Age and height dependence of lung clearance index and functional residual capacity

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Abstract (200 words)

Rationale: The lung clearance index(LCI) is more sensitive than spirometry in detecting abnormal lung function in children with cystic fibrosis. LCI is thought to be independent of age, but recent evidence suggests that the upper limit of normal is higher in infants and preschool children than in older subjects. This study examines whether LCI remains independent of body size throughout childhood.

Methods: Multiple breath washout data from healthy children and adolescents were collated from three centres using the Mass Spectrometer system and inert gas SF_6.   Reference equations for LCI and functional residual capacity (FRC) were constructed using the LMS (Lambda, Mu, Sigma) method.

Results: Data were available from 497 subjects (2weeks to 19 years old) tested on 659 occasions. LCI was dependent on body size, decreasing in a non-linear pattern as height increases. Changes were particularly marked in the first five years of life. Height, age and sex were all independent predictors of FRC. Minimal between-centre differences allowed unified reference equations to be developed.

Conclusions:LCI is not independent of body size. Although a constant upper normal limit would suffice for cross-sectional clinical assessments from six years of age, appropriate reference equations are essential for accurate interpretation of results during early childhood.

Keywords: children; Functional Residual Capacity; Lung Clearance Index, Reference range; ventilation inhomogeneity

INTRODUCTION

Measuring the efficiency of ventilation distribution within the lung offers exciting potential to detect early disease processes missed by conventional flow-based lung function techniques such as spirometry. Multiple breath inert gas washout (MBW) is a non-invasive tidal breathing test, feasible across all ages, which offers improved sensitivity compared to spirometry to detect early cystic fibrosis lung disease throughout childhood [1-3].

It is generally thought that LCI is independent of body size. However, cross-sectional studies in infants have suggested that upper limit of normal for global measures of ventilation distribution inhomogeneity, such as lung clearance index (LCI), may be higher compared to older subjects [3,4]. Such differences, which could arise from developmental changes or differences in measurement conditions, may preclude identification of early lung disease in both cross-sectional and longitudinal studies. Furthermore appropriate normative data for Functional Residual Capacity derived from MBW (FRC_{MBW}) spanning the paediatric age range is lacking. The aim of this study was to investigate the relationship between body size and LCI from infancy to young adulthood and establish reference equations for both LCI and FRC_{MBW} across this age range.

METHODS

MBW was measured in healthy children at three specialised paediatric centres in the UK, Sweden and Canada as described previously [3,5-10]. Briefly, during wash-in, a dry air mixture containing 4% sulfur hexafluoride (SF_6) was inspired until inspiratory and expiratory SF_6 concentrations were stable for a minimum of 5 breaths. During washout, room air was inhaled until end tidal SF_6 concentration was consistently below 0.1% (i.e. $1/40^{th}$ of starting concentration). Mean FRC and LCI were reported from three technically acceptable runs. Data were collected between 2000 and 2010 using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark) with identical MBW system design and analysis software (developed by PG) and protocols in all centres [11]. Please see online supplement for details of equipment and software indicators. The inclusion criteria for healthy children were the same across all three sites [2,3,7,8,10,12]. Subjects were free from respiratory illness for at least 3 weeks. Infants and young children under 2 years of age were studied supine and in quiet sleep following light sedation with chloral hydrate using a facemask. Preschool (3-6 years) and school age (>6 years) children were tested sitting upright and awake using a facemask and mouthpiece respectively. Data from London and Sweden were collected using a Fleisch pneumotachometer (PNT) while a Hans Rudolf PNT was used in Toronto (See online supplementary material for details of methodological differences between centres). In London and Toronto, healthy children were followed longitudinally as part of observational studies. The timing of repeated measurements varied according to study protocol, and ranged from 2 weeks to 6 years. All research studies were approved by local Research Ethics Committees of participating hospitals, and informed written consent obtained from all parents and assent from older children.

Statistical analysis

Population characteristics were summarised using descriptive statistics. Centre differences were explored using ANOVA or chi-squared analysis where appropriate. The relationship between body size and MBW outcomes was initially explored by visual inspection of data. Reference equations for both LCI and FRC_{MBW} were constructed as described previously [13-15] using the LMS

(Lambda, Mu, Sigma) method [16]. This method is an extension of regression analysis which includes three components: 1) skewness (**L**ambda), which models the departure of variables from normality using a Box-Cox transformation, 2) median (**M**u) or predicted value, and 3) coefficient of variation (**S**igma), which models the spread of values around the median and adjusts for any non-uniform dispersion, hence LMS. The three quantities (LMS) are allowed to change with height and/or age, to reflect changes in the distribution as children grow. Together L, M and S coefficients are combined algebraically to convert individual observations to z-scores [16].

$$\text{z-score} = [(\text{Measurement}/M)^L - 1] / [L*S]$$
$$\text{Upper Limit of Normal (ULN, 97.5}^{th}\text{ percentile)} = M*(1.96*S*L +1)^{1/L}$$

We applied the LMS method using the GAMLSS package [17] in R (Version 2.6.1; R Foundation, http://www.r-project.org). In these analyses we also used the random function to adjust for repeated measurements in individuals. Fractional polynomials [18], whereby a combination of integer or fractional power terms are fitted to produce a polynomial equation, were used to fit each curve to explain body size related changes. Goodness of fit was assessed using the Schwarz Bayesian Criterion (SBC), which compares consecutive models directly while adjusting for increased complexity to determine the simplest model with best fit [19].

Although conventionally the ULN is at 1.64 z-score, however, as this is an epidemiological study, where the cost and consequences of false-positive and false-negative test results are over-riding, an ULN corresponding to the 97.5[th] centile (ULN 97.5%, z-score 1.96) is used as recommended by the Global lung function initiative [20].

Data storage and management at ICH, London were undertaken using Re-Base[(tm)] software (Re-Base Ltd, London, UK).

RESULTS

Population characteristics

LCI data were available from 497 subjects on 659 test occasions from 2 weeks to 19 years of age (Table 1). There were 201 observations from infants and children <2 y of age (49% boys); 138 between 3-6 y (44% boys) and 320 from those >6 years of age (47% boys). Although the majority of subjects were measured on a single test occasion, 22% had 2 observations, 11% had 3-4 observations, while 8 children (2%) had 5-6 observations. When compared against subjects from London or Toronto, Swedish subjects were older (p < 0.0001 for both centres) and after adjusting for sex and age [21], significantly heavier (p = 0.002 and p=0.03 respectively).

Table 1: Population characteristics

Background details	London, UK	Skovde, Sweden	Toronto, Canada	Total population
N (% male)	293 (47%)	102 (45%)	102 (52%)	497 (48%)
White (%)	220 (75%)	100%	76 (75%)	398 (80%)
No of test occasions	443	102	114	659
Age (y)	5.2 (0.05 – 16.0)	17.9 (4.5 – 18.7)	1.6 (0.23 – 18.9)	5.8 (0.05 – 18.9)
Weight (kg)	19.1 (3.7 – 71.9)	63.0 (20.5 –101.0)	11.1 (5.2 – 98.7)	20.7 (3.7 -101.0)
Weight (z-score) [#§]	0.22 (1.00)	0.59 (0.93)	0.24 (1.14)	0.28 (1.02)
Height (cm)	110.5 (52.5 – 183.0)	169.3 (113.2 -196.5)	82.8 (60.0 – 185.5)	114.7 (52.5 – 196.5)
Height (z-score) [#§]	0.39 (1.04)	0.59 (0.93)	0.32 (1.02)	0.41 (1.03)

Data presented as median (range) unless otherwise specified; # denotes Mean (SD); § adjusted according to British 1990 growth charts [21]

Lung Clearance Index

LCI decreased non-linearly with increasing age and height, with no significant association with sex. In a multivariable regression model, after adjusting for height, age was no longer an independent predictor of LCI. The simplest model that explained the greatest variability of LCI was used to define the reference equation for LCI, and hence in the final model only height was included (Table 2). The relationship between height and LCI is presented in Figure 1, as the fitted reference equation (50th centile) together with upper (97.5th centile) and lower (2.5th centile) limits of normal. Table 3 shows examples of predicted and ULN for LCI according to height. After correction for height, no significant inter-subject differences were found between LCI measurements made supine (i.e. sedated infants) and older subjects who were studied sitting (adjusted $\beta = 0.14$ (95% CI -0.07; 0.34; see E Fig 1, online supplement), nor between those obtained with a mask (infants and preschool children) or mouthpiece in older children (adjusted $\beta = -0.11$ (95% CI -0.23; 0.01). After the best model was determined, centre differences were tested as fixed effects within the model. There were small, albeit statistically significant, differences between centres, with Toronto having slightly lower LCI results (mean difference (95% CI): -0.35 (-0.45; -0.24)) than either London or Sweden. Sensitivity analysis found the same non-linear relationship with height and minimal effect (<1%) on reported upper limit of normal if Toronto data were excluded.

The majority (80%) of children were of white European origin, the remainder being Black African or Caribbean (5.6%); Asian (7.3%) or of a variety of other ethnic origins (7.1%). When the sample was dichotomised into "White" and "non-White", no statistical differences in LCI were observed between groups (mean difference (95% CI): 0.05 (-0.17; 0.07)). The sample size for different ethnic groups was too small to investigate changes in LCI in relation to height within each group separately (<40 in three groups and <10 in the remaining three).

Table 2: Paediatric reference equations for LCI and FRC$_{MBW}$

LCI

L	**-0.81**
M (Predicted)	**5.99+(73.85*height^{-1}[cm])**
S	**0.08**
ULN	**Predicted***((1.96***0.08***-0.**81**) +1)$^{1/-0.\mathbf{81}}$

FRC

L	0.21
M (Predicted)	exp (-11.07 +(2.12*lnHeight) + (0.27*age$^{0.5}$[y]) + (0.04*sex))
S	exp(-1.67 + 159.61*height^{-2}[cm])
ULN	**Predicted***((1.96***S***0.21) +1)$^{1/0.21}$

Abbreviations: exp: exponential; lnHeight: natural log for Height; sex: boy=1
*Footnote: Based on these equations, the ULN for LCI can be simplified to Predicted LCI * 1.18; whereas that for FRC$_{MBW}$ becomes (0.36*S+1) to the power 5.37 * Predicted FRC.*
For simplicity, the factors are displayed to 2 decimal places. For calculation of Z scores and ULN, the full equations presented in E Table 3 (online supplement material should be used).

Table 3: Examples of Predicted and ULN for LCI according to height

Height (cm)	Pred LCI	ULN LCI
55	7.33	8.67
60	7.22	8.54
65	7.13	8.43
70	7.04	8.33
80	6.91	8.17
100	6.73	7.96
120	6.60	7.81
140	6.52	7.71
160	6.45	7.63

Footnote: As a broad approximation, 55cm represents average height (50th centile) ~at 5 weeks, 60 cm at 3 months, 70 cm at 9 months, 80 cm at 18 months, 100 cm at 4 years, 120cm at 7 years and 140 at 10 years. After the age of 10 years, height becomes highly dependent on sex as well as age, the average height of an 18 year old male and female being 177 and 164 cm respectively

LCI in children above 6 years of age.

As can be seen from figure 1, despite a continuing small reduction in LCI throughout the entire paediatric range, changes were minimal once the child attained a height of around 115 cm (approximately 6 years of age). LCI data from children over 6 years of age were therefore examined separately. These were available from 255 subjects on 316 test occasions of which 55 (17%) datasets were collected using a facemask. Within this sub-group, there was no significant association between LCI and either height or age nor whether a mask or mouthpiece was used. Mean (SD) LCI for children 6-19 years of age was 6.54 (0.51), with an ULN (ULN =Mean ± 1.96SD) for this age group of 7.56 if a fixed threshold were to be used (Fig 2). A similar pattern was seen plotting LCI against height (data not shown).

FRC_{MBW}

FRC_{MBW} data were available from 469 subjects on 631 test occasions, as FRC had not been originally recorded separately for 28 Swedish subjects and could not be retrieved subsequently due to software and equipment upgrades. Since FRC was heavily right skewed, log transformed FRC values were used in the models. Using multi-variable models, height, age and sex were all independent predictors of FRC. In addition, variability around the mean varied with height and there was residual skewness, both of which were adjusted for in the models. The relationship between FRC and height is presented in Figure 3. There was, on average, a 30- fold increase in FRC during the first 19 years of life. After adjustment for height, age and sex, children from Sweden and Toronto had slightly higher values of FRC compared with London: mean difference (95% CI) 0.05L (-0.02; 0.12) and 0.05(0.01; 0.09), respectively. No significant differences in FRC were detected between non-White children compared with White children [mean difference (95% CI) 0.01 (-0.02; 0.05)]. By contrast, type of equipment and posture were both significant in the FRC model: mean (95% CI) values being 0.12L (0.07; 0.17) higher when a mask rather than mouthpiece was used and 0.14L (0.08; 0.21) lower in the supine than sitting posture, after adjusting for height, age and sex.

Table 4: Examples of predicted values and ULN for FRC$_{MBW}$ according to height and sex

Height (cm)	Boys		Girls	
	Pred FRC$_{MBW}$	ULN FRC$_{MBW}$	Pred FRC$_{MBW}$	ULN FRC$_{MBW}$
55	0.085	0.123	0.082	0.119
60	0.107	0.154	0.103	0.149
65	0.132	0.189	0.128	0.184
70	0.162	0.232	0.158	0.226
80	0.237	0.338	0.229	0.327
100	0.463	0.660	0.447	0.636
110	0.622	0.885	0.598	0.851
120	0.816	1.161	0.784	1.115
140	1.339	1.903	1.276	1.814
160	2.024	2.874	1.966	2.792

FRC$_{MBW}$ values are presented in litres. To take age into account, results have been calculated by using the age at which the quoted values for height represent the 50th centile. Thus 70 cm represents the 50th centile for height at 0.64 years in boys but 0.75 years in girls, whereas 160 cm represents the 50th centile for height at 13.7 years in boys but 14.1 years in girls [21].

Predicted FRC from the current study was fairly similar to that derived from He dilution equations [22], despite use of different techniques and inert gas (see E Table 2, online supplement).

DISCUSSION

Contrary to the widely held belief that LCI remains constant in health, significant reductions in LCI occur with growth, which are particularly marked in the first 5 years of life. Use of a fixed upper limit of normal (ULN) creates two sources of error with clinical implications: over-diagnosis of abnormal ventilation distribution in healthy infants and young children, and poor sensitivity to detect worsening lung function (relative to the true change in ULN) when absolute values either remain stationary over time or decline by a smaller than the predicted amount. It is well recognised that relative independence of LCI from body size, due to the intrinsic correction for FRC, is of considerable advantage when interpreting results, particularly in children with respiratory disease who may have accompanying impairments of somatic growth [1,23]. Magnitude of change in LCI over the first 19 years of life is minimal when compared with the 30-fold increase observed for lung volumes and airflows over this period [24]. Nevertheless, observed changes in LCI during early childhood, with an absolute decrease in ULN by >0.8 between one month and 5 years of age (~110cm), are large enough to be of clinical significance and could bias attempts to track lung function between infancy, the preschool years and later childhood.

Despite frequent reference to the stability of LCI in health across age ranges, close inspection of the literature reveals a range of values according to the age of the population studied, with the ULN ranging from between 7.8 (using Mass spectrometry; SF_6) to 8.2 (using an ultrasonic flowmeter; SF_6) during infancy [1,3,25,26] 7.4 to7.8 in preschoolers [1,27] and 7.2 to 7.4 in older school children [2,5], which is consistent with the significant inverse association of LCI with height across the paediatric age range found in this study. Nevertheless, beyond six years of age (~115cm height), LCI did stabilise with <0.3 further reduction in ULN until early adulthood. Indeed, when analysis was restricted to children above 6 years of age, no significant relationship with height or age was observed.

The use of a fixed LCI ULN of 7.53 in older children (Figure 2) would increase the risk of over-diagnosis of abnormality in children below 10 years of age (i.e. below~140cm), with differences of up to 0.3 between the fixed and 'true' ULN derived from all subjects (Table 3). However, given the marked elevations in LCI reported in CF once school age is reached [2,5,28,29], this is likely to have minimal clinical impact in cross sectional measurements. By contrast, when interpreting longitudinal changes within the same child at any age, the use of LCI expressed as z-scores is advised as the ULN changes with body size.

Although predicted LCI and FRC values are likely to be both device and inert gas specific [23], the changes in LCI that we have observed during early childhood are likely to be relevant irrespective of the precise technique used. When using ultrasonic flowmeter technology in infants, an ULN > 8 has been reported [26,30]. Furthermore, Chakr et al recently demonstrated that gas mixing within the lung becomes more homogeneous with increasing age early in life using the LCI and phase III slopes [31]. This is in keeping with our current findings based on Mass Spectrometry. Likewise, the ULN for LCI reported for subjects above 5 years of age when using photo acoustic SF_6 gas analyser and Hans-Rudolph PNT [29] or ultrasonic flowmeter technology [28,32] is similar to that reported here. While Fuchs et al found LCI to be independent of growth between 5-18 years [28,32], LCI was found to *increase* slightly in healthy subjects between 6-58 years of age [29], a pattern also observed by Robinson et al when comparing published data across a wide age range [23]. Recently, age dependency of LCI during adulthood (between 25 and 65 years of age) has been reported, showing that gas mixing becomes more heterogeneous with age [33]. This suggests that gas mixing is more heterogeneous both in the very young and in elderly adults , although formal longitudinal studies across wide age ranges is required to confirm this.

Measurement conditions did influence FRC_{MBW}, with higher FRC_{MBW} values in facemask tests (despite attempts to minimise apparatus deadspace and correction for such deadspace) and in the

sitting position. These influences are inevitable when transitioning from early to late childhood and must be reflected in the prediction equations used. The magnitude of effect is unclear in early childhood and a number of factors may act in different directions. Increased equipment deadspace may cause compensatory increase in tidal volume, and dynamic elevation of end expiratory level, especially during infancy [34]. Minimisation of deadspace is recommended because in young children, increased deadspace and hypercapnia leads to deeper VT and increased tendency to dynamic hyperinflation of FRC. This may explain the inter-centre differences (ICH vs. Toronto) in LCI and FRC results observed during infancy [35]. Equipment deadspace is larger with a facemask as opposed to a mouthpiece, and increased anatomical dead space, in relation to lung volume, especially in infants and young children could adversely affect gas mixing efficiency. However, the magnitude of effect has been investigated recently in infants, by using alveolar based indices corrected for deadspace, and did not account for the increased LCI pattern seen in early life [36].

In the current study, choice of interface did not have a significant effect on children > 6 years, where numbers were large enough to allow comparison. Minimal atelectasis in the dependent lung regions of supine infants and the effects of gravity may in theory increase LCI, but would not explain the growth-related changes observed *during* infancy. We speculate that these growth related changes in part reflect the rapid alveolarisation that occurs during infancy and early childhood [3,37], with more heterogeneous gas distribution due to asymmetrical branching within the acinar regions in younger subjects.

A small study of 6 healthy adults using nitrogen washout, suggested that LCI increased significantly in the supine and head-down postures, supporting the view that gas distribution is less uniform in such postures than when upright [38,39]. However, preliminary data from CF subjects, possibly due to the reduced FRC and/or due to previously less ventilated apical lung regions becoming more ventilated, this effect is not seen in young sedated infants and toddlers with CF [40]. Imaging studies in children demonstrate atelectasis in dependent lung regions when supine [41,42]. Effect of

sedation on ventilation distribution inhomogeneity remains unclear, although values in healthy unsedated infants [26,30] are similar to the results reported here in sedated infants, despite different systems. Chloral hydrate sedation has been shown to have minimal effects on breathing patterns, strength of Hering Breuer inflation reflex or oxygen saturation in healthy infants during the first two years of age [43,44].

Irrespective of the exact contributions of these underlying mechanisms, it is evident that LCI values are elevated during early childhood, and that prediction equations which reflect the changes occurring with growth are required. The reference equations presented in this paper are unique and for the first time contain sufficient numbers across a wide enough age range to provide smoothly changing curves describing the transition between infancy, preschool and school-age children without arbitrary break points at different ages or body size, thereby eliminating the need to switch between equations [45,46]. This was achieved by collating data from three centres using almost identical equipment. The lower LCI observed in Toronto may have been due to the relatively large equipment deadspace (E Table 1) resulting in higher FRC_{MBW} when compared with ICH data. Despite small inter-centre-differences, the ability to combine these data to form unified equations suggests that they can be generalised to other populations using similar equipment and analysis protocols. The height dependency of LCI observed in this dataset are likely to be relevant irrespective of techniques used but the extent to which these equations are applicable to newer commercially available equipment [32] especially those based on Nitrogen washout [47-49] will need to be determined.

In conclusion, LCI is not constant throughout childhood and whilst the use of fixed upper limits of normal for assessments on single occasions in children above 6 years of age may not have any significant clinical impact on interpretation, this is not appropriate for younger children or those in whom serial measurements are being undertaken at any age. Expression of results as z-scores, using

prediction equations reflecting the developmental changes occurring across childhood, will allow

more accurate interpretation of LCI results in children.

Reference List

1. Aurora P, Kozlowska W, Stocks J. Gas mixing efficiency from birth to adulthood measured by multiple-breath washout. *Respir Physiol Neurobiol* 2005; 148: 125-139.

2. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003; 22: 972-979.

3. Lum S, Gustafsson P, Ljungberg H, Hulskamp G, Bush A, Carr SB, Castle R, Hoo AF, Price J, Ranganathan S, Stroobant J, Wade A, Wallis C, Wyatt H, Stocks J. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. *Thorax* 2007; 62: 341-347.

4. Sinhal S, Galati J, Baldwin DN, Stocks J, Pillow JJ. Reproducibility of multiple breath washout indices in the unsedated preterm neonate. *Pediatr Pulmonol* 2010; 45: 62-70.

5. Aurora P, Gustafsson P, Bush A, Lindblad A, Oliver C, Wallis CE, Stocks J. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. *Thorax* 2004.

6.   Brown M, Balkovec S, Keast C, Rampersad N, Grewal R, Subbarao P, Ratjen F. Lung clearance index as a marker of peripheral airways disease in children under 5 years of age. Am J Respir Crit Care Med 181, A5968. 2010.

7.   Brown M, Jensen R, Balkovec S, Rampersad N, Grewal R, Ratjen F, Subbarao P. Effects of forced expiratory maneuvers and bronchodilator on lung clearance index in infants. Am J Respir Crit Care Med 183, A6311. 2011.

8. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, Gustafsson PM. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 2010; 65: 1045-1052.

9. Sonnappa S, Bastardo CM, Wade A, Saglani S, McKenzie SA, Bush A, Aurora P. Symptom-pattern phenotype and pulmonary function in preschool wheezers. *J Allergy Clin Immunol* 2010; 126: 519-526.

10. Aurora P, Stanojevic S, Wade A, Oliver C, Kozlowska W, Lum S, Bush A, Price J, Carr SB, Shankar A, Stocks J. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2011; 183: 752-758.

11. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis, online supplement (http://ajrccm.atsjournals.org/cgi/data/171/3/249/DC1/1). *Am J Respir Crit Care Med* 2005; 171: 249-256.

12. Lum S, Kirkby J, Welsh L, Marlow N, Hennessy E, Stocks J. Nature and severity of lung function abnormalities in extremely pre-term children at 11 years of age. *Eur Respir J* 2011; 37: 1199-1207.

13. Cole TJ, Stanojevic S, Stocks J, Coates AL, Hankinson JL, Wade AM. Age- and size-related reference ranges: a case study of spirometry through childhood and adulthood. *Stat Med* 2009; 28: 880-898.

14. Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, Rosenthal M, Corey M, Lebecque P, Cole TJ. Reference ranges for spirometry across all ages: a new approach. *Am J Respir Crit Care Med* 2008; 177: 253-260.

15. Stanojevic S, Wade A, Cole TJ, Lum S, Custovic A, Silverman M, Hall GL, Welsh L, Kirkby J, Nystad W, Badier M, Davis S, Turner S, Piccioni P, Vilozni D, Eigen H, Vlachos-Mayer H, Zheng J, Tomalak W, Jones M, Hankinson JL, Stocks J. Spirometry centile charts for young Caucasian children: the Asthma UK Collaborative Initiative. *Am J Respir Crit Care Med* 2009; 180: 547-552.

16. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992; 11: 1305-1319.

17. Rigby RA, Stasinopoulos DM. Smooth centile curves for skew and kurtotic data modelled using the Box-Cox power exponential distribution. *Stat Med* 2004; 23: 3053-3076.

18. Royston P, Altman DG. Regression Using Fractional Polynomials of Continuous Covariates: Parsimonious Parametric Modelling. *Applied Statistics* 1994; 43: 429-467.

19. Schwarz G. Estimating the dimension of a model. *Ann Stat* 1978; 6: 461-464.

20. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver B, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J. Multi-ethnic reference values for spirometry for the 3-95 year age range: the global lung function 2012 equations. *Eur Respir J* 2012.

21. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 1998; 17: 407-429.

22. Stocks J, Quanjer PH. Reference values for residual volume, functional residual capacity and total lung capacity. *Eur Respir J* 1995; 8: 492-506.

23. Robinson PD, Latzin P, Gustafsson PM. Multiple-breath washout. In: Frey U, Merkus PJ, editors. Paediatric Lung Function. European Respiratory Society Journals Ltd., 2010: 87-104.

24. Lum S, Stocks J. Forced expiratory manoeuvres. In: Merkus P, Frey U, editors. Paediatric lung function. ERS Journals Ltd, 2010: 46-65.

25. Fuchs O, Latzin P, Thamrin C, Stern G, Frischknecht P, Singer F, Kieninger E, Proietti E, Riedel T, Frey U. Normative data for lung function and exhaled nitric oxide in unsedated healthy infants. *Eur Respir J* 2011; 37: 1208-1216.

26. Hulskamp G, Lum S, Stocks J, Wade A, Hoo AF, Costeloe K, Hawdon J, Deeptha K, Pillow JJ. Association of prematurity, lung disease and body size with lung volume and ventilation inhomogeneity in unsedated neonates: a multicentre study. *Thorax* 2009; 64: 240-245.

27. Sonnappa S, Bastardo C, Stafler P, Bush A, Aurora P, Stocks J. Ethnic differences in FeNO and lung function in healthy young children. *Chest* 2011; 140: 1325-1331.

28. Fuchs SI, Sturz J, Junge S, Ballmann M, Gappa M. A novel sidestream ultrasonic flow sensor for multiple breath washout in children. *Pediatr Pulmonol* 2008; 43: 731-738.

29. Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, Davies JC, Cunningham S, Alton EW, Innes JA. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. *Thorax* 2008; 63: 135-140.

30. Latzin P, Roth S, Thamrin C, Hutten GJ, Pramana I, Kuehni CE, Casaulta C, Nelle M, Riedel T, Frey U. Lung volume, breathing pattern and ventilation inhomogeneity in preterm and term infants. *PLoS One* 2009; 4: e4635.

31. Chakr VC, Llapur CJ, Sarria EE, Mattiello R, Kisling J, Tiller C, Kimmel R, Poindexter B, Tepper RS. Ventilation homogeneity improves with growth early in life. *Pediatr Pulmonol* 2012; 47: 373-380.

32. Fuchs SI, Eder J, Ellemunter H, Gappa M. Lung clearance index: Normal values, repeatability, and reproducibility in healthy children and adolescents. *Pediatr Pulmonol* 2009; 44: 1180-1185.

33. Verbanck S, Thompson BR, Schuermans D, Kalsi HS, Biddiscombe MF, Stuart-Andrews et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. Thorax. DOI: 10.1136/thoraxjnl-2011-201484

34. Stocks J, Hislop AA. Structure and function of the respiratory system: Developmental aspects and their relevance to aerosol therapy. In: Bisgaard H, O'Callaghan C, Smaldone GC, editors. Drug Delivery to the Lung: Clinical Aspects. New York: Marcel Dekker, Inc., 2001: 47-104.

35. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E, Pillow JJ, Seddon PC, Silverman M, Sly PD, Stocks J, Tepper RS, Vilozni D, Wilson NM. An official american thoracic society/european respiratory society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007; 175: 1304-1345.

36. Haidopoulou K, Lum S, Stocks J, Turcu S, Guinard C, Aurora P, Sonnappa S. Alveolar LCI vs. standard LCI in detecting early CF lung disease. *Respir Physiol Neurobiol* 2012; 180: 247-251.

37. Schulzke SM, Hall GL, Nathan EA, Simmer K, Nolan G, Pillow JJ. Lung volume and ventilation inhomogeneity in preterm infants at 15-18 months corrected age. *J Pediatr* 2010; 156: 542-549.

38. Bouhuys A, van Lennep HJ. Effect of body posture on gas distribution in the lungs. *J Appl Physiol* 1962; 17: 38-42.

39. Gronkvist M, Bergsten E, Gustafsson PM. Effects of body posture and tidal volume on inter- and intraregional ventilation distribution in healthy men. *J Appl Physiol* 2002; 92: 634-642.

40. Aljassim F, Lindblad A, Gustafsson PM. Effects of body posture on FRC and LCI in CF chilldren. Am J Respir Crit Care Med 179, A1776. 2009.

41. Lutterbey G, Wattjes MP, Doerr D, Fischer NJ, Gieseke J, Jr., Schild HH. Atelectasis in children undergoing either propofol infusion or positive pressure ventilation anesthesia for magnetic resonance imaging. *Paediatr Anaesth* 2007; 17: 121-125.

42. Sarria EE, Mattiello R, Rao L, Wanner MR, Raske ME, Tiller C, Kimmel R, Tepper RS. Computed tomography score and pulmonary function in infants with chronic lung disease of infancy. *Eur Respir J* 2011; 38: 918-923.

43. Jackson EA, Rabbette PS, Dezateux CA, Hatch DJ, Stocks J. The effect of triclofos sodium sedation on respiratory rate, oxygen saturation and heart rate in infants and young children. *Pediatr Pulmonol* 1991; 10: 40-45.

44. Rabbette PS, Dezateux CA, Fletcher ME, Costeloe KL, Stocks J. Influence of sedation on the Hering-Breuer Inflation Reflex in healthy infants. *Pediatr Pulmonol* 1991; 11: 217-222.

45. Kirkby J, Aurora P, Spencer H, Rees S, Sonnappa S, Stocks J. 'Stitching and Switching': The Impact of discontinuous lung function reference equations. *Eur Respir J* 2012; 39: 1256-1257.

46. Stanojevic S, Wade A, Stocks J. Reference values for lung function: past, present and future. *Eur Respir J* 2010; 36: 12-19.

47. Fuchs SI, Buess C, Gappa M. In vvitro validation of nitrogen multiple breath washout using ultrasonic equipment. Eur Respir J 38[Suppl 55], 355s. 2011.

48. Singer F, Abbas C, Stern G, Kieninger E, Fuchs O, Regamey N, Gustafsson P, Casaulta C, Frey U, Latzin P. Double tracer gas single breath washout - comparison with conventional lung function tests in children with and without cystic fibrosis. Eur Respir J 38[Suppl 55], 354s. 2011.

49. Viklund E, Lindblad A, Robinson PD, Houltz B, Bergh L, Rosberg M, Gustafsson P. Comparison of a new nitrogen multiple breath washout method to mass spectrometer SF6 washout in cystic fibrosis subjects. Eur Respir J 38[Suppl 55], 354s. 2011.

FIGURE LEGENDS

Figure 1: LCI from infancy to 19 years of age.

Footnote: Solid line denotes the predicted (50th centile) LCI for height and the dashed lines denote the upper (ULN 97.5[th] centile) and lower limits of the normal (LLN 2.5[th] centile) range.
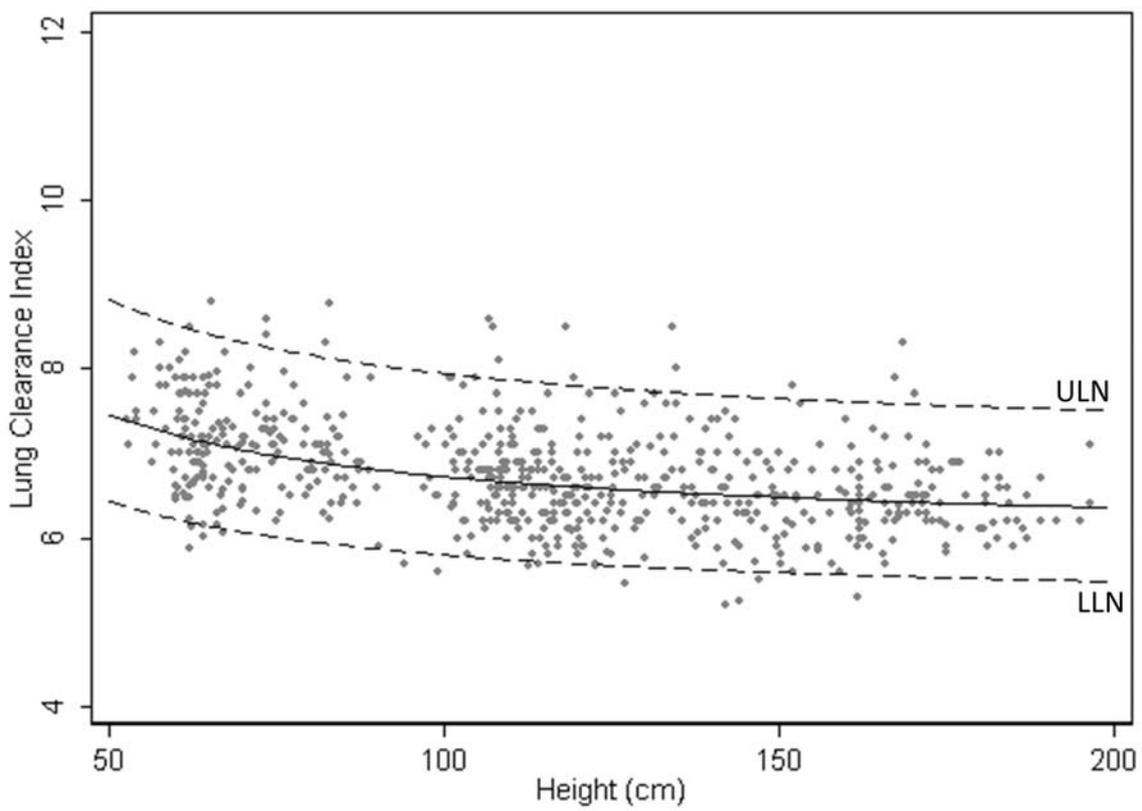

Figure 1

Figure 2: LCI plotted against age in subjects above 6 years of age

Footnote: when analysis was limited to children older than 6 years, LCI was independent of both age and height, such that a constant ULN of 7.56 could be used for cross-sectional assessments between 6-19 years.
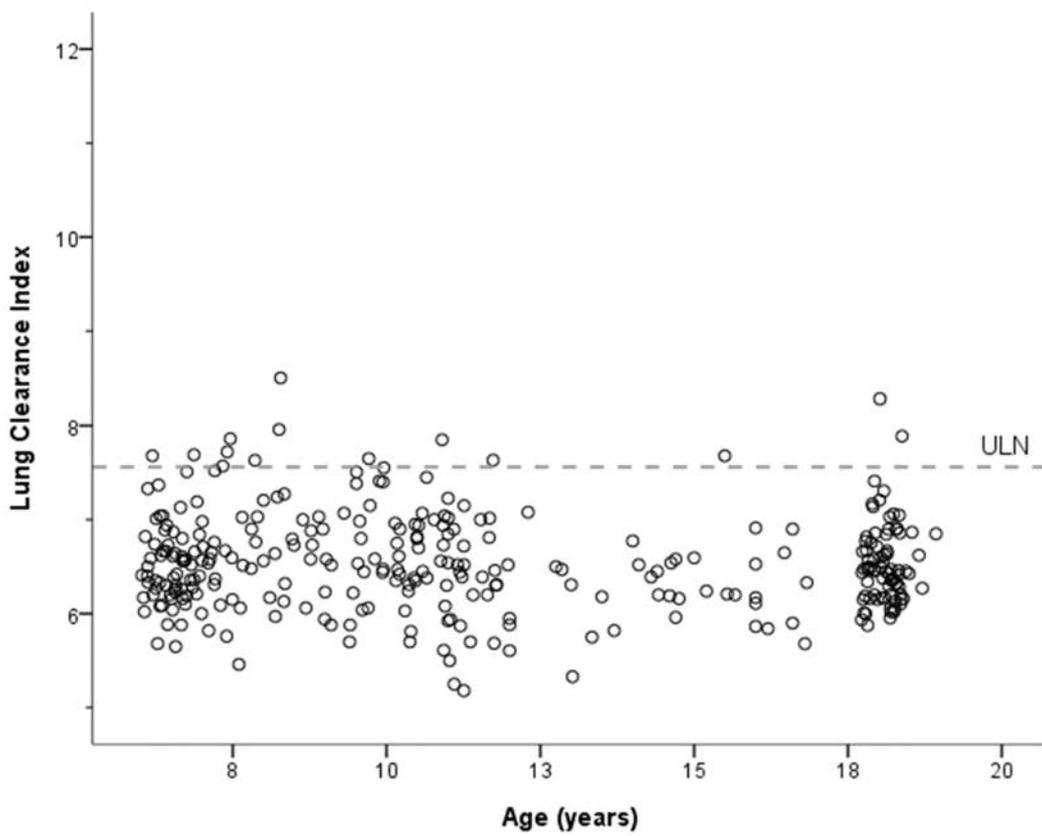
Figure 2

Figure 3: Relationship between FRC and body size

Figure 3