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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.

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% O_2 inhalation was commenced. The CB protocol required participants to breathe at a RR between 8-12 breaths.min^{-1} and V_T 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 N_2 phase III slope was observed; calculated S_{cond} and S_{acin} were adjusted for V_T, 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 V_T) 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 FEV_1 89.2±19.2%predicted and FEV_1/FVC 74.4±8.35 %. Compared to the CB protocol, the FB protocol had smaller mean V_T (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±1.04 vs LCI_{FB} 7.46±1.17, 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±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 that observed in health. S_{cond} was not significantly different between the CB and FB protocols (S_{condCB} 0.033±0.018 L^{-1} vs S_{condFB} 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, Fig. 1D). In contrast, S_{acin} was significantly different between the protocols (S_{acinCB} 0.086±0.05 vs S_{acinFB} 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=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_{acin}. However, neither between-protocol differences in S_{cond} nor in S_{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_{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_{cond}, 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 Sacin 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_{cond}, -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}, -0.024(-0.156, 0.108) L^{-1} for S_{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

1.   King GG. Cutting edge technologies in respiratory research: lung function testing. Respirology. 2011;16(6):883-90.
2.   Farah CS, King GG, Brown NJ, Peters MJ, Berend N, Salome CM. Ventilation heterogeneity predicts asthma control in adults following inhaled corticosteroid dose titration. Journal of Allergy and Clinical Immunology. 2012;130(1):61-8.
3.   Tang FSM, Rutting S, Farrow CE, Tonga KO, Watts J, Dame-Carrol JR, et al. Ventilation heterogeneity and oscillometry predict asthma control improvement following step-up inhaled therapy in uncontrolled asthma. Respirology. 2020;25(8):827-35.
4.   Farah CS, Badal T, Reed N, Rogers PG, King GG, Thamrin C, et al. Mepolizumab improves small airway function in severe eosinophilic asthma. Respir Med. 2019;148:49-53.
5.   Verbanck S, Thompson BR, Schuermans D, Kalsi H, Biddiscombe M, Stuart-Andrews C, et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. Thorax. 2012;67(9):789-95.
6.   Verbanck S, Van Muylem A, Schuermans D, Bautmans I, Thompson B, Vincken W. Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults. Eur Respir J. 2016;47(1):166-76.
7.   Kjellberg S, Houltz BK, Zetterström O, Robinson PD, Gustafsson PM. Clinical characteristics of adult asthma associated with small airway dysfunction. Respir Med. 2016;117:92-102.
8.   Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. European Respiratory Journal. 2013;41(3):507-22.
9.   Handley BM, Jeagal E, Schoeffel RE, Badal T, Chapman DG, Farrow CE, et al. Controlled versus free breathing for multiple breath nitrogen washout in healthy adults. ERJ Open Research. 2020:00435-2020.
10.   Verbanck S, Schuermans D, Paiva M, Robinson PD, Vanderhelst E. Mitigating increased variability of multiple breath washout indices due to tidal breathing. European Respiratory Journal. 2021;57(2):2002765.
11.   Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. 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):1324-43.
12.   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.
13.   Ratjen F, Jensen R, Klingel M, McDonald R, Moore C, Benseler N, et al. Effect of changes in tidal volume on multiple breath washout outcomes. PLOS ONE. 2019;14(7):e0219309.
14.   Prisk GK, Petersen GM, Geier ET, Sá RC. Ventilatory heterogeneity in the normal human lung is unchanged by controlled breathing. J Appl Physiol (1985). 2020;129(5):1152-60.
15.   Short C, Saunders C, Davies JC. Utility of lung clearance index in CF: What we know, what we don't know and musings on how to bridge the gap. Journal of Cystic Fibrosis. 2020;19(6):852-5.
16.   Gonem S, Corkill S, Singapuri A, Gustafsson P, Costanza R, Brightling CE, et al. Between-visit variability of small airway obstruction markers in patients with asthma. European Respiratory Journal. 2014;44(1):242-4.
17.   Sandvik RM, Gustafsson PM, Lindblad A, Robinson PD, Nielsen KG. Improved agreement between N2 and SF6 multiple-breath washout in healthy infants and toddlers with improved EXHALYZER D sensor performance. Journal of Applied Physiology. 2021;131(1):107-18.
18.   Wyler F, Oestreich M-AH, 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.2021.
19.   Bates JHT, Peters U. A model-based approach to interpreting multibreath nitrogen washout data. J Appl Physiol (1985). 2018;124(5):1155-63.
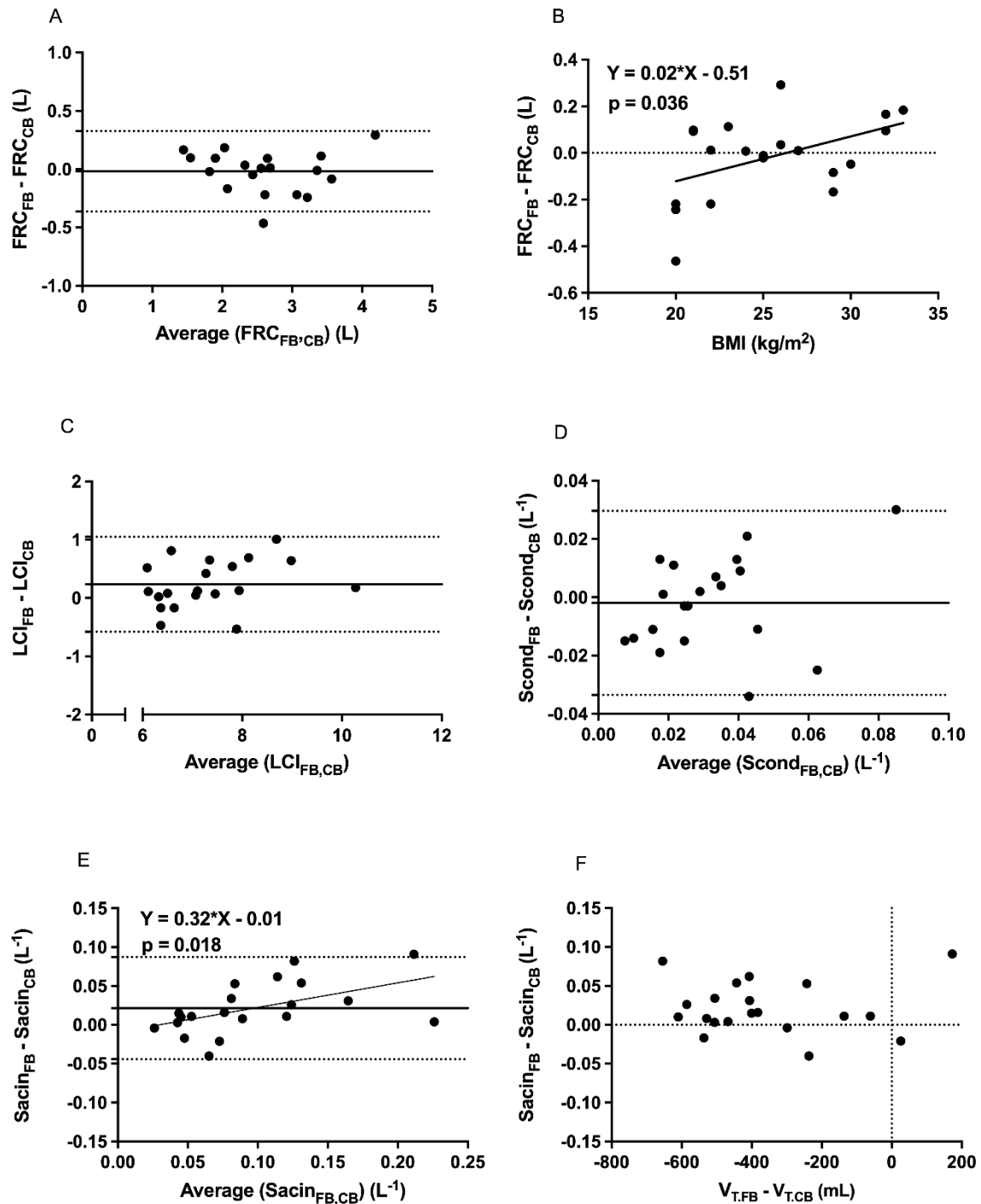
Figure 1 – Differences between controlled (CB) and free breathing (FB) protocols, and associated factors. (A) Functional residual capacity (FRC), showing no significant differences between the two protocols (mean difference (95% limits of agreement) -0.019 (-0.364, 0.327) L, p=0.64) and no proportional 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).