

# Multiple Breath Washout With a Sidestream Ultrasonic Flow Sensor and Mass Spectrometry: A Comparative Study<sup>†,‡</sup>

Susanne I. Fuchs, MD,<sup>1</sup> Christian Buess, PhD,<sup>2</sup> Sooky Lum, PhD,<sup>3</sup> Wanda Kozłowska,<sup>3</sup>  
Janet Stocks, PhD,<sup>3</sup> and Monika Gappa, MD<sup>1\*</sup>

**Summary.** Over recent years, there has been renewed interest in the multiple breath wash-out (MBW) technique for assessing ventilation inhomogeneity (VI) as a measure of early lung disease in children. While currently considered the gold standard, use of mass spectrometry (MS) to measure MBW is not commercially available, thereby limiting widespread application of this technique. A mainstream ultrasonic flow sensor was marketed for MBW a few years ago, but its use was limited to infants. We have recently undertaken intensive modifications of both hardware and software for the ultrasonic system to extend its use for older children. The aim of the current in vivo study was to compare simultaneous measurements of end-tidal tracer gas concentrations and lung clearance index (LCI) from this modified ultrasonic device with those from a mass spectrometer. Paired measurements of three MBW, using 4% sulfur hexafluoride (SF<sub>6</sub>) as the tracer gas and the two systems in series, were obtained in nine healthy adult volunteers. End-tidal tracer gas concentrations (n = 675 paired values) demonstrated close agreement (95% CI of difference -0.23; -0.17%, r<sup>2</sup> = 1). FRC was slightly higher from the MS (95%CI 0.08; 0.17 L), but there was no difference in LCI (95%CI -0.10; 0.3). We conclude, that this ultrasonic prototype system measures end-tidal tracer gas concentration accurately and may therefore be a valid tool for MBW beyond early childhood. This prototype system could be the basis for a commercial device allowing more widespread application of MBW in the near future. **Pediatr Pulmonol.** 2006; 41:1218–1225.

© 2006 Wiley-Liss, Inc.

**Key words:** multiple breath washout; validation; mass spectrometer; molar mass; lung clearance index; comparison; ultrasonic flow sensor; child; ventilation inhomogeneity.

## INTRODUCTION

While the potential use of the multiple breath inert gas wash-out technique (MBW) as a sensitive measure of small airways disease has been recognized for many years,<sup>1–6</sup> clinical applications, particularly in children, have been limited. During recent years, advances in

computer technology, combined with increased awareness of the need to detect and treat signs of early lung disease as soon as possible if lung health is to be optimized throughout life has led to renewed interest in the use of this technique.<sup>7–16</sup> MBW is a non-invasive technique, which is performed during tidal breathing and, as such, is potentially applicable from birth to old age. In addition to

<sup>‡</sup>Results were presented at the annual meeting of the European Respiratory Society 2005 in Copenhagen and the Gesellschaft Paediatrische Pulmologie 2006 in Frankfurt am Main, Germany.

Grant sponsor: Mukoviszidose e.V. Germany; Grant sponsor: CF Trust UK (to W.K.).

\*Correspondence to: Prof. Monika Gappa, M.D., Department of Paediatric Pulmonology and Neonatology, Medizinische Hochschule Hannover, D- 30623 Hannover.

E-mail: gappa.monika@mh-hannover.de; monika.gappa@web.de

Received 7 March 2006; Revised 9 August 2006; Accepted 10 August 2006.

DOI 10.1002/ppul.20524

Published online in Wiley InterScience  
(www.interscience.wiley.com).

<sup>1</sup>Department of Paediatric Pulmonology and Neonatology, Medizinische Hochschule Hannover, Germany.

<sup>2</sup>ndd Medical Technologies, Zurich, Switzerland.

<sup>3</sup>UCL, Institute of Child Health, Portex Unit; Respiratory Physiology, London, United Kingdom.

<sup>†</sup>The study was planned and initiated at Hannover and realized in London. S.F. and C.B. travelled to London to assess the feasibility of performing such a study and to prepare the equipment for the comparative measurements. S.F. and M.G. spent several days in London for the comparative measurements.

measuring functional residual capacity (FRC), various indices of ventilation inhomogeneity (VI) such as the lung clearance index (LCI) can be calculated from MBW. In contrast to most conventional lung function parameters, LCI does not change with age,<sup>3,17</sup> thus facilitating longitudinal assessment across all age groups.<sup>11,12</sup> Calculation of any parameter of VI requires accurate breath-by-breath gas concentrations throughout the wash-out. Although the respiratory mass spectrometer (MS) has been the most commonly used gas analyser for these assessments, and is still considered to be the gold standard, there are no commercially available systems that incorporate appropriate software for data collection and analysis.<sup>7</sup> In addition, mass spectrometry is expensive, non-portable and requires considerable technical skills, thereby limiting its potential use for routine clinical application.

In an attempt to meet this need, a commercial device (Exhalyzer D, Eco Medics AG, Duernten, Switzerland) which uses a mainstream ultrasonic device (Spirosion<sup>®</sup>, nnd Medical Technologies, Zurich, Switzerland) to measure molar mass (MM) of the respired gas breath by breath as a reflection of the tracer gas concentration was marketed several years ago for use in infants and very young children.<sup>16,18</sup> Attempts to adapt the Spirosion<sup>®</sup> with the Exhalyzer D equipment for use in older children revealed various problems with respect to both the mainstream ultrasonic signal and the underlying algorithms.<sup>19,20</sup> Furthermore, the existing hardware, particularly the bias flow, could not be adapted for use in older subjects with higher airflows.<sup>21</sup> Consequently, a new ultrasonic flow sensor (USFS) prototype system based on the Spirosion<sup>®</sup>, was developed by S.F., M.G., and C.B. This included the introduction of a sidestream ultrasonic flow sensor (which, unlike the mainstream device, is not influenced by temperature and humidity of the respired gas) and a valve system to adapt the bypass flow. The software was also changed to allow (a) longer sampling times necessary in older subjects, (b) more accurate identification of end of wash-in and wash-out periods, (c) correction for exhaled carbon dioxide (CO<sub>2</sub>), and (d) detailed validation and adaptation of the algorithms used for calculating FRC and LCI.

This prototype system has subsequently been used to perform MBW in children with and without lung disease, yielding results comparable with those published using mass spectrometry.<sup>22</sup>

Several published studies have used the Exhalyzer D equipment for MBW in infants,<sup>16,18,23–25</sup> Only one of these studies has attempted to validate the system against a MS in vivo; and this comparison was based on sequential measurements without any direct comparisons of end-tidal tracer gas concentrations.<sup>18</sup> In vitro studies using calibrated syringes or a lung model are not appropriate when validating the US system, since analysis of the molar

mass signal involves correction for temperature and humidity.

The aim of the present study was to (a) make direct in vivo comparisons of end-tidal tracer gas concentrations using the mass spectrometer and USFS prototype system, and (b) to see the potential impact of any differences by comparing values of FRC and LCI derived from each system.

## MATERIALS AND METHODS

### Ultrasonic Technology

The ultrasonic technology has been described previously,<sup>26,27</sup> and is based on the principle that sound, travelling through a streaming medium, is sped up or slowed down by the movement of the medium.

The mainstream ultrasonic flow sensor (Spirosion<sup>®</sup>, nnd Medical Technologies, Zurich, Switzerland) contains two ultrasonic transducers which are mounted on opposite sides of the flow tube. Both transducers emit ultrasonic pulses travelling through the streaming inspired and expired air. The moving air causes an increase in the upstream and a decrease in the downstream transit time of the ultrasound. The change in transit times is related to the gas velocity of the air flow thus allowing measurement of flow and volume. In addition, molar mass (MM) of the inspired and expired air is computed simultaneously breath by breath from the transit times, which is the prerequisite for assessment of parameters of ventilation inhomogeneity. MM values are directly proportional to the density of the gas mixture and should thus reflect tracer gas concentration.

Initial attempts to adapt the existing infant equipment for use in older subjects proved unsuccessful. Substantial changes in both hardware and software (S.F., C.B., and M.G.) resulted in the USFS prototype system used in the present study. *Changes to the hardware* included introduction of a sidestream ultrasonic transducer connected via a nafion tube that allows the exhaled air to equilibrate with room air, thereby precluding the need to correct for changes in temperature and humidity. Introduction of a CO<sub>2</sub> analyser (DUET ET CO<sub>2</sub> Module, Welch Allyn OEM Technologies, Beaverton, OR) into the system allowed simultaneous measurement of exhaled CO<sub>2</sub> and subsequent correction of the molar mass signal for influences of CO<sub>2</sub>. This was necessary because, in contrast to mass spectrometry which directly measures gas concentrations, the molar mass signal represents a sum signal of all gases in the respired air.

The existing Exhalyzer D equipment for infants allows a maximal bias flow of 250 ml/sec. Adaptation of the bias flow system for use in older subjects by using an admixture of sulfurhexafluoride (SF<sub>6</sub>) to room air proved unsuccessful, with highly unstable tracer gas concentrations both within and between tests, such that meaningful analysis of

the data was impossible. The prototype system has a bias flow system that delivers a constant mixture of tracer gas during wash-in, containing 4% SF<sub>6</sub>, 21% oxygen and balance nitrogen from a premixed gas cylinder. A three-way valve system provides valve-controlled tracer gas delivery during wash-in and a supply of room air during wash-out.

WBreath, the software developed and adapted for the current USFS equipment, was used for data acquisition, storage and analysis (nnd Medical Technologies, Switzerland). *WBreath software modifications* included post-data collection correction for the time delay of the new side-stream signals; calculation of FRC by subtraction of the external pre-capillary dead space; calculation of cumulative expired volume (CEV) whereby external dead space outside the lips is subtracted from each expired breath volume; calculation of the LCI as the CEV divided by the FRC as described for the mass spectrometer;<sup>28</sup> and increased sampling time. The latter was necessary for older subjects and patients with obstructive changes, due to the prolonged period required for completion of wash-in and wash-out compared with infants.

One major issue with using the molar mass signal from USFS devices is that it does not measure absolute gas concentration: The original software algorithms assumed the starting concentration of the tracer gas at start of wash-out to be 100% (i.e., 4% SF<sub>6</sub>), even if wash-in was incomplete such that maximal inspired tracer gas concentration remained below 4%. However, this leads to false calculation of FRC and parameters of VI.

Following introduction of the side-stream transducer and the new bias flow system, the signal quality was improved such that end of wash-in and wash-out periods could be identified much more clearly.

The Portex Respiratory Unit at the Institute of Child Health in London has been applying MBW with a mass spectrometer for several years.<sup>11,12,17,18</sup> As the USFS prototype system is portable, this collaborative study was undertaken in London.

Configuration of the MS system in London and the USFS prototype system from Hannover remained essentially unchanged during the simultaneous comparative in vivo measurements, except that they were connected by an adapter. The Spiroson<sup>®</sup> includes an integrated mouthpiece which is part of the disposable hygienic breathing tube of the equipment (Spirette). The adapter, which was mounted on the distal part of the Spiroson<sup>®</sup> was fitted with two sampling needles allowing simultaneous gas sampling from the mid sensor point for the sidestream molar mass (MMss) signal and the MS.

For the MMss an automatic gas pump sampled respired air (2–4 ml/sec) through a nafion tube into an external ultrasonic transducer working as described above (nnd Medical Technologies, Zurich, Switzerland). The corre-

sponding changes in flow and volume were still assessed from the mainstream ultrasonic flow sensor, since these signals are unaffected by changes of temperature and humidity.

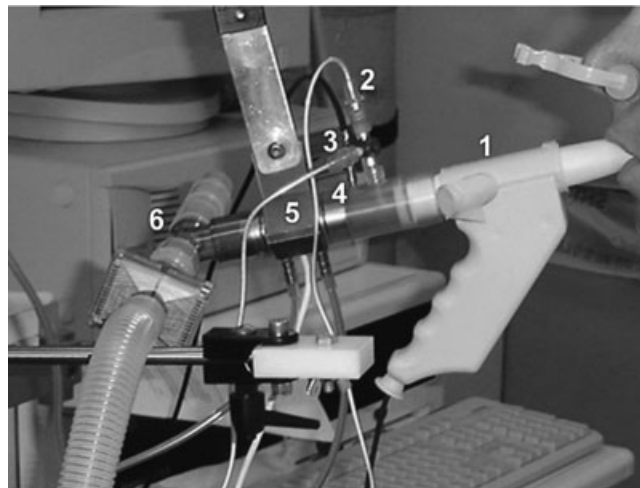
In order to correct the molar mass signals for exhaled CO<sub>2</sub> concentrations, an additional sampling tube, leading to an infrared analyzer (DUET ETCO<sub>2</sub> Module, Welch Allyn OEM Technologies, Beaverton, OR), was attached via a three way adapter to the the sampling tube for MMss.

For the MS, an automatic gas pump sampled respired air (20 ml/sec) through a tube into the MS (Amis 2000, Innovision A/S, Odense, Denmark). Thus, side stream molar mass with corresponding ultrasonic flow measurement, together with MS tracer gas and exhaled CO<sub>2</sub> concentrations, were sampled at the mid sensor point, near to the airway opening (Fig. 1). As shown in Figure 2, mainstream molar mass and the imported CO<sub>2</sub> signal from the mass spec were also recorded in WBreath, but were not analyzed for the present study.

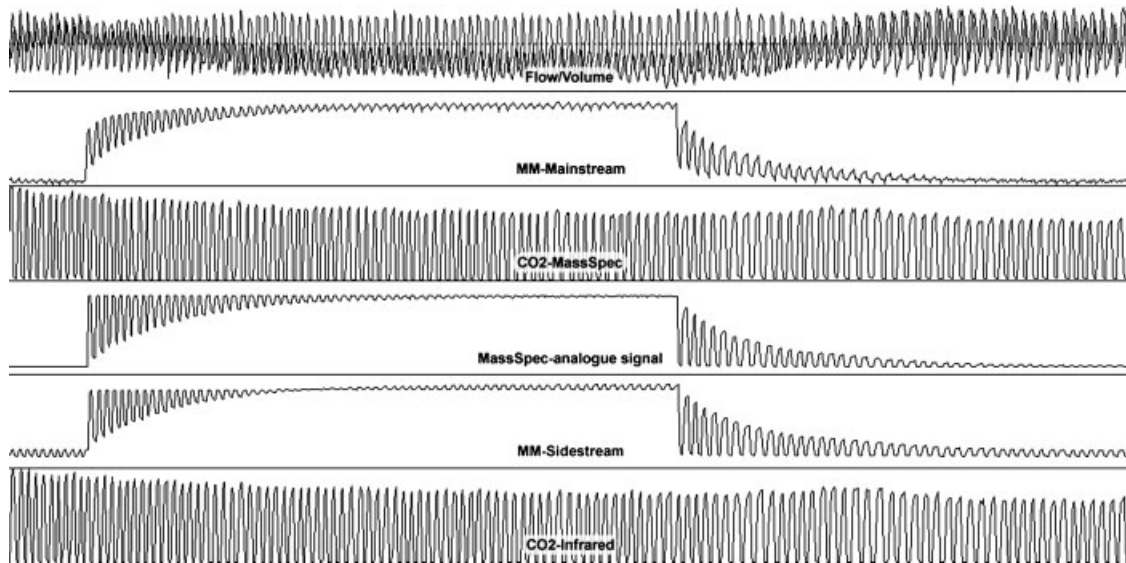
A heated Fleisch No.1 pneumotachograph (PNT) (Metabo SA, Epalinges, Switzerland), measuring flow for MS analysis, was attached distal to the adapter. The gas bias flow system was connected to the distal end of the PNT, providing valve-controlled tracer gas delivery during wash in and a supply of room air during wash-out.

Calibration of both systems was performed as described previously<sup>11,13,18</sup> and checked prior to each test occasion. Before starting the measurements, the gas bias flow system was flushed with tracer gas to ensure the first breath of the wash-in contained 4% SF<sub>6</sub>.

Data acquisition, storage and analysis was performed with WBreath for the USFS prototype system (nnd



**Fig. 1.** Study set up for comparative multiple breath wash-out (MBW) using ultrasonic technology and mass spectrometry, showing mainstream flow sensor (1), sampling tube to side-stream flow sensor (2), sampling tube to CO<sub>2</sub> sensor (3), sampling needle for Mass Spectrometer (4), PNT (5), bias flow system (6).



**Fig. 2.** Computer screen shot of a MBW measurement with the study set up in WBreath. MM-mainstream, molar mass measured with the mainstream ultrasonic flow sensor; CO<sub>2</sub>-MassSpec, CO<sub>2</sub> signal imported from the mass spectrometer; MassSpec-analog signal, imported analog signal of tracer gas concentration from the mass spectrometer; MM-sidestream, molar mass measured with the sidestream ultrasonic flow sensor; CO<sub>2</sub>-infrared, CO<sub>2</sub> signal from the infrared-CO<sub>2</sub>-sensor.

Medical Technologies, Switzerland). For the MS system, custom written software based on a commercially available data acquisition software pack (Test Point, Capital Equipment Corp., Billerica, MA) was used as described previously.<sup>13</sup>

### Subjects and Protocol

For practical reasons, healthy adult volunteers were recruited to this study. The study was approved by the local ethics committees both in Hannover (S.F., M.G.) and London (S.L., W.K., J.S.). Informed written consent was obtained before the tests.

Following preliminary inter-laboratory visits to inform practical issues such as circuitry design for simultaneous data collection, the Hannover USFS prototype system was brought to London. Measurements with the MS and USFS prototype system in series were performed in triplicate in the respiratory function laboratory at the Institute of Child Health. Subjects were measured in a sitting position, wearing a nose clip, breathing through a mouthpiece while (optionally) watching a video.

Measurements started with approximately 10 breaths of room air. Switching to the tracer gas at end expiration was controlled by the WBreath software. End of wash-in was defined as a visibly stable plateau of the MMss curve. Switching back to room air was also controlled by the software. The wash-out phase was stopped when the tracer gas concentration had fallen below 2.5% of the starting level (Fig. 2, example of measurement screen shot).

### Statistics

For comparison of end-tidal tracer gas concentrations, paired values from MS and USFS prototype system were plotted throughout the wash-out period for each breath from each measurement to observe the relationship between the techniques ( $r^2$ ); agreement was assessed according to the method described by Bland and Altman.<sup>29</sup> For each subject mean, standard deviation and intra-subject coefficient of variation (CV %) for FRC and LCI were calculated for MS and USFS, and agreement between the two methods was assessed.<sup>29</sup>

It was estimated that paired measurements in nine subjects (yielding 27 sets of data) would give a power of 99% to detect a difference equivalent to one standard deviation between techniques. The software package SPSS v13.0 for Windows was used for all analyses.

### RESULTS

Attempts were made to collect data in 14 healthy adult volunteers (11 female). Measurements from three males had to be excluded because their flows were higher than had been anticipated and exceeded the bias flow. Measurements from a further two subjects had to be excluded because saliva obstructed the rather narrow sampling needle used for the MS measurements. Three sets of technically satisfactory paired data were obtained from each of nine subjects for final analysis.

End-tidal tracer gas concentrations from 27 measurements (675 paired values) showed almost perfect correla-

**TABLE 1—Difference (MS-USFS) in End-Tidal Tracer Gas Concentrations Derived From the MS and the USFS for All Nine Subjects (Three Paired Measurements Each)**

| Subjects (n = 9) | Mean diff (%) | SD      | CI 95%   |          | P     | r <sup>2</sup> |
|------------------|---------------|---------|----------|----------|-------|----------------|
| 1                | -0.17616      | 0.22496 | -0.23020 | -0.12212 | 0.000 | 1              |
| 2                | 0.40766       | 0.28947 | 0.34737  | 0.46795  | 0.000 | 1              |
| 3                | -0.09218      | 0.38195 | -0.18534 | 0.00099  | 0.052 | 1              |
| 4                | 0.03213       | 0.29185 | -0.04603 | 0.11029  | 0.414 | 1              |
| 5                | 0.29894       | 0.31014 | 0.24087  | 0.35701  | 0.000 | 1              |
| 6                | 0.53822       | 0.32045 | 0.46346  | 0.61299  | 0.000 | 1              |
| 7                | 0.23578       | 0.30938 | 0.17938  | 0.29218  | 0.000 | 1              |
| 8                | 0.25288       | 0.46856 | 0.08674  | 0.41902  | 0.004 | 1              |
| 9                | 0.11727       | 0.46080 | -0.00613 | 0.24067  | 0.062 | 1              |

tion between the two devices ( $r^2 = 1.00$  in all subjects, see Table 1 and Fig. 3), although values were fractionally higher when using the MS (mean difference (95% CI)  $-0.2\%$  ( $-0.23; -0.17\%$ )  $P < 0.001$ , Fig. 4).

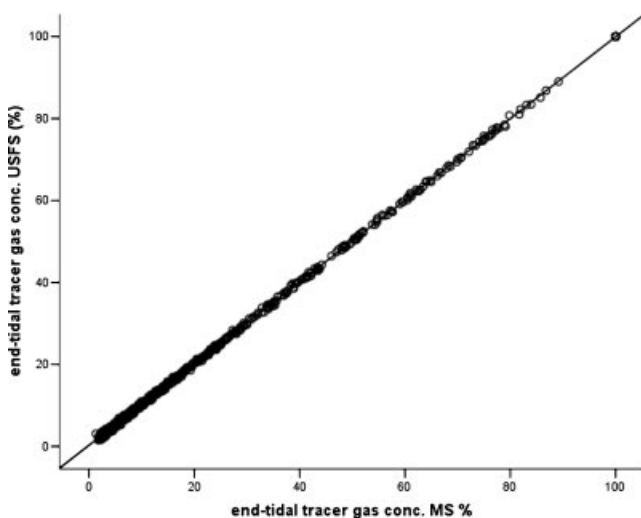
Values for FRC and LCI are summarised in Table 2. A similarly low intra-subject variability ( $CV < 5\%$ ) was observed for both devices. Values of LCI were similar regardless of technique (95% CI of difference [MS-US]  $-0.01; 0.33$ , Fig. 5a) and in keeping with those previously reported from mass spectrometry.<sup>11,13,17</sup> FRC was slightly higher when measured with the MS: (mean (95% CI) difference  $0.124$  L ( $0.08; 0.17$ ), Fig. 5b). While this was statistically significant, on average it represented a difference of only  $2.38\%$  (range  $-0.76$  to  $4.66$ )

## DISCUSSION

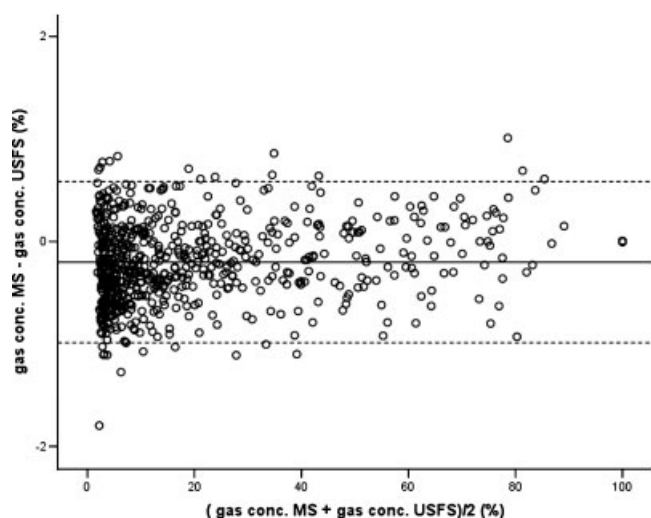
The present study demonstrates that end-tidal tracer gas concentrations measured with the sidestream USFS prototype system are comparable to those measured with

the current gold standard of mass spectrometry. In addition, calculated values of FRC and LCI were of similar magnitude and variability. Thus, this USFS prototype system could provide a valuable basis for developing a commercial device that would allow more widespread application of MBW in all age groups.

Accurate measurement of end-tidal tracer gas concentrations is an essential prerequisite for accurate calculation of FRC and parameters of VI derived from MBW. We have demonstrated that there is an excellent correlation between the sidestream USFS prototype system and MS. The small mean difference of  $0.2\%$  is probably due to different calibration procedures and should not affect further computations. This in vivo comparison was necessary as the ultrasonic molar mass signal does not simply reflect gas concentrations, but is influenced by temperature, humidity, and  $CO_2$ . With the side-stream position of the ultrasonic transducers in the current prototype system, the influence of temperature and humidity should be negligible. Simultaneous measure-



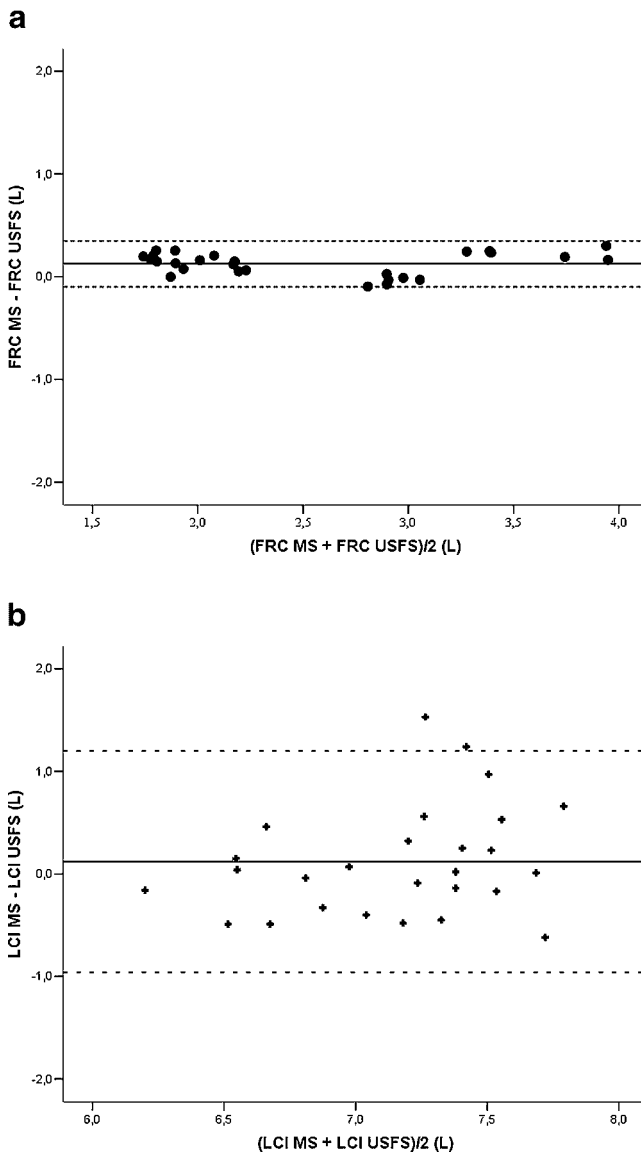
**Fig. 3.** Correlation between end-tidal tracer gas concentrations from 27 paired measurements derived from mass spectrometry (MS) and sidestream ultrasonic flow sensor (USFS) (675 paired values).



**Fig. 4.** Agreement between end-tidal tracer gas concentrations derived from mass spectrometry (MS) and sidestream ultrasonic flow sensor (USFS); mean difference [MS-USFS]:  $-0.2\%$  (95% limits of agreement  $0.58; -0.98$ ).

**TABLE 2—Mean, Standard Deviation (SD) and Intrasubject Coefficient of Variation (CV %) of FRC and LCI for the Group (n = 9) Derived From the Mass Spectrometer (MS) and Side Stream Ultrasonic Flow Sensor (USFS)**

|                   | MS          | MM USFS     |
|-------------------|-------------|-------------|
| FRC mean (SD) [L] | 2.60 (0.09) | 2.48 (0.10) |
| FRC CV%           | 3.46        | 4.47        |
| LCI mean (SD)     | 7.21 (0.26) | 7.10 (0.30) |
| LCI CV%           | 3.59        | 4.15        |



**Fig. 5. Agreement between (a) FRC and (b) LCI when measured with mass spectrometry (MS) and sidestream ultrasonic flow sensor (USFS): The black line represents the mean difference of all measurements. The dotted lines represent plus and minus two standard deviations of this mean difference (i.e., 95% limits of agreement).**

ment of CO<sub>2</sub> allowed correction of the molar mass signal for influences of exhaled CO<sub>2</sub> before analysis. Our results demonstrate that the resultant molar mass signal is a valid reflection of end-tidal tracer gas concentration in MBW allowing more confident use and further development of the ultrasonic flow technology.

The secondary aim of the present study was to compare parameters calculated from MBW measurements. When calculating FRC and LCI, deadspace definitions that are currently being used in London<sup>11,12,17</sup> were applied for both systems, as the MS system was the gold standard for these comparisons. LCI was chosen as a representative parameter of ventilation inhomogeneity because it is a robust parameter that has been reported in all recent studies and, unlike many other VI indices, there is reasonable consensus regarding method of calculation. The small observed difference in FRC (124 ml in an average FRC of 2.60 L, which equated to a difference of <5%) is unlikely to be of any clinical or physiological relevance, but may reflect the different algorithms used to compute flow and volume for the two techniques. In addition, the algorithms for calculating FRC were slightly different in the two systems: in the MS system, breaths used for calculation of FRC and LCI are only included until 2.5% of the initial tracer gas concentration are reached<sup>28</sup>, whereas USFS analysis includes all breaths recorded during the wash-out.

Although, ideally, we would have recruited children to participate in this methodological study, measurement of end-tidal tracer gas concentrations merely reflects technical accuracy of the measurement technology and can thus be extrapolated to application of this device to other age groups. Recruitment of a sufficient number of children for a purely methodological study has to be justified on ethical grounds and would have been impractical in the current collaborative study due to time constraints when both sets of equipment could be in the same country. Furthermore, although the combined deadspace necessitated by the serial set-up of equipment for the present study was reasonable for adults, it might have caused problems if comparative measurements had been undertaken in young subjects, since minimization of external deadspace is crucial for valid MBW measurements.<sup>7</sup> However, the excess deadspace in the current investigation was purely due to the experimental set-up for the comparative study and would not be an issue if the device was used “as intended.” To further clarify how deadspace influences computations from MBW measurements, breath by breath correction will be an important task for future investigations.

The high drop-out rate experienced in this study was unexpected since both laboratories have a very high success rate when undertaking such measurements in infants and young children. This was primarily due to the fact that lack of experience working with adults meant that

**TABLE 3—Mean Differences of FRC and LCI for Each Individual Measurement (n = 27) Comparing Mass Spectrometer (MS) and Side Stream Ultrasonic Flow Sensor (USFS)**

|                | FRC diff (L) | LCI diff    |
|----------------|--------------|-------------|
| MS versus USFS | 0.124        | 0.12        |
| p              | <0.001       | 0.267       |
| 95% CI         | 0.08; 0.7    | −0.10; 0.33 |

we underestimated the high air flows that some males would exhibit. The maximal bias flow allowed by the valves in the current USFS prototype system is 1.5 L/sec, which should be more than adequate for children of any age group, in whom much lower flows are encountered. Obstruction of the narrow sampling needle is a recognised potential problem during MS measurements, but one that we usually avoid when testing children by careful positioning of the mouthpiece and equipment. Unfortunately our adult volunteers were given more freedom to adopt a comfortable position which may have contributed to these errors. Fortunately, since we were still able to collect valid paired data in nine subjects, there was sufficient power of study to detect any differences with confidence, as demonstrated by the fact that the extremely small differences between expired gas concentrations and FRC were statistically significant (Fig. 4, Table 3).

In conclusion, we have demonstrated that the side stream USFS prototype system measures end-tidal tracer gas concentrations comparable to those obtained using a mass spectrometer, and that similar values of FRC and LCI from MBW measurements can also be obtained. Further work is required to investigate reasons for the small, though clinically insignificant, discrepancies when calculating FRC, which probably relate to differences in software algorithms between the two systems. Based on these data, this USFS prototype system could provide the basis for developing a commercial device that would allow more widespread application of MBW for the detection of early lung disease and as a measure of treatment efficacy throughout childhood.

## ACKNOWLEDGMENTS

Susanne Fuchs, Sooky Lum, Wanda Kozłowska, Janet Stocks, and Monika Gappa have no declared conflict of interest. C.B. is R&D engineer at nnd Medical Technologies, Zurich, Switzerland. The manuscript describes a new technology currently not available in a commercial product. S.F. is supported by Mukoviszidose e.V. Germany.

## REFERENCES

- Hutchison AA, Sum AC, Demis TA, Erben A, Landau LI. Moment analysis of multiple breath nitrogen washout in children. *Am Rev Respir Dis* 1982;125:28–32.

- Kraemer R, Meister B. Fast real-time moment-ratio analysis of multibreath nitrogen washout in children. *J Appl Physiol* 1985;59:1137–1144.
- Kraemer R, Zehnder M, Meister B. Intrapulmonary gas distribution in healthy children. *Respir Physiol* 1986;65:127–137.
- Saniie J, Saidel GM, Chester EH. Real-time moment analysis of pulmonary nitrogen washout. *J Appl Physiol* 1979;46:1184–1190.
- Wall MA. Moment analysis of multibreath nitrogen washout in young children. *J Appl Physiol* 1985;59:274–279.
- Wall MA, Misley MC, Brown A. Changes in ventilation homogeneity from preschool through young adulthood as determined by moment analysis of nitrogen washout. *Pediatr Res* 1988;23:68–71.
- Gustafsson PM. Inert gas washout in preschool children. *Paediatr Respir Rev* 2005;6:239–245.
- Verbanck S, Schuermans D, Meysman M, Paiva M, Vincken W. Noninvasive assessment of airway alterations in smokers: the small airways revisited. *Am J Respir Crit Care Med* 2004;170:414–419.
- Verbanck S, Schuermans D, Paiva M, Vincken W. Nonreversible conductive airway ventilation heterogeneity in mild asthma. *J Appl Physiol* 2003;94:1380–1386.
- 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.
- Aurora P, Gustafsson P, Bush A, Lindblad A, Oliver C, Wallis CE, Stocks J. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. *Thorax* 2004;59:1068–1073.
- Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. *Am J Respir Crit Care Med* 2005;171:249–256.
- Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003;22:972–979.
- Gustafsson PM, Kallman S, Ljungberg H, Lindblad A. Method for assessment of volume of trapped gas in infants during multiple-breath inert gas washout. *Pediatr Pulmonol* 2003;35:42–49.
- Kraemer R, Blum A, Schibler A, Ammann RA, Gallati S. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. *Am J Respir Crit Care Med* 2005;171:371–378.
- Schibler A, Schneider M, Frey U, Kraemer R. Moment ratio analysis of multiple breath nitrogen washout in infants with lung disease. *Eur Respir J* 2000;15:1094–1101.
- Aurora P, Kozłowska W, Stocks J. Gas mixing efficiency from birth to adulthood measured by multiple-breath washout. *Respir Physiol Neurobiol* 2005;148:125–139.
- Pillow JJ, Ljungberg H, Hulskamp G, Stocks J. Functional residual capacity measurements in healthy infants: ultrasonic flow meter versus a mass spectrometer. *Eur Respir J* 2004;23:763–768.
- Fuchs S, Gerlach S, Gappa M. Multiple breath washout (MBW) for assessment of functional residual capacity (FRC) using an ultrasonic flowmeter—a pilot study. *Eur Respir J* 2003;22:383s.
- Fuchs S, Gerlach S, Gappa M. Variability of multiple breath washout (MBW) assessment of functional residual capacity (FRC) in cystic fibrosis (CF) patients using an ultrasonic device. *Eur Respir J* 2003;22:129s.
- Fuchs S, Gerlach S, Gappa M. Multiple breath inert gas washout (MBW) in healthy children using an ultrasonic flowmeter. *Eur Respir J* 2004;24:376s.
- Fuchs S, Sturz J, Buess C, Gappa M. Clinical application of the inert gas multiple breath washout technique (MBW) in children

- with cystic fibrosis (CF) using a prototype ultrasonic side stream flow sensor. *Eur Respir J* 2006;28:704s.
23. Schibler A, Hall GL, Businger F, Reinmann B, Wildhaber JH, Cernelc M, Frey U. Measurement of lung volume and ventilation distribution with an ultrasonic flow meter in healthy infants. *Eur Respir J* 2002;20:912–918.
  24. Schibler A, Henning R. Measurement of functional residual capacity in rabbits and children using an ultrasonic flow meter. *Pediatr Res* 2001;49:581–588.
  25. Schibler A, Henning R. Positive end-expiratory pressure and ventilation inhomogeneity in mechanically ventilated children. *Pediatr Crit Care Med* 2002;3:124–128.
  26. Buess C, Pietsch P, Guggenbuhl W, Koller EA. A pulsed diagonal-beam ultrasonic airflow meter. *J Appl Physiol* 1986; 61:1195–1199.
  27. Buess C, Pietsch P, Guggenbuhl W, Koller EA. Design and construction of a pulsed ultrasonic air flowmeter. *IEEE Trans Biomed Eng* 1986;33:768–774.
  28. Gustafsson PM, Johansson HJ, Dahlback GO. Pneumotachographic nitrogen washout method for measurement of the volume of trapped gas in the lungs. *Pediatr Pulmonol* 1994;17:258–268.
  29. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.