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RESPIRATORY IMPEDANCE AND BRONCHODILATOR RESPONSE IN HEALTHY ITALIAN PRESCHOOL CHILDREN

Claudia Calogero, Niccolò Parri, Antonella Baccini, Barbara Cuomo, Massimo Palumbo, Elio Novembre, Paolo Morello, Chiara Azzari, Maurizio de Martino, Peter Sly, et al.

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**RESPIRATORY IMPEDANCE AND BRONCHODILATOR
RESPONSE IN HEALTHY ITALIAN PRESCHOOL CHILDREN**



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Keywords:	forced oscillations, children, reference values, bronchodilator



1
2
3 Prof. V. Chernick
4 Editor
5 PEDIATR PULMONOL
6

May 19, 2010

7
8 Dear Prof. Chernick,
9

10 enclosed please find the new revised version of the manuscript entitled "RESPIRATORY
11 IMPEDANCE AND BRONCHODILATOR RESPONSE IN HEALTHY ITALIAN
12 PRESCHOOL CHILDREN" which we are resubmitting to be considered for publication. A point-
13 by-point reply to the reviewers is also enclosed.
14

15
16 None of the authors have a conflict of interest with the data presented.
17

18 Part of this work has been presented at the American Thoracic Society meeting 2009 and the
19 European Respiratory Society meetings 2008 and 2009. Neither this work nor any part of its
20 essential substances, tables, or figures have been or will be published or submitted to another
21 scientific journal nor are being considered for publication elsewhere.
22
23

24 Thank you in advance for your attention.
25

26 Best regards,
27

28
29 Claudia Calogero
30

31 Reviewers' Comments to Author:
32

33
34 Reviewer: 2
35 Comments to the Author
36 No further comments
37

38
39 Reviewer: 1
40 Comments to the Author

41 In the revised version of this manuscript, the authors have addressed most of the issues which
42 were problematic in the previous version. There are some outstanding minor issues which I have
43 detailed below.
44

45 - Reply: *We thank the reviewer for the positive comments.*
46

47 P10 L23: Having clarified what the authors meant, their clarified statement is not supported by the
48 evidence. The evidence that FOT is more able to detect a difference between healthy and diseased
49 children than other preschool techniques is patchy and contradictory? Even in this section (P11
50 L20) the authors present data suggesting that BDR in wheezy children was less than that recorded
51 elsewhere in healthy children. Doesn't this suggest that the technique is poor at distinguishing?
52 This paper does not itself present any data which address the issue: I would suggest this sentence
53 is omitted.
54

55 -Reply: *the paragraph has been deleted as suggested by the reviewer. The references have been*
56 *changed accordingly.*
57

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59 P12 L8: parents were sent...
60

-Reply: *Modified as suggested by the reviewer.*

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4 P13 L18: talking or glottal closure

5 -Reply: *Modified as suggested by the reviewer.*
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7

8 P13 L43: 15 minutes

9 -Reply: *Modified as suggested by the reviewer.*
10

11 P14 L12: Using an asterisk to mean “multiplied by” is accepted in some contexts (eg spreadsheet
12 functions) but is potentially ambiguous. (Editor: Does the journal have a policy on this?)

13 -Reply: *We haven’t found if the Journal has a policy on this particular issue however the asterisk
14 has been replaced by the symbol “×”.*
15
16

17 P16 L20 onwards: this paragraph, and particularly the long string of results within the brackets,
18 has now become very complicated and difficult to follow. Could these data not be more elegantly
19 presented in table form, either as a new table or extending an existing table?

20 -Reply: *Table 5 has been added as suggested by the reviewer.*
21
22

23 P16 L47: layout is odd and needs attention – would be better if manuscript text is left-justified.

24 -Reply: *Modified as suggested by the reviewer.*
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27 P20 L28: for Italian children

28 -Reply: *Modified as suggested by the reviewer.*
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2 **RESPIRATORY IMPEDANCE AND BRONCHODILATOR RESPONSE IN HEALTHY**
3 **ITALIAN PRESCHOOL CHILDREN**
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5
6 C Calogero¹, N Parri¹, A Baccini¹, B Cuomo², M Palumbo², E Novembre¹, P Morello³, C Azzari¹, M de
7 Martino¹, PD Sly⁴, E Lombardi¹
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15 ⁴ Division of Clinical Sciences, Telethon Institute for Child Health Research and Centre for Child
16 Health Research, University of Western Australia, Perth, Western Australia
17

18
19 Partially presented at the American Thoracic Society meeting 2009 and the European Respiratory
20 Society meetings 2008 and 2009.
21
22

23
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35 **Key words:** forced oscillations, children, reference values, bronchodilator
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38 **Short title:** Respiratory Impedance in Healthy Preschoolers
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Summary

Objective – To define normal values for respiratory resistance (R_{rs}) and reactance (X_{rs}) and bronchodilator response (BDR) in a population of healthy Italian preschool children using a commercially available forced oscillation device.

Methods – R_{rs} and X_{rs} were measured in kindergartens in Viterbo, Italy. Regression analysis was performed taking into account height, weight, age, gender and reference equations calculated. The coefficient of repeatability (CR) between two tests performed 15 minutes apart was calculated in a subset of children. BDR was assessed by repeating the measurements 15 minutes after the administration of 200 μ g of inhaled salbutamol and calculated as an absolute change in R_{rs} and X_{rs} at 8Hz, as a percent change in baseline, and as a change in Z-score calculated from the reference equations.

Results – Lung function was attempted in 175 healthy children and successful in 163 (81 male, median age 4.8, range 2.9-6.1 yr). R_{rs} and X_{rs} at 6, 8 and 10 Hz were related to height but not other variables. The CR was 1.53 hPa.s.L⁻¹ for R_{rs8} and 0.91 hPa.s.L⁻¹ for X_{rs8} . The 5th percentile for absolute R_{rs8} BDR was -3.16 hPa.s.L⁻¹, while the 95th percentile for absolute X_{rs8} BDR was 2.25 hPa.s.L⁻¹. These cut-off values corresponded to a change in the Z-score of -1.88 and 2.48, respectively.

Conclusions - We have established reference equations for R_{rs} and X_{rs} in healthy Italian preschool children using forced oscillations. We recommend a change in Z-score of -1.88 for R_{rs8} and 2.48 for X_{rs8} as cut-off values for a positive BDR.

Abstract word count: 249

INTRODUCTION

Lung function testing is important in evaluating patients with respiratory diseases. Adults and cooperative older children, usually from school age, can perform tests that require specific respiratory manoeuvres, such as spirometry which requires maximal inspiration and maximal forced expiration, to measure lung function. In preschool children such manoeuvres are frequently not possible. Respiratory impedance (Z_{rs}) can be measured with the forced oscillation technique (FOT). This is a useful tool in evaluating lung function in young children with limited ability to cooperate [1] and older children [2-4]. FOT has been shown to be applicable in the clinical evaluation of preschool children with cystic fibrosis [5] as well as older children with cystic fibrosis during a respiratory tract exacerbation [6]. Lung function measured with FOT has also been evaluated in children born prematurely with or without neonatal chronic lung diseases [7-9]. FOT has also been shown to be a valid tool in the assessment of bronchodilator response in different populations of patients, especially asthmatic or wheezing children [4,10-14].

Deleted: Furthermore, FOT seems to be more able to detect differences in lung function between children with lung disease and controls compared to other tests, such as the interrupter technique or spirometry [4,8,10]

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Before FOT can be introduced into clinical practice as a routine test, appropriate reference values from healthy populations are needed. While a number of data sets from children have been published these are likely to be specific to the equipment used and to the study population. Other reference data sets from large samples of children have been previously published using the pseudorandom noise as the forcing function [2,15-18]. Hall et al. [18] have previously reported Z_{rs} reference values in a population of Australian preschoolers using a commercially available FOT device that uses a pseudo-random noise as the forcing function. The Australian equation was compared to other equations previously published [18]. To date, no assessment of whether these data are applicable to other populations has been published.

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2 For the clinical application of the technique, knowing the repeatability of the test and the
3 bronchodilator response (BDR) in healthy subjects is also necessary. The between-measurement
4 coefficient of repeatability (CR, i.e. twice the standard deviation of the difference between two
5 measurements) for FOT has been reported in healthy children to be between 1.1 and 2.6 hPa.s.L⁻¹ for
6 respiratory resistance (R_{rs}) and between 1.2 and 1.7 hPa.s.L⁻¹ for respiratory reactance (X_{rs}) [18-20].
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12 Thamrin et al. [10] have shown BDR calculated as a percent change with respect to baseline of -37%
13 for R_{rs8} and 67% for X_{rs8} in healthy Australian children. Oostven et al. have reported a decrease in
14 resistance on average of 22% in wheezing children [14]. In the present study, we measured lung
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MATERIAL AND METHODS

Study subjects

Children attending kindergartens in Viterbo, Italy were approached. Parents were sent a consent form and questionnaire by the education authorities and asked to return these to the child's kindergarten. The Ethics Committee of the Local Health Authority in Viterbo approved the study and written consent was obtained from the parents.

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Respiratory history

Respiratory history was assessed using a standardized questionnaire (ISAAC modified) [21]. Subjects born at less than 36 weeks of gestational age or who had received oxygen after birth for more than 30 days were excluded. Subjects were defined as healthy, and thus included into the analysis, if they had never had a doctor diagnosis of asthma and had no more than 3 episodes of wheezing ever, with no episodes of wheezing during the previous 12 months. A personal history of atopy was defined as positive if the child had ever had allergic rhinitis and/or eczema. Also, parental history of atopy was considered to be positive if the parents had ever had allergic rhinitis or eczema or asthma and children were considered to be exposed to environmental tobacco smoke (ETS) if any family member smoked. All subjects were free of respiratory symptoms and signs at the time of testing.

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Lung function assessment

Subjects underwent FOT measurements in the kindergartens in Viterbo. R_{rs} and X_{rs} were measured according to American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations [1], using a commercial device (I2M; Chess Medical, Belgium, marketed by Cosmed, Italy), which uses a pseudo-random noise signal between 4 and 48 Hz. The device was calibrated every day before

1
2 the testing using a calibration device of known impedance. The impedance of the mouthpiece and
3
4 bacterial filter (Cosmed, Rome, Italy) was measured and subtracted by the equipment software
5
6 automatically. During the test subjects sat in an upright position with a neutral position of the head and
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8 were connected to the oscillation device by the mouthpiece incorporating a bacterial filter. They wore a
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10 nose clip and they were instructed to breathe normally at tidal volume. The cheeks and the floor of the
11
12 mouth of the subject were supported during all measurements by one of the investigators.
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14 Measurements were excluded if a sudden drift on the volume tracing (suggesting an incomplete seal
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16 around the mouth) or a reduction/interruption of the flow oscillations (suggesting movement of the
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18 mouth or tongue, swallowing, talking, or glottal closure) were detected by visual control on the screen
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20 [1]. The validity of the measurements at individual frequencies was assessed automatically by the
21
22 software using the coherence function, which describes the statistical relationship between the input
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24 and output signals. A coherence function ≥ 0.95 is taken as an acceptable limit for Z_{rs} measurements [1].
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26 Individual frequencies that had a coherence function of < 0.95 were excluded and the entire
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28 measurement was discarded if three or more individual frequencies were unacceptable. Between three
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30 and five five data epochs lasting 8 seconds each, were recorded to obtain at least 3 acceptable
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32 measurements [1]. R_{rs} and X_{rs} were calculated for each frequency and the mean of the obtained
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34 measurements reported for each child at the frequencies of 6, 8 and 10 Hz.
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37 The short-term repeatability of the test was investigated in a subset of preschoolers. For this purpose
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39 two sets of measurements were taken 15 minutes apart.
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43 Bronchodilator response (BDR) was assessed by measuring again Z_{rs} 15 minutes after the
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45 administration of 200 μg of inhaled salbutamol administered at tidal breathing via MDI with a spacer
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47 (AeroChamber Plus, Trudell Medical International, London, ON, Canada).
48

Statistical Analysis

Data are reported as mean and standard deviation (SD). Individual within-test variations for Z_{rs} variables are expressed as coefficients of variation (CV), calculated as: $CV = (SD/mean) \times 100$, where SD is the standard deviation and the mean is the mean of all technically acceptable measurements. The coefficient of repeatability (CR) for two repeated sets of measurements was calculated as absolute value ($2 \times SD$ of the difference between the repeated measurements) and as relative value ($2 \times SD$ of the difference between repeated measurements as a percentage of the mean of the two measurements).

Stepwise multiple regression analysis was performed to take into account height, weight, age and gender and potential effects of personal and parental history of atopy, and ETS exposure. Regression equations and the residual standard deviation (RSD) were determined for R_{rs} and X_{rs} at the frequencies of 6, 8 and 10 Hz. BDR was assessed using three indices: absolute change in R_{rs8} and X_{rs8} ; percent change in R_{rs8} and X_{rs8} with respect to baseline; and as change in Z-score (post-salbutamol Z-score – baseline Z-score) for R_{rs8} and X_{rs8} calculated from the baseline data. SPSS for Windows (version 16.0) was used for the analysis. A two-tailed p value of less than 0.05 was considered as statistically significant.

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RESULTS

Questionnaires were distributed to families of 690 children and 324 were returned. Two children with neurological problems were excluded and consent for lung function testing was not obtained from 47 children. Eighty four children had a diagnosis of asthma or had recurrent respiratory symptoms and were excluded from the current analysis. Measurements could not be attempted in 16 children due to equipment failure. Lung function measurements were attempted in 175 healthy children and data successfully obtained from 163 children aged 2.9 to 6.1 years. Demographic characteristics of the population are shown in Table 1. The success rate of FOT increased with age: being 66% (2/3) for children < 3 years old; 90.6 % (29/32) for children from 3 to <4 years; 94.4% (67/71) for children from 4 to <5 years; 93.8% (61/65) for children aged 5 to <6 years and 100% (4/4) for children older than 6 years of age.

Group mean data for R_{rs} and X_{rs} at 6, 8 and 10 Hz as well as the within test variability are reported in Table 2. A smaller number of acceptable measurements for R_{rs6} were obtained due to coherence < 0.95 (n=143). There were no significant sex differences in any variable. The prediction equations for the relationship between height (H) and respiratory impedance were determined and the standard deviation of residuals (RSD) calculated. The result for the frequencies of 6, 8 and 10 Hz is shown in Table 3. Weight, age, personal and parental history of atopy, as well as ETS exposure did not significantly affect Z_{rs} after taking height into account. The relationship between height and R_{rs} and X_{rs} at 8 Hz for study population is shown in Figure 1.

Short-term repeatability of the test was assessed in 44 children. A smaller number of acceptable measurements for R_{rs6} were obtained due to coherence < 0.95 (n=37). CR for R_{rs} and X_{rs} at 6, 8 and 10

Hz is shown in Table 4. The difference between the two repeated measurements was not correlated to the mean values of the two measurements (data not shown).

BDR was assessed in 154 children. R_{rs8} and X_{rs8} changed significantly after salbutamol (baseline R_{rs8} 8.49 hPa.s.L⁻¹ vs post salbutamol R_{rs8} 7.40 hPa.s.L⁻¹, $p < 0.001$; baseline X_{rs8} -2.44 hPa.s.L⁻¹ vs post salbutamol X_{rs8} -1.73 hPa.s.L⁻¹, $p < 0.001$). The 5th percentile for the absolute R_{rs8} BDR was -3.16 hPa.s.L⁻¹, while the 95th percentile for the absolute X_{rs8} BDR was 2.25 hPa.s.L⁻¹. These cut-off values corresponded to a percent change with respect to baseline of -34% and 61%, respectively, and a change in the Z-score of -1.88 and 2.48, respectively. The changes in all three indices of bronchodilation were related to the magnitude of the baseline measurement for both R_{rs8} and X_{rs8} (Table 5, Figure 2). BDR, expressed as a percent of baseline, was correlated with height for R_{rs8} ($r = 0.18$, $b = -0.386$, $p = 0.029$), but not for X_{rs8} ($r = 0.13$, $b = 0.712$, $p = 0.112$). No significant correlations were found between height and absolute BDR ($r = 0.09$, $b = -0.017$, $p = 0.287$ for R_{rs8} ; $r = 0.20$, $b = -0.002$, $p = 0.805$ for X_{rs8}) or change in Z-score ($r = 0.09$, $b = -0.010$, $p = 0.287$ for R_{rs8} ; $r = 0.02$, $b = -0.003$, $p = 0.805$ for X_{rs8}).

Deleted: R_{rs8} absolute change: $r = 0.49$, $b = -0.317$, $p < 0.001$; X_{rs8} absolute change: $r = 0.67$, $b = -0.523$, $p < 0.001$; R_{rs8} change in percent baseline: $r = 0.30$, $b = -2.249$, $p < 0.001$; X_{rs8} change in percent baseline: $r = 0.33$, $b = -11.525$, $p < 0.001$; R_{rs8} change in Z-score: $r = 0.49$, $b = -0.189$, $p < 0.001$; X_{rs8} change in Z-score: $r = 0.67$, $b = -0.574$, $p < 0.001$

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Using the Australian equations [18] to calculate Z-scores for Italian children and comparing then with Z-scores calculated using the Italian equations from the present study showed minor differences for R_{rs10} (0.23, $p = 0.036$), X_{rs8} (0.32, $p = 0.012$) and X_{rs10} (0.65, $p < 0.001$). No significant differences were found for R_{rs6} (-0.02, $p = 0.82$), R_{rs8} (0.10, $p = 0.43$) and X_{rs6} (-0.02, $p = 0.68$). The equations for R_{rs8} and X_{rs8} for both the Australian and the Italian populations are shown in Figure 3.

DISCUSSION

The results of the present study confirm that the forced oscillation technique is feasible and is a potentially useful tool for evaluating lung function in preschool children. With this study we provide reference data for Italian children. In addition, we have shown that the technique is repeatable and appropriate for assessing bronchodilation as change in R_{rs} or X_{rs} Z-score.

According to the most recent ATS/ERS recommendations [1] the measurements should be considered acceptable primarily based on the coefficient of variability (CV) of the individual frequencies. In the present study measurements in which three or more individual frequencies had a coherence of <0.95 were excluded. Rejecting data at a single frequency where the coherence is <0.95 is important as this provides a recognised standard for quality control and is built into the software of the I2M FOT device. Data with lower coherence suggests low signal to noise ratio and a violation of the underlying assumption of a linear relationship between the input and output signals. We recommend reporting R_{rs} and X_{rs} at 8 Hz as these data can be obtained from most children and are reproducible in the short term (Table 2).

From a clinical point of view it is very important to have appropriate reference data for a given population. FOT can be used in young children or older children not able to perform spirometry. Knowing the reference equation it is possible to detect abnormal lung function and, as previously mentioned, FOT can then be used in the follow-up of children with lung diseases such as asthma, neonatal chronic lung disease or cystic fibrosis or asthma [5, 7-9, 13-14]. The reference equations calculated from the healthy Italian children in the present study are very similar to those published by Hall et al. [18] from Australian children who have used a different commercial implementation of the same basic equipment and methodology. Hall et al. [18] have already compared the Australian Z_{rs}

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2 equation to those published previously from other large populations of children measured with different
3 equipment; showing that even when performed under different conditions, the different reference
4 values were very similar. Using the Australian equations [18] to calculate Z-scores for Italian children
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6 and comparing them with Z-scores calculated using the Italian equations from the present study, no
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8 significant difference for R_{rs8} and only a minor difference for X_{rs8} was found. Both study populations
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10 were largely Caucasian and of similar age and height range. The Italian data were collected in the field
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12 whereas the Australian data were largely collected in a specialized pulmonary function laboratory. The
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14 similarity in the equations, as shown in Figure 3, encourages an effort to combine data sets from
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16 different parts of the world to provide an international reference data set, as has been done for other
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18 techniques. Such a data set would also allow appropriate examination of potential influences on lung
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20 function, such as sex, atopy, family history of asthma and atopy, that are rarely significant in individual
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22 data sets due to limited sample size.
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27 For the clinical interpretation of the measurements it is necessary to consider the repeatability of the
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29 test. In the current study between-measurement repeatability was defined as absolute CR ($2 \times$ SD of the
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31 difference between the repeated measurements made 15 minutes apart), according to the international
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33 recommendations [1], and relative CR ($2 \times$ SD of the difference between repeated measurements as a
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35 percentage of the mean of the two measurements). We have reported an absolute and a relative CR for
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37 R_{rs8} and for X_{rs8} (as shown in Table 4) that are similar to other previously published between-
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39 measurement repeatability values [18-20]. This short term CR describes the variability introduced by
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41 having the child make two sets of measurements 15 minutes apart. Sources of variability that may
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43 contribute to this CR include: different breathing pattern; different level of engagement and co-
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45 operation by the child; and any minute to minute fluctuations in airway calibre due to changes in
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47 airway tone. It is reasonable to expect that true changes in the physiological state of the lungs will be
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2 minimal in healthy children over this time interval. Knowledge of these short term changes in measured
3 lung function without any therapeutic intervention are vital to interpreting true changes due to
4 salbutamol inhalation. However, in the present study we have found that the change in lung function in
5 healthy preschool children after salbutamol was higher than the short-term variability of the test,
6 reflecting a true BDR and highlighting the importance of cut-off for BDR based on measurements in
7 healthy preschoolers.
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15 In the present study we assessed BDR using R_{rs} and X_{rs} at 8 Hz as these were previously recommended
16 by Thamrin et al. [10]. Our data confirm their earlier data that the change in both R_{rs} and X_{rs} following
17 bronchodilator was related to the magnitude of the baseline values. In our study we have found a
18 correlation between BDR expressed as a percent of baseline and height for R_{rs} . BDR, as measured by
19 FOT, is an expression of the change in airway caliber following inhalation of a bronchodilator. The
20 change in R_{rs} is directly related to the increase in airway caliber of conducting airways, that is, those
21 airways in which gas movement occurs by bulk flow. While X_{rs} primarily reflects the elastic and
22 inertive properties of the respiratory system, the mechanical properties of small airways, that is those in
23 which gas movement occurs by facilitated diffusion, are also included. Thus the change in X_{rs} with
24 bronchodilator is more complex. The airways become larger and the respiratory system becomes less
25 stiff with growth and development, reflected here by both baseline lung function and height. Thus it is
26 not surprising that we found a relationship between the magnitude of the BDR and both baseline lung
27 function and height. Oostveen et al. [14] have recently argued that using absolute change in lung
28 function to express BDR avoids hiding differences in baseline bronchomotor tone, which they implied
29 underlies the difference in baseline lung function in children with different wheeze phenotypes.
30 However, as argued by Thamrin et al. [22] correcting for differences in airway caliber due to size rather
31 than underlying bronchomotor tone is critical to getting the clinical interpretation correct. Normalizing
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1 lung function to the child's height, as occurs by using Z-score or expressing lung function as a percent
2 of the predicted value, should remove height-dependence of the BDR. Indeed, in the present study by
3 using Z-scores to represent R_{rs8} and X_{rs8} the change in lung function following bronchodilator was no
4 longer related to the child's height. This finding is similar to that reported by the Australian group [13].

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5 The major difference between our study and the one from Thamrin et al. [10] is the use of 600 μ g of
6 salbutamol to assess BDR whereas 200 μ g was used in the present study. Despite this difference, the
7 change in both R_{rs8} and X_{rs8} with salbutamol was similar, being 40% and 35% respectively for R_{rs8} and
8 65% and 61% respectively for X_{rs8} . The similarities in BDR despite the substantial difference in
9 salbutamol dose show that 200 μ g is sufficient for healthy children and likely reflects a relatively flat
10 dose-response curve in such children. Other studies have reported similar changes in lung function
11 following bronchodilatation measured with FOT in asthmatic children [13] or with different wheezing
12 phenotypes [14].

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13 In conclusion, the data from the present study give the reference equation for R_{rs} and X_{rs} for Italian
14 children between 2 and 6 years of age. The data also demonstrate that healthy Italian preschool children
15 have similar lung function, as assessed by the FOT using the similar commercially available
16 equipment, to healthy Australian preschool children. In addition, we have demonstrated that the most
17 appropriate index for determine BDR is a decrease in R_{rs8} Z-score of ≥ 1.88 or an increase in X_{rs8} Z-
18 score of ≥ 2.48 in preschool children.

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2 **FIGURE LEGENDS**
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6 **Figure 1** - Relationship between height and respiratory impedance Z_{rs} at 8 Hz in preschool children.
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8 The single values are expressed as circles, the regression line and the 95% confidence intervals are
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10 shown as solid lines. (A) respiratory resistance at 8 Hz (R_{rs8}); (B) respiratory reactance at 8 Hz (X_{rs8}).
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13 **Figure 2** – Bronchodilator response as a function of baseline values expressed as A) percent of baseline
14 R_{rs8} and X_{rs8} ; B) absolute change in R_{rs8} and in X_{rs8} ; and C) change in Z-score for R_{rs8} and X_{rs8} .
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19 **Figure 3** – Predicted values for respiratory resistance (R_{rs8}) and reactance (X_{rs8}) at 8 Hz using the
20 equations by Hall et al. [18] (dashed lines) and the equations reported in the present study (full lines).
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3 **RESPIRATORY IMPEDANCE AND BRONCHODILATOR RESPONSE IN HEALTHY**
4 **ITALIAN PRESCHOOL CHILDREN**
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8 C Calogero¹, N Parri¹, A Baccini¹, B Cuomo², M Palumbo², E Novembre¹, P Morello³, C Azzari¹, M de
9 Martino¹, PD Sly⁴, E Lombardi¹
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20 Health Research, University of Western Australia, Perth, Western Australia
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24 Partially presented at the American Thoracic Society meeting 2009 and the European Respiratory
25 Society meetings 2008 and 2009.
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44 **Key words:** forced oscillations, children, reference values, bronchodilator
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47 **Short title:** Respiratory Impedance in Healthy Preschoolers
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Summary

Objective – To define normal values for respiratory resistance (R_{rs}) and reactance (X_{rs}) and bronchodilator response (BDR) in a population of healthy Italian preschool children using a commercially available forced oscillation device.

Methods – R_{rs} and X_{rs} were measured in kindergartens in Viterbo, Italy. Regression analysis was performed taking into account height, weight, age, gender and reference equations calculated. The coefficient of repeatability (CR) between two tests performed 15 minutes apart was calculated in a subset of children. BDR was assessed by repeating the measurements 15 minutes after the administration of 200 μ g of inhaled salbutamol and calculated as an absolute change in R_{rs} and X_{rs} at 8Hz, as a percent change in baseline, and as a change in Z-score calculated from the reference equations.

Results – Lung function was attempted in 175 healthy children and successful in 163 (81 male, median age 4.8, range 2.9-6.1 yr). R_{rs} and X_{rs} at 6, 8 and 10 Hz were related to height but not other variables. The CR was 1.53 hPa.s.L⁻¹ for R_{rs8} and 0.91 hPa.s.L⁻¹ for X_{rs8} . The 5th percentile for absolute R_{rs8} BDR was -3.16 hPa.s.L⁻¹, while the 95th percentile for absolute X_{rs8} BDR was 2.25 hPa.s.L⁻¹. These cut-off values corresponded to a change in the Z-score of -1.88 and 2.48, respectively.

Conclusions - We have established reference equations for R_{rs} and X_{rs} in healthy Italian preschool children using forced oscillations. We recommend a change in Z-score of -1.88 for R_{rs8} and 2.48 for X_{rs8} as cut-off values for a positive BDR.

Abstract word count: 249

INTRODUCTION

Lung function testing is important in evaluating patients with respiratory diseases. Adults and cooperative older children, usually from school age, can perform tests that require specific respiratory manoeuvres, such as spirometry which requires maximal inspiration and maximal forced expiration, to measure lung function. In preschool children such manoeuvres are frequently not possible. Respiratory impedance (Z_{rs}) can be measured with the forced oscillation technique (FOT). This is a useful tool in evaluating lung function in young children with limited ability to cooperate [1] and older children [2-4]. FOT has been shown to be applicable in the clinical evaluation of preschool children with cystic fibrosis [5] as well as older children with cystic fibrosis during a respiratory tract exacerbation [6]. Lung function measured with FOT has also been evaluated in children born prematurely with or without neonatal chronic lung diseases [7-9]. FOT has also been shown to be a valid tool in the assessment of bronchodilator response in different populations of patients, especially asthmatic or wheezing children [4,10-14].

Before FOT can be introduced into clinical practice as a routine test, appropriate reference values from healthy populations are needed. While a number of data sets from children have been published these are likely to be specific to the equipment used and to the study population. Other reference data sets from large samples of children have been previously published using the pseudorandom noise as the forcing function [2,15-18]. Hall et al. [18] have previously reported Z_{rs} reference values in a population of Australian preschoolers using a commercially available FOT device that uses a pseudo-random noise as the forcing function. The Australian equation was compared to other equations previously published [18]. To date, no assessment of whether these data are applicable to other populations has been published.

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3 For the clinical application of the technique, knowing the repeatability of the test and the
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5 bronchodilator response (BDR) in healthy subjects is also necessary. The between-measurement
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7 coefficient of repeatability (CR, i.e. twice the standard deviation of the difference between two
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9 measurements) for FOT has been reported in healthy children to be between 1.1 and 2.6 hPa.s.L⁻¹ for
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11 respiratory resistance (R_{rs}) and between 1.2 and 1.7 hPa.s.L⁻¹ for respiratory reactance (X_{rs}) [18-20].
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14 Thamrin et al. [10] have shown BDR calculated as a percent change with respect to baseline of -37%
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16 for R_{rs} and 67% for X_{rs} in healthy Australian children. Oostven et al. have reported a decrease in
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18 resistance on average of 22% in wheezing children [14]. In the present study, we measured lung
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20 function using FOT in 163 preschool aged Italian children to produce reference equations for R_{rs} and
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22 X_{rs} and to compare these with the Australian data. We also aimed to evaluate the repeatability of the
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24 test and to determine appropriate methods for assessing response to inhaled bronchodilator.
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MATERIAL AND METHODS

Study subjects

Children attending kindergartens in Viterbo, Italy were approached. Parents were sent a consent form and questionnaire by the education authorities and asked to return these to the child's kindergarten. The Ethics Committee of the Local Health Authority in Viterbo approved the study and written consent was obtained from the parents.

Respiratory history

Respiratory history was assessed using a standardized questionnaire (ISAAC modified) [21]. Subjects born at less than 36 weeks of gestational age or who had received oxygen after birth for more than 30 days were excluded. Subjects were defined as healthy, and thus included into the analysis, if they had never had a doctor diagnosis of asthma and had no more than 3 episodes of wheezing ever, with no episodes of wheezing during the previous 12 months. A personal history of atopy was defined as positive if the child had ever had allergic rhinitis and/or eczema. Also, parental history of atopy was considered to be positive if the parents had ever had allergic rhinitis or eczema or asthma and children were considered to be exposed to environmental tobacco smoke (ETS) if any family member smoked. All subjects were free of respiratory symptoms and signs at the time of testing.

Lung function assessment

Subjects underwent FOT measurements in the kindergartens in Viterbo. R_{rs} and X_{rs} were measured according to American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations [1], using a commercial device (I2M; Chess Medical, Belgium, marketed by Cosmed, Italy), which uses a pseudo-random noise signal between 4 and 48 Hz. The device was calibrated every day before

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3 the testing using a calibration device of known impedance. The impedance of the mouthpiece and
4 bacterial filter (Cosmed, Rome, Italy) was measured and subtracted by the equipment software
5 automatically. During the test subjects sat in an upright position with a neutral position of the head and
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10 were connected to the oscillation device by the mouthpiece incorporating a bacterial filter. They wore a
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13 nose clip and they were instructed to breathe normally at tidal volume. The cheeks and the floor of the
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the testing using a calibration device of known impedance. The impedance of the mouthpiece and bacterial filter (Cosmed, Rome, Italy) was measured and subtracted by the equipment software automatically. During the test subjects sat in an upright position with a neutral position of the head and were connected to the oscillation device by the mouthpiece incorporating a bacterial filter. They wore a nose clip and they were instructed to breathe normally at tidal volume. The cheeks and the floor of the mouth of the subject were supported during all measurements by one of the investigators. Measurements were excluded if a sudden drift on the volume tracing (suggesting an incomplete seal around the mouth) or a reduction/interruption of the flow oscillations (suggesting movement of the mouth or tongue, swallowing, talking, or glottal closure) were detected by visual control on the screen [1]. The validity of the measurements at individual frequencies was assessed automatically by the software using the coherence function, which describes the statistical relationship between the input and output signals. A coherence function ≥ 0.95 is taken as an acceptable limit for Z_{rs} measurements [1]. Individual frequencies that had a coherence function of < 0.95 were excluded and the entire measurement was discarded if three or more individual frequencies were unacceptable. Between three and five five data epochs lasting 8 seconds each, were recorded to obtain at least 3 acceptable measurements [1]. R_{rs} and X_{rs} were calculated for each frequency and the mean of the obtained measurements reported for each child at the frequencies of 6, 8 and 10 Hz.

The short-term repeatability of the test was investigated in a subset of preschoolers. For this purpose two sets of measurements were taken 15 minutes apart.

Bronchodilator response (BDR) was assessed by measuring again Z_{rs} 15 minutes after the administration of 200 μg of inhaled salbutamol administered at tidal breathing via MDI with a spacer (AeroChamber Plus, Trudell Medical International, London, ON, Canada).

Statistical Analysis

Data are reported as mean and standard deviation (SD). Individual within-test variations for Z_{rs} variables are expressed as coefficients of variation (CV), calculated as: $CV = (SD/mean) \times 100$, where SD is the standard deviation and the mean is the mean of all technically acceptable measurements. The coefficient of repeatability (CR) for two repeated sets of measurements was calculated as absolute value ($2 \times SD$ of the difference between the repeated measurements) and as relative value ($2 \times SD$ of the difference between repeated measurements as a percentage of the mean of the two measurements).

Stepwise multiple regression analysis was performed to take into account height, weight, age and gender and potential effects of personal and parental history of atopy, and ETS exposure. Regression equations and the residual standard deviation (RSD) were determined for R_{rs} and X_{rs} at the frequencies of 6, 8 and 10 Hz. BDR was assessed using three indices: absolute change in R_{rs8} and X_{rs8} ; percent change in R_{rs8} and X_{rs8} with respect to baseline; and as change in Z-score (post-salbutamol Z-score – baseline Z-score) for R_{rs8} and X_{rs8} calculated from the baseline data. SPSS for Windows (version 16.0) was used for the analysis. A two-tailed p value of less than 0.05 was considered as statistically significant.

RESULTS

Questionnaires were distributed to families of 690 children and 324 were returned. Two children with neurological problems were excluded and consent for lung function testing was not obtained from 47 children. Eighty four children had a diagnosis of asthma or had recurrent respiratory symptoms and were excluded from the current analysis. Measurements could not be attempted in 16 children due to equipment failure. Lung function measurements were attempted in 175 healthy children and data successfully obtained from 163 children aged 2.9 to 6.1 years. Demographic characteristics of the population are shown in Table 1. The success rate of FOT increased with age: being 66% (2/3) for children < 3 years old; 90.6 % (29/32) for children from 3 to <4 years; 94.4% (67/71) for children from 4 to <5 years; 93.8% (61/65) for children aged 5 to <6 years and 100% (4/4) for children older than 6 years of age.

Group mean data for R_{rs} and X_{rs} at 6, 8 and 10 Hz as well as the within test variability are reported in Table 2. A smaller number of acceptable measurements for R_{rs6} were obtained due to coherence < 0.95 (n=143). There were no significant sex differences in any variable. The prediction equations for the relationship between height (H) and respiratory impedance were determined and the standard deviation of residuals (RSD) calculated. The result for the frequencies of 6, 8 and 10 Hz is shown in Table 3. Weight, age, personal and parental history of atopy, as well as ETS exposure did not significantly affect Z_{rs} after taking height into account. The relationship between height and R_{rs} and X_{rs} at 8 Hz for study population is shown in Figure 1.

Short-term repeatability of the test was assessed in 44 children. A smaller number of acceptable measurements for R_{rs6} were obtained due to coherence < 0.95 (n=37). CR for R_{rs} and X_{rs} at 6, 8 and 10

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3 Hz is shown in Table 4. The difference between the two repeated measurements was not correlated to
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5 the mean values of the two measurements (data not shown).
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10 BDR was assessed in 154 children. R_{rs8} and X_{rs8} changed significantly after salbutamol (baseline R_{rs8}
11 8.49 hPa.s.L⁻¹ vs post salbutamol R_{rs8} 7.40 hPa.s.L⁻¹, $p < 0.001$; baseline X_{rs8} -2.44 hPa.s.L⁻¹ vs post
12 salbutamol X_{rs8} -1.73 hPa.s.L⁻¹, $p < 0.001$). The 5th percentile for the absolute R_{rs8} BDR was -3.16
13 hPa.s.L⁻¹, while the 95th percentile for the absolute X_{rs8} BDR was 2.25 hPa.s.L⁻¹. These cut-off values
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15 corresponded to a percent change with respect to baseline of -34% and 61%, respectively, and a change
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17 in the Z-score of -1.88 and 2.48, respectively. The changes in all three indices of bronchodilation were
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19 related to the magnitude of the baseline measurement for both R_{rs8} and X_{rs8} (Table 5, Figure 2). BDR,
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21 expressed as a percent of baseline, was correlated with height for R_{rs8} ($r = 0.18$, $b = -0.386$, $p = 0.029$), but
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23 not for X_{rs8} ($r = 0.13$, $b = 0.712$, $p = 0.112$). No significant correlations were found between height and
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25 absolute BDR ($r = 0.09$, $b = -0.017$, $p = 0.287$ for R_{rs8} ; $r = 0.20$, $b = -0.002$, $p = 0.805$ for X_{rs8}) or change in Z-
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27 score ($r = 0.09$, $b = -0.010$, $p = 0.287$ for R_{rs8} ; $r = 0.02$, $b = -0.003$, $p = 0.805$ for X_{rs8}).
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36 Using the Australian equations [18] to calculate Z-scores for Italian children and comparing then with
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38 Z-scores calculated using the Italian equations from the present study showed minor differences for
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40 R_{rs10} (0.23, $p = 0.036$), X_{rs8} (0.32, $p = 0.012$) and X_{rs10} (0.65, $p < 0.001$). No significant differences were
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42 found for R_{rs6} (-0.02, $p = 0.82$), R_{rs8} (0.10, $p = 0.43$) and X_{rs6} (-0.02, $p = 0.68$). The equations for R_{rs8} and
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44 X_{rs8} for both the Australian and the Italian populations are shown in Figure 3.
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DISCUSSION

The results of the present study confirm that the forced oscillation technique is feasible and is a potentially useful tool for evaluating lung function in preschool children. With this study we provide reference data for Italian children. In addition, we have shown that the technique is repeatable and appropriate for assessing bronchodilation as change in R_{rs} or X_{rs} Z-score.

According to the most recent ATS/ERS recommendations [1] the measurements should be considered acceptable primarily based on the coefficient of variability (CV) of the individual frequencies. In the present study measurements in which three or more individual frequencies had a coherence of <0.95 were excluded. Rejecting data at a single frequency where the coherence is <0.95 is important as this provides a recognised standard for quality control and is built into the software of the I2M FOT device. Data with lower coherence suggests low signal to noise ratio and a violation of the underlying assumption of a linear relationship between the input and output signals. We recommend reporting R_{rs} and X_{rs} at 8 Hz as these data can be obtained from most children and are reproducible in the short term (Table 2).

From a clinical point of view it is very important to have appropriate reference data for a given population. FOT can be used in young children or older children not able to perform spirometry. Knowing the reference equation it is possible to detect abnormal lung function and, as previously mentioned, FOT can then be used in the follow-up of children with lung diseases such as asthma, neonatal chronic lung disease or cystic fibrosis or asthma [5, 7-9, 13-14]. The reference equations calculated from the healthy Italian children in the present study are very similar to those published by Hall et al. [18] from Australian children who have used a different commercial implementation of the same basic equipment and methodology. Hall et al. [18] have already compared the Australian Z_{rs}

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3 equation to those published previously from other large populations of children measured with different
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5 equipment; showing that even when performed under different conditions, the different reference
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7 values were very similar. Using the Australian equations [18] to calculate Z-scores for Italian children
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9 and comparing them with Z-scores calculated using the Italian equations from the present study, no
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11 significant difference for R_{rs8} and only a minor difference for X_{rs8} was found. Both study populations
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13 were largely Caucasian and of similar age and height range. The Italian data were collected in the field
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15 whereas the Australian data were largely collected in a specialized pulmonary function laboratory. The
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17 similarity in the equations, as shown in Figure 3, encourages an effort to combine data sets from
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19 different parts of the world to provide an international reference data set, as has been done for other
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21 techniques. Such a data set would also allow appropriate examination of potential influences on lung
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23 function, such as sex, atopy, family history of asthma and atopy, that are rarely significant in individual
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25 data sets due to limited sample size.
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34 For the clinical interpretation of the measurements it is necessary to consider the repeatability of the
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36 test. In the current study between-measurement repeatability was defined as absolute CR ($2 \times$ SD of the
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38 difference between the repeated measurements made 15 minutes apart), according to the international
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40 recommendations [1], and relative CR ($2 \times$ SD of the difference between repeated measurements as a
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42 percentage of the mean of the two measurements). We have reported an absolute and a relative CR for
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44 R_{rs8} and for X_{rs8} (as shown in Table 4) that are similar to other previously published between-
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46 measurement repeatability values [18-20]. This short term CR describes the variability introduced by
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48 having the child make two sets of measurements 15 minutes apart. Sources of variability that may
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50 contribute to this CR include: different breathing pattern; different level of engagement and co-
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52 operation by the child; and any minute to minute fluctuations in airway calibre due to changes in
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54 airway tone. It is reasonable to expect that true changes in the physiological state of the lungs will be
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3 minimal in healthy children over this time interval. Knowledge of these short term changes in measured
4 lung function without any therapeutic intervention are vital to interpreting true changes due to
5 salbutamol inhalation. However, in the present study we have found that the change in lung function in
6 healthy preschool children after salbutamol was higher than the short-term variability of the test,
7 reflecting a true BDR and highlighting the importance of cut-off for BDR based on measurements in
8 healthy preschoolers.
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11 In the present study we assessed BDR using R_{rs} and X_{rs} at 8 Hz as these were previously recommended
12 by Thamrin et al. [10]. Our data confirm their earlier data that the change in both R_{rs} and X_{rs} following
13 bronchodilator was related to the magnitude of the baseline values. In our study we have found a
14 correlation between BDR expressed as a percent of baseline and height for R_{rs8} . BDR, as measured by
15 FOT, is an expression of the change in airway caliber following inhalation of a bronchodilator. The
16 change in R_{rs} is directly related to the increase in airway caliber of conducting airways, that is, those
17 airways in which gas movement occurs by bulk flow. While X_{rs} primarily reflects the elastic and
18 inertive properties of the respiratory system, the mechanical properties of small airways, that is those in
19 which gas movement occurs by facilitated diffusion, are also included. Thus the change in X_{rs} with
20 bronchodilator is more complex. The airways become larger and the respiratory system becomes less
21 stiff with growth and development, reflected here by both baseline lung function and height. Thus it is
22 not surprising that we found a relationship between the magnitude of the BDR and both baseline lung
23 function and height. Oostveen et al. [14] have recently argued that using absolute change in lung
24 function to express BDR avoids hiding differences in baseline bronchomotor tone, which they implied
25 underlies the difference in baseline lung function in children with different wheeze phenotypes.
26 However, as argued by Thamrin et al. [22] correcting for differences in airway caliber due to size rather
27 than underlying bronchomotor tone is critical to getting the clinical interpretation correct. Normalizing
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3 lung function to the child's height, as occurs by using Z-score or expressing lung function as a percent
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5 of the predicted value, should remove height-dependence of the BDR. Indeed, in the present study by
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7 using Z-scores to represent R_{rs8} and X_{rs8} the change in lung function following bronchodilator was no
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9 longer related to the child's height. This finding is similar to that reported by the Australian group [18].
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11 The major difference between our study and the one from Thamrin et al. [10] is the use of 600 μ g of
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13 salbutamol to assess BDR whereas 200 μ g was used in the present study. Despite this difference, the
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15 change in both R_{rs8} and X_{rs8} with salbutamol was similar, being 40% and 35% respectively for R_{rs8} and
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17 65% and 61% respectively for X_{rs8} . The similarities in BDR despite the substantial difference in
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19 salbutamol dose show that 200 μ g is sufficient for healthy children and likely reflects a relatively flat
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21 dose-response curve in such children. Other studies have reported similar changes in lung function
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23 following bronchodilatation measured with FOT in asthmatic children [13] or with different wheezing
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25 phenotypes [14].
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34 In conclusion, the data from the present study give the reference equation for R_{rs} and X_{rs} for Italian
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36 children between 2 and 6 years of age. The data also demonstrate that healthy Italian preschool children
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38 have similar lung function, as assessed by the FOT using the similar commercially available
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40 equipment, to healthy Australian preschool children. In addition, we have demonstrated that the most
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42 appropriate index for determine BDR is a decrease in R_{rs8} Z-score of ≥ 1.88 or an increase in X_{rs8} Z-
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44 score of ≥ 2.48 in preschool children.
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For Peer Review

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FIGURE LEGENDS

Figure 1 - Relationship between height and respiratory impedance Z_{rs} at 8 Hz in preschool children.

The single values are expressed as circles, the regression line and the 95% confidence intervals are shown as solid lines. (A) respiratory resistance at 8 Hz (R_{rs8}); (B) respiratory reactance at 8 Hz (X_{rs8}).

Figure 2 – Bronchodilator response as a function of baseline values expressed as A) percent of baseline R_{rs8} and X_{rs8} ; B) absolute change in R_{rs8} and in X_{rs8} ; and C) change in Z-score for R_{rs8} and X_{rs8} .

Figure 3 – Predicted values for respiratory resistance (R_{rs8}) and reactance (X_{rs8}) at 8 Hz using the equations by Hall et al. [18] (dashed lines) and the equations reported in the present study (full lines).

Table 1 - Characteristics of the population of children. Data are expressed as median (range) for age, mean (SD) for height and weight, and n (%) for gender, personal history of atopy, parental history of atopy, and environmental tobacco smoke (ETS) exposure.

	n=163
Age years	4.8 (2.9-6.1)
Male n	81 (49.7)
Height cm	107.8 (6.5)
Weight Kg	19.9 (3.5)
Personal history of atopy n	59 (37.1)
Parental history of atopy n	37 (24.7)
ETS exposure n	86 (54.1)

Table 2 – Group mean and SD of R_{rs} and X_{rs} and the within test variability in the study population

(Legend: SD = standard deviation; CV = coefficient of variation).

	Mean	SD	CV (%)	n
R_{rs6} hPa.s.L ⁻¹	8.40	0.74	8.8	143
R_{rs8} hPa.s.L ⁻¹	8.49	0.49	5.9	163
R_{rs10} hPa.s.L ⁻¹	8.16	0.51	6.3	163
X_{rs6} hPa.s.L ⁻¹	-3.24	0.55	17.2	143
X_{rs8} hPa.s.L ⁻¹	-2.45	0.43	18.8	163
X_{rs10} hPa.s.L ⁻¹	-2.23	0.38	18.6	163

Table 3 - Regression relationship between respiratory impedance and height in the study population.

(Legend: RSD = standard deviation of residuals, Ht = height in centimetres).

	Equation	RSD	R square	p value
R_{rs6}	23.452 - (0.139xHt)	1.79	0.204	<0.001
R_{rs8}	22.692 - (0.132xHt)	1.70	0.202	<0.001
R_{rs10}	21.453 - (0.123xHt)	1.52	0.216	<0.001
X_{rs6}	-11.990 + (0.081xHt)	0.84	0.280	<0.001
X_{rs8}	-9.584 + (0.066xHt)	0.91	0.182	<0.001
X_{rs10}	-9.001 + (0.063xHt)	0.94	0.158	<0.001

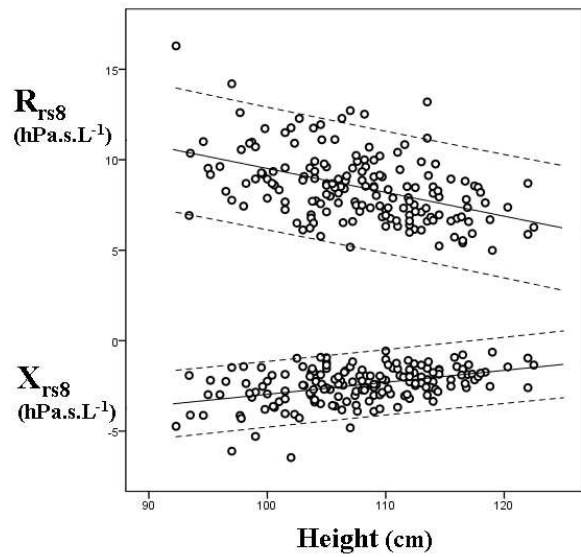
Table 4 – Mean absolute and relative coefficient of repeatability (CR) for R_{rs} and X_{rs} at 6, 8 and 10 Hz.

	Absolute CR (hPa.s.L ⁻¹)	Relative CR (%)	n
R_{rs6}	2.18	26.90	37
R_{rs8}	1.53	18.78	44
R_{rs10}	1.29	16.45	44
X_{rs6}	1.17	40.85	37
X_{rs8}	0.91	39.22	44
X_{rs10}	0.89	43.37	44

Table 5 – Different indices of bronchodilation for R_{rs} and X_{rs} at 8 Hz and their relationship with the magnitude of the baseline measurement expressed as r (correlation coefficient) and b (regression coefficient).

	r	b	p
R_{rs8} absolute change	0.49	-0.317,	<0.001
X_{rs8} absolute change	0.67	-0.523	<0.001
R_{rs8} change in percent baseline	0.30	-2.249	<0.001
X_{rs8} change in percent baseline	0.33	-11.525	<0.001
R_{rs8} change in Z-score	0.49	-0.189	<0.001
X_{rs8} change in Z-score	0.67	-0.574	<0.001

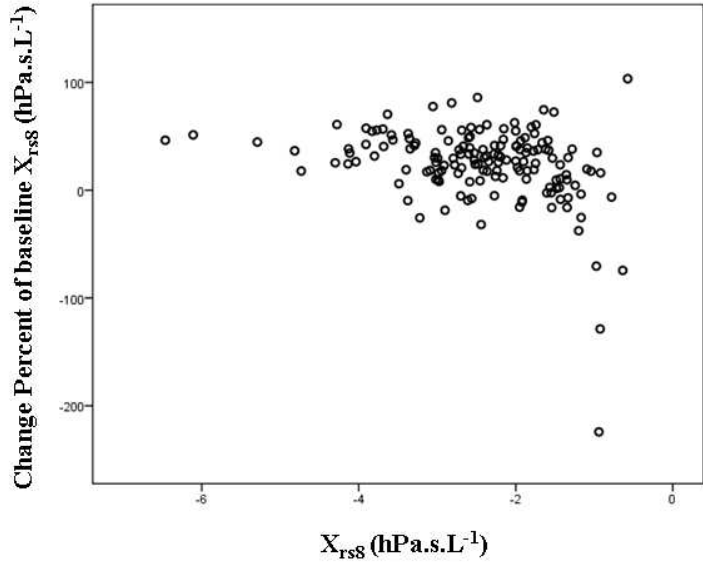
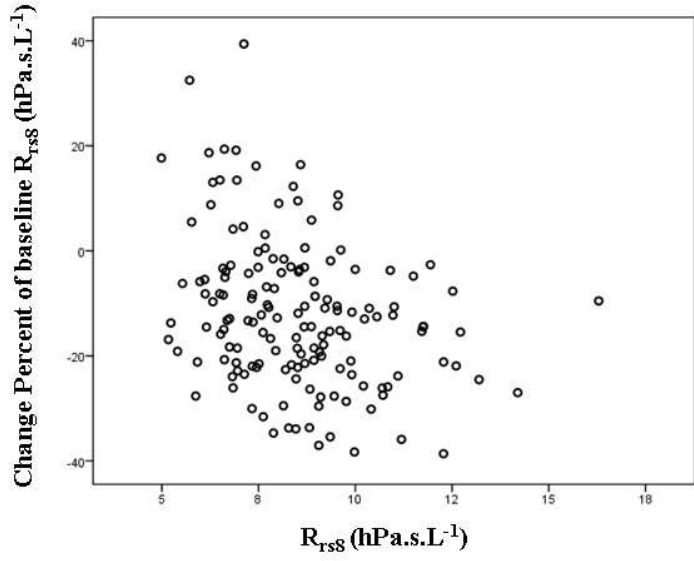
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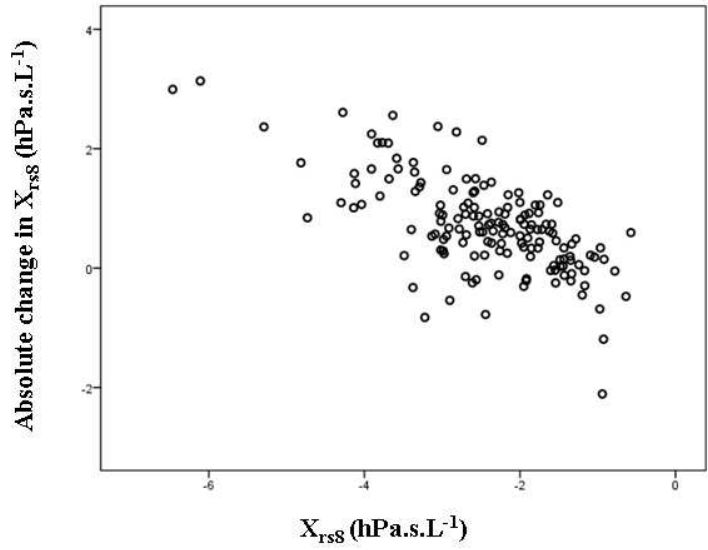
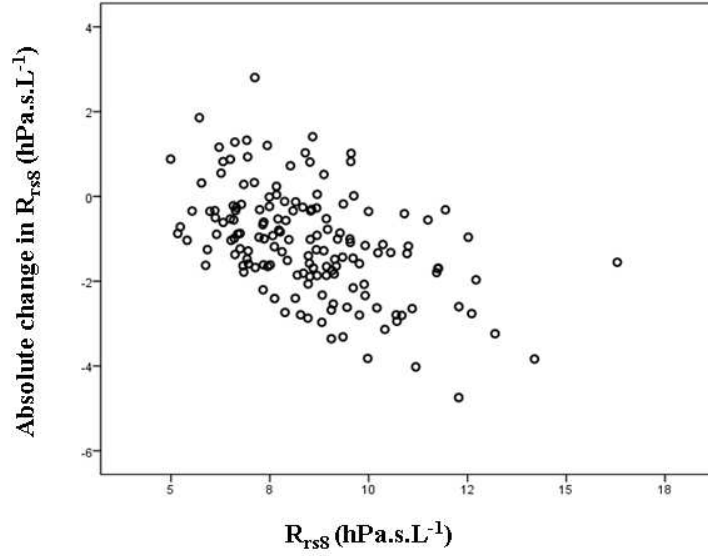
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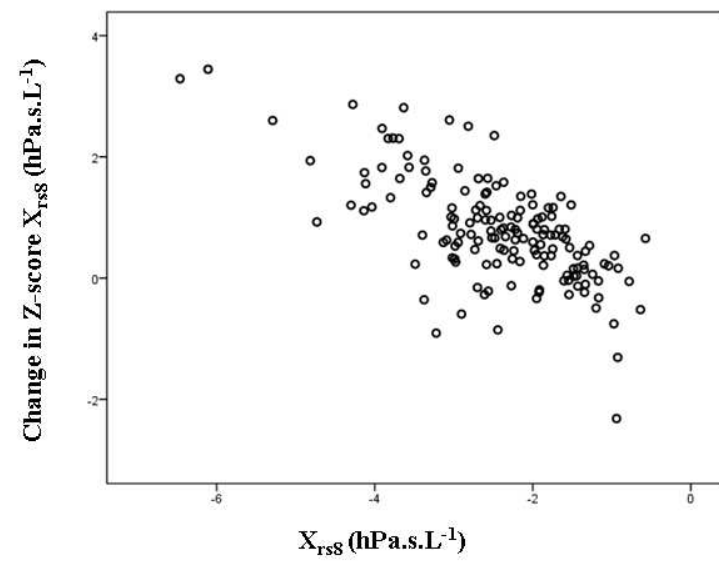
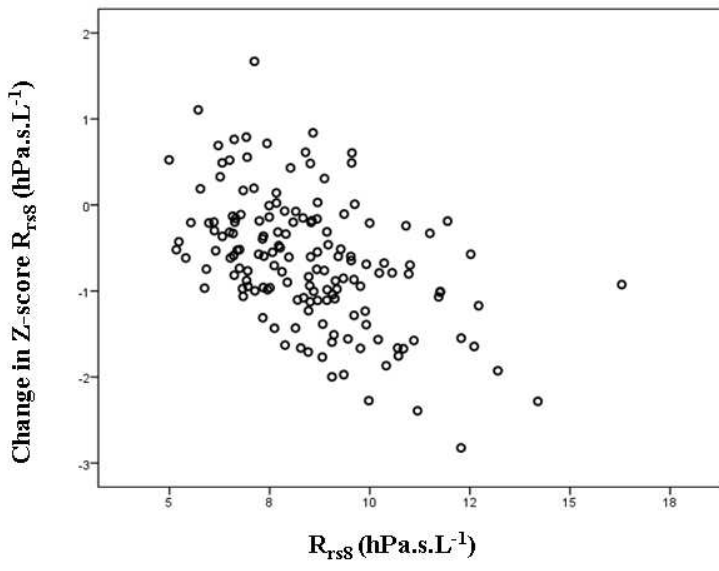
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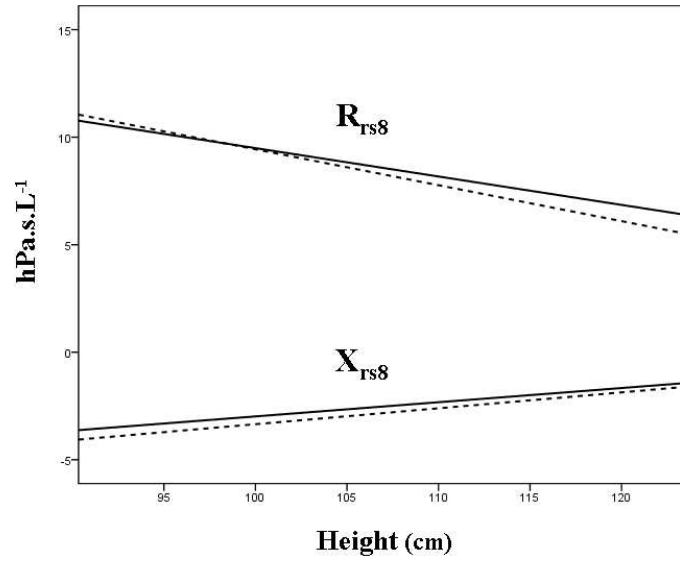
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