



HAL
open science

METHACHOLINE INDUCED LUNG FUNCTION CHANGES MEASURED WITH INFANT BODY PLETHYSMOGRAPHY

Leo Pekka Malmberg, Lauri von Wright, Anne Kotaniemi-Syrjänen, Kristiina Malmström, Anna Pelkonen, Mika Mäkelä

► **To cite this version:**

Leo Pekka Malmberg, Lauri von Wright, Anne Kotaniemi-Syrjänen, Kristiina Malmström, Anna Pelkonen, et al.. METHACHOLINE INDUCED LUNG FUNCTION CHANGES MEASURED WITH INFANT BODY PLETHYSMOGRAPHY. *Pediatric Pulmonology*, 2010, 46 (4), pp.362. 10.1002/ppul.21375 . hal-00599820

HAL Id: hal-00599820

<https://hal.science/hal-00599820>

Submitted on 11 Jun 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**METHACHOLINE INDUCED LUNG FUNCTION CHANGES
MEASURED WITH INFANT BODY PLETHYSMOGRAPHY**

Journal:	<i>Pediatric Pulmonology</i>
Manuscript ID:	PPUL-10-0127.R2
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	03-Sep-2010
Complete List of Authors:	Malmberg, Leo; Helsinki University Central Hospital, Department of Allergy von Wright, Lauri; Helsinki University Kotaniemi-Syrjänen, Anne; Helsinki University Central Hospital, Department of Allergology Malmström, Kristiina; Helsinki University Central Hospital, Department of Allergology Pelkonen, Anna; Helsinki University Central Hospital, Department of Allergology Mäkelä, Mika; Helsinki University Hospital, Allergology
Keywords:	Bronchial provocation tests, Forced expiratory flow, Body plethysmography, Infant



ew

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

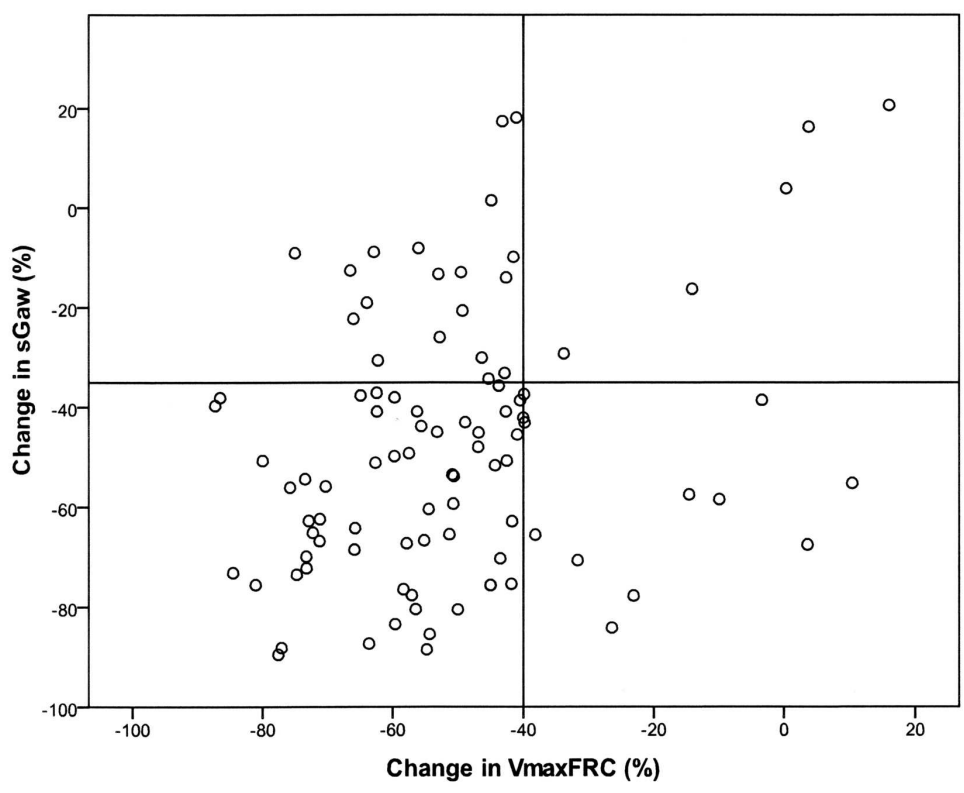


Figure 1. The relationship between methacholine induced post-challenge changes in sGaw and VmaxFRC. The vertical and horizontal lines represent corresponding cutoffs of significant change.
72x59mm (600 x 600 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

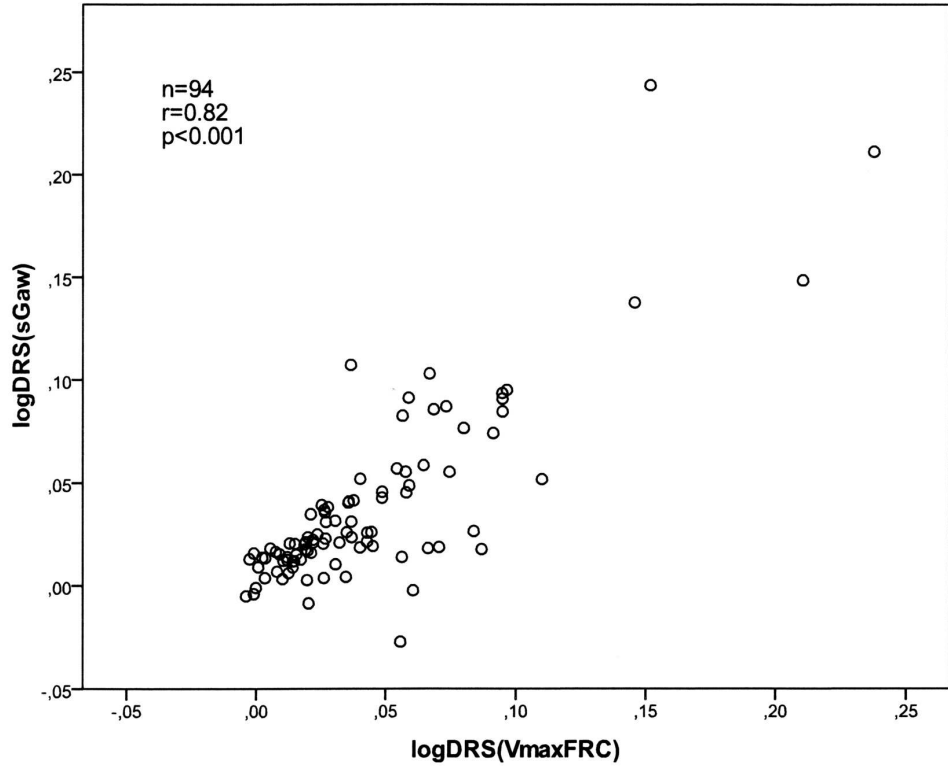


Figure 2. The relationship between dose response slopes (DRS) by sGaw and VmaxFRC during methacholine airway challenge.
73x60mm (600 x 600 DPI)

Table 1. Demographic data of the study infants.

N	94
Boys	72 (77 %)
Age, mo	14.0 (6.0 - 30.0)*
Gestational age, wk	39.7 (28.4 - 42.0)*
Length, cm	78.3 (63.5 - 91.5)*
Weight, kg	10.7 (6.5 - 18.2)*
Atopy [#]	44 (47 %)
Family history of atopy or asthma	80 (85 %)
Parental smoking	31 (33 %)
Dominant respiratory symptom	
-cough or excess sputum	52 (55 %)
-wheezy breathing	23 (25 %)
-dyspnoe or laborious breathing	19 (20 %)
Physician confirmed wheeze	74 (79 %)

* median (range); [#]SPT positive or atopic eczema

Table 2. Mean (SD) baseline and postchallenge lung function, and change from baseline expressed as absolute units (abs), percentage change (%) and as multiples of within subject variation (standard deviation) at baseline (SD_{ws}).

	Baseline	Postchallenge	Change (abs)	Change (%)	Change (SD_{ws})
FRC, ml	240 (55)	279 (69)*	39 (36) [#]	17 (17) [#]	5.6 (7.4) [#]
sGaw, $kPa^{-1}s^{-1}$	3.3 (2.8)	1.4 (1.0)*	-1.9 (2.4) [#]	-48 (27) [#]	-5.0 (4.1) [#]
V_{max} FRC, ml/s	237 (124)	116 (88)*	-121 (92) [#]	-51 (21) [#]	-23.0 (43.3) [#]
SaO ₂ , %	96 (1)	93 (2)*	-3 (2) [#]	-3 (2) [#]	

* $p < 0.001$ compared to baseline; [#] $p < 0.001$, one sample t-test

For Peer Review

Table 3. The relationship (correlation coefficients) of postchallenge percentage changes in sGaw, FRC, SaO₂ and V_{max}FRC.

	sGaw	FRC	SaO ₂
V _{max} FRC	0.30 (p=0.004)	-0.27 (p=0.008)	0.33 (p=0.001)
sGaw		-0.12 (p=0.24)	0.21 (p=0.04)
FRC			-0.09 (p=0.41)

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

METHACHOLINE INDUCED LUNG FUNCTION CHANGES MEASURED
WITH INFANT BODY PLETHYSMOGRAPHY

L. Pekka Malmberg, MD, PhD, Lauri von Wright, BM, Anne Kotaniemi-Syrjänen,
MD, PhD, Kristiina Malmström, MD, PhD, Anna S. Pelkonen, MD, PhD,
Mika J. Mäkelä, MD, PhD
Department of Allergy, Helsinki University Central Hospital, Helsinki, Finland

Correspondence

Dr. L. Pekka Malmberg, MD, PhD

Department of Allergy, Helsinki University Central Hospital

PoBox 160, 00029 HUS

Helsinki, Finland

Tel. +358 9 47186217

Fax. +358 9 47186280

email. pekka.malmberg@hus.fi

Grants: The study was financially supported by Helsinki University Central Hospital
Research Fund, Sigrid Juselius Foundation, Foundation for Paediatric Research in
Finland, Nummela Sanatorium Foundation, and Finska Läkaresällskapet. An
unrestricted research grant was received from Merck Sharp & Dohme Ltd.

Abbreviated title: AR measured by infant body plethysmography

SUMMARY

Several techniques have been applied to measure airway responsiveness (AR) in infants, but there are limited data on lung function changes measured by body plethysmography during induced bronchoconstriction. The aim of this study was to compare changes in maximum forced expiratory flow measured at functional residual capacity ($V'_{\max\text{FRC}}$) by rapid thoracoabdominal compression (RTC) technique with plethysmographic measurements of specific airway conductance (sG_{aw}), and to investigate whether changes in FRC occur during methacholine induced bronchoconstriction in infants.

We examined 94 infants with recurrent airway symptoms using methacholine airway challenge test including RTC and plethysmographic measurements. A significant association between changes in $V'_{\max\text{FRC}}$ and sG_{aw} ($r=0.30$; $p=0.004$) was observed, but after adjustments with baseline variability the changes in $V'_{\max\text{FRC}}$ were greater and showed a closer association with changes in oxygen saturation. At the point of maximal airway obstruction, there was a poor agreement between $V'_{\max\text{FRC}}$ and sG_{aw} to indicate a significant methacholine induced bronchoconstriction. Airway challenge was also associated with a significant increase in FRC ($p<0.001$), with decreasing $V'_{\max\text{FRC}}$.

We conclude that in infants undergoing airway challenge with methacholine, plethysmographic measurements of sG_{aw} correlate with the changes in $V_{\max\text{FRC}}$, but the agreement is poor and the methods cannot be used interchangeably. $V_{\max\text{FRC}}$ is also more sensitive to detect airway obstruction than sG_{aw} . However, methacholine induced bronchoconstriction was associated with significant increases in FRC, which may affect the validity of $V_{\max\text{FRC}}$ measurements during the test.

KEYWORDS

Bronchial provocation tests, forced expiratory flow, body plethysmography, infants

INTRODUCTION

Increased airway responsiveness (AR) is considered one of the basic pathophysiological manifestations of asthma. In infants, the relationship between wheezing lower respiratory tract illnesses and AR is more complex. While associated with clinical features such as atopy, current airway symptoms and the viral aetiology of wheeze,¹ the predictive value of AR in infancy is still unclear. Two longitudinal studies of randomly ascertained birth cohorts have suggested an association with abnormal airway function, persistence of lower respiratory symptoms and emergence of asthma later in life,^{2,3} but in a four-year prospective study of wheezy children, the level of AR showed no predictive value.⁴ While the contribution of AR measurements in the clinical assessment of infants with recurrent airway symptoms may be questioned,⁵ they afford an important tool for research of the pathophysiology, outcome and interventions of wheezing disorders in infancy.

The measurement of AR in infants requires a method to administer bronchoconstrictive agents (such as methacholine or histamine) and lung function tests to measure response to the test agonist. Rapid thoracoabdominal compression (RTC) is one of the lung function techniques which is standardized⁶ and can be applied in the measurement of AR in infants. It enables the assessment of maximal flow at functional residual capacity ($V'_{\max\text{FRC}}$) and requires several squeeze maneuvers to be conducted. During airway challenge tests, however, it is unclear whether repeated forceful compression of the thoracic cage affects infant bronchial tone as such, and whether induced bronchoconstriction may cause changes in the functional residual capacity (FRC) that could affect the validity of serial $V'_{\max\text{FRC}}$ measurements.

Plethysmographic method for measuring FRC and airway resistance (R_{aw}) was introduced in the late 1950s and has been later modified and standardized for use in young children and infants.⁷ Theoretically, plethysmography can be used to monitor infant lung function during bronchial provocation testing by measuring changes in R_{aw} , with the advantage of following simultaneous changes in FRC and conducting measurements during tidal breathing, without forced manouevres. However, so far there have been no attempts to **compare lung function measurements by body plethysmography with those of RTC** during airway challenge testing in infants.

1
2
3
4
5 The aim of the study was to compare methacholine induced changes in lung function
6 by body plethysmography, with those measured by more conventional RTC-
7 technique.¹ Specifically, we aimed to test the agreement between the changes in
8 $V'_{\max\text{FRC}}$ and the change in plethysmographic measurements of specific airway
9 conductance (sG_{aw}), both indexes of airway patency, and additionally to investigate
10 whether changes in FRC occur during methacholine induced bronchoconstriction.
11 Therefore, in a series of 94 infants undergoing airway challenge testing with the RTC
12 technique, baseline and post-challenge measurements with infant body
13 plethysmography were included.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

METHODS

Study subjects

The study consists of data of 94 infants, who had been referred to a tertiary center (Dept of Allergology, Helsinki University Central Hospital, Helsinki, Finland) due to recurrent airway symptoms and underwent a complete and successful airway challenge test between May 12, 2004 and June 20, 2007. These infants constituted part of the recruitment cohort of a prospective intervention study into infant asthma. **The design of the current study was observational, and the number of infants undergoing the challenge test was powered only for the prospective study.** The intervention study, including the performance of the baseline lung function and airway challenge tests during the recruitment phase, was approved by the local ethics committee. The tests were conducted after informed consent to conduct these tests had been received from the parents. The parents gave also their consent for the use of the lung function and airway challenge test results for research purposes. The demographic data of the study infants have been shown in Table 1. The mean (SD) duration of the infants' respiratory symptoms (cough, **dyspnea** or wheeze) was 7.5 (3.8) months, and 79 % of the infants had a history of physician confirmed wheeze. 42 % had atopic dermatitis, and a family history of asthma or allergy was found in 85 %. Five of the infants had born preterm (gestational age <36 weeks), and one of them (gestational age 28.4 weeks) needed ventilatory support and continuous oxygen supply for 2 weeks after birth due to respiratory distress syndrome (RDS).

Lung function tests

Before lung function measurements, the infants were clinically examined to exclude current respiratory infection or clinical obstruction. β_2 -agonists were withheld for 12 h prior to testing. The infants were sedated with orally administered chloralhydrate (50 – 100 mg / kg; maximum dose 1000 mg). Oxygen saturation (SaO₂) and heart rate were continuously monitored during lung function tests and airway challenge with a pulse oximeter (Biox 3700e; Ohmeda, Louisville, KY, USA).

1
2
3 During lung function testing, the infant was lying supine with the head supported in
4 the midline and the neck slightly extended to minimise airway or glottic obstruction.
5 All measurements were recorded and calculations performed with a commercial
6 paediatric pulmonary function equipment (Babybody Masterscreen, Jaeger GmbH,
7 Würzburg, Germany). The functional residual capacity (FRC) and specific airway
8 conductance (sG_{aw}) were measured by whole body plethysmography employing an
9 electronic algorithm to compensate for differences of temperature and humidity
10 between inspired and expired air.⁸ FRC was measured by selecting artifact free mouth
11 vs. box pressure tracings during endinspiratory occlusion which were not preceded by
12 pressure drift within the box, and did not show signs of leakage (such as change in
13 endexpiratory level after occlusion). Accordingly, pressure-flow tracings were
14 recorded during stable tidal breathing. Airway resistance was measured from the
15 linear portion of the pressure-flow curve **between 50 % of the maximum inspiratory
16 and expiratory tidal flow, respectively**. Loops without distortion due to artifacts and
17 with minimal thermal drift were chosen. At the baseline, two sets of measurements
18 including 3-5 occlusions and pressure-flow tracings were recorded to calculate the
19 mean FRC and sG_{aw} and their baseline within-subject variation (SD_{ws}).⁹
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34
35 The baseline measurements with body plethysmography were followed by the
36 assessment of partial expiratory flow volume curves.⁶ The maximum flow at FRC
37 (V'_{maxFRC}) was determined using the RTC technique. The jacket was wrapped around
38 the infant's chest and abdomen with the arms extended outside the jacket. Flow was
39 measured at the infant's nose and mouth with a pneumotachometer attached to a face
40 mask. A rim of silicone putty was applied around the mouth and nose and to the face
41 mask to provide an airtight seal. The compression pressure was progressively
42 increased until there was no further increase in V'_{maxFRC} . The flow volume curves
43 were considered acceptable when the peak flow occurred prior to expiring 50% of the
44 tidal volume, there were no transients in the tracings in the region of FRC, and forced
45 expiration lasted long enough to allow recording of flow at FRC. **According to our
46 protocol, which is similar to that used in the study of the reference values for
47 V'_{maxFRC} ,**¹⁰ three acceptable maximal partial expiratory flow volume curves were
48 recorded to calculate the mean V'_{maxFRC} and its baseline within-subject variation
49 (SD_{ws}).
50
51
52
53
54
55
56
57
58
59
60

Methacholine airway challenge

The details of the methacholine airway challenge have been described earlier.¹ After baseline measurements of $V'_{\max\text{FRC}}$, increasing doses of methacholine chloride were administered by using an inhalation-synchronized dosimeter (Spira Electro 2, Spira Respiratory Care Centre Ltd, Hämeenlinna, Finland). The dosimeter was set to be triggered by an inhaled volume of 20 ml, after which a methacholine chloride dose of 50 μg was nebulised within 0.2 seconds in an air volume of 25 ml, in each breath. The dosimeter incorporates an indicator for inspiratory flows and volumes, which were carefully monitored during the administration of methacholine to ensure sufficient tidal flows (>100 ml/s) and volumes (>50 ml). By calculating the number of breaths with nebulised methacholine, a rapid dosage scheme with four non-cumulative dose steps was applied (0.1, 0.3, 0.9 and 1.8 mg), with $V'_{\max\text{FRC}}$ being recorded after each dose (mean of 2-3 acceptable curves). The procedure was continued until a 40% decrease in $V'_{\max\text{FRC}}$ was observed or the maximum dose of methacholine had been administered. At each phase, the applied compression pressure was the same as that which achieved the highest flows at baseline. After the final measurement of $V'_{\max\text{FRC}}$, a plethysmographic measurement was performed to determine methacholine induced changes in FRC and sG_{aw} . A similar set of measurements were recorded as in the baseline, to assess postchallenge mean FRC and sG_{aw} . The lowest postchallenge value for SaO₂ was also recorded. Only infants with a complete, uninterrupted methacholine challenge test including successful baseline and post challenge measurements of FRC, sG_{aw} and $V'_{\max\text{FRC}}$ were included in the study.

The provocative dose of methacholine causing a 40% fall in $V'_{\max\text{FRC}}$ ($\text{PD}_{40} V'_{\max\text{FRC}}$) was determined from the dose-response curves. In addition, the degree of AR was estimated by calculating the dose response slope (DRS),¹¹ by dividing the maximum percentage change in lung function with the maximum dose of methacholine administered.

Following the challenge test, the infants received inhaled salbutamol (0.6 mg, 0.1 $\text{mg}\cdot\text{dose}^{-1}$; Ventoline Evohaler®; GlaxoSmithKline, Middlesex, UK) via nebunette® (AstraZeneca, Södertälje, Sweden), and the measurement of $V'_{\max\text{FRC}}$ was repeated 15 min after the salbutamol inhalation.

Statistics

For statistical analysis, SPSS 17.0 for Windows (SPSS Inc. Chicago, IL, USA) was used. Mean values, within subject standard deviation (SD_{ws}) and coefficient of variation (CV) were calculated for each of the baseline variables. Post-challenge changes in lung function were expressed as absolute units, percentage changes and as multiples of SD_{ws} -units, the latter indicating the change in absolute units divided by the within subject SD at baseline.

Scatter plots of the change in FRC, sG_{aw} and SaO_2 were formed as a function of the change in V'_{maxFRC} and the corresponding relationships were determined using linear regression analysis. The response data were divided into binomial classes according to the cutoff which was considered as a significant change indicating methacholine induced bronchoconstriction. For V'_{maxFRC} , a decrease of 40% or more was considered significant. Regression analysis was used to define a corresponding cutoff value for the change in sG_{aw} and the data was classified accordingly. Kappa-analysis was applied to assess agreement between these new binomial class variables of V'_{maxFRC} and sG_{aw} .

DRSs were calculated for each infant, based on the maximum dose of methacholine, and pre- and postchallenge results of V'_{maxFRC} and sG_{aw} . Due to distribution of DRS data, log10-based transformation was used, and the degree of airway responsiveness thereby assessed by using V'_{maxFRC} and sG_{aw} data, were compared with regression analysis.

RESULTS

Table 2 presents a summary of the baseline and post-challenge measurements and the changes in FRC, sG_{aw} , V'_{maxFRC} and SaO_2 during methacholine challenge. The median within subject CVs in baseline FRC, V_{maxFRC} and sG_{aw} were 3.3, 4.4 and 10.1 %, respectively. As a response to methacholine, sG_{aw} , V_{maxFRC} and SaO_2 decreased, and FRC increased significantly. The post-challenge percentage changes in sG_{aw} and V'_{maxFRC} were similar, but expressed as multiples of the baseline within-subject variation, V'_{maxFRC} showed a greater change, indicating better sensitivity to assess methacholine induced bronchoconstriction.

Figure 1 shows the scatter plot between postchallenge changes in sG_{aw} and V'_{maxFRC} , indicating a loose association. V'_{maxFRC} showed also an correlation of similar degree with postchallenge changes in airway resistance (R_{aw}) ($r=-0.23$; $p=0.025$). Almost all of the studied infants responded to methacholine challenge with decreases in both V'_{maxFRC} and sG_{aw} . The correlation coefficients between postchallenge changes in FRC, sG_{aw} , SaO_2 and FRC have been presented in Table 3. The changes in V'_{maxFRC} were significantly associated with changes in FRC and SaO_2 , but sG_{aw} showed a weaker relationship with SaO_2 and no correlation to FRC.

Based on regression analysis between V'_{maxFRC} and sG_{aw} , a 40% decrease in V'_{maxFRC} was approximately comparable to a 35% decrease in sG_{aw} . Accordingly the changes in V'_{maxFRC} and sG_{aw} were divided into two classes (significant or non-significant), indicated in Figure 1 as horizontal and vertical lines. Crosstabulation and kappa-analysis of these binomial classes gave the κ -value of 0.057 ($p=0.56$) indicating poor agreement between the V'_{maxFRC} and sG_{aw} as measures of methacholine induced bronchoconstriction.

In the current study group of infants with recurrent airway symptoms, the median (range) $PD_{40}V'_{maxFRC}$ was 0.54 (0.05 - >1.8) mg of methacholine. Figure 2 shows a significant association of the estimated AR in terms of the logDRS using V_{maxFRC} and sG_{aw} data, the value of 0 indicating no response, and higher values increasing responsiveness.

DISCUSSION

In infants undergoing airway challenge with methacholine, an expected association between $V'_{\max\text{FRC}}$ and plethysmographic measurements of sG_{aw} , both indices of airway patency, was observed. However, this relationship was rather weak and there was a poor agreement between these variables to indicate a significant methacholine induced bronchoconstriction. Interestingly, challenging infants with methacholine have also significant effects to their FRC levels, determined by body plethysmography. This may have implications to the validity of $V_{\max\text{FRC}}$ measurements during the test.

Difficulties in standardizing the bronchial challenge tests in infants have been acknowledged previously.¹² In particular, the dose of the test agonist is difficult to adjust for children of different size. We have chosen a direct-acting bronchoconstrictor methacholine, which was administered using a rapid dosimetric method found safe and well tolerated by infants.¹ Furthermore, different lung function methods are available to assess the response to the bronchoconstricting agent. The original approaches to evaluate airway responsiveness to histamine or methacholine in infants applied partial expiratory flow volume curves by using the RTC technique.¹³⁻¹⁵ Subsequently, a methodology using the raised volume forced expiration technique was reported, which enables measurements of forced expiratory volumes as indices of airway patency.¹⁶ There are a limited number of studies evaluating other lung function techniques to assess histamine or methacholine induced bronchoconstriction, than forced expiration. Benoist et al.¹⁷ compared the ability of $V_{\max\text{FRC}}$, tidal breathing parameters and measures of passive respiratory mechanics to assess a response to methacholine determined by a decrease in transcutaneous oxygen tension. They concluded that all these noninvasive lung function tests could be used to detect minor or moderate airway obstruction. Springer et al.¹⁸ studied thoracoabdominal asynchrony in 17 infants by using respiratory inductance plethysmography during a methacholine challenge, and found the method sensitive enough to measure bronchial reactivity in most (82 %) of the children. In a large sample of infants, measurements of transcutaneous oxygen tension and forced expiratory volume in 0.5 second by raised volume RTC technique were superior in sensitivity during induced airway obstruction, compared to auscultation or tidal breathing indexes.¹⁹ By using low

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

frequency forced oscillation it has been also shown that inhaled methacholine alters both airway and respiratory tissue mechanics in infants.²⁰ Only a few studies have applied infant whole body plethysmography during airway challenge testing.²¹⁻²³ In principle, this method allows rapid assessment of changes in airway resistance with simultaneous measurements of lung volumes (FRC). The potential advantage over forced expirations is that the measurements take place during normal tidal breathing, without interfering the breathing pattern, bronchial tone or resting lung volumes levels.²⁴ Although some comparative studies have been published in awake preschool children,^{25,26} so far, there have been no attempts to compare whole body plethysmography and RTC technique in assessing airway responsiveness in sedated infants.

The present results in infants during airway challenge demonstrate that plethysmographic measurements of sG_{aw} result in an estimate of airway responsiveness that closely correlates with that based on the response in V'_{maxFRC} . However, at the point of maximal and similar degree of airway obstruction, there seems to be a poor agreement between the two lung function methods, indicating that their physiological determinants are different. Little is known of the structure/function relationships of infants' airways, but it is assumed that measures of airway resistance reflect mainly changes in the larger airways, and forced expiratory flows at the end expiratory level are more sensitive to peripheral airway obstruction.⁵ As the precise target of the test agonist in the bronchial tree is largely unknown and possibly varies between individual infants, the significance of these physiological mechanisms remain hypothetical. Factors associated with the infant's status (such as posture, changing breathing pattern, sleep state, glottis closure) as well as method-specific sources of error increase the inherent variability and probably also contribute to the disagreement between the two estimates of airway obstruction.^{6,7} In the current settings, the within-subject variation of V_{maxFRC} was significantly lower than that of sG_{aw} . This resulted in a more pronounced postchallenge change in V_{maxFRC} than in sG_{aw} , when expressed as multiples of baseline SD_{ws} , suggesting that the former is a more sensitive measure of methacholine induced bronchoconstriction. Closer association of SaO_2 with V_{maxFRC} also favours its use over sG_{aw} in the assessment of response during airway challenge.

1
2
3
4 In the present study infants, methacholine induced airway obstruction was associated
5 with a significant increase in FRC level, determined by body plethysmography. The
6 prior repeated forced expirations should not significantly alter FRC,²⁷ therefore
7 methacholine induced dynamic changes in breathing pattern or air trapping are more
8 likely mechanisms. The finding is important, since the measurements of $V_{\max\text{FRC}}$ are
9 dependent on the actual end expiratory lung volume, and an increase in FRC may
10 result in an underestimation of the decrease in $V_{\max\text{FRC}}$ during the challenge test. The
11 observation is also a strong argument to favor raised volume RTC technique in
12 bronchial challenge testing of infants, since forced expiratory volumes after full lung
13 inflation are not effected by changes in FRC level. Dynamic instability of FRC in
14 infants is a major source of variability of $V_{\max\text{FRC}}$ measurements.²⁸ However, in a
15 study of infants comparing tidal and raised volume RTC measurements during
16 methacholine airway challenge, the agreement between the two methods was good
17 and neither showed any obvious advantages over the other.¹⁶

18
19
20
21
22
23
24
25
26
27
28
29
30 Changes in lung function were induced by methacholine, which has a limited time of
31 action. The postchallenge measurements of lung function were assessed in a fixed
32 sequence, as the decrease of $V'_{\max\text{FRC}}$ was followed as an endpoint **crit**erion of the
33 challenge test. Time dependent alleviation of airway obstruction would thus favour
34 sensitivity of the RTC method over that of sGaw, and affect the association between
35 them as well as with SaO₂. However, studies on the time course of the methacholine
36 induced bronchoconstriction have shown that, even though the peak action usually
37 occurs within 4 minutes, it is followed by a plateau phase where significant
38 bronchoconstriction is sustained for over 15 minutes.²⁹ In a similar comparative study
39 of four different lung function methods in preschool children, Klug and Bisgaard²⁵
40 found relatively stable airway obstruction for at least 10 minutes after methacholine
41 inhalation. This time frame was found to be sufficient in the current study to make all
42 the postchallenge measurements ($V'_{\max\text{FRC}}$, FRC and sGaw). Accordingly, they should
43 not be **severely** affected by time dependent changes of airway obstruction.

44
45
46
47
48
49
50
51
52
53
54
55
56
57 We conclude that in infants undergoing airway challenge with methacholine,
58 plethysmographic measurements of sG_{aw} correlate with the changes in $V_{\max\text{FRC}}$, but
59 the agreement is poor and the methods cannot be used interchangeably. $V_{\max\text{FRC}}$ is
60 also more sensitive to detect airway obstruction than sG_{aw}. However, methacholine

1
2
3 induced bronchoconstriction was associated with significant increases in FRC, which
4 may affect the validity of $V_{\max\text{FRC}}$ measurements during the test.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5 ACKNOWLEDGEMENTS
6
7

8 We wish to thank Tuija Rito, RN, and Helena Punkari, RN, for their skill and care
9 with the infants and their parents. The personnel of Spira Respiratory Care Center
10 Ltd, is acknowledged for their help in the development of the inhalation-synchronized
11 dosimetric equipment used in the methacholine challenge.
12
13
14
15
16
17

18
19
20
21 CONFLICTS OF INTEREST
22
23

24 None of the authors has any competing interests to declare.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FIGURE LEGENDS

Figure 1. The relationship between methacholine induced post-challenge changes in sGaw and VmaxFRC. The vertical and horizontal lines represent corresponding cutoffs of significant change.

Figure 2. The relationship between dose response slopes (DRS) by sGaw and VmaxFRC during methacholine airway challenge.

For Peer Review

REFERENCES

1. Kotaniemi-Syrjänen A, Malmberg LP, Pelkonen A, Malmström K, Mäkelä M. Airway responsiveness: associated features in infants with recurrent respiratory symptoms. *Eur Resp J* 2007;30:1150-1157.
2. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Le Souef PN. Airway responsiveness in early infancy predicts asthma, lung function and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001;163:37-42.
3. Turner SW, Young S, Goldblatt J, Landau LI, Le Souef PN. Childhood asthma and increased airway responsiveness. A relationship that begins in infancy. *Am J Respir Crit Care Med* 2008;179:98-104.
4. Delacourt C, Benoist MR, Waernessyckle S, Rufin P, Brouard JJ, de Blic J, Scheinmann P. Relationship between bronchial responsiveness and clinical evolution in infants who wheeze. *Am J Respir Crit Care Med* 2001;164:1382-1386.
5. Frey U. Clinical applications of infant lung function testing: does it contribute to clinical decision making ? *Pediatr Respir Rev* 2001;2:126-130.
6. Sly PD, Tepper RS, Henschen M, Gappa M, Stocks J. ERS/ATS Task force on standards for infant respiratory function testing. Tidal forced expirations. *Eur Resp J* 2000;16:741-748.
7. Stocks J, Godfrey S, Beardsmore C, Bar-Yishay E, Castile E. Plethysmographic measurements of lung volume and airway resistance. *Eur Resp J* 2001;17:302-312.
8. Reinmann B, Stocks J, Frey U. Assessment of an infant whole-body plethysmograph using an infant lung model. *Eur Resp J* 2001;17:765-772.
9. Malmberg LP, Pelkonen A, Hakulinen A, Hero M, Pohjavuori M, Skyttä J, Turpeinen M. Intraindividual variability of infant whole body plethysmographic measurements: effects of age and disease. *Pediatr Pulmonol* 1999;28:356-362.
10. Hoo A-F, Dezateux C, Hanrahan JP, Cole TJ, Tepper R, Stocks J. Sex-specific prediction equations for V'_{maxFRC} in infancy. A multicenter study. *Am J Respir Crit Care Med* 2002;165:1085-1092.

11. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine: an approach suitable for population studies. *Am Rev Respir Dis* 1987;136:1412-1417.
12. Le Souef PN. Validity of methods used to test airway responsiveness in children. *Lancet* 1992;339:1282-1284.
13. Prendiville A, Green S, Silverman M. Bronchial responsiveness to histamine in wheezy infants. *Thorax* 1987;42:92-99.
14. Tepper R. Airway reactivity in infants: a positive response to methacholine and metaprotenol. *J Appl Physiol* 1987;62:1155-1159.
15. Le Souef PN, Geelhoed GC, Turner DJ, Morgan SEG, Landau LI. Response of normal infants to inhaled histamine. *Am Rev Respir Dis* 1989;139:62-66.
16. Hayden MJ, Devadason SG, Sly PD, Wildhaber J, Le Souef PN. Methacholine responsiveness using the raised volume forced expiration technique in infants. *Am J Respir Crit Care Med* 1997;155:1670-1675.
17. Benoist MR, Brouard JJ, Rufin P, Delacourt C, Waernessyckle S, Scheinmann P. Ability of new lung function tests to assess methacholine-induced airway obstruction in infants. *Pediatr Pulmonol* 1994;18:308-316.
18. Springer C, Godfrey S, Vilozi D, Bar-Yishay E, Noviski N, Avital A. Comparison of respiratory inductance plethysmography with thoracoabdominal compression in bronchial challenges in infants and young children. *Am J Respir Crit Care Med* 1996;154:665-669.
19. Loland L, Buchvald F, Brydensholt Halkjaer L, Anhoj J, Hall G, Persson T, Krause TG, Bisgaard H. Sensitivity of bronchial responsiveness measurements in young infants. *Chest* 2006;129:669-675.
20. Hall G, Hantos Z, Wildhaber J, F P, Sly PD. Methacholine responsiveness in infants assessed with low frequency forced oscillation and forced expiration techniques. *Thorax* 2001;56:42-47.
21. Gutkowski P. Airway responsiveness following wheezy bronchitis in infants. *Eur Resp J* 1990;3:807-811.
22. O'Callaghan C, Milner AD, Webb MS, Swarbrick A. Nebulized water as a bronchoconstricting agent in infancy. *Arch Dis Child* 1991;66:948-951.
23. Yuksel B, Greenough A. Nebulized sodium cromoglycate in preterm infants - protection against water challenge-induced bronchoconstriction. *Respir Med* 1993;87:37-42.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
24. Lum S, Hulskamp G, Hoo A-F, Ljungberg H, Stocks J. Effect of raised lung volume technique on subsequent measures of VmaxFRC in infants. *Pediatr Pulmonol* 2004;38:146-154.
 25. Klug B, Bisgaard H. Measurement of lung function in awake 2-4-year old asthmatic children during methacholine challenge and acute asthma. *Pediatr Pulmonol* 1996;21:290-300.
 26. Klug B, Bisgaard H. Measurement of the specific airway resistance by plethysmography in young children accompanied by an adult. *Eur Respir J* 1997;10:1599-1605.
 27. Lanteri CJ, Raven JM, Sly PD. Effect of expiration on thoracic gas volume in wheezy infants. *Pediatr Pulmonol* 1990;9:220-223.
 28. Delacourt C, Benoist MR, Waernessyckle S, Rufin P, Brouard JJ, de Blic J, Scheinmann P. Repeatability of lung function tests during methacholine challenge in wheezy infants. *Thorax* 1998;53:933-938.
 29. Cartier A, Malo J-C, Begin P, Sestier M, Martin RR. Time course of the bronchoconstriction induced by inhaled histamine and methacholine. *J Appl Physiol* 1983;54:821-826.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

METHACHOLINE INDUCED LUNG FUNCTION CHANGES MEASURED
WITH INFANT BODY PLETHYSMOGRAPHY

L. Pekka Malmberg, MD, PhD, Lauri von Wright, BM, Anne Kotaniemi-Syrjänen,
MD, PhD, Kristiina Malmström, MD, PhD, Anna S. Pelkonen, MD, PhD,
Mika J. Mäkelä, MD, PhD
Department of Allergy, Helsinki University Central Hospital, Helsinki, Finland

Correspondence

Dr. L. Pekka Malmberg, MD, PhD

Department of Allergy, Helsinki University Central Hospital

PoBox 160, 00029 HUS

Helsinki, Finland

Tel. +358 9 47186217

Fax. +358 9 47186280

email. pekka.malmberg@hus.fi

Grants: The study was financially supported by Helsinki University Central Hospital
Research Fund, Sigrid Juselius Foundation, Foundation for Paediatric Research in
Finland, Nummela Sanatorium Foundation, and Finska Läkaresällskapet. An
unrestricted research grant was received from Merck Sharp & Dohme Ltd.

Abbreviated title: AR measured by infant body plethysmography

SUMMARY

Several techniques have been applied to measure airway responsiveness (AR) in infants, but there are limited data on lung function changes measured by body plethysmography during induced bronchoconstriction. The aim of this study was to compare changes in maximum forced expiratory flow measured at functional residual capacity ($V'_{\max\text{FRC}}$) by rapid thoracoabdominal compression (RTC) technique with plethysmographic measurements of specific airway conductance (sG_{aw}), and to investigate whether changes in FRC occur during methacholine induced bronchoconstriction in infants.

We examined 94 infants with recurrent airway symptoms using methacholine airway challenge test including RTC and plethysmographic measurements. A significant association between changes in $V'_{\max\text{FRC}}$ and sG_{aw} ($r=0.30$; $p=0.004$) was observed, but after adjustments with baseline variability the changes in $V'_{\max\text{FRC}}$ were greater and showed a closer association with changes in oxygen saturation. At the point of maximal airway obstruction, there was a poor agreement between $V'_{\max\text{FRC}}$ and sG_{aw} to indicate a significant methacholine induced bronchoconstriction. Airway challenge was also associated with a significant increase in FRC ($p<0.001$), with decreasing $V'_{\max\text{FRC}}$.

We conclude that in infants undergoing airway challenge with methacholine, plethysmographic measurements of sG_{aw} correlate with the changes in $V_{\max\text{FRC}}$, but the agreement is poor and the methods cannot be used interchangeably. $V_{\max\text{FRC}}$ is also more sensitive to detect airway obstruction than sG_{aw} . However, methacholine induced bronchoconstriction was associated with significant increases in FRC, which may affect the validity of $V_{\max\text{FRC}}$ measurements during the test.

KEYWORDS

Bronchial provocation tests, forced expiratory flow, body plethysmography, infants

INTRODUCTION

Increased airway responsiveness (AR) is considered one of the basic pathophysiological manifestations of asthma. In infants, the relationship between wheezing lower respiratory tract illnesses and AR is more complex. While associated with clinical features such as atopy, current airway symptoms and the viral aetiology of wheeze,¹ the predictive value of AR in infancy is still unclear. Two longitudinal studies of randomly ascertained birth cohorts have suggested an association with abnormal airway function, persistence of lower respiratory symptoms and emergence of asthma later in life,^{2,3} but in a four-year prospective study of wheezy children, the level of AR showed no predictive value.⁴ While the contribution of AR measurements in the clinical assessment of infants with recurrent airway symptoms may be questioned,⁵ they afford an important tool for research of the pathophysiology, outcome and interventions of wheezing disorders in infancy.

The measurement of AR in infants requires a method to administer bronchoconstrictive agents (such as methacholine or histamine) and lung function tests to measure response to the test agonist. Rapid thoracoabdominal compression (RTC) is one of the lung function techniques which is standardized⁶ and can be applied in the measurement of AR in infants. It enables the assessment of maximal flow at functional residual capacity ($V'_{\max\text{FRC}}$) and requires several squeeze maneuvers to be conducted. During airway challenge tests, however, it is unclear whether repeated forceful compression of the thoracic cage affects infant bronchial tone as such, and whether induced bronchoconstriction may cause changes in the functional residual capacity (FRC) that could affect the validity of serial $V'_{\max\text{FRC}}$ measurements.

Plethysmographic method for measuring FRC and airway resistance (R_{aw}) was introduced in the late 1950s and has been later modified and standardized for use in young children and infants.⁷ Theoretically, plethysmography can be used to monitor infant lung function during bronchial provocation testing by measuring changes in R_{aw} , with the advantage of following simultaneous changes in FRC and conducting measurements during tidal breathing, without forced manouevres. However, so far there have been no attempts to compare lung function measurements by body plethysmography with those of RTC during airway challenge testing in infants.

1
2
3
4
5 The aim of the study was to compare methacholine induced changes in lung function
6 by body plethysmography, with those measured by more conventional RTC-
7 technique.¹ Specifically, we aimed to test the agreement between the changes in
8 $V'_{\max\text{FRC}}$ and the change in plethysmographic measurements of specific airway
9 conductance (sG_{aw}), both indexes of airway patency, and additionally to investigate
10 whether changes in FRC occur during methacholine induced bronchoconstriction.
11 Therefore, in a series of 94 infants undergoing airway challenge testing with the RTC
12 technique, baseline and post-challenge measurements with infant body
13 plethysmography were included.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

METHODS

Study subjects

The study consists of data of 94 infants, who had been referred to a tertiary center (Dept of Allergology, Helsinki University Central Hospital, Helsinki, Finland) due to recurrent airway symptoms and underwent a complete and successful airway challenge test between May 12, 2004 and June 20, 2007. These infants constituted part of the recruitment cohort of a prospective intervention study into infant asthma. The design of the current study was observational, and the number of infants undergoing the challenge test was powered only for the prospective study. The intervention study, including the performance of the baseline lung function and airway challenge tests during the recruitment phase, was approved by the local ethics committee. The tests were conducted after informed consent to conduct these tests had been received from the parents. The parents gave also their consent for the use of the lung function and airway challenge test results for research purposes. The demographic data of the study infants have been shown in Table 1. The mean (SD) duration of the infants' respiratory symptoms (cough, dyspnea or wheeze) was 7.5 (3.8) months, and 79 % of the infants had a history of physician confirmed wheeze. 42 % had atopic dermatitis, and a family history of asthma or allergy was found in 85 %. Five of the infants had born preterm (gestational age <36 weeks), and one of them (gestational age 28.4 weeks) needed ventilatory support and continuous oxygen supply for 2 weeks after birth due to respiratory distress syndrome (RDS).

Lung function tests

Before lung function measurements, the infants were clinically examined to exclude current respiratory infection or clinical obstruction. β_2 -agonists were withheld for 12 h prior to testing. The infants were sedated with orally administered chloralhydrate (50 – 100 mg / kg; maximum dose 1000 mg). Oxygen saturation (SaO₂) and heart rate were continuously monitored during lung function tests and airway challenge with a pulse oximeter (Biox 3700e; Ohmeda, Louisville, KY, USA).

1
2
3 During lung function testing, the infant was lying supine with the head supported in
4 the midline and the neck slightly extended to minimise airway or glottic obstruction.
5 All measurements were recorded and calculations performed with a commercial
6 paediatric pulmonary function equipment (Babybody Masterscreen, Jaeger GmbH,
7 Würzburg, Germany). The functional residual capacity (FRC) and specific airway
8 conductance (sG_{aw}) were measured by whole body plethysmography employing an
9 electronic algorithm to compensate for differences of temperature and humidity
10 between inspired and expired air.⁸ FRC was measured by selecting artifact free mouth
11 vs. box pressure tracings during endinspiratory occlusion which were not preceded by
12 pressure drift within the box, and did not show signs of leakage (such as change in
13 endexpiratory level after occlusion). Accordingly, pressure-flow tracings were
14 recorded during stable tidal breathing. Airway resistance was measured from the
15 linear portion of the pressure-flow curve between 50 % of the maximum inspiratory
16 and expiratory tidal flow, respectively. Loops without distortion due to artifacts and
17 with minimal thermal drift were chosen. At the baseline, two sets of measurements
18 including 3-5 occlusions and pressure-flow tracings were recorded to calculate the
19 mean FRC and sG_{aw} and their baseline within-subject variation (SD_{ws}).⁹
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34
35 The baseline measurements with body plethysmography were followed by the
36 assessment of partial expiratory flow volume curves.⁶ The maximum flow at FRC
37 (V'_{maxFRC}) was determined using the RTC technique. The jacket was wrapped around
38 the infant's chest and abdomen with the arms extended outside the jacket. Flow was
39 measured at the infant's nose and mouth with a pneumotachometer attached to a face
40 mask. A rim of silicone putty was applied around the mouth and nose and to the face
41 mask to provide an airtight seal. The compression pressure was progressively
42 increased until there was no further increase in V'_{maxFRC} . The flow volume curves
43 were considered acceptable when the peak flow occurred prior to expiring 50% of the
44 tidal volume, there were no transients in the tracings in the region of FRC, and forced
45 expiration lasted long enough to allow recording of flow at FRC. According to our
46 protocol, which is similar to that used in the study of the reference values for
47 V'_{maxFRC} ,¹⁰ three acceptable maximal partial expiratory flow volume curves were
48 recorded to calculate the mean V'_{maxFRC} and its baseline within-subject variation
49 (SD_{ws}).
50
51
52
53
54
55
56
57
58
59
60

Methacholine airway challenge

The details of the methacholine airway challenge have been described earlier.¹ After baseline measurements of $V'_{\max\text{FRC}}$, increasing doses of methacholine chloride were administered by using an inhalation-synchronized dosimeter (Spira Electro 2, Spira Respiratory Care Centre Ltd, Hämeenlinna, Finland). The dosimeter was set to be triggered by an inhaled volume of 20 ml, after which a methacholine chloride dose of 50 μg was nebulised within 0.2 seconds in an air volume of 25 ml, in each breath. The dosimeter incorporates an indicator for inspiratory flows and volumes, which were carefully monitored during the administration of methacholine to ensure sufficient tidal flows (>100 ml/s) and volumes (>50 ml). By calculating the number of breaths with nebulised methacholine, a rapid dosage scheme with four non-cumulative dose steps was applied (0.1, 0.3, 0.9 and 1.8 mg), with $V'_{\max\text{FRC}}$ being recorded after each dose (mean of 2-3 acceptable curves). The procedure was continued until a 40% decrease in $V'_{\max\text{FRC}}$ was observed or the maximum dose of methacholine had been administered. At each phase, the applied compression pressure was the same as that which achieved the highest flows at baseline. After the final measurement of $V'_{\max\text{FRC}}$, a plethysmographic measurement was performed to determine methacholine induced changes in FRC and sG_{aw} . A similar set of measurements were recorded as in the baseline, to assess postchallenge mean FRC and sG_{aw} . The lowest postchallenge value for SaO₂ was also recorded. Only infants with a complete, uninterrupted methacholine challenge test including successful baseline and post challenge measurements of FRC, sG_{aw} and $V'_{\max\text{FRC}}$ were included in the study.

The provocative dose of methacholine causing a 40% fall in $V'_{\max\text{FRC}}$ ($\text{PD}_{40} V'_{\max\text{FRC}}$) was determined from the dose-response curves. In addition, the degree of AR was estimated by calculating the dose response slope (DRS),¹¹ by dividing the maximum percentage change in lung function with the maximum dose of methacholine administered.

Following the challenge test, the infants received inhaled salbutamol (0.6 mg, 0.1 $\text{mg}\cdot\text{dose}^{-1}$; Ventoline Evohaler®; GlaxoSmithKline, Middlesex, UK) via nebunette® (AstraZeneca, Södertälje, Sweden), and the measurement of $V'_{\max\text{FRC}}$ was repeated 15 min after the salbutamol inhalation.

Statistics

For statistical analysis, SPSS 17.0 for Windows (SPSS Inc. Chicago, IL, USA) was used. Mean values, within subject standard deviation (SD_{ws}) and coefficient of variation (CV) were calculated for each of the baseline variables. Post-challenge changes in lung function were expressed as absolute units, percentage changes and as multiples of SD_{ws} -units, the latter indicating the change in absolute units divided by the within subject SD at baseline.

Scatter plots of the change in FRC, sG_{aw} and SaO_2 were formed as a function of the change in V'_{maxFRC} and the corresponding relationships were determined using linear regression analysis. The response data were divided into binomial classes according to the cutoff which was considered as a significant change indicating methacholine induced bronchoconstriction. For V'_{maxFRC} , a decrease of 40% or more was considered significant. Regression analysis was used to define a corresponding cutoff value for the change in sG_{aw} and the data was classified accordingly. Kappa-analysis was applied to assess agreement between these new binomial class variables of V'_{maxFRC} and sG_{aw} .

DRSs were calculated for each infant, based on the maximum dose of methacholine, and pre- and postchallenge results of V'_{maxFRC} and sG_{aw} . Due to distribution of DRS data, log10-based transformation was used, and the degree of airway responsiveness thereby assessed by using V'_{maxFRC} and sG_{aw} data, were compared with regression analysis.

RESULTS

Table 2 presents a summary of the baseline and post-challenge measurements and the changes in FRC, sG_{aw} , V'_{maxFRC} and SaO_2 during methacholine challenge. The median within subject CVs in baseline FRC, V_{maxFRC} and sG_{aw} were 3.3, 4.4 and 10.1 %, respectively. As a response to methacholine, sG_{aw} , V_{maxFRC} and SaO_2 decreased, and FRC increased significantly. The post-challenge percentage changes in sG_{aw} and V'_{maxFRC} were similar, but expressed as multiples of the baseline within-subject variation, V'_{maxFRC} showed a greater change, indicating better sensitivity to assess methacholine induced bronchoconstriction.

Figure 1 shows the scatter plot between postchallenge changes in sG_{aw} and V'_{maxFRC} , indicating a loose association. V'_{maxFRC} showed also an correlation of similar degree with postchallenge changes in airway resistance (R_{aw}) ($r=-0.23$; $p=0.025$). Almost all of the studied infants responded to methacholine challenge with decreases in both V'_{maxFRC} and sG_{aw} . The correlation coefficients between postchallenge changes in FRC, sG_{aw} , SaO_2 and FRC have been presented in Table 3. The changes in V'_{maxFRC} were significantly associated with changes in FRC and SaO_2 , but sG_{aw} showed a weaker relationship with SaO_2 and no correlation to FRC.

Based on regression analysis between V'_{maxFRC} and sG_{aw} , a 40% decrease in V'_{maxFRC} was approximately comparable to a 35% decrease in sG_{aw} . Accordingly the changes in V'_{maxFRC} and sG_{aw} were divided into two classes (significant or non-significant), indicated in Figure 1 as horizontal and vertical lines. Crosstabulation and kappa-analysis of these binomial classes gave the κ -value of 0.057 ($p=0.56$) indicating poor agreement between the V'_{maxFRC} and sG_{aw} as measures of methacholine induced bronchoconstriction.

In the current study group of infants with recurrent airway symptoms, the median (range) $PD_{40}V'_{maxFRC}$ was 0.54 (0.05 - >1.8) mg of methacholine. Figure 2 shows a significant association of the estimated AR in terms of the logDRS using V_{maxFRC} and sG_{aw} data, the value of 0 indicating no response, and higher values increasing responsiveness.

DISCUSSION

In infants undergoing airway challenge with methacholine, an expected association between $V'_{\max\text{FRC}}$ and plethysmographic measurements of sG_{aw} , both indices of airway patency, was observed. However, this relationship was rather weak and there was a poor agreement between these variables to indicate a significant methacholine induced bronchoconstriction. Interestingly, challenging infants with methacholine have also significant effects to their FRC levels, determined by body plethysmography. This may have implications to the validity of $V_{\max\text{FRC}}$ measurements during the test.

Difficulties in standardizing the bronchial challenge tests in infants have been acknowledged previously.¹² In particular, the dose of the test agonist is difficult to adjust for children of different size. We have chosen a direct-acting bronchoconstrictor methacholine, which was administered using a rapid dosimetric method found safe and well tolerated by infants.¹ Furthermore, different lung function methods are available to assess the response to the bronchoconstricting agent. The original approaches to evaluate airway responsiveness to histamine or methacholine in infants applied partial expiratory flow volume curves by using the RTC technique.¹³⁻¹⁵ Subsequently, a methodology using the raised volume forced expiration technique was reported, which enables measurements of forced expiratory volumes as indices of airway patency.¹⁶ There are a limited number of studies evaluating other lung function techniques to assess histamine or methacholine induced bronchoconstriction, than forced expiration. Benoist et al.¹⁷ compared the ability of $V_{\max\text{FRC}}$, tidal breathing parameters and measures of passive respiratory mechanics to assess a response to methacholine determined by a decrease in transcutaneous oxygen tension. They concluded that all these noninvasive lung function tests could be used to detect minor or moderate airway obstruction. Springer et al.¹⁸ studied thoracoabdominal asynchrony in 17 infants by using respiratory inductance plethysmography during a methacholine challenge, and found the method sensitive enough to measure bronchial reactivity in most (82 %) of the children. In a large sample of infants, measurements of transcutaneous oxygen tension and forced expiratory volume in 0.5 second by raised volume RTC technique were superior in sensitivity during induced airway obstruction, compared to auscultation or tidal breathing indexes.¹⁹ By using low

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

frequency forced oscillation it has been also shown that inhaled methacholine alters both airway and respiratory tissue mechanics in infants.²⁰ Only a few studies have applied infant whole body plethysmography during airway challenge testing.²¹⁻²³ In principle, this method allows rapid assessment of changes in airway resistance with simultaneous measurements of lung volumes (FRC). The potential advantage over forced expirations is that the measurements take place during normal tidal breathing, without interfering the breathing pattern, bronchial tone or resting lung volumes levels.²⁴ Although some comparative studies have been published in awake preschool children,^{25,26} so far, there have been no attempts to compare whole body plethysmography and RTC technique in assessing airway responsiveness in sedated infants.

The present results in infants during airway challenge demonstrate that plethysmographic measurements of sG_{aw} result in an estimate of airway responsiveness that closely correlates with that based on the response in V'_{maxFRC} . However, at the point of maximal and similar degree of airway obstruction, there seems to be a poor agreement between the two lung function methods, indicating that their physiological determinants are different. Little is known of the structure/function relationships of infants' airways, but it is assumed that measures of airway resistance reflect mainly changes in the larger airways, and forced expiratory flows at the end expiratory level are more sensitive to peripheral airway obstruction.⁵ As the precise target of the test agonist in the bronchial tree is largely unknown and possibly varies between individual infants, the significance of these physiological mechanisms remain hypothetical. Factors associated with the infant's status (such as posture, changing breathing pattern, sleep state, glottis closure) as well as method-specific sources of error increase the inherent variability and probably also contribute to the disagreement between the two estimates of airway obstruction.^{6,7} In the current settings, the within-subject variation of V_{maxFRC} was significantly lower than that of sG_{aw} . This resulted in a more pronounced postchallenge change in V_{maxFRC} than in sG_{aw} , when expressed as multiples of baseline SD_{ws} , suggesting that the former is a more sensitive measure of methacholine induced bronchoconstriction. Closer association of SaO_2 with V_{maxFRC} also favours its use over sG_{aw} in the assessment of response during airway challenge.

1
2
3
4 In the present study infants, methacholine induced airway obstruction was associated
5 with a significant increase in FRC level, determined by body plethysmography. The
6 prior repeated forced expirations should not significantly alter FRC,²⁷ therefore
7 methacholine induced dynamic changes in breathing pattern or air trapping are more
8 likely mechanisms. The finding is important, since the measurements of $V_{\max\text{FRC}}$ are
9 dependent on the actual end expiratory lung volume, and an increase in FRC may
10 result in an underestimation of the decrease in $V_{\max\text{FRC}}$ during the challenge test. The
11 observation is also a strong argument to favor raised volume RTC technique in
12 bronchial challenge testing of infants, since forced expiratory volumes after full lung
13 inflation are not effected by changes in FRC level. Dynamic instability of FRC in
14 infants is a major source of variability of $V_{\max\text{FRC}}$ measurements.²⁸ However, in a
15 study of infants comparing tidal and raised volume RTC measurements during
16 methacholine airway challenge, the agreement between the two methods was good
17 and neither showed any obvious advantages over the other.¹⁶

18
19
20
21
22
23
24
25
26
27
28
29
30 Changes in lung function were induced by methacholine, which has a limited time of
31 action. The postchallenge measurements of lung function were assessed in a fixed
32 sequence, as the decrease of $V'_{\max\text{FRC}}$ was followed as an endpoint criterion of the
33 challenge test. Time dependent alleviation of airway obstruction would thus favour
34 sensitivity of the RTC method over that of sGaw, and affect the association between
35 them as well as with SaO₂. However, studies on the time course of the methacholine
36 induced bronchoconstriction have shown that, even though the peak action usually
37 occurs within 4 minutes, it is followed by a plateau phase where significant
38 bronchoconstriction is sustained for over 15 minutes.²⁹ In a similar comparative study
39 of four different lung function methods in preschool children, Klug and Bisgaard²⁵
40 found relatively stable airway obstruction for at least 10 minutes after methacholine
41 inhalation. This time frame was found to be sufficient in the current study to make all
42 the postchallenge measurements ($V'_{\max\text{FRC}}$, FRC and sGaw). Accordingly, they should
43 not be severely affected by time dependent changes of airway obstruction.

44
45
46
47
48
49
50
51
52
53
54
55
56
57 We conclude that in infants undergoing airway challenge with methacholine,
58 plethysmographic measurements of sG_{aw} correlate with the changes in $V_{\max\text{FRC}}$, but
59 the agreement is poor and the methods cannot be used interchangeably. $V_{\max\text{FRC}}$ is
60 also more sensitive to detect airway obstruction than sG_{aw}. However, methacholine

1
2
3 induced bronchoconstriction was associated with significant increases in FRC, which
4 may affect the validity of $V_{\max\text{FRC}}$ measurements during the test.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

ACKNOWLEDGEMENTS

We wish to thank Tuija Rito, RN, and Helena Punkari, RN, for their skill and care with the infants and their parents. The personnel of Spira Respiratory Care Center Ltd, is acknowledged for their help in the development of the inhalation-synchronized dosimetric equipment used in the methacholine challenge.

CONFLICTS OF INTEREST

None of the authors has any competing interests to declare.

FIGURE LEGENDS

Figure 1. The relationship between methacholine induced post-challenge changes in sGaw and VmaxFRC. The vertical and horizontal lines represent corresponding cutoffs of significant change.

Figure 2. The relationship between dose response slopes (DRS) by sGaw and VmaxFRC during methacholine airway challenge.

For Peer Review

REFERENCES

1. Kotaniemi-Syrjänen A, Malmberg LP, Pelkonen A, Malmström K, Mäkelä M. Airway responsiveness: associated features in infants with recurrent respiratory symptoms. *Eur Resp J* 2007;30:1150-1157.
2. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Le Souef PN. Airway responsiveness in early infancy predicts asthma, lung function and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001;163:37-42.
3. Turner SW, Young S, Goldblatt J, Landau LI, Le Souef PN. Childhood asthma and increased airway responsiveness. A relationship that begins in infancy. *Am J Respir Crit Care Med* 2008;179:98-104.
4. Delacourt C, Benoist MR, Waernessyckle S, Rufin P, Brouard JJ, de Blic J, Scheinmann P. Relationship between bronchial responsiveness and clinical evolution in infants who wheeze. *Am J Respir Crit Care Med* 2001;164:1382-1386.
5. Frey U. Clinical applications of infant lung function testing: does it contribute to clinical decision making ? *Pediatr Respir Rev* 2001;2:126-130.
6. Sly PD, Tepper RS, Henschen M, Gappa M, Stocks J. ERS/ATS Task force on standards for infant respiratory function testing. Tidal forced expirations. *Eur Resp J* 2000;16:741-748.
7. Stocks J, Godfrey S, Beardsmore C, Bar-Yishay E, Castile E. Plethysmographic measurements of lung volume and airway resistance. *Eur Resp J* 2001;17:302-312.
8. Reinmann B, Stocks J, Frey U. Assessment of an infant whole-body plethysmograph using an infant lung model. *Eur Respir J* 2001;17:765-772.
9. Malmberg LP, Pelkonen A, Hakulinen A, Hero M, Pohjavuori M, Skyttä J, Turpeinen M. Intraindividual variability of infant whole body plethysmographic measurements: effects of age and disease. *Pediatr Pulmonol* 1999;28:356-362.
10. Hoo A-F, Dezateux C, Hanrahan JP, Cole TJ, Tepper R, Stocks J. Sex-specific prediction equations for V'maxFRC in infancy. A multicenter study. *Am J Respir Crit Care Med* 2002;165:1085-1092.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
11. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine: an approach suitable for population studies. *Am Rev Respir Dis* 1987;136:1412-1417.
12. Le Souef PN. Validity of methods used to test airway responsiveness in children. *Lancet* 1992;339:1282-1284.
13. Prendiville A, Green S, Silverman M. Bronchial responsiveness to histamine in wheezy infants. *Thorax* 1987;42:92-99.
14. Tepper R. Airway reactivity in infants: a positive response to methacholine and metaprotenol. *J Appl Physiol* 1987;62:1155-1159.
15. Le Souef PN, Geelhoed GC, Turner DJ, Morgan SEG, Landau LI. Response of normal infants to inhaled histamine. *Am Rev Respir Dis* 1989;139:62-66.
16. Hayden MJ, Devadason SG, Sly PD, Wildhaber J, Le Souef PN. Methacholine responsiveness using the raised volume forced expiration technique in infants. *Am J Respir Crit Care Med* 1997;155:1670-1675.
17. Benoist MR, Brouard JJ, Rufin P, Delacourt C, Waernessyckle S, Scheinmann P. Ability of new lung function tests to assess methacholine-induced airway obstruction in infants. *Pediatr Pulmonol* 1994;18:308-316.
18. Springer C, Godfrey S, Vilozni D, Bar-Yishay E, Noviski N, Avital A. Comparison of respiratory inductance plethysmography with thoracoabdominal compression in bronchial challenges in infants and young children. *Am J Respir Crit Care Med* 1996;154:665-669.
19. Loland L, Buchvald F, Brydensholt Halkjaer L, Anhoj J, Hall G, Persson T, Krause TG, Bisgaard H. Sensitivity of bronchial responsiveness measurements in young infants. *Chest* 2006;129:669-675.
20. Hall G, Hantos Z, Wildhaber J, F P, Sly PD. Methacholine responsiveness in infants assessed with low frequency forced oscillation and forced expiration techniques. *Thorax* 2001;56:42-47.
21. Gutkowski P. Airway responsiveness following wheezy bronchitis in infants. *Eur Resp J* 1990;3:807-811.
22. O'Callaghan C, Milner AD, Webb MS, Swarbrick A. Nebulized water as a bronchoconstricting agent in infancy. *Arch Dis Child* 1991;66:948-951.
23. Yuksel B, Greenough A. Nebulized sodium cromoglycate in preterm infants - protection against water challenge-induced bronchoconstriction. *Respir Med* 1993;87:37-42.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
24. Lum S, Hulskamp G, Hoo A-F, Ljungberg H, Stocks J. Effect of raised lung volume technique on subsequent measures of VmaxFRC in infants. *Pediatr Pulmonol* 2004;38:146-154.
 25. Klug B, Bisgaard H. Measurement of lung function in awake 2-4-year old asthmatic children during methacholine challenge and acute asthma. *Pediatr Pulmonol* 1996;21:290-300.
 26. Klug B, Bisgaard H. Measurement of the specific airway resistance by plethysmography in young children accompanied by an adult. *Eur Respir J* 1997;10:1599-1605.
 27. Lanteri CJ, Raven JM, Sly PD. Effect of expiration on thoracic gas volume in wheezy infants. *Pediatr Pulmonol* 1990;9:220-223.
 28. Delacourt C, Benoist MR, Waernessyckle S, Rufin P, Brouard JJ, de Blic J, Scheinmann P. Repeatability of lung function tests during methacholine challenge in wheezy infants. *Thorax* 1998;53:933-938.
 29. Cartier A, Malo J-C, Begin P, Sestier M, Martin RR. Time course of the bronchoconstriction induced by inhaled histamine and methacholine. *J Appl Physiol* 1983;54:821-826.