±0.60 L), as may 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 ($y=0.02x+0.51$, $p=0.036$, Fig. 1B), but not with age, sex, height, mean $V_T$, or mean RR. 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 ($LCI_{CB} 7.23\pm1.04 \text{ vs } LCI_{FB} 7.46\pm1.17$, $p=0.02$), 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 ($y=0.12x – 0.64$, $p=0.18$, Fig. 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 (CEV); 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 $0.23\pm0.41$ 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 that observed in health. $S_{cond}$ was not significantly different between the CB and FB protocols ($S_{condCB} 0.033\pm0.018 \text{ L}^{-1} \text{ vs } S_{condFB} 0.031\pm0.022 \text{ L}^{-1}$, $p=0.59$), with significant correlation between the two ($r=0.70$, $p=0.0006$), and no evidence of proportional bias ($p=0.20$, Fig. 1D). In contrast, $S_{acin}$ was significantly different between the protocols ($S_{acinCB} 0.086\pm0.05 \text{ vs } S_{acinFB} 0.108\pm0.07$, $p=0.01$), with significant correlation between the two ($r=0.87$, $p<0.0001$), but evidence of proportional bias.
\( y = 1.17x + 0.007, p < 0.0001, \) Fig. 1E. These findings are consistent with the larger differences expected from the proportional bias observed in health (9, 10), particularly for \( S_{\text{acin}} \). However, neither between-protocol differences in \( S_{\text{cond}} \) nor in \( S_{\text{acin}} \) had any associations with age, sex, height, BMI, mean \( V_T \), or mean RR. This lack of dependence on breathing pattern in asthma is contrary to what we observed in health for \( S_{\text{acin}} \), 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 (Fig. 1E).

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 \( S_{\text{cond}} \), 0.0215(-0.044, 0.087) \( L^{-1} \) for \( S_{\text{acin}} \)) were similar in range to those published in health (0.0002(-0.030, 0.030) \( L^{-1} \) for \( S_{\text{cond}} \) and 0.029(-0.045, 0.103) \( L^{-1} \) in \( S_{\text{acin}} \))(9), despite the larger \( S_{\text{cond}} \) and \( S_{\text{acin}} \) values. A possible explanation may again be that the degree of abnormal ventilation distribution due to asthma is a stronger contributor to the measured \( S_{\text{cond}} \) and \( S_{\text{acin}} \) 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 \( S_{\text{cond}} \) and \( S_{\text{acin}} \) close to zero render any variations proportionately larger.

We do not have data on between-session repeatability in these patients, though 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 \( S_{\text{cond}} \), -0.002(-0.039, 0.034) \( L^{-1} \) for \( S_{\text{acin}} \), over 2-10 weeks)(9), but less than the between-session repeatability in asthma (0.004(-0.072, 0.079) \( L^{-1} \) for \( S_{\text{cond}} \), -0.024(-0.156, 0.108) \( L^{-1} \) for \( S_{\text{acin}} \), over 2 weeks)(16), reflecting contributions from protocol differences, test variability, as well as disease.
The limitations of this study include the small sample size and the high proportion of participants who had undergone lung function testing before, though 15/20 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 Exhalyser 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[REF]. 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.
REFERENCES

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 (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).