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RESEARCH LETTER

# Mitigating increased variability of multiple breath washout indices due to tidal breathing

*To the Editor:*

Multiple breath washout (MBW) tests have gained interest for clinical use, with normal values being published for the lung clearance index (LCI) in the paediatric [1, 2] and adult range [3, 4]. In adults, the greater interest in mechanisms of acinar and conductive ventilation heterogeneity (reflected in $S_{acin}$ and $S_{cond}$, respectively) drove the adoption of a 1 L breathing protocol to ensure a clear estimate of phase III slope. Alternatively, natural breathing protocols aimed to enhance feasibility in paediatric settings [5] have also been used in some adult studies [6]. Since $S_{acin}$ or $S_{cond}$ are indirect measures of lung structure and its potential abnormality in disease [7, 8], the manoeuvre (natural breathing or not) by which these indices are obtained does not directly alter the underlying process, but merely affects the magnitude of its estimates. In specific age groups, or in patient groups of any age with severe lung disease, a 1 L tidal volume ($V_T$) may not be achievable or may result in an altered functional residual capacity (FRC). $V_T$ increases from 0.5 to 1.3 L have led to FRC decrease by 17% in children [9] and by 7% in adults [10]; corresponding LCI increases were observed in children, but not in adults. In natural breathing studies, $S_{acin}$ and $S_{cond}$ are usually compensated by $V_T$ multiplication to account for differences in lung size (multiplication by FRC) and in breathing pattern (multiplication by $V_T$/FRC) [5]. In the present work we systematically studied the effect of natural tidal breathing ($MBW_{nat}$), 1 L breathing ($MBW_{1L}$) and 1.5 L breathing ($MBW_{1.5L}$) on MBW indices LCI, $S_{acin}$, $S_{cond}$ and the $V_T$ compensation of the latter two, with an aim to distil a breathing modality that may enhance clinical utility of MBW in the future.

40 healthy young adults (20 males, 20 females) were recruited (local ethics committee BUN143201836071) and all nitrogen MBW testing was performed as previously described [11]. Subjects performed nine MBW tests (three sets of three trials, always performing the first set during natural tidal breathing, to avoid a potential impact on this from any larger $V_T$ breathing before it). Friedman test and multiple regression were performed using MedCalc (version16.4.3, Mariakerke, Belgium).

In the study cohort (mean±SD age 24.4±3.4 years), mean±SD z-scores for FRC, LCI, $S_{acin}$ and $S_{cond}$ obtained with the $MBW_{1L}$ test were 0.1±0.8, −0.1±1.1, −0.1±0.8 and −0.1±1.7, respectively, using equipment-specific reference equations [11]; Global Lung Function Initiative-based z-score for forced expiratory volume in 1 s was 0.3±0.09 [12]. Mean±SD trial durations were 191±41 s for $MBW_{nat}$, 124±26 s for $MBW_{1L}$ and 98±21 s for $MBW_{1.5L}$. Figure 1 illustrates the effects of $V_T$ on raw value FRC, LCI, $S_{acin}$ and $S_{cond}$. Compared to $MBW_{1L}$, natural tidal breathing induced statistically significant but small LCI increases, and a considerable $S_{acin}$ increase, on average from 0.070 $L^{-1}$ ($MBW_{1L}$) to 0.255 $L^{-1}$ ($MBW_{nat}$). When $V_T$-compensated as per guidelines [5], a significant difference persisted but mean $S_{acin}\cdot V_T$ increased relatively less, from 0.077 ($MBW_{1L}$) to 0.150 ($MBW_{nat}$). While $S_{cond}$ did not show a significant difference ($MBW_{1L}$ 0.030 $L^{-1}$ *versus* $MBW_{nat}$ 0.029 $L^{-1}$), it reached significance for $S_{cond}\cdot V_T$ ($MBW_{1L}$ 0.033 *versus* $MBW_{nat}$ 0.017), suggesting overcompensation by $V_T$. Interestingly, $V_T$ compensation works better between $MBW_{1L}$ and $MBW_{1.5L}$, where $V_T$ multiplication better neutralises the consistent $S_{acin}$ and $S_{cond}$ decreases observed with increased $V_T$ (mean $S_{acin}\cdot V_T$ 0.069 for $MBW_{1.5L}$ *versus* 0.077 for $MBW_{1L}$, and $S_{cond}\cdot V_T$ 0.036 for $MBW_{1.5L}$ *versus* 0.033 for $MBW_{1L}$).

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**By studying tidal volume dependence of diagnostic multiple breath washout (MBW) indices, it can be determined how breathing patterns can be modulated to obtain less variable MBW outcomes and shorten MBW test duration in the clinical routine** https://bit.ly/32GxRWj

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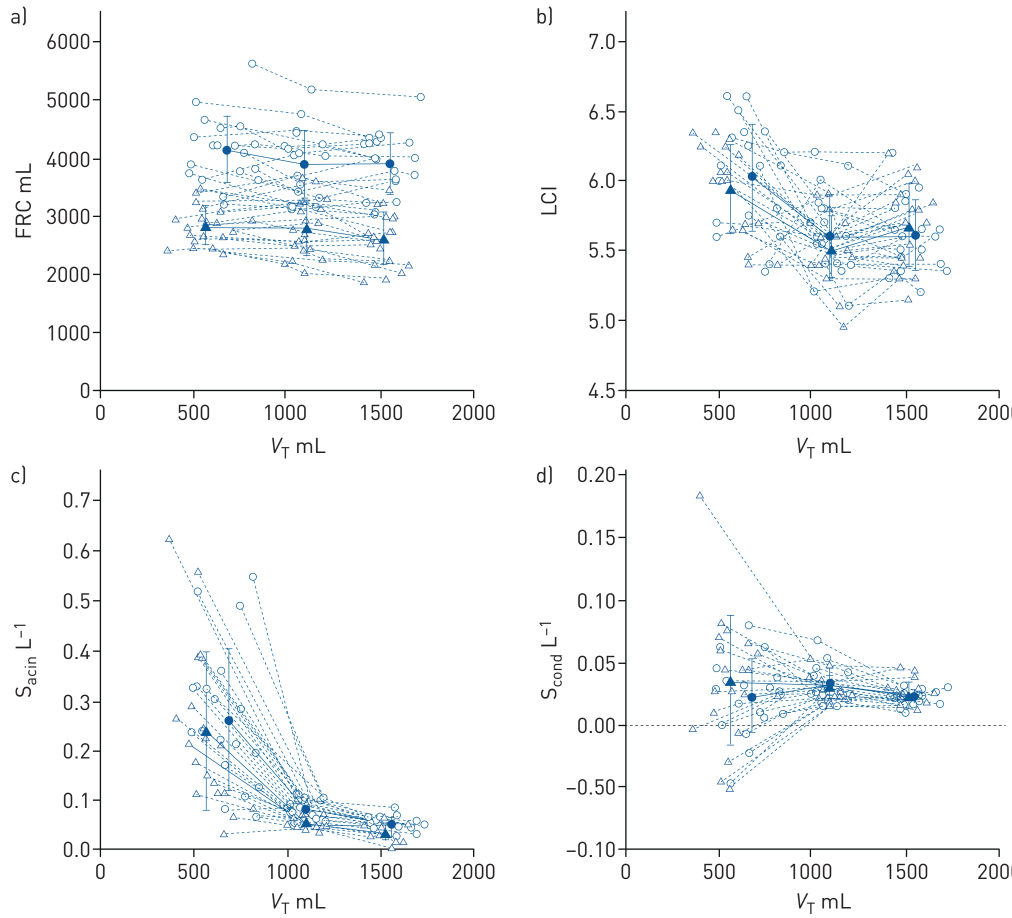


FIGURE 1 Multiple breath washout (MBW)-derived indices as a function of tidal volume ($V_T$) for male (circles) and female (triangles) normal subjects. Closed symbols are mean±SD and positioned at mean $V_T$ values corresponding to target volumes of respectively $MBW_{nat}$, $MBW_{1L}$ and $MBW_{1.5L}$. Open symbols are individual data points *versus* individual $V_T$ values, connecting $MBW_{nat}$, $MBW_{1L}$ and $MBW_{1.5L}$ data sets for any given subject (dotted lines). a) Functional residual capacity (FRC); b) lung clearance index (LCI); c) acinar ventilation heterogeneity ($S_{acin}$); d) conductive ventilation heterogeneity ($S_{cond}$).

To investigate whether the experimentally observed degree of $V_T$ dependence of $S_{acin}$ could be replicated based on the underlying model of diffusion–convection interaction, simulations were performed with an adult lung model recently used for simulation of $S_{acin}$ increases typically seen in COPD [7]. With the model in its normal baseline state (and simulated FRC of 3000 mL), this obtained simulated $S_{acin}$ values of 0.134 $L^{-1}$ (750 mL), 0.085 $L^{-1}$ (1000 mL), 0.060 $L^{-1}$ (1500 mL); with $V_T$ compensation, corresponding simulated $S_{acin}{\cdot}V_T$ values were 0.101, 0.085 and 0.090.

A striking $V_T$ effect in figure 1 is its impact on inter-subject variability. While inter-subject coefficient of variation for this young adult cohort was similar across the studied $V_T$ range for FRC ($MBW_{nat}$ 23%; $MBW_{1L}$ 25%; $MBW_{1.5L}$ 25%) and LCI ($MBW_{nat}$ 5.8%; $MBW_{1L}$ 4.8%; $MBW_{1.5L}$ 4.9%), this was not the case for $S_{cond}$ ($MBW_{nat}$ 147%; $MBW_{1L}$ 37%; $MBW_{1.5L}$ 32%) nor for $S_{acin}$ ($MBW_{nat}$ 59%; $MBW_{1L}$ 30%; $MBW_{1.5L}$ 36%). Also with $V_T$ compensation, inter-subject $S_{acin}{\cdot}V_T$ and $S_{cond}{\cdot}V_T$ variability for $MBW_{nat}$ were 58% and 128%, respectively, and only in the case of $S_{acin}{\cdot}V_T$ could this be partly accounted for by inter-subject variability in $V_T$ and FRC ($R^2_{adjusted}$=0.38; $V_T$: $r_{partial}$=−0.35, p=0.03; FRC: $r_{partial}$=0.64, p<0.001). What is also apparent from figure 1, is that for any individual with a given FRC, there is a steep dependence of $S_{acin}$ on $V_T$ near natural breathing, such that small variations in $V_T$ can result in large $S_{acin}$ variations. Hence, if subjects are allowed to freely use their natural breathing, instead of a weight- or height-based fixed tidal $V_T$, this will be detrimental to variability of study outcomes. Also, if a treatment were to increase natural $V_T$, a $S_{acin}$ decrease could signal a treatment effect or a purely volumetric effect, or both.

We show here quantitatively, a $V_T$ effect that has long been known to affect ventilation distribution, with increasing $V_T$ generally decreasing phase III slopes [13, 14]. The $V_T$ compensation is similar to what is done when comparing species, *e.g.* phase III slopes from humans (in $L^{-1}$) and rats (in $mL^{-1}$) [15], where both $V_T$ and FRC are scaled by a factor 1000. The present experimental data show that the $V_T$ compensation did attenuate dependency of both $S_{acin}$ and $S_{cond}$ on $V_T$ in the 1.0−1.5 L $V_T$ range, but that it could not fully compensate $S_{acin}$, and even overcompensated $S_{cond}$ in the case of $MBW_{nat}$. In the case of $S_{acin}$, this was supported by model simulations where the purely volumetric effect on diffusion–convection interaction in the lung periphery could be assessed. By contrast, $S_{cond}$ and the conductive ventilation heterogeneity portion of LCI, cannot be readily simulated unless complex patient-specific models are constructed [8].

Besides the increase of $S_{acin}$ with natural tidal breathing, its variability also increases considerably. Whilst the limits of normal for healthy reference data will take care of this inherent variability, it is tempting to suggest that encouragement to achieve slightly deeper breaths than their natural breathing may benefit MBW measurement variability. $S_{acin}$ values would indeed become smaller with greater $V_T$, but the $V_T$ compensation would work better as one moves away from natural tidal $V_T$, as is seen here in the higher $V_T$ range. The recent consensus statement suggested that when assessing $S_{acin}$ and $S_{cond}$, "an initial $V_T$ range of 10–15 $mL{\cdot}kg^{-1}$ can be used but may need to be adjusted for the individual patient depending on the expirogram seen" (table 5 in [5]). Our study, where $V_T$ was 9±2 $mL{\cdot}kg^{-1}$ for $MBW_{nat}$ and 16±3 $mL{\cdot}kg^{-1}$ for $MBW_{1L}$, shows the benefit of the larger $V_T$ in reducing $S_{acin}$ and $S_{cond}$ variability. Of note, the estimated population-based $V_T$ ranges encountered in recent normative studies were ∼13 $mL{\cdot}kg^{-1}$ in children [1] and ∼14 $mL{\cdot}kg^{-1}$ in adults [4], suggesting that this phenomenon may be less problematic in that paediatric age range (6–18 years) and in existing adult data. As an additional benefit, our data show that the larger $V_T$ reduces MBW test duration, *i.e.* time to achieve the end-of-test concentration threshold for LCI computation.

In conclusion, the quality control required for MBW indices is more complicated than for conventional lung function tests, partly because of $V_T$ effects, which may be amplified during natural breathing. In interventional studies, alterations in breathing pattern should be scrutinised when interpreting reported changes in $S_{acin}$ and $S_{cond}$, and identifying actual treatment effects. Adoption of procotols with a fixed $V_T$ (or higher $V_T$ range) rather than natural breathing in study populations where this is feasible, would be expected to decrease $S_{acin}$ and $S_{cond}$ variability, improve accuracy of any $V_T$ compensation applied, and also shorten MBW test duration. LCI variability is less affected by $V_T$ and is the more robust index, suggesting it may be more suitable across widely varying patient populations. Future research, within other age ranges and also in the setting of severe lung disease, are needed to examine the generalisability of these findings.

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