

Respiratory Impedance and Bronchodilator Responsiveness in Healthy Children Aged 2–13 Years

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Summary. Background: The forced oscillation technique (FOT) can be used in children as young as 2 years of age and in those unable to perform routine spirometry. There is limited information on changes in FOT outcomes in healthy children beyond the preschool years and the level of bronchodilator responsiveness (BDR) in healthy children. We aimed to create reference ranges for respiratory impedance outcomes collated from multiple centers. Outcomes included respiratory system resistance (R_{rs}) and reactance (X_{rs}), resonant frequency (F_{res}), frequency dependence of R_{rs} (F_{dep}), and the area under the reactance curve (AX). We also aimed to define the physiological effects of bronchodilators in a large population of healthy children using the FOT. Methods: Respiratory impedance was measured in 760 healthy children, aged 2–13 years, from Australia and Italy. Stepwise linear regression identified anthropometric predictors of transformed R_{rs} and X_{rs} at 6, 8, and 10 Hz, F_{res} , F_{dep} , and AX. Bronchodilator response (BDR) was assessed in 508 children after 200 μg of inhaled salbutamol. Results: Regression analysis showed that R_{rs} , X_{rs} , and AX outcomes were dependent on height and sex. The BDR cut-offs by absolute change in R_{rsB} , X_{rsB} , and AX were $-2.74 \text{ hPa s L}^{-1}$, $1.93 \text{ hPa s L}^{-1}$, and -33 hPa s L^{-1} , respectively. These corresponded to relative and Z-score changes of -32% ; -1.85 for R_{rsB} , 65% ; 1.95 for X_{rsB} , and -82% ; -2.04 for AX. Conclusions: We have established generalizable reference ranges for respiratory impedance and defined cut-offs for a positive bronchodilator response using the FOT in healthy children. **Pediatr Pulmonol.** 2013; 48:707–715. © 2012 Wiley Periodicals, Inc.

Key words: forced oscillation technique; lung function; children; reference values; bronchodilator.

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INTRODUCTION

The forced oscillation technique (FOT) enables the evaluation of the mechanical properties of the respiratory system and is particularly useful for determining lung function in young children who are unable to perform traditional assessments of lung function due to the high levels of cooperation required. Successful FOT measurements have been reported in children as young as 2 years.^{1,2} FOT outcomes (respiratory system resistance (R_{rs}) and reactance (X_{rs})) at varying frequencies have been reported to be altered in children with chronic lung disease of prematurity,^{3,4} cystic fibrosis,^{5,6} recurrent wheeze, and asthma.⁷⁻⁹ In addition, the FOT has been successfully utilized in investigations of airway hyper-responsiveness using both direct¹⁰⁻¹² and indirect^{13,14} challenge agents and in the assessment of bronchodilator responsiveness (BDR).^{8,15,16} More recently the area under the reactance curve (AX: the area under the X_{rs} -frequency curve below the resonant frequency) has been suggested to be sensitive to peripheral airway obstruction and thus may provide additional useful information about early lung disease.^{17,18}

In order for the FOT to be integrated into clinical practice as a reliable diagnostic test, appropriate reference ranges and well defined limits of BDR using commercially available equipment are required. To date reference ranges for the FOT have tended to be in relatively small populations derived from impulse oscillometry (IOS)^{2,19-21} with only two studies reporting reference ranges for young children at multiple frequencies generated using pseudo-random forcing signals between 4 and 48 Hz.^{1,22} Ideally, reference ranges should be established using a large number of healthy children however this is often difficult for a single center to achieve. Recently, several research groups have collated data from multiple centers to create unified reference ranges for spirometry,²³ gas transfer,²⁴ the interrupter technique,²⁵ and specific airway resistance.²⁶ In the present study we aimed to create robust generalizable reference ranges for a wide range of FOT outcomes including resistance and reactance at multiple frequencies as well as the area under the X_{rs} curve in healthy young children and adolescents using commercially available FOT equipment. In addition, we aimed to define the normal limits of BDR within the healthy population by all FOT outcomes.

MATERIALS AND METHODS

Study Participants

Healthy children were recruited for various research studies in both Perth, Western Australia and Viterbo, Italy. The Perth population performed FOT measurements in the lung function laboratory of the Respiratory

Medicine Department, Princess Margaret Hospital for Children, Perth as well as in primary schools as part of a field study. Children participating in the study from Viterbo performed measurements at local kindergartens and schools. A preschool aged subset of both the Perth and Viterbo populations have been previously published.^{1,22} All participants included into the analysis were Caucasian.

Respiratory symptoms were assessed using a modified respiratory questionnaire (ISAAC).²⁷ Participants born earlier than 36 completed weeks of gestational age, or who had received oxygen at birth, were excluded from the analysis. Additionally, children were excluded if they had ever been diagnosed with asthma, had more than three episodes of wheezing ever, or reported wheeze in the preceding 12 months. All children had been free of respiratory signs and symptoms for the 3 weeks preceding testing. Parentally reported history of eczema, hay fever, and environmental tobacco smoke (ETS) exposure were recorded. Participants in Perth were assessed for sensitization using skin prick allergen testing to seven allergens including: cow's milk, egg white, rye grass, mixed grass, cat hair, dog hair, house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), alternaria, and aspergillus. Histamine, 10 mg/ml concentration, was used as a positive control. A positive response to an allergen was defined as a weal equal to or greater than the positive control.

The study was approved by the Princess Margaret Hospital Human Ethics Committee in Perth, WA and by the Ethics Committee of the Local Health Authority in Viterbo, Italy. Written parental consent was obtained from parents and/or guardians for participation in the study.

Lung Function Assessment

Respiratory impedance was measured according to international recommendations^{28,29} using the same commercial device (I2M; Chess Medical, Gent, Belgium, marketed in Italy by Cosmed, Rome, Italy), which uses a pseudo-random noise forcing signal between 4 and 48 Hz. The device was verified daily using a known resistance. Each child performed the test sitting upright with their head in a neutral position and was connected to the oscillation device via a mouthpiece incorporating a bacterial filter (Suregard, BirdHealthcare, Melbourne, Australia or Cosmed, Rome, Italy), the impedance of which was subtracted from the child's measurement. Children were instructed to breathe normally while wearing a nose clip with the cheeks and floor of the mouth supported by an investigator. Measurements were excluded if leak, mouth or tongue movement, swallowing, glottal closure, talking or audible noise

occurred during the test. Respiratory impedance (Z_{rs}) measurements in which three or more individual frequencies had a coherence (between input and output signal) of <0.95 were excluded. Three to five acceptable measurements of Z_{rs} were obtained for each child. R_{rs} and X_{rs} were calculated at each frequency and the mean of the total acceptable measurements was reported. The frequency dependence of the R_{rs} between 4 and 24 Hz and the resonant frequency (the point at which X_{rs} is zero) were determined from the average Z_{rs} of all acceptable measurements. The area under the reactance curve (AX) was calculated using the trapezoidal rule to approximate the integral, or area, of n trapezoids between the averaged reactance at each frequency and zero for equally spaced 2 Hz frequencies from 6 Hz up to the resonant frequency (being the frequency at which the X_{rs} curve equals zero). The response to bronchodilator was assessed by repeating the measurements 15 min after 200 μg of salbutamol (Glaxo-SmithKline, Melbourne, Australia) administered via a valve holding chamber (Volumatic, GlaxoSmithKline, Australia and AeroChamber Plus, TrudellMedical International, London, ON, Canada).

Statistical Analysis

The relationships between the respiratory function variables (R_{rs6} , R_{rs8} , R_{rs10} , X_{rs6} , X_{rs8} , X_{rs10} , resonant frequency (Fres), frequency dependence 4–24 Hz (Fdep) and AX), and the anthropometric data (age, sex, height, and weight) were examined using backward stepwise linear regression. Transformation was necessary for all respiratory function variables except Fres and Fdep to achieve approximate normality. For R_{rs} at 6, 8, and 10 Hz, the natural logarithm was used in order to achieve normality while for X_{rs} at 6, 8, and 10 Hz the square root ($|X_{rs} - 10|$) was used. Prediction models were determined for transformed R_{rs} , X_{rs} , and AX (square root) and are presented with the standard error of the estimate (SEE) to enable Z-score calculation ($Z\text{-score} = (\text{measured value} - \text{predicted value})/\text{SEE}$). The reference ranges for Fdep were calculated within two height categories, <140 cm and ≥ 140 cm, using

the 2.5th and 97.5th centile of the data. Absolute, relative and Z-score changes in R_{rs} , X_{rs} , and AX were calculated and the upper limit of normal defined from the 5th/95th centiles of the healthy population.

The possible effects of center, environmental tobacco smoke (ETS) exposure and atopy were assessed using t -tests after calculation of Z-scores for the FOT variables. Statistical analyses were performed using SPSS for Windows, Version 19.0 (Chicago, IL). Statistical significance was considered at $P < 0.05$.

RESULTS

Baseline FOT Outcomes

Impedance spectra were obtained in 760 healthy children (335 male) aged 2–13 years, and with height ranging between 90 and 160 cm. Demographic characteristics of the children are shown in Table 1. The area under the reactance curve (AX) was calculated in 671 of these children, with 89 children excluded from the analysis due to unacceptable X_{rs6} measurements ($N = 4$) or Fres above 48 Hz ($N = 85$).

The relationship between height and baseline R_{rs8} , X_{rs8} , and AX is presented in Figure 1. Stepwise regression analysis indicated that all transformed R_{rs} , X_{rs} outcomes were associated with height and sex, with girls demonstrating larger respiratory system impedance than boys. Fres was significantly associated with height, but not sex, while Fdep was independent of all demographics. The inclusion of weight or age into the models (if found to be significant) only added an additional 1–2% to the explanation of variance after accounting for height and sex, and were therefore excluded from the predictive models. The standard error of the regression models was also not altered after the exclusion of weight or age from the models. Prediction equations for transformed X_{rs} , R_{rs} , and AX, as well as Fres, are provided in Table 2. The upper and lower limits of normal for frequency dependence were 0.04 and -0.27 hPa $\text{s}^2 \text{L}^{-1}$ for children less than 140 cm and 0.02 and -0.24 hPa $\text{s}^2 \text{L}^{-1}$ for children taller than 140 cm.

TABLE 1—Characteristics of the Study Population

| | Perth (n = 547) | Viterbo (n = 213) | Total (n = 760) |
|-------------|----------------------------|----------------------------|----------------------------|
| Age (years) | 7.7 (2.5) 2.7–12.9 | 5.7 (2.2) 2.9–12.5 | 7.2 (2.6) 2.7–12.9 |
| Male (n, %) | 233 (42.5) | 102 (47.9) | 335 (44) |
| Height (cm) | 126.7 (15.7) 94.0–159.0 | 113.5 (13.7) 92.3–158.4 | 123.1 (16.3) 92.3–159.0 |
| Weight (kg) | 29.0 (11.0) 13.0–68.0 | 22.9 (8.2) 14.0–58.0 | 27.3 (10.7) 13.0–68.0 |

Data are expressed as mean (SD) and range for age, height and weight and as n (%) for sex.

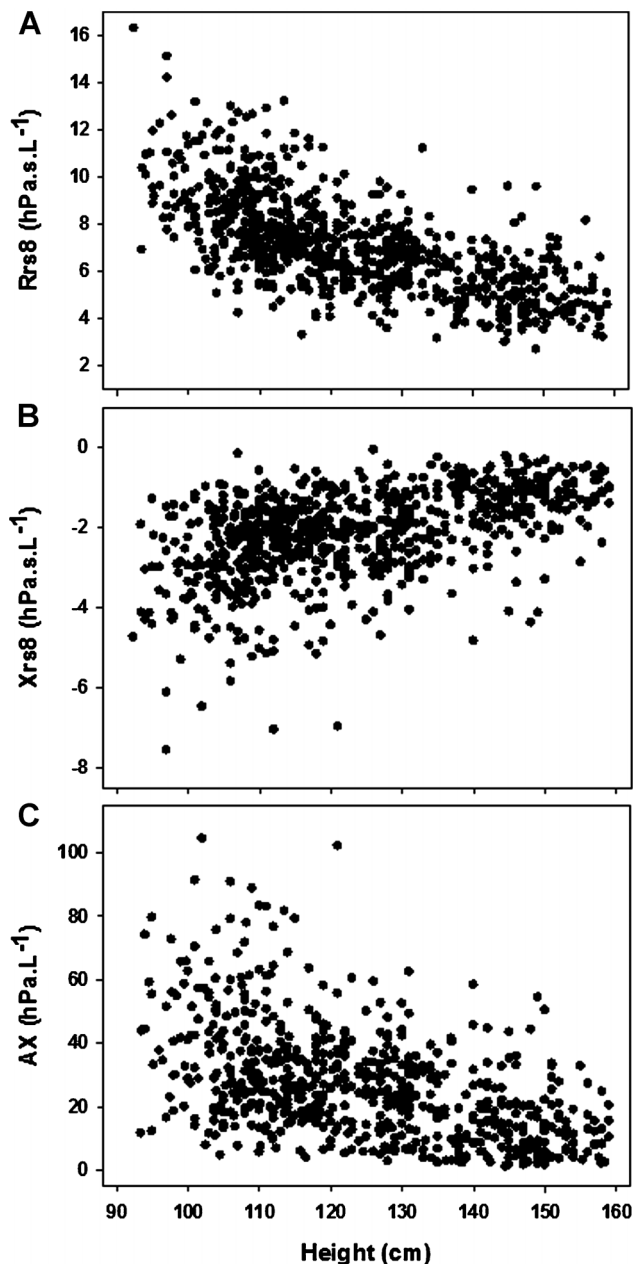


Fig. 1. Baseline respiratory resistance at 8 Hz (R_{rs8}) (A), reactance at 8 Hz (X_{rs8}) (B), and area under the reactance curve (AX) between 6 Hz and the resonant frequency (C) against height in healthy children.

Bronchodilator Response

The BDR was assessed in 508 children. The absolute magnitude of R_{rs} , X_{rs} , AX, and F_{res} significantly decreased after administration of salbutamol ($P < 0.001$ for all FOT outcomes). The 5th percentiles for absolute change in R_{rs8} and AX were $-2.74 \text{ hPa s L}^{-1}$ and -33 hPa s L^{-1} respectively, while the 95th percentile for absolute change in X_{rs8} postbronchodilation was $1.94 \text{ hPa s L}^{-1}$. These cut off values corresponded to

relative and Z-score changes of -32% and -1.85 for R_{rs8} , -82% and -2.04 for AX, and 65% and 1.93 for X_{rs8} , as presented in Table 3. Changes by all three indices of BDR were related to baseline lung function for R_{rs8} , X_{rs8} , and AX, though only weakly by relative change with standardized coefficients of -0.555 , -0.385 , and -0.313 , respectively (see Fig. 2).

Effects of Center, ETS Exposure, and Atopy on Baseline Lung Function and BDR

Center differences were detected in all baseline FOT variables, except R_{rs10} , with the mean Z-score difference between centers ranging from 0.1 for X_{rs6} (t -test; $P = 0.035$) to 0.6 for AX ($P < 0.001$) (Italian children -0.4 AX Z-scores from 0). In general, the Italian children had lower absolute magnitudes of R_{rs} , X_{rs} , and AX when compared to the children from Australia, with the Italian children displaying an R_{rs8} Z-score 0.2 ($P = 0.002$) and X_{rs8} Z-score 0.4 ($P < 0.001$) lower than the Australian children.

In this study, children who had been exposed to environmental tobacco smoke ($N = 250$) did not have altered baseline lung function by any of the FOT variables ($P > 0.1$). Similarly, the response to bronchodilator, was not altered in the ETS exposure group ($N = 200$; $P > 0.1$) when compared to children who are not exposed to tobacco smoke in the home. In addition, height, and sex ($P > 0.05$) were not significantly associated with the relative magnitude of the bronchodilator response (BDR).

We examined atopy in two different ways with 396 children undergoing skin prick testing (SPT) and 629 completing a questionnaire to determine atopic status (current eczema and/or hay fever). Of the children who underwent SPT, 272 children participated in the BDR aspect of the study, as did 446 of the children who completed the questionnaire, with some overlap between the two groups. There was no difference in baseline lung function by any of the FOT variables in children who had a positive SPT ($N = 147$; $P > 0.1$). However, children with a parentally reported history of atopy had a significantly lower magnitude of X_{rs} at 8 Hz (Z-score difference 0.21; $P = 0.01$) and 10 Hz (0.30; $P = 0.007$), but no differences in AX or any of the R_{rs} outcomes. The response to bronchodilator was not different in children with a parentally reported history of atopy ($P > 0.1$). Children with a positive SPT had a larger response to bronchodilator by X_{rs8} (relative difference 10%; $P = 0.03$) but not by any other FOT outcome.

DISCUSSION

This study is the first to report reference ranges and BDR for FOT outcomes, derived from a large multi-

TABLE 2—Regression Data for Transformed (T) Respiratory Impedance Variables in Healthy Children

| Transformed (T) variable | Covariates | Estimate of regression coefficients | P-value | Adjusted R-squared | SEE |
|----------------------------------|------------|-------------------------------------|---------|--------------------|-------|
| Fres (N = 675) | Sex | — | — | 0.248 | 5.147 |
| | Height | −0.17763 | <0.001 | | |
| | Constant | 45.68724 | <0.001 | | |
| T (R _{rs6}) (N = 756) | Sex | −0.04157 | 0.011 | 0.417 | 0.223 |
| | Height | −0.01155 | <0.001 | | |
| | Constant | 3.37377 | <0.001 | | |
| T (R _{rs8}) (N = 760) | Sex | −0.03942 | 0.011 | 0.470 | 0.213 |
| | Height | −0.01228 | <0.001 | | |
| | Constant | 3.44422 | <0.001 | | |
| T (R _{rs10}) (N = 760) | Sex | −0.03211 | 0.030 | 0.503 | 0.202 |
| | Height | −0.01248 | <0.001 | | |
| | Constant | 3.40885 | <0.001 | | |
| T (X _{rs6}) (N = 756) | Sex | −0.03134 | 0.002 | 0.294 | 0.137 |
| | Height | −0.00537 | <0.001 | | |
| | Constant | 4.23244 | <0.001 | | |
| T (X _{rs8}) (N = 760) | Sex | −0.03243 | 0.001 | 0.248 | 0.133 |
| | Height | −0.00463 | <0.001 | | |
| | Constant | 4.06745 | <0.001 | | |
| T (X _{rs10}) (N = 760) | Sex | −0.02565 | 0.011 | 0.257 | 0.138 |
| | Height | −0.00494 | <0.001 | | |
| | Constant | 4.08552 | <0.001 | | |
| T (AX) (N = 671) | Sex | −0.32673 | 0.004 | 0.292 | 1.432 |
| | Height | −0.05518 | <0.001 | | |
| | Constant | 11.90851 | <0.001 | | |

Transformations are natural logarithm for respiratory system resistance (R_{rs}) at 6, 8, and 10 Hz, square root (absolute (reactance − 10)) for respiratory system reactance (X_{rs}) at 6, 8, and 10 Hz and square root for the area under the reactance curve (AX). Units for impedance measures are hPa s L^{−1}, except for AX (hPa L^{−1}). Height in centimetres. Sex: 0 = female, 1 = male.

center population of healthy Australian and Italian children. We provide relationships between anthropometric factors and respiratory impedance variables, creating a robust data set for Caucasian children. We also quantified the response to bronchodilator in healthy children by FOT variables including AX, the integrated sum of X_{rs}.

FOT provides a simple measure of respiratory mechanics, possible across a large age range. The extended reference equations provided herein are intended for use in young children who routinely perform FOT, however, may also be of value in examining the progression of lung function changes in health and disease beyond the years that FOT is traditionally performed. Follow up studies from preschool to adolescence may provide

a meaningful longitudinal assessment, particularly in children with cystic fibrosis, asthma, chronic lung disease of prematurity or neuromuscular disease. With this in mind, we initially included children above 160 cm and 13 years in the analysis. However, there were insufficient numbers of participants above these cut-offs (n = 66) to describe the complex changes in respiratory impedance that occur through puberty and therefore the reference equation presented here are limited to children <13 years and <160 cm.

We report that all R_{rs} and X_{rs} outcomes are dependent on height and sex, with girls having higher respiratory system impedance than boys. Previous studies in adult subjects have also reported higher R_{rs} values for females compared to males,³⁰ however, the majority of

TABLE 3—Values for a Significant Bronchodilator Response by Absolute Change, Relative Change and Change in Z-Score for Area Under the Reactance Curve (AX), Resonant Frequency (Fres), Resistance at 6 (R_{rs6}), 8 (R_{rs8}) and 10 (R_{rs10}) Hz (5th Percentiles) and Reactance at 6 (X_{rs6}), 8 (X_{rs8}), and 10 (X_{rs10}) Hz (95th percentile)

| | AX | Fres | R _{rs6} | R _{rs8} | R _{rs10} | X _{rs6} | X _{rs8} | X _{rs10} |
|---|-------|-------|------------------|------------------|-------------------|------------------|------------------|-------------------|
| Absolute change (hPa s L ^{−1}) ¹ | −33 | −12 | −2.91 | −2.74 | −2.39 | 1.80 | 1.93 | 1.90 |
| Relative change (% of baseline) | −81 | −47 | −34 | −32 | −31 | 50 | 65 | 74 |
| Z-score change | −2.04 | −2.35 | −1.85 | −1.83 | −1.85 | 1.86 | 1.95 | 1.92 |

¹Units for Absolute Change are hPa s L^{−1}, Except for AX (hPa L^{−1}).

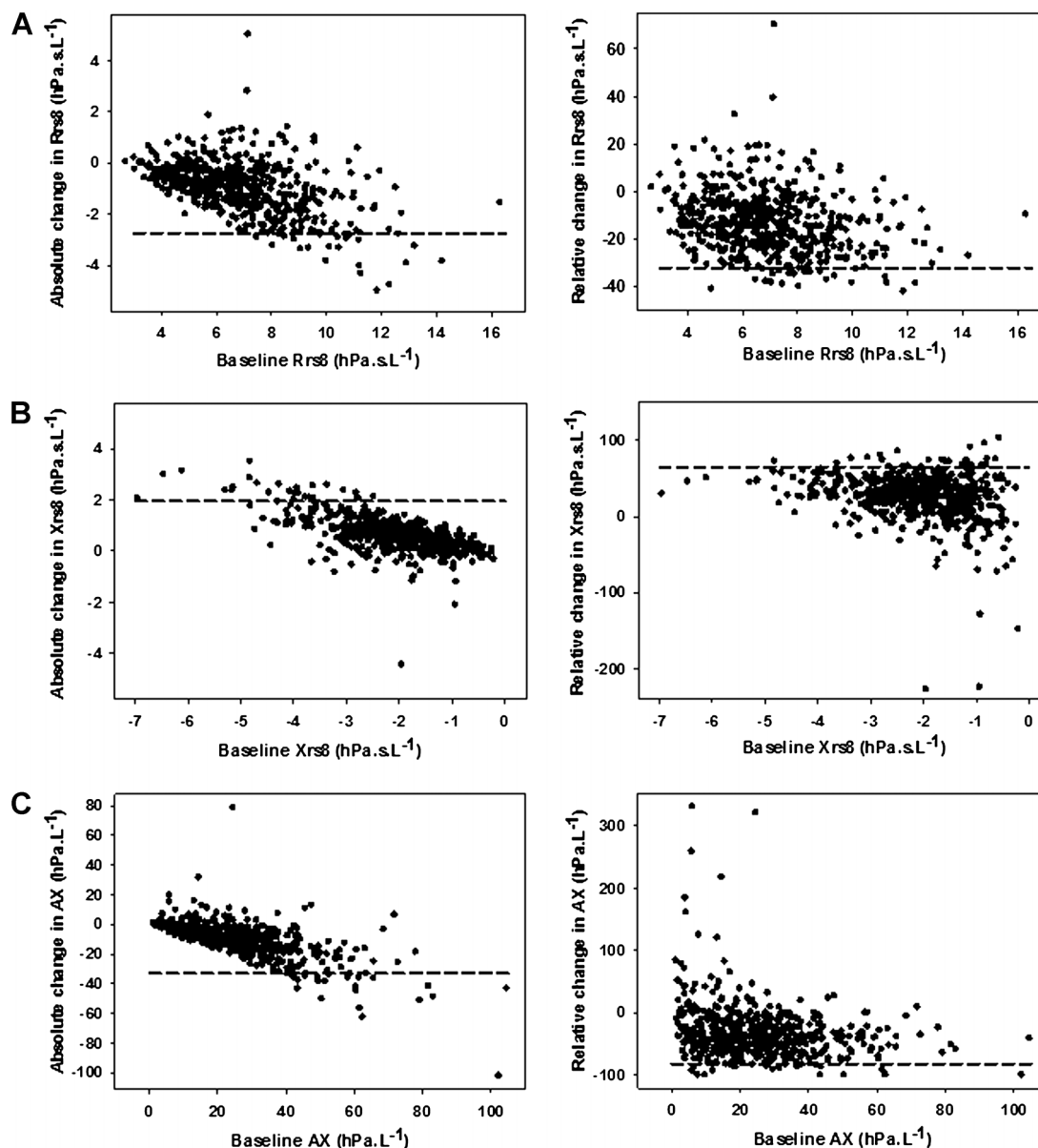


Fig. 2. Bronchodilator response (BDR) as a function of baseline values. BDR is expressed as absolute change (left) or percent of baseline (right) for respiratory system resistance (R_{rs8}) (A) and reactance (X_{rs8}) at 8 Hz (B), and area under the reactance curve (AX) (C). Dashed lines indicate the cut of value for a significant bronchodilator response.

studies in children have published regression equations with height as the only significant predictor of FOT outcomes.^{1,20,31,32} These sex dependent differences are likely the result of comparatively smaller lung volumes³³ and narrower upper airways in females than males.³⁴ Some recent studies with larger sample sizes (360–621 children) have included additional associations for R_{rs} and X_{rs} with sex,^{19,21} age,¹⁹ and weight.² Differences in prediction variables and values obtained from prediction equations (see Fig. 3) likely arise from

differences in sample size, ethnicity, height distribution, input signal, data normalization, and the frequency reported.

In addition to reference equations for R_{rs} and X_{rs} , we also determined reference values for AX, using data from 671 healthy children. Measures of AX could not be obtained in 89 children, generally the younger children and predominantly a consequence of F_{res} being greater than 48 Hz, thus limiting its utility in this age group. In addition, some caution should be applied

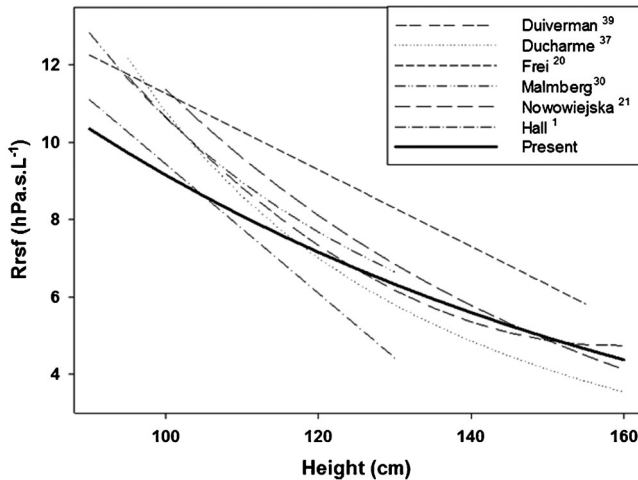


Fig. 3. Respiratory resistance reference equation compared with previous studies in healthy children.

when interpreting AX in very young children due to the increased effects of the shunt properties of the upper airways at higher oscillation frequencies,³⁵ even in the presence of good cheek support. Goldman¹⁷ proposed AX, an index of respiratory system elastance that is sensitive to changes in the degree of airflow obstruction,³⁶ to improve the X_{rs} signal–noise ratio at the lower frequencies. To our knowledge, only two other studies have provided reference equations for AX, with AX predicted solely by height in small studies of 177 Canadian children²⁰ aged 3–10 years and 119 Korean children aged 3–6 years.³² These studies obtained AX data using impulse oscillation systems and to date there are no reports of reference ranges for AX obtained using commercially available equipment that utilizes a pseudorandom forcing signal. The ability of AX to predict peripheral airway disease is not clear at this time and the development of reference ranges for AX will allow further investigation into the clinical utility of this FOT outcome.

Previous studies have shown that the FOT is able to identify BDR in young children (reviewed in Oostveen²⁸). However, from a clinical viewpoint, the criteria for a positive BDR by FOT outcomes must take into account the normal physiological response to salbutamol in healthy children. The average response to bronchodilator in this healthy population was defined as a decrease in the absolute magnitude of: R_{rs8} by 12.9%; X_{rs8} by 24.9%; and AX by 31.9%. These values for R_{rs} are in keeping with other studies using similar amounts of salbutamol,^{37,38} while Thamrin et al.,¹⁶ Malmberg et al.,³¹ and Oostveen et al.⁸ reported larger average response to bronchodilator (19–22%). Despite this, the relative cut-off values derived from this study (–32% for R_{rs8}) were similar to those previously reported for

R_{rs} (–28% to –42%),^{7,15,32,37,38} regardless of the salbutamol dose (200–600 μg). The similarities in BDR despite salbutamol dose indicate that 200 μg is likely sufficient for a maximum response in healthy children.

The use of relative change in FOT variable after bronchodilator has recently received some attention with our research group advocating its use,³⁹ while others advocate the use of absolute change.⁸ Oostveen et al.⁸ argued that the expression of change relative to baseline blunts the differences in bronchomotor tone that underlie respiratory diseases like asthma. In contrast, Thamrin et al.³⁹ reasoned that the absolute response is generally dependent on baseline lung function, a result of airway size rather than bronchomotor tone. In this study, we present absolute, relative and Z-score change in lung function after inhaled salbutamol. Change expressed by any of these indices is dependent on baseline lung function, though least strongly with relative change. The persistence of a negative relationship between relative change and baseline lung function may well extend the observation that airway sensitivity and reactivity decreases with age from infancy to adolescence,^{40,41} and is perhaps consistent with the clinical observation of decreased wheezy illness with increasing age. While expressing BDR as relative change may lead to a decrease in the number of asthmatic responders, using absolute change will lead to an overestimation of responders in children who have a higher baseline resistance since even healthy children with higher baseline resistances have more improvement after bronchodilator. Further research including both healthy and asthmatic children is required to explore which expression of BDR is most capable of discriminating between healthy and asthmatic children and how the BDR evolves through development.

Quantifying the BDR by AX may magnify the clinical signal as changes in AX will include differences in individual X_{rs} frequencies, the resonant frequency and the shape of the X_{rs} -frequency curve. Oostveen et al.⁸ reported that baseline AX discriminated between children with different wheezing phenotypes better than X_{rs} at a single frequency and that decreases in AX following bronchodilator inhalation were larger in children who had wheezed than those who had never wheezed. Therefore, with the caveats around interpretation in young children, AX may provide further clinical knowledge in the detection of reversible airways disease.

Our study combined data from two centers (Western Australia and Italy), with both centers collecting some data in the pulmonary function laboratory and the remainder in the field. Both centers followed ATS/ERS guidelines^{28,29} and used same commercial equipment in the collection of this data, though the data was collected using different versions of acquisition software in each center. One advantage of having data collected at

multiple centers is the creation of reference ranges that are more robust and generalizable, however, this also has the potential to increase the population variance and therefore widen the limits of normal. When examining the preschool population alone, Calogero et al.²² reported no difference between the Australian and Italian children. However, in the current extended study, the Italian children had lower absolute magnitude of resistance, reactance, and AX when compared to their Australian counterparts after accounting for anthropometric factors. However, it should be noted that there were fewer older Italian children, above 120 cm, which may contribute to the explanation of center differences. Although statistically significant, differences of 0.2 Z-scores, as is the case for R_{rs8} , are unlikely to be physiologically relevant, with previous multicenter studies (using spirometry) arbitrarily considering a Z-score difference of >0.5 to be clinically significant.⁴² AX was the only outcome to demonstrate a center difference greater than 0.5 Z-scores, with differences likely magnified since any systematic difference at individual frequencies will be additive in the calculation of AX. Clinical interpretation of FOT results is unlikely to be affected by the possible decrease in the sensitivity of these equations, particularly if emphasis is placed on how a patient's lung function tracks with time, rather than simply whether a result falls outside of the normal range.

We have shown that children who were exposed to tobacco smoke in the house, or had a personal history of atopy, did not have altered baseline lung function by any of the FOT outcomes. Increased BDR was observed by ΔX_{rs8} in children with a positive SPT, but was not different by any other FOT outcome. Other studies have either carefully selected children with no history of ETS exposure or atopy for reference groups,^{20,32,43} or have shown no influence of tobacco smoke exposure or atopy on baseline FOT variables. We did not have information on prenatal exposure to tobacco smoke, or whether this exposure was current and therefore, it is likely that a broad range of ETS exposures is observed within this group.

In summary, we have established international reference equations for respiratory resistance, reactance, and area under the reactance curve in a large group of healthy children aged 2–13 years using the FOT. In addition, the physiological response to bronchodilator by respiratory impedance outcomes was defined in this healthy population. These data, obtained using commercially available equipment, provide a comprehensive reference for the use of oscillatory mechanics in the clinical setting. While a multi-center study design enables the recruitment of a large reference population to identify determinants of airway growth and reversibility, we encourage validation of reference values

particularly in light of the center differences observed in this study.

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