1. Multiple-breath washout measurements using a ultrasonic flowmeter.

Ultrasonic flow meters allow for measuring simultaneously airflow and tracer gas concentration, employing a single sensor. The measuring principle of an ultrasonic flow meter, as shown in Fig. S1, is based on two physical laws: (i) sound travelling through a streaming medium is accelerated or slowed down by the movement of the medium and (ii) sound speed (c) in an ideal gas is given by

\[
c = \sqrt{\gamma \cdot \frac{R \cdot T}{MM}}
\]  

(S1)

where T is the temperature, R the molar gas constant, MM the molar mass of the gas and \( \gamma \) the adiabatic index. Thus, the flow rate \( V'(t) \) is directly proportional to the difference between both transit times \( t_1 \) and \( t_2 \) (Fig. S1) while the molar mass of the gas can be calculated from the sum of \( t_1 \) and \( t_2 \). The computation of the molar mass, however, requires the knowledge of the precise temperature along the sound transmission path. As shown in Fig. S1 there is a distinct temperature gradient between body and ambient temperature and there are also changes in temperature and humidity of the breathing gas during tidal breathing. This requires adjustments of the raw measurement signal for humidity and temperature. Due to the complexity of the temperature along the sound transmission path the manufacturer of the ultrasonic flowmeter developed a temperature simulation model which accounts for the changes in temperature along the flow sensor and changes in the MM signal during inspiration and expiration. This model divided the total volume in 5 compartments (2 body volumes and 3 dead space volumes) with special temperature adjustments. While the dead space volumes VolSens 1, 2 and 3 (Fig. 1s) are real volumes, both body volumes are only
theoretical model parameters and cannot be allocated to an exactly defined anatomical volume. In a clinical study, optimized temperature and dead space settings were derived to improve the stability and robustness of FRC and LCI measurements in infants (Latzin et al. 2007).

An important problem of any ultrasonic flow meter arises from positions of the sound transducers outside the flow path (Fig S1.) resulting in an uneven gas mixing within the flow meter during tracer gas washin and washout. Accordingly, the step response of the molar mass signal is delayed and requires a further numerical signal correction being part of the software.

![Ultrasonic flow meter diagram with face mask and temperature profile](image)

**Fig. S1:** Ultrasonic flow meter with face mask and the temperature profile of the breathing gas: $t_1, t_2$ - ultrasound transit times, VolSen1 - 3 - ultrasonic sensor dead space volumes

Beside the commonly used BTPS (body temperature, ambient pressure, saturated with water vapor) correction of the displayed volumes, a ultrasonic flow meter requires several numerical corrections and parameter adjustments of the raw measuring signal to obtain
reliable MBW measurements. In our study we have performed all measurements with the parameter adjustments provided by the manufacturer including the use of the updated temperature model (Latzin et al 2007).

2. Results

2.1. MBW measurements

Table S1: Patient characteristics of the total study population (N=150) during the neonatal period and at the time of measurement

|                                | No (%)          | Median (IQR)          |
|--------------------------------|-----------------|-----------------------|
| **Neonatal period**            |                 |                       |
| Male                           | 88 (57%)        |                       |
| Gestational age (weeks)        |                 | 28 (26 - 31)          |
| Birth weight (g)               |                 | 950 (755 - 1470)      |
| Birth weight z-score           |                 | -0.18 (-0.86 - 0.37)  |
| Birth weight <1000 g           | 87 (58%)        |                       |
| Fetal lung maturation<sup>1)</sup> | 109/148 (74%)  |                       |
| Surfactant administration<sup>1)</sup> | 96/146 (66%) |                       |
| Mechanical ventilation >24h<sup>1)</sup> | 76/149 (51%) |                       |
| Duration of mechanical ventilation (days) | 14.5 (7 - 26) |                       |
| **At day of measurement**      |                 |                       |
| Age (days)                     | 137 (104 - 166) |                       |
| Postmenstrual age (weeks)      | 49.2 (43.4 - 51.7) |                      |
| Body weight (g)                | 4605 (4000– 5405) |                      |
| Body weight z-score            | -0.36 (-1.17 - 0.4) |                      |
| Body length (cm)               | 56.0 (53.0 – 60.0) |                      |
| Body length z-score            | -0.12 (-1.11 - 0.57) |                      |

Data represent median (interquartile range) or n (%).
<sup>1)</sup> Total number is reduced because some data of outpatients were incomplete
**Table S2**: Results of MBW measurements of all enrolled infants (N=150)

| MBW measurements |          |          |
|------------------|----------|----------|
| $V_D$ (mL)       | 4.94     | (3.2 - 8.1) |
| $V_D$ (mL/kg)    | 1.10     | (0.79 - 1.61) |
| $V_T$ (mL)       | 36.8     | (27.5 - 48.4) |
| $V_T$ (mL/kg)    | 7.77     | (6.50 - 9.59) |
| FRC (mL)         | 98.5     | (84.2 - 125.1) |
| FRC (mL/kg)      | 22.1     | (18.4 - 25.8) |
| $V_D/V_T$        | 0.15     | (0.11 - 0.20) |
| $V_D$/FRC        | 0.051    | (0.031 - 0.081) |
| $V_T$/FRC        | 0.36     | (0.30 - 0.47) |
| LCI              | 6.13     | (5.64 - 6.83) |
| Predicted LCI by modeling | | |
| LCI\_predicted | 5.28     | (4.88 - 5.79) |

**Blood gases**

|          |          |
|----------|----------|
| $p_{O_2}$ (mmHg) | 61.8     | (54.0 - 73.0) |
| $p_{CO_2}$ (mmHg) | 44.7     | (42.1 - 49.6) |

Data represent median (interquartile range).
2.2. Multiple linear regression analysis

A multiple linear regression analysis was used to investigate the relationship between LCI and the different influencing factors. For the initial regression model all patient characteristics, lung volumes and their volume ratios were considered.

**Table S3.** Linear regression analysis using patient characteristics, lung volumes and derived parameters (initial model with 15 independent variables).

|                     | LCI                        | T - Statistics | p-value |
|---------------------|----------------------------|----------------|---------|
| Gestational age (weeks) | 1.372                      | 0.172          |         |
| Birth weight (g)     | 1.447                      | 0.150          |         |
| Birth weight z-score | 1.285                      | 0.201          |         |
| Age (days)           | 1.150                      | 0.252          |         |
| Postmenstrual age (weeks) | 0.997                    | 0.321          |         |
| Body weight (g)      | 0.352                      | 0.725          |         |
| Body weight z-score  | 0.376                      | 0.708          |         |
| Body length (cm)     | 0.611                      | 0.542          |         |
| Body length z-score  | 0.615                      | 0.540          |         |
| V_D (mL)             | 1.509                      | 0.134          |         |
| V_T (mL)             | 2.884                      | **0.005**      |         |
| FRC (mL)             | 2.767                      | **0.007**      |         |
| V_D/V_T              | 5.015                      | <0.001         |         |
| V_D/FRC              | 2.529                      | **0.013**      |         |
| V_T/FRC              | 5.573                      | <0.001         |         |
| Constant             | 0.710                      | 0.479          |         |

Significant p-values are printed in bold.
In multivariate regression analysis, of the 15 included variables only the lung volumes and their volume ratios showed a significant impact. The $R^2$ statistic indicates that the model as fitted explains 81.8% of the variability in LCI. A backward stepwise selection was used to obtain a final model with the most important variables that were significantly associated with the LCI (cut off p-value < 0.01). The results are shown in table S4.

Table S4. Results of the backward stepwise selection (final model)

|                | LCI                      | T - Statistics | p-value |
|----------------|--------------------------|----------------|---------|
| $V_T/FRC$      |                          | 14.417         | <0.001  |
| $V_D/V_T$      |                          | 10.489         | <0.001  |
| Constant       |                          | 23.248         | <0.001  |

Significant p-values are printed in bold.

Of the 15 investigated variables only the two volume ratios $V_T/FRC$ and $V_D/V_T$ remained in the final model. The coefficient of determination ($R^2$) of the final model was 78.5%.

2.2. Multivariate Analysis of Variance

To investigate the impact of patient characteristics at birth and at the day of measurements on the LCI difference between infants without and with wheezing, a multivariate analysis of variance (MANOVA) was performed. The results are shown in Table S4. There was no statistical significant impact of the patient characteristics on the LCI while the differences between non-wheezers and wheezers remained statistically significant.
**Table S4:** Multivariate analysis of variance (MANOVA) for LCI considering patient characteristics as covariates

| Source                      | F-ratio | p-value |
|-----------------------------|---------|---------|
| **COVARIATES**              |         |         |
| Gestational age             | 0.37    | 0.543   |
| Birth weight                | 0.41    | 0.524   |
| Birth weight z-score        | 0.18    | 0.675   |
| Age                         | 0.47    | 0.494   |
| Postmenstrual age           | 0.37    | 0.543   |
| Body weight                 | 0.53    | 0.467   |
| Body weight z-score         | 0.25    | 0.621   |
| Body length                 | 0.00    | 0.992   |
| Body length z-score         | 0.05    | 0.828   |
| **MAIN EFFECTS**            |         |         |
| Expiratory wheezing         | **4.96**| **0.029**|

All F-ratios are based on the residual mean square error. The P-values test the statistical significance of each of the factors.