Eur Respir J
1992, 5, 879–886

Lung volumes measured by the forced rebreathing technique in children with airways obstruction

P.J.F.M. Merkus*, S. Verver*, E.E.M. van Essen-Zandvliet**, E.J. Duiverman***, K.F. Kerrebijn**, Ph.H. Quanjer*

Lung volumes measured by the forced rebreathing technique in children with airways obstruction. P.J.F.M. Merkus, S. Verver, E.E.M. van Essen-Zandvliet, E.J. Duiverman, K.F. Kerrebijn, Ph.H. Quanjer.

ABSTRACT: Forced rebreathings may recruit trapped gas into the mixing process. Therefore, we assessed the validity and reproducibility of measurements of residual volume (RV_{N_2}) by forced rebreathing in a closed circuit using N_2 as indicator gas (N_2FR) in children with airways obstruction.

Validity was studied from measurements of RV obtained by N_2FR, by helium dilution during resting ventilation, and by body plethysmograph at low panting frequency in young patients (8–18 yrs, 13 with asthma, forced expiratory volume in one second (FEV_1) 93.0±22.8 % pred; 12 with cystic fibrosis (CF), FEV_1 80.4±16.4 % pred). Reproducibility of RV_{N_2} was assessed from duplicate measurements in 73 patients with asthma before and after bronchodilation (FEV_1 81.4±13.7 and 99.6±11.5 % pred, respectively), and in nine patients with CF; the total lung capacity (TLC) was unaffected by bronchodilation; 3,797±830 ml and 3,807±843 ml, respectively.

Gas dilution methods gave comparable results in all subjects but gave lower values than plethysmography in patients with cystic fibrosis. Reproducibility was satisfactory, median differences between duplicate measurements of RV_{N_2} and TLC_{N_2} varying between 13 and 46 ml, respectively.

We conclude that N_2FR is quickly performed and well-tolerated. Lung volumes are highly reproducible and agree well with those obtained with the helium dilution method. Deep inspirations do not seem to overcome gas trapping in patients with CF.

Eur Respir J., 1992, 5, 879–886.

* Dept of Physiology, Leiden University, The Netherlands. ** Sophia Children's Hospital, Division of Pulmonary Medicine, Erasmus University, Rotterdam, The Netherlands *** Juliana Children's Hospital, The Hague, The Netherlands.

Correspondence: P.J.F.M. Merkus
Physiology Dept
P.O. Box 9604
2300 RC Leiden
The Netherlands

Keywords: Airways obstruction
asthma
cystic fibrosis
nitrogen multiple-breath washout technique
reproducibility
validity.

Received: July 17 1991
Accepted after revision March 9 1992.

Supported by a grant from the Netherlands' Health Research Promotion Programme (SGO).

Measurements of total lung capacity (TLC) and residual volume (RV) are an important ingredient in assessing lung growth and lung disorders. They are also indispensable in interpreting *e.g.* measurements of airways resistance, ventilatory flows, lung recoil and gas transfer. However, such measurements are not routinely performed, in part because of the time and cost involved. When BUIST and ROSS [1] introduced a single-breath nitrogen determination of RV, which was subsequently applied in an epidemiological setting [2], this held promise that the technique could be easily applied on a large scale. However, it appeared to systematically underestimate the total lung capacity, in particular in the presence of obstructive lung disease [3]. From this, STERK *et al.* [3] developed a forced rebreathing method, washing N_2 out of the lungs at large tidal volumes, requiring only 12–20 breaths, and validated it in adults using a double tracer technique [4]. This N_2 forced rebreathing multiple-breath wash-out technique (N_2FR) was then successfully applied in epidemiological studies on healthy adults and adolescents with good reproducibility [5, 6]. N_2FR has been validated in adult patients with chronic obstructive pulmonary disease [7], but it has not been used in children with airways obstruction, even though repeated measurements can be done easily and quickly.

In this study, we looked into the validity of measurements of RV by the N_2FR technique by comparing it to the helium dilution method (RV_{He}) and whole body plethysmography (RV_{pleth}) in children with asthma and cystic fibrosis. We hypothesized that because of the forced rebreathings, trapped gas might be recruited into the process of gas mixing [8]. Subsequently, we assessed the reproducibility of measurements of RV_{N_2} and TLC_{N_2} in children with cystic fibrosis and asthma. In asthmatic children, duplicate measurements were carried out before and after bronchodilation to assess the influence of airway calibre on the reproducibility of measurements.

Methods

Patients

For validation purposes, measurements of RV by N_2FR (RV_{N_2}), helium dilution and body plethysmography were compared in 13 subjects with asthma

(8–18 yrs, FEV_1 61–148 % pred) and 12 subjects with cystic fibrosis (8–17 yrs, FEV_1 52–105 % pred) (table 1). They were patients from the out-patient clinic of the division of respiratory medicine at the Sophia Children's Hospital, who were not familiar with N_2FR, but were accustomed to performing spirometric lung function tests. RVN_2, RV_{He} and RV_{pleth} were obtained in random order. The difference between RV_{He} and RV_{pleth} was used as a measure of trapped gas.

The reproducibility of RVN_2 and TLC by N_2FR ($TLCN_2$) was studied from duplicate measurements in nine children with cystic fibrosis from the group mentioned above, and in 49 boys and 24 girls with asthma. In the asthmatics, RVN_2 and $TLCN_2$ were obtained twice before and twice after inhalation of 1 mg salbutamol *via* a spacing device. They were recruited from the out-patient clinics of the divisions of respiratory medicine of Sophia and Juliana Children's Hospitals in Rotterdam and The Hague, respectively. They were familiar with N_2FR, so that learning effects would probably be minimal. Their mean age was 12 yrs (range 8–18 yrs), mean standing height 153 cm (range 129–184 cm). All pulmonary medication (β_2-agonists and/or corticosteroids by inhalation) was discontinued at least 8 h prior to the measurements.

Nitrogen forced rebreathing technique

The test was performed according to previously published standards [3, 5, 6], where the forced rebreathings were preceded by the single-breath nitrogen test. The seated subject, wearing a noseclip, was connected to a bag-in-box system at the level of RV; the bag had been previously filled with a known volume of oxygen. Subsequently, oxygen was slowly inhaled to the level of TLC. Without a respiratory pause, the subject then performed a slow, full expiration.

Table 1. – Data on 25 children in whom the validity of measurements of RVN_2 was studied

	Age yrs	Height cm	Sex	FEV_1 % pred	FEV_1/FVC %	RVN_2 ml	RV_{He} ml	RV_{pleth} ml
Asthma								
1	18	174	M	61	45	2000	1800	2880
2	15	161	M	67	61	1430	1395	1480
3	16	182	M	90	65	1855	1845	1660
4	10	131	M	92	68	760	805	910
5	10	141	M	83	74	795	775	880
6	11	134	F	71	80	590	685	570
7	10	147	F	83	81	700	885	920
8	10	147	F	96	83	845	1010	840
9	16	173	F	102	83	935	1015	1320
10	8	131	M	102	85	585	560	690
11	12	155	M	96	85	1175	965	1140
12	12	164	F	148	90	1070	1085	1190
13	13	164	F	118	93	875	1005	610
Mean	12.4	154		93.0	76.4	1047	1064	1161
SD	3.0	17.1		22.8	13.4	456	394	613
Cystic fibrosis								
1	17	178	M	52	47	1705	1620	3030
2	15	163	F	75	56	1315	795	1800
3	11	147	F	69	64	810	750	990
4	16	164	F	85	64	1285	1700	1460
5	14	165	M	65	68	1190	1105	1790
6	12	163	F	78	70	820	885	1320
7	12	146	F	77	71	720	710	950
8	12	147	F	71	76	820	680	1340
9	8	126	M	105	82	335	455	530
10	9	130	M	105	86	555	480	820
11	15	166	F	101	86	841	840	1140
12	13	149	F	82	89	634	515	1260
Mean	12.8	154		80.4	71.6	919	878	1369
SD	2.7	15.5		16.4	12.9	383	409	641

RVN_2: residual volume (RV) measured by forced rebreathing in a closed circuit using N_2 as indicator gas; RV_{He}: RV obtained by helium dilution during resting ventilation; RV_{pleth}: RV obtained by plethysmography at low panting frequency; FEV_1: forced expiratory volume in one second; FEV_1/FVC: FEV_1 as a percentage of forced vital capacity.

Flow during the single-breath manoeuvre was voluntarily held below 0.5 $l \cdot s^{-1}$ [9], as was practised prior to the manoeuvre and verified by visual inspection of the volume-time recordings. After the single-breath manoeuvre, further gas mixing was achieved by forced rebreathing at large tidal volumes at a rate of about 20 breaths·min^{-1} during 80 s. Near the end of the measurement, an inspiratory vital capacity was obtained for control purposes.

The nitrogen concentration was measured at the mouth with a nitrogen analyser (Hewlett Packard, Vertek series), and a volume signal obtained from a water-sealed spirometer and delayed by 100 ms to account for the instrumental delay of the nitrogen analyser. A computer sampled the volume signal and the nitrogen concentration at the mouth.

In the case of complete gas mixing and mass balance, residual volume follows from:

$$RV = V_B \cdot F_B/(F_A - F_B)$$

where V_B=volume of oxygen in the bag (including the deadspace); F_A=alveolar nitrogen concentration at the start; and F_B=concentration when gas mixing is complete. The above equation can be used to compute an "apparent volume" after each breath (fig. 1). Gas mixing accounts for a marked initial increase of this apparent volume; upon completion of gas mixing there is a slight linear rise which reflects the continuous oxygen uptake, decreasing CO_2 release and some release of N_2 from blood and tissues. Gas mixing is established at about the fifth breath in healthy subjects [3] and about five breaths later in patients with airways obstruction (own observations). A mass balance of nitrogen actually does not apply [10], and volume changes during the manoeuvre [4], but these effects are generally small, compared to the effects of gas mixing.

In healthy subjects, STERK *et al.* [3], by employing a double tracer technique [4], established that on average at the eighth breath total CO_2 release and oxygen consumption balances, so that the lung volume computed from the gas concentration at breath 8 represents RV; this is on average three breaths more than required to reach gas mixing. STERK *et al.* [3] obtained RV_{N_2} at breath number 8 by interpolation from the linear regression line (fig. 1).

In the present study, RV_{N_2} was obtained by interpolation at the third breath after the phase of rapid gas mixing was completed, as judged by eye. In obstructive patients with poorly mixing gas compartments, one might underestimate RV. In such cases, delayed gas mixing or increased oxygen cost of breathing would show up in an abnormally steep slope of the regression line depicted in figure 1. In order to see whether this in fact happened, slopes of regression lines in patients were compared with those of 264 healthy adolescents studied previously [6].

Helium dilution and body plethysmography

The seated subject was connected to a water-sealed spirometer, filled with a known volume of helium, at the level of the functional residual capacity (FRC). Helium was allowed to wash-in for at least 5 min during normal tidal breathing until a stable concentration was obtained; during measurements CO_2 was absorbed and the O_2 concentration held constant. The measurements were preceded and ended with an inspiratory vital capacity manoeuvre; RV was obtained by subtracting expiratory reserve volume from FRC.

A constant volume whole body plethysmograph was used to assess thoracic gas volume according to recommended procedures [11, 12]. Panting frequency

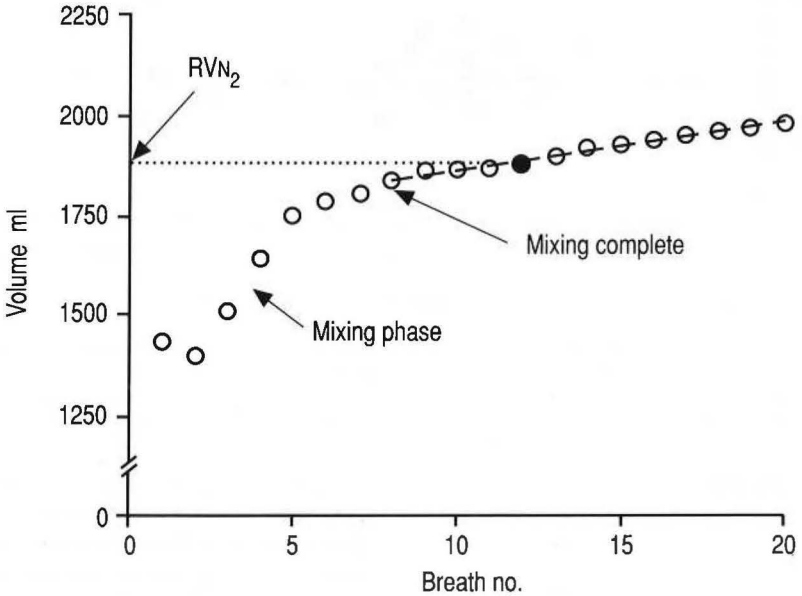

Fig. 1. – Example of volume calculated per breath in a subject with asthma. A rapid gas mixing phase is followed by a slow increase in apparent lung volume, for which a line is fitted using linear regression, as explained in the text. RV is obtained by interpolation at the third breath (●) after completion of gas mixing. RV_{N_2}: residual volume measured by forced rebreathing in a closed circuit using N_2 as the indicator gas.

during measurements was <1·s^{-1} [14, 15] and six measurements of thoracic gas volume were averaged for the analysis. Inspiratory reserve volume was assessed by integration of airflow at the mouth. RV was obtained by subtracting the inspiratory vital capacity from TLC.

All volumes were corrected to body temperature and pressure, saturated with water vapour (BTPS); reference values were those according to ZAPLETAL *et al.* [16]. The equipment and procedures were in accordance with recommendations [12, 13]; between measurements 10 min were allowed for rest and for normalization of the alveolar gas concentrations. The experimental procedures were carried out with the informed consent of both children and parents, and were approved by the local Medical Ethics Committee.

The validity and reproducibility of measurements was analysed from the relationship between differences and means of paired data [17]. Differences were tested applying the paired t-test or Wilcoxon's signed rank test, when appropriate.

adolescents. In asthmatics and patients with cystic fibrosis, it took on average (±SD) 11±4 and 14±4 breaths, respectively, to establish gas mixing. The slopes of the lines of "apparent volume" regressed on breath number were comparable to those from 264 healthy adolescents [6]: median slopes in patients with asthma and cystic fibrosis were respectively, 4 ml·breath^{-1} and 7 ml·breath^{-1} as compared to 6 ml·breath^{-1} in healthy subjects.

In order to test the hypothesis that trapped gas would be recruited by forced rebreathings, we compared the differences between RV_{pleth} and RV_{He} with those between RV_{pleth} and RVN_2. The mean difference was 11 ml, (95% confidence interval: -59 to 82 ml). Hence, the gas dilution methods showed good agreement in all patients (fig. 2, p=0.72). In the patients with cystic fibrosis there was a systematic difference between RV_{pleth} and RVN_2 (fig. 2, p=0.001), and this difference was inversely related to FEV_1 % pred (fig. 3; r=-0.69, p<0.01). This was mainly explained by patient no. 1 with severe airways obstruction. When he was

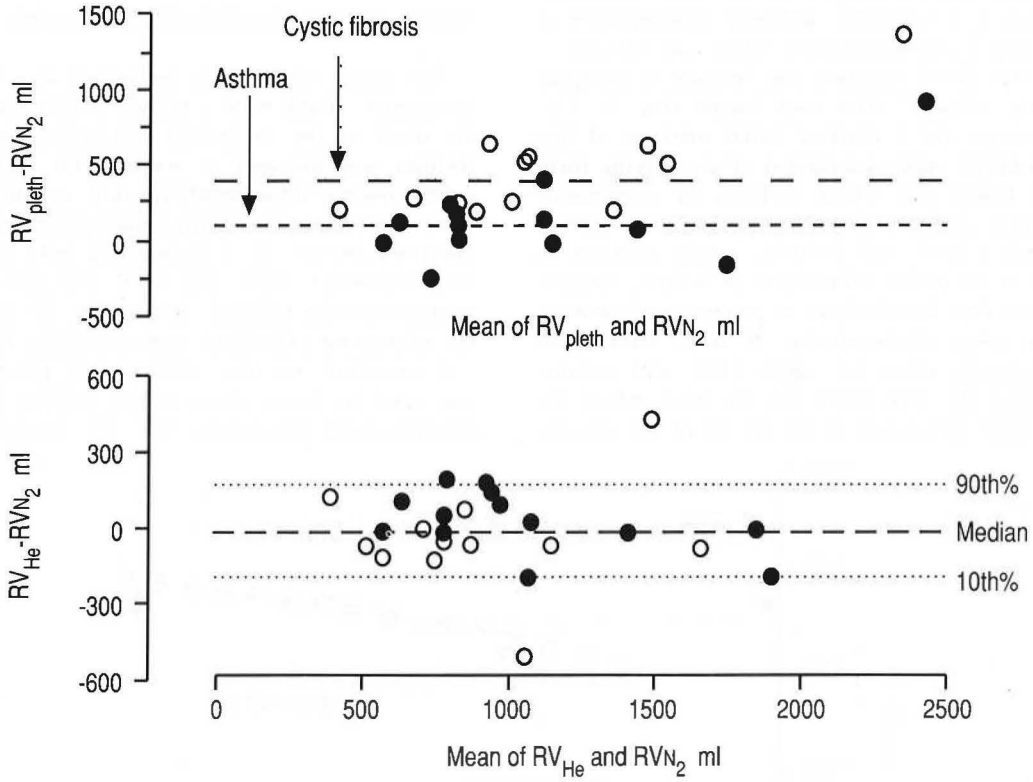

Fig. 2. – Validity of measurements of residual volume by gas dilution methods and body plethysmography in patients with asthma (●) and cystic fibrosis (○). The upper and lower line in the top panel refer to the medians of patients with cystic fibrosis and asthma, respectively. The medians and percentiles in the lower panel refer to all patients. RV_{He}: residual volume obtained by helium dilution during resting ventilation; RV_{pleth}: RV obtained by plethysmograph at low panting frequency. For further abbreviations see legend to figure 1.

Results

Validity

Anthropometric data, RVN_2, RV_{He}, and RV_{pleth} of 25 patients with asthma and cystic fibrosis are listed in table 1. N_2FR took 4–8 more breaths to achieve gas mixing in obstructed patients than in healthy

excluded from the analysis, this relationship lost significance (r=-0.48, p=0.14). In the asthmatic children differences between RVN_2 and RV_{pleth} were due to chance (fig. 2, p=0.18), and not related to FEV_1 % pred (fig. 3; r=-0.35, p>0.10). For a similar degree of airways obstruction in subjects with cystic fibrosis, RVN_2 showed a systematic difference with RV_{pleth}, whereas those with asthma did not (fig. 3).

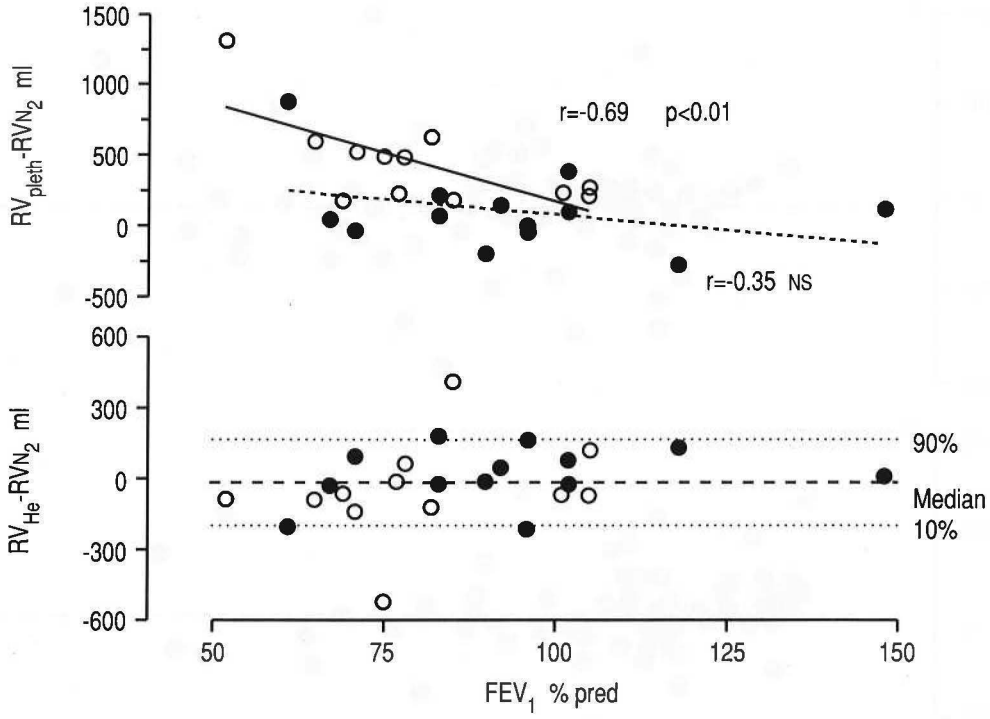

Fig. 3. – Relationship between FEV_1 (% pred) and measurements of RV by gas dilution methods and body plethysmography in patients with asthma (●) and cystic fibrosis (○). The difference between RVN_2 and RV_{pleth} was negatively correlated with FEV_1 (% pred) in children with cystic fibrosis (solid line, r=-0.69, p<0.01). This relationship was not significant when patient No. 1 (FEV_1=52 % pred) was excluded from analysis. No significant correlation was found in children with asthma (dotted line). FEV_1: forced expiratory volume in one second. For further abbreviations see legend to figures 1 and 2.

Table 2. – Ventilatory function (mean±SD) of 49 boys and 24 girls with stable asthma, before and after inhalation of 1 mg of salbutamol (metered dose inhaler)

		Before salbutamol	After salbutamol	t-test p
RVN_2	ml	1006±289	829±271	<0.001
RVN_2	% pred	114.6±28.0	94.4±26.5	<0.001
$TLCN_2$	ml	3797±830	3807±843	NS
$TLCN_2$	% pred	94.3±16.4	94.6±17.0	NS
RV/TLC	%	26.6±5.1	21.8±4.9	<0.001
FEV_1	% pred	81.4±13.7	99.6±11.5	<0.001
FVC	% pred	93.2±11.2	98.4±11.3	<0.01
FEV_1/FVC	%	73.3±8.8	85.3±6.5	<0.001

$TLCN_2$: total lung capacity by forced breathing in a closed circuit using nitrogen as indicator gas; RV/TLC: residual volume as a percentage of TLC; NS: nonsignificant. For further abbreviations see legend to table 1.

Table 3. – Difference within subjects of duplicate measurements of RVN_2 and $TLCN_2$ obtained in children with asthma (before and after bronchodilation) and in children with cystic fibrosis

	RVN_2 ml	$TLCN_2$ ml
Asthma (n=73)		
Before salbutamol	-24(-169 to 99)	-13(-171 to 148)
After salbutamol	21(-73 to 154)	46(-91 to 197)
Cystic Fibrosis (n = 9)		
Baseline	15(-85 to 150)	20(-54 to 197)

Values shown are medians and 10th to 90th percentiles in parenthesis. For abbreviations see legends to table 1 and 2.

Influence of airway calibre on RVN_2 and $TLCN_2$ in patients with asthma

Following bronchodilation, RVN_2 decreased (mean = -177 ml, SEM=46 ml, p<0.001), whilst mean $TLCN_2$ remained almost identical (p=0.44). Its mean difference was -10 ml, (95% confidence interval: -36 to 16 ml). After bronchodilation gas mixing was complete at about the fifth breath, (as in healthy adults and adolescents [5, 6]) compared to breath ten before bronchodilation; the more rapid gas mixing runs parallel with restoration of FEV_1 and FVC to normal predicted values (table 2). RVN_2 was subsequently obtained by interpolation on average at breath number 13 before bronchodilation and at number 8 after bronchodilation. Baseline FEV_1 % pred ranged 50–111% with a mean±SD of 81±14%.

Reproducibility

The reproducibility of measurements is summarized in table 3. Differences between duplicate measurements were not normally distributed and best described by median values with 10th and 90th percentiles.

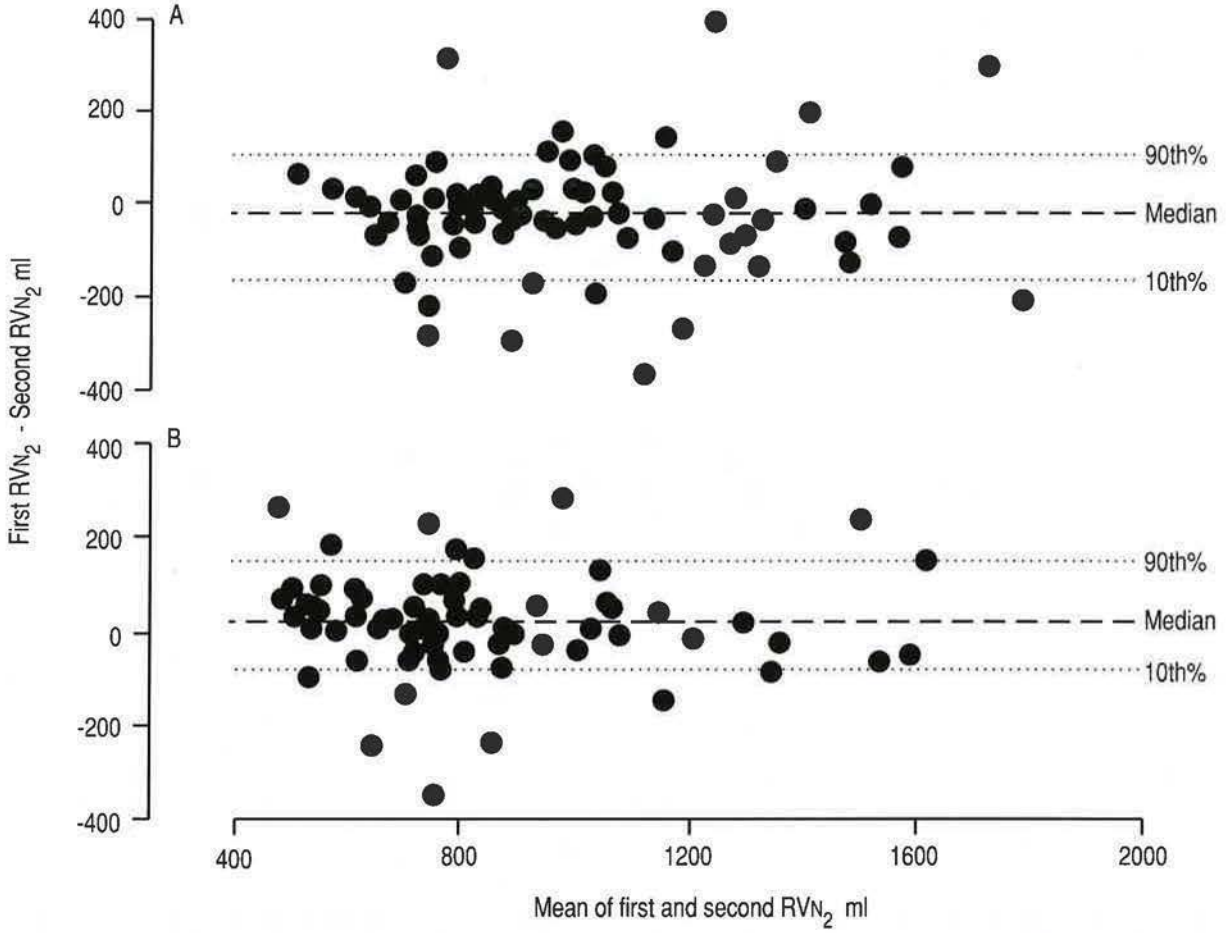

Fig. 4. – Reproducibility of RV assessed by forced rebreathing N_2 wash-out method in 73 asthmatic children: A) before bronchodilation; and B) after bronchodilation. For abbreviations see legend to figure 1.

These were similar for RV and TLC, with no evidence for learning effects. In the nine patients with cystic fibrosis, reproducibility was comparable with that found in asthmatics. In the asthmatic children, bronchodilation did not affect $TLCN_2$ (table 2) or its reproducibility (table 3). There was a weak correlation between baseline FEV_1 % pred and the absolute difference between duplicate measurements of RVN_2 (r=-0.33, p=0.004); no correlation was found between baseline FEV_1 % pred and the absolute difference between duplicate measurements of $TLCN_2$ (r=0.05, p=0.68). In general, differences between two measurements were unrelated to the means of measurements before and after bronchodilation (fig. 4).

Discussion

We have shown that N_2FR provides estimates of RV and TLC that are generally well reproducible and that show good agreement with those obtained using the helium dilution method in children with asthma and cystic fibrosis. In the asthmatic children there was no significant difference between RV determined by N_2FR, the helium wash-in method or body plethysmography. This held irrespective of airflow limitation with the exception of subject No. 1, who appeared to have trapped gas. Good agreement between the gas dilution technique and plethysmography in asthmatic children with moderate degree of airways obstruction was also found by others [18], suggesting that trapped gas does not play a role in moderate asthma.

In the 12 children with cystic fibrosis, trapped gas was present, as described in the literature [19]. We had hypothesized that forced rebreathing at large tidal volumes would open up hitherto closed airways and thus minimize the amount of trapped gas. This was based on studies in adults with chronic obstructive pulmonary disease (COPD), in whom helium concentrations which had stabilized during quiet breathing have been shown to drop after each deep inspiration. After such inspiratory manoeuvres, results with the helium dilution technique did not differ from those obtained by body plethysmography in these patients [8], suggesting that hitherto unventilated compartments were recruited by the deep inspirations. If this also applies to patients with cystic fibrosis, RVN_2 and RV_{pleth} should give similar results, or RVN_2 should be intermediate between RV_{He} and RV_{pleth}, but this was not the case. It is conceivable that in cystic fibrosis, even after deep inspirations, trapped gas cannot be recruited when airways are plugged with sticky mucus. An alternative

explanation for the difference between body plethysmography and gas dilution technique in patients with cystic fibrosis is erroneous overestimation with the former technique due to compliant extrathoracic airways, so that in severe airways obstruction mouth pressure during panting against an occluded airway is less than alveolar pressure [20]. Such errors can be minimized by panting at a frequency of $<1 \cdot s^{-1}$ [14, 15], a procedure adopted in this study. Furthermore, erroneous overestimation seems unlikely as the explanation for the difference in results of both methods, since for the same degree of airflow limitation as in these patients with cystic fibrosis, asthmatic children showed good agreement between gas dilution and plethysmography. The results in children with cystic fibrosis are similar to those obtained in adults with severe COPD [7]; in that study it was also found that helium dilution and forced rebreathing methods gave the same values for RV which were lower than those with plethysmography, even when changes in alveolar pressure were estimated from an oesophageal balloon in order to circumvent the artifact described above.

Both in children with asthma and cystic fibrosis the increase in computed volume per breath after the phase of rapid gas mixing was very small and comparable with that of healthy adolescents, confirming that the results were not influenced by gas mixing problems or by extra oxygen cost of breathing. This indicates that in children with asthma before and after bronchodilation and in children with cystic fibrosis, the end of the gas mixing phase can be easily identified by eye. Also, errors made by interpolation one breath too late or too early can introduce only minor errors in the estimation of RV_{N_2}.

Theoretically, the calculations of RV are complicated because a mass balance of nitrogen actually does not apply: small quantities of nitrogen evolving from blood and tissues are added to the system when the alveolar nitrogen concentration falls. Because of the very low solubility, the net nitrogen transfer in about 1 min is very small, and a correction procedure is mainly of theoretical interest [10]. Furthermore, the nitrogen concentration is also affected by a change in the volume of the lung-bag system due to a change in the respiratory exchange ratio. The first inhalation of oxygen dilutes alveolar gas, causing considerable CO_2 release from pulmonary blood, whilst the oxygen uptake is affected little, if at all. As rebreathing continues, progressively less CO_2 evolves, whilst O_2 uptake continues. Thus, a quick initial volume increase is followed by a slower, progressive decrease; even when gas mixing is complete, the nitrogen concentration will continuously rise. NUNNELEY *et al.* [4] estimated that the volume change in adults may be in the order of 100 ml. Thus, in healthy subjects, the effects of changing quantities of nitrogen and of volume on the calculation of RV_{N_2} are small compared to the effects of gas mixing. Based on the data of validation and of reproducibility, this was apparently also the case in the patients in the present study.

Our findings demonstrate that in children with and without trapped gas the procedure of calculating RV from the nitrogen concentration at the third breath after completion of gas mixing is valid. Reproducibility was equally good in the 73 asthmatic children, who had no trapped gas, as in the nine children with cystic fibrosis, in whom trapped gas could be demonstrated. In general, differences between two measurements of RV and TLC were unrelated to the means of measurements before and after bronchodilation. Because of the lack of such a proportionality in our data, the coefficient of variation is not an appropiate measure of reproducibility. When the mean coefficient of variation of RV_{N_2} is calculated, nevertheless, for comparison with the literature, it is 6.3% (median 4.4%), which is lower than the 8.1% reported in healthy adolescents [6], in spite of any possible negative effect of airflow limitation on reproducibility. These more favourable results may be partly due to the fact that our subjects were familiar with the technique, and that pulmonary patients in general are more used to performing spirometry. In the 73 asthmatic children, the reproducibility of RV_{N_2} was only slightly affected by severity of airways obstruction, whilst that of TLC_{N_2} was not. Apparently, the extent of airways obstruction influences the reproducibility of RV and of vital capacity. Following bronchodilation in these patients, airway calibre normalized and gas mixing occurred as rapidly as in healthy adolescents [5, 6]. Furthermore, TLC_{N_2} did not change, indicating that no trapped gas was present before bronchodilation, and that the validity of N_2FR is not influenced by changes of airway calibre, even though FEV_1 was sometimes severely disturbed (range 50–110 % pred).

We conclude that the forced rebreathing N_2 method is a valid technique, which gives reproducible estimates of RV and TLC, irrespective of severity of airways obstruction in children with stable asthma; it is easily applicable and can be implemented usefully in clinical practice from the age of 8 yrs. However, when trapped gas is present, as appears to be the case in cystic fibrosis, it is not recruited into the mixing process, so that RV and TLC may be systematically underestimated. Hence, calculated volumes are similar to those obtained using the helium dilution method, but the procedure is quicker and the first ("single") breath yields extra information.

Acknowledgements: The authors thank the participants and the laboratory personnel of the hospitals for their contributions to this study.

References

1. Buist AS, Ross BB. – Predicted values for closing volume using a modified single-breath nitrogen test. *Am Rev Respir Dis*, 1973; 107: 744–752.
2. Becklake MR, Leclerc M, Strobach H, Swift J. – The N_2 closing volume test in population studies: sources of variation and reproducibility. *Am Rev Respir Dis*, 1975; 111: 141–147.
3. Sterk PJ, Quanjer PhH, Van der Maas LLJ, Wise ME, van der Lende R. – The validity of the single-breath

nitrogen determination of residual volume. *Bull Eur Physiopathol Respir,* 1980; 16: 195–213.
4. Nunneley SA, Flynn Jr, Camporesi EM. – Two-tracer method for rapid determination of residual volume. *J Appl Physiol,* 1974; 37: 286–289.
5. Sterk PJ, Quanjer PhH, van Zomeren BC, Wise ME, van der Lende R. – The single-breath nitrogen test in epidemiological surveys: an apppraisal. *Bull Eur Physiopathol Respir,* 1981; 17: 381–397.
6. DeGroodt EG, Quanjer PhH, Wise ME. – Short- and long–term variability of indices from the single and multiple–breath nitrogen test. *Bull Eur Physiopathol Resp,* 1984; 20: 271–277.
7. Mashalla YJS. – Measurement of lung volumes in healthy subjects and in subjects with airflow limitation. Emphasis on patients with airways obstruction resulting in gas mixing disorders. PhD Thesis, Dar es Salaam, Tanzania, 1987.
8. Reichel G. – Differences between intrathoracic gas measured by the body plethysmograph and functional residual capacity determined by gas dilution method. *In*: DuBois A., van de Woestijne K.P., eds. Progress in Respiration Research. Body Plethysmography. Basel, Karger, 1969; pp. 188–193.
9. DeGroodt EG, Quanjer PhH, Wise ME. – Influence of external resistance and minor flow variations on single-breath nitrogen test and residual volume. *Bull Eur Physiopathol Respir,* 1983; 19: 267–272.
10. Harris EA, Whitlock RML. – Correction of inert gas wash-in or wash-out for gas solubility in blood. *J Appl Physiol,* 1988; 65: 1598–1603.
11. DuBois AB, Botelho SY, Bedell GN, Marshall R, Comroe JH Jr. – A rapid plethysmographic method for measuring thoracic gas volume. A comparison with a nitrogen wash-out method for measuring functional residual capacity in normal subjects. *J Clin Invest,* 1956; 35: 322–326.
12. Quanjer PhH (ed). – Standardized lung function testing. Report Working Party Standardization of Lung Function Tests. European Community for Coal and Steel. *Bull Eur Physiopathol Respir,* 1983; 19 (Suppl. 5): 7–27.
13. Sixt R. – The single-breath nitrogen wash-out test. Standardization of lung function tests in paediatrics. *Eur Respir J,* 1989; 2 (Suppl. 4): 167s–170s.
14. Shore S, Milic-Emili J, Martin JG. – Reassessment of body plethysmographic technique for the measurement of thoracic gas volume in asthmatics. *Am Rev Respir Dis,* 1982; 126: 515–520.
15. Bohadana AB, Peslin R, Hannhart B, Teculescu D. – Influence of panting frequency on plethysmographic measurements of thoracic gas volume. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1982; 52: 739–747.
16. Zapletal A, Motoyama EK, van de Woestijne KP, Hunt VR, Bouhuys A. – Maximum expiratory flow-volume curves and airway conductance in children and adolescents. *J Appl Physiol,* 1969; 26: 308–316.
17. Bland JM, Altman DG. – Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet,* 1986; 307–310.
18. Peslin R, Marchal F, Gallina C, Oswald M, Crance JP. Assessment of thoracic gas volume by low frequency ambient pressure changes in children. *Eur Respir J,* 1988; 1: 594–599.
19. Wood RE, Boat TF, Doershuk CF. – Cystic fibrosis. State of the art. *Am Rev Respir Dis,* 1976: 113; 833–878.
20. Stanescu DC, Rodenstein D, Cauberghs M, van de Woestijne KP. – Failure of body plethysmography in bronchial asthma. *J Appl Physiol: Respirat Environ Exercise Physiol,* 1982; 52: 939–948.