

## Repeatability and bronchodilator reversibility of lung function in young children

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**ABSTRACT:**

**Introduction:** Knowledge of short and longer-term repeatability of lung function in health and disease is essential to determine bronchodilator reversibility (BDR) thresholds and to recognise if changes in lung function represent disease progression, therapeutic intervention or normal variability.

**Methods:** Multiple-breath washout (MBW) indices (lung clearance index [LCI], conductive ventilation inhomogeneity [ $S_{\text{cond}}$ ]) and specific airways resistance ( $sR_{\text{aw}}$ ) were measured in healthy children and stable wheezers. Measurements were performed at baseline and after 20 minutes without intervention to assess repeatability and determine BDR thresholds. BDR was assessed by repeating baseline measurements 20 minutes after inhaled salbutamol.

**Results:** Twenty-eight healthy controls, mean age 6.1 (SD 0.7)y and 62 wheezers 5.4 (0.6)y were tested. Baseline variability in MBW indices and  $sR_{\text{aw}}$  was not significantly different between wheezers and healthy controls. Significant BDR was only observed in wheezers for  $S_{\text{cond}}$  (16%); but in both wheezers (37%) and healthy controls (20%) for  $sR_{\text{aw}}$ . Some wheezers and healthy controls demonstrated increases in MBW indices post-bronchodilator.

**Conclusions:** LCI and  $sR_{\text{aw}}$  demonstrate low baseline variability in health and disease. Neither MBW indices nor  $sR_{\text{aw}}$  are ideal for assessing BDR in young children with stable wheeze. These findings will help interpret effect of therapeutic interventions in children with respiratory diseases.

**Keywords:** Bronchodilator reversibility, multiple-breath washout (MBW), lung clearance index (LCI); preschool children, repeatability, specific airways resistance ( $sR_{aw}$ )

## INTRODUCTION

Multiple-breath washout (MBW) and plethysmographic specific airways resistance ( $sR_{aw}$ ) are popular techniques for measuring lung function in young children.[1,2] Lung clearance index (LCI) derived from MBW is being increasingly recommended as a sensitive outcome measure to assess the efficacy of early therapeutic intervention in children with cystic fibrosis.[3,4] However, the short-term variability of repeated measurements for MBW indices and  $sR_{aw}$  in this age group is unknown. It is essential to know the short and longer-term variability of lung function indices in health and disease to establish thresholds for bronchodilator reversibility (BDR) and to determine if changes in lung function are due to disease progression, therapeutic intervention or normal fluctuation.

Short-term repeatability reflects the variability of the measuring instrument and the biological variability in health and disease.[5] Only change greater than the short-term repeatability can be attributed to disease progression or a pharmacological intervention such as bronchodilator administration. Although BDR assessment is increasingly applied to assess the reversible component of airways obstruction in young children with wheeze, there is currently no consensus on what constitutes significant BDR with MBW indices and  $sR_{aw}$  measurements.[1]

The main aim of the present study was to establish short-term repeatability and determine thresholds for BDR for MBW indices and  $sR_{aw}$  in healthy children and clinically stable children with doctor-diagnosed recurrent wheeze aged 4-6 years. A secondary aim was to determine longer-term repeatability by determining the

between visit variability of baseline measurements. Some of the results in this study have been previously reported. [6]

## **SUBJECTS AND METHODS**

This prospective cross-sectional study was conducted at UCL Institute of Child Health (ICH), London, UK from October 2006 to August 2009. The Joint UCL/UCLH Ethics Committees approved the study (Ref. 05/Q0505/76). Parents gave informed written consent for their child to participate.

### **Subjects**

Healthy children and children with doctor-diagnosed recurrent wheeze aged 4-6 years were recruited to the study as described previously.[6-8]

### **Methods**

Children underwent either the repeatability or the BDR protocol, in random order on two separate occasions. All children had anthropometry measured and a clinical respiratory examination to ensure the absence of acute wheeze or URTI during the two test visits to the respiratory laboratory. For assessment of short-term repeatability, MBW indices (lung clearance index [LCI], conductive airways inhomogeneity [ $S_{\text{cond}}$ ], acinar airways inhomogeneity [ $S_{\text{acin}}$ ], and  $sR_{\text{aw}}$ ) were measured and repeated after 20 minutes, as described previously [6]. BDR was assessed by measuring MBW indices and  $sR_{\text{aw}}$  at baseline and 20 minutes after administration of inhaled salbutamol 200mcg via a spacer device. In wheezers, short-acting and long-acting bronchodilators were withdrawn for 8 hours and 24 hours, respectively, at the

time of the test. The baseline MBW and  $sR_{aw}$  measurements on the two different test occasions were used to assess the longer-term repeatability of these indices.

MBW was performed as previously described in young children.[6,9] Sulphur hexafluoride was the inert marker gas used for calculation of gas mixing indices reported in this study, as measured by a respiratory mass spectrometer (AMIS 2000; Innovision A/S, Odense, Denmark). LCI was calculated by dividing the cumulative expired volume by the functional residual capacity;  $S_{cond}$  and  $S_{acin}$  were estimated by calculating phase III slopes, as described previously.[10] The mean LCI,  $S_{cond}$  and  $S_{acin}$  from three technically acceptable wash-outs are reported.  $sR_{aw}$  was measured with a constant volume body plethysmograph (Master Screen Body Plethysmograph; VIASYS Healthcare, Hochberg, Germany), version 5.02. Children sat alone in the plethysmograph wearing a nose-clip. They were guided to breathe gently at a rate of 30-45 breaths per minute through the mouthpiece; three trials of 10 loops each were recorded. Results were excluded if fewer than five technically acceptable loops were obtained. The median total  $sR_{aw}$  from the three trials are reported. [11]

### **Statistical analyses**

The standard deviation (SD) of the within-child differences between 2 tests in the absence of any intervention on the same occasion was used to calculate the coefficient of repeatability ( $CoR = 1.96 \times SD$ ) in healthy children and those with wheeze. Bland-Altman limits of agreement (mean difference  $\pm$  CoR) were estimated and the lower limits used to determine thresholds for reversibility in health and disease.[12]

Paired t-tests were used to compare the differences between repeated baseline measurements, between pre- and post-bronchodilator measurements and also, for children who had both sets of measurements, the within-child differences between change during BDR and repeatability in absence of intervention. These tests were performed separately for the healthy controls and wheezers. Two sample t-tests were used to compare the differences between healthy controls and wheezers.

The within-child changes were analysed within multilevel models with factors for repeatability/BDR, healthy control/wheezers and the interaction between these. These models utilised the within-child pairing of measurements where they existed, but also allowed all available measures to contribute to the estimates of differences attributable to BDR or wheeze. Furthermore, the interaction terms provided formal comparison of whether any BDR/repeatability differences were significantly different between healthy controls and wheezers.

The limits of agreement, all differences and model coefficients are presented with 95% confidence intervals to illustrate the precision of our estimates (determined by the sample size).[12] The level of significance was set at  $p < 0.05$ . Data analyses were performed using SPSS software for Windows (version 15, SPSS Inc, IL, USA), MLWin v2.20 and GraphPad Prism (version 5, GraphPad Software, San Diego, CA, USA). The graphs were created using GraphPad.

**Power of study:** When we first set out to conduct the study, there were no studies in preschool or older children that had determined the repeatability of MBW indices in health or disease. The sample size of the current study was based on the primary outcome i.e. LCI and was informed by published data from our centre in healthy preschool children (mean age 4.1 years), in whom mean $\pm$ SD was 6.8 $\pm$ 0.4 for LCI [9] .

It was planned to recruit at least 30 wheezy children and 30 healthy controls to establish BDR thresholds. This would allow the limits of agreement to be estimated to within  $\pm 0.62SD$  and to detect differences of  $0.85SD$  with 90% power at the 5% significance level.

## RESULTS

Sixty-two children with recurrent wheeze (mean age 5.4 (SD 0.6) years; 39 males) and 28 healthy controls (mean age 6.1 (SD 0.7) years; 13 males) were recruited. Of those recruited, repeatability of lung function was assessed in 30 wheezers and 18 healthy controls (Table 1); BDR in 62 wheezers and 26 healthy controls (Table 2). Both sets of repeatability and BDR measurements were available in 30 wheezers and 16 healthy controls (Table 3). The interval between BDR and repeatability measurements for wheezers was median (IQR) 5.3 (2.2 – 7.8) months and for healthy controls 9 (2 – 11) months.

### Repeatability

The short-term variability of repeat lung function measurements in wheezers and healthy controls is shown in Table 1. There were no significant differences between the two sets of measurements in either group for any of the lung function outcomes (Table 1; Figure 1), nor any differences in change between the two sets of measurements according to health status (Table 1). Although the CoR was similar between wheezers and healthy controls for LCI and  $sR_{aw}$ , it was insignificantly larger for  $S_{cond}$  and  $S_{acin}$  in wheezers compared with healthy controls (Tables 1 and 3; Figure 1).

The longer-term variability of repeat lung function measurements in wheezers and healthy controls is shown in Table S1 (Online supplement). Other than a significant variability in  $S_{\text{cond}}$  measurements in wheezers, there were no significant differences between the two sets of measurements in either group for the lung function indices or any differences in change between the two sets of measurements according to health status (Table S1).

**Table 1: Repeatability measurements in all wheezers and healthy controls with Bland-Altman thresholds for reversibility**

Variable	Wheezers (n=30)				Healthy Controls (n=18)				Comparison of wheezers and controls
	Test 1 (T <sub>1</sub> )	Test 2 (T <sub>2</sub> )	(T <sub>2</sub> -T <sub>1</sub> )	Threshold for reversibility	Test 1 (T <sub>1</sub> )	Test 2 (T <sub>2</sub> )	(T <sub>2</sub> -T <sub>1</sub> )	Threshold for reversibility	Mean difference
<b>LCI</b>	7.15 (1.05)	7.22 (1.03)	0.07 (0.36) [-0.07, 0.20]	-0.63 [-0.40, -0.86]	6.50 (0.42)	6.51 (0.40)	0.01 (0.38) [-0.18, 0.19]	-0.74 [-0.43, -1.00]	-0.06 [-0.28, 0.17]
<b>S<sub>cond</sub></b>	0.022 (0.020)	0.030 (0.022)	0.008 (0.022) [-0.000, 0.016]	-0.035 [-0.025, -0.045]	0.007 (0.010)	0.010 (0.010)	0.003 (0.014) [-0.004, 0.011]	-0.024 [-0.010, -0.030]	-0.005 [-0.015, 0.006]
<b>S<sub>acin</sub></b>	0.060 (0.041)	0.064 (0.040)	0.004 (0.048) [-0.014, 0.021]	-0.089 [-0.059, -0.119]	0.053 (0.044)	0.049 (0.043)	-0.004 (0.027) [-0.018, 0.019]	-0.056 [-0.036, -0.076]	-0.008 [-0.029, 0.014]
<b>sR<sub>aw</sub> (kPa.s)</b>	1.14 (0.34)	1.13 (0.29)	-0.01 (0.11) [-0.05, 0.03]	-0.23 [-0.16, -0.30]	1.08 (0.19)	1.07 (0.20)	-0.01 (0.15) [-0.08, 0.07]	-0.30 [-0.17, -0.4]	-0.01 [-0.08, 0.09]

*Footnote: Results are expressed as mean (SD) or mean [95% Confidence interval]; The SD of within-child differences was used to calculate the coefficient of repeatability (CoR = 1.96 × SD). Bland-Altman limits of agreement (mean difference ± CoR) were estimated and the lower limit determined as the threshold for reversibility. If expressed as percentages, the thresholds for wheezers would be LCI -9%, S<sub>cond</sub> -159%, S<sub>acin</sub> -148%, sR<sub>aw</sub> -20% and for healthy controls LCI -11%, S<sub>cond</sub> -343%, S<sub>acin</sub> -106%, sR<sub>aw</sub> -28%. The last column compares the difference in thresholds for reversibility between the two groups. None of the differences were statistically significant.*

### **Bronchodilator reversibility**

Responses to bronchodilator in wheezers and healthy controls are shown in Table 2. Wheezers demonstrated a significant decrease in post-bronchodilator  $S_{\text{cond}}$  and  $sR_{\text{aw}}$  (Table 2), with 10 (16%) and 23 (37%) wheezers demonstrating BDR larger than the determined thresholds of reversibility for  $S_{\text{cond}}$  and  $sR_{\text{aw}}$ , respectively (Figure 2). Healthy controls also showed a significant decrease in post-bronchodilator  $sR_{\text{aw}}$  (Table 2), with 4 (20%) children demonstrating BDR larger than the determined threshold of reversibility (Figure 2). The wheezers showed a strong correlation between the baseline values and BDR for all MBW indices and  $sR_{\text{aw}}$ : Spearman's Rho for LCI was 0.64 ( $p < 0.005$ ),  $S_{\text{cond}}$  0.51 ( $p < 0.005$ ),  $S_{\text{acin}}$  0.426 ( $p < 0.005$ ) and  $sR_{\text{aw}}$  0.737 ( $p < 0.005$ ). A statistically significant, increase in post-bronchodilator LCI was seen in the healthy controls (Table 2), the increase in one child being above the upper 95% limit of agreement (LA) of 0.75 for healthy controls (Figure 2). Although there was no significant group change among the wheezers, an increase in LCI above the upper 95% LA (0.78 for wheezers) occurred in 6 (10%) wheezers (Figure 2). The healthy controls showed a strong correlation between the baseline values and BDR for LCI and  $sR_{\text{aw}}$ , but not for  $S_{\text{cond}}$  and  $S_{\text{acin}}$ : Spearman's Rho for LCI was 0.55 ( $p < 0.05$ ),  $S_{\text{cond}}$  -0.09 ( $p = 0.72$ ),  $S_{\text{acin}}$  0.01 ( $p < 0.98$ ) and  $sR_{\text{aw}}$  0.76 ( $p < 0.005$ )

**Table 2: Bronchodilator reversibility measurements in all wheezers and healthy controls**

Variable	Wheezers (n=62)			Healthy Controls (n=26)			Comparison of wheezers and controls
	Baseline	Post - BD	Mean difference	Baseline	Post-BD	Mean difference	Mean difference
<b>LCI</b>	7.14 (1.00)	7.05 (0.80)	-0.09 [-0.26, 0.09]	6.47 (0.47)	6.66 (0.51)	<b>0.19 [0.04, 0.33]*</b>	<b>0.28 [0.04, 0.49]*</b>
<b>S<sub>cond</sub></b>	0.040 (0.032)	0.030 (0.024)	<b>-0.010 [-0.018, -0.004]**</b>	0.018 (0.017)	0.015 (0.019)	-0.003 [-0.015, 0.008]	0.007 [-0.005, 0.021]
<b>S<sub>acin</sub></b>	0.062 (0.038)	0.055 (0.041)	-0.007 [-0.018, 0.006]	0.043 (0.029)	0.043 (0.024)	-0.000 [-0.014, 0.013]	0.007 [-0.012, 0.024]
<b>sR<sub>aw</sub></b> <b>(kPa.s)</b>	1.17 (0.29)	0.98 (0.24)	<b>-0.19 [-0.24, -0.14]**</b>	1.05 (0.22)	0.89 (0.18)	<b>-0.16 [-0.22, -0.09]*</b>	0.03 [-0.04, 0.11]

Footnote: Results are expressed as mean (SD) or mean [95% Confidence interval]; Significant results in **bold**; \*p<0.05; \*\* p<0.005. The last column compares the difference between the two groups in response to bronchodilator.

### **Comparison of differences between pairs of repeatability and BDR measurements in wheezers and healthy controls**

The data in Table 3 are restricted to the 46 children who had complete sets of data (i.e. those with both repeatability and BDR assessments). As when examining the whole group, a significant post-BDR reduction in mean  $sR_{aw}$  was seen in both wheezers and healthy controls with paired measurements. Similarly, a significant paradoxical increase in LCI was seen amongst the healthy controls but not wheezers. The difference between repeatability and BDR measurements did not, however, differ significantly between wheezers and controls for any indices (Table 3).

These data were further analysed by multilevel modelling. The multilevel regression model combined the analyses shown in Tables 1-3 and utilised all available measurements in each estimate, hence allowing greater precision. The results were similar to those above and therefore are not presented here in detail. In summary, there were no significant differences between repeated measurements for either wheezers or healthy controls, nor in the repeatability between each of these groups. The only significant overall difference observed post-BDR was with respect to  $sR_{aw}$  for which there was, on average, a reduction of 0.141 (0.057, 0.225) kPa.s when compared to repeatability data ( $p=0.001$ ), but this did not differ significantly between wheezers and controls ( $p=0.63$ ).

Table 3: Comparison of differences between pairs of repeatability and BDR measurements in wheezers and healthy controls

Variable	Wheezers (30)			Healthy Controls (16)			Comparison of wheezers and healthy controls
	Repeatability measurements	BDR measurements	Mean difference	Repeatability measurements	BDR measurements	Mean difference	Mean difference
<b>LCI</b>	0.07 (0.36)	-0.08 (0.65)	-0.14 [-0.45, 0.16]	-0.04 (0.34)	0.25 (0.37)	<b>0.29 [0.01, 0.57]*</b>	0.43 [-0.03, 0.89]
<b>S<sub>cond</sub></b>	0.008 (0.022)	-0.006 (0.024)	<b>-0.014 [-0.024, -0.003]*</b>	0.005 (0.014)	0.001 (0.023)	-0.004 [-0.019, 0.012]	0.010 [-0.008, 0.029]
<b>S<sub>acin</sub></b>	0.004 (0.048)	-0.008 (0.050)	-0.012 [-0.036, 0.012]	-0.008 (0.024)	-0.007 (0.022)	0.001 [-0.018, 0.021]	0.012 [-0.017, 0.043]
<b>sR<sub>aw</sub> (kPa.s)</b>	-0.01 (0.11)	-0.15 (0.16)	<b>-0.14 [-0.20, -0.08]**</b>	-0.01 (0.13)	-0.11 (0.13)	<b>-0.12 [-0.23, -0.01]*</b>	0.02 [-0.09, 0.14]

Footnote: Results are expressed as mean (SD) or mean [95% Confidence interval]; Significant results in **bold**; \* $p < 0.05$ ; \*\*  $p < 0.005$ . The last column compares differences between the two groups with respect to the response to bronchodilator over and above natural variability observed in the absence of any intervention.

## **DISCUSSION**

This is the first study to determine the normal variability of MBW indices and  $sR_{aw}$  in health and disease and establish thresholds for significant BDR in young children. We found that the coefficients of repeatability and the thresholds for reversibility for MBW indices and  $sR_{aw}$  were not materially different between healthy controls and stable wheezers. Significant BDR was seen only in some of the indices measured i.e., wheezers demonstrated significant BDR for  $S_{cond}$ ; and both wheezers and controls demonstrated significant BDR for  $sR_{aw}$ .

### **Repeatability**

The good short-term and longer-term repeatability and similarity in variability of repeat measures in wheezers and healthy controls for LCI suggests that variability of LCI is unaffected by health status during periods of clinical stability in wheezers. Although repeat measurements of  $S_{cond}$  and  $S_{acin}$  were not significantly different in either wheezers or healthy controls, the wide 95% CIs, particularly in wheezers, suggest that these indices are influenced more than LCI by the uneven gas distribution. The large baseline variability of these indices increases the threshold for determining significant BDR and reduces their capacity to discriminate between health and disease in terms of bronchial responsiveness. A number of physiological factors such as lung volume, inhomogeneous airway closure and changing tidal volume affect ventilation distribution, particularly those indices derived from phase III slope analysis.[13-15] Moreover, in obstructive airways disease the relationship between airways obstruction and ventilation inhomogeneity is heterogeneous, thus varying from one subject to another.[16] It is difficult to examine the effects of single variables on gas mixing as the variability between wheezers and healthy subjects is

probably multifactorial. It is speculated that this relates to inter-subject differences in site and extent of airways obstruction leading to differences in regional and inter-lobar distribution of time constants.[16]

Despite LCI being endorsed as a suitable tool to assess early intervention strategies in children with cystic fibrosis, there is a paucity of data on repeatability of MBW indices in children. Repeatability of MBW indices (LCI,  $S_{\text{cond}}$  and  $S_{\text{acin}}$ ) in a small group of adults with and without asthma showed good repeatability in both groups, albeit a higher variability of  $S_{\text{cond}}$  in the asthmatics compared to healthy controls, which is similar to our data.[17] Similarly, Fuchs et al showed low variability in repeat LCI measurements using the side-stream ultrasonic flow sensor in healthy subjects aged 5-20 years.[18] The hardware and software for MBW used in this study is identical to that used in the majority of published research studies in children, and at the time of our data collection, MBW by mass spectrometry was considered the gold standard. We are aware that two commercial nitrogen washout MBW devices are now available and that validation studies comparing these devices with mass spectrometer based systems are on-going. If the validations are successful, the findings of this study would be potentially applicable to these other devices.

There was a low variability in repeat  $sR_{\text{aw}}$  measurements in both wheezers and healthy controls. These results are in keeping with those of Klug et al who showed good short-term repeatability of  $sR_{\text{aw}}$  with an intra-class correlation coefficient of 0.84 in healthy children aged 2-7 years.[19] There are no studies assessing short-term repeatability of  $sR_{\text{aw}}$  in young wheezers or in older children with asthma.

The applicability of our results is limited by the smaller number of healthy controls studied compared to wheezers. A further limitation is that the range of within-subject variability varied between the selected indices of airway function and the sample sizes obtained were sometimes smaller than the planned 30, the smallest group being 16. Hence the confidence intervals for the thresholds were wider than expected. In addition, the power to detect the specified difference (0.85 SD) was reduced to 77% although differences of 1 SD or more were detected with 90% power, with the current sample size. Considering the age range targeted, it was logistically difficult to bring back the same children on two separate occasions, when they were free from upper respiratory tract infections for at least 3 weeks, within the time restraints of the study. Nonetheless, increased confidence in the results was obtained by undertaking multilevel modelling so that all available measurements could be taken into account; the results of this analysis supporting the findings from the multiple tests undertaken. The repeatability data facilitates better interpretation of BDR. A further strength is that the assessment of short-term repeatability was performed in healthy young children and wheezers of similar ages, on the same laboratory visit, by the same personnel over a short period of time, thereby eliminating operator variability.

### **Bronchodilator reversibility**

Among the MBW indices, although a statistically significant BDR in wheezers was seen only for  $S_{\text{cond}}$ , only 16% demonstrated significant BDR larger than the determined threshold. Furthermore, the large baseline variability in repeat measures of  $S_{\text{cond}}$ , and the time required to obtain these measurements [20] does not make this a robust index to assess BDR. Of potential interest, slightly more individuals than

expected showed an increase in post bronchodilator MBW indices outside the determined BDR thresholds. It has been suggested that inhaled bronchodilator aerosols have a primary effect on low-resistance pathways and are thus mainly delivered to well-ventilated lung units.[21,22] This distribution of the aerosol would result in further increase of ventilation inhomogeneity, even though airway resistance might fall.[22] These results suggest that bronchodilator administration may have favourable or unfavourable effects on gas mixing, making MBW an unsuitable technique to assess BDR.

Significant BDR for  $sR_{aw}$  was seen in both wheezers and healthy controls, undermining the discriminating capacity of this technique in young children. Several studies have shown that changes in lung function after bronchodilator administration does not significantly differ between healthy and clinically stable asthmatic children in the younger ages.[23,24]

It is known that BDR is significantly influenced by the degree of baseline impairment in lung function [25] and similarly the wheezers with poorer lung function in our study demonstrated greater BDR in all the indices. We also found this relation in healthy controls, except for the slope indices.

The main limitation of BDR assessment in this study is that the effect of bronchodilator was not compared to placebo, to eliminate effects of using the inhaler and spacer and any possible effects of the propellant. It could be argued that 200 mcg of salbutamol is insufficient to fully reverse the baseline inefficient gas mixing in wheezers. Ideally dose response curves should have been used, but this is not feasible in young children. Nevertheless, a number of studies in children and adults with stable asthma have demonstrated up to 20% improvement in forced expiratory

flow volumes on spirometry with salbutamol doses of 100 mcg [26] and 200 mcg.[27,28] Besides, studies that have investigated the effect of bronchodilation on  $sR_{aw}$  in young children have demonstrated reversibility with 500 mcg of inhaled terbutaline (equivalent to 200 mcg of salbutamol).[25,29] However, in the absence of dose response curves, it is not possible to exclude the possibility that the degree of reversibility has been underestimated. Allowing for these limitations, our study describes novel data on the effects of bronchodilator on MBW indices and  $sR_{aw}$  in young healthy children and those with wheeze.

In conclusion,  $sR_{aw}$  and LCI have low variability in health and disease, but  $S_{cond}$  and  $S_{acin}$  show higher baseline variability in disease compared to health. MBW indices are not ideal to assess BDR because of large baseline variability of repeat measurements particularly in wheezers and unpredictability of gas mixing and distribution after bronchodilator administration. The capacity of  $sR_{aw}$  to discriminate between healthy young children and children with stable wheeze according to BDR is doubtful because of the large overlap in response between the two groups. These findings will inform choice of outcomes when attempting to assess BDR in young children and help interpret the effect of therapeutic interventions in children with respiratory diseases.

## **ACKNOWLEDGEMENTS**

The authors thank Dr Per Gustafsson for his on-going support with the MBW system and all the parents and children who participated in the study.

## **COMPETING INTERESTS**

None

## **FUNDING**

This study was supported by Asthma UK, the European Respiratory Society and Smiths Medical UK. AB was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College, London.

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