Lung function and clinical risk factors for asthma in infants and young children with recurrent wheeze

Luis Miguel Borrego¹, MD; Janet Stocks², PhD; Paula Leiria-Pinto¹, MD; Isabel Peralta¹; Ana Margarida Romeira¹, MD; Nuno Neuparth¹, MD, PhD; José E Rosado-Pinto¹, MD; Ah-Fong Hoo², PhD

¹ Serviço de Imunoalergologia, Centro Hospitalar Lisboa Central -Hospital de Dona Estefania, Rua Jacinta Marto, 1169-045 Lisboa, Portugal
² Portex Respiratory Unit, UCL Institute of Child Health & Great Ormond Street Hospital NHS Trust, 30 Guilford Street, London WC1N 1EH, UK

Corresponding author:
Dr Luis Miguel Borrego.
Serviço de Imunoalergologia, Centro Hospitalar Lisboa Central -Hospital de Dona Estefania, Rua Jacinta Marto, 1169-045 Lisboa, Portugal
email: miguel.borrego@sapo.pt
Tel: 00 351 213 126 600
Fax: 00 351 213 126 654

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Running title: Lung function in recurrently wheezing young children
ABSTRACT

**Background:** Although several risk factors for asthma have been identified in infants and young children with recurrent wheeze, the relevance of assessing lung function in this group remains unclear. We assessed whether lung function was reduced during the first 2 years in recurrently wheezy children, with and without clinical risk factors for developing subsequent asthma (i.e., parental asthma, personal history of allergic rhinitis, wheezing without colds and/or eosinophil level >4%), when compared with healthy controls.

**Methods:** Forced expiratory flows and volumes in steroid naïve young children with ≥ 3 episodes of physician-confirmed wheeze and healthy controls, aged 8-20 months, were measured using the tidal and raised-volume rapid thoraco-abdominal compression manoeuvres.

**Results:** Technically acceptable results were obtained in 50 wheezy children and 30 controls using tidal RTC, and 44 wheezy children and 29 controls with the raised-volume technique. After adjustment for sex, age, body length at test and maternal smoking, significant reductions in Z-scores for FEV$_{0.5}$ (mean difference [95% CI]: -1.0 [-1.5; -0.5]), FEF$_{75}$ (-0.6 [-1.0; -0.2]) and FEF$_{25-75}$ (-0.8 [-1.2; -0.4]) were observed in those with recurrent wheeze when compared with controls. Wheezy children with risk factors for asthma (n=15) had significantly lower Z-scores for FVC (-0.7 [-1.4; -0.04]) and FEF$_{25-75}$ (-0.6 [-1.2; -0.1]) than those without such risk factors (n=29).

**Conclusions:** When compared to healthy controls, airway function is reduced in young children with recurrent wheeze, particularly those at risk for subsequent asthma. These findings provide further evidence for associations between clinical risk factors and impaired respiratory function in early life.
Introduction
Recurrent wheeze is a common symptom during infancy and early childhood.[1,2] Although the majority of children will outgrow their symptoms, some go on to develop asthma.[3,4] Early onset of asthma has been associated with persistence of symptoms and reduced lung function that continues into adulthood.[5-7] A recently described clinical index considers young children with recurrent wheezing in the first 3 years of life to be at high risk of developing asthma if there is parental history of asthma or personal history of eczema, or if two of the following are present: personal history of allergic rhinitis, wheezing without a cold and/or serum eosinophil level >4%.[8] Other predictive indices additionally take immunological measurements [1] and clinical parameters into account.[9] but these indices cannot be easily applied to daily practice.

Besides clinical risk factors, lung function evaluation may contribute to the assessment of wheezing phenotypes during early life. A reduction in pre-morbid lung function has been associated with increased risk of wheezing in the first years.[3,10-12] Tracking of lung function (whereby those with lower lung function initially continue to do so thereafter) has been well documented during infancy,[5,13,14] childhood[15,16] and adulthood[5,7,17]. However, the association between wheezing phenotypes and early airway function remains unclear. The Tucson study reported a link between reduced maximal forced expiratory flow at functional residual capacity ($V'_{maxFRC}$) from the tidal rapid thoraco-abdominal compression (RTC) technique, in the first weeks of life prior to onset of respiratory symptoms, and transient wheeze but not with persistent wheeze.[2] Similarly, data from Wilson et al found no association between wheezing beyond 4y and reduced $V'_{maxFRC}$ at 4w of age.[18] By contrast, the Perth group reported that persistent wheeze/asthma at 11y was associated with reduced pre-morbid $V'_{maxFRC}$ at 1m.[16] A further study observed that children with transient wheeze had lower $V'_{maxFRC}$ at 17m compared to those with persistent wheeze.[19] Others have suggested that asymptomatic children with evidence of flow-limitation during tidal breathing in the first days of life are likely to have a higher risk of subsequent asthma.[20]

In recent years, several investigators have reported that, compared to tidal RTC manoeuvres, the raised-volume RTC technique is more sensitive in distinguishing lung function between infants with and without respiratory disease.[21-24] The raised-volume technique has been used in children with [24,25] and without recurrent wheezing [26] but not, to our knowledge, to compare lung function in wheezy young children according to risk of developing asthma.

This study aimed to assess whether lung function was reduced during the first 2y of life in recurrently wheezy children with a high risk of developing subsequent asthma[8], when compared with age matched “low-risk” wheezy children and healthy controls.

Methods

Subjects
Infants and young children, aged between 8-20m, with recurrent wheeze (≥3 episodes of medically-diagnosed wheeze) but prior to receiving any inhaled corticosteroid or anti-leukotriene agents, were recruited from the out-patient clinic in Hospital Dona Estefania, Lisbon (September 2005 to September 2007). Children requiring hospitalisation for exacerbations were ineligible for study. Children were stratified into high and low-risk for developing asthma.[8]

Age-matched healthy children without prior history of lower respiratory illness, wheeze or allergic disorders (food allergy, eczema) or parental asthma, were recruited from those attending routine developmental screening appointments. Control children meeting the inclusion criteria, and who had been recruited to epidemiological studies at the UCL Institute of Child Health, London, UK,[14] were also included in the study population.

All children were born ≥37 gestational weeks with birthweight >10th percentile, and any with cardiac, metabolic, neurological or gastrointestinal diseases, and upper airway pathology were excluded.

The Ethics Committees at the Lisbon and London institutions approved the study. Parents of participating children gave informed written consent and were present during measurements.
Respiratory function tests
At time of assessments, infants were free of respiratory symptoms or illness for at least 3w. Weight and crown-heel length were measured using digital scales and a calibrated stadiometer, and values expressed as Z-scores.[27] Lung function measurements were undertaken in supine position during quiet sleep following oral administration of chloral hydrate (60–75mg/kg). Prior to sedation, the child’s heart-rate and oxygen saturation level were recorded, and continuous monitoring maintained throughout the test period. Identical equipment (VIASYS Healthcare Masterscreen BabyBody, version 4.6, Hochberg, Germany) and standardised techniques, which adhered to international guidelines,[28,29] were used in both laboratories. The London team provided training for the Lisbon group and on-going supervision throughout the study through inter-laboratory visits; all datasets being cross-analysed anonymously. A lung inflation pressure of 30 cmH₂O was used during the raised-volume test.[28] At least 2 technically satisfactory and reproducible (within 10%) partial and raised-volume forced expiratory flow-volume (FEFV) curves were collected.[28,29] Detailed descriptions of both techniques, including quality control criteria, are available in the online supplement. Results were reported as Z-scores[30,31], values being considered abnormal if below -1.96 Z-scores. A questionnaire was completed with parents to document smoking habits, family history of allergic illness, and their child’s respiratory symptoms or illnesses since birth. Peripheral blood samples for eosinophil levels were obtained in those with recurrent wheeze when seen at outpatient clinics.[8]

Sample size and statistical analysis
Power calculations indicated that 35 children per group would provide 80% certainty of detecting differences of 0.67 Z-scores at the 5% significance level for the selected outcome variables between wheezy infants and controls. For subgroup analysis according to wheezing phenotype, 20/group would provide 80% power to detect differences of 0.9 Z-score.[32] Comparisons of group characteristics and lung function between study groups were performed using independent sample t tests with 95% confidence intervals (CI) and chi-square tests. The extent to which recurrent wheeze is associated with lung function was also examined using multiple linear regression (MLR) (SPSS for Windows, v.15, Chicago, Illinois) after adjustment for sex, age, body weight and length, and effects of potential confounding factors, such as maternal smoking.

Results
Parents of 82/94 (87%) eligible Lisbon wheezy children gave consent for lung function measurements and of these, 55/82 (67%) attended for tests (Figure 1). Technically satisfactory $V'_{\text{maxFRC}}$ flow-volume curves were obtained in 50 wheezy infants, whereas 44 had acceptable raised-volume FEFV curves. By contrast, among the eligible Lisbon healthy children, 21/59 (36%) families gave consent and of these, 14 (24%) attended for tests. Technically acceptable data were obtained in 12 healthy Lisbon children for $V'_{\text{maxFRC}}$ and 11 for the raised-volume technique. Technically satisfactory data using both partial and raised-volume manoeuvres were collected in 18 healthy children in London.

At birth, there was no significant difference in gestational age, weight or prevalence of smoking during pregnancy between the Lisbon and London controls, nor were there any significant differences in either age or body size at time of tests or lung function results (Table E1, online supplement). Consequently, data-sets from the two healthy sub-groups were combined for comparison with wheezy children.

Comparison of wheezy and healthy groups
Gestational age of the wheezy group was 0.6w lower than that of controls (p<0.02) but there were no significant differences in sex, birth-weight or maternal smoking during pregnancy (Table 1). At time of tests, wheezy children were somewhat older, heavier and longer than the healthy controls, primarily due to deferment of testing following upper respiratory infections in the
wheezy infants. After expressing weight and length as Z-scores to adjust for age and sex, the wheezy group remained significantly heavier than controls, with no difference in length (Table 1). There was a non-significant tendency for weight gain since birth to be greater among wheezy children (mean [95% CI] change in weight Z-score: 0.4 [-0.1; 1.0]).

Z-scores for FVC, FEV$_{0.5}$, FEF$_{75}$ and FEF$_{25-75}$ were all significantly lower in wheezy than in healthy children (Figure 2) but no differences were observed in $V'_{\text{maxFRC}}$ Z-score, respiratory rate, tidal volume or tPTEF:tE between groups (Table 1).

Associations between lung function and wheezing phenotype were also examined after adjusting for factors that influenced lung function on univariable analysis (sex, birth weight Z-score, age and test length and smoking during pregnancy). After adjustment for these co-variants, significant reductions [mean (95% CI), wheeze-controls] were observed for FVC: -42 (-78; -6) mL; FEV$_{0.5}$ -30 (-54; -6) mL; FEF$_{75}$: -42 (-75; -10) and FEF$_{25-75}$: -70 (-117; -23) mL·s$^{-1}$. Addition of change in weight Z-score since birth had minimal effect on these relationships.

After adjustment for other factors, FVC was 57 (21; 92) mL higher in boys than girls and 26 (5; 48) mL higher per unit increment in birth weight Z-score. Maternal smoking was not significantly associated with any outcome on MLR. There was no association between $V'_{\text{maxFRC}}$ and wheeze on MLR analysis.

**Wheezing sub-groups**

Among the 17 infants at high risk for subsequent asthma[8] (Table 1), 13 (76%) had a history of parental asthma, 5 (29%) a personal history of rhinitis, 12 (71%) had wheezed without colds and 9 (53%) had serum eosinophil levels >4%. When dichotomised into wheezy subgroups according to these risk factors, there were no significant differences in background characteristics between the 3 groups, apart from controls being more slightly more mature at birth (Table 1). After adjusting for age and sex, both wheezing subgroups were significantly heavier than controls at time of test (mean [95% CI] difference in weight Z-score, low-risk wheezers – controls: 0.6 [0.04; 1.1]; high-risk wheezers – controls: 1.0 [0.3; 1.8]).

FEV$_{0.5}$, FEF$_{75}$ and FEF$_{25-75}$ Z-scores were all significantly lower in both wheezing subgroups compared to healthy controls; FVC Z-score also being lower in the high, but not the low-risk group (Table 2, Figure 2). When compared with low-risk wheezers, FVC and FEF$_{25-75}$ were significantly reduced in high-risk young children (Table 2, Figure 2). A significant difference in $V'_{\text{maxFRC}}$ Z-score was only observed between the high-risk wheezers and healthy controls. No significant differences were seen in respiratory rate, tidal volume or tPTEF:tE between the 3 groups (Table 2).

The associations between lung function and wheezing phenotype were also examined using MLR after adjusting for factors found to be significant on univariable analysis (sex, birth weight Z-score, age and length at test, and maternal smoking). After adjustment for these co-variants, FVC was significantly reduced in the high versus low-risk group by -45 (-89; -2) mL whereas, despite a trend towards lower values among high-risk children, there were no significant differences in FEV$_{0.5}$ (-25 [95% CI: -56; 7]) mL or FEF$_{25-75}$ (-47 [-115; 20] mL·s$^{-1}$). Among the wheezy children, FEF$_{25-75}$ was significantly lower [-78 [-149; -8] mL·s$^{-1}$] in those whose mothers had smoked during pregnancy.

**Discussion**

Findings from our study demonstrated that, after adjustment for sex, age, length and maternal smoking, forced flows and volumes from the raised-volume technique, but not $V'_{\text{maxFRC}}$ or any of the tidal breathing variables, were significantly reduced during the first 2 years of life in young children with recurrent physician-confirmed wheeze when compared with prospective healthy controls. Amongst recurrently wheezy infants, those with a positive clinical index for asthma had significantly reduced FVC and FEF$_{25-75}$ than those without such risk factors.
**Strengths and limitations**

Interpretation of these findings and their clinical relevance depends on factors such as accuracy of measurements, lack of bias and the extent to which results can be generalised, as discussed below.

**Population**

Our sample included 59% of eligible wheezy young children who presented at clinic during a 2-year recruitment period. In an attempt to study a relatively homogenous group and collect essential baseline data prior to long-term interventions, young children with recurrent wheeze were only eligible if they had not yet received inhaled corticosteroids or anti-leukotriene therapy, nor been hospitalised during exacerbations. Similarly, since our research question specifically related to risk factors for wheezing, healthy controls were only eligible if there was no prior history of lower respiratory illness, wheeze, allergic disorders or parental asthma. Children born prematurely or small-for-gestational age were excluded from index and control groups. While these stringent criteria inevitably excluded a number of children who would otherwise have been eligible, they allowed us to undertake a comparison of steroid-naïve, recurrently wheezy young children versus healthy controls, without additional bias related to preterm delivery[13] or intrauterine growth restriction[14], both of which can impact negatively on subsequent lung development.

Among the wheezy children enrolled, 28% could not be tested: either because they became too old for inclusion, or due to hospitalisation/treatment with corticosteroids/anti-leukotrienes before they could be tested. Results from this study may therefore under-estimate the true magnitude of difference between groups, since some of the more severely affected children were excluded. Despite this, when using the raised-volume technique, clear differences were evident not only between wheezy infants and healthy controls, but within the wheezing group according to risk factors.

Index children were slightly older than controls at time of test (Table 1), due both to the requirement for at least three episodes of physician-confirmed wheeze prior to recruitment and postponements of tests due to respiratory illness, particularly in those with viral-associated wheeze. After adjustment for age and sex, the wheezy infants were significantly heavier, though not longer, than healthy controls. While the reason for this is unclear, by expressing lung function as Z-scores or adjusting for age and length when using MLR, such differences were accounted for when ascertaining the effect of wheeze on lung function. Turner et al [33] reported a negative association between postnatal weight gain and change in length-adjusted $V'_{\text{maxFRC}}$ in some infants between 1 and 12m of age. They speculated that, in infants with reduced birthweight but accelerated “catch-up” during infancy, somatic growth may outstrip that of the lungs, resulting in reduced lung function. Although we excluded any infants with low birthweight for gestational age, a similar phenomenon might have occurred in those with rapid weight gain. However, we did not observe any significant relationship between change in weight and lung function, once other co-variants had been adjusted for.

Ideally we would have assessed bronchodilator responsiveness in this study, but this would have prolonged test duration and potentially jeopardised success rates. Furthermore, although airway obstruction may be largely reversible in older subjects with asthma, bronchodilator response is far more variable among wheezy infants, many of whom may show no improvement or even paradoxical responses.[22]

**Power of study**

This study was well powered to identify clinically significant differences (0.67 Z-scores) between wheezy children and controls. Original power calculations were based on 35 in each group. The final study population consisted of 73 children (44 wheezy, 29 controls). Retrospective power calculations confirmed that with a 1.5:1 imbalance between groups, there would be the same power as 35/group. Similarly, although there was an imbalance of 1.9:1 between the wheezy subgroups (29 low; 15 high-risk), the total of 44 subjects provided the same power as 20/group.
Nevertheless, the lower numbers meant that there was only 60% power of detecting differences as small as 0.67 Z-scores (80% for detecting 0.9 Z-score), increasing the risk of Type II errors during subgroup analysis, especially during MLR.

**Lung function testing and results**

The standardised approach to data collection and analysis between the 2 centres was one of the strengths of this study. The London team provided intensive training to the Lisbon principal investigator (LMB), followed by the establishment of identical infant lung function equipment and measurement protocols in Lisbon. In addition, regular inter-laboratory site visits and cross-analysis of data were undertaken throughout the duration of the study to minimise bias.

Results from this study suggest that while lung function is reduced in infants with recurrent wheeze but low-risk for subsequent asthma, these differences are less marked than in those at high-risk. The reduction in FVC in the wheezy children - which was accompanied by reduced flows - was more evident in the high-risk group, probably reflects small airway obstruction and airway closure at low volumes during forced expiration, rather than any alteration in lung growth or gas trapping during spontaneous breathing. While reductions in FVC can also occur due to gastric distension during the RVRTC,[28] we took particular care to exclude this possibility by checking that there was no systematic fall in FVC between the first and last manoeuvre. Similarly, data from manoeuvres where there was any indication of early termination of forced expiration were excluded (see OLS).

We were not able to assess resting lung volume in this study, due to the limited duration of sleep in many of the subjects, but had there been any dynamic elevation of functional residual volume in those who wheezed, measures of $V_{\text{maxFRC}}$ would have been obtained at a higher volume than in healthy controls, thereby minimising differences between groups.[21] This, together with the intrinsic variability of end-expiratory level during infancy, could contribute to the reduced sensitivity of the partial compared with raised-volume technique in differentiating between those with and without prior wheeze. Although some studies have reported reductions in $V_{\text{maxFRC}}$ in wheezy infants,[34,35] recent publications suggest that the raised-volume technique is more discriminative.[21-24]

It has been reported that tidal breathing indices such as tPTEF:tE may be associated with subsequent wheezing or asthma in later life.[20,36] Significant associations between tPTEF:tE and subsequent outcome have, however, largely been limited to large epidemiological studies with pre-morbid assessments of tidal breathing measured shortly after birth, a time when modulation of expiratory flows and timing is most active. In this study, tPTEF:tE was slightly, but not significantly, lower in wheezy infants; this relative lack of discrimination of tidal breathing parameters in older infants with prior wheeze being in keeping with previous reports.[37] In contrast to previous publications,[2,37] we did not find a significant effect of maternal smoking during pregnancy, although flows tended to be lower in those exposed. This may reflect the relatively small subgroups, the highly selected nature of the population or some interaction between the effects of wheezing and maternal smoking on measured flows. In support of this contention, when analysis was limited to wheezy infants, flows were significantly lower in those whose mothers had smoked.

**Conclusions**

We have demonstrated that lung function is reduced in infants and young children with recurrent wheeze, and that these changes are most marked in those at high risk of subsequent asthma. Findings from this study suggest that the raised-volume technique is able to identify diminished lung function in wheezy infants compared with controls, and between wheezy subgroups according to clinical risk factors. Given the overlap between groups, it is, however, unlikely that such tests would be able to predict persistent wheeze within individuals.

While further work is still required to establish short and long term repeatability, use of such physiological measures in combination with clinical symptoms and risk factors could potentially influence therapeutic interventions.
Acknowledgement

We thank the parents who consented for their infants and young children to participate in this study and staff at the Hospital de Dona Estefânia, Lisbon, and Portex Respiratory Unit, UCL Institute of Child Health, London, for their support. We thank Dr Sooky Lum for her assistance in providing technical training, and Schering Plough's financial support towards travel expenses which enabled collaborative work between the two institutions.

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References


Table 1. Comparison of wheezy and healthy children: background characteristics and lung function results

<table>
<thead>
<tr>
<th>Background details</th>
<th>High-risk† wheezy children (n=17)</th>
<th>Low-risk† wheezy children (n=33)</th>
<th>All wheezy children (n=50)</th>
<th>Healthy controls (n=30)</th>
<th>Mean difference (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>38.9 (1.1)</td>
<td>39.0 (0.9)</td>
<td>39.0 (1.0)</td>
<td>39.6 (1.0)</td>
<td>-0.6 (-1.0; -0.09)</td>
<td>0.02</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.4 (0.5)</td>
<td>3.3 (0.4)</td>
<td>3.3 (0.4)</td>
<td>3.3 (0.4)</td>
<td>0.02 (-0.2; 0.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Birth weight Z-score</td>
<td>0.3 (0.9)</td>
<td>0.1 (0.8)</td>
<td>0.1 (0.9)</td>
<td>-0.2 (0.8)</td>
<td>0.3 (-0.1; 0.7)</td>
<td>0.1</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>9 (53%)</td>
<td>23 (70%)</td>
<td>32 (64%)</td>
<td>18 (60%)</td>
<td>4% (-17%; 25%)</td>
<td>0.7 §</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy, n (%)</td>
<td>6 (35%)</td>
<td>8 (24%)</td>
<td>14 (28%)</td>
<td>5 (17%)</td>
<td>11% (-9%; 28%)</td>
<td>0.2 §</td>
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<tr>
<td>At time of Test</td>
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<tr>
<td>Postnatal age, weeks</td>
<td>60.6 (16.9)</td>
<td>64.6 (16.6)</td>
<td>63.2 (16.6)</td>
<td>55.0 (17.0)</td>
<td>8.1 (0.4;15.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>11.2 (2.2)</td>
<td>11.0 (1.6)</td>
<td>11.1 (1.8)</td>
<td>9.7 (1.2)</td>
<td>1.4 (0.7;2.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Weight Z-score</td>
<td>0.7 (1.6)</td>
<td>0.3 (1.1)</td>
<td>0.4 (1.3)</td>
<td>-0.3 (1.0)</td>
<td>0.7 (0.2; 1.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Length, cm</td>
<td>79.8 (5.6)</td>
<td>79.9 (5.4)</td>
<td>79.8 (5.4)</td>
<td>76.7 (4.6)</td>
<td>3.2 (0.8; 5.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Length Z-score</td>
<td>1.0 (1.4)</td>
<td>0.6 (1.2)</td>
<td>0.7 (1.2)</td>
<td>0.5 (1.2)</td>
<td>0.2 (-0.3; 0.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Lung function indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FEV_{0.5} Z-score</td>
<td>-1.8 (1.0) a</td>
<td>-1.1 (1.1) b</td>
<td>-1.4 (1.1) c</td>
<td>-0.4 (1.0) d</td>
<td>-1.0 (-1.5; -0.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FEF_{25-75} Z-score</td>
<td>-2.2 (1.1) a</td>
<td>-1.6 (0.8) b</td>
<td>-1.80 (0.9) c</td>
<td>-1.00 (0.8) d</td>
<td>-0.8 (-1.2; -0.4)</td>
<td>0.0001</td>
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<tr>
<td>FEF_{75} Z-score</td>
<td>-1.7 (1.2) a</td>
<td>-1.3 (0.7) b</td>
<td>-1.4 (0.9) c</td>
<td>-0.8 (0.8) d</td>
<td>-0.6 (-1.0; -0.2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>FVC Z-score</td>
<td>-1.2 (1.0) a</td>
<td>-0.5 (1.0) b</td>
<td>-0.7 (1.1) c</td>
<td>-0.03 (1.3) d</td>
<td>-0.7 (-1.2; -0.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>V’_{maxFRC} Z-score</td>
<td>-2.2 (1.0)</td>
<td>-1.7 (1.0)</td>
<td>-1.9 (1.0)</td>
<td>-1.5 (0.9)</td>
<td>-0.4 (-0.9; 0.1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Respiratory rate, bpm</td>
<td>30.4 (8.3)</td>
<td>29.7 (6.0)</td>
<td>29.9 (6.8)</td>
<td>30.2 (5.6)</td>
<td>-0.3 (-3.2; 2.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>VT/kg, mL</td>
<td>10.0 (1.5)</td>
<td>9.8 (1.1)</td>
<td>9.9 (1.2)</td>
<td>9.9 (1.5)</td>
<td>-0.1 (-0.7; 0.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>t_{PETF/RE}</td>
<td>0.24 (0.1)</td>
<td>0.26 (0.1)</td>
<td>0.25 (0.1)</td>
<td>0.29 (0.1)</td>
<td>-0.03 (-0.1; 0.01)</td>
<td>0.2</td>
</tr>
</tbody>
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Data shown as mean (SD) for continuous and n (%) for categorical variables.

Symbols:
§ statistical significance was calculated using χ² test.
† “High-risk” denotes wheezy children with parental history of asthma, or wheezing without colds and serum eosinophilia above 4%; “Low-risk” denotes wheezy children without such clinical features.

\[ a \text{ } n=15; \quad b \text{ } n=29; \quad c \text{ } n=44; \quad d \text{ } n=29 \]

Abbreviations:
CI: confidence intervals; FVC: forced vital capacity; \( \text{FEV}_{0.5} \): forced expiratory volume at 0.5 second; FEF_{75}: forced expired flow after 75% FVC has been exhaled; FEF_{25-75}: average forced expired flow over the mid 50% of FVC; \( V'_{\text{maxFRC}} \): maximal forced flows at functional residual capacity; bpm: breath per minute; \( V_T \): tidal volume; \( t_{\text{pTEF/tE}} \): ratio of time taken to reach peak tidal expiratory flow to total expiratory time
## Table 2. Mean difference (95% CI) in lung function variables: wheezy sub-groups† versus healthy controls

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>FEV(_{0.5}) Z-score</td>
<td>-1.4 (-2.1; -0.8) p &lt; 0.001</td>
<td>-0.8 (-1.3; -0.3) p &lt; 0.01</td>
<td>-0.7 (-1.3; 0.01) p=0.1</td>
</tr>
<tr>
<td>FEF(_{25-75}) Z-score</td>
<td>-1.2 (-1.8; -0.6) p &lt; 0.001</td>
<td>-0.6 (-1.0; -0.2) p &lt; 0.01</td>
<td>-0.6 (-1.2; -0.1) p &lt; 0.05</td>
</tr>
<tr>
<td>FEF(_{75}) Z-score</td>
<td>-1.0 (-1.5; -0.3) p &lt; 0.01</td>
<td>-0.4 (-0.8; -0.04) p &lt; 0.05</td>
<td>-0.5 (-1.1; 0.1) p=0.1</td>
</tr>
<tr>
<td>FVC Z-score</td>
<td>-1.2 (-1.9; -0.4) p &lt; 0.01</td>
<td>-0.5 (-1.1; 0.2) p &lt; 0.05</td>
<td>-0.7 (-1.4; -0.04) p &lt; 0.05</td>
</tr>
<tr>
<td>V′(_{\text{maxFRC}}) Z-score</td>
<td>-0.7 (-1.3; -0.1) p &lt; 0.05</td>
<td>-0.2 (-0.7; 0.3) p=0.2</td>
<td>-0.5 (-1.1; 0.1) p=0.1</td>
</tr>
<tr>
<td>Respiratory rate, bpm</td>
<td>0.2 (-3.9; 4.3) p=0.9</td>
<td>-0.5 (-3.4; 2.4) p=0.7</td>
<td>0.7 (-3.4; 4.8) p=0.7</td>
</tr>
<tr>
<td>V(_t)/kg, mL</td>
<td>0.1 (-0.8; 1.1) p=0.8</td>
<td>-0.1 (-0.8; 0.5) p=0.7</td>
<td>0.3 (-0.5; 1.0) p=0.5</td>
</tr>
<tr>
<td>t(_{PTEF}/t_E)</td>
<td>-0.04 (-0.10; 0.01) p=0.1</td>
<td>-0.03 (-0.08; 0.02) p=0.2</td>
<td>-0.01 (-0.10; 0.04) p=0.6</td>
</tr>
</tbody>
</table>

Symbols: † Wheezy sub-groups: “High risk” denotes wheezy children with history of parental history of asthma or wheezing without colds and serum eosinophilia > 4%; “Low risk” denotes wheezy children without such clinical history.

Abbreviations: please see footnote for Table 1
Figure 1.

Title: Flow diagram of recruitment process for lung function measurements in the wheezy children.

Total eligible index children (n=94)

Declined participation n=12 (13%)

Consented for lung function n=82 (87%)

LA tests not performed
a) repeat deferments due to lower respiratory illness / hospitalisation / commencement on inhaled steroid or anti-leukotriene agents: n=23 (28%)  
b) did not attend for appointments: n=4 (5%)

55 attended for LFT (67% of consented, 59% of all potentially eligible)

Incomplete LF test: (woke early: n=3)

Technically unacceptable test results: tidal RTC (n=2); raised-volume RTC (n=8)

Available LF results: tidal RTC (n=50); raised-volume RTC (n=44)
**Figure 2.**

**Title:** Comparison of FEV$_{0.5}$, FEF$_{75-25}$ and FVC Z-scores in healthy controls and wheezy subgroups.

**Footnote:** Results from individual child are shown with mean value (indicated by the horizontal bar) for each subgroup. For mean difference and 95% CI of the difference between subgroups, please refer to Table 2.
Figure 2.

A) FEV\(_{1.0}\) Z-score

B) FEF\(_{75-25}\) Z-score

C) FVC Z-score

- Healthy controls
- Low-risk wheezy children
- High-risk wheezy children

P-values:
- A) P < 0.01
- B) P = 0.1
- C) P = 0.2
- P < 0.001
- P < 0.01
- P < 0.05
- P = 0.005
Lung function and clinical risk factors for asthma in infants and young children with recurrent wheeze

Luis Miguel Borrego MD, Janet Stocks PhD, Paula Leiria-Pinto MD, Isabel Peralta, Ana Margarida Romeira MD, Nuno Neuparth MD PhD, José E Rosado-Pinto MD, Ah-Fong Hoo PhD

Online Data supplement
Online supplement

Methods

Pulse oximetry

Prior to administration of choral hydrate, a spot check of the young child's oxygen saturation level (SpO₂), respiratory and heart rate was undertaken. When the infant or young child had settled (ideally before falling asleep), a flexible oximeter probe was attached either to the large toe or outer aspect of the foot. Immediately after the child had fallen asleep, continuous oxygen saturation (SpO₂) and heart rate recording was commenced, prior to attaching the mask to the face. Vital sign monitoring was maintained throughout the test period.

Forced expiratory manoeuvres

All measurements were undertaken in accordance with international guidelines.[1,2] During the tests, the infant breathed through a face-mask (Rendell Baker Soucek, size 2; Rusch UK Ltd, High Wycombe, UK) placed over the mouth and nose. A ring of therapeutic putty, secured around the rim of the face-mask, was used to ensure a leak-free seal. A minimum of 25 regular tidal breaths were collected prior to undertaking the partial and raised-volume forced expiratory manoeuvres.

a) Application of the rapid thoraco-abdominal compression (RTC) jacket

On each test occasion, the jacket was adjusted to fit the young child snugly, allowing sufficient space at the sternum to accommodate insertion of at least two or three adult fingers.[2] Once asleep, the child was placed supine in the cot, with the head supported in the midline and neck and shoulders slightly extended. The schematic figure below (Figure E1) shows placement of the inflatable bladder over the top of the chest and abdomen, and encased within the outer jacket by fastening the Velcro strips at the front. The arms remained outside the jacket to avoid splinting of the chest.[3] The jacket extended from the level of axillae to symphysis pubis.
Lung function in recurrently wheezing young children

Figure E1. Schematic diagram showing the apparatus used for the tidal RTC

At least 5-10 regular breaths were recorded to establish a stable end-expiratory baseline, followed by an occlusion test to assess the face mask seal. Measurements using the tidal RTC method were performed before the raised-volume RTC manoeuvres. All measurements were performed during epochs of behaviourally determined quiet (non-rapid eye movement) sleep.

b) Acquisition of tidal RTC curves

Once a stable end-expiratory level had been established, the jacket was inflated at end-inspiration and compression of the chest and abdomen was held until all volume had been exhaled. An initial jacket compression pressure of 30 cmH$_2$O was used, and progressively increased in steps of 5-10 cmH$_2$O until no further increase in maximal forced flow ($V'_{maxFRC}$) was seen. A minimum of three technically satisfactory partial forced expiratory flow-volume curves (Figure E2), with the highest values for $V'_{maxFRC}$, were obtained. The mean of three best $V'_{maxFRC}$ values (within 10% or 10 mL/s) was reported for each child.
Figure E2. A tidal RTC curve showing the calculation of $V'_{\text{maxFRC}}$

For further details pertaining to the tidal RTC (or tidal “Squeeze”) manoeuvres, readers are referred to the ERS/ATS standardisation article published by Sly et al [2].

c) Acquisition of raised-volume RTC curves

For the collection of raised-volume RTC data, additional items were added to the equipment set-up for the tidal RTC tests shown in Figure E1. A Neopuff infant resuscitator (pressure relief valve system: RD 1000), allowed the setting of a predetermined pressure and enabled a positive lung inflation pressure of 30 cmH$_2$O, using a flow of 10-12 L/min of air to be delivered to young children to inflate lung volume towards total lung capacity. The T-connector was attached to the Neopuff system and distal end of the pneumotachometer, the remaining opening being available for subsequent intermittent occlusions in order to effect lung inflations (Figure E3). The jacket pressure used for the raised-volume RTC was the optimal compression pressure determined during the preceding tidal RTC manoeuvres for each child, i.e. that at which the highest $V'_{\text{maxFRC}}$ had been obtained.
Figure E3. Schematic diagram showing the apparatus used for the raised-volume RTC

Repeated occlusions of the expiratory limb of the T-connector at a rate approximating the infant’s respiratory frequency resulted in inflations and deflations of the respiratory system. Four to five such inflations were administered to induce respiratory muscle relaxation, prior to triggering jacket inflation (by a second operator) to effect jacket compression at the end of the subsequent augmented inspiration as shown in the time-based trace (Figure E4a). To aid relaxation and induce the Hering Breur Reflex, inflations were held until a plateau was observed on the pressure recording (Figure E4a). Jacket compression was maintained until expiration was complete, as indicated by attainment of zero flow. Following the release of compressive pressure by venting to atmosphere, a respiratory pause was often observed (Figure E4a). A technically satisfactory raised-volume flow-volume curve is illustrated in Figure E4b.

Further details pertaining to the raised-volume RTC technique have been described previously.[1,4]
Figure E4a. A time-based trace illustrating augmented breath inflations and timing of jacket compression during a raised-volume forced expiratory manoeuvre.

Footnote: The upward slope along the volume trace denotes inspiration and downward slope denotes expiration. In this study, a positive airway inflation pressure of 30 cmH$_2$O was used to augment lung volume towards total lung capacity.

Figure E4b. Assessment of forced vital capacity and forced expiratory flows from the raised-volume flow-volume curve derived from the time-based data shown in Figure E4a.
Quality control for RVRTC data

The main criteria for accepting raised-volume RTC data were:

- Absence of leaks around the facemask or pneumotachometer
- A stable end-expiratory baseline prior to execution of jacket inflation at end inspiratory phase
- Jacket pressure reaching a pressure plateau within 100 millisecond of the start of inflation
- Jacket inflation was maintained throughout the entire forced expiration
- No evidence of early inspiration following forced expiratory manoeuvre, i.e., forced expiratory continued towards residual volume, and
- Absence of glottic narrowing or flow transients on visual inspection of the expiratory flow-volume curve
Lung function in recurrently wheezing young children

Table E1. Lisbon and London healthy control children: background details and lung function results

<table>
<thead>
<tr>
<th>Background details</th>
<th>Lisbon (n=12)</th>
<th>London (n=18)</th>
<th>Mean difference (95% CI) Lisbon-London</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>39.3 (1.1)</td>
<td>39.8 (1.0)</td>
<td>-0.5 (-1.2; 0.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.2 (0.3)</td>
<td>3.4 (0.5)</td>
<td>-0.2 (-0.5; 0.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>Birth weight Z-score</td>
<td>-0.4 (0.7)</td>
<td>-0.1 (0.9)</td>
<td>-0.3 (-0.9; 0.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (83%)</td>
<td>8 (44%)</td>
<td>39% (3%; 62%)</td>
<td>0.01 §</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy, n (%)</td>
<td>2 (17%)</td>
<td>3 (17%)</td>
<td>0.0 (-0.3; 0.3)</td>
<td>1.0 §</td>
</tr>
</tbody>
</table>

| At the time of test                                      |              |               |                                       |         |
| Postnatal age, weeks                                    | 58.6(10.9)   | 52.7(20.2)    | 5.8 (-7.3;18.9)                       | 0.4     |
| Test Weight, kg                                         | 10.0 (0.9)   | 9.5 (1.3)     | 0.5 (-0.4;1.4)                        | 0.3     |
| Test Weight Z-score                                     | -0.5 (0.9)   | -0.2 (1.0)    | -0.3 (-1.0;0.4)                       | 0.4     |
| Test Length, cm                                         | 77.5 (1.7)   | 76.0 (5.1)    | 1.5 (-2.1;4.9)                        | 0.4     |
| Test Length Z-score                                     | 0.1 (0.8)    | 0.7 (1.2)     | -0.7 (-1.6;0.3)                       | 0.2     |

| Lung function variables                                  |              |               |                                       |         |
| FVC Z-score                                              | 0.5 (0.8) a  | -0.3 (1.5)    | 0.8 (-0.2; 1.8)                       | 0.1     |
| FEV_{0.5} Z-score                                        | -0.6 (1.0) a | -0.2 (1.0)    | -0.4 (-1.1; 0.5)                      | 0.4     |
| FEF_{75} Z-score                                         | -0.6 (0.67) a| -0.9 (0.88)   | 0.3 (-0.3;1.0)                        | 0.3     |
| FEF_{25-75} Z-score                                      | -1.2 (0.79) a| -0.9 (0.82)   | -0.3 (-0.9; 0.4)                      | 0.4     |
| V'_{maxFRC} Z-score                                      | -1.5 (0.9)   | -1.5 (0.9)    | -0.1 (-0.6; 0.7)                      | 0.8     |
| Respiratory rate, bpm                                    | 29.7 (1.4)   | 30.4 (6.0)    | -0.7 (-5.0; 3.7)                      | 0.8     |
| VT/kg, mL                                               | 10.4 (5.2)   | 9.6 (1.6)     | 0.9 (-0.3; 2.0)                       | 0.1     |
| t\_PTEF/t\_E                                           | 0.31 (0.09)  | 0.27 (0.08)   | 0.04 (-0.24; 0.10)                    | 0.2     |

Data shown as mean (SD) for continuous and n (%) for categorical variables

§ statistical significance calculated using $\chi^2$ test

CI: confidence intervals; FVC: forced vital capacity; FEV_{0.5}: forced expiratory volume at 0.5 second; FEF_{75}: forced expired flow after 75% FVC has been exhaled; FEF_{25-75}: average forced expired flow over the mid 50% of FVC; V'_{maxFRC}: maximal forced flows at functional residual capacity; bpm: breath per minute; VT: tidal volume; t\_PTEF/t\_E: ratio of time taken to reach peak tidal expiratory flow to total expiratory time.
References


