

Spirometry and Forced Oscillations in the Detection of Airway Hyperreactivity in Asthmatic Children

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Summary. Background: Provocation tests are routinely used to detect airway hyperreactivity (AH) in the diagnosis of asthma. We compared the sensitivities of the forced oscillation technique (FOT) and spirometry in the detection of AH in asthmatic children. Methods: FOT and spirometry were performed in 20 asthmatic children (aged 5–18 years) following aerosolized histamine and methacholine at an interval of 2 weeks. The respiratory system input impedance was measured by FOT; the resistance at 6 Hz (R_6), the average resistance between 4 and 24 Hz, the area under the reactance curve (AX) and the resonant frequency were extracted from these recordings. Spirometry was used to obtain forced expiratory volumes and flow parameters. Results: Following provocation with the two agonists, the FOT detected the airway response as early as spirometry. When the greater variability of the impedance parameters was taken into account, the two methods were observed to have similar sensitivities. Among the lung function parameters studied, AX and R_6 were the most sensitive for the demonstration of AH. Conclusions: Our findings demonstrate that FOT is as suitable as spirometry for the measurement of bronchoconstriction and thus it may be considered for the detection of AH in asthmatic children. Since its application requires minimal cooperation, this approach imposes less stress and may be particularly favorable in the diagnosis of asthma at a young age.

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Key words: lung function; bronchoconstriction; airway resistance; histamine; methacholine.

Funding source: Hungarian Basic Scientific Research, Number: OTKA K81179, Bolyai Janos Research Fellowship, European Union, European Regional Fund.

INTRODUCTION

The airway hyperreactivity (AH) during bronchoprovocation tests plays a key role in the diagnosis of asthma.^{1–4} The early diagnosis and adequate treatment of asthma in early childhood significantly improve the prognosis and the quality of life.⁵ In older children or adults, the AH can be detected indirectly by performing spirometry during airway challenges with an inhaled bronchoconstrictor agonist. However, the measurement of lung function and the subsequent detection of AH in young children are limited by their inability to perform the close cooperation necessary for spirometry.

The forced oscillation technique (FOT) has gained increasing attention for the measurement of pulmonary mechanics in children because this approach requires no special breathing maneuvers.^{6,7} Moreover, in contrast with spirometry, the FOT provides direct information on the mechanical properties of the airways and the respiratory tissues. These beneficial properties of the FOT have resulted in numerous previous studies in which the lung function was assessed in children with no respiratory disease,^{8–10} or with various lung diseases such as cystic fibrosis,^{9,11–14} neonatal chronic lung disease,^{9,15} wheezy bronchitis,^{9,16–18} or asthma.^{1–3,9,10}

Despite the potential of the FOT to detect early bronchoconstriction during different challenges, the sensitivity of this method has not been systematically evaluated with the *gold standard* of spirometry in asthmatic children. The aims of the present study were therefore (i) to compare the sensitivities of the FOT and spirometry during airway challenges involving the inhalation of different direct stimuli commonly used in clinical practice (histamine [His] and methacholine [MCh]) and

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Conflict of interest: None

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Received 2 August 2011; Accepted 29 December 2011.

DOI 10.1002/ppul.22551

Published online in Wiley Online Library (wileyonlinelibrary.com).

(ii) to establish which FOT parameter and which provocation test is most appropriate for the detection of AH in asthmatic children.

MATERIALS AND METHODS

Subjects

Twenty asthmatic children (5 girls and 15 boys, age range 5–18 years) were enrolled into the study. The diagnosis of asthma, the degree of its severity, and the level of control were categorized according to the GINA (Global Initiative for Asthma) guidelines,¹⁹ based on the previous history of recurrent coughing, wheezing, shortness of breath (at rest or following exercise), and the symptomatic improvement following short-acting bronchodilator administration. Each child and/or their guardian completed the informed consent document, which was approved by the Clinical Ethics Committee of the University of Szeged (Ref No. WHO2803). Seventeen children did not receive any regular asthma medication during one month prior to the study; they used only inhaled salbutamol (Sal) occasionally, as needed (GINA level 1). Three children used inhaled corticosteroid (ICS) as maintenance (budesonide 200 µg twice a day) and Sal (200 µg as needed) as rescue therapy (GINA level 2). All children were controlled during the study period. Only patients who did not need Sal during 8 hr before the visit were included in the study.

Forced Oscillations

The FOT measurements were performed in accordance with the ERS guidelines.⁶ To measure the input impedance of the respiratory system (Zrs) during spontaneous breathing, a commercially available device (i2m, Chess Medica, Oostakker, Belgium) was applied. Briefly, a loudspeaker was used to generate the pseudo-random broad-band oscillatory pressure signals containing integer multiple frequencies between 4 and 48 Hz. The forcing signal was applied to the child via a disposable bacterial filter and a mouthpiece (Type PBF-30, Piston Ltd., Budapest, Hungary). Zrs was obtained from 8-sec measurements while the child performed normal breathing during the oscillatory measurements. The equipment was calibrated by measuring known impedances prior to the measurements. The additional impedance of the bacterial filter and mouthpiece was determined and the Zrs spectra were corrected for this equipment component.

During the 8-sec data recording periods, the children sat upright, wearing a noseclip, and breathing quietly through the mouthpiece. They were asked to support their cheeks with their palms during these maneuvers in order to minimize upper airway shunting. The Zrs

recordings were accepted if the coherence functions between the pressure and flow signals were generally >0.95, no leak was noted around the mouthpiece, and no technical artifact occurred due to coughing, swallowing or glottis closure.

The changes in the resistive properties of the respiratory system were evaluated by calculating the average resistance between 4 and 24 Hz (R_{4-24}) and by extracting the resistance and reactance at 6 Hz (R_6 and X_6 ; the lowest frequency at which the impedance was reliably measured). The elasticity of the respiratory system was assessed by calculating the sum of all negative values in the imaginary part of Zrs from 4 Hz, which corresponds to the area above the reactance curve and the x-axis (AX). The resonance frequency of the respiratory system (F_{res}), that is, the frequency at which the reactance was zero, was determined by linear interpolation.

Spirometry

Spirometry was performed in accordance with the ATS/ERS recommendations.²⁰ Forced expiratory flow-volume curves were measured by means of a commercial spirometer (Type PDD-301/s, Piston Ltd., Budapest, Hungary) including a screen pneumotachograph equipped with a differential pressure transducer. The flow signal was integrated to obtain changes in lung volume during the forced expiratory maneuvers. The forced expiratory volume in the first second of expiration (FEV_1), the forced vital capacity (FVC), and the forced expired flow between 25% and 75% of the volume expired (FEF_{25-75}) were extracted from the recordings. Three technically acceptable reproducible measurements were performed, and the highest values of the spirometric parameters were extracted from the maneuvers for the final analyses.

Measurement Protocol and Bronchial Challenges

On the first study day, three-to-five reproducible Zrs data epochs were recorded, which were followed by three consecutive technically acceptable forced expiratory maneuvers in order to establish the baseline values of the FOT and spirometric lung functional parameters. Since the FOT does not require any special breathing maneuvers, this technique was always performed first. The deep inspirations and forced expirations necessary for spirometry may affect FOT measurements.^{13,21} This sequence guaranteed the lack of influence of the assessments themselves on the measured values. Increasing doses of aerosolized (Voyage Mefar jet nebulizer, Italy) His (Sigma–Aldrich Ltd., Budapest, Hungary) was then administered (airflow 8 L/min, mass median aerodynamic diameter 1.9 µm, minimum output 0.3 ml/min) to the children for 2 min via a face mask, in doubling doses ranging from 0.5 to 16 mg/ml. A set of Zrs

recordings was first collected, including measurements of three 8-sec data epochs at 10, 50, and 90 sec after the end of the inhalation. This was followed by three forced expiratory maneuvers for spirometry at 2, 2.5, and 3 min after the completion of each dose. An interval of at least 5 min was allowed between two consecutive doses. The study was terminated before the highest dose of His, or if FEV₁ decreased by more than 20% and/or clinical symptoms of wheeze or persistent cough were noted.^{22,23} To detect the reversibility of the bronchoconstriction, the challenge protocol was terminated in all children by administering 400 µg inhaled Sal (Ventolin, GlaxoSmithKline, Greenford, UK) followed by the final assessment of Zrs and spirometry.

The children revisited the lung function laboratory 2 weeks later. The baseline FOT and spirometry were performed identically as detailed above. Aerosolized saline as the solvent of the agonists was then administered for 2 min, and both the FOT and spirometry were repeated. The bronchoprovocation tests with increasing doses of MCh (0.5–8 mg/ml for 2 min) and the broncholysis with Sal were then repeated in the same manner as detailed above.

The deep inspirations and forced expirations necessary for spirometry may affect FOT measurements.^{13,21} Accordingly, to test whether the variability of the FOT parameters was altered when this lung function test was performed alone, another 8 asthmatic children similar to the main study population were studied (three girls and five boys, age range 6–16 years, weight range 27–74 kg, height range 122–164 cm). Two sets of Zrs recordings by the FOT were collected in these children at times 5 min apart.

Statistical Analyses

Short-term variability in the spirometric and FOT parameters was defined as the standard deviation of percentage changes between measurements before and after NaCl inhalations, while long-term variability was determined by calculating the standard deviation of percentage changes between the two baseline values at the two visits. The Z score for each parameter was calculated as the difference between the post-test and the basal value divided by the standard deviation of the expected value of the change (i.e., the short-term variability). This Z score is the number of standard deviations by which a lung function parameter responded to the constrictor agonists as compared with inhalation of the solvent. Responses were considered statistically significant when the Z scores for each parameter were outside the –1.96 and +1.96 critical levels. The Z-test comparing two proportions was applied to detect statistical significance between the lung function parameters. To express group means and deviations, the values are reported as

means ± SE. The Kolmogorov–Smirnov test was used to test data for normality. The changes in the group means of the FOT and spirometric Z score parameters were assessed by using one-way ANOVA tests on repeated measures. The relationships between the continuous variables (i.e., the FOT and spirometry indices) were examined by means of Pearson correlation tests. Pairwise comparisons were performed by using the Student–Newman–Keuls multiple comparison procedures. Statistical significance was accepted at the level $P < 0.05$.

RESULTS

The children had normal basal lung function parameters, with a mean percentage predicted value for FEV₁ of 106% (range 93–136%) and a mean Z score of 0.54 (ranging between –1.0 and +1.43). The mean FVC was 105% (67–121%), with a Z score of 0.48 (ranging between –1.3 and +1.75). FEF_{25–75} was also normal, with a mean percentage predicted value of 106% (77–124%) and a mean Z score of 0.62 (ranging between –2.05 and +3.04).²⁴ The mean respiratory resistance between 4 and 24 Hz (R_{4–24}) was 91% of the percentage predicted (range 74–105%).

Data on the short- and long-term variabilities in the spirometric and FOT parameters are demonstrated in Table 1. The smallest variabilities were found for FEV₁ and FVC, those for FEF_{25–75}, R_{4–24}, R₆ were higher, and the highest were observed for X₆, AX and Fr in the children who participated in both the FOT and spirometry. Substantial decreases in the short-term variability were observed for the FOT parameters in the eight additional

TABLE 1—Percentages of Short- and Long-Term Variability of the Lung Function Parameters Determined With Spirometry or Forced Oscillations in the Main Study Group of 20 Children (5 Girls and 15 Boys, Age Range 5–18 Years)

	Percentage short-term variability	Percentage long-term variability
Spirometry		
FEV ₁	4.3	9.3
FEF _{25–75}	7.1	15.3
FVC	4.4	8.2
Forced oscillation		
R _{4–24}	10.3 (6.1)	13.8
R ₆	11.3 (6.2)	12.2
X ₆	15.41 (7.2)	18.7
AX	25.6 (17.0)	31.9
Fr	16.7 (9.2)	25.7

Values in parentheses: values obtained in eight additional children where the FOT was performed without spirometry (three girls and five boys, age range 6–16 years). Variability was defined as the standard deviation of the percentage changes between the data obtained in the first and second tests.

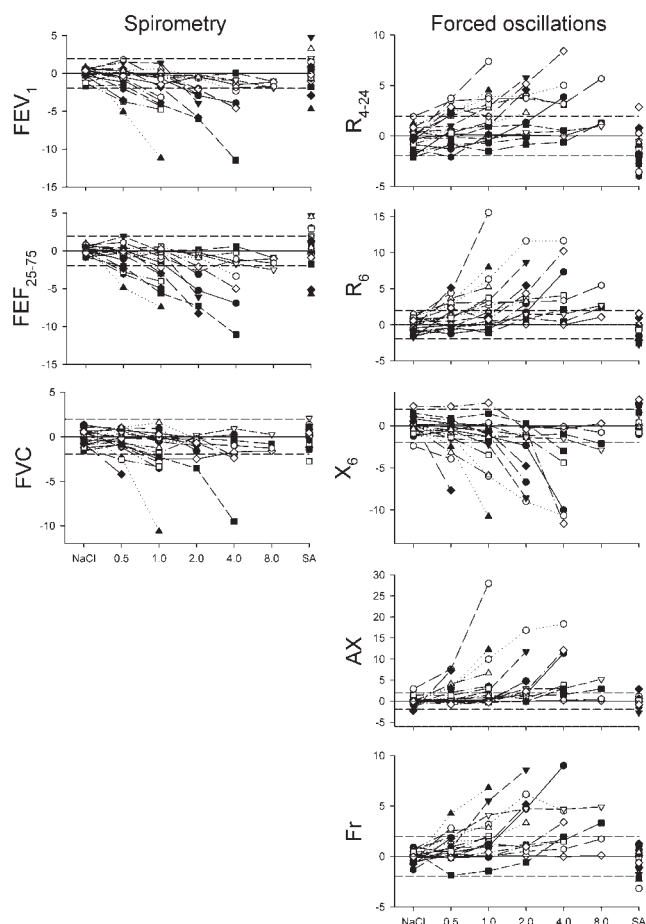


Fig. 1. The Z scores of the lung function parameters obtained with spirometry (left) or forced oscillations (right) in individual asthmatic children under the baseline condition (BL), following inhalations of normal saline (NaCl) and increasing doses of methacholine (0.5–8.0 mg/ml), and after inhalation of salbutamol (SA). Horizontal dashed lines indicate the limits of statistical significance (Z score 1.96).

children where the FOT data were collected without spirometry.

Figure 1 illustrates the Z scores for the spirometric and FOT parameters during the MCh challenge protocol. Inhalation of saline following the baseline recordings did not cause significant changes in any of the

lung function parameters ($P = 0.47, 0.34, 0.75, 0.98, 0.84, 0.78, 0.94$, and 0.55 for FEV_1 , FEF_{25-75} , FVC , R_{4-24} , R_6 , X_6 , AX , and Fr , respectively). Inhalation of increasing doses of MCh resulted in dose-dependent decreases in FEV_1 , FEF_{25-75} ($P < 0.001$ for both) and FVC ($P = 0.002$). All parameters obtained with the FOT displayed dose-dependent significant increases following MCh inhalation ($P < 0.001$ for all). Inhalation of Sal after these bronchial provocations totally abolished the bronchoconstrictor effects of MCh. Similar to His, the resistance parameters obtained by FOT were able to detect significant decreases in the airway tone after Sal inhalations ($P = 0.002$ for R_{4-24} , $P = 0.006$ for R_6), while these changes were not reflected in the spirometric indices. Statistical results on these findings are presented in Table 2. The final concentration of MCh caused significant changes in the spirometric and forced oscillatory indices in the majority of the children; Z-tests revealed no significant difference between forced expiratory flow parameters (FEV_1 and FEF_{25-75}) and lung function indices obtained by forced oscillations.

The Z scores of the spirometric and FOT parameters during His challenges are depicted in Figure 2. Increasing doses of aerosolized His caused statistically significant decreases in the lung volume/flow indices determined by spirometry ($P < 0.001$ for FEV_1 and FEF_{25-75} ; $P = 0.011$ for FVC). The parameters extracted from the real and imaginary parts of the Zrs spectra exhibited dose-dependent elevations after His provocation ($P < 0.001$ for R_{4-24} , R_6 and Fr , $P = 0.005$ for X_6 and AX). The lung function parameters obtained with spirometry after Sal inhalation were not significantly different from those measured at the beginning of the study, but administration of Sal led to significant decreases in R_6 ($P < 0.005$), X_6 ($P < 0.005$), and AX ($P < 0.05$), indicating that the decreases in the airway tone by the β_2 agonist may be reflected in the FOT indices. Table 3 contains the results of the Z-test on the data in Figure 2. The majority of the children responded to His at the final concentration; Z-tests revealed no significant difference between the forced expiratory flow parameters (FEV_1 and FEF_{25-75}) and the resistance parameters obtained by forced oscillations.

TABLE 2—P Values Resulting From the Z-Tests Comparing Two Proportions for the Lung Function Parameters Obtained With Spirometry and Forced Oscillations During the Methacholine Challenges

	R_{4-24} (13/7)	R_6 (17/3)	X_6 (16/4)	AX (16/4)	Fr (13/7)
FEV_1 (14/6)	0.73	0.26	0.46	0.46	0.73
FEF_{25-75} (14/6)	0.73	0.26	0.46	0.46	0.73
FVC (8/12)	0.11	0.003	0.01	0.01	0.11

Values in parentheses: number of children with significant change to the highest dose of histamine (defined as the Z score for each parameter exceeding the critical levels of -1.96 or $+1.96$) over the number of children with no significant change.

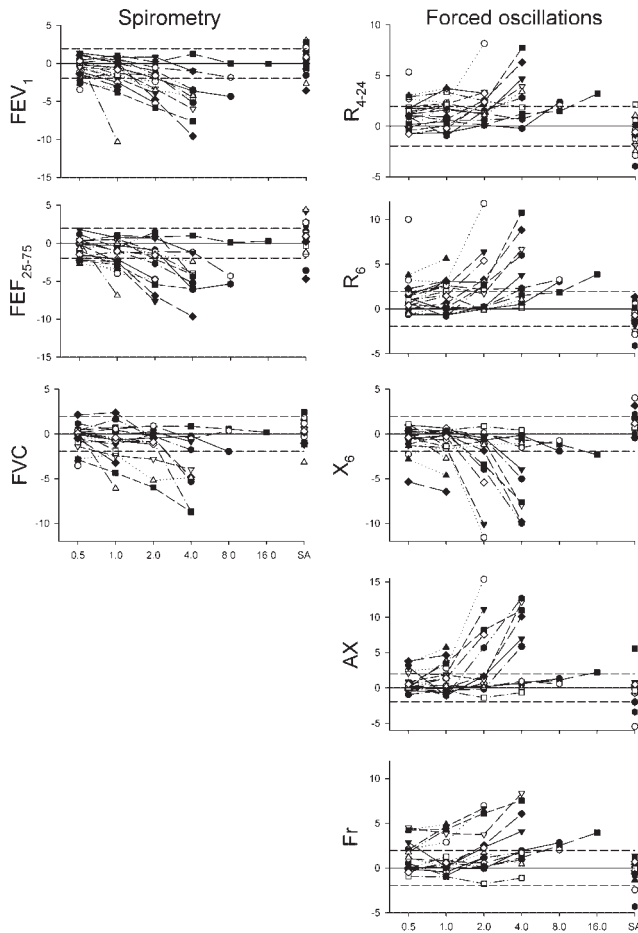


Fig. 2. The Z scores of the lung function parameters obtained with spirometry (left) or forced oscillations (right) in individual asthmatic children under the baseline condition (BL), following inhalations of increasing doses of histamine (0.5–16 mg/ml) and after inhalation of salbutamol (SA). Horizontal dashed lines indicate the limits of statistical significance (Z score 1.96).

To compare the sensitivities of spirometry and the FOT in the detection of airway narrowing, the absolute values of the Z scores for the lung function parameters during the His and MCh challenges (determined with spirometry and the FOT) are presented in Figure 3. Data are shown for the lower part of the dose-response

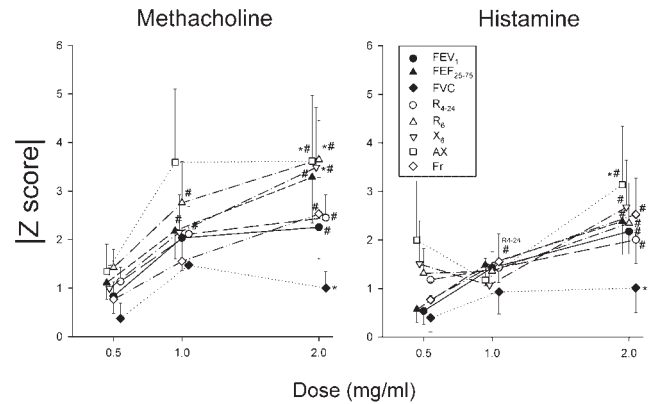


Fig. 3. The absolute Z scores for the lung function parameters obtained with spirometry (filled symbols) and forced oscillations (open symbols) following histamine or methacholine challenges. * $P < 0.05$ versus FEV_1 within a dose, # $P < 0.05$ versus zero level within a parameter.

curve, where measurements were made for the majority of the children (i.e., prior to a 20% decrease in FEV_1 or the appearance of clinical symptoms). The sensitivities of the FOT parameters related to the low-frequency part of the Zrs spectra were among the greatest during both His and MCh challenges. R_6 and FEF_{25-75} exhibited similarly high sensitivities in the detection of His- or MCh-induced airway narrowing. The parameters X_6 , AX, Fr, FEV_1 , or FEF_{25-75} were also able to detect bronchoconstriction after His and MCh challenges, but only after the second doses of the constrictor agonists.

Since the variability in the FOT parameters was greater than that in the spirometric indices, we examined the magnitude of the changes in the resistance parameters where the His and MCh provocation tests became positive according to the ATS/ERS guidelines (a 20% decrease in FEV_1 or the appearance of clinical symptoms). These data are displayed in Table 4. When a positive response was detected during the bronchial challenge tests, the changes in R_{4-24} were about twice as high as those in the FEV_1 for both of the constrictor agonists studied, while the elevations in R_6 were even more pronounced.

The FEV_1 values obtained with spirometry and the resistance values in the medium frequency range

TABLE 3—P Values Resulting From the Z-Tests Comparing Two Proportions for the Lung Function Parameters Obtained With Spirometry and Forced Oscillations During the Histamine Challenges

	R_{4-24} (17/3)	R_6 (18/2)	X_6 (14/6)	AX (13/7)	Fr (12/8)
FEV_1 (18/2)	0.63	1	0.42	0.058	0.028
FEF_{25-75} (16/4)	0.67	0.38	0.32	0.29	0.17
FVC (8/12)	0.003	0.001	0.09	0.11	0.21

Values in parentheses: number of children with significant change to the highest dose of histamine (defined as the Z scores for each parameter exceeding the critical levels of -1.96 or $+1.96$) over the number of children with no significant change.

TABLE 4—Percentage Changes in the Resistance Parameters Where the Histamine and Methacholine Provocation Tests Became Positive According to the ATS/ERS Guidelines (a 20% Decrease in FEV₁ or the Appearance of Clinical Symptoms)

	Histamine			Methacholine		
	R _{4–24}	R ₆	Response	R _{4–24}	R ₆	Response
1	38.1	59.4	W	82.0	172.8	W
2	48.4	80.9	SW	80.9	77.6	SW
3	53.6	124.1	W	56.9	110.4	SW
4	16.1	24.9	SW	38.7	80.1	SW
5	37.0	53.4	SW	39.7	51.6	SW
6	41.5	–1.5	W	30.8	38.8	W
7	64.9	107.8	SW	53.6	76.1	SW
8	46.8	48.3	SW	28.9	36.8	SW
9	66.9	116.8	SW	7.1	20.0	SW
10	25.8	46.7	SW	5.2	17.3	W
11	12.3	27.3	SW	–5.3	5.94	SW
12	20.7	40.4	W	40.8	54.4	W
13	5.9	5.5	SW	–2.7	19.8	SW
14	16.3	31.0	SW	30.8	62.4	SW
15	27.7	23.6	W	2.9	11.3	W
16	34.8	11.1	SW	24.1	41.0	SW
17	38.6	58.0	SW	11.4	8.1	SW
18	23.4	23.1	W	12.8	9.9	SW
19	7.0	23.2	SW	10.7	28.3	SW
20	15.5	40.9	W	46.9	43.4	SW
Mean	32.1	48.9		27.6	49.7	
SE	4.1	8.7		5.4	10.1	

SW, positive response (20% decrease in FEV₁ and the appearance of wheeze); W, wheeze, clinical symptoms appeared.

(4–24 Hz) obtained with the FOT are primarily determined by the geometry and the mechanical properties of the central conducting airways. Conversely, the contributions of the small airways appear in the spirometric FEF_{25–75} and the low-frequency resistance values, such as R₆.^{7,25} Accordingly, we characterize the associations between these corresponding spirometric and FOT indices during the His and MCh challenges in Figure 4. During His challenges, strong and statistically significant correlations were observed between FEV₁ and R_{4–24} ($R^2 = 0.6$, $P < 0.0001$) and between FEF_{25–75} and R₆ ($R^2 = 0.47$, $P < 0.0001$). The correlations between these parameters during MCh challenges were somewhat weaker but their associations were still close and highly significant ($R^2 = 0.48$, $P = 0.0001$ between FEV₁ and R_{4–24} and $R^2 = 0.5$, $P < 0.0001$ between FEF_{25–75} and R₆).

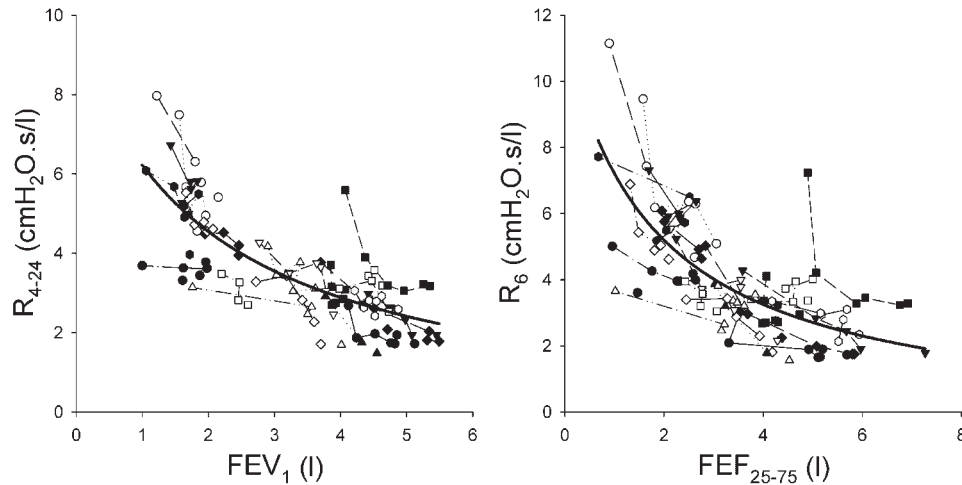
DISCUSSION

The results of the present study demonstrated concomitant changes in the lung function parameters determined with spirometry and the FOT during different bronchial challenges in asthmatic children, with significant correlations between the corresponding parameters. Inhalation of increasing doses of His or MCh caused bronchoconstriction, which was detected similarly from

both the spirometric and the FOT parameters. Expressing the changes in Z scores, which take into account the different variabilities in the lung function parameters, revealed that the sensitivities of the lung function indices are comparable in spirometry and the FOT for the detection of bronchoconstriction induced by His and MCh in asthmatic children.

Since the introduction of the FOT by Dubois in 1956²⁶ and its extension toward the application of a wide frequency range of pseudorandom pressure excitation,²⁷ the popularity of this lung mechanical measurement technique has increased, particularly as concerns examinations of patients with a limited ability to cooperate.^{1–3,7–9,11–15,17,18,28–30} This beneficial profile of the FOT allows the characterization of lung function abnormalities in children with various respiratory diseases, such as cystic fibrosis,^{9,11–14} neonatal chronic lung disease,^{9,15} wheezy bronchitis,^{9,16–18} or asthma.^{1–3,9,10} Furthermore, the FOT has been used successfully to characterize bronchodilator responsiveness^{9,18,28} and to detect lung function changes following the inhalation of a bronchoconstrictor agonist in children.^{2,3,16,17} However, we are aware of only a few previous studies in which the sensitivities of the FOT and spirometry were compared with regard to the detection of AH in asthmatic children,^{1,29,30} when the bronchial challenges are performed in accordance with the ATS guidelines.²³ In

Responses to histamine



Responses to methacholine

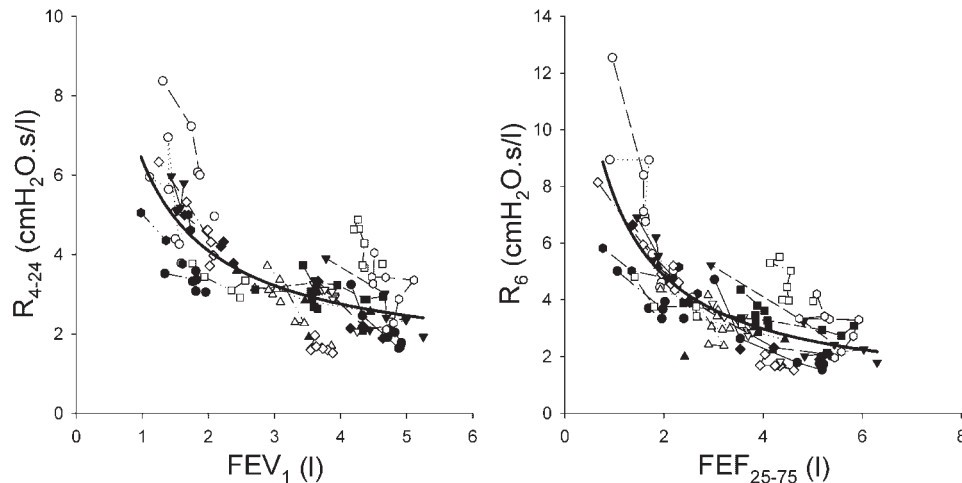


Fig. 4. Correlations observed between spirometric and resistance parameters obtained with forced oscillations following aerosolized histamine (top) or methacholine (bottom) challenges in individual asthmatic children.

agreement with the present findings (Fig. 4), significant correlations between forced expiratory and impedance parameters were found in these previous reports following inhalation of His³⁰ or MCh.²⁹ The sensitivity of the resistance and reactance parameters of the respiratory impedance spectra for the detection of bronchoconstriction in asthmatic children following MCh inhalation also accords with previous findings.¹ These earlier studies lack a comprehensive analysis of the sensitivities of the lung function parameters involving a consideration of the differences in variability of the techniques applied. Since spirometry is a routine procedure in lung function laboratories for the detection of AH in the diagnosis of asthma, demonstration of the similar ability

of the FOT is of major importance, since this technique can be carried out reliably in a much younger age group (as low as 2 years of age^{3,10}). Nevertheless, children of school age were enrolled in the present study, in order to allow a comparison with spirometry. The diagnosis of asthma via the detection of AH would require the involvement of an age-matched healthy control group. This would have required the enrolment of healthy children in a similar age range for the two bronchoprovocation tests with His and MCh, but this was not feasible at our institution. Despite our having the limitation the lack of a healthy control group, however, the comparisons of the changes in the FOT with the spirometric indices during bronchoprovocation tests still provides

valuable information concerning the ability of the former approach to detect airway narrowing. This similar ability suggests that AH can be detected reliably in asthmatic children by using the FOT.

Strong and highly significant correlations were observed between the corresponding FOT and spirometric parameters relating to the larger (FEV_1 vs. R_{4-24}) and smaller airways (FEF_{25-75} vs. R_6), which suggests that airway narrowing is faithfully reflected by both techniques. Nevertheless, the correlation analysis does not take into account the variability of the measured parameters, which is of great importance in a comparison of the ability of a lung function test to measure bronchoconstriction with subsequent promise to detect AH. Previous studies have established that lung function parameters obtained by measurement techniques utilized during tidal breathing exhibit somewhat greater variability than those based on forced expiration.¹¹ In accordance with that finding, we experienced greater short- and long-term variabilities for the FOT parameters than those measured with spirometry. This implies that greater magnitudes of changes can be regarded as positive responses in the FOT parameters (Table 4), which corresponds to previous observations^{2,17,22} and recommendations for higher acceptance limits of clinically important changes.²²

In order to take the greater magnitudes of the changes and the somewhat higher short-term variability in the FOT parameters into account, the alterations in the lung function indices during the His and MCh challenges were also expressed in Z scores. This analysis revealed comparable Z scores for the lung function parameters determined with the FOT and spirometry in the detection of airway narrowing during both the His and the MCh challenges. However, in agreement with previous findings,¹ there was a tendency to a greater sensitivity of the FOT parameters relating to the low-frequency part of the Zrs spectra, which was particularly evident for R_6 . It is also noteworthy that the reactance parameter X_6 and AX reflected the lung function changes after both His and MCh inhalation. Since these parameters are extracted from the low-frequency reactance, they primarily reflect changes in the elasticity of the total respiratory system. It, therefore, seems likely that, besides inducing a constriction of the conducting airways manifested in elevated forced expiratory lung volumes and resistance parameters, the inhalation of the bronchoconstrictor agonists also led to a stiffening of the respiratory tissues. The most probable mechanism responsible for such a change in the respiratory elasticity is the loss of lung volume via the development of air trapping, due to the uneven deposition of the bronchoprovocation agonists, which was earlier clearly demonstrated during challenges with aerosolized His.^{21,31} The lack of a significant decrease in FVC despite this loss

of lung volume may be related to the forced deep inspiration to the total lung capacity prior to the expiration, which has been shown to be likely to reopen the compromised lung regions.²¹

As concerns the greater variabilities in the parameters determined with the FOT than those with spirometry, the sequence of the tests in the present experimental setting warrants consideration. Since the FOT requires normal quiet breathing only, it has no influence on the spirometric outcome. This sequence of the tests ensured the minimal interaction between the two lung function tests. Nevertheless, the deep inspiration and subsequent forced expiration maneuvers necessary for spirometry may affect the lung mechanics, which may be manifested even 5 min later in the FOT recordings. In addition to the potential diminution of the lung functional changes that results from the deep inspirations homogenizing the lung periphery,²¹ these forced breathing maneuvers may also widen the short-term variability of the FOT parameters obtained in the present study, which then ultimately affect the Z scores extracted from the Zrs spectra, and hence the sensitivity of the tests. To check on this hypothesis, we recruited eight asthmatic children of school age on whom two sets of baseline FOT recordings were performed 5 min apart, without spirometry between them. The short-term variability of each FOT parameter decreased substantially (Table 1), suggesting that the sensitivity of the FOT test for the detection of AH can be potentially doubled if the measurements are performed without additional lung function tests requiring special breathing maneuvers. The short-term variability of Rrs in this subgroup of children fits well within the range reported in earlier studies (5–11%), where FOT was performed without spirometry.^{3,8} The somewhat greater variabilities in the reactance parameters also agree with previous results.^{1,3,8} The deep inspiration (DI) itself required for spirometry may also affect the FOT during bronchial challenges. DI alleviates the airway narrowing by decreasing the enhanced bronchial smooth muscle tone and advancing lung recruitment. However, in asthmatics, this reduction may not be present³² or DI may even enhance the airway tone.³³ Since our subjects were classified as mild intermittent and mild persistent asthmatics, it can be anticipated that the bronchoprotective effect of DI predominated. Thus, it is possible that the His and MCh effects would have been somewhat greater if the FOT had have performed alone to assess the lung function changes.

A further important feature of our study protocol is the lack of randomization of the FOT and spirometric measurements. While this order minimized the interference between the measurement techniques since FOT does not modify the bronchial tone, this timing may have biased our findings if the bronchoconstriction was

not stable over the measurement period (approximately 3 min after completing each challenge). To exclude the potential bias due to the diminished effect of His or MCh with time, we performed measurements on five additional children, where the lung function was assessed by spirometry at 1, 3, and 5 min after MCh inhalation at a single dose of 4 mg/ml for 2 min. These results demonstrated that, within the time frame covered by the spirometry and forced oscillations, no significant reduction in the bronchial tone was observed (FEV_1 decreases were stable with values of $70.3 \pm 22\%$, $68.3 \pm 24\%$, $68.4 \pm 19\%$ of the pre-MCh level at 1, 3, and 5 min after MCh inhalation, respectively, $P = 0.74$). We are, therefore, certain that our protocol allows an exact comparison of the two techniques, even in the presence of an elevated airway tone.

In conclusion, in asthmatic children subjected to bronchoprovocation tests with His and MCh, the resistance and reactance parameters determined with the FOT detected the airway constriction similarly as for the parameters assessed by standard spirometry. The Z scores, which take into account the greater variability in the changes in the FOT parameters when the FOT was performed concomitantly with spirometry revealed that the two lung function measurement methods have similar sensitivities in the assessment of bronchoconstriction. If the FOT was performed alone, the short-term variability of the resistance parameters decreases markedly and approaches that observed for the spirometric indices. Since the FOT requires less cooperation from the patient than in the case of spirometry, use of the FOT may impose less stress on the children and may lead to a decrease in the age at which airway narrowing can be detected during bronchoprovocation tests. This implies that FOT is feasible for the detection of AH in children and thus its use may contribute to the diagnosis of asthma in the preschool age range.

ACKNOWLEDGMENTS

The authors thank Balla Mária, Kissné Klimász Zsuzsanna, and Szakácsné Szabó Ágota for their excellent technical assistance. The cooperation of the children is greatly appreciated.

REFERENCES

1. Bisgaard H, Klug B. Lung function measurement in awake young children. *Eur Respir J* 1995;8:2067–2075.
2. Hall GL, Gangell C, Fukushima T, Horak F Jr, Patterson H, Stick SM, Sly PD, Franklin PJ. Application of a shortened inhaled adenosine-5'-monophosphate challenge in young children using the forced oscillation technique. *Chest* 2009;136:184–189.
3. Klug B, Bisgaard H. Measurement of lung function in awake 2–4-year-old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique, and transcutaneous measurement of oxygen versus whole-body plethysmography. *Pediatr Pulmonol* 1996;21:290–300.
4. Cockcroft DW. Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 1988;93:24S–24S.
5. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003;349:1414–1422.
6. Oostveen E, MacLeod D, Lorino H, Farre R, Hantos Z, Desager K, Marchal F. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 2003;22:1026–1041.
7. Frey U. Forced oscillation technique in infants and young children. *Paediatr Respir Rev* 2005;6:246–254.
8. Hall GL, Sly PD, Fukushima T, Kusel MM, Franklin PJ, Horak F Jr, Patterson H, Gangell C, Stick SM. Respiratory function in healthy young children using forced oscillations. *Thorax* 2007;62:521–526.
9. Thamrin C, Gangell CL, Udomittipong K, Kusel MM, Patterson H, Fukushima T, Schultz A, Hall GL, Stick SM, Sly PD. Assessment of bronchodilator responsiveness in preschool children using forced oscillations. *Thorax* 2007;62:814–819.
10. Nielsen KG, Bisgaard H. Lung function response to cold air challenge in asthmatic and healthy children of 2–5 years of age. *Am J Respir Crit Care Med* 2000;161:1805–1809.
11. Gangell CL, Hall GL, Stick SM, Sly PD. Lung function testing in preschool-aged children with cystic fibrosis in the clinical setting. *Pediatr Pulmonol* 2010;45:419–433.
12. Gangell CL, Horak F Jr, Patterson HJ, Sly PD, Stick SM, Hall GL. Respiratory impedance in children with cystic fibrosis using forced oscillations in clinic. *Eur Respir J* 2007;30:892–897.
13. Lebecque P, Stanescu D. Respiratory resistance by the forced oscillation technique in asthmatic children and cystic fibrosis patients. *Eur Respir J* 1997;10:891–895.
14. Ren CL, Brucker JL, Rovitelli AK, Bordeaux KA. Changes in lung function measured by spirometry and the forced oscillation technique in cystic fibrosis patients undergoing treatment for respiratory tract exacerbation. *Pediatr Pulmonol* 2006;41:345–349.
15. Udomittipong K, Sly PD, Patterson HJ, Gangell CL, Stick SM, Hall GL. Forced oscillations in the clinical setting in young children with neonatal lung disease. *Eur Respir J* 2008;31:1292–1299.
16. Wilson NM, Bridge P, Silverman M. Bronchial responsiveness and symptoms in 5–6 year old children: a comparison of a direct and indirect challenge. *Thorax* 1995;50:339–345.
17. Wilson NM, Bridge P, Phagoo SB, Silverman M. The measurement of methacholine responsiveness in 5 year old children: three methods compared. *Eur Respir J* 1995;8:364–370.
18. Oostveen E, Dom S, Desager K, Hagendorens M, De Backer W, Weyler J. Lung function and bronchodilator response in 4-year-old children with different wheezing phenotypes. *Eur Respir J* 2010;35:865–872.
19. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143–178.
20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
21. Monfraix S, Bayat S, Porra L, Berruyer G, Nemoz C, Thomlinson W, Suortti P, Sovijarvi AR. Quantitative measurement of regional lung gas volume by synchrotron radiation computed tomography. *Phys Med Biol* 2005;50:1–11.

22. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007;175:1304–1345.
23. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161:309–329.
24. Stanojevic S, Wade A, Cole TJ, Lum S, Custovic A, Silverman M, Hall GL, Welsh L, Kirkby J, Nystad W, et al. Spirometry centile charts for young Caucasian children: the asthma UK collaborative initiative. *Am J Respir Crit Care Med* 2009;180:547–552.
25. Lebecque P, Kiakulanda P, Coates AL. Spirometry in the asthmatic child: is FEF 25-75 a more sensitive test than FEV1/FVC? *Pediatr Pulmonol* 1993;16:19–22.
26. Dubois AB, Brody AW, Lewis DH, Burgess BF Jr. Oscillation mechanics of lungs and chest in man. *J Appl Physiol* 1956;8: 587–594.
27. Landser FJ, Nagles J, Demedts M, Billiet L, van de Woestijne KP. A new method to determine frequency characteristics of the respiratory system. *J Appl Physiol* 1976;41:101–106.
28. Mazurek HK, Marchal F, Derelle J, Hatahet R, Moneret-Vautrin D, Monin P. Specificity and sensitivity of respiratory impedance in assessing reversibility of airway obstruction in children. *Chest* 1995;107:996–1002.
29. Duiverman EJ, Neijens HJ, Van der Snee-van Smaalen M, Kerrebijn KF. Comparison of forced oscillometry and forced expirations for measuring dose-related responses to inhaled methacholine in asthmatic children. *Bull Eur Physiopathol Respir* 1986;22:433–436.
30. Lebecque P, Spier S, Lapierre JG, Lamarre A, Zinman R, Coates AL. Histamine challenge test in children using forced oscillation to measure total respiratory resistance. *Chest* 1987; 92:313–318.
31. Popa V, Chandnani PC, Reardon M. The relationship between conductance and functional residual capacity during drug-induced bronchoconstriction. *Chest* 1990;97:831–839.
32. Brusasco V, Crimi E, Barisione G, Spanevello A, Rodarte JR, Pellegrino R. Airway responsiveness to methacholine: effects of deep inhalations and airway inflammation. *J Appl Physiol* 1999; 87:567–573.
33. Gayraud P, Orehek J, Grimaud C, Charpin J. Mechanisms of the bronchoconstrictor effects of deep inspiration in asthmatic patients. *Thorax* 1979;34:234–240.