Methodology:

Multiple-breath washout equipment:

The gas analyser used in this study was an AMIS 2000 respiratory mass spectrometer (Innovision, Odense, Denmark). The AMIS 2000 is a quadropole mass spectrometer, which operates by identifying gases according to their mass-charge ratio. The AMIS 2000 was two-point calibrated once daily using certified concentration gas (alpha-gravimetric standard, British Oxygen Company, Guildford, UK) containing 3.97% He, 3.98% SF_6, 7.04% CO_2, 21% O_2 and 64% N_2. A signal-noise ratio of 100 or greater was deemed acceptable. This full calibration included an automatically performed re-optimisation of atomic mass unit peaks. A short one-point calibration was performed prior to each measurement.
Analogue outputs from the demodulator and from the mass spectrometer were recorded at 100Hz by a personal computer (Dell Computers, Round Rock, TX, USA) through a 16-channel AD-conversion board (Model RS485, Keithley Metrabyte, Taunton, MA, USA) using custom written software based on a commercially available data acquisition software pack (TestPoint, Capital Equipment Corp., Billerica, MA, USA).

The pneumotachometer was calibrated prior to use with separate calibration constants for inspiratory and expiratory flows using a 241ml precision syringe. Recorded inspiratory and expiratory flows and volumes were converted to BTPS conditions. Gas samples and flow signals were aligned in time. Delay to the gas signal was measured using a custom-made delay switch (manufactured by Mr E Bergsten, Swedish Defense Research Agency, Department of Defense Medicine, Linköping, Sweden). This system measured the delay between gas appearing at the capillary inlet of the mass spectrometer (enabled by opening the switch) and that gas bolus being recorded by the software. Once daily a series of 20 delay recordings were performed, and the median delay and rise times obtained were used to align flow and gas signals from subsequent recordings. The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. Prior to use the pneumotachometer was heated to 37°C (Heater model FWS4D, Hugo Sachs Elektronik, March, Germany).

Analysis of washout curve

The *functional residual capacity* (FRC) was determined from the cumulative exhaled SF_6 divided by the difference in end-tidal SF_6 concentration at the start of the washout and end-tidal SF_6 concentration at completion of the washout. A proportion of the marker gas expired at each breath remained within the post-capillary apparatus dead-space at the end of expiration and was re-inspired on the next breath. This re-inspired marker gas was measured, and the calculation of cumulative exhaled marker gas was corrected accordingly. The calculated FRC therefore excludes the post-capillary apparatus dead-space, but includes the pre-capillary apparatus dead-space.

The *cumulative expired volume* (CEV) from the beginning to the end of the washout was calculated by integration of the flow signal. The post-capillary apparatus dead-space was subtracted from each breath, so that the CEV, like the FRC, was corrected for post-capillary apparatus dead-space, but not for pre-capillary apparatus dead-space.

The *lung clearance index* (LCI) was calculated by dividing the CEV by the FRC. LCI therefore represents the number of FRC turnovers (TO) required to dilute the inspired SF_6 to $1/40^{th}$ of its starting concentration.

$$LCI = \frac{CEV}{FRC}$$

Where

LCI = Lung Clearance Index

CEV = Cumulative Expired Volume

FRC = Functional Residual Capacity

The FRC and LCI were calculated, by the method described, using custom-written software (TestPoint, Capital Equipment Corp., Billerica, MA, USA). FRC variability had to be less than

10% between runs on the same test occasion, to be accepted. The child had to perform 3 acceptable runs to calculate all MBW indices and for slope analysis had to have at least 6 lung volume turnovers.

Calculation of phase III slopes

To calculate phase III slopes, for each breath the SF_6 concentration was plotted as a function of expired volume. A least-square fit of the slope of the alveolar phase (phase III slope, SIII) was done. The margins of the regressed portion were manually adjusted to avoid phase II or phase IV elements, the influence of cardiogenic oscillations or signal noise obviously distorting the slopes. The SIII was then normalised by the mean tracer gas concentration over the phase III interval of interest, to account for dilution, giving the normalised phase III slope (SnIII) for SF_6.
S_{acin} and S_{cond} were calculated by the method described by Verbanck et al [1] and modified for application in preschool children in our laboratory.[2] The SnIII for each breath was plotted against turnover (TO). In each case an aggregate SnIII versus TO plot was produced from results obtained from 3 MBW runs. S_{cond} is defined as the normalised slope difference per unit TO over the portion of the MBW where only conducting airways contribute to the generation of ventilation inhomogeneity. S_{acin} was determined by subtracting that part attributable to the conducting airways from the slope of the first breath.

The phase III slopes to calculate S_{cond} and S_{acin} were analysed according to the following criteria:

- A minimum expired breath volume from which SnIII could commence and minimum total breath volume as defined according to subject current weight (kg) were met.

  Minimum starting point = (2 × Wt. + 12.5 [precapillary dead-space]) × 2 (ml)

  Minimum breath volume = (3.5 × Wt. + 12.5 [precapillary dead-space]) × 2 (ml)

- SnIII was not reported from any breaths where it was not clearly visible, whether because of inadequate breath volume or signal noise.

  If the first breath from a run could not be reported, or if more than one third of breaths over the S_{cond} regression interval could not be reported, then that MBW was excluded from analysis. S_{cond} and S_{acin} are only reported if results from three MBW runs were available, after normalising for tidal volume.

Table S1: Comparison of longer-term repeatability measurements between wheezers and healthy controls

Variable	Wheezers (n=30)			Healthy Controls (n=16)			Comparison of wheezers and controls
	Test 1 (T_1)	**Test 2 (T_2)**	**(T_2-T_1)**	**Test 1 (T_1)**	**Test 2 (T_2)**	**(T_2-T_1)**	**Mean difference**
LCI	7.15 (1.05)	7.20 (0.97)	0.05 (1.15) [-0.48, 0.38]	6.50 (0.42)	6.48 (0.43)	-0.02 (0.5) [-0.27, 0.31]	0.07 [-0.44, 0.58]
S_{cond}	0.022 (0.020)	0.035 (0.032)	**0.012 (0.031) [-0.024, 0.001]***	0.007 (0.010)	0.013 (0.012)	0.006 (0.017) [-0.015, 0.035]	0.006 [-0.007, 0.021]
S_{acin}	0.060 (0.041)	0.064 (0.043)	0.004 (0.052) [-0.023, 0.014]	0.053 (0.044)	0.052 (0.026)	-0.001 (0.049) [-0.024, 0.031]	0.003 [-0.025, 0.040]
sR_{aw} (kPa.s)	1.14 (0.34)	1.19 (0.32)	0.05 (0.32) [-0.17, 0.07]	1.08 (0.19)	1.00 (0.22)	-0.08 (0.23) [-0.05, 0.21]	0.13 [-0.03, 0.31]

*Footnote: Results are expressed as mean (SD) or mean [95% Confidence interval]. Significant results in **bold;** *p<0.05. The last column compares the difference between the mean differences of the two groups. None of the differences were statistically significant.*

References

1. Verbanck S, Schuermans D, Noppen M, Van Muylem A, Paiva M, Vincken W. Evidence of acinar airway involvement in asthma. *Am J Respir Crit Care Med* 1999; 159: 1545-1550.

2. Aurora P, Kozlowska W, Stocks J. Gas mixing efficiency from birth to adulthood measured by multiple-breath washout. *Respir Physiol Neurobiol* 2005; 148: 125-139.