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Multiple-breath inert gas washout to detect inhomogeneity of ventilation distribution in preschool children with cystic fibrosis

A thesis submitted by
Paul Aurora
For the Degree of Doctor of Philosophy

From the Portex Department of Anaesthesia,
Intensive Therapy and Respiratory Medicine
Institute of Child Health
University College London Medical School
University of London
2005
Abstract

Measurement of lung function in preschool children (those aged two to five years) is notoriously difficult. The aims of this thesis were to determine whether multiple-breath inert gas washout (MBW) could be performed successfully in preschool children, and whether MBW indices were more sensitive for detecting cystic fibrosis (CF) lung disease in preschool children than spirometry indices.

First, quality control criteria for data collection and interpretation in children with and without CF were examined. 75% of preschool children successfully performed spirometry at first attempt, but adult criteria for start and end of test and reproducibility were inappropriate. Similarly, 79% of preschool children completed MBW at first attempt. The primary outcome measure from MBW (Lung Clearance Index [LCI]) was independent of subject characteristics in healthy preschool and school-age children. Analysis of the progression of the phase III slope through MBW allowed derivation of indices representing conducting zone ($S_{\text{cond}}$) and acinar zone ($S_{\text{acin}}$) inhomogeneity. Volume correction of these indices facilitated comparison between groups.

MBW and spirometry results were compared in 55 school-age and 60 preschool children. Whilst group differences were seen for both tests, 11/22 (50%) of school-age children with CF had normal spirometry, whilst only one (5%) had normal LCI. 26/30 (87%) of preschool children with CF had normal spirometry, whilst only eight (27%) had normal LCI. Virtually all children with CF had raised $S_{\text{cond}}$, with no age relationship seen. $S_{\text{acin}}$ was normal in most younger children with CF, and raised in most aged 10 years and older.

MBW detected lung disease in preschool children with CF more frequently than spirometry. Most young children with CF had evidence of conducting airway disease, whilst acinar zone involvement was predominantly seen in older school-age children. These findings support the hypothesis that MBW will have value as a clinical measure in this patient group.
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Acknowledgements

I am greatly indebted to my two outstanding supervisors, Professors Janet Stocks and Andrew Bush, who assisted me in obtaining funding and facilities to start this project, and who gave me clear and constructive guidance as it developed. Between them, they encouraged me to combine precise methodology with physiological interpretation and clinical application. I am equally indebted to my collaborator and ‘unofficial supervisor’, Dr Per Gustafsson, now of the Queen Silvia Children’s Hospital, Gothenburg. Dr Gustafsson has spent many years developing the multiple-breath washout (MBW) technique, and without his assistance much of this work would have been impossible. Dr Gustafsson assisted me in setting up the MBW apparatus in London, provided me with the necessary software, and expended great effort in teaching me the skills of successful data collection. Just as importantly, he pointed me to the most important unanswered questions regarding interpretation of MBW, and so helped me to focus my work.

This work would not have been possible without the co-operation and assistance of the children who were tested for this study, and their families. I was often awed by their generosity of spirit.

I also thank Ms Cara Oliver, who assisted me for the first two years of data collection, and Ms Clare Saunders, who assisted for the final 12 months. Collecting data from preschool subjects often requires two investigators, and the research presented here would not have been possible without their hard work. In addition, both Cara and Clare assisted greatly with subject recruitment, project administration, and data interpretation. Dr Angie Wade advised me with regard to power calculations, and also with the analyses presented in Section 3.5.6. From late 2002 Ms Lucy Robinson helped with administration. Drs Wanda Kozlowska and Sooky Lum joined the preschool lung function team in 2003, and have provided useful insight regarding data collection and analysis as I wrote up this work. Before them, Drs Anders Lindblad, Henrik Ljungberg, and Greg Chaziparasidis passed through the department, and filled similar roles. More recently, Aidan Laverty, and my great friend Deniz Huseyin meticulously proof-read the penultimate version of this thesis. I would like to thank the clinicians at Great Ormond Street Hospital, who allowed me to recruit many subjects from their cystic fibrosis clinic, and also
thank the other members of the London Collaborative Cystic Fibrosis Study, who also recruited subjects for me. They are: Beryl Adler, Ian Balfour Lynn, Siobhan Carr, Jane Davies, Bob Dinwiddie, Adam Jaffe, John Price, Mark Rosenthal, Gary Ruiz, John Stroobant, Colin Wallis, and Hilary Wyatt.

I also thank the Dunhill Medical Foundation for providing essential funding for me to perform this work, and the British Lung Foundation and the Cystic Fibrosis Trust for funding Cara and Clare respectively.

In January 2002, just 16 months into this PhD study, I was appointed to a consultant post at Great Ormond Street Hospital for Children. Completion of this thesis has only been possible because of the kindness, understanding, and flexibility of all my clinical colleagues in the Cardiothoracic Transplant Team, and in the Respiratory Unit. I would particularly like to thank Professor Martin Elliott, for his official sanction, encouragement, and support, and Dr Mike Burch and Sister Pauline Whitmore, both of whom worked harder than they should have done, simply so that I could complete this work.

There have been tragedies in my life and work whilst I have been working on this project. Rosemary Castle and Gail Slade were both friends as well as colleagues, and both passed away unexpectedly during this period. Soon after I started this project my close friend Graham Lee was killed in an accident. His approach to life was different to my own, as he valued time outside work so greatly. However, he would have understood that I started this research because I wanted to, and completed it for the same reason, despite the obstacles.

There is more to life than work. I got married to Narita very soon after starting this project, and my greatest regret of the last 4½ years is that our evenings and weekends have not been our own. She has been exceptionally patient, as have both our families, and our friends. I am not going to list them all, or apologise to them, but I will try to make it up to them now. In particular, Narita and I will try to rediscover what weekends are for. After completion of data collection it took me many months to reanalyse all the data, and many months more to write this thesis. During this last period my wife has repeatedly offered to toss it into the fire. Having finally completed it, I dedicate it to her, and hope that it survives the flames.
Declaration

Data from 55 school-age children and 84 preschool children are presented in this study. All were tested between September 2000 and November 2003. I undertook measurements in 51 of the school-age children, with the assistance of Dr Anders Lindblad, Ms Cara Oliver and Ms Gail Slade for many. Dr Anders Lindblad undertook measurements in the other four school-age children. I undertook measurements in 78 of the preschool children. For all these measurements I received assistance from either Ms Cara Oliver, Ms Clare Saunders, Dr Anders Lindblad, or Ms Gail Slade. The remaining six children were measured by Dr Wanda Kozlowska (two children), and Drs Henrik Ljungberg, Anders Lindblad, Sooky Lum and Greg Chaziparasidis (one each). These investigators were assisted by Ms Cara Oliver or Ms Clare Saunders. I performed all the MBW data interpretation, and interpreted the spirometry data jointly with Ms Cara Oliver or Ms Clare Saunders. I performed all the statistical analyses presented in this thesis. Figure 1.4 and Figures 1.7 to 1.11 are adapted from original Figures supplied by Professor Manuel Paiva. Figures 1.6, 1.12 and 1.13 are adapted from Figures supplied by Dr Per Gustafsson. Unless indicated otherwise, I prepared all other Figures myself. This work has not been presented in any previous application for a degree.
Publications arising from this thesis


Papers 1-3 are original papers, based on data presented in Chapters 3 and 5, which were peer reviewed. Paper 4 is an invited submission, which nevertheless contains some original data (most of which is presented in Chapter 4) and which was peer reviewed. All publications are presented in the Appendix, in portable document format.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AUC&lt;sub&gt;ROC&lt;/sub&gt;</td>
<td>Area under the receiver-operator characteristic curve</td>
</tr>
<tr>
<td>CDI</td>
<td>Convection dependent inhomogeneity</td>
</tr>
<tr>
<td>CEV</td>
<td>Cumulative expired volume</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CoV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DCDI</td>
<td>Diffusion-convection dependent inhomogeneity</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;T&lt;/sub&gt;</td>
<td>Forced expired volume in &lt;i&gt;T&lt;/i&gt; seconds</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>LCI</td>
<td>Lung Clearance Index</td>
</tr>
<tr>
<td>ln</td>
<td>natural logarithm</td>
</tr>
<tr>
<td>MBW</td>
<td>Inert gas multiple-breath washout</td>
</tr>
<tr>
<td>MEF&lt;sub&gt;25&lt;/sub&gt;</td>
<td>Maximal expiratory flow when 25% of FVC remains to be expired</td>
</tr>
<tr>
<td>MMMEF</td>
<td>Maximum mid-expiratory flow</td>
</tr>
<tr>
<td>MR</td>
<td>Mixing ratio</td>
</tr>
<tr>
<td>RR</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt;</td>
<td>Acinar component of ventilation inhomogeneity</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin_corr&lt;/sub&gt;</td>
<td>Volume corrected S&lt;sub&gt;acin&lt;/sub&gt;</td>
</tr>
<tr>
<td>SBW</td>
<td>Inert gas single-breath washout</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt;</td>
<td>Conductive component of ventilation inhomogeneity</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond_corr&lt;/sub&gt;</td>
<td>Volume corrected S&lt;sub&gt;cond&lt;/sub&gt;</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SIII</td>
<td>Phase III slope</td>
</tr>
<tr>
<td>SnIII</td>
<td>Normalised phase III slope</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TO</td>
<td>Lung volume turnover (calculated as number of FRCs)</td>
</tr>
<tr>
<td>V&lt;sub&gt;D&lt;/sub&gt;</td>
<td>Dead-space volume</td>
</tr>
<tr>
<td>V&lt;sub&gt;Dapp&lt;/sub&gt;</td>
<td>Apparatus dead-space volume</td>
</tr>
<tr>
<td>V&lt;sub&gt;Daw&lt;/sub&gt;</td>
<td>Airway dead-space volume</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>( V_{\text{exp}} )</td>
<td>Expired volume</td>
</tr>
<tr>
<td>( V_T )</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>z-score</td>
<td>Standard deviation score</td>
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</table>
Chapter 1: Introduction

1.1 Lung function testing in preschool children with cystic fibrosis

1.1.1 Introduction

The preschool years, defined as the years between a child’s second and sixth birthdays, have been termed “the silent years” with regard to measurement of lung function. Measurement techniques commonly employed in older children and adults require levels of co-operation and co-ordination that are not possible from preschool subjects. Techniques employed in infants that require subjects to be sedated are similarly impractical in the preschool years. Since the 1980s a few reports have described modifications of lung function techniques for use in preschool children. The majority concluded that the effort required to obtain usable results could not be justified.

This consensus is now changing. The last decade has witnessed a number of studies describing measurement of lung function in preschool children, with some utilising lung function measures as outcomes to differentiate between groups, or to evaluate interventions. The commonest techniques reported have been measures of airway resistance, either measured by body plethysmography or by the interrupter technique, but there have also been recent reports of successful spirometric measurements in this age group. A variety of other techniques, including inert gas multiple-breath washout (MBW), and measures from forced oscillation, have also been reported.

This work is only beginning. Significant hurdles remain before preschool lung function measures can be reliably employed as research tools, and certainly before they can be employed in routine clinical practice. Specifically, it is now necessary to establish age-appropriate standards for data collection and interpretation; and to identify which techniques are of the most value in distinguishing health from disease.
1.1.2 Clinical importance of measuring lung function in preschool children with CF

Cystic fibrosis (CF) is the commonest life-threatening inherited disorder in Caucasians, with an incidence of approximately 1 in 2500 live births. The disease is caused by mutations in a gene on chromosome seven, encoding cystic fibrosis transmembrane conductance regulator (CFTR), which is a polypeptide sited on the apical membrane of epithelial cells. The primary cause of death for people with CF is respiratory failure resulting from chronic suppurative lung disease. CF usually presents in the first two years of life with respiratory symptoms (cough, wheeze, or respiratory failure), failure to thrive, and steatorrhoea. Less common presentations are bowel obstruction (meconium ileus), rectal prolapse, or electrolyte disturbance. A minority of individuals present late, usually with mild disease, and an even smaller minority present with a borderline phenotype, where the diagnosis of CF is uncertain.

The major cause of progressive lung damage is neutrophil-driven chronic bronchocentric inflammation, secondary to chronic bacterial infection. The most common infecting organisms are *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Chronic infection with *P. aeruginosa* is progressively more common as subjects get older, and is the primary infecting organism by the teenage years. It is now recognised that inflammation secondary to *P. aeruginosa* infection is the main cause of CF lung disease, but the reason for the association between abnormal CFTR function and chronic *P. aeruginosa* infection is less clear. Although the CF lung is histologically normal at birth, and appears to remain so for the first few weeks after birth, it is now clear that infants with CF have evidence of airway inflammation as early as 4 weeks after birth.

Recent studies suggest that young children with CF have a more intense inflammatory response to bacterial infection than non-CF subjects. It is still unclear whether this enhanced inflammatory response is present in the absence of bacterial infection. This inflammation can cause airway obstruction, either from intraluminal mucus, or as a result of airway wall thickening, increase in airway smooth muscle, or effects on airway-parenchymal tethering. Studies of pulmonary function in infants with CF have demonstrated abnormal airway function early in life, even in
those infants with no history of respiratory symptoms\textsuperscript{10-15}. These studies are addressed in more detail later in this chapter.

Eradication of early \textit{P. aeruginosa} infection has been shown to increase life expectancy in CF. Once chronic \textit{P. aeruginosa} infection is established, regular control of infection load, and treatment of infective exacerbations improves prognosis\textsuperscript{2,3}. This is achieved by regular use of nebulised anti-pseudomonal antibiotics, and by courses of intravenous anti-pseudomonal antibiotics. The progress of CF lung disease can also be slowed by interventions that improve mucus clearance, such as chest physiotherapy, and inhaled DNase; and by interventions that reduce inflammation, such as oral corticosteroids, or oral ibuprofen. Oral azithromycin has been demonstrated to improve lung function in subjects with CF\textsuperscript{16,17}, but its mode of action is unclear, and may be related to an anti-inflammatory effect rather than an antibiotic effect.

Regular measurement of lung function, usually by spirometry, is an essential component of clinical monitoring of adults and school-age children with CF. Specifically, the forced expired volume in one second (FEV\textsubscript{1}), or its rate of decline is commonly used to monitor disease progression in individuals, to predict prognosis in subjects with advanced lung disease\textsuperscript{18,19}, and as an outcome measure in clinical trials\textsuperscript{16}. Over the last two decades there has been a shift towards closer monitoring and more aggressive treatment of early CF lung disease\textsuperscript{20-22}, and, as a consequence, two major disadvantages to the use of FEV\textsubscript{1} in children with CF have become apparent. First, many school-age children with CF now have FEV\textsubscript{1} within the normal range, even though they probably have lung disease\textsuperscript{23}. Second, reliable forced expiratory manoeuvres are difficult to obtain in children under the age of 5 years, with testing in the infant and preschool age groups being largely confined to specialist laboratories\textsuperscript{24,25}. As interest in monitoring younger patients increases, so does the need for alternative, more sensitive measures of lung function that can be obtained in children of all ages.

One possible reason for the relative insensitivity of FEV\textsubscript{1} for detecting early lung disease in CF is that CF lung disease may commence in the peripheral airways, which contribute little to total airway resistance. For this reason, a variety of alternative lung function measures have been investigated, including measurement
of the ratio of residual volume to total lung capacity, as a marker of hyperinflation; maximal expiratory manoeuvres using gases of different density, the slope of the alveolar plateau from the single-breath inert gas washout, and a variety of indices derived from MBW.

1.1.3 Aim of thesis

The broad aim of this thesis is to investigate whether the efficiency of gas mixing, measured by the inert gas multiple-breath washout technique, could be employed as a measure of lung function in preschool children with CF. Prior to considering this aim, it is necessary to ask a series of questions:

- What do we require of a lung function measure in this population, and how do we assess whether a test meets these criteria?
- Can school-age children perform MBW, and, in those with CF, does it provide more information than spirometry?
- What modifications to data collection and analysis are required for the preschool age group?

The remainder of this chapter discusses what is meant by a lung function test, and how such tests can be modified for the preschool age group. Spirometry and MBW are then described in detail. Finally, the above broad aim is refined into specific hypotheses, aims, and objectives, and the outline of the body of the thesis is presented. A basic description of respiratory physiology, and of the pathophysiology of CF lung disease is provided in the Appendix. Readers who are unfamiliar with these areas are encouraged to read this appendix before addressing the remainder of this chapter.

1.2 What is a lung function test, and what can it tell us?

1.2.1 What is the purpose of a lung function test?

An ideal lung function test should be easy to obtain, and involve no risk to the subject. In order to be of value, it should be able to help diagnosis, assist prognosis, monitor disease progress or measure the effect of therapeutic interventions.
For the researcher, a test should be able to identify differences in lung function between groups, describe longitudinal changes in health and disease, and identify group responses to interventions. For the clinician, it is important to know whether lung function testing is helpful in distinguishing health from disease and is capable of monitoring disease progress in individuals. Ideally, a clinical test should be able to identify an exacerbation of lung disease, and identify whether a therapy has been of benefit. The requirements for clinically useful tests are therefore more stringent than those for research tests, and a test that can identify group differences may be of little value in individual subjects.

1.2.2 Precision and repeatability

To properly interpret any measurement of lung function, the precision of the measurement should be known in health and in disease. This is usually described by the coefficient of variation (CoV). When several measurements are made in succession, the within-subject CoV is calculated as

$$\text{CoV} (%) = 100 \cdot \frac{\text{standard deviation (SD)}}{\text{mean}}$$

To evaluate change following an intervention, the repeatability of the test should be known over the time course of the intervention. If this time period is short enough to encompass a single testing session (e.g. following bronchodilator inhalation) then this is described as the within-occasion repeatability. If the time period encompasses more than one test session, then this is described as the between-occasion repeatability. Both within-occasion and between-occasion repeatability are assessed by calculation of the limits of agreement (LA) of two measurements, where

$$\text{LA} = \text{mean} \pm 2 \cdot \text{SD of the differences between measurements}$$

If a difference between two lung function measurements lies between these limits then it cannot be said with 95% certainty that there has been a change in lung function.

For lung function testing, it is assumed that both the CoV and the within-occasion repeatability measure the stability of the apparatus: and, for tests that require technical expertise on the part of the subject or the operator, the short-term consistency of this expertise. Between-occasion repeatability also measures
biological variability. In subjects where airway lability is part of the clinical condition and where manoeuvres involving rapid changes in lung volume can themselves change airway properties, biological factors can also contribute to instability between measurements on the same occasion. The *between-subject variability* is a measure of differences between individuals within a group. It can be expressed as the group SD. For the researcher studying group differences, knowledge of the precision of a measurement, as well as the between-subject variability, is important for calculating power of study, and for avoiding type 2 error. For the clinician, knowledge of the between-occasion repeatability is essential for interpreting changes over time in the individual.

1.3 Special considerations for lung function testing in the preschool age group

1.3.1 Co-operation or passivity, incentive or distraction?

The majority of lung function tests employed in adults and school-age children (those aged six to 16 years) require a large measure of active co-operation, and a degree of co-ordination. Neither are readily available when studying younger children. In infants and in very young children up to the age of two years this problem can be overcome by performing measurements when the child is asleep. It is then possible to take measurements of tidal breathing, measurements of lung volumes by body plethysmography, or even measures of forced expiration. In preschool children daytime sleep is uncommon, particularly in a laboratory environment, and may be difficult to obtain even with sedation. There are two main methods by which lung function measurements can be obtained in the preschool age group. For measurements that can be obtained during tidal breathing, it may be sufficient to distract the child, for example with music or an entertainment video. In this situation the child is passive whilst the investigator obtains measurements. For measurements that require a degree of co-operation, such as spirometry, some form of incentive is required. In essence the investigator
encourages the child to perform the appropriate manoeuvres by incorporating them into play.

1.3.2 Lung function measurement techniques available for use in preschool children

There is value in classifying the lung function measurement techniques available for use in preschool children into two groups: those either commonly or rarely used in older children and adults.

A. Techniques commonly used in older children and adults:
   -Spirometry
   -Body plethysmography

Techniques in this group have a detailed literature arising from studies of older children and adults. This allows development of standardised methodology for obtaining measurements in older age groups; development of commercially manufactured laboratory equipment, which aids standardisation between centres; and calculation of reference ranges for normal (older) populations. The standardised methodology and commercially available equipment may be modified for use in this younger age group. Reference data from normal older children and adults cannot be extrapolated to normal preschool children, and this issue will also be addressed in this thesis.

The other important feature of these techniques is familiarity, which is particularly important for clinicians who are not specialists in respiratory physiology.

B. Techniques rarely used in older children and adults:
   -Inert gas washout
   -Impulse oscillation technique (IOT), also known as forced oscillation technique (FOT)
   -Interrupter technique (Rint)
   -Tidal breathing analysis
   -Transcutaneous oximetry
   -Objective measurement of breath sounds

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None of these techniques have well established reference ranges for normal populations of adults and older children. The investigations reported in this thesis employ spirometry and multiple-breath inert gas washout. Spirometry is chosen because this is the main tool for monitoring lung function of older children and adults with CF, both in the clinic and in clinical research studies. MBW is selected because there is reason to believe that it may be a more sensitive measure of mild CF lung disease than spirometry. These two techniques are discussed in detail below, whilst the other techniques listed are described more briefly.

1.4 Spirometry

1.4.1 Principles of spirometry

Spirometry is the term applied to the analysis of the volume \textit{versus} time (volume-time) and flow \textit{versus} volume (flow-volume) curves produced when a subject exhales with maximum effort from total lung capacity. The principles of spirometry are described in the Appendix for readers who are unfamiliar with the technique. The reproducibility of spirometry is a result of the physiological phenomenon of flow limitation, first demonstrated by Hyatt and Fry in 1958\textsuperscript{32}. By placing oesophageal catheters in adult volunteers these researchers were able to plot the relationship between intra-thoracic pressure and expiratory flow at different lung volumes. They demonstrated that at all lung volumes expiratory flow became effort independent above a certain minimum intra-thoracic pressure. This maximal flow varied with lung volume, being greater at higher lung volumes, and less at lower lung volumes (Figure 1.1). By plotting the maximal flow against lung volume, it is possible to obtain a normal flow-volume plot, where at the beginning of expiration flow rapidly increases to a peak, but then steadily falls as intra-thoracic volume falls, or expired volume increases. As long as the mechanical properties of the lungs are unchanged, the starting volume of the expiration is unchanged, and the subject generates enough positive intra-thoracic pressure to achieve flow limitation, then the flow-volume and volume-time curves should be identical on repeated measurement.
An explanation for flow limitation has been provided by wave-speed theory\textsuperscript{33}. This "tube-law" states that mean flow through a flexible tube cannot exceed the speed at which the pressure driving that flow can be propagated along that tube. The velocity of pressure propagation is described as the tube wave-speed. During flow limitation the speed of gas molecules flowing in the tube at the site of flow limitation (this site is termed the choke point) equals the wave speed. Therefore, bulk flow is the product of the wave-speed and the cross-sectional area at the choke point. Forced expiratory flows limited by this mechanism are inversely proportional to gas density, and directly proportional to airway wall elastance and cross-sectional area at the choke point,

\[
\text{Bulk flow at wave speed} = (A \cdot 8 \cdot \text{Ptm} \cdot 8 \cdot A^{-1})^{1/2} \cdot (P^{-1})^{1/2} \cdot A^{3/2}
\]
Where $A$ is the cross-sectional area, $\rho$ is the gas density, and $\delta P_{tm} \delta A^{-1}$ is a measure of the tube wall elastance. $P_{tm}$ is determined by the extrinsic properties of the lung:

$$P_{tm} = (P_{alv} - P_{pl}) - (P_{alv} - P_{ib})$$

Where $P_{alv}$ is the alveolar pressure, $P_{pl}$ is pleural pressure, and $P_{ib}$ is the intrabronchial pressure at the choke point. $(P_{alv} - P_{pl})$ represents the elastic recoil pressure of the lungs. $(P_{alv} - P_{ib})$ represents the gas pressure difference from the alveolus to the choke point. This second term is zero under static conditions, meaning that $P_{tm}$ is determined by elastic recoil only. During expiration $P_{alv}$ is greater than $P_{ib}$, $P_{tm}$ decreases, and thus wave-speed decreases. The opposite is the case during inspiration. This explains why flow is more likely to be limited during expiration than inspiration, and also demonstrates that as expiratory flow develops the wave speed decreases until at some point (the initial choke point) the wave speed will begin to limit the real flow. The initial choke point is in the proximal airways (where $P_{tm}$ is lower, and the total cross-sectional area smaller, than at the alveoli), and as forced expiration continues, moves peripherally as the wave-speeds in these airways diminish. Flow measured at the mouth represents the integrated output of all airways. Further exploration of this theory is beyond the scope of this chapter.

A number of technical factors can impact upon reliable measurement of FVC, FEV$\textsubscript{n}$, and maximal expiratory flows. The most important are: ensuring the subject takes a rapid full inspiration prior to expiration; ensuring that flow limitation is achieved; and ensuring that the expiration continues down to residual volume. In addition, it is essential that the measuring equipment is standardised and correctly calibrated. Both the European Respiratory Society and the American Thoracic Society have published standards for the collection and analysis of spirometry manoeuvres$^{34,35}$.

### 1.4.2 Spirometry and lung disease

Characteristic changes to the volume-time and flow-volume curves are produced by intra- and extra-thoracic airway obstruction, as well as by restrictive lung disease and by respiratory muscle weakness.
With mild intra-thoracic airway obstruction, the earliest change seen is development of curvilinearity of the maximal expiratory flow-volume curve, convex to the volume axis. This is seen as a result of reduced expiratory flow, particularly at lower lung volumes, which is also recorded as reduction in maximal expiratory flows. In normal subjects, PEF reflects the characteristics of the largest intra-thoracic airways, while MEF_{50} and MEF_{25} are more influenced by the smaller airways. When airway resistance is increased by disease, PEF, MEF_{50}, and MEF_{25} are all reduced. With moderate or severe intra-thoracic airway obstruction, the reduction in expiratory flow reduces the volume that can be exhaled during any time t, so that FEV\textsubscript{1} is reduced. FVC is relatively preserved, so the ratio FEV\textsubscript{1}/FVC is also reduced. In the healthy adult, approximately 70% of FVC is exhaled during the first second of a maximal forced expiration, so that FEV\textsubscript{1}/FVC in a healthy adult is approximately 70%. In adult clinical practice, FEV\textsubscript{1} has been accepted as the primary outcome measure from spirometry, as it is both reproducible and sensitive to moderate airway obstruction.

FEV\textsubscript{1} is also used as the primary lung function measure in the clinical care of school-age children with CF, and as the primary outcome measure in intervention studies investigating possible treatments for CF lung disease.

Detailed description of the effects of extra-thoracic airway obstruction, restrictive lung disease, and respiratory muscle weakness are beyond the scope of this chapter.

1.4.3 How sensitive is spirometry to mild airway disease?

The rationale for this thesis is that spirometry is insensitive to early CF airway disease in preschool children. The basis for this argument is that airway disease in CF is known to be non-uniformly distributed. First, if flow measured during forced expiration is the integrated output of all airways (see above), then it is possible that minor alterations of airway calibre in some lung regions may be masked by normal function in other regions. Second, airway obstruction in CF is partly due to intraluminal mucus, and this may be dislodged by a forced expiratory manoeuvre. Third, spirometry is insensitive to changes in the peripheral airways, as the large total cross-sectional area in the lung periphery prevents flow limitation in this
region, even in the presence of extensive disease. This concept will be discussed further below. The rationale for this thesis is presented schematically in Figure 1.2.

**Figure 1.2 How sensitive is spirometry?**

- **Legend:** Traditionally, the severity of CF lung disease has been defined by spirometry results, i.e. subjects with slightly abnormal spirometry are defined as having mild lung disease, subjects with poorer spirometry results defined as having moderate disease etc. This approach may have been practical, but it is not logical, as there is no reason to assume that children with normal spirometry results must have normal airways. What if some of these children with normal spirometry results have early or mild disease that would benefit from intervention? These children (shaded area) are completely excluded from the spirometry dose-response curve.
1.5 Spirometric measurement in preschool children

1.5.1 Assessing flow limitation

Spirometry requires the child to inhale deeply, and then exhale, at maximum effort, down to residual volume. The precision of forced expiratory manoeuvres is a result of flow limitation. Achieving flow limitation requires a degree of co-ordinated effort from the child, which may be difficult for preschool children.

Comparison of FVC and FEV₁ results between different expiratory manoeuvres and calculation of variability gives some idea of whether flow limitation has been achieved. Alternatively, for assessment of a single manoeuvre, the transpulmonary pressure during a forced expiration can be measured using an oesophageal catheter. This is an invasive measurement, which is poorly tolerated in children. Another method is to apply a short pulse of negative expiratory pressure (NEP) at the airway opening during a forced expiration. If this increases flow, then flow limitation was not present during the manoeuvre. If this technique is applied to all forced expirations the results obtained from each expiration can then be assessed retrospectively. If flow limitation is not achieved for any expiration, then the results from that expiration can be disregarded. This technique has been applied to a small group of three to five year old children and is reported to be well tolerated, although it is not yet widely employed.

1.5.2 Previous studies in preschool children

There have been a number of publications in the last two decades describing spirometry in the preschool age group. Kanengiser described a retrospective review of all forced expiratory manoeuvres performed by 3 to 5 year old children in one paediatric lung function laboratory between January 1992 and August 1993. 98 children had attempted spirometry during this period. Of these, 88 were able to produce six expiratory manoeuvres that were recorded. However, only 71% of 3 year olds, 48% of 4 year olds, and 62% of 5 year olds were able to produce FVC measurements that met ATS reproducibility criteria, namely that the largest FVC and the second largest FVC varied by 5% or less. Very few of the children could produce reproducible FEV₁ measurements (none of the 3 year olds, 38% of the 4
year olds, and 36% of the 5 year olds). The poor success rate for FEV₁ measurement may indicate that this measure is of limited value in very young children, many of whom have completed a forced expiration in less than 1 second. This explanation is supported by the authors' observation that none of the 3 year olds, 14% of the 4 year olds, and 27% of the 5 year olds were able to exhale for at least 1 second on all 6 of the recorded expiratory manoeuvres.

Crennesse described a retrospective review of 473 children, aged 3 to 5 years, who attempted spirometry for the first time between September 1993 and December 1997. Of these, 267 (56%) were able to produce 2 or more technically acceptable curves. Only 26 of these children were aged less than 4 years. The forced expiratory time (FET) was equal to or less than 1 second in 21% of all tested children. Zapletal described a population of 173 healthy children aged 3 to 6 years, and reported that 62% were able to produce technically acceptable curves.

Two recent studies have described results of spirometry in large populations of healthy young children. Eigen tested 259 healthy children aged 3 to 6 years (mean age 5.0 years) in the community. Of these, 214 (83%), were able to produce satisfactory curves with FET greater than 1 second. From these data, the authors were able to report regression equations for peak flow (PEFR), FVC, FEV₁, MMEF, FEV₁/FVC, and MMEF/FVC. Nystad tested 652 children aged 3-6 years, in the community, of whom 603 (92%) were able to produce technically acceptable curves. Regression equations for PEFR, FVC, FEV₁, and FEV₀.₅ were calculated from these data.

1.5.3 Use of incentive software

Success in achieving maximal forced expiration in preschool children may be increased by the use of incentive spirometry computer programs. These programs display interactive cartoon games in which the task of the game is achieved if the subject produces a forced expiration. Animations can be designed to encourage deep inspiration, rapid expiration, and prolongation of expiration. Manufacturers recommend first teaching the child how to use a mouthpiece, then using an incentive that encourages rapid expiration, then using an incentive that encourages prolonged expiration. These programs do not measure whether flow limitation has
been achieved: this must still be estimated from repeatability of expirations. Nystad used two Jaeger Masterscreen incentive programs to encourage expiration. These were the candles program, in which a high peak flow results in candles being blown out, and the balloon program in which a prolonged expiration causes a balloon to be blown up. Vilozni compared the Jaeger candle incentive alone with a home designed “Spirogame” program. The Spirogame is a complex animation that encourages tidal inspiratory and expiratory breathing, deep inspiration, rapid expiration, and prolonged expiration. The study population was a group of 79 healthy children aged 3 to 6 years, of whom the majority were aged 5 years and older. Success in producing PEFR and \( \text{FEV}_{0.5} \) was similar with both incentive programs (84% and 87% respectively), although success in producing FVC was higher for the Spirogame than for the candle blowing game. This may be because the candle blowing game is designed to encourage rapid expiration rather than prolonged expiration, and would not be expected to promote a full forced expiration when used alone. Interestingly, PEFR and \( \text{FEV}_{0.5} \) results were significantly higher with the candle incentive than with the Spirogame. Gracchi compared spirometry results with and without use of incentive programs in 88 children aged 4 to 8 years, and reported that children produced more reproducible \( \text{FEV}_1 \) and FVC results without incentives. The PEFR results obtained with incentives were significantly higher than those obtained without incentives. However, in the majority of subjects the Jaeger candle program alone was used as an incentive, possibly explaining these results.

1.5.4 Quality control

Many school-age children are unable to meet the quality control criteria published by the European Respiratory Society and the American Thoracic Society, and they are likely to be inappropriate for preschool children also. This issue has never been studied.

1.5.5 Spirometric measurements in this thesis

Spirometry is the gold standard for quantifying lung disease in older children and adults with cystic fibrosis, and spirometric measurements will therefore be employed in this thesis.
However:

a) It will first be necessary to examine whether spirometry can be performed reliably by preschool children, and whether modifications to published quality control criteria are necessary for this age group.

b) It is assumed that spirometry is a poor measure of mild CF lung disease, and other lung function techniques may be better. Ideally, spirometry should be compared with numerous other measures of lung function. In practice it is unlikely that preschool children will tolerate a lengthy protocol, and some selection is required. The rationale for selecting MBW as the most promising alternative technique is now presented.

1.6 Measurement of gas mixing by inert gas washout

1.6.1 The phase III slope

The uniformity of ventilation distribution can be assessed by analysis of exhaled N\textsubscript{2} following inhalation of a single breath of 100% O\textsubscript{2} (Figure 1.3). When exhaled N\textsubscript{2} concentration is plotted against exhaled volume, the resulting curve has three distinct phases. The first two phases represent mixing of inhaled O\textsubscript{2} with resident N\textsubscript{2} in the conducting airways. Phase III represents mixing of inhaled O\textsubscript{2} with resident N\textsubscript{2} in the respiratory zone of the lung. If gas mixing within this zone is homogenous, phase III will have a slope of zero. In human subjects, phase III has a positive slope, indicating inhomogeneity of ventilation distribution.
Figure 1.3 The single-breath nitrogen washout curve

Legend: The plot shows three phases. Phases I and II represent incomplete mixing of expired gas with resident gas in the conducting airways. In a perfectly mixing lung, Phase III represents mixing of inspired gas and resident gas in the lung periphery. In human subjects, mixing of gas in this zone is inhomogeneous, even in health, and the Phase III slope also reflects some gas mixing in conducting airways.

One possible explanation for this positive slope is presented in Figure 1.4. The observation that phase III slopes obtained from human subjects are always positive has a direct implication: whatever the anatomical origin of these units, the best ventilated ones (left unit in Figure 1.4, where $N_2$ concentration is lower) necessarily empty more (with respect to the less well ventilated units) at the beginning of the expiration.
Figure 1.4 Two compartment model of ventilation inhomogeneity

Legend: This schematic diagram provides one possible explanation for the positive slope of phase III during a single breath nitrogen washout. The two balloons represent lung units with differing time-constants for filling and emptying. Prior to the inspiration, both have equal volumes, and equal $N_2$ concentration. During inspiration, the unit on the left expands more quickly, and hence finishes inspiration with a lower $N_2$ concentration than the unit on the right. During expiration the unit on the left empties more rapidly, and hence contributes more to the early part of the phase III slope than the slowly emptying unit on the right. The exhaled $N_2$ concentration therefore continues to slowly rise through the expiration.

It is also possible to perform a single-breath inert gas washout using an inhaled, poorly soluble, inert gas, such as helium, argon, or sulphur hexafluoride. In this case, the shape of the washout curve will be inverted, and the phase III will have a negative slope (Figure 1.5).
Figure 1.5 Single-breath SF₆ washout

Legend: SF₆ concentration plotted against expired volume following a single inhalation of a gas mixture containing SF₆. At the beginning of the expiration the SF₆ concentration in the expired gas is the same as that in the inhaled gas. This falls rapidly as the SF₆ that was inhaled into the periphery of the lung has been diluted with the resident gas. The slope of phase III is negative.

1.6.2 The multiple-breath washout curve

The inert gas multiple-breath washout (MBW) describes the analysis of changes in gas concentration over a series of expirations. Again, this can be performed using resident N₂ as the marker gas, or alternatively an inhaled marker gas can be employed. In the former case, washout is performed with 100% oxygen, which can be provided via a bias flow or demand valve. In the latter case it is first necessary to “wash-in” a tracer gas. Washout can then be performed with air. The most straightforward method for analysing a multiple-breath washout is to study the
progression of the end-tidal marker gas concentration, plotted either against time; breath number; or against an index of cumulative expired volume. This plot is displayed in Figure 1.6.

Figure 1.6 Plot of end-tidal N₂ concentration against cumulative expired volume (expressed as lung volume turnovers) for a multiple-breath nitrogen washout

Legend: CetN₂ = end-tidal N₂ concentration as % of starting concentration.
TO = number of lung volume turnovers, calculated by dividing the cumulative expired volume (CEV) by the functional residual capacity (FRC).
For a perfectly mixing compartment the plot shows exponential decay, and if logCetN₂ is plotted against TO, a linear relationship is seen.

The washout curve in Figure 1.6 is derived from a N₂ MBW performed with 100% O₂. The curve obtained is identical to that obtained if an inert marker gas is first washed in, and washout is then performed with air. A number of indices to describe this curve have been proposed (all of which are essentially measuring similar aspects of airway function)⁴⁷,⁴⁸:
The Lung Clearance Index (LCI) is the number of lung volume turnovers required to reduce end tidal gas concentration to \(1/40^{th}\) of starting value, so that

\[ \text{LCI} = \frac{\text{CEV}}{\text{FRC}} \]

CEV = cumulative expired volume, FRC = functional residual capacity

The Mixing Ratio (MR) is the ratio between the observed and the predicted number of breaths required to reduce the end tidal tracer concentration to \(1/40^{th}\) of starting value, where the predicted number of breaths is that which would be needed to complete the washout in ideal mixing conditions.

The Becklake Index is the number of TO required to wash 90% of FRC free from tracer gas, divided by 0.9

The Multiple Breath Alveolar Mixing Inefficiency (MBAMI) is given by the formula

\[ 100 \cdot (1 - \frac{\text{TO}_{\text{ideal}}}{\text{TO}_{\text{actual}}}) \]

where:  
\[ \text{TO}_{\text{ideal}} = \text{ideal number of TO required to wash 90\% of FRC free of tracer gas} \]

and:  
\[ \text{TO}_{\text{actual}} = \text{actual number of TO required to wash 90\% of FRC free of tracer gas} \]

Moment ratios represent the ratio between the first and zeroth moment of the washout \((M_1 / M_0)\) or the ratio between the second and zeroth moment of the washout \((M_2 / M_0)\)

Where

i) \( M_0 \) is the area under the \([\text{gas}] \) versus \( \text{TO} \) plot

ii) \( M_1 \) is the area under the \([\text{gas}] \cdot \text{TO} \) versus \( \text{TO} \) plot

iii) \( M_2 \) is the area under the \([\text{gas}] \cdot \text{TO}^2 \) versus \( \text{TO} \) plot.

Pulmonary clearance delay (PCD) is given by the formula

\[ 100 \cdot \frac{\text{actual average time a tracer gas molecule remains in the lung} - \text{the ideal time}}{\text{the ideal time}} \]
There have been few studies comparing these indices directly to determine which gives the most information. From the limited published data available, Lung Clearance Index, Mixing Ratio, and moment ratios appear to have similar ability to discriminate between health and disease states.

1.6.3 The progression of the phase III slope during MBW

In the traditional analysis of MBW, only end or mean expired N\(_2\) concentrations are used. When plotted as a function of breath number or lung turnover they are referred to as the expired N\(_2\) washout curve. However, diverse alterations in anatomy or lung function can lead to similar alterations of the N\(_2\) washout curve. In particular, washout curves do not provide information about the mechanisms involved in the production of ventilation inhomogeneity, or the magnitude of lung units involved. However, it has been suggested\(^{49,50}\) that the identification of specific mechanisms could be obtained from the MBW if the phase III slope is also computed for each breath.

The simplest model allowing a quantitative description of transport in parallel units is represented in Figure 1.4, and consists of two compartments. A MBW simulated with the two-compartment model is shown in Figure 1.7. Change in N\(_2\) concentration through the washout can be calculated for each of the two compartments of this model. The washout curve for each unit follows an exponential decay (dashed lines), and the expired N\(_2\) washout curve resulting from the two units combined (solid line) therefore shows a bi-exponential N\(_2\) decay. If the logarithm of the N\(_2\) concentration is plotted against TO, a linear relationship will be seen.
Figure 1.7 Washout curve from simulated MBW based on two compartment model

Legend: This plot is mathematically derived from two compartment model presented in Figure 1.4. The dashed lines represent the exponential decay of gas concentration in each compartment. The solid line is the bi-exponential decay obtained by combining them.

\[ \text{LnN}_2 = \text{natural logarithm of end-tidal } N_2 \text{ concentration} \]
\[ \text{TO} = \text{lung volume turnover} \]

The projected phase III slopes derived from this model are dependent upon the expiratory flow profile of each unit upon emptying. In this respect, the phase III slope not only gives information on spatial, but also on temporal ventilation inhomogeneities. If the two units empty synchronously, the phase III slope is flat, whatever the concentration difference between them.

Figure 1.8 displays simulated phase III slopes as a function of TO for this parallel unit model. The slopes have been corrected for mean mid-phase III gas concentration, to produce normalised phase III slopes (SnIII). Increase of SnIII through the washout can be understood from Figure 1.4. The calculation of the SnIII compares the concentration differences between units to their mean value. As the concentrations decrease exponentially, the relative concentration difference increases progressively. It is maximal when the best-ventilated unit is empty (N₂ concentration equal to zero in that unit).
The model described in Figure 1.4 assumes that ventilation inhomogeneity is generated by differing gas flows to and from parallel units, as a result of differences in time constants. This model predicts that SnIII will increase linearly through the course of an MBW.

**Figure 1.8 Simulated SnIII versus turnover for the two compartment model**

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**Legend:**

TO = Lung volume turnover  
SnIII = phase III slope divided by mean gas concentration over phase III slope

### 1.6.4 The Paiva-Engel model

An alternative model for the generation of ventilation inhomogeneity has been proposed by a series of theoretical and experimental studies reported by Paiva, Engel, and colleagues. This model, based upon morphometric data reported by Weibel, consists of two asymmetric 'trumpets'. The trumpets meet at a branch point, and above this share a common airway (Figure 1.9). Each trumpet increases in cross-sectional area with increasing distance from the branch point, with this cross-sectional area representing both conducting airways and alveoli. Convection and diffusion take place simultaneously in each trumpet.

During a multiple-breath washout of this asymmetrical two-trumpet model, the concentration of inspired gas is higher (and therefore the concentration of resident, tracer gas lower) in the terminal alveoli of the smaller trumpet, as the inspired gas is
able to reach the terminal alveoli of the smaller unit more rapidly by diffusion. The smaller unit is thus 'better ventilated' despite the inspiratory flow (per unit volume) to the two units being equal.

Figure 1.9 The two-trumpet model

Legend: $V_0 =$ resting lung volume (FRC); $V_T =$ tidal volume;
$V_D =$ airway dead-space

The model is based upon two units of differing size, but equal time-constants. Initially, diffusion results in the concentration of tracer gas falling more rapidly in the smaller unit than in the larger unit. As the MBW progresses, the tracer gas concentration at the mouth of the larger unit during expiration ($S_2$) is higher than the concentration within the smaller unit ($S_1$). Tracer gas molecules therefore diffuse from the larger unit into the smaller unit, and the difference in gas concentration between the units no longer rises.

SnIII can be related to the end expiratory $N_2$ concentration difference between the two trumpet units. As the washout progresses this difference initially increases, but then remains constant as the concentration gradient between inspired gas and the resident gas in the two units falls (Figure 1.9). The mechanism for this is a form of
pendelluft. During expiration, tracer gas (e.g. N₂) concentration at the mouth of the larger unit will be higher than tracer gas concentration within the smaller unit. Tracer gas molecules will therefore diffuse into the smaller unit, reducing the difference in gas concentration between the two units. The prediction from this model is that the slope of the phase III will initially rise, but reach asymptote after 4-5 breaths, or 1.5 lung volume turnovers.

This diffusion-convection interaction in an asymmetric acinus results in inhomogeneity of gas concentrations, and a sloping plateau, even when the simulated acinar expansion is uniform. Inequality of airway cross-sectional diameter within the lung periphery also results in intra-acinar inhomogeneity and a sloping plateau. Paiva and Engel proposed that both convection and diffusion-convection interaction generate ventilation inhomogeneity. A washout curve from a MBW simulated with the diffusion-convection interaction model is shown in Figure 1.10.

Figure 1.10 Simulated washout curve based on the two-trumpet model

Legend: LnN₂ = natural logarithm of end-tidal N₂ concentration
TO = lung volume turnover. As for a single compartment model (Figure 1.7), there is exponential decay of the end tidal N₂ concentration. In other words, analysis of the washout curve does not differentiate between inhomogeneity created by the two-compartment model presented in Figure 1.4, and the diffusion-convection interaction model presented in Figure 1.9.
Figure 1.11 displays simulated SnIII as a function of TO for the two-trumpet model.

**Figure 1.11 Simulated SnIII versus TO plot based on the two-trumpet model**

![Graph showing simulated SnIII as a function of TO](image)

*Legend: TO = Lung volume turnover
SnIII = phase III slope divided by mean gas concentration over phase III slope
SnIII increases over the first 1.5 TO, then reaches asymptote*

In summary, the model described by Paiva and Engel describes two mechanisms by which ventilation inhomogeneity is generated during an MBW. Convection dependent inhomogeneity (CDI) is generated by temporal and spatial differences in filling and emptying between parallel units. The transport mechanism bringing inspired O₂ to these lung units is convection, and this mandates that these units should not be too small, otherwise the concentration would equilibrate by molecular diffusion. In adult man, under normal breathing conditions, CDI is thought to take place between lung units at least as big as acini, *i.e.* the set of units subtended by a terminal bronchiole.

The diffusion-convection front arises because of the dramatic increase in total cross-sectional area with increasing airway generation (Figure 1.12). During inspiration, air is initially transported peripherally by convection. As the cross-sectional area increases, so the forward velocity of the gas falls. Eventually,
diffusion takes over as the dominant mechanism of gas transport. The region in which diffusion and convection both play major roles in gas transport is termed the diffusion-convection front. Proximal to this front, convection is the dominant mechanism of gas transport. Distal to this front, diffusion is the dominant mechanism of gas transport.

**Figure 1.12 The diffusion-convection front**

Legend: With increasing airway generation, the total cross-sectional area of the bronchial tree increases. As the cross-sectional area increases convection plays a lesser role in gas mixing, until a point where diffusion plays as large a role in transport of gas molecules as convection. This region is termed the diffusion-convection front.
Diffusion-convection dependent inhomogeneity (DCDI) arises as a consequence of interactions between diffusion and convection in the region of the diffusion-convection front. This inhomogeneity is generated by size differences in units subtended at branch points within the diffusion-convection front, and by differences in airway calibre at these branch points. Any inhomogeneous gas distribution distal to the diffusion-convection front is rapidly eliminated by diffusion.

Crawford et al suggested that DCDI only increases for the first 5 breaths, or 1.5 TO of an MBW\textsuperscript{49}. Therefore, after the fifth breath the increase in SnIII is diffusion independent and represents a measure of CDI. Subtraction of this component from the overall slope leaves DCDI. (Figure 1.13).

The phase III slope of the first breath is almost entirely due to DCDI inhomogeneities. The DCDI component of the phase III slope obtained from breath 1 (which has been labelled S\textsubscript{acin} as an indicator that it represents inhomogeneity generated in or at the entrance to the acinus), is computed by subtracting the CDI contribution. This CDI contribution is computed from the linear portion of the SnIII versus TO relationship.

The advantage of using TO instead of breath number is mainly for MBW comparisons with different lung and/or tidal volumes. S\textsubscript{acin} is therefore an index of diffusion-convection dependent ventilation inhomogeneity, generated in the region of the diffusion-convection front. The CDI contribution to the SnIII versus TO plot, calculated as the normalised slope difference per TO determined by linear regression between TO=1.5 and TO=6, can be used as an index of convection dependent inhomogeneity. This index has been labelled S\textsubscript{cond} as an indicator that it represents inhomogeneity generated in the conducting airways. S\textsubscript{cond} is therefore an index of convection dependent ventilation inhomogeneity, generated proximal to the diffusion-convection front. The derivation of these indices is illustrated in Figure 1.13.
Legend: According to the Paiva-Engel model, CDI has a linear relationship with expired volume (whether expressed as breath number or TO). DCDI rises for the first 5 breaths, or 1.5 turnovers, and then is unchanged. These characteristics allow separation of the $S_{nIII}$ versus breath number (or $S_{nIII}$ versus TO plot) into the two components. The $S_{cond}$ index is calculated as the slope of the $S_{nIII}$ versus turnover CDI relationship. $S_{acin}$ is the DCDI component of the first breath $S_{nIII}$, calculated by subtracting the CDI component (see inset equation).

### 1.6.5 Evidence to support the Paiva-Engel model

When $N_2$ multiple breath washouts were performed in healthy subjects, the observed results matched the diffusion-convection model more closely than they matched the sequential emptying model. Further evidence comes from studies that attempted to reduce or eliminate the contribution of convection dependent inhomogeneity. Crawford et al performed MBW with two tracer gases: sulphur
hexafluoride (SF$_6$) and helium (He). Whilst convection of SF$_6$ and He is similar, the lower molecular weight of He allows it to diffuse more readily than SF$_6$. Any difference in inhomogeneity recorded during simultaneous SF$_6$ and He washouts can be attributed to differences in diffusion-convection interaction, and the impact of this diffusion-convection interaction component on overall inhomogeneity. Crawford demonstrated that the difference between the SnIII for SF$_6$ and He increased for the first five breaths, and then remained constant for the rest of the washout$^{49}$. 

Animal studies, performed in rats$^{56,62}$, and steers$^{63}$, produced SnIII versus TO relationships different from those seen in man. In rats, SnIII increased for the first 3 breaths of the washout and then remained constant, suggesting that CDI plays an insignificant role in the generation of ventilation inhomogeneity in this animal. The anatomy of the rat lung has been described$^{64}$, and this information was used for modelling studies generating predicted SnIII versus TO plots, based on the Paiva-Engel model$^{57}$. As in humans, there was close concordance between results predicted by the model and those obtained experimentally. A recently published study measured the effect of oleic acid infusion into the right atrium of ventilated dogs upon CDI and DCDI during MBW of He. Total ventilation inhomogeneity increased, largely due to increasing DCDI, an observation consistent with the pulmonary oedema that oleic acid infusion induces$^{65}$. 

When single-breath washout (SBW) or MBW is performed in healthy adults, the SnIII increases with increasing pre-inspiratory lung volume, but decreases with breathholding, suggesting that diffusion plays some role in the production of ventilation inhomogeneity$^{54,66,67}$. MBW studies suggest that increasing tidal volume reduces the inhomogeneity due to DCDI, but increases the inhomogeneity due to CDI$^{68}$. 

As the majority of convection dependent inhomogeneity is generated by the effect of gravity, with (in adults) dependent regions being better ventilated, further studies were performed in microgravity. Multiple-breath washout experiments performed in healthy adults during sustained microgravity (during the Spacelab flight Spacelab Life Sciences-2) showed that the plot of SnIII against lung turnover was not
significantly different from that obtained in 1G, suggesting that the mechanical characteristics of the compartments are not gravity dependent. 69.

**1.6.6 Effect of lung disease upon gas washout indices**

Any process that increases resistance of airways or reduces the compliance of the lung will increase the time taken for inhaled gas to equilibrate with the resident gas of the lung. Any process that has uneven effects upon airway resistance or lung compliance (i.e. affecting some areas of the lung more than others) will increase inhomogeneity of ventilation distribution. In both cases, but particularly the latter, the predicted effect is a prolongation of the washout curve. This will result in an increase in the number of lung volume turnovers required to clear resident gas, and an increase in LCI, MR, and moment ratios. 47, 48. Increased inhomogeneity of ventilation distribution will also result in an increase in the phase III slope. 52. The Paiva-Engel model has been applied to study the effects of histamine challenge in asymptomatic non-smoking adults 45 (Figure 1.14) and ventilation inhomogeneity in adults with well controlled asthma, before and after bronchodilator. 70. These studies suggest that asymptomatic individuals who will respond to histamine challenge have abnormal baseline $S_{acin}$ but that histamine challenge predominantly affects $S_{cond}$. Stable asthmatic patients have abnormal $S_{cond}$ and $S_{acin}$, and both these indices improve with bronchodilator. Factor analysis of $S_{acin}$ and $S_{cond}$ alongside a variety of more established lung function measurements in patients with chronic obstructive pulmonary disease (COPD) suggests that $S_{cond}$ is linked to specific airway conductance ($sG_{aw}$) and forced expiratory flows, whereas $S_{acin}$ is linked to diffusion capacity measurements. 71.
Figure 1.14 Effect of histamine challenge on $S_{\text{acin}}$ and $S_{\text{cond}}$

![Figure 1.14 Effect of histamine challenge on $S_{\text{acin}}$ and $S_{\text{cond}}$]

Legend: Closed markers represent SnIII prior to histamine challenge, open circles represent SnIII post challenge. There is little change in $S_{\text{acin}}$ (total SnIII for breath 1 does not change) but marked increase in $S_{\text{cond}}$. From Verbanck et al.51.

1.7 Measurement of gas mixing by multiple-breath inert gas washout in preschool children

1.7.1 MBW studies in preschool children

The use of this technique in children is largely confined to the measurement of indices from MBW rather than SBW. There have been three previous studies of MBW involving preschool children. In 1985, Couriel reported MBW in 58 healthy children and 24 children with CF aged 3.9 to 6.8 years.72 The test was performed...
using a mouthpiece and nose-clip apparatus, and the authors reported great
difficulty in collecting adequate measurements in this age group\textsuperscript{72}. Acceptable
results were obtained in only 40 children (49\%) of whom only 10 were aged less
than 5 years (29\% of the 34 children in this age group who attempted the test).
Moment ratios obtained in children with CF were significantly higher than those
obtained in controls, and moment ratios obtained in children with CF who had
evidence of severe lung disease were significantly higher than those obtained in
children with fewer symptoms. In the same year, Wall reported measurements in 36
healthy children and 10 children with CF aged 3-6 years. Again, a mouthpiece and
nose-clip apparatus was employed, but in this study the children were distracted by
means of a portable music system and headphones. Success rates from this study
were not presented. Children with CF had significantly higher moment ratios than
healthy children, and there was a negative relationship ($r^2 = 0.49$) between $M_1/M_0$
and Shwachman score in the children with CF. In the discussion of these results, the
author speculated that use of a facemask system, sealed with putty, would allow
MBW measurement in even younger children. Recently Gustafsson has reported
MBW and spirometry in 43 children with CF and 28 healthy children\textsuperscript{27}. Eight of
these children were aged 6 years or less, and used a facemask apparatus rather than
the mouthpiece and nose-clip used by the older children. Main outcome measures
were MR and LCI from MBW, and FEV\textsubscript{1} and MEF\textsubscript{25} from spirometry. The
majority of children with CF had normal spirometry results, but many of these had
abnormal LCI or MR.

There are no data on SnIII analysis from MBW in children.

\textbf{1.7.2 MBW studies in older children with CF}

In addition to the above, several previous studies, mostly performed during the
1980s and early 1990s, have investigated ventilation distribution in school-age and
adult CF subjects using this or related methods. These studies have already shown
that indices of ventilation inhomogeneity are raised in CF subjects compared with
healthy subjects\textsuperscript{26,28,29,72-75}, that ventilation inhomogeneity is correlated to airway
resistance\textsuperscript{28} and FEV\textsubscript{1}\textsuperscript{75} in CF subjects. MBW has also been performed in healthy
pre-term infants, and in infants with lung disease\textsuperscript{15,76}.
1.7.3 SBW studies in children

Three studies of SBW analysis in school-age children have been reported. These studies were performed in healthy children\textsuperscript{77}, children with asthma\textsuperscript{78}, and children with CF\textsuperscript{26}. In this last study, younger children with CF were noted to have reversal of the normal SF\textsubscript{6}-He phase III slope relationship (i.e. the slope of phase III was steeper for He than for SF\textsubscript{6}) indicating that airways in the region of the He diffusion convection front were involved in the pathological process.

1.8 Other methods for measurement of lung function in preschool children

1.8.1 Body plethysmography

The mechanical properties of the lung can be described by the resistance (R), and the compliance (C). The reciprocal of the resistance is the conductance (G). A suffix is added to describe which component of the respiratory system is measured (\textsubscript{aw} for airway, \textsubscript{L} for lung, and \textsubscript{rs} for respiratory system). Airway resistance (R\textsubscript{aw}) and thoracic gas volume (TGV) may be calculated from the relationship between airway flow and plethysmographic volume. The subject is required to sit inside a body plethysmograph, whilst breathing heated and humidified air. The airflow at the mouth is measured by a pneumotachograph, whilst the change in volume and pressure inside the plethysmograph is measured. The principle behind the measurement is that movement of gas from lungs to box results in no change in total volume (provided there is no change in temperature), and so the change in box pressure during breathing is related to volume change of the lungs. The total gas volume can be calculated via a Pfueger manoeuvre, where the airway is temporarily occluded with a shutter and variations in mouth pressure and in thoracic volume are recorded. The thoracic volume can be derived from Boyle’s Law:

\[
P_1 \times V_1 = P_2 \times V_2
\]

where \(P_1\) and \(V_1\) denote the pressure and volume respectively of the gas at condition 1, and \(P_2\) and \(V_2\) denote the pressure and volume respectively of the gas at condition 2. If the volume of thoracic gas is known, the observed change in box
pressure can be used to calculate the change in alveolar pressure. Dividing change
in alveolar pressure by change in flow at the mouth gives $R_{aw}$. Measurements at
different lung volumes in a body plethysmograph have demonstrated that $R_{aw}$ has a
curvilinear negative relationship with lung volume, increasing greatly as RV is
approached. If the result of the measurement is specific to the lung volume at which
it is measured, then the term ‘specific’ is added to the description, and the prefix ‘s’
added to the abbreviation. Thus specific airway resistance is denoted $sR_{aw}$.
Calculation of TGV and $R_{aw}$ can be difficult in young children, who may have
difficulty with breathing against the airway occlusion. More than 20 years ago Dab
and Alexander\textsuperscript{79,80} proposed a simplified method for calculating specific airway
resistance ($sR_{aw}$) which does not require the measurement of TGV, and which can
be measured during tidal breathing through a mouthpiece:

$$sR_{aw} = \frac{\Delta P_{box}}{\Delta V'} \times (P_{bar} - P_{H2O})$$

where $\Delta P_{box}$ and $\Delta V'$ represent changes in plethysmographic pressure and volume
flow respectively; and $P_{bar} - P_{H2O}$ is barometric pressure minus water vapour
pressure in the lung. Many modern plethysmographs allow electronic compensation
for temperature, pressure, and humidity, and this has been proposed as an
alternative to using heated humidified air or to asking the subject to pant.
A number of reports have employed measurement of $sR_{aw}$ as an outcome measure
in short term intervention studies in preschool children\textsuperscript{81-83}, sometimes in
combination with other lung function tests (below).

\textbf{1.8.2 Impulse oscillation technique (IOT)}

This technique is also known as the forced oscillation technique (FOT). The
technique is based upon the observation that the mechanical characteristics of a
system may be calculated by relating the stress applied to the system to the resultant
deforestation. The mechanical characteristics of the lung can therefore be calculated
by applying a stress to the lung (in the form of a pressure signal) and measuring the
deforation (the change in size and shape) that results. This deforation can be
measured as a flow signal at the mouth. The technique is analogous to listening for
an echo, except that as the lung is a compliant rather than a rigid structure, the echo
is in the form of a flow signal rather than a pressure signal.
During breathing, pressure is being generated by the respiratory muscles to produce deformation of the lung, and flow at the airway. Rather than interrupt breathing to obtain IOT measurements, the applied transpulmonary pressure can be varied in a frequency domain different from that of respiratory muscle activity. This allows the flow signals arising from the respiratory muscle activity and the applied pressure to be separated, and the mechanics related to the applied transpulmonary pressure to be studied.

In practice, the frequency employed for IOT measurements is 6Hz or greater. This signal is generated by computer and delivered through one or more loudspeakers placed at the mouth or at the chest, or alternatively is delivered through a headbox. The angular velocity and frequency of applied pressure and resultant flow are measured. These measurements allow calculation of the respiratory resistance $(R_{rs})$ and the respiratory reactance. The latter measure is related to the respiratory impedance and reflects the apparent elasticity of the respiratory system.

A number of methodological issues with this technique have yet to be resolved, and as yet the methodology and equipment for performing the IOT have not been standardised. There are no reference data for normal children. However, a number of centres have compared IOT with plethysmography and other techniques in young children following methacholine challenge$^{81,84}$, cold air challenge$^{85}$ or administration of bronchodilator$^{86}$. Data from adults in which IOT measurements (and Rint measurements) were compared with the presumed gold standards of total airway resistance $(R_{aw})$ measured during panting manoeuvres in a body plethysmograph, and total lung resistance measured with an oesophageal catheter, suggest that both $R_{rs}$ and Rint (described below) are less sensitive at detecting induced airways narrowing than the more established methods$^{87}$.

1.8.3 Interrupter technique (Rint)

This technique is based upon the assumption that change in transpulmonary pressure observed immediately after sudden occlusion of the airway is entirely explained by cessation of flow. Resistance of the respiratory system can then be calculated from change in pressure during the interruption and flow immediately preceding the interruption. If this technique is to provide an accurate measure of
airways resistance it is assumed that pressure measured at the mouth represents pressure throughout the airways, i.e. that pressure at the mouth equilibrates with pressure at the alveoli within the time that the shutter is closed. As the duration of the occlusion (in most studies) is 100ms, this assumption is probably invalid, particularly in the presence of airway obstruction. However, as with other techniques described here, the true value of the technique to the clinician is whether it can distinguish between health and disease, not whether a single factor of respiratory physiology is accurately represented. Early studies of this technique were performed in animals, and in awake and anaesthetised human adults. However, the technique has never been widely used in adults, and it was only with the realisation that it may be of value in performing measurements in infants and young children that research interest increased. Early studies in children suggested that $R_{\text{int}}$ correlates with $R_{\text{aw}}$ and $\text{FEV}_1$ in children with asthma and cystic fibrosis and that the technique was well tolerated even in 3 year old children. $R_{\text{int}}$ appears able to discriminate between asthmatic children, children with chronic cough, and control children both at baseline and following bronchodilator administration, and is able to detect response to exercise challenge in children with asthma. Reference data for $R_{\text{int}}$ in preschool children have been published, and data on between-occasion repeatability are also available.

Studies comparing $R_{\text{int}}$ with alternative measures of lung function during methacholine challenge have produced conflicting results. There is currently great interest in this technique, largely due to the recent introduction of a portable, commercially available interrupter device. However, the potential clinical applications of the technique have not yet been fully determined.

1.8.4 Analysis of tidal breathing

This technique is based upon the observation that a characteristic pattern of expiratory flow is seen in airways obstruction, even during tidal breathing. The first detailed analysis of this technique was reported in 1981, though the technique had been described earlier. The technique has been extensively studied in sleeping infants. In preschool children, the technique is reported to be well tolerated,
and the ratio of time needed to reach maximal tidal expiratory flow to the total
expiratory time (T\textsubscript{MEF}/T\textsubscript{E}) is able to distinguish between normal children and
children with CF or asthma\(^{102}\). Further data are awaited.

1.8.5 Transcutaneous measurement of oxygen tension

The transcutaneous measurement of partial pressure of oxygen in the capillaries
(tcPO\(_2\)) is easy to perform and well tolerated in young children. The measure is a
sensitive and repeatable means of detecting changes following methacholine
challenge in young asthmatic children\(^{92,96,103}\). The technique does not simply
measure changes in airway resistance during bronchoconstriction, and correlates
poorly with other measures of lung function\(^{92,104}\). It is likely that a fall in tcPO\(_2\)
represents an increase in ventilation perfusion inequality (VQ mismatch) following
bronchodilator challenge.

1.8.6 Objective measurement of breath sounds

Breath sounds have long been used by clinicians to detect changes in airway calibre
during acute exacerbations of asthma, or during bronchodilator challenge\(^{105,106}\).
Objective analysis of breath sounds involves recording sounds at the chest wall and
analysing frequency and power spectra. Data thus far have been mixed, but some
reports suggest that changes in vesicular breath sounds are of value in detecting
changes in airway calibre during methacholine challenge in children with asthma\(^{107}\).

1.8.7 Lung function studies in preschool children with CF

Spirometry and MBW studies of preschool children with CF have been described in
Sections 1.5.2, and 1.7.1. In addition, there have been a small number of studies
utilising other lung function techniques.

In 2002, Beydon\(^{108}\) described measurement of FRC (by He dilution) and Rint in 40
preschool children with CF, and 79 healthy controls. Thirty-nine children were able
to complete the measurements. Children with CF had significantly higher Rint, and
significantly lower interrupter conductance (G\textsubscript{int}), but FRC results were identical
for the CF and control groups. Children with CF who had a history of respiratory
symptoms (n=31) had significantly lower G\textsubscript{int}, and significantly higher FRC
standard deviation score (z-score), than those children with CF who had no history
of respiratory symptoms (n=8). When individual data were analysed, 9 of the 39 children with CF (23%) had a Rint z-score greater than +2. sRaw has been utilised as an outcome measure in one longitudinal study of young children with CF, published by Nielsen in 2004. In this latter study, 30 children with CF - of whom 13 were aged 6.0 years or less at the start of the study - were repeatedly tested over a four-year period. Seventeen of the 30 children (57%) in Nielsen’s study had raised sRaw at commencement, whereas none had raised Rint, only one had raised respiratory reactance, and only two had raised respiratory resistance (both latter measured by the impulse oscillation technique). Group mean sRaw was persistently abnormal over the four-year period, whereas the other measures tested were not consistently abnormal.

Comparison of these studies with those described earlier is complicated by differences in the lung function tests employed, and by differences in the study populations. The care of children with CF has changed in the last two decades, and populations measured in the early 1990s cannot be compared with populations studied in the last few years. In addition, the term preschool, though widely used, does not have a universally accepted definition. Although PubMed defines a preschool child as one aged between two to five years, in the studies quoted above 3 groups studied children aged three to six years but did not define them as preschool, one group studied children aged three to eight years and described them as preschool, whilst one studied children aged two to eight years, but did not describe them as preschool.

1.9 MBW as an outcome measure in young children with CF

There are a number of alternative measures of lung function that could have been examined in this thesis. MBW was chosen as the main outcome measure because:

a) CF is a heterogeneous disease. Whilst most lung function measures listed above will not detect inhomogeneous changes in lung function (indeed, abnormalities in some regions can be masked by normal function in other regions), MBW is predicted to be a sensitive marker of heterogeneous change.
b) Whilst most of the measures described above are determined by large, central airway function, CF lung disease is also known to affect the small, peripheral airways. MBW is predicted to detect heterogeneous change in the lung periphery, as distal as the location of the diffusion-convection front.

c) MBW is measured during tidal breathing, which should not dislodge intraluminal mucus. In CF the presence of mucus is of clinical importance, as it is the cycle of infection and inflammatory response to infection that leads to bronchiectasis.

1.10 Rationale for study, aims and hypotheses

1.10.1 Starting aim of study

In Section 1 of this chapter, the starting aim for this study was presented as: “to investigate whether the efficiency of gas mixing, measured by the inert gas multiple-breath washout technique, could be employed as a measure of lung function in preschool children with CF.” This general aim must now be distilled into an answerable study question, informed by the review of data presented in this chapter.

1.10.2 Choice of outcome measures, and quality control

The FEV₁, obtained from spirometry, is by far the most commonly used lung function measure in children and adults with CF. Recent reports have suggested that spirometry is possible in preschool children. It is therefore necessary that MBW in preschool children be measured against spirometry.

Although a small number of studies have been published, the application of spirometry in the preschool age group has not been studied critically. In particular, published standards for spirometry data collection and analysis are specific to adults, and have already been demonstrated to be unsuitable for school-age children. A detailed analysis of quality control criteria for spirometry in preschool children has never been undertaken.

Similarly, almost all previous MBW studies have been performed in school-age children or adults. The Paiva-Engels analysis has never been tested in the preschool age group.
Specific outcome measures from spirometry will be selected after quality control analysis is completed, as one of the hypotheses (below) is that FEV₁ may not be a suitable outcome measure in this age group. For MBW, the Lung Clearance Index (LCI) is selected as the basic outcome measure from washout curve analysis (Section 1.6.2). LCI is selected because it is easier to comprehend than the other indices described, and because in a preliminary analysis, presented in the Appendix, it was as sensitive in discriminating between school-age children with CF and healthy school-age children as the Mixing Ratio or Moment Ratios. It is also proposed that S₁ and S₂ be calculated in preschool children, but it will first be necessary to investigate whether some of the assumptions of this analysis are applicable to the preschool age group.

1.10.3 Study design, and method of comparison

To fully answer the general aim of this study, it is necessary to determine that preschool children can perform MBW; and that indices from MBW are more sensitive than spirometry indices for detecting early CF lung disease. Although the first question is relatively straightforward, the second is complicated by the lack of a “gold standard” for defining CF lung disease. If the presence or absence of lung disease cannot be confirmed, then it is not possible to determine whether MBW is able to detect disease more reliably than spirometry. The best means of answering this question is by a series of studies, investigating:

- Whether indices derived from MBW are more commonly abnormal in children with CF than spirometry indices
- The relationship between MBW indices and spirometry indices
- The relationships between these lung function indices and clinical information (i.e. symptoms, clinical examination, radiology findings)
- The relationship between lung function indices and measures of inflammation or infection
- The effect of CF treatment upon MBW indices
- The change in MBW indices over time

Given that these studies must be preceded by pilot studies to establish methodology, and by quality control analyses, it is clear that this series of studies is beyond the
scope of a single research thesis. It is therefore proposed that this thesis will first address methodology and quality control, and then establish the relationship between spirometry and MBW. Studies will be performed first in school-age children, to establish the technique, and then in preschool children. Relationships between lung function measures and clinical status will be performed as a secondary analysis. The possible design of future longitudinal and intervention studies will be discussed at the end of the thesis.

1.10.4 Study hypotheses, aims, and objectives

Primary hypotheses:

- More children with CF will have abnormal washout results than will have abnormal spirometry results.
- Children with CF with abnormal washout curve results will also have abnormal phase III slope results. These children will have evidence of inhomogeneity arising both from convective flow (i.e. $S_{\text{cond}}$), and from the interaction between diffusion and convection (i.e. $S_{\text{acin}}$). Both measures of inhomogeneity will be greater in older children.

Prior to undertaking these investigations, it is necessary to undertake a series of quality control analyses for both spirometry and MBW:

Preliminary hypotheses:

- Children aged two to five years are unable to meet some of the quality control criteria for spirometry, but alternative criteria may be feasible.
- In healthy subjects, the LCI is independent of subject age, age group (i.e. preschool or school-age), sex, and body size. In all subjects, the LCI is independent of variations in respiratory rate, tidal volume, and functional residual capacity seen during spontaneous tidal breathing.
- In healthy subjects, the phase III slope is negatively related to tidal volume, but otherwise independent of subject age, age group, sex, and body size. In all subjects, the phase III slope is negatively related to tidal volume, but is
independent of variations in respiratory rate and functional residual capacity seen during spontaneous tidal breathing.

**Aims:**

- To determine whether preschool children can perform spirometry, and how best to analyse the data
- To determine in preschool children the precision of LCI and the phase III slope, and to determine any modifications to the $S_{cond}$ and $S_{acin}$ analysis necessary in this age-group
- To compare the sensitivity of MBW and spirometry for detecting abnormal lung function in preschool children with CF
- To determine whether the predominant mechanism for ventilation inhomogeneity in preschool children with CF is that related to convection \((i.e. \ CDI, \ or \ S_{cond})\), or to the interaction between diffusion and convection \((i.e. \ DCDI, \ or \ S_{acin})\), or whether both mechanisms are equally involved

**Objectives:**

- To analyse results from spirometry performed by preschool children against quality control criteria recommended for such measures in adults; and to identify possible modifications, which could be applicable in preschool children
- To examine, in school-age and preschool children, the precision of the Lung Clearance Index and the first breath phase III slope, over three repeated measures; and to investigate whether subject age, age group, sex, body size, and spontaneous variations in breathing pattern influence these indices
- To determine which, if any, modifications to the $S_{cond}$ and $S_{acin}$ MBW analysis are necessary to calculate these indices in spontaneously breathing children
- To determine whether the Lung Clearance Index is more frequently abnormal in preschool children with CF than indices derived from spirometry
• To calculate $S_{\text{cond}}$ and $S_{\text{acin}}$ for both school-age and preschool children, and
determine which mechanism predominates in which age-group

1.10.5 Structure of thesis

This thesis is presented in seven chapters. Equipment and data processing are
described in Chapter 2. The next four chapters describe the following studies:

• Analysis of quality control for spirometry in preschool children
• Analysis of effect of subject characteristics and breathing pattern upon
  MBW indices
• Comparison of LCI and spirometry results in school-age and preschool
  children
• Comparison of $S_{\text{cond}}$ and $S_{\text{acin}}$ results in school-age and preschool children

Other than the description of equipment and data processing, each results chapter is
self-contained, with a detailed description of aims and hypotheses (including
secondary aims and hypotheses), a description of the study population and
statistical methods, results, and an interpretation of these results.
The thesis concludes with a discussion of the implications of the thesis findings and
suggestions for further investigation.
Chapter 2: Methods

2.1 Summary of study design

This methods chapter describes the equipment and data processing methods employed in these investigations. The study structure for preschool children is summarised in Figures 2.1 and 2.2. Children with CF were primarily recruited from the CF clinic at Great Ormond Street Hospital for Children, though a minority were recruited from other CF centres in London. Healthy children were recruited from the community. If preschool children were able to complete the study protocol on the first laboratory visit, they were discharged. Preschool children who were unable to complete the protocol were asked to return for another attempt approximately six months later. No school-age children were recalled for repeat attempts.

Figure 2.1 Summary of study pathway for preschool children
Figure 2.2 Preschool subjects included in main study

Legend: The two panels describe recruitment of preschool children with CF, and healthy preschool children. In addition, two preschool children attempted spirometry at the beginning of the study period, before the MBW system was set up. Their results are included in Chapter 3 only.
Recruitment of children to the preschool study is summarised in Figure 2.2. The aim was to obtain lung function results in 30 children with CF, and 30 healthy control children who were matched for age, sex, and ethnicity. On original recruitment the populations were not matched by ethnicity. The original control population was therefore used to calculate success rates for the lung function tests, but a further five healthy white children were also tested and included in the control population for comparison of lung function results. The last 6 non-white subjects were excluded from this population. Further details on hypotheses, study design, and subject recruitment are presented in the four results chapters (Chapters 3 to 6).

2.2 Test Procedure

On arrival at the laboratory the procedures were explained to the parents and to the children. The parents were then asked to complete and sign a study consent form approved by the Research Ethics Committee of Great Ormond Street Hospital and The Institute of Child Health (Appendix). School-age children signed an assent form (Appendix). They had already read information leaflets sent by post (Appendix). Prior to testing the parent was asked to complete a symptom questionnaire for the child, with questions being administered by the author. The questionnaires differed for school-age and preschool children, and are presented in the Appendix. The primary outcome measures from these questionnaires are detailed in Chapter 5.

Height without shoes was measured in cm to 1 decimal place by a calibrated wall-mounted Harpenden stadiometer (Holtain, Crymych, UK). Weight was measured in kg to 1 decimal place with calibrated electronic scales (Tanita HD-305, Tanita Corporation, Tokyo, Japan), with the child wearing minimal clothing. Weight measurements were performed in duplicate, and only accepted if results were identical.

Physical examination of the child was performed by the author, and whether wheeze or crackles were heard was noted. Measurement of transcutaneous oxygen saturation was performed using an Ohmeda Biox pulse oximeter (Model 3740, Ohmeda, Louisville, USA).
School-age children then performed MBW, followed by spirometry. Preschool children performed MBW, followed by body plethysmography, followed by spirometry. Details of equipment and data collection for spirometry and MBW are presented below. Body plethysmography results are not presented in this thesis. Children with CF were isolated according to the hospital infection control policy.

2.3 Spirometry

2.3.1 Spirometry equipment and calibration

Spirometry was performed with a Jaeger MasterScope spirometer (VIASYS Healthcare GmbH, Höchberg, Germany). Calibration was performed once daily, according to manufacturers' instructions, using a 1L calibration syringe. The Jaeger Masterscope system consists of a PC based system incorporating a heated screen pneumotachometer. Manufacturers' specifications give a flow accuracy of +/- 2% over a flow range of 0.2 to 12 L·sec⁻¹, and a frequency response of 100Hz, which is filtered to 60Hz to reduce noise.

Following each testing session the spirometer was cleaned according to a set protocol (Appendix). Collection of spirometry data is described below.

2.3.2 Spirometry data collection, inspection and reporting in school-age children

All measurements were performed with the child wearing a nose-clip and in the seated position. The child was encouraged to sit upright, not slumped. Spirometry was performed with the child blowing through a mouthpiece and filter. In all cases expiration was encouraged using a Jaeger incentive program that promoted prolonged expiration. During each expiration, the flow-volume curve was monitored on the screen so the investigator could interpret the effort. Between three and five expirations were recorded for each subject. Recording was stopped when the investigator assessed that maximal results had been obtained, or if the child was showing signs of fatigue or restlessness.

Recordings were accepted for further analysis if they met the following criteria on visual inspection: the flow-volume trace showed a rapid rise to peak flow, and a smooth descending limb, with no evidence of cough or glottic closure; and the
volume-time trace "went around the corner", i.e. the trace was approaching a horizontal plateau. All other traces were excluded. Children were required to produce two repeatable FVC and two repeatable FEV₁ results, with the lower result being within 5% of the higher. If only one technically acceptable curve was obtained the results were not reported. Data were automatically stored on the Jaeger Masterscope system. The highest values for FVC, FEV₁, and MEF₂⁵ were transferred manually to a SPSS data file (Statistical Program for Social Sciences, SPSS Inc, Chicago, USA). Following all data entry, data were verified by selecting a sample of 10 cases and cross-checking values entered on the SPSS file against those stored on the Jaeger Masterscope system.

2.3.3 Spirometry data collection and recording in preschool children

Prior to performing spirometry, children were instructed in what was expected by means of descriptions and a story. They were asked to demonstrate that they had understood the instructions by blowing into a mouthpiece. Spirometry was then performed (Figure 2.3). The first expirations were encouraged using the Jaeger ‘Candles’ incentive program, which encourages a rapid expiration. When the child had mastered rapid expiration the program was changed to display an incentive that encourages prolonged expiration. In most cases this was the Jaeger ‘Bowling’ program, though some children responded better to other incentives (Figures 2.4 and 2.5)

The difficulty (i.e. the flow or volume target required to successfully complete the game) of the incentive program was altered between expirations. The aim of this adjustment was to allow the child to score highly enough to remain motivated, but not to achieve the maximum score or to complete the task, as either achievement would result in the child ceasing their effort prematurely. At the end of the test session the difficulty level was adjusted again so that the child was allowed to achieve the maximum score or to complete the task.
Figure 2.3 Four-year old child performing spirometry

Legend: Note that the nose-clip and mouthpiece are well tolerated. The pneumotachometer is connected to the mouthpiece via a bacterial filter to prevent cross-infection. The child is watching a computer-generated animation on a monitor (off left of picture), which promotes rapid or prolonged expiration.

During each expiration the flow-volume curve was monitored on the screen so the investigator could interpret the effort. The Jaeger Masterscope program allows only 5 manoeuvres to be recorded per test file. Additional test files were therefore created as necessary, and a maximum of 25 expiratory manoeuvres were recorded. Recording was stopped when the investigator assessed that maximal results had been obtained, or if the child was showing signs of fatigue or restlessness.
Figure 2.4 Spirometry incentive animations from the Jaeger Masterscope system for initial training, or for school-age children

Legend: The animation on the left is the 'Candles' incentive. The higher the expiratory flow generated by the child, the more candles are blown out. There is no incentive to prolong expiration. This incentive is suitable for early training only, or to encourage a peak flow. The right animation is the 'Flying Toaster' incentive. A prolonged expiration causes the toaster to fly into the air, catch the toast, loop-the-loop, burn the toast, and spit it out. Although school-age children enjoy this incentive, many preschool children are baffled by it.

2.3.4 Spirometry data inspection and reporting in preschool children

All traces were initially exported from the Jaeger Masterscope system to a separate PC workstation. This procedure was necessary because standard Jaeger software does not allow

a) reporting of all necessary parameters
b) data to be excluded but saved for future re-analysis.

Use of a workstation allowed data to be ‘passed’ and then exported for further analysis, or excluded but saved for future re-analysis. All volume-time and flow-volume curves were then visually inspected by the author and by at least one other investigator (C Oliver or C Saunders). Borderline traces were also examined by Prof J Stocks.
Figure 2.5 Spirometry incentive animations from the Jaeger Masterscope system suitable for full forced expiratory measurement in preschool children

Legend: The animation on the left is the 'Balloon' incentive. A prolonged expiration fills the balloon up and causes it to rise up to the spike, where it bursts. The animation on the right is the 'Bowling' incentive. A prolonged expiration causes the ball to roll down the lane and knock down the skittles. The common feature of these incentives is that they encourage prolonged expiration, and that they are simple to understand, being based upon common children's games. It is necessary to adjust the difficulty of the game between attempts, so that the child does not achieve the objective too easily, but also does not become discouraged. Used with care, these are suitable animations for encouraging maximal expiratory manoeuvres in preschool children.

Criteria for visual inspection of curves were identical to those applied for school-age children and described above (Section 2.3.2). Duration of expiration was recorded, but was not a criterion for acceptance. Examples of acceptable and unacceptable recordings are provided in Figures 2.6 to 2.10.

Results were exported from the workstation into an Access relational database (Microsoft Corporation, Redmond, WA, USA) in which demographic and clinical information on the study subjects was also stored.
Figure 2.6 Acceptable flow-volume and volume-time traces from a healthy boy, aged five years.

Legend: For Figures 2.6 to 2.10: The flow-volume trace is on the left, and the volume-time trace is on the right. For the flow-volume trace, flow is presented in $L \cdot s^{-1}$, and volume (labelled Vol) is presented in L. F/V in identifies the inspiratory limb. F/V ex identifies the expiratory limb. For the volume-time trace, volume (labelled Vol) is presented in L, and time is presented in seconds (s).

For Figure 2.6: Note a) that the inspiratory loop is of smaller volume than the expiratory loop (this is partly because the boy started inspiration before putting his mouth on the mouthpiece); b) that the expiratory duration is less than one second, but that the volume-time trace ‘turns the corner’ indicating a complete expiration. FVC, FEV$_{0.5}$, FEV$_{0.75}$, MMEF are reportable from this effort. FEV$_1$ is not reportable.
Figure 2.7 Trace from four-year old girl with CF, first attempt.

Legend: Initial expiratory effort using the Jaeger 'Candle' incentive is inadequate. Although there is a satisfactory start to the test, there is no peak flow and there is early termination. This effort was excluded on visual inspection.

Figure 2.8 Trace from four-year old girl with CF, later attempt.

Legend: Same child as in Figure 2.7, same test occasion, 8th attempt. The child is now using the Jaeger 'Bowling' incentive and is able to produce a full forced expiration. This effort was passed on visual inspection.
Figure 2.9 Trace from five-year old boy with CF.

Legend: This effort was excluded on visual inspection because it does not show an adequate peak flow.

Figure 2.10 Trace from four-year old boy with CF.

Legend: The interruption to expiratory flow is due to cough. This curve was excluded on visual inspection.
The method for calculating back-extrapolated volume (VBE) is described in Figure 2.11. VBE was recorded for every technically acceptable expiration.

**Figure 2.11 Schematic diagram of volume-time trace illustrating calculation of back-extrapolated volume.**

Legend: Axis orientation is identical to Figures 2.6-2.9. The heavy solid line represents the recorded volume-time trace. A regression line from maximum flow is back-extrapolated to the starting volume (broken line), and the starting point of the expiration (vertical axis) is defined as the point at which this back-extrapolated line reaches the starting volume (A). All timed expired volumes are measured from this point. The back-extrapolated volume (VBE) is the difference between the starting volume A, and the point at which the volume-time trace crosses the vertical axis.
2.4 Multiple-breath washout

2.4.1 Multiple-breath washout equipment: pneumotachometer-transducer-demodulator system

The flow meter used in this study was a pneumotachometer-transducer-demodulator system. The pneumotachometer used was a Fleisch number 1 (Fleisch, Lausanne, Switzerland) and is displayed in Figure 2.13. One side of the pneumotachometer was labelled the inspiratory port, and this orientation was used for all calibrations and all measurements. The pneumotachometer was connected to a Validyne variable reluctance pressure transducer (Model MP45-14-871, Validyne Corp, Northridge, USA) via equal lengths of stiff polyethylene tubing (Tricoflex SA, Hozelock Tricoflex, Vitry-le-François, France). The output from the pressure transducer was connected to a high gain carrier demodulator plug-in module (Model CD19, Validyne Corp, Northridge, USA), which was part of a multi-channel modular transducer system (Model MC1, Validyne Corp, Northridge, USA). Prior to connection of the pneumotachometer, the transducer and demodulator were calibrated and balanced by use of an output voltmeter as recommended by the manufacturer.

Linearity of the pneumotachometer-transducer-demodulator system was tested at the beginning of the study (September 2000) and repeated at the conclusion of the study (August 2003). This was performed using a wall-mounted calibrated rotameter, providing flows up to 60 L·sec⁻¹. Results of linearity checks are presented in Figure 2.12. There was a change in linearity of the system over the course of the study, but even at the end of the study the system was linear up to a flow of 40 L·min⁻¹, or 667mL·sec⁻¹, which is consistent with manufacturers specifications, and satisfactory for this application, where flows of greater than 600mL·sec⁻¹ are not expected (Section 2.4.5).
Figure 2.12 Pre- and post-study linearity checks for pneumotachometer system

Legend: Voltage polarity on inspiratory flow has been reversed for the purpose of this analysis.

At the beginning of the study, the system was linear up to 60 L·min⁻¹ for both inspiratory flow and expiratory flow.

At the end of the study, the system was linear up to 40 L·min⁻¹ for both inspiratory flow and expiratory flow, but nonlinear at flows above this.

Slope of regression line = 6.1mV·(L·min⁻¹)⁻¹

2.4.2 Multiple-breath washout equipment: respiratory mass spectrometer

The gas analyser used in this study was an AMIS 2000 respiratory mass spectrometer (Innovision, Odense, Denmark), which is displayed in Figure 2.13.
The AMIS 2000 is a quadrupole mass spectrometer, which operates by identifying gases according to their mass-charge ratio. A gas mixture is drawn into a vacuum chamber along a narrow-bore capillary tube. Upon contact with an electrical filament, positively charged ions are created, which are accelerated along a voltage gradient towards a receptor. An electromagnetic field generated across this pathway allows only ions of a preset mass-charge ratio to reach the receptor. The mass-charge ratio is determined by the molecular mass of the gas, so only gases of one mass-charge ratio (i.e. in most cases, only one gas) can reach the receptor at any one time. The resultant electrical signal can be amplified. Although the mass spectrometer can identify only one gas at any time, rapid cycling of the accepted
mass-charge ratio allows multiple gases to be identified quasi-simultaneously. The AMIS 2000 is capable of identifying 12 gases, up to a molecular mass of 200 atomic mass units, at a cycling frequency of 16.7Hz. For the current application the AMIS 2000 was programmed to recognise helium (He), nitrogen (N2), oxygen (O2), carbon dioxide (CO2), and sulphur hexafluoride (SF6). Sampling time for SF6 was set at 10 milliseconds, and for the other gases at 5 milliseconds, giving a cycling time of 30 milliseconds and a cycling frequency of 33.3Hz. The gas sampling rate of the AMIS 2000 is 15mL·minute⁻¹.

Atomic mass unit peaks for the five test gases were manually optimised when the mass spectrometer was set up in September 2000. The AMIS 2000 was two-point calibrated once daily using a certified concentration calibration gas (alpha-gravimetric standard, British Oxygen Company, Guildford, UK) containing 3.97% He, 3.98% SF6, 7.04% CO2, 21.00% O2, and 64.01% N2. A signal-noise ratio of 100 or greater was deemed acceptable. This full calibration included an automatically performed re-optimisation of atomic mass unit peaks. Short calibration was performed prior to each measurement.

2.4.3 Multiple-breath washout apparatus: facemask and connectors

School-age children used a mouthpiece and nose-clip apparatus. Preschool children used a Rendell-Baker size 2 facemask (Ambu International, Bath, Avon, UK) which was applied to the face using therapeutic putty (North Coast Medical, Morgan Hill, California, USA) to form an airtight seal. The mouthpiece or facemask were connected to the inspiratory port of the pneumotachometer by means of a custom made connector, labelled ‘connector 1’. This connector had a side port to accommodate the capillary inlet of the AMIS 2000 mass spectrometer. The expiratory port of the pneumotachometer was connected via a second custom-made connector (labelled ‘connector 2’) to a T-piece (Intersurgical, Wokingham, UK) to which large bore anaesthetic breathing-circuit tubing (elephant tubing) was attached (Mallinckrodt DAR, Mirandola, Italy). Mr R Taylor, of the Department of Biomedical Engineering, Great Ormond Street Hospital for Children, manufactured both connectors, using Acetal plastic tubing and Nitrile rubber O-rings. The afferent limb of the elephant tubing was connected to a cylinder of medical grade gas,
containing 4% SF₆, 4% He, 21% O₂, and balance N₂ (British Oxygen Company, Guildford, UK). The efferent limb of the elephant tubing was open to room air. The connector 2 / T-piece / elephant tubing assembly were collectively labelled the bias flow apparatus, and this term is used for the remainder of this thesis. The facemask apparatus is displayed in Figures 2.14 and 2.15.

Dead-space of the facemask was measured as 20mL by water displacement. It was estimated that 50% of this dead-space would be occupied by the child’s face when the mask was applied, giving an estimated facemask dead-space of 10mL during use. Dead-space of the other components was also measured by water displacement.

**Figure 2.14 Components of facemask-pneumotachometer apparatus**

*Legend: Note the side-port on connector 1. This accommodates the capillary inlet of the AMIS 2000 mass spectrometer. The white cable from the pneumotachometer is the heater cable.*
The system dead-space was separated into two components:

- The pre-capillary dead-space was defined as the dead-space between the child's lips and the capillary inlet, and was estimated as 12.5mL for the facemask system, and 5mL for the mouthpiece system.
- The post-capillary dead-space was defined as the dead-space between the capillary inlet and the end of the expiratory port of the pneumotachometer, and was measured as 15mL.

**Figure 2.15 Facemask-pneumotachometer apparatus, assembled**

Legend: Pre-capillary dead-space and post-capillary dead-space are marked. The post-capillary dead-space excludes connector 2 and the T-piece as these are removed during washout.

**2.4.4 Multiple-breath washout apparatus: calibration, data acquisition and processing**

Analogue outputs from the demodulator and from the mass spectrometer were recorded at 100Hz by a personal computer (Dell Computers, Round Rock, TX, USA) through a 16-channel AD-conversion board (Model RS485, Keithley Metrabyte, Taunton, MA, USA) using custom written software based on a
commercially available data acquisition software pack (TestPoint, Capital Equipment Corp., Billerica, MA, USA).

The pneumotachometer was calibrated prior to use with separate calibration constants for inspiratory and expiratory flows using a 241mL precision syringe (manufactured by Mr R Taylor, Department of Biomedical Engineering, Great Ormond Street Hospital for Children). Recorded inspiratory and expiratory flows and volumes were converted to BTPS conditions. Gas samples and flow signals were aligned in time. Delay to the gas signal was measured using a custom-made delay switch (manufactured by Mr E Bergsten, Swedish Defense Research Agency, Department of Defense Medicine, Linköping, Sweden). This system measured the delay between gas appearing at the capillary inlet of the mass spectrometer (enabled by opening the switch) and that gas bolus being recorded by the software. Once daily a series of 20 delay recordings were performed, and the median delay and rise times obtained were used to align flow and gas signals from subsequent recordings. The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. Prior to use the pneumotachometer was heated to 37°C (Heater model FWS4D, Hugo Sachs Elektronik, March, Germany).

2.4.5 Data collection in school-age children

The participants were investigated in the sitting position during tidal breathing. During the MBW tests the subjects watched an entertainment video on a television screen (Aiwa integrated television/video cassette recorder model VX-D2120K, Sony Corporation, Tokyo, Japan), whilst the investigator watched the tidal volume trace on a computer screen. If the subject’s tidal volume fell below 8mL·kg⁻¹ or rose above 15mL·kg⁻¹ body weight the subject was asked to increase or decrease their tidal volume respectively. Other than this, subjects were encouraged to breathe comfortably.

Each MBW ‘run’ consisted of two phases. During the wash-in phase the subject inspired a dry gas mixture containing 4% SF₆, 4% He, 21% O₂ and balance N₂. The gas was provided using a bias flow applied on the expiratory port of the pneumotachometer (Section 2.4.3). The bias flow was set at a level greater than the
maximum inspiratory flow produced by the subject, so that rebreathing did not occur. In most cases this was between 400 and 600mL·sec$^{-1}$. Wash-in was continued until the inspiratory and expiratory SF$_6$ concentrations were stable and equal plus another 15 seconds. At this moment the wash-in was stopped by disconnecting the bias flow assembly during expiration, and washout was started. The washout phase continued until the end-tidal SF$_6$ concentration was below 0.1% (i.e. 1/40th of the starting concentration). The output obtained during washout is displayed in Figure 2.17. Irregularities of breathing pattern, such as pauses or sighs, were accepted. A minimum of three wash-in-washout runs was recorded on each test occasion.

2.4.6 Data collection in preschool children

The participants were investigated in the sitting position. During the MBW test the room was darkened and the child was encouraged to watch a children’s entertainment video, which they had previously selected. The aim of the video was to distract the child and encourage tidal breathing. One investigator (C Oliver or C Saunders) held the mask to the child’s face throughout the washout, ensuring a good seal and keeping the child settled. The second investigator (the author) monitored the flow and gas signal outputs in real time on a computer screen. All participants breathed through a Fleisch No.1 pneumotachometer (PNT) via a Rendell-Baker facemask, which was applied to the child’s face using therapeutic putty (Section 2.4.3). Figure 2.16 displays the set-up for the wash-in phase of MBW in a preschool child.

Each test consisted of two phases. During the wash-in phase the subject inspired a dry gas mixture containing 4% SF$_6$, 4% He, 21% O$_2$ and balance N$_2$. The bias flow was set at a level greater than the maximum inspiratory flow produced by the subject, so that rebreathing did not occur. In most cases this was between 250 and 400mL·sec$^{-1}$. Wash-in was continued until the inspiratory and expiratory SF$_6$ concentrations were stable and equal plus another 10 seconds. At this moment the wash-in was stopped by disconnecting the bias flow assembly during expiration, and washout was started. The washout phase continued until the end-tidal SF$_6$ concentration was below 0.1% (i.e. 1/40th of the starting concentration). The output obtained during washout is displayed in Figure 2.17.
Figure 2.16 A three-year old child performing the wash-in phase of multiple-breath washout

Legend: The investigator is supporting the back of the child's head with her right hand, and holding the facemask apparatus to the child's face with her left hand. This ensures an airtight seal, which must be maintained throughout the wash-in and washout. The child is distracted by an entertainment video playing on a television screen off the left of the picture. The second investigator is able to monitor flow and gas concentration on the computer screen at the top left of the picture. At the end of wash-in the bias flow is disconnected and the washout phase commences.

A recording was considered technically acceptable as long as there was no evidence of leak during the latter part of the wash-in or at any stage during the washout. If the child adopted a 'panting' breathing pattern, the recording was stopped, and the child distracted and settled before restarting. Other irregularities of breathing pattern, such as pauses or sighs, were accepted. A minimum of three wash-in-washout manoeuvres were recorded on each test occasion.
Figure 2.17 Washout curve

Legend: The black trace represents flow (left axis). The green trace represents SF$_6$ concentration (right axis). A is the end of the wash-in, where SF$_6$ concentration during inspiration and expiration is identical, and equal to the SF$_6$ concentration in the test gas cylinder. The bias flow is disconnected at point B, and from this point the SF$_6$ concentration during inspiration is zero, as the child is inhaling air. The SF$_6$ concentration on expiration falls with each breath (C), as the resident gas of the lung is progressively diluted by the inspired air.

2.4.7 Analysis of washout curve

The functional residual capacity (FRC) was determined from the cumulative exhaled SF$_6$ divided by the difference in end-tidal SF$_6$ concentration at the start of the washout and end-tidal SF$_6$ concentration at completion of the washout. A proportion of the marker gas expired at each breath remained within the post-capillary apparatus dead-space at the end of expiration and was re-inspired on the next breath. This re-inspired marker gas was measured, and the calculation of cumulative exhaled marker gas was corrected accordingly. The calculated FRC therefore excludes the post-capillary apparatus dead-space, but includes the pre-capillary apparatus dead-space.

The cumulative expired volume (CEV) from the beginning to the end of the washout was calculated by integration of the flow signal. The post-capillary apparatus dead-space was subtracted from each breath, so that the CEV, like the FRC, was corrected for post-capillary apparatus dead-space, but not for pre-capillary apparatus dead-space.
The lung clearance index (LCI) was calculated by dividing the CEV by the FRC. LCI therefore represents the number of FRC turnovers (TO) required to dilute the inspired SF$_6$ to $1/40^{th}$ of its starting concentration.$^{48}$

\[
\text{LCI} = \frac{\text{CEV}}{\text{FRC}}
\]

Where

- LCI = Lung Clearance Index
- CEV = Cumulative Expired Volume
- FRC = Functional Residual Capacity

The FRC and LCI were calculated, by the method described, using custom-written software (TestPoint, Capital Equipment Corp., Billerica, MA, USA). This data acquisition and processing software was written by Mr. E Bergsten (Swedish Defense Research Agency, Department of Defense Medicine, Linköping, Sweden).

2.4.8 Calculation of phase III slopes

For each breath during the washout the SF$_6$ and He concentrations were plotted as a function of expired volume. The phase III slope was calculated for both gases for each breath by a least square fit over the interval of 65\% to 95\% of the expired volume. All breaths were visually inspected, and if the 65\% to 95\% interval appeared to include the phase II slope or the phase IV slope, or if the regression line was distorted by cardiogenic oscillations or signal noise, then the regression line was manually refitted by adjusting the interval.$^{45,49}$ The phase III slope was normalised by the mean tracer gas concentration over the phase III interval, to give the normalised phase III slope (SnIII).

Figure 2.18 presents a phase III slope calculated automatically over 65-95\% of expired volume. Figures 2.19 and 2.20 show traces where a prominent phase IV is present. If the regression is performed over 65-95\% of expired volume then an erroneously high value for SnIII is obtained.
Figure 2.18 Phase III slope fitted over 65% to 95% of expired volume

Legend: Phase III slope in a healthy 3-year old child. The black trace is tracer gas concentration plotted against expired volume. The blue line represents least-square regression over the interval 65-95% of expired volume. The red line is continuation of the regression line. The slope of the regression, i.e. the slope of phase III, is 0.28 L\(^{-1}\).

Figure 2.19 Phase III slope fitted over 65% to 95% of expired volume, in presence of pronounced phase IV

Legend: Plot from a 14-year old girl with CF. There is a pronounced phase IV, so regression over the 65-95% interval over-estimates SnIII, giving a result of 1.01 L\(^{-1}\).
Figure 2.20 Phase III slope fitted over 55% to 85% of expired volume, in presence of pronounced phase IV

Legend: Same trace as Figure 2.18. Regression over 55-85% of expired volume gives an SnIII of 0.80 L⁻¹.

In previous studies of adult subjects reported by the Paiva-Engels group, subjects were required to maintain a fixed tidal volume during MBW. This volume was most commonly defined as 0.75 or 1.0 L. During pilot studies for the current investigation it was noted that children are not always able to maintain the same tidal volume throughout MBW. Two issues relating to this were identified. First, if tidal volume falls below a certain minimum, it will not be possible to identify a phase III slope (Fig 2.21). Second, even if a phase III slope can be identified, the value may be influenced by changes in tidal volume.

A minimum breath size was therefore stipulated for phase III slope to be reported. This was expressed as a minimum starting point for phase III, and a total minimum breath size. The first point was determined by the equation:

\[
\text{Phase III starting volume} = (2 \cdot \text{body weight [kg]} + \text{precapillary dead-space}) \cdot 2
\]

(in mL)

The rationale for this minimum starting point for regression of phase III is that the apparatus and airway dead-space must be cleared (i.e. phase I and II must be completed) before phase III commences. Precapillary dead-space has been
estimated by water displacement (see above), whilst the airway dead-space (in mL) is predicted by the formula $2 \cdot \text{body weight (kg)}$. Minimum total breath size was determined by the equation:

$$\text{Minimum breath size} = (3.5 \cdot \text{body weight [kg]} + \text{precapillary dead-space}) \cdot 2$$

(in mL)

The rationale for this total breath volume is that a minimum phase III duration of $3\text{mL/kg bodyweight}$ is required to obtain an adequate regression. It is accepted that this figure is arbitrary, but it is argued that some minimum regression duration must be set.

**Figure 2.21 Calculation of SnIII from a small volume expiration**

![Graph showing the calculation of SnIII from a small volume expiration](image)

Legend: On first inspection there does appear to be a phase III slope, although it appears rather late in expiration, and is steep ($SnIII = 4.13$). However, this child is 3-years old, and weighs 16 kg. The total expired volume of this breath is just $7.5\text{mL} \cdot \text{kg}^{-1}$, and it is unlikely that a true phase III slope is visible. To emphasise the point, this breath is from the same MBW, in the same child, as that presented in Figure 2.17, with this being the second breath of MBW, and 2.17 being the third breath. Note the difference in expired volume and calculated SnIII between the two plots.
Phase III slopes from breaths of inadequate volume were excluded from $S_{\text{cond}}$ and $S_{\text{acin}}$ analysis, but were retained on the database so that values could be compared with those obtained from breaths of adequate volume (Chapter 4). The relationship between $S_{\text{III}}$ and tidal volume in breaths where phase III can be calculated is explored in Chapter 4.

2.4.9 Calculation of $S_{\text{acin}}$ and $S_{\text{cond}}$

$S_{\text{acin}}$ and $S_{\text{cond}}$ were calculated by the method of Verbanck et al.\textsuperscript{45} The normalised phase III slope for each breath was plotted against turnover (TO). In each case an aggregate $S_{\text{III}}$ versus TO plot was produced from results obtained from 3 MBW runs. $S_{\text{cond}}$ is defined as the normalised slope difference per unit TO over the portion of the MBW where only conducting airways contribute to the generation of ventilation inhomogeneity. In adults this portion is theoretically predicted\textsuperscript{52,54} and experimentally confirmed\textsuperscript{49} as being between 1.5 and 6.0 TO (Figure 2.14). The analysis from which prediction of the linear portion of the $S_{\text{III}}$ versus TO plot is derived is based upon morphological data from the adult lung\textsuperscript{58}. Similar data describing the paediatric lung are not available, and the current study is the first attempt to experimentally test this model in young children. $S_{\text{cond}}$ was therefore calculated by two methods:

a) as the normalised slope difference per unit TO over the period 1.5 to 6.0 TO

b) as the normalised slope difference per unit TO over the period 2.5 to 6.0 TO

For each method $S_{\text{cond}}$ was calculated by least square regression over the specified interval. For the method (b) the breaths closest to 1.5 and 2.0 TO were identified, and the residual from the regression line was calculated for these breaths. By this method the linear portion of the $S_{\text{III}}$-TO relationship was identified.

$S_{\text{acin}}$ was determined by subtracting that part attributable to the conducting airways from the slope of the first breath, as described by the equation:

$$S_{\text{acin}} = S_{\text{III B1}} - S_{\text{cond}} \cdot TO_{\text{B1}}$$

Where

$S_{\text{III B1}} = $ Total phase III slope of first breath

$TO_{\text{B1}} = $ Expired volume of first breath as function of FRC
If the first breath from an MBW was excluded (e.g. because of inadequate breath volume, or signal noise), then this MBW run was excluded from analysis. If more than one third of the breaths over the period of $S_{\text{cond}}$ regression were excluded, then this MBW run was also excluded from analysis. The impact of excluding breaths from the SnIII versus TO plot is presented in Figures 2.22 and 2.23.

Figure 2.22 SnIII versus TO plot in school-age child with cystic fibrosis, aggregated raw data

Legend: The closed circles represent values of SnIII (averaged from three MBW runs) plotted against TO. The open triangles represent the slope of $S_{\text{cond}}$ calculated by least-square regression over the 1.5 to 6.0 TO interval. There is no clear pattern, and a negative $S_{\text{cond}}$ is calculated.
Figure 2.23 SnIII versus TO plot in school-age child with cystic fibrosis, aggregated data after exclusion of breaths of inadequate volume

Legend: These are the same data as presented in Figure 2.22. Note the amendment to the y-axis, otherwise labelling between the two figures is identical. SnIII results calculated from breaths of inadequate volume, or where there was excessive signal noise (i.e. breaths where SnIII was probably not visible), have been excluded. No other corrections have been performed. There is now a clear relationship between SnIII and TO, with a positive $S_{\text{cond}}$.

2.4.10 Summary of quality control criteria for calculation of $S_{\text{cond}}$ and $S_{\text{acin}}$

The quality criteria for analysing phase III slopes and calculating $S_{\text{acin}}$ and $S_{\text{cond}}$ are summarised below:

- SnIII is calculated over 65-95% of expired volume whenever possible
- All slopes are visually inspected. If visual inspection indicates that the 65-95% interval is inappropriate then this interval is altered manually
- For any breath, the interval over which He SnIII is calculated is the same as that over which the SF$_6$ SnIII is calculated
• A minimum volume from which SnIII can commence, and minimum total 
  breath volumes are defined according to subject weight
• SnIII is not reported from any breaths where it is not (or is unlikely to be) 
  clearly visible, whether because of inadequate breath volume or signal noise
• $S_{\text{cond}}$ and $S_{\text{acin}}$ are calculated according to the method of Verbanck et al
• If the first breath from a run cannot be reported, or if more than one third of 
  breaths over the $S_{\text{cond}}$ regression interval cannot be reported, then that MBW 
  is excluded from analysis. $S_{\text{cond}}$ and $S_{\text{acin}}$ can only be reported if results from 
  three MBW runs are available.
Chapter 3: Feasibility and quality control for spirometry in preschool children

3.1 Introduction

Spirometry is the most frequently used method for measuring lung function. The reliability of this test is dependent upon using standardised methodology, particularly with regard to how quickly the subject increases flow at the beginning of the expiration, sustained effort throughout the expiration, the duration of the expiratory manoeuvre, and repeatability. Detailed criteria for data collection and interpretation have been published by the American Thoracic Society (ATS), and by the European Respiratory Society (ERS)\textsuperscript{34,35}. Application of these criteria when collecting and interpreting spirometry data is considered mandatory in adult pulmonary function laboratories.

Spirometry is not only employed in adults; it is also commonly performed in paediatric pulmonary function laboratories. The test was previously limited to school-age children (those aged 6 to 16 years), but recent reports have confirmed that preschool children (those aged 2 to 5 years) are also able to attempt these manoeuvres\textsuperscript{24,36,40-42}. Both the ATS and ERS guidelines were written for adult patients, and Arets and colleagues have already demonstrated that many school-age children have difficulty meeting some of the quality control criteria\textsuperscript{44}. Recent reports of spirometry in preschool children have focused upon feasibility\textsuperscript{24,40,41}; use of incentive devices\textsuperscript{41}; and recording of reference values in healthy populations\textsuperscript{24,40}, rather than upon the issue of quality control.

3.2 Hypothesis and aims

3.2.2 Hypothesis

Children aged 2 to 5 years are unable to meet some of the quality control criteria for spirometry, but alternative criteria may be feasible.
3.2.2 Aims and objectives

The primary aims and objectives of this investigation were

a) To analyse results from spirometry performed by preschool children against quality control criteria recommended for such measures in adults
b) To identify which of these criteria are easily achieved by preschool children, and which are not
c) To determine whether success in meeting these criteria is affected by presence of lung disease or by subject age
d) To identify possible modifications which could be applicable in preschool children.

The secondary aims and objectives were to compare results obtained in healthy children at the Institute of Child Health with those previously reported from other centres, and to assess whether reference ranges published by those centres apply to the current study population.

3.3 Subjects and Methods

3.3.1 Subjects

All subjects attending the CF clinic at Great Ormond Street Hospital who met the inclusion and exclusion criteria below were identified and approached. This recruitment method was judged unlikely to yield the number of subjects required, so the other four CF centres participating in the London Collaborative Cystic Fibrosis Study (LCCFS) were also approached. The LCCFS is a recently completed study of the effect of CF upon lung function in infancy. The participating centres are Great Ormond Street Hospital for Children, King’s College Hospital, Royal Brompton Hospital, The Royal London Hospital, and University Hospital, Lewisham. Subjects with CF who were tested in infancy as part of the LCCFS were in most cases too young to enter the current study. The LCCFS centres therefore identified a small number of preschool children with CF who met the inclusion and exclusion criteria below, and recruited them to this study. No attempt was made to recruit random or representative samples of children from these four CF centres.
Healthy control children were primarily recruited from local schools and playgroups. Local Education Authorities were contacted for permission to approach schools and playgroups. Head teachers were then contacted for permission to approach parents. Parents were then contacted by letter in the first instance. Any parents expressing an interest in the study received a follow-up telephone call and recruitment form to determine whether their child met the eligibility criteria for the study. In a small number of cases friends and siblings of children with CF were recruited as controls. In all cases, for healthy children and those with CF, verbal and written information was provided to the parents or guardians prior to the day of testing, and written consent was obtained.

Copies of letters sent to Local Education Authorities and schools, recruitment letters and screening forms, and consent forms and information sheets for this study are provided in appendices D-F.

Inclusion criteria for subjects with CF:
- CF diagnosed by a positive sweat test and/or two known CF disease-producing mutations identified by DNA analysis
- Subject aged ≥ 2.0 years and <6.0 years at time of testing.

Exclusion criteria for subjects with CF:
- Congenital cardiac disease that requires medical therapy, has previously required surgical therapy, or is awaiting surgical therapy
- Preterm birth, defined as birth before 34 weeks gestational age
- Neuromuscular or bone disease likely to result in weakness of respiratory muscles or restriction of the thorax.

Inclusion criteria for healthy control children
- Subject aged ≥ 2.0 years and <6.0 years at time of testing.

Exclusion criteria for healthy control children
- Any previous hospitalisation for a respiratory condition
- More than 5 courses of antibiotics for respiratory symptoms (including upper respiratory symptoms) in the previous 12 months
- Any physician diagnosis of asthma or reactive airways disease at any time in the past
• Use of anti-asthma medication on more than one occasion in the previous 12 months
• Any history of chronic productive cough, recurrent wheezing, or shortness of breath within the previous 12 months
• Congenital cardiac disease that requires medical therapy, has previously required surgical therapy, or is awaiting surgical therapy
• Preterm birth, defined as birth before 34 weeks gestational age.
• Low birthweight, defined as birthweight less than 2.3kg
• Neuromuscular or bone disease likely to result in weakness of respiratory muscles or restriction of the thorax.

3.3.2 Study design
This was a cross-sectional study, with data limited to spirometry attempts made during the child’s first laboratory visit.

3.3.3 Data collection
Collection and analysis of spirometry data, including criteria for accepting or discarding results, is described in Section 2.3.

3.4 Analysis

3.4.1 Outcome measures
The following measures were reported from each test:
• The two highest technically acceptable forced vital capacity (FVC) measurements
• The two highest technically acceptable measurements of forced expired volume in one second, 0.75 seconds, and 0.5 seconds (FEV₁, FEV₀.₇₅, FEV₀.₅)
• The duration of the expiration that resulted in the largest FVC (forced expiratory time [FET])
• The back-extrapolated volume (VBE) calculated as per ATS guidelines\(^3\)\(^4\) (Figure 2.10), reported as an absolute value, and as a percentage of FVC (VBE/FVC\%).

• The mean forced expired flow between 25 and 75% of expired volume (MMEF) was reported from the expiration that had the highest sum of FVC and FEV\(_{0.75}\), unless FET was less than 0.75 seconds, in which case this flow was reported from the expiration that had the highest sum of FVC and FEV\(_{0.5}\).

3.4.2 Comparison with previously published data

Data obtained in healthy children were compared with published reference values. This was achieved in two ways. First, original data collected by Eigen and colleagues from 214 healthy children tested in Indianapolis, USA\(^2\)\(^4\) were obtained from the authors. FVC, FEV\(_1\), and FEF\(_{25-75}\) results from Indianapolis were plotted against subject height along with data obtained in this study. Data were plotted separately for all healthy children at both centres, and for white children only. Second, reference equations derived from the Indianapolis population, and equations derived from a separate study of 603 preschool children in Oslo, Norway\(^4\)\(^0\), were used to calculate z-scores for our healthy population. The Indianapolis reference equations were calculated from the 184 white children in their study. Ethnic data were not available for the Oslo study.

3.4.3 Statistical analysis

The CF and control populations were compared for age, weight, and height. Success in producing FVC, FEV\(_1\), FEV\(_{0.75}\), FEV\(_{0.5}\), and FEF\(_{25-75}\) was compared by age group, and by diagnosis. FET was plotted against age and height. Start of test was examined by plotting VBE and VBE/FVC against age. Repeatability was examined by calculating the difference between the two highest FEV\(_{0.75}\) readings (ΔFEV\(_{0.75}\)), and between the two highest FVC readings (ΔFVC). Timed expired volumes as fractions of FVC (FEV\(_{0.5}\)/FVC, FEV\(_{0.75}\)/FVC, and FEV\(_1\)/FVC) were calculated. All parameters were compared by age group and by diagnosis.
Summary statistics are presented as mean and standard deviation (SD) if normally distributed, and median and interquartile range (IQR) if non-normally distributed. Proportions were compared by Chi² test. T-tests or Mann-Whitney tests were employed for comparison of two groups; ANOVA and post-hoc analysis by Tukey's honestly significant difference (HSD) were used to compare three or more groups. Where interaction between multiple factors was suspected this was investigated by multiple regression. Summary data for z-scores for our population were calculated separately for all healthy children, and for white children only. For all analyses a p-value of 0.05 is regarded as significant.

3.4.4 Power of study

The power calculation for this sample was based upon the requirements of the analysis presented in Chapter 5, where results obtained from the different lung function techniques are compared for CF and control groups. For this study, a sample size of 50 subjects in each group was calculated to be sufficient to detect a difference of 0.5 SD in sRan, FEV₀.₅, FEF₂₅-₇₅, LCI and CV_LCI between groups. Interim analysis performed in 2002 indicated that the difference between populations for the primary outcome measures was likely to be greater than 0.5 SD, and the study population was therefore recalculated as 30 subjects per group. For the current study, all subjects meeting the entry criteria and attempting spirometry on their first visit to the laboratory were included. Post hoc power calculation demonstrated that for the tests of published prediction equations, a sample size of nine subjects would detect a difference from predicted values of one z-score with 90% at the 5% significance level, whilst a sample size of 16 subjects would detect a difference from predicted values of 0.75 z-scores with 90% at the 5% significance level.
3.5 Results

3.5.1 Subjects

Forty-two children with CF and 37 healthy children were recruited. The study population is summarised in Table 3.1.

Table 3.1 Characteristics of study population, summarised by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Cystic Fibrosis (n = 42)</th>
<th>Healthy Control (n = 37)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>45%</td>
<td>60%</td>
<td>-15 (-36, 8)</td>
</tr>
<tr>
<td>Ethnicity (% white)</td>
<td>88%</td>
<td>70%</td>
<td>18 (0, 36)*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.14 (0.90)</td>
<td>4.14 (0.85)</td>
<td>0 (-0.39, 0.40)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>100.9 (7.3)</td>
<td>104.2 (7.9)</td>
<td>-3.3 (-6.6, 0.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>16.7 (2.9)</td>
<td>18.6 (3.8)</td>
<td>-1.9 (-3.4, -0.4)*</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.42 (1.13)</td>
<td>0.26 (1.23)</td>
<td>-0.68 (-1.22, -0.13)*</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>-0.01 (1.11)</td>
<td>0.71 (1.23)</td>
<td>-0.72 (-1.26, -0.18)*</td>
</tr>
</tbody>
</table>

Legend: Results are presented as mean (SD) unless otherwise stated.
Difference calculated as CF – control, * = p<0.05.

Although matched for age, the children with CF were significantly lighter and shorter than the control children.
3.5.2 Success rates

The median number of spirometry manoeuvres attempted was 12 (range 6-22). Success in producing a reportable FVC, FEV\textsubscript{0.5}, FEV\textsubscript{0.75}, FEV\textsubscript{1}, and FEF\textsubscript{25-75} is presented in Table 3.2.

Table 3.2 Success rates in obtaining forced expiratory parameters

<table>
<thead>
<tr>
<th></th>
<th>All children (n=79)</th>
<th>Cystic Fibrosis (n=42)</th>
<th>Healthy Controls (n=37)</th>
<th>2 - &lt;4 years (n=39)</th>
<th>4 - &lt;5 years (n=25)</th>
<th>5 - 6 years (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>59</td>
<td>30</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(71%)</td>
<td>(78%)</td>
<td>(64%)</td>
<td>(84%)</td>
<td>(87%)</td>
</tr>
<tr>
<td>FEV\textsubscript{0.5}</td>
<td>59</td>
<td>30</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(71%)</td>
<td>(78%)</td>
<td>(64%)</td>
<td>(84%)</td>
<td>(87%)</td>
</tr>
<tr>
<td>FEV\textsubscript{0.75}</td>
<td>53</td>
<td>28</td>
<td>25</td>
<td>20</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(67%)</td>
<td>(67%)</td>
<td>(68%)</td>
<td>(51%)</td>
<td>(80%)</td>
<td>(87%)</td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>46</td>
<td>26</td>
<td>20</td>
<td>16</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(58%)</td>
<td>(62%)</td>
<td>(54%)</td>
<td>(41%)</td>
<td>(72%)</td>
<td>(80%)</td>
</tr>
<tr>
<td>MMEF</td>
<td>59</td>
<td>30</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(75%)</td>
<td>(71%)</td>
<td>(78%)</td>
<td>(64%)</td>
<td>(84%)</td>
<td>(87%)</td>
</tr>
</tbody>
</table>

Legend: Results are expressed as number of children and percentage, summarised by age group and diagnosis. As only six of the children were aged less than three years, children aged 2-<4 years were analysed together.

Fifty-nine of the 79 children were able to produce acceptable spirometry loops (Figure 2.6). Nine of these children could produce only two acceptable loops, while the remaining 50 children produced between three and nine acceptable loops each (median was four). Of the 29 healthy children who completed spirometry, 18 were white, three were black and eight were of mixed ethnic group. Of the 30 children with CF who completed spirometry, 25 were white, one was black, two were South Asian, and two were of mixed ethnic group. During visual inspection of the spirometry curves it was discovered that the Jaeger Masterscope software can report timed expired volumes inappropriately, so that
some children appeared to have a FEV₁ equal to FEV₀.75 or a FEV₀.75 equal to FEV₀.5. In such cases the FET was invariably greater than 1 second. After communication with the manufacturer it was determined that the Jaeger system determines end of expiration by detecting absolute cessation of expiratory flow. If there is a slight expiratory offset on the flow signal, and the child removes their mouth from the apparatus before their next inspiration, then expiration can continue to be recorded. This is explained further in Figure 3.7. Verification of the data was therefore undertaken. First, all reports where FET was less than one second, and/or where FEV₀.75 or FEV₁ were equal to FVC, were identified. These curves were then visually inspected and timed volumes that had been inappropriately reported were excluded. By this method, six children who had produced satisfactory FEV₀.5, but from whom FEV₀.75 should not have been reported, and seven children who had produced satisfactory FEV₀.5 and FEV₀.75, but from whom FEV₁ should not have been reported, were identified.

There was no significant difference between the CF and control groups in success rate for any of the parameters. Children aged 2-<4 years were significantly less likely to produce FEV₀.75 or FEV₁ than children aged 4-<5 years or children aged 5-6 years. No other differences in success rates by age group were seen.

3.5.3 Start of test criteria

Values for back-extrapolated volume (VBE) and VBE/FVC are summarised in Table 3.3. Mean (SD) VBE for all children was 65 (17) mL; VBE/FVC was 7.2 (2.8)%.
### Table 3.3 Start of test criteria and repeatability criteria

<table>
<thead>
<tr>
<th></th>
<th>All children</th>
<th>Healthy Controls</th>
<th>Cystic Fibrosis 2-&lt;4 years</th>
<th>4 - &lt;5 years</th>
<th>5 - &lt;6 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>VBE (mL)</td>
<td>65 (17)</td>
<td>63 (20)</td>
<td>67 (14)</td>
<td>64 (16)</td>
<td>67 (12)</td>
</tr>
<tr>
<td>VBE/FVC (%)</td>
<td>7.2 (2.8)</td>
<td>6.4 (2.3)</td>
<td>7.9 (3.0)</td>
<td>7.7 (2.7)</td>
<td>7.2 (2.8)</td>
</tr>
<tr>
<td>FET (sec)</td>
<td>1.5 (1.1, 2.2)</td>
<td>1.4 (1.1, 1.8)</td>
<td>1.5 (1.2, 2.2)</td>
<td>1.3 (1.1, 2.1)</td>
<td>1.6 (1.1, 2.2)</td>
</tr>
<tr>
<td>AFVC (mL)</td>
<td>34 (16, 63)</td>
<td>34 (14, 77)</td>
<td>33 (16, 58)</td>
<td>34 (14, 63)</td>
<td>31 (18, 73)</td>
</tr>
<tr>
<td>AFVC (%)</td>
<td>3.5 (1.7, 7.0)</td>
<td>4.2 (1.6, 7.6)</td>
<td>3.1 (1.7, 6.5)</td>
<td>4.3 (1.5, 7.6)</td>
<td>3.3 (1.8, 7.1)</td>
</tr>
<tr>
<td>ΔFEV$_{0.75}$ (mL)</td>
<td>32 (11, 53)</td>
<td>38 (15, 62)</td>
<td>23 (10, 44)</td>
<td>23 (11, 40)</td>
<td>36 (9, 59)</td>
</tr>
<tr>
<td>ΔFEV$_{0.75}$ (%)</td>
<td>3.4 (1.4, 5.2)</td>
<td>4.0 (1.5, 5.6)</td>
<td>3.1 (1.4, 5.1)</td>
<td>3.2 (2.3, 4.3)</td>
<td>3.9 (1.0, 6.2)</td>
</tr>
</tbody>
</table>

**Legend:** Results are summarised by age group and diagnosis

- VBE = back extrapolated volume
- FET = duration of manoeuvre producing the best FVC
- AFVC = difference between best and second best FVC
- ΔFEV$_{0.75}$ = difference between best and second best FEV$_{0.75}$

VBE and VBE/FVC results are presented as mean (SD). All other results are presented as median (IQR).

There was no relationship between VBE and height (Figure 3.1) or VBE and age (Figure 3.2), and all but 4/59 children (7%) produced a VBE less than or equal to 80mL.
Figure 3.1 Back-extrapolated volume (VBE) plotted against subject height.

Legend for Figures 3.1 and 3.2: Children with CF plotted as closed circles, healthy children as open circles. Broken line represents a possible quality control cut-off of 80mL. There is no relationship between VBE and height or VBE and age.

Figure 3.2 Back-extrapolated volume plotted against age
Figure 3.3 demonstrates the relationship between VBE/FVC and height ($r^2 = 0.27$, p<0.0005). Although younger children tended to have higher VBE/FVC this difference was not significant (Figure 3.4). Only 16/59 children (27%) were able to produce VBE/FVC <5%. Seven children (12%) produced a VBE/FVC greater than 10%, of whom two produced a VBE/FVC greater than 12.5%.

**Figure 3.3 Back-extrapolated volume / FVC plotted against subject height**

Legend: Children with CF plotted as closed circles, healthy children as open circles. The four children with back extrapolated volume greater than 80mL are marked with arrows.

Broken line represents a possible quality control cut-off of 12.5%.

There was no relationship between diagnosis and VBE. Children with CF had a significantly higher VBE/FVC. After correction for height, the diagnosis of CF was no longer significantly related to VBE/FVC (partial coefficient for diagnosis vs.
VBE/FVC 0.19 %, p = 0.1; partial coefficient for height vs. VBE/FVC 0.49 % cm⁻¹, p<0.0005).

Figure 3.4 Back-extrapolated volume / FVC plotted against age

Legend: Children with CF plotted as closed circles, healthy children as open circles.

Curves with VBE greater than 80mL and curves with VBE/FVC greater than 12.5% were re-examined, and compared with curves that had been excluded on visual inspection (Figures 3.5 to 3.7).
Figure 3.5 Trace from three-year old girl with CF

Legend: This curve was excluded on visual inspection because of slow start. The back extrapolated volume was 110mL, and the back extrapolated volume / FVC was 18.2%.

Figure 3.6 Trace from healthy three-year old boy

Legend: Although this curve was passed on visual inspection the back extrapolated volume is 120mL, and the back extrapolated volume / FVC is 11.6%.
Figure 3.7 Trace from five-year old girl with CF

Legend: Although this curve was passed on visual inspection as having a satisfactory start to expiration, the back extrapolated volume is 120mL and the back extrapolated volume / FVC is 12.7%.

The Jaeger Masterscope system continues to record expiration until absolute cessation of expiratory flow is detected. This feature allows complete recording of expiration in subjects with severe airflow limitation. However, if there is a slight expiratory offset on the flow signal, expiration will continue to be recorded until the subject takes an inspiration. This child removed her mouth from the mouthpiece at the end of expiration, prior to taking her next inspiration. In such circumstances (see Section 3.5.4) the spirometer continues recording until terminated manually by the operator. The forced expiratory time is therefore recorded as 2.3s rather than 0.9s (A). There is a small offset at zero flow, so the FVC is recorded as 0.92L rather than 0.89L. The MMEF will also be inaccurate. The child produced a satisfactory effort later in the test session.

3.5.4 Duration of manoeuvre

FET, summarised by age group and diagnosis, is also presented in Tables 3.3 and 3.4. Twelve of the 59 children produced a FET of less than one second.
Table 3.4 Expiratory duration of longest FVC (FET) cross-tabulated against age group

<table>
<thead>
<tr>
<th></th>
<th>2-&lt;4 years</th>
<th>4-&lt;5 years</th>
<th>5-6 years</th>
<th>All children</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.75s</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>0.75-&lt;1s</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>1-&lt;2s</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>&gt;2s</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Totals</td>
<td>26</td>
<td>20</td>
<td>13</td>
<td>59</td>
</tr>
</tbody>
</table>

Legend: An error in the Jaeger program regarding recording of FET was noted. This error led to some of the values being over-estimated.

Table 3.5 presents timed forced expired volumes expressed as a proportion of FVC, summarized by age group.

Table 3.5 Timed forced expired volumes as a proportion of forced vital capacity

<table>
<thead>
<tr>
<th></th>
<th>All children</th>
<th>Healthy Controls</th>
<th>Cystic Fibrosis</th>
<th>2-&lt;4 years</th>
<th>4-&lt;5 years</th>
<th>5-6 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV0.5/FVC (%)</td>
<td>81 (72, 87)</td>
<td>84 (73, 89)</td>
<td>78 (71, 87)</td>
<td>82 (73, 88)</td>
<td>81 (70, 88)</td>
<td>74 (71, 82)</td>
</tr>
<tr>
<td>FEV0.75/FVC (%)</td>
<td>91 (85, 97)</td>
<td>92 (84, 98)</td>
<td>91 (85, 96)</td>
<td>92 (85, 95)</td>
<td>91 (84, 98)</td>
<td>86 (83, 97)</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>94 (91, 98)</td>
<td>94 (90, 97)</td>
<td>94 (89, 98)</td>
<td>96 (93, 98)</td>
<td>93 (85, 98)</td>
<td>93 (90, 98)</td>
</tr>
</tbody>
</table>

Legend: Results are summarised by age group and diagnosis. Results are presented as median (IQR). For the majority of children FEV1/FVC was greater than 90%, irrespective of age group or diagnosis. By contrast, for the majority of children FEV0.5/FVC was less than 90%.
Individual data are presented in Figures 3.8 to 3.10.

**Figure 3.8 FEV\(_{0.5}\)/FVC plotted against subject height**

Legend for Figures 3.8 to 3.10: Children with CF presented as closed circles, healthy children as open circles. Note the relationships between FEV\(_{0.5}\)/FVC and height \((r^2 = 0.22, p = 0.009, \text{Figure 3.8})\), and between FEV\(_{0.75}\)/FVC and height \((r^2 = 0.27, p = 0.008, \text{Figure 3.9})\) in the healthy population. There is no relationship between FEV\(_1\)/FVC and height in the healthy population (Figure 3.10).

In healthy children there was a significant negative relationship between FEV\(_{0.5}\)/FVC and height \((r^2 = 0.22, p = 0.009, \text{Figure 3.8})\), and FEV\(_{0.75}\)/FVC and height \((r^2 = 0.27, p = 0.008, \text{Figure 3.9})\) but no relationship between FEV\(_1\)/FVC and height (Figure 3.10). There was no relationship between FEV/FVC parameters and height in the CF population.
Figure 3.9 FEV$_{0.75}$/FVC plotted against subject height

Figure 3.10 FEV$_1$/FVC plotted against subject height
3.5.5 Within occasion repeatability

Differences between the two highest values of FVC (ΔFVC), and FEV\textsubscript{0.75} (ΔFEV\textsubscript{0.75}) are displayed in Table 3.3 and Figures 3.11 and 3.12. The population median for all age groups was less than 4% for ΔFEV\textsubscript{0.75}, and less than 5% for ΔFVC, with no significant difference for either parameter by age group. Twenty-four children (41%) had ΔFVC greater than 5%, of whom five (9%) had ΔFVC greater than 10%. Fifteen children (28%) had ΔFEV\textsubscript{0.75} greater than 5%, of whom three (6%) had ΔFEV\textsubscript{0.75} greater than 10%.

**Figure 3.11 Difference between the two best FVC, as a percentage of best FVC, plotted against subject height**

Legend: Five children had ΔFVC of greater than 10% (broken line).

ΔFVC = difference between the two best FVC, as a percentage of best FVC
Figure 3.12 Difference between the two best FEV$_{0.75}$, as a percentage of best FEV$_{0.75}$, plotted against subject height

![Graph showing data points and a line indicating 10% difference.]

Legend: Three children had ΔFEV$_{0.75}$ greater than 10% (broken line).
ΔFEV$_{0.75}$ = difference between the two best FEV$_{0.75}$, as a percentage of best FEV$_{0.75}$

3.5.6 Comparison with published reference data
Figures 3.13 to 3.18 present FVC, FEV$_1$, and MMEF results from Indianapolis and ICH. Results are presented for healthy children of all ethnic groups, and separately for all white children. When white children alone were plotted there was good concordance between results obtained in the two centres.
Figure 3.13 FVC plotted against height in healthy children of all ethnic groups

Legend (for Figures 3.13 to 3.18): Children measured at ICH plotted as black closed markers. Children measured in Indianapolis plotted as red open markers.

Figure 3.14 FVC plotted against height in healthy white children
Figure 3.15 FEV₁ plotted against height in healthy children of all ethnic groups

Figure 3.16 FEV₁ plotted against height in healthy white children
Figure 3.17 MMEF plotted against height in healthy children of all ethnic groups

Figure 3.18 MMEF plotted against height in healthy white children
Z-scores for spirometry parameters were calculated for the healthy children. Of the 29 children who produced reportable results, 18 were white; 3 were black, and 8 were mixed race. Summary results are presented for the whole control population, and for the white children alone in Table 3.6.

<table>
<thead>
<tr>
<th></th>
<th>All healthy children (n=29)</th>
<th>Healthy white children (n=18)</th>
<th>95% CI of difference from zero, for healthy white children§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FVC z-score (Oslo)</strong></td>
<td>0.18 (0.70)</td>
<td>0.43 (0.52)</td>
<td>0.17, 0.67**</td>
</tr>
<tr>
<td><strong>FVC z-score (Indianapolis)</strong></td>
<td>-0.76 (1.21)</td>
<td>-0.30 (0.79)</td>
<td>-0.70, 0.09</td>
</tr>
<tr>
<td><strong>FEV_{0.5} z-score (Oslo)</strong></td>
<td>-0.11 (0.84)</td>
<td>0.08 (0.77)</td>
<td>-0.30, 0.46</td>
</tr>
<tr>
<td><strong>FEV_{1} z-score (Oslo)</strong></td>
<td>0.17 (0.66)</td>
<td>0.31 (0.47)</td>
<td>0.02, 0.59*</td>
</tr>
<tr>
<td><strong>FEV_{1} z-score (Indianapolis)</strong></td>
<td>0.13 (1.1)</td>
<td>0.37 (0.71)</td>
<td>-0.06, 0.80</td>
</tr>
<tr>
<td><strong>MMEF z-score (Indianapolis)</strong></td>
<td>-0.35 (1.44)</td>
<td>-0.29 (1.30)</td>
<td>-0.94, 0.36</td>
</tr>
</tbody>
</table>

Legend: Results presented as mean (SD unless otherwise stated.
§ Comparison between z-scores obtained in healthy white children vs zero, by one-sided t-test. *p<0.05, **p<0.005. Provided the published reference equations are accurate, and the populations are comparable, all z-scores should be close to zero, with a standard deviation of one. Note that this does not hold here for all z-scores tested, and also that the total population has mean z-scores lower than the white population. This will be investigated further below.

Z-scores for healthy white children measured at ICH, calculated from Indianapolis\textsuperscript{24} and Oslo\textsuperscript{40} reference equations, and plotted against age, are presented in Figures 3.19 to 3.24.
Figure 3.19 FVC z-score (Oslo) for healthy white children measured at ICH, plotted against height

Legend for Figures 3.19 to 3.24: Z-scores calculated from reference equations derived from healthy children measured in Oslo, Norway (Figures 3.19, 3.21, and 3.23), or healthy children measured in Indianapolis, USA (Figures 3.20, 3.22, 3.24). Broken lines represent mean and predicted limits of normality (+/- 1.96 z-scores). Z-scores obtained in children measured at ICH are significantly greater than zero for FVC and FEV1 z-scores calculated from Oslo reference data, but are not significantly different from zero for all other parameters (Table 3.6).
Figure 3.20 FVC z-score (Indianapolis) for healthy white children measured at ICH, plotted against height.

Figure 3.21 FEV₁ z-score (Oslo) for healthy white children measured at ICH, plotted against height.
Figure 3.22 FEV₁ z-score (Indianapolis) for healthy white children measured at ICH, plotted against height.

Figure 3.23 FEV₀.₅ z-score (Oslo) for healthy white children measured at ICH, plotted against height.
3.5.7 Comparison of results by ethnic group

Comparison of z-scores obtained in healthy white children with those obtained in healthy children of all other ethnic groups is presented in Table 3.7. Non-white children had significantly lower FVC z-scores, and tended to have lower FEV\textsubscript{0.5} and FEV\textsubscript{1} z-scores, though these differences were not statistically significant.
Table 3.7 Spirometry z-scores in healthy children, analysed by ethnic group

<table>
<thead>
<tr>
<th></th>
<th>White children (n=18)</th>
<th>Non-white children (n=11)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC z-score (Oslo)</td>
<td>0.43 (0.52)</td>
<td>-0.23 (0.78)</td>
<td>0.65 (0.16, 1.15)*</td>
</tr>
<tr>
<td>FVC z-score (Indianapolis)</td>
<td>-0.30 (0.79)</td>
<td>-1.52 (1.42)</td>
<td>1.22 (0.38, 2.06)**</td>
</tr>
<tr>
<td>FEV_{0.5} z-score (Oslo)</td>
<td>0.08 (0.77)</td>
<td>-0.43 (0.88)</td>
<td>0.51 (-0.13, 1.14)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} z-score (Oslo)</td>
<td>0.31 (0.47)</td>
<td>-0.09 (0.91)</td>
<td>0.40 (-0.24, 1.04)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} z-score (Indianapolis)</td>
<td>0.37 (0.71)</td>
<td>-0.32 (1.45)</td>
<td>0.69 (-0.31, 1.70)</td>
</tr>
<tr>
<td>MMEF z-score (Indianapolis)</td>
<td>-0.29 (1.30)</td>
<td>-0.46 (1.69)</td>
<td>0.17 (-0.97, 1.32)</td>
</tr>
</tbody>
</table>

Legend: Results presented as mean (SD) unless otherwise stated. Difference calculated as white – non-white.

* p<0.05

** p<0.01
3.6 Discussion

3.6.1 Summary of results
In this study results from spirometry attempts in 79 two to five year old children were analysed in detail, in order to determine whether the results met published quality control criteria for adult spirometry. All flow-volume and volume-time traces were first visually inspected and passed or rejected. Forced expired volumes and flows obtained in healthy children were similar to those reported by other groups. The criteria tested were: start of test, assessed by back extrapolation; duration of expiration, and the timed expired volumes that are influenced by this; and repeatability. For all of these parameters, modifications to quality control criteria appear necessary for application to preschool children.

3.6.2 Visual inspection
Visual inspection of the flow-volume curve and the volume-time curve is a mandatory first step for quality control of spirometry. Some of the faults that require results to be excluded are self-evident. These include: cough or glottic closure; double expiration (seen as a double peak on the flow-volume curve); and failure to produce an adequate peak flow. Previous studies of spirometry in this age group have used similar criteria for accepting or discarding loops\textsuperscript{24,40,41}. Evaluation of end of test by visual inspection is more difficult. In older children and adults the volume-time trace asymptotes to horizontal towards the end of expiration\textsuperscript{34}. In preschool children, particularly the youngest, this asymptote is frequently not seen, even in children who appear to have produced a complete expiration. The abrupt cessation of expiration in very young children complicates assessment of end of test, as curves such as that shown in Figure 2.6 should not be excluded inappropriately. Eigen and colleagues (from the Indianapolis group) have suggested excluding curves where expiratory flow abruptly ceases from a point greater than 25\% of peak flow\textsuperscript{24}; Marostica and colleagues (also from the Indianapolis group) have proposed excluding curves where expiratory flow abruptly ceases from a point greater than 10\% of peak flow\textsuperscript{39}; whilst Vilozni and colleagues
have suggested excluding curves where cessation occurs abruptly from a flow of 300 mL·s\(^{-1}\) or more\(^{41}\). All suggestions have merit, particularly if consensus could be reached, but many spirometry software systems do not provide such information automatically. The current study design, where all curves are inspected by at least two experienced researchers, and "borderline" curves by at least three, is not possible in daily practice. It is therefore suggested that manufacturers should modify software, so that information on change in rate of lung emptying towards the end of expiration is easily available, with automatic identification and display of critical cut-off points as suggested above. In those curves where incomplete expiration is identified, timed expired volumes and peak flows may still be reportable\(^{110}\), even though FVC and forced expiratory flows cannot be reported from such manoeuvres.

3.6.3 Quantitative start of test criteria

ATS guidelines recommend that start of test be assessed quantitatively by calculating the volume of back extrapolation (VBE), and that this volume should be no greater than 5% of FVC, or an absolute volume of 150mL, whichever is the greater\(^3\)\(^4\). ERS criteria use 5% of FVC, or absolute volume of 100mL as the cut-offs\(^3\)\(^5\). Although the majority of school-age children can achieve the 5% criterion\(^4\)\(^4\), this does not appear to be the case amongst preschool children. In this study only 16 (27%) children produced a VBE/FVC of less than 5%, suggesting that this cut-off is too strict, whereas a cut-off of 150mL is too high to be of any value in a preschool population. In Figure 3.3 the four children who produced a VBE greater than 80mL have been marked: all of these children had VBE/FVC less than 12.5%.

All of the curves included in this analysis had been passed visually as showing acceptable rise to peak flow. After re-inspection of curves with high VBE or VBE/FVC, it was concluded that the initial visual inspection had included and excluded curves correctly. It is therefore suggested that expirations with VBE greater than 80mL or VBE/FVC greater than 12.5% should be visually re-inspected to ensure acceptable rise to peak flow, but should not necessarily be excluded.
3.6.4 Quantitative end of test criteria and timed expired volumes

ATS criteria recommend that expiration continues until there is a clear plateau on the volume-time trace, and that FET should be at least 6 seconds, or that there should be no volume change for one second. Although these ATS recommendations state that a shorter exhalation time is acceptable in children they do not specify what this time should be. ERS criteria do not quantify duration of expiration for quality control. A previous study has demonstrated that a 6-second forced expiration is impossible even for school-age children to achieve\textsuperscript{44}, and this study intended to examine this in our preschool population. Unfortunately, the FET results obtained from the Jaeger system were found to be unreliable.

Duration of expiration determines which time limited volumes are reportable. If FET is less than one second, then FEV\textsubscript{1} cannot be reported from that expiration. Success in producing FEV\textsubscript{1} was dependent on age in this study, with only 41% of children younger than 4 years able to produce a FEV\textsubscript{1}, compared with 80% of those over 5 years of age. There was no difference in the success rates of the healthy and CF groups. Previous researchers have reported similar findings. Eigen and co-workers only reported results from children who were able to expire for at least one second, but still recorded a creditable success rate of 83% in a slightly older population than that reported here (mean (SD) age 5.0 (0.9) years)\textsuperscript{24}. Crenesse and co-workers reported results in 473 children aged 3 to 5 years. Seventy-five percent (355) were able to produce at least one acceptable forced expiratory manoeuvre. Of these, only 75% of 3-<4 year olds, 73% of 4-<5 year olds, and 87% of 5-<6 year olds were able to produce a FET of at least one second\textsuperscript{36}. Nystad and colleagues reported results from 630 children aged 3-6 years, and found that 10% were unable to expire for 1 second\textsuperscript{40}. It is therefore recommended that spirometry software should calculate FEV\textsubscript{0.5} and FEV\textsubscript{0.75}, and that all laboratories should analyse and report both these parameters in addition to FEV\textsubscript{1} for children aged under 6 years, in order to allow comparison with others of similar age. For many 2-4 year old children, only the shorter timed volumes will be reportable. The Jaeger software currently allows timed expired volumes to be reported incorrectly. Operators should ensure that their own software does not contain similar errors. Any timed volume that is reported as equal to FVC should be considered potentially erroneous.
Even when FEV₁ is attainable, the clinical value of this parameter in this age group is questionable. Compared with older children and adults, infants and preschool children have large airways relative to their lung volume, and therefore empty their lungs more rapidly. As a result, the FEV₁/FVC ratio is over 90% in the majority of preschool children, including those aged 5 to 6 years, and regardless of whether these children have lung disease. In support of this point, it is noted that there is no relationship between FEV₁/FVC and height in healthy preschool children, whereas negative relationships between FEV₀.₇₅/FVC and height and FEV₀.₅/FVC and height were demonstrated. Thirty years ago, a report by Cogswell and colleagues identified FEV₀.₇₅ as an appropriate outcome measure in school-age children. Reporting of FEV₀.₄ or FEV₀.₅ in infants is now accepted, and assessment of FEV₀.₅ or FEV₀.₇₅ may be more clinically relevant than FEV₁ in the preschool age group.

3.6.5 Repeatability

ATS criteria recommend that the difference between the two highest values of FVC and FEV₁ should ideally be less than 200mL, whereas ERS criteria stipulate that such differences should be less than 100mL, or 5% of the best effort, whichever is the greater. In the present study, the repeatability of FVC and FEV₀.₇₅ were assessed. All but five children had ΔFVC less than 100mL, and all had a ΔFEV₀.₇₅ less than 100mL. However, as FVC and FEV₀.₇₅ are much lower in this age group, use of absolute values as measures of repeatability may not be appropriate. Arets and colleagues have previously reported that 88% of school-age children can produce a second FVC within 5% of their highest, and 87% can produce a second FEV₁ within 5% of their highest. In comparison, only 59% of the current study population were able to produce a second FVC and 72% a second FEV₀.₇₅ within 5% of the highest. However, for both parameters, almost all were able to produce a second effort within 10% of the highest. These figures are similar to those previously reported by Nystad and colleagues. It is therefore suggested that either an absolute value of 100mL, or 10% of best effort, would be achievable repeatability targets for FVC and FEV₀.₇₅ in this age group. As recommended for
adults, failure to meet repeatability criteria should not necessarily invalidate the manoeuvre.\(^3\)\(^4\).

### 3.6.6 Comparison with previously published data

In order to test whether results in healthy children were similar to those reported by other groups, these data were plotted alongside those previously reported by Eigen and colleagues\(^2\)\(^4\), and z-scores were calculated from published reference equations\(^2\)\(^4\),\(^40\). Original data were not available from the Oslo group, so it was not possible to plot the current data alongside those obtained in Oslo, nor was it possible to calculate composite reference equations from data collected in both Oslo and Indianapolis. Furthermore, there were differences in the exclusion criteria for the two reported studies, with the Indianapolis group excluding children with a prior history of asthma, whilst the Oslo group included such children. However, the Oslo group reported that their results were similar to those reported from Indianapolis, and also reported that children with a history of asthma did not have significantly different results from those with no history of asthma\(^40\).

The population measured in the current study had a higher proportion of non-white children (11 out of 29 successful children, or 38%) than that reported by Eigen and colleagues (30 out of 214, or 14%)\(^2\)\(^4\). This may explain why concordance between the two populations is better for white children only than when data from children of all ethnic groups is combined. From data presented in Figures 3.13 to 3.16 and Table 3.6, it appears that non-white children have lower FVC and lower timed expired volumes than white children of the same height. Small numbers and the heterogeneity of the non-white population prevent further conclusions being drawn from the data currently available. However, this finding is consistent with reports from older children.

Ethnic group was not specified by the Oslo group, but Oslo has a very small non-white population. Summary statistics were therefore calculated for the entire healthy population and separately for the healthy white children. Z-scores for the control population should be zero, with a standard deviation of 1, for all parameters, provided that the equipment and methodology are similar to that used by previous groups, and that the control population is similar to those from whom the reference
equations were derived\textsuperscript{112}. For the white population in the current study, this was true for FEV\textsubscript{0.5} z-scores calculated from Oslo data, for FVC and FEF\textsubscript{25-75} z-scores calculated from Indianapolis data, and arguably true for FEV\textsubscript{1} z-scores calculated from Indianapolis data. FVC and FEV\textsubscript{1} results did not fit the reference equations reported by the Oslo group.

3.6.7 Methodological issues and strengths and limitations

When FET results obtained from the database were compared with volume-time traces for the corresponding expirations it was apparent that FET had been overestimated in some cases (Figure 3.7). The reason for this is that some children removed their mouths from the mouthpiece immediately after ceasing expiration, rather than taking another inspiration through the mouthpiece. The Jaeger Masterscope system continues to record expiration until absolute cessation of expiratory flow is detected. This feature allows complete recording of expiration in subjects with severe airflow limitation. However, if there is a slight expiratory offset on the flow signal, expiration will continue to be recorded until the subject takes an inspiration.

In this study children were tested in the seated position wherever possible, and asked to wear nose-clips. Very young children may rebel against such instructions and in such cases these criteria should be relaxed if this is the only means of obtaining results. ATS guidelines suggest that subjects can be tested either seated or standing, but that this should be reported with the data\textsuperscript{34}. A recent report from school-age children who were tested with and without nose-clips found no systematic effect upon FEV\textsubscript{1} or FVC \textsuperscript{113}.

Incentive software can be distracting for older children\textsuperscript{43}, but in this study it was found to be helpful. The Jaeger incentive programme (which is routinely available with Jaeger spirometry software) was used for all children. Vilozni and colleagues have reported that only 2\% of 3 to 6 year old children are able to produce a FEV\textsubscript{1} with the Jaeger incentive software, as compared to 49\% of children who could produce a FEV\textsubscript{1} with their own Spirogame incentive software\textsuperscript{41}. It is noted that in Vilozni's study only the Jaeger 'Candle' incentive (which encourages rapid expiration) was used, and that, unlike in this study, the investigators did not move
on to alternative incentives that encourage prolongation of expiration. This methodological difference may explain the marked discrepancy between the current results (in a younger population) and those previously reported by Vilozni’s group. On a related matter, children in the current study performed a maximum of 22 expiratory manoeuvres, rather than the eight recommended by the ATS\textsuperscript{34}. The reason for this is that many younger children initially produced incomplete expirations, particularly when using the ‘Candle’ incentive. The Jaeger system does not allow easy recording of repeated manoeuvres, and does not allow borderline or excluded traces to be stored for later reanalysis. Overcoming these hurdles required considerable effort, including the use of custom written software, and is a strength of this study.

The most notable weakness of the current study is that children were tested towards the end of a two-hour laboratory visit. It is likely that some children were fatigued by the time they performed spirometry, and success rates may have been adversely affected. It is unlikely that other parameters would have been influenced by this methodological issue. By contrast, one factor that may have increased the success rate was the use of a specially adapted preschool laboratory, with data collection performed by trained operators. This set-up is not always available in the clinical setting.

Suggestions for alternative quality control criteria for preschool children are discussed further in Chapter 7.

3.6.8 Conclusion

In conclusion, data presented in this chapter have demonstrated that children aged 2 to 5 years are unable to meet some of the quality control criteria for spirometry recommended for adults, but alternative criteria may be feasible. These alternative criteria are discussed further in Chapter 7.
Chapter 4: Feasibility and quality control for multiple-breath inert gas washout

4.1 Introduction

Early studies using the MBW technique were performed in adults and older children, and required subjects to breathe in a controlled manner, with a regular respiratory rate and tidal volume. This methodology is not possible in younger children, where measurements must be performed during natural tidal breathing. During spontaneous tidal breathing, subjects may vary their respiratory rate (RR), tidal volume \( V_T \) and/or functional residual capacity (FRC).

A small number of studies in adults have examined the effect of changes in RR, \( V_T \) and FRC upon MBW indices such as the lung clearance index (LCI), the mixing ratio (MR) and the phase III slope. The breathing patterns employed during these studies were beyond the limits of normal physiological tidal breathing. Furthermore, these studies have all been performed in adults, and it is known that somatic growth can greatly influence lung function parameters (Chapter 3).

Prior to examining whether the LCI and indices derived from phase III slope analysis can be used to distinguish health from disease, it is necessary to examine the effect of subject characteristics and breathing pattern variability upon these parameters.

4.2 Aims and hypotheses

4.2.1 Hypotheses

a) In healthy subjects, the LCI is independent of subject age, age group (i.e. preschool or school-age), sex, and body size. In all subjects, the LCI is independent of variations in RR, \( V_T \), and FRC seen during spontaneous tidal breathing
b) In healthy subjects, the phase III slope is negatively related to tidal volume and FRC, but otherwise independent of subject age, age group, sex, and body size.

4.2.2 Aims and objectives

The aim of this study was to examine the effect of breathing pattern and various analysis methods upon MBW indices. The specific objectives of this study were:

a) To examine the precision of the Lung Clearance Index and the first breath phase III slope, over three repeated measures
b) To investigate whether subject age, age group, sex, and body size influence the Lung Clearance Index and the phase III slope
c) To investigate whether spontaneous variations in RR, V_t, and FRC during normal tidal breathing are sufficient to influence the Lung Clearance Index and the phase III slope
d) To examine the effect of excluding SnIII results from breaths of inadequate volume (Chapter 2) upon the SnIII versus lung volume turnover relationship
e) To determine whether the SnIII versus lung volume turnover relationship is linear over 1.5 to 6.0 turnovers, in spontaneously breathing children.

4.3 Subjects and methods

4.3.1 Subject recruitment

The study was performed in both school-age and preschool children, with and without CF.

School-age children with CF were recruited from the CF clinic at Great Ormond Street Hospital. All subjects were approached by the author. Subjects were recruited opportunistically: i.e. no attempt was made to recruit all children with CF who were eligible for participation in the study, or to recruit a random or representative sample from the clinic. The study was explained verbally, and written information leaflets were also provided. Written informed consent was obtained from parents, and from all children aged 12 years or greater. Younger children gave verbal assent.
Children with CF were asked to bring one or more friends with them on the day of testing to act as healthy controls. Additional controls were recruited through the families of CF children participating in this study and from friends and family of members of laboratory staff. In all cases, verbal and written information was provided prior to the day of testing, and written consent was obtained as for the children with CF.

**Inclusion criteria for school-age subjects with CF:**

- CF diagnosed by a positive sweat test and / or two known CF disease-producing mutations identified by DNA analysis
- Subject aged ≥ 6.0 years and <17.0 years at time of testing.

**Exclusion criteria for school-age subjects with CF:**

- Congenital cardiac disease that requires medical therapy, has previously required surgical therapy, or is awaiting surgical therapy
- Neuromuscular or bone disease likely to result in weakness of respiratory muscles or restriction of the thorax.

**Inclusion criteria for school-age healthy control children:**

- Subject aged ≥ 6.0 years and <17.0 years at time of testing.

**Exclusion criteria for school-age healthy control children:**

- Any previous hospitalisation for a respiratory condition
- Use of anti asthma medication on more than one occasion in the previous 12 months
- Any history of chronic productive cough, recurrent wheezing, or shortness of breath within the previous 12 months
- Congenital cardiac disease that requires medical therapy, has previously required surgical therapy, or is awaiting surgical therapy
- Children with neuromuscular or bone disease likely to result in weakness of respiratory muscles or restriction of the thorax.

Recruitment of preschool subjects is described in Section 3.3.1.

Copies of consent forms and information sheets for both school-age and preschool children are provided in Appendices B-D.
4.3.2 Study design

This was a cross-sectional study. All school-age subjects successfully completing spirometry and MBW were included in the analyses presented in this chapter. No school-age subjects were recalled for a second visit. Preschool subjects were participating in a study comparing spirometry, body plethysmography, and MBW results. Subjects who did not complete all three measurements were asked to return to the laboratory six months after their first visit for repeat measurements. The study was continued until 30 subjects with CF and 30 healthy subjects had completed all three measurements. MBW results from these 60 children are presented in this chapter.

4.3.3 Data collection

Collection and analysis of MBW data, including criteria for accepting or discarding results; and derivation of the indices listed below, are described in Chapter 2. Some breaths were considered to be of inadequate volume for estimation of SnIII (Section 2.4.8). Nevertheless, for these breaths, the SnIII was calculated over 65-95% of expired volume, and the results recorded to allow comparison with breaths of larger volume.

4.4 Analysis

4.4.1 Outcome measures

The following measures were reported from each MBW run (note: these values will be identical for SF₆ and He analyses):

- The mean respiratory rate during the washout (RR\text{mean})
- The mean expired volume during the washout (V_{T\text{mean}})
- The expired volume of the first breath of the washout (first breath V_{exp}).

Both V_{T\text{mean}} and first breath V_{exp} were corrected for apparatus dead-space.

The following measures were reported from each SF₆ MBW run:

- The Lung Clearance Index (LCI)
- The Functional Residual Capacity (FRC).

The following measure was reported separately for each SF₆ and He run:
• The normalised phase III slope (SnIII) calculated from the first breath of the washout.

For all the above measures the mean value from three runs was also calculated and reported.

The following measures were calculated from mean data collected from three SF6 and He MBW runs, and reported separately for SF6 and He data:

• The slope of the regression of SnIII versus lung volume turnover (TO) over 1.5 to 6.0 TO:
  a) calculated from all SnIII data (raw $S_{\text{cond}}$, [$S_{\text{condR}}$])
  b) calculated after excluding SnIII results obtained from breaths of inadequate volume (see chapter 2, corrected $S_{\text{cond}}$, [$S_{\text{condC}}$])

• The slope of the regression of SnIII versus lung volume turnover (TO) over 2.5 to 6.0 TO, calculated after excluding SnIII results obtained from breaths of inadequate volume ($S_{\text{cond2.5}}$)

• The square of the correlation coefficient ($r^2$) for the regressions of $S_{\text{condR}}$ and $S_{\text{condC}}$.

4.4.2 Analysis

Subject characteristics (age, sex, weight, height, and weight and height z-scores) were compared by diagnosis and by age group.

The precision of LCI was determined by calculating the coefficient of variation (CoV) as $100 \cdot \text{SD} \div \text{mean} \cdot 100$ (%) and by Bland-Altman analyses of the mean LCI calculated over three runs (LCI mean), versus the mean LCI calculated from the first two runs (LCI 1,2), and the LCI calculated from the first run only (LCI 1).

The relationships between subject characteristics and LCI were examined by regression analyses, with LCI mean as the dependent variable. The relationships between LCI and breath characteristics were then examined, with data from individual MBW runs (rather than mean data from three runs) entered into the regression models. These analyses were performed for four populations: healthy preschool children; preschool children with CF; healthy school-age children; and school-age children with CF. For each population, a series of univariate analyses was performed first, followed by multivariate analysis. As the final stage in these
analyses, data from the entire study population was entered into a regression model, with subject characteristics, breath characteristics, and diagnosis included as predictor variables.

The effect of breathing pattern upon LCI was examined further by a within-subject comparison. This was performed by calculating: the difference between the LCI obtained from the first run with the LCI obtained from the third run ($\Delta$LCI$_{1,3}$), and relating this to the difference in FRC between the first and third run ($\Delta$FRC$_{1,3}$); the difference in $V_{T\text{mean}}$ between the first and third run ($\Delta V_{T\text{mean}1,3}$); and the difference in RR$_{\text{mean}}$ between the first and the third run ($\Delta RR_{\text{mean}1,3}$), by scatter plot and regression analysis.

The effect of small breath volume on SnIII was assessed by comparing SnIII results from breaths considered to be of adequate volume against SnIII results obtained from breaths of inadequate volume. This was performed by identifying children in whom either one or two of the three first breaths had been identified as small volume. In the former case, a mean SnIII value and a mean breath volume was calculated for the two breaths of adequate volume. In the latter case, a mean SnIII value and mean breath volume was calculated for the two breaths of inadequate volume. SnIII values for breaths of adequate and inadequate volume could then be compared within subject for this population. Examples of phase III slopes calculated from breaths of small volume are presented in Chapter 2. Precision of SnIII was assessed as for LCI, i.e. by calculating the CoV, and by Bland-Altman analysis of the SnIII results from three runs (SnIII$_{\text{mean}}$) against the mean SnIII result from the first two runs (SnIII$_{1,2}$) and the SnIII result from the first run only (SnIII$_{1}$). The effect of subject characteristics and breathing pattern upon SnIII was examined as for LCI: i.e. by univariate and multivariate regression analyses examining the relationships between subject characteristics and first breath SnIII$_{\text{mean}}$; the relationships between breath characteristics (FRC and first breath $V_{\text{exp}}$) and first breath SnIII from individual runs; and within subject comparisons examining change in first breath SnIII, FRC and first breath $V_{\text{exp}}$ from the first breath of the first run to the first breath of the third run.

The effect of excluding breaths of inadequate volume upon the calculation of $S_{\text{cond}}$ was examined by comparing the fit ($r^2$) of the $S_{\text{cond}}$ regression line over 1.5 to 6.0
lung volume turnovers before and after the exclusion process, and by comparing $S_{\text{condR}}$ and $S_{\text{condC}}$, separately for SF$_6$ and He data. The origin of the linear portion of the SnIII versus TO relationship was examined by comparing $S_{\text{condC}}$ with $S_{\text{cond2.5}}$, separately for SF$_6$ and He data.

Group results were expressed as mean (SD) or median (IQR). Group comparisons were by one or two-sided t-test, or by their non-parametric equivalents. T test results were expressed as mean difference (95% confidence interval of difference) as well as by p-values. Comparison of results obtained by two methods was by Bland-Altman analysis. Regression model fit was expressed by $r^2$, whilst partial regression coefficients were calculated to demonstrate contribution of predictor variables to multivariate models. For each model the residual standard deviation (RSD) was also calculated. A p-value equal to or less than 0.05 was regarded as statistically significant.

4.4.3 Power of study

The power calculation for this sample was based upon the requirements of the analysis presented in Chapter 5, where results obtained from MBW and spirometry are compared for CF and control groups (see section 3.4.4). For Chapter 5, sample sizes of 22 school-age subjects in each group, and 30 preschool subjects in each group were predicted to be sufficient to detect significant differences in lung function parameters. Full details of this analysis are presented in Section 5.4.4.

4.5 Results: washout curve analysis

4.5.1 Study population

Fifty-seven school-age children were studied. From two children with CF the spirometry results failed to meet the criteria laid out in the methods. Results from these children were therefore excluded from further analysis. All subjects completed three successful washouts.

Information regarding the remaining 55 children is summarised in Table 4.1. There were 22 subjects with CF, aged 6.4 to 16.5 years, and 33 control subjects, aged 5.9 to 16.8 years. CF and control subjects were well matched for age, height and
gender. The children with CF were significantly shorter and significantly lighter than the control subjects.

### Table 4.1 School-age subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>CF (n = 22)</th>
<th>Controls (n = 33)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>50%</td>
<td>42%</td>
<td>8% (-18, 42)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.5 (3.2)</td>
<td>11.3 (3.1)</td>
<td>0.2 (-1.6, 2.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>36.9 (11.8)</td>
<td>41.1 (14.7)</td>
<td>-4.2 (-11.8, 3.3)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>-0.41 (0.96)</td>
<td>0.37 (0.78)</td>
<td>-0.78 (-1.2, -0.3)*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>142.5 (18.6)</td>
<td>147.4 (18.3)</td>
<td>-4.9 (-15.1, 5.2)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.61 (1.20)</td>
<td>0.50 (0.74)</td>
<td>-1.11 (-1.6, -0.6)*</td>
</tr>
</tbody>
</table>

*Legend: Except for sex, results are given as mean (SD). Mean difference calculated as CF - control. * \( p<0.05 \)*

Of the 22 school-age children with CF, 15 were homozygous for the \( \Delta F508 \) mutation, whereas the other seven had one \( \Delta F508 \) mutation and one other mutation. Forty preschool children with CF and 37 healthy preschool children were recruited. Although matched for age, the children with CF were significantly lighter and significantly shorter than the control children (Table 4.2).
**Table 4.2 Characteristics of initial preschool study population**

<table>
<thead>
<tr>
<th></th>
<th>CF (n = 40)</th>
<th>Controls (n = 37)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>45%</td>
<td>61%</td>
<td>-16 (-36, 6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.13 (0.90)</td>
<td>4.22 (0.87)</td>
<td>-0.09 (-0.48, 0.30)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>16.6 (2.8)</td>
<td>18.8 (3.7)</td>
<td>-2.1 (-3.6, -0.65)**</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>-0.04 (1.11)</td>
<td>0.70 (1.16)</td>
<td>-0.74 (-1.24, -0.24)**</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>100.8 (7.4)</td>
<td>104.8 (8.0)</td>
<td>-4.0 (-7.4, -0.6)*</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.42 (1.13)</td>
<td>0.29 (1.14)</td>
<td>-0.71 (-1.21, -0.21)**</td>
</tr>
</tbody>
</table>

Legend: Except for sex, results are given as mean (SD). Mean difference calculated as CF - control.

* p<0.05, ** p<0.01

Success in completing MBW measurements at first laboratory visit is presented in Table 4.3. There was no significant difference in success by age or diagnosis for LCI.

**Table 4.3 Success in completing MBW measurements on first visit: all preschool children**

<table>
<thead>
<tr>
<th></th>
<th>All children (n=77)</th>
<th>Cystic Fibrosis (n=40)</th>
<th>Healthy Controls (n=37)</th>
<th>2 - 3 years (n=6)</th>
<th>3 - 4 years (n=32)</th>
<th>4 - 5 years (n=24)</th>
<th>5 - 6 years (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBW</td>
<td>61 (79%)</td>
<td>30 (75%)</td>
<td>31 (84%)</td>
<td>3 (50%)</td>
<td>25 (78%)</td>
<td>20 (83%)</td>
<td>13 (87%)</td>
</tr>
</tbody>
</table>

Legend: Results presented as number (%). Comparisons between groups by Chi squared or Fisher's exact test are presented in the text.

In addition to completing MBW measurement, preschool children were required to successfully complete spirometry and body plethysmography measurements. On the
first visit, 27 healthy children and 23 children with CF were able to complete this protocol (see Figure 2.2). On repeat visits a further four healthy controls and seven children with CF were able to complete the protocol, giving a total study population of 31 healthy children and 30 children with CF. However, it was noted that the CF and control populations were not matched for ethnicity, and so the study was continued until a further five white children had completed the protocol. The three healthy black children who had completed the protocol and the last three healthy children of mixed race who had completed the protocol were excluded from the control group for this study. Summary data for the final preschool study population are presented in Table 4.4.

Table 4.4 Characteristics of final preschool study population

<table>
<thead>
<tr>
<th></th>
<th>CF (n=30)</th>
<th>Healthy controls (n=30)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (%M)</td>
<td>43%</td>
<td>60%</td>
<td>-17% (-42, 8)</td>
</tr>
<tr>
<td>Ethnicity (% white)</td>
<td>87%</td>
<td>80%</td>
<td>7% (-12, 25)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.43 (0.77)</td>
<td>4.31 (0.84)</td>
<td>0.13 (-0.29, 0.55)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>17.7 (2.6)</td>
<td>18.8 (3.4)</td>
<td>-1.2 (-2.7, 0.4)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.15 (1.07)</td>
<td>0.69 (1.19)</td>
<td>-0.57 (-1.15, 0.02)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>103.9 (6.3)</td>
<td>105.3 (7.7)</td>
<td>-1.4 (-5.0, 2.2)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.24 (1.15)</td>
<td>0.27 (1.25)</td>
<td>-0.49 (-1.11, 0.13)</td>
</tr>
</tbody>
</table>

Legend: Except for sex, results are given as mean (SD). Mean difference calculated as CF - control.

Comparison of subject characteristics for preschool and school-age groups is presented in Table 4.5. As preschool children were younger, they were also significantly shorter and lighter. There was no significant difference in weight z-scores or height z-scores between the two groups.
Table 4.5 Characteristics of total study population, compared by age group.

<table>
<thead>
<tr>
<th></th>
<th>Preschool (n = 60)</th>
<th>School-age (n = 55)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>52%</td>
<td>45%</td>
<td>6 (-12, 24)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.37 (0.80)</td>
<td>11.34 (3.17)</td>
<td>-6.97 (-7.81, -6.12)***</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>104.6 (6.9)</td>
<td>145.4 (18.4)</td>
<td>-40.8 (-45.9, -35.7)***</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.2 (3.1)</td>
<td>39.4 (13.7)</td>
<td>-21.2 (-24.8, -17.6)***</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.02 (1.21)</td>
<td>-0.03 (0.82)</td>
<td>0.05 (-0.97, 1.02)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.42 (1.11)</td>
<td>-0.01 (0.86)</td>
<td>0.43 (-0.49, 1.35)</td>
</tr>
</tbody>
</table>

Legend: Except for sex, results are given as mean (SD). Mean difference calculated as preschool – school-age. *** p<0.001

There was no significant difference in weight z-scores or height z-scores between the two groups.

4.5.2 Precision of Lung Clearance Index

The relationship between CoV_{LCl}, age, and diagnosis is presented in Figure 4.1. Only 10 of the 115 children had CoV_{LCl} greater than 10%, of whom six were preschool children with CF.

CoV_{LCl} was compared by age group and diagnosis. Children with CF had significantly higher CoV_{LCl} than healthy children: (7.18% [4.46] versus 5.18% [2.56], mean difference -1.99% [-3.31, -0.68]). Preschool children tended to have higher CoV_{LCl} than school-age children, but this difference was not significant (6.43% [4.33] versus 5.71% [2.76], mean difference 0.72% [-0.64, 2.07]).
Figure 4.1 Coefficient of variation for LCI, plotted against age, in all children

Legend: $CoV_{LCI}$ = Coefficient of variation for LCI. Children with CF presented as closed circles, healthy children as open circles. Broken line is at $CoV_{LCI}$ of 10%. Only 10 of the 115 children had $CoV_{LCI}$ greater than 10%, of whom six were preschool children with CF.

A Bland-Altman comparison of LCI results obtained from the first two runs ($LCI_{1,2}$) with those obtained from all three runs ($LCI_{mean}$) is presented in Figure 4.2. There was no significant group difference in LCI results obtained from two versus three runs, either for the population as a whole, or for the preschool, school-age, healthy and CF subgroups (Table 4.6). Limits of agreement were narrow, being $-0.54, 0.49$ for the population as a whole (Table 4.6). Results presented later in this chapter and in Chapter 5 demonstrate that these differences are smaller than those seen between CF and control groups.
Figure 4.2 Bland-Altman plot of mean LCI result obtained from two runs, compared with mean LCI result obtained from three runs, in all children.

Legend:  
$LCI_{1,2} = \text{LCI result obtained as mean of first two runs.}$

$LCI_{\text{mean}} = \text{LCI result obtained as mean of all three runs.}$

Difference calculated as $LCI_{1,2} - LCI_{\text{mean}}$. Children with CF presented as closed circles, healthy children as open circles. Broken lines represent 95% limits of agreement, calculated as mean $\pm$ 1.96SD, from full population (CF and control, see Table 4.6).
Table 4.6 Bland-Altman analysis of mean LCI result obtained from two runs, compared with mean LCI result obtained from three runs

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=115)</td>
<td>-0.03 (0.26)</td>
<td>-0.08, 0.02</td>
<td>-0.54, 0.49</td>
</tr>
<tr>
<td>All children with CF (n=52)</td>
<td>-0.06 (0.35)</td>
<td>-0.16, 0.03</td>
<td>-0.74, 0.62</td>
</tr>
<tr>
<td>All healthy children (n=63)</td>
<td>0.00 (0.16)</td>
<td>-0.04, 0.04</td>
<td>-0.31, 0.31</td>
</tr>
<tr>
<td>All preschool children (n=60)</td>
<td>-0.01 (0.31)</td>
<td>-0.09, 0.07</td>
<td>-0.61, 0.59</td>
</tr>
<tr>
<td>All school-age children (n=55)</td>
<td>-0.05 (0.21)</td>
<td>-0.10, 0.01</td>
<td>-0.45, 0.36</td>
</tr>
</tbody>
</table>

Legend: The 95% CI of difference of the mean is calculated by one-sided t-test, against zero, as mean difference +/- (1.96 · Standard Error of the mean). This analysis indicates whether there is a significant group difference between the results. The 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control) indicate the agreement between the two methods. Both these values are discussed further in Section 4.7.

Difference calculated as $LCI_{1,2} - LCI_{mean}$

There was no significant group difference between $LCI_{1,2}$ and $LCI_{mean}$.

A Bland-Altman comparison of LCI results obtained from the first run with those obtained from all three runs is presented in Figure 4.3.
Figure 4.3 Bland-Altman plot of LCI result obtained from the first run, compared with mean LCI result obtained from three runs, in all children.

Legend:  

\[ LCI_1 = \text{LCI result obtained from the first run only.} \]

\[ LCI_{\text{mean}} = \text{LCI result obtained as mean of all three runs.} \]

Difference calculated as \( LCI_1 - LCI_{\text{mean}} \). Children with CF presented as closed circles, healthy children as open circles. Broken lines represent 95% limits of agreement, calculated as mean ± 1.96SD, from full population (CF and control, see Table 4.7).

There was a trend for lower results for LCI\(_1\) compared with LCI\(_{\text{mean}}\). This difference was significant when school-age children were analysed as a subgroup (Table 4.7). This phenomenon is examined further in section 4.5.6.
Table 4.7 Bland-Altman analysis of LCI result obtained from one run, compared with mean LCI result obtained from three runs

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=115)</td>
<td>-0.10 (0.57)</td>
<td>-0.21, 0.00*</td>
<td>-1.22, 1.01</td>
</tr>
<tr>
<td>All children with CF</td>
<td>-0.14 (0.75)</td>
<td>-0.35, 0.07</td>
<td>-1.62, 1.34</td>
</tr>
<tr>
<td>All healthy children</td>
<td>-0.07 (0.36)</td>
<td>-0.16, 0.02</td>
<td>-0.77, 0.63</td>
</tr>
<tr>
<td>All preschool children</td>
<td>-0.05 (0.60)</td>
<td>-0.20, 0.11</td>
<td>-1.23, 1.13</td>
</tr>
<tr>
<td>All school-age children</td>
<td>-0.16 (0.54)</td>
<td>-0.31, -0.02*</td>
<td>-1.22, 0.90</td>
</tr>
</tbody>
</table>

Legend: Difference calculated as $LCI_1 - LCI_{mean}$

* $p=0.056$, ** $p<0.05$

There was a trend for lower results for $LCI_1$ compared with $LCI_{mean}$. This difference was significant when school-age children were analysed as a subgroup.

4.5.3 Effect of subject characteristics on Lung Clearance Index

For all analyses presented in this section, the mean LCI result calculated from three washouts ($LCI_{mean}$) is the dependent variable. Figure 4.4 demonstrates the relationship between age and $LCI_{mean}$ in healthy children.
Figure 4.4 LCI_{mean} plotted against age in healthy children

Legend: The broken line marks the division between the preschool and school-age groups. There is a weak negative relationship between LCI_{mean} and age, but it is not clear from the Figure whether this relationship is linear, or whether there is a step change between the two age groups. This relationship is examined further by regression analysis (see text).

The relationships between LCI_{mean} and subject characteristics in healthy children were examined further by regression analyses. By univariate analyses, there were significant negative relationships between height and LCI_{mean}, between weight and LCI_{mean}, and between age and LCI_{mean}. There was no relationship between sex and LCI_{mean}. When age group was tested as a categorical variable, with preschool coded as zero, and school-age as one, there was a significant negative relationship. Results of multivariate analysis of LCI_{mean} against subject characteristics are presented in Table 4.8.
Table 4.8 Results of multivariate regression of subject characteristics against LCImean in healthy children

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (B)</th>
<th>Standard Error of B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>7.86</td>
<td>1.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.171</td>
<td>0.071</td>
<td>0.02</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.0255</td>
<td>0.121</td>
<td>0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.0182</td>
<td>0.014</td>
<td>0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.0240</td>
<td>0.015</td>
<td>0.1</td>
</tr>
<tr>
<td>Age group</td>
<td>-0.646</td>
<td>0.244</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. Age group is coded as zero for preschool and one for school-age. After correction for other variables, both age and age group make a significant contribution to the model fit, but the greatest contribution is made by the constant. For the model as a whole, the RSD is 0.463, and the $r^2$ is 0.241, indicating that 24.1% of the variability of LCImean in healthy children is explained by this regression model.

Figure 4.5 demonstrates the relationship between age and LCImean in children with CF. There is a positive relationship between age and LCImean in the school-age population, but this relationship does not appear to extend to the preschool population.
Legend: The broken line marks the division between the preschool and school-age groups. Note the difference in y-axis scale compared with Figure 4.4. There is a positive relationship between LCI_{mean} and age in the school-age CF population, but this relationship does not appear to extend to the preschool population. The relationship is examined further by regression analysis.

For children with CF, by univariate analyses, there were significant positive relationships between age and LCI_{mean}, height and LCI_{mean}, weight and LCI_{mean} and age group and LCI_{mean}. There was no relationship between sex and LCI_{mean}. Results of multivariate analysis of LCI_{mean} against subject characteristics are presented in the Appendix. None of the entered variables were significant independent predictors of LCI_{mean}. The $r^2$ for the model was 0.218, indicating that only 21.8% of the variability of LCI_{mean} in children with CF is explained by the model.

As the last step in these analyses, results for all children in the study were examined by multivariate analysis, first modelling subject characteristics as predictor
variables, and then adding diagnosis (as a categorical variable) to this model. The first model included age, sex, height, weight and age group as predictor variables. For this model, the RSD was 2.40, and the $r^2$ was 15.4%.

Table 4.9 presents the results of the same regression analysis after including diagnosis as a predictor variable. Addition of diagnosis to the model increased the $r^2$ from 15.4% to 57.1%. The regression coefficient for diagnosis was 3.55, indicating that children with CF had an LCI 3.55 higher than healthy children, after adjustment for other variables.

Table 4.9 Results of multivariate regression of subject characteristics and diagnosis against LCI$_{\text{mean}}$ in all children

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (B)</th>
<th>Standard Error of B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>4.76</td>
<td>2.92</td>
<td>0.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.486</td>
<td>0.169</td>
<td>0.005</td>
</tr>
<tr>
<td>Sex</td>
<td>0.320</td>
<td>0.323</td>
<td>0.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.0118</td>
<td>0.036</td>
<td>0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.100</td>
<td>0.044</td>
<td>0.03</td>
</tr>
<tr>
<td>Age group</td>
<td>-1.17</td>
<td>0.654</td>
<td>0.08</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>3.55</td>
<td>0.344</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female and one for male. Age group is coded as zero for preschool and one for school-age. Diagnosis is coded as zero for healthy, and one for CF. Diagnosis is by far the most important predictor of LCI$_{\text{mean}}$ within this model, with a regression coefficient of 3.55, indicating that children with CF had an LCI 3.55 higher than healthy children, after adjustment for other variables. For the model as a whole, the RSD was 1.71, and the $r^2$ was 0.571, indicating that 57.1% of the variability of LCI$_{\text{mean}}$ is explained by this regression model.
4.5.4 Effect of breathing pattern upon Lung Clearance Index: between subject comparisons

For these analyses, the FRC, $V_{\text{mean}}$, and $R_{\text{R mean}}$ measured for each individual run was tested against the LCI calculated from each individual run. For these analyses, both breathing pattern (intra-subject variability), and subject characteristics (between-subject variability) will be expected to influence LCI. The results of these analyses are presented in detail in the Appendix. In summary, a weak negative relationship between LCI and $V_{\text{mean}}$ was noted in healthy children, and a weak positive relationship between LCI and FRC was noted in children with CF. When all children were included in the analyses, a model including FRC, $V_{\text{mean}}$, and $R_{\text{R mean}}$ explained only 4.1% of the variability of LCI. Addition of age, age group, sex, height, and weight to the model increased the $r^2$ to 23.0%, whilst further addition of diagnosis to this model increased the $r^2$ to 58.3%.

4.5.5 Change in LCI over three consecutive MBW runs

It was noted in section 4.5.2, that LCI results from the third run tended to be higher than those obtained from the first run, particularly in children with CF. This was examined further. A Bland-Altman plot of LCI results obtained from the first run with those obtained from the third run is presented in Figure 4.6. The difference between these values is $\Delta LCI_{1-3}$.
Figure 4.6 Bland-Altman plot of LCI result obtained from first run, compared with LCI result obtained from third run, all children

Legend: $LCI_1 =$ LCI result obtained from the first run. $LCI_3 =$ LCI result obtained from the third run.

Difference calculated as $LCI_1 - LCI_3$. Children with CF presented as closed circles, healthy children as open circles. Dashed lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from control population; dotted lines represent 95% limits of agreement calculated from CF population (Table 4.10).

$LCI_1$ was significantly lower than $LCI_3$ for the population as a whole. By subgroup analysis this trend was seen in all populations, but the difference was only significant in the school-age subgroup (Table 4.10).
Table 4.10 Bland-Altman analysis of LCI results obtained from first run, compared with LCI results obtained from third run

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=115)</td>
<td>-0.17 (0.89)</td>
<td>-0.34, -0.01 *</td>
<td>-1.91, 1.57</td>
</tr>
<tr>
<td>All children with CF (n=52)</td>
<td>-0.28 (1.16)</td>
<td>-0.61, 0.04</td>
<td>-2.55, 1.99</td>
</tr>
<tr>
<td>All healthy children (n=63)</td>
<td>-0.08 (0.58)</td>
<td>-0.22, 0.07</td>
<td>-1.22, 1.06</td>
</tr>
<tr>
<td>All preschool children (n=60)</td>
<td>-0.10 (0.93)</td>
<td>-0.34, 0.14</td>
<td>-1.92, 1.72</td>
</tr>
<tr>
<td>All school-age children (n=55)</td>
<td>-0.25 (0.86)</td>
<td>-0.48, -0.02 *</td>
<td>-1.94, 1.44</td>
</tr>
</tbody>
</table>

Legend:  
* Difference calculated as LCI₁ - LCI₃.  
* p<0.05  

LCI₁ was significantly lower than LCI₃ for the population as a whole. By subgroup analysis this trend was seen in all populations, but the difference was only significant in the school-age subgroup. Note that limits of agreement differ for control and CF groups.

The largest difference between LCI₁ and LCI₃ were seen for the school-age subgroup, and the CF subgroup. The relationship between ΔLCI₁,3 and age is presented in Figure 4.7. No significant relationship was seen.
Figure 4.7 Scatter plot of ΔLCl₁₃ against age, all children

Legend: Children with CF presented as closed circles, healthy children as open circles. Broken lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control, see Table 4.10).

4.5.6 Effect of breathing pattern on Lung Clearance Index: within subject comparisons

The relationship between breathing pattern and LCl was examined further by examining change in FRC, V₁mean, RR₁mean and LCI from the first run to the third run, within subjects. These breathing pattern characteristics will be expected to vary between the first and third runs, whilst the subject characteristics will remain unchanged. Effect of breathing pattern upon LCI can therefore be examined, with the confounding effect of between-subject variability eliminated. The relationship between ΔLCl₁₃ and ΔFRC₁₃ is presented in Figure 4.8. There was a weak negative relationship between ΔLCl₁₃ and ΔFRC₁₃ ($r^2 = 0.11$).
Figure 4.8 Scatter plot of ΔLCI₁.₃ against ΔFRC₁.₃, all children

Legend: Children with CF presented as closed circles, healthy children as open circles. There was a weak negative relationship between ΔLCI₁.₃ and ΔFRC₁.₃ ($r^2 = 0.11$).

Figure 4.8 suggests that many children with CF have ΔLCI₁.₃ less than zero, i.e. the LCI result obtained from the third run was higher than the result obtained from the first run. This is explored further in Section 4.5.6.

There was no relationship between ΔLCI₁.₃ and ΔV_{Tmean₁.₃} (Figure 4.9).
Figure 4.9 Scatter plot of $\Delta LC_{1,3}$ against $\Delta V_{T\text{mean}1-3}$, all children

Legend: Children with CF presented as closed circles, healthy children as open circles. There was no relationship between $\Delta LC_{1,3}$ and $\Delta V_{T\text{mean}1-3}$.

There was no relationship between $\Delta LC_{1,3}$ and $\Delta RR_{\text{mean}1-3}$ (Figure 4.10).
Figure 4.10 Scatter plot of $\Delta LCI_{1,3}$ against $\Delta RR_{mean 1-3}$, all children

Legend: Children with CF presented as closed circles, healthy children as open circles. There was no relationship between $\Delta LCI_{1,3}$ and $\Delta RR_{1,3}$.

The relationships between $\Delta FRC_{1,3}$, $\Delta V_{mean 1-3}$, $\Delta RR_{mean 1-3}$, and $\Delta LCI_{1,3}$ were explored further by regression analyses. Comparisons were first performed separately for healthy children and children with CF. These results are presented in detail in the Appendix. In summary, $\Delta FRC_{1,3}$ was a significant predictor of $\Delta LCI_{1,3}$ in both healthy children and those with CF. However, by multivariate analyses, all three variables only explained 12.7% of the variability of $\Delta LCI_{1,3}$ in healthy children, and 27.9% of the variability of $\Delta LCI_{1,3}$ in children with CF. When all children were studied together, the multivariate model predicted 16.1% of the variability of $\Delta LCI_{1,3}$ (Table 4.11). For this model, the regression coefficient for $\Delta FRC_{1,3}$ was -0.315, indicating that a 10% fall in FRC would be expected to produce a 3.2% increase in LCI. The coefficient for $\Delta RR_{mean 1-3}$ was 0.143, indicating that a 10% rise in RR would be expected to produce a 1.4% increase in
LCI. Addition of diagnosis and age group to this analysis had no significant effect upon model fit.

**Table 4.11 Results of multivariate regression against ΔLCI_{1.3}, in all children**

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (B)</th>
<th>Standard error of B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-1.96</td>
<td>0.913</td>
<td>0.03</td>
</tr>
<tr>
<td>ΔFRC_{1.3} (%)</td>
<td>-0.315</td>
<td>0.073</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΔV_{Tmean1.3} (%)</td>
<td>0.085</td>
<td>0.061</td>
<td>0.2</td>
</tr>
<tr>
<td>ΔRR_{mean1.3} (%)</td>
<td>0.143</td>
<td>0.047</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Legend: For the model as a whole, the RSD was 0.548, and the $r^2$ was 0.161, indicating that for healthy children studied under this protocol, 83.9% of the variability of ΔLCI_{1.3} was unrelated to changes in FRC, $V_{Tmean}$, or $RR_{mean}$. 

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4.6 Results: Phase III slope analysis

4.6.1 Exclusion of first breaths during quality control

Phase III slope analysis criteria included a mechanism for excluding breaths that were of such small volume that the phase III slope was unlikely to be visible (Figure 2.20). The effect of this approach was examined in first breaths, as these are essential for calculation of \( S_{acim} \), and as they are less affected by breathing pattern than subsequent breaths in the MBW. In 10 children, the first breath was excluded in one of the three runs. In six children, the first breath was excluded in two of the three runs. In one child, the first breath was excluded in all three runs. In the remaining 98 children, the first breath was of adequate volume in all three runs.

Mean \( S_{nIII} \) values and mean breath volume for breaths of adequate and inadequate volume were calculated for the 16 children in the first two groups, as described in Section 4.4.2.

By paired Wilcoxon test, \( S_{nIII} \) values for adequate volume breaths were significantly lower than \( S_{nIII} \) values for inadequate volume breaths (Table 4.12).

| Table 4.12 Comparison of \( S_{nIII} \) and breath volume between first breaths of adequate volume and first breaths of inadequate volume |
|---------------------------------|--------------------------|-----------------|---------|--------|
|                                  | Adequate volume          | Inadequate volume | Z-score | p-value |
| \( S_{nIII} \) (L⁻¹)            | 0.23 (0.18, 0.38)        | 1.38 (0.65, 3.09) | -3.5    | 0.001  |
| Breath volume (L)               | 0.30 (0.22, 0.47)        | 0.15 (0.11, 0.31) | -3.5    | <0.001 |

Legend: Results presented as median (IQR). Comparison by Wilcoxon signed ranks test.

A Bland-Altman plot of the paired \( S_{nIII} \) results is presented in Figure 4.11. Due to the skewed nature of these data, limits of agreement could not be calculated. However, this plot demonstrates that in 13 of the 16 children, the adequate volume
SnIII – inadequate volume SnIII result was less than -0.3 L⁻¹. In other words, in the majority of cases where small first breaths could be identified, the SnIII calculated from these breaths was at least 0.3 L⁻¹ greater than the SnIII calculated from first breaths of adequate volume obtained during the same test session.

Figure 4.11 Bland-Altman plot of first breath SF₆ SnIII results from breaths of adequate and inadequate volume

Legend: Children with CF presented as closed circles, healthy children as open circles. 1st breath SnIII, ad – inad = SnIII from breaths of adequate volume – SnIII from breaths of inadequate volume. 1st breath SnIII, mean of ad, inad = mean of SnIII from breaths of adequate volume and SnIII from breaths of inadequate volume. The data are skewed, so Limits of Agreement cannot be calculated.

These analyses were not repeated for the first breath He SnIII data, as there was a close correlation between the SnIII results obtained from SF₆ traces, and those obtained from the corresponding He traces (Section 4.6.3). For the analyses presented below, only breaths of adequate volume are considered. However, the effect of breath volume upon SnIII is addressed further in Chapter 6.
4.6.2 Precision of first breath SnIII

All three first breaths were of adequate volume in 49 healthy children (21 preschool, 28 school-age), and in 45 children with CF (26 preschool, 19 school-age). The precision of first breath SF₆ SnIII results was assessed in these 94 children. The median (IQR) CoV_snIII for the entire population was 42.5% (24.6, 62.8). The relationship between the coefficient of variation for first breath SF₆ SnIII (CoV_snIII) and age is presented in Figure 4.12.

Figure 4.12 Coefficient of variation for first breath SF₆ SnIII, plotted against age

Legend: CoV_snIII = Coefficient of variation for first breath SF₆ SnIII. Children with CF presented as closed circles, healthy children as open circles.

CoV_snIII was compared by age group and diagnosis, using Mann-Whitney U Test. Children with CF had significantly lower CoV_snIII than healthy children: (30.6% [14.9, 50.7] vs. 45.1% [36.2, 57.9], Z -2.99, p 0.003). There was no significant difference in CoV_snIII between preschool and school-age children (44.5% [23.8, 53.8], vs. 38.4 [21.2, 56.4], Z -0.6, p 0.6).
A Bland-Altman comparison of first breath SF$_6$ SnIII results obtained from the first two runs (SnIII$_{1,2}$) with those obtained from all three runs (SnIII$_{mean}$) is presented in Figure 4.13.

**Figure 4.13 Bland-Altman plot of mean first breath SF$_6$ SnIII result obtained from two runs, compared with mean first breath SF$_6$ SnIII result obtained from three runs, all children**

Legend: $SnIII_{1,2}$ = First breath SF$_6$ SnIII result obtained as mean of first two runs.  
$SnIII_{mean}$ = First breath SF$_6$ SnIII result obtained as mean of all three runs.  
Difference calculated as $SnIII_{1,2} - SnIII_{mean}$. Children with CF presented as closed circles, healthy children as open circles.

There was no significant group difference in first breath SF$_6$ SnIII results obtained from two versus three runs, either for the population as a whole, or for the preschool, school-age, healthy and CF subgroups. However, it can be seen from
Figure 4.13 that the data are widely scattered. The data distribution is heteroscedastic, i.e. as the mean first breath SnIII increased, so the magnitude of the difference between SnIII\textsubscript{1,2} and SnIII\textsubscript{mean} also increased. However, the polarity of this difference was not consistent. The heteroscedastic distribution prevents calculation of limits of agreement.

A Bland-Altman comparison of first breath SF\textsubscript{6} SnIII results obtained from the first run only (SnIII\textsubscript{1}) with those obtained from all three runs (SnIII\textsubscript{mean}) is presented in Figure 4.14.

**Figure 4.14 Bland-Altman plot of first breath SF\textsubscript{6} SnIII result obtained from first run, compared with mean first breath SF\textsubscript{6} SnIII result obtained from three runs, all children**

Legend: 
SnIII\textsubscript{1} = First breath SF\textsubscript{6} SnIII result obtained from first run.
SnIII\textsubscript{mean} = First breath SF\textsubscript{6} SnIII result obtained as mean of all three runs.

Difference calculated as SnIII\textsubscript{1,2} - SnIII\textsubscript{mean}. Children with CF presented as closed circles, healthy children as open circles.
There was no significant group difference in first breath SF₆ SnIII results obtained from one versus three runs, either for the population as a whole, or for the preschool, school-age, healthy and CF subgroups. However, limits of agreement were wide. As for Figure 4.13, the data distribution displayed in Figure 4.14 is heteroscedastic, and limits of agreement cannot be calculated.

4.6.3 Relationships between first breath SnIII and subject characteristics: between subject comparisons

For the 94 subjects who had three first breaths of adequate volume, the mean first breath SnIII was calculated from the SF₆ trace, and from the He trace. There was close correlation between these results \( r^2 = 0.90, p<0.001 \), Figure 4.15.

Figure 4.15 mean first breath SF₆ SnIII plotted against mean first breath He SnIII, all subjects

Legend: Children with CF plotted as closed circles, healthy children plotted as open circles. There was close correlation between the SF₆ and He results \( r^2 = 0.90, p<0.001 \).
For all further analyses in this chapter, SF$_6$ data alone are analysed. Further comparisons of SF$_6$ and He SnIII are presented in Chapter 6.

Mean first breath SF$_6$ SnIII was related to subject characteristics. Only subjects who had three first breaths of adequate volume were included in the analyses presented in this section. In healthy children, preschool children had significantly higher first breath SF$_6$ SnIII than school-age children (0.46 [0.26] vs. 0.18 [0.12] L$^{-1}$, mean [95% CI] difference 0.29 L$^{-1}$ [0.17, 0.40, p<0.001]).

In healthy preschool children, by univariate analyses, and by multivariate analysis: age, sex, height and weight were not significant predictors of first breath SnIII, either independently or in combination ($r^2$ for multivariate model 0.03, p 0.5).

In healthy school-age children, by univariate analyses, age, height, and weight were all significant predictors of first breath SnIII (all negative relationships), whereas sex was not a predictor. By multivariate analysis, none of the four variables were significant independent predictors of first breath SnIII, and the multivariate model was a weak but statistically significant predictor ($r^2$ 0.22, p 0.05, model presented in the Appendix).

When all healthy children were studied together, with age group also modelled as an explanatory variable, none of the five variables were significant independent predictors of first breath SnIII. However, the multivariate model was a significant predictor ($r^2$ 0.38, p<0.001, Table 4.13).
**Table 4.13 Results of multivariate regression against mean first breath SnIII in all healthy children**

<table>
<thead>
<tr>
<th>Coefficient (B)</th>
<th>Standard Error of B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.521</td>
<td>0.540</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.0533</td>
<td>0.034</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.0110</td>
<td>0.058</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.0123</td>
<td>0.007</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>$4.16 \cdot 10^{-4}$</td>
<td>0.007</td>
</tr>
<tr>
<td>Age-group</td>
<td>-0.14</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. Age-group is coded as zero for preschool, and one for school-age. By multivariate analysis, none of the variables were independent predictors. The multivariate model was a significant predictor of first breath SnIII ($RSD 0.191, r^2 0.38, p<0.001$).

Results for children with CF are presented in the Appendix. In summary, none of the variables were predictors of first breath SF$_6$ SnIII by multivariate analysis. Finally, results from all children were modelled together: first without and then with diagnosis included as an explanatory variable. The first multivariate model was a significant predictor of first breath SF$_6$ SnIII ($r^2 0.27, p<0.001$). Addition of diagnosis to the model increased the $r^2$ to 0.33. After correction for other variables, diagnosis was the most significant explanatory variable, though height and age were also significant predictors (Table 4.14).
Table 4.14 Results of multivariate regression against mean first breath SF₆ SnIII in all children, including diagnosis as a predictor

<table>
<thead>
<tr>
<th>Coefficient (B)</th>
<th>Standard Error of B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.553</td>
<td>0.507</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.0744</td>
<td>0.029</td>
</tr>
<tr>
<td>Sex</td>
<td>0.0285</td>
<td>0.054</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.0134</td>
<td>0.006</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.0026</td>
<td>0.008</td>
</tr>
<tr>
<td>Age-group</td>
<td>-0.143</td>
<td>0.106</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>0.178</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. Age-group is coded as zero for preschool, and one for school-age. Diagnosis is coded as zero for control, and one for CF. By multivariate analysis, the diagnosis of CF was the most important predictor of first breath SF₆ SnIII, though subject age and height were also significant independent predictors. The multivariate model was a significant predictor of first breath SnIII (RSD 0.260, $r^2$ 0.33, $p<0.001$).

The regression coefficient for diagnosis was 0.178, indicating that children with CF had a first breath SF₆ SnIII 0.178 L⁻¹ higher than healthy children, after adjustment for other variables. Further comparison of SnIII results between CF and healthy populations is presented in Chapter 6.

4.6.4 Relationships between first breath SF₆ SnIII and breath characteristics: between subject comparisons

In healthy children, the relationships between first breath SF₆ SnIII, and breath characteristics were examined for the 171 first breaths that were of adequate volume. Figure 4.16 displays the relationship between first breath SF₆ SnIII and subject age. A negative relationship was noted ($r^2$ 0.23, $p<0.001$).
Figure 4.16 First breath SF₆ SnIII plotted against subject age for all runs in all healthy children.

Legend: Only breaths of adequate volume are included. There is a negative relationship between first breath SF₆ SnIII and age ($r^2 0.23$, $p<0.001$).

Figure 4.17 displays the relationship between first breath SF₆ SnIII and breath volume. A hyperbolic relationship was noted.
Figure 4.17 First breath SF₆ SnIII plotted against expired volume for all runs in all healthy children

Legend: Only breaths of adequate volume are included. There is a hyperbolic relationship between first breath SF₆ SnIII and expired volume.

Figure 4.18 displays the relationship between first breath SF₆ SnIII as a product of expired volume, and subject age. There was no significant relationship between the two variables ($r^2 0.004$, $p=0.4$).

The relationship between subject characteristics and first breath SF₆ SnIII as a product of expired volume was examined further by multiple regression in healthy children. To allow direct comparison with the regression analysis presented in Table 4.13, the first breath SF₆ SnIII as a product of expired volume was calculated for each run, the mean of the three results was calculated, and this mean was the dependent variable for the regression analysis. Only subjects who had three first breaths of adequate volume were included in the analysis. None of the five
explanatory variables (age, sex, height, weight, or age-group) was a significant predictor of the mean first breath SF₆ SnIII as a product of mean expired volume, either independently or in combination ($r^2$ for multivariate model 0.017, $p$ 0.3).

**Figure 4.18 First breath SF₆ SnIII as a product of expired volume, plotted against subject age for all runs in all healthy children**

*Legend: Only breaths of adequate volume to allow SnIII estimation are included. There was no significant relationship between the two variables ($r^2$ 0.004, $p$=0.4).*

Finally, the first breath SF₆ SnIII as a product of expired volume was calculated for breaths that were excluded from the above analyses because of inadequate breath volume. Eighteen such breaths were identified in healthy children. The first breath SF₆ SnIII as a product of expired volume was significantly higher for these ‘excluded’ breaths than for the 171 ‘included’ breaths that had been considered of adequate volume for phase III slope estimation (0.191 [0.115] versus 0.076 [0.042], mean difference excluded – included 0.115 [0.058, 0.173] $p$=0.001). Figure 4.19
suggests that for some of these excluded breaths the phase III slope may have been estimated correctly, whilst for others the calculated phase III slope was clearly too high.

**Figure 4.19 First breath SF₆ SnIII as a product of expired volume, plotted against subject age for all runs in all healthy children, with excluded breaths also plotted**

Legend: Breaths that were considered to be of inadequate volume for phase III slope estimation are presented as closed markers, whilst breaths that were of adequate volume are presented as open markers. There was a significant difference between the two groups (see text).
4.6.5 Relationships between first breath SnIII and breath characteristics: within subject comparisons

The relationships between first breath SF₆ SnIII and breath characteristics were examined further by examining change in SnIII (Δ1st breath SnIII₁₋₃), FRC (ΔFRC₁₋₃) and expired volume (Δ1st breath Vₑₓᵖ₁₋₃) from the first breath of the first run to the first breath of the third run, within subjects. A priori primary outcome measures were change in value expressed as a percentage. However, the mean value of SnIII was so small for healthy children that Δ₁₋₃ values were very large for some children. For one healthy child Δ₁st breath SnIII₁₋₃ was 2534%. Analyses were therefore repeated using absolute change in value between first and third runs as outcome measures.

There was no relationship between Δ₁st breath SnIII₁₋₃ and ΔFRC₁₋₃ (Figure 4.20).

Figure 4.20 Scatter plot of Δ₁st breath SnIII₁₋₃ against ΔFRC₁₋₃, all children

Legend: Children with CF presented as closed circles, healthy children presented as open circles. There was no relationship between the two variables.
There was a weak negative relationship between Δ1st breath SnIII$_{1.3}$ and Δ1st breath V$_{\text{exp}1.3}$ ($r^2 = 0.11$, $p = 0.001$, Figure 4.21).

**Figure 4.21 Scatter plot of Δ1st breath SnIII$_{1.3}$ against Δ1st breath V$_{\text{exp}1.3}$, all children**

![Scatter plot](image)

**Legend:** Children with CF presented as closed circles, healthy children presented as open circles. There was a weak negative relationship between the two variables ($r^2 = 0.11$, $p = 0.001$).

These relationships were explored further by regression analyses, performed separately for healthy children and for those with CF. In healthy children, neither ΔFRC$_{1.3}$, nor Δ1st breath V$_{\text{exp}1.3}$ were significant predictors of Δ1st breath SnIII$_{1.3}$, either separately, or in combination ($r^2$ for multivariate model 0.01, $p = 0.3$). For children with CF, ΔFRC$_{1.3}$ was not a significant predictor of Δ1st breath SnIII$_{1.3}$, but Δ1st breath V$_{\text{exp}1.3}$ was (negative relationship, $r^2 = 0.26$, RSD 0.240, $p<0.001$). This relationship was not altered by the addition of ΔFRC$_{1.3}$ to the regression model.
4.6.6 Effect of small breaths upon the $S_{cond}$ regression

The effect of excluding breaths of inadequate volume upon the calculation of $S_{cond}$ was examined by comparing the fit of the $S_{cond}$ regression line over 1.5 to 6.0 lung volume turnovers before and after the exclusion process. For this analysis, exclusion of all small breaths over the Scond regression interval was examined, rather than first breaths. Of the 94 children in this analysis, 22 did not have any breaths excluded in any of the three runs. The median number of breaths excluded over three runs was two (one, six). There was a trend for children with CF to have fewer breaths excluded than healthy children (1 vs. 3, $Z$ = -1.94, $p$ = 0.05), and for preschool children to have fewer breaths excluded than school-age children (1.5 vs. 2, $Z$ = -1.46, $p$ = 0.2).

Results from SF$_6$ washout are presented in Table 4.15. For all subject groups the regression fit, as measured by $r^2$, was significantly better after exclusion of small breaths.

Table 4.15 Comparison of fit of $S_{cond}$ regression from SF$_6$ washout, before and after exclusion of small breaths

<table>
<thead>
<tr>
<th></th>
<th>Raw $r^2$</th>
<th>Corrected $r^2$</th>
<th>$Z$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td>0.186</td>
<td>0.410</td>
<td>-4.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(n=94)</td>
<td>(0.029, 0.643)</td>
<td>(0.055, 0.745)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All children with CF</td>
<td>0.551</td>
<td>0.729</td>
<td>-3.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(n=45)</td>
<td>(0.243, 0.761)</td>
<td>(0.502, 0.827)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All healthy children</td>
<td>0.044</td>
<td>0.090</td>
<td>-2.32</td>
<td>0.02</td>
</tr>
<tr>
<td>(n=49)</td>
<td>(0.015, 0.169)</td>
<td>(0.017, 0.348)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>0.423</td>
<td>0.552</td>
<td>-2.71</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>(0.067, 0.747)</td>
<td>(0.128, 0.780)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>0.096</td>
<td>0.254</td>
<td>-3.48</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(0.015, 0.370)</td>
<td>(0.033, 0.602)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: Raw $r^2$ calculated from all data. Corrected $r^2$ calculated after excluding breaths of inadequate volume. $r^2$ results presented as median (IQR). Comparison between groups is by Wilcoxon signed ranks test.
Results from He washout are presented in Table 4.16. For all subject groups the regression fit, as measured by $r^2$, was better after exclusion of small breaths, though this difference was not significant when healthy children were analysed as a subgroup.

Table 4.16 Comparison of fit of $S_{\text{cond}}$ regression from He washout, before and after exclusion of small breaths

<table>
<thead>
<tr>
<th></th>
<th>Raw $r^2$</th>
<th>Corrected $r^2$</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=94)</td>
<td>0.166</td>
<td>0.269</td>
<td>-4.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.036, 0.545)</td>
<td>(0.059, 0.651)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All children with CF (n=45)</td>
<td>0.523</td>
<td>0.659</td>
<td>-4.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.181, 0.721)</td>
<td>(0.516, 0.812)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All healthy children (n=49)</td>
<td>0.065</td>
<td>0.075</td>
<td>-1.37</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>(0.020, 0.165)</td>
<td>(0.023, 0.232)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>0.225</td>
<td>0.546</td>
<td>-3.34</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>(0.094, 0.642)</td>
<td>(0.130, 0.752)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>0.074</td>
<td>0.154</td>
<td>-2.55</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(0.022, 0.299)</td>
<td>(0.028, 0.596)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: Raw $r^2$ calculated from all data. Corrected $r^2$ calculated after excluding breaths of inadequate volume. $r^2$ results presented as median (IQR). Comparison between groups is by Wilcoxon signed ranks test.

The effect of exclusion of small breaths upon the value of $S_{\text{cond}}$ was examined by Bland-Altman analysis. A plot of $S_{\text{cond}}$ results obtained from raw SF$_6$ data with those obtained from corrected SF$_6$ data is presented in Figure 4.22. There were no significant group differences between $S_{\text{cond}}$ results calculated by the two methods (Table 4.17). The majority of children had $S_{\text{condR}} - S_{\text{condC}}$ close to zero. Limits of agreement were broad, but this was because of a small number of outliers. There was a positive relationship between the difference and the mean for these data. This is discussed in Section 4.7.
Figure 4.22 Bland-Altman plot of $S_{\text{cond}}$ results obtained from raw SF$_6$ data with those obtained from corrected SF$_6$ data

Legend:  
$S_{\text{condR}} = S_{\text{cond}}$ result obtained from raw data. 
$S_{\text{condC}} = S_{\text{cond}}$ result obtained from corrected data.

Difference calculated as $S_{\text{condR}} - S_{\text{condC}}$. Children with CF presented as closed circles, healthy children as open circles. Two healthy children had $S_{\text{condR}}$ less than −2, but $S_{\text{condC}}$ of greater than zero. These two outliers are not displayed on the plot. The majority of children had $S_{\text{condR}} - S_{\text{condC}}$ close to zero, but limits of agreement were broad, due to the effect of outliers. These limits of agreement are not therefore presented. There is a positive relationship between the difference and the mean for these data.
Table 4.17 Bland-Altman analysis of $S_{\text{cond}}$ results obtained from raw SF$_6$ data with those obtained from corrected SF$_6$ data

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=94)</td>
<td>-0.049 (0.591)</td>
<td>-0.170, 0.072</td>
</tr>
<tr>
<td>All children with CF (n=45)</td>
<td>0.033 (0.278)</td>
<td>-0.051, 0.116</td>
</tr>
<tr>
<td>All healthy children (n=49)</td>
<td>-0.123 (0.770)</td>
<td>-0.344, 0.098</td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>-0.117 (0.830)</td>
<td>-0.361, 0.127</td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>0.020 (0.084)</td>
<td>-0.005, 0.045</td>
</tr>
</tbody>
</table>

Legend: Difference calculated as $S_{\text{condR}} - S_{\text{condC}}$.

There was no significant group difference between $S_{\text{condR}}$ and $S_{\text{condC}}$.

Limits of agreement are distorted by outliers, and are not therefore presented.

A plot of $S_{\text{cond}}$ results obtained from raw He data with those obtained from corrected He data is presented in Figure 4.23. There were no significant group differences between $S_{\text{cond}}$ results calculated by the two methods (Table 4.18). As for the SF$_6$ data, the majority of children had $S_{\text{condR}} - S_{\text{condC}}$ close to zero. Again, limits of agreement were broad, but this was because of a small number of outliers. There was a positive relationship between the difference and the mean for these data. This is discussed in Section 4.7.
Figure 4.23 Bland-Altman plot of $S_{\text{cond}}$ results obtained from raw He data with those obtained from corrected He data.

Legend: $S_{\text{condR}} = S_{\text{cond}}$ result obtained from raw data.

$S_{\text{condC}} = S_{\text{cond}}$ result obtained from corrected data.

Difference calculated as $S_{\text{condR}} - S_{\text{condC}}$. Children with CF presented as closed circles, healthy children as open circles. Two healthy children had $S_{\text{condR}}$ less than −2, but $S_{\text{condC}}$ of greater than zero. These two outliers (the same children who were outliers for SF$_6$ data) are not displayed on the plot. As for the SF$_6$ data (Figure 4.22), the majority of children had $S_{\text{condR}} - S_{\text{condC}}$ close to zero, but limits of agreement were broad, due to the effect of outliers. There is a positive relationship between the difference and the mean for these data. Limits of agreement are not presented, as these are distorted by outliers.
Table 4.18 Bland-Altman analysis of $S_{\text{cond}}$ results obtained from raw He data with those obtained from corrected He data

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=94)</td>
<td>-0.079 (0.778)</td>
<td>-0.170, 0.072</td>
</tr>
<tr>
<td>All children with CF (n=45)</td>
<td>0.017 (0.381)</td>
<td>-0.097, 0.132</td>
</tr>
<tr>
<td>All healthy children (n=49)</td>
<td>-0.167 (1.012)</td>
<td>-0.458, 0.124</td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>-0.171 (1.091)</td>
<td>-0.361, 0.127</td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>-0.014 (0.123)</td>
<td>-0.022, 0.050</td>
</tr>
</tbody>
</table>

Legend: Difference calculated as $S_{\text{condR}} - S_{\text{condC}}$.  
There was no significant group difference between $S_{\text{condR}}$ and $S_{\text{condC}}$.  
Limits of agreement are distorted by outliers, and are not therefore presented.

4.6.7 Calculation of $S_{\text{cond}}$ over 1.5 to 6 turnovers vs. 2.5 to 6 turnovers

$S_{\text{cond}}$ results calculated over 1.5 to 6.0 turnovers were compared with results calculated over 2.5 to 6.0 turnovers by Bland-Altman analysis, separately for SF$_6$ and He data. Corrected data (Section 4.6.6) were employed for these analyses. A Bland-Altman plot of the SF$_6$ data is presented in Figure 4.24. There were no significant group differences between $S_{\text{cond}}$ results calculated by the two methods (Table 4.19). The majority of children had $S_{\text{condC}} - S_{\text{cond2.5}}$ close to zero, with no relationship between the difference and the mean.
Figure 4.24 Bland-Altman plot of SF$_6$ $S_{cond}$ results calculated over 1.5 to 6.0 turnovers with those calculated over 2.5 to 6.0 turnovers

Legend:
$S_{condC} = S_{cond}$ result obtained from corrected data, over 1.5 to 6.0 TO.
$S_{cond2.5} = S_{cond}$ result obtained from corrected data, over 2.5 to 6.0 TO.
Children with CF presented as closed circles, healthy children as open circles.
Broken lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control, see Table 4.19). The majority of children had SF$_6$ $S_{condC} - S_{cond2.5}$ close to zero, and there was no relationship between the difference and the mean.
Table 4.19 Bland-Altman analysis of SF$_6$ $S_{\text{cond}}$ results calculated over 1.5 to 6.0 turnovers with those calculated over 2.5 to 6.0 turnovers

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=94)</td>
<td>-0.014 (0.104)</td>
<td>-0.020, 0.017</td>
<td>-0.220, 0.192</td>
</tr>
<tr>
<td>All children with CF (n=45)</td>
<td>0.008 (0.100)</td>
<td>-0.022, 0.039</td>
<td>-0.190, 0.206</td>
</tr>
<tr>
<td>All healthy children (n=49)</td>
<td>0.018 (0.107)</td>
<td>-0.013, 0.049</td>
<td>-0.194, 0.230</td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>-0.018 (0.131)</td>
<td>-0.021, 0.056</td>
<td>-0.277, 0.241</td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>0.010 (0.067)</td>
<td>-0.010, 0.030</td>
<td>-0.143, 0.123</td>
</tr>
</tbody>
</table>

Legend: Difference calculated as $S_{\text{condC}} - S_{\text{cond2.5}}$.

There were no significant group differences between $S_{\text{condC}}$ and $S_{\text{cond2.5}}$.

There was no relationship between SF$_6$ $S_{\text{condC}} - S_{\text{cond2.5}}$ and age (Figure 4.25).

A Bland-Altman plot of the He data is presented in Figure 4.26.
Figure 4.25 Difference between $\text{SF}_6$ $S_{\text{cond}}$ results calculated over 1.5 to 6.0 turnovers and those calculated over 2.5 to 6.0 turnovers, plotted against age, all children.

$S_{\text{condC}} = S_{\text{cond}}$ result obtained from corrected data, over 1.5 to 6.0 TO.

$S_{\text{cond2.5}} = S_{\text{cond}}$ result obtained from corrected data, over 2.5 to 6.0 TO.

Children with CF presented as closed circles, healthy children as open circles.

Broken lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control, see Table 4.19). There was no relationship between $\text{SF}_6$ $S_{\text{condC}} - S_{\text{cond2.5}}$ and age.
Figure 4.26 Bland-Altman plot of He $S_{\text{cond}}$ results calculated over 1.5 to 6.0 turnovers with those calculated over 2.5 to 6.0 turnovers

Legend:

$S_{\text{cond}C} = S_{\text{cond}}$ result obtained from corrected data, over 1.5 to 6.0 TO.

$S_{\text{cond}2.5} = S_{\text{cond}}$ result obtained from corrected data, over 2.5 to 6.0 TO.

Children with CF presented as closed circles, healthy children as open circles.

Broken lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control, see Table 4.20). The majority of children had He $S_{\text{cond}C} - S_{\text{cond}2.5}$ close to zero. Some children with a high mean $S_{\text{cond}}$ appeared to have a negative difference, suggesting that the slope of $S_{\text{cond}2.5}$ is greater than the slope of $S_{\text{cond}C}$ for these children. The author is unable to provide an explanation for this finding.

There were no significant group differences between $S_{\text{cond}}$ results calculated by the two methods (Table 4.20). The majority of children had $S_{\text{cond}C} - S_{\text{cond}2.5}$ close to zero. Some children with a high mean $S_{\text{cond}}$ appeared to have a negative difference,
suggesting that the slope of $S_{\text{cond}2.5}$ is greater than the slope of $S_{\text{cond}C}$ for these children. This is discussed further in Section 4.7. There was no relationship between $He_{S_{\text{cond}C}} - S_{\text{cond}2.5}$ and age (Figure 4.27).

Table 4.20 Bland-Altman analysis of $He_{S_{\text{cond}}}$ results calculated over 1.5 to 6.0 turnovers with those calculated over 2.5 to 6.0 turnovers

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) difference</th>
<th>95% CI of difference</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children (n=94)</td>
<td>-0.001 (0.090)</td>
<td>-0.020, 0.017</td>
<td>-0.179, 0.177</td>
</tr>
<tr>
<td>All children with CF (n=45)</td>
<td>-0.006 (0.016)</td>
<td>-0.040, 0.026</td>
<td>-0.038, 0.026</td>
</tr>
<tr>
<td>All healthy children (n=49)</td>
<td>0.004 (0.069)</td>
<td>-0.016, 0.024</td>
<td>-0.133, 0.141</td>
</tr>
<tr>
<td>All preschool children (n=47)</td>
<td>-0.014 (0.115)</td>
<td>-0.048, 0.020</td>
<td>-0.242, 0.214</td>
</tr>
<tr>
<td>All school-age children (n=47)</td>
<td>0.012 (0.057)</td>
<td>-0.005, 0.028</td>
<td>-0.101, 0.125</td>
</tr>
</tbody>
</table>

Legend: Difference calculated as $S_{\text{cond}C} - S_{\text{cond}2.5}$.

There was no significant group difference between $S_{\text{cond}C}$ and $S_{\text{cond}2.5}$. 

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Figure 4.27 Difference between He $S_{\text{cond}}$ results calculated over 1.5 to 6.0 turnovers and those calculated over 2.5 to 6.0 turnovers, plotted against age, all children.

**Legend:**

1. $S_{\text{condC}} = S_{\text{cond}}$ result obtained from corrected data, over 1.5 to 6.0 TO.
2. $S_{\text{cond2.5}} = S_{\text{cond}}$ result obtained from corrected data, over 2.5 to 6.0 TO.

Children with CF presented as closed circles, healthy children as open circles. Broken lines represent 95% limits of agreement, calculated as mean +/- 1.96SD, from full population (CF and control, see Table 4.20). There was no relationship between He $S_{\text{condC}} - S_{\text{cond2.5}}$ and age.
4.7 Discussion

4.7.1 Summary of most important results

In this study, the relationships between subject characteristics, breathing pattern and MBW indices were studied. All school-age children were asked to adopt a comfortable tidal breathing pattern during three consecutive washouts. Preschool children were measured whilst distracted by an entertainment video. In healthy children, LCI was remarkably constant across age groups, despite a wide range of lung volumes and breathing patterns. When children with CF were included in multivariate analyses, the diagnosis of CF was by far the most important determinant of LCI. In healthy children, the first breath SnIII was negatively related to breath volume, age, and to a lesser extent, to body size. However, if the first breath SnIII was expressed as a product of breath volume, then this index was independent of subject characteristics (Figures 4.17 and 4.18). When children with CF were included in multivariate analyses, diagnosis was the most important determinant of first breath SnIII.

The analyses presented in this chapter are pragmatic, i.e. they are based upon data that were obtained during normal tidal breathing. These results therefore have practical value for the clinical researcher, but it is accepted that further insights into physiological mechanisms could be obtained from controlled breathing studies. In justification, the primary purpose of this thesis was to explore the use of MBW in preschool children, where controlled breathing experiments are impossible.

Analysis of changes observed during spontaneous variation of breathing pattern is the only practical approach.

4.7.2 Precision of MBW indices

Precision of LCI was good, with only 10 of 115 children having $\text{CoV}_{\text{LCI}}$ greater than 10%. In comparison, the mean $\text{CoV}_{\text{SnIII}}$ in healthy children was 45.1%, and in children with CF 30.6%. This $\text{CoV}_{\text{SnIII}}$ is distorted by the relatively small mean values for SnIII, particularly in healthy children. Bland-Altman analyses were employed to compare results from one run, and mean results from two runs, with
the mean results from three runs. The limits of agreement, for the whole population, for LCI_{1,2} versus LCI_{mean} were -0.54 and 0.49. If the difference in LCI between the populations to be studied (e.g. healthy children versus those with CF) is greater than 0.54, then it is possible to detect these differences using LCI calculated from two runs, rather than three. Similar arguments apply to the Bland-Altman analyses presented in Table 4.7. This issue will be examined further in Chapters 5 and 6, when the MBW results between populations will be presented in more detail. It is noted at this point that the Bland-Altman relationships for SnIII_{mean} and SnII_{1,2} and SnIII_{mean} and SnIII_{1} are distributed heteroscedastically. In other words, the difference between results obtained by the methods increases as the value of first breath SnIII increases. This should be noted when comparing results between individuals who have high SnIII.

From the results presented in Figure 4.3, Section 4.5.5, and 4.5.6, it is clear that some children have higher LCI results from their third MBW run than from their first MBW run. This finding was not consistent between individuals, and a significant group difference was only seen in the school-age subgroup (Table 4.19). One possible explanation for these results is that school-age children were not given sufficient time between the three MBW runs, and so commenced the third run without having completely cleared all SF6 inspired during the previous two runs.

4.7.3 Effect of subject characteristics and breathing pattern upon MBW indices

This study has demonstrated that LCI is largely independent of subject characteristics and breathing pattern. In healthy children aged two to 16 years, only 30.2% of the variability of LCI is explained by subject age, sex, height and weight (Table 4.8). If children with CF are also included in this multivariate analysis, then only 19.1% of the variability of LCI is explained by this model, whilst addition of subject diagnosis to the model increases the r^2 to 59.3% (Table 4.9). Similar results are obtained by multivariate analyses of breathing pattern, with FRC, RR_{mean}, V_{T,mean} explaining only 5.0% of the variability of LCI in all children (Table 4.14). Addition of subject characteristics to this model increased the r^2 to 24.8% (Table 4.15), and addition of diagnosis and subject characteristics increased the r^2 to 59.4% (Table 4.16).
The relationship between breathing pattern and LCI was examined further by
within-subject comparisons (Section 4.5.5). Although a weak negative relationship
between FRC and LCI was noted, changes in breathing pattern accounted for only
10.8% of the within-subject variability of LCI.

Similar techniques were employed to study the relationships between subject
characteristics, breathing pattern, and first breath SnIII. The most important finding
from these analyses was the negative relationship between SnIII and expired
volume (Figure 4.18). Multivariate analyses suggest that this relationship is
responsible for the apparent effect of subject characteristics upon SnIII. When
subject characteristics were modelled against SnIII in healthy children, 38% of the
variability of SnIII was explained. However, the product of SnIII and expired
volume was independent of subject characteristics in healthy children ($r^2$ 6%,
Figure 4.20).

4.7.4 How can these results be explained?

Most measures of lung function are partly dependent upon lung (and therefore
subject) size, and upon breathing pattern. The LCI is calculated in such a way that
differences in body size, and within subject alterations in FRC and $V_T$ should be
compensated.

As described previously

$$LCI = \frac{CEV}{FRC}$$

Any increase in FRC will increase the volume of tracer gas that needs to be washed
out of the lung, and thus the CEV (the numerator in this equation) will also be
increased. Including the FRC as the denominator in the equation to calculate LCI
should compensate this effect. Any increase in $V_T$ is likely to reduce the number of
breaths required for the subject to reach the end-point of the washout. However, as
these breaths will be of larger volume, the CEV should not be affected. Apparatus
dead-space is subtracted from any calculation of CEV, but airway dead-space is not.

Any subject who breathes at a very low tidal volume, such that the majority of
ventilation is directed at airway dead-space may, theoretically, produce a high LCI.
This effect was not seen in the spontaneously breathing children studied here,
presumably because none of them spontaneously adopted such a small tidal volume.
SnIII is the slope of the expired tracer gas concentration versus expired volume relationship, over the portion of the expiration that represents expired alveolar gas. It is therefore expected that there should be a negative relationship between SnIII and expired volume. In theory, this would be best corrected by multiplying the SnIII by the volume over which it is calculated. However, correcting the first breath SnIII by the total first breath expired volume appears to correct for subject characteristics. This is investigated in more detail in Chapter 6, and discussed further in Chapter 7. It should also be expected that changes in breathing pattern will have physiological effects, altering the extent of ventilation inhomogeneity, and thereby impacting upon MBW indices. There have been several previous studies of the effect of \( V_T \) upon ventilation inhomogeneity, but these have produced conflicting results. Some studies have suggested that increases in \( V_T \) result in decreased inhomogeneity (as measured by the phase III slope) \(^{67,114}\), whilst others have suggested the reverse \(^{115}\). Crawford and colleagues \(^{68}\) proposed that increases in \( V_T \) result in an increase in the convection-dependent component of ventilation inhomogeneity, but a decrease in inhomogeneity arising from interactions between diffusion and convection. This hypothesis has been supported by more recent data \(^{46}\) and could explain discrepancies in the literature. It is notable that many of these earlier studies used tidal volumes that were outside the normal physiological range. Thus, while Crawford and colleagues required healthy adults to breathe at a \( V_T \) ranging from 0.6 to 1.5L (i.e. up to 200% above normal values), the spontaneous within-subject changes observed in this study were considerably smaller.

By contrast, the data presented here suggest that spontaneous changes in FRC between washouts may have a minor effect upon LCI, with a weak negative relationship seen. There are two possible explanations for this finding. Given that LCI should be compensated for FRC for each washout, this observation may reflect the physiological effects of altering FRC. In other words, when healthy subjects breathe at a higher FRC, the ventilation distribution would be expected to become more homogenous, and vice versa. Alternatively, these findings may reflect the way in which LCI is calculated. Edelman has suggested that in a uniformly ventilated lung, an increase in FRC (with no accompanying change in \( V_T \)) will produce a decrease in LCI due to the relative effect of airway dead-space \(^{47}\).
Published data on the effect of FRC upon ventilation inhomogeneity are limited, because of the difficulty of controlling FRC in the laboratory. One study which aimed to investigate the effect of airway closure on ventilation inhomogeneity found increasing inhomogeneity below closing volume both for the slope of phase III and for alveolar mixing efficiency. In the range between closing capacity and FRC the normalised slope of phase III consistently increased with higher volumes while the alveolar mixing efficiency gave more variable results. In the current study, spontaneous changes of FRC were much smaller, and it is unlikely that any subject was breathing at or below their closing capacity.

No relationship between RR and LCI was seen. It has been shown that breath-holding decreases the phase III slope in single breath washouts, presumably because gas mixing continues by diffusion during the pause. Theoretically a faster RR would leave less time for gas mixing within small lung units, but within the population studied here, spontaneous changes in RR were not sufficient to affect LCI.

4.7.5 Exclusion of small breaths for SnIII analysis

Some breaths were of inadequate volume for estimation of SnIII (Section 2.4.8). These breaths were identified by predetermined criteria, rather than by the SnIII calculated. Nevertheless, for these breaths, the SnIII was calculated over 65-95% of expired volume, and the results recorded to allow comparison with breaths of larger volume. The results presented in Section 4.6.1 demonstrate that SnIII calculated from breaths of inadequate volume is significantly higher than for breaths of adequate volume. The effect of excluding breaths upon the fit of the $S_{\text{cond}}$ regression was examined by comparison of $r^2$ values. The value of $r^2$ was significantly better after exclusion of SnIII results from small breaths, both in healthy children and in those with CF. When results calculated by the two methods were compared by Bland-Altman analysis, a positive relationship between the difference (raw minus corrected) and mean $S_{\text{cond}}$ was seen. The explanation for this is that corrected $S_{\text{cond}}$ values were closer to the group mean results than the corresponding raw $S_{\text{cond}}$, providing further evidence that exclusion of SnIII results from small breaths improves the fit of the $S_{\text{cond}}$ regression.
It is noted that there was a trend for preschool children to have fewer breaths excluded than school-age children, and for children with CF to have fewer breaths excluded than healthy children. The former trend can be explained by the larger deadspace of the mask apparatus, when compared to the facemask apparatus, which would be expected to result in an increase in tidal volume. The latter trend may be the result of children with CF adopting a higher tidal volume to compensate for poorer ventilation distribution or because children with CF are more accustomed to following instructions during lung function testing.

4.7.6 Identification of the linear portion of the SnIII versus lung volume turnover relationship

The final analyses presented in this chapter examined whether the linear portion of the SnIII versus TO relationship commences by 1.5 TO in spontaneously breathing children. This was determined by calculating \( S_{\text{cond}} \) over 1.5 to 6.0 TO and again over 2.5 to 6.0 TO. If the linear portion of this relationship commences after 1.5 TO, then the value for \( S_{\text{cond}C} \) should be greater than the value of \( S_{\text{cond}2.5} \). No significant group differences were seen, but in a small number of children with high He \( S_{\text{cond}} \) the opposite phenomenon was seen, i.e. the value of \( S_{\text{cond}C} \) was less than the value of \( S_{\text{cond}2.5} \) (Figure 4.27). This pattern was only seen in a very small number of children, and the author is unable to provide an explanation for this finding.

4.7.7 Conclusion

The results of this analysis are highly encouraging. It has been demonstrated that LCI is almost independent of subject characteristics and breathing pattern, and that SnIII is dependent upon expired volume, but not upon other subject or breath characteristics. It can be concluded that measurements of LCI or SnIII indices can be obtained satisfactorily in children during spontaneous tidal breathing without the need to control breathing pattern. This finding supports the contention that MBW has potential for measurement of lung function in preschool children, where control of breathing is not possible.
Chapter 5: Comparison of washout curve results with spirometry results

5.1 Introduction

As described in Chapter 1, regular lung function testing is an essential part of clinical care for children with CF. Over the last two decades, there has been a shift towards closer monitoring and more aggressive treatment of early CF lung disease \(^{20-22,118}\). As interest in monitoring younger patients increases, so does the need for alternative, more sensitive measures of lung function that can be obtained in children of all ages.

The results presented in Chapters 3 and 4 demonstrate that preschool children can successfully perform both spirometry and MBW, and that repeatable results can be obtained with both techniques. The purpose of this chapter is to compare results obtained from washout curve analysis and from spirometry in children with CF and in matched healthy children. Results obtained from school-age children and preschool children are presented separately.

5.2 Aims and hypothesis

5.2.1 Hypothesis

- More children with CF will have abnormal washout results than will have abnormal spirometry results.

5.2.2 Aims

The primary aims of this study were:

a) To investigate the relationship between parameters derived from MBW washout curve analysis and from forced expiration in a population of healthy British children and children with CF, aged from two to 16 years

b) To compare MBW and spirometry results from school-age British children with those recently reported for Swedish children.
The secondary aims of this investigation were to relate MBW results, obtained in children with CF, to clinical status, as measured by past medical history, cough frequency, sputum microbiology, and audible signs on chest auscultation.

5.3 Subjects and methods

5.3.1 Subject recruitment
This is described in Section 4.3.1 for school-age children, and Section 3.3.1 for preschool children.

5.3.2 Study design
This was a cross-sectional study. All school-age subjects successfully completing spirometry and MBW were included in the analyses presented in this chapter. No school-age subjects were recalled for a second visit. Preschool subjects were participating in a study comparing spirometry, body plethysmography, and MBW results. Subjects who did not complete all three measurements were asked to return to the laboratory six months after their first visit for repeat measurements. The study was continued until 30 subjects with CF and 30 healthy subjects had completed all three measurements. MBW results from these 60 children are presented in this chapter.

5.3.3 Data collection
Collection and analysis of spirometry data, including criteria for accepting or discarding results, and derivation of the indices listed below, are described in detail in Chapters 2 and 3. Collection and analysis of MBW data are described in detail in Chapters 2 and 4.

5.3.4 Additional clinical information, and recording of clinical data
For both school-age and preschool children with CF, clinical information was obtained from three sources. First, the senior paediatrician (consultant) responsible for the child's clinical CF care was sent a questionnaire in the month prior to the child’s visit. Second, at the time of visit the child’s parent was asked to complete a
symptom questionnaire, which was directly administered by the author. Third, the
child was examined by the author, and data obtained was analysed. The
questionnaires differed for school-age and preschool children, and are presented in
Appendices E. Some of the data collected were to aid future analyses, and will not
be presented in this thesis. Those data that were required for the current study are
highlighted below:

Consultant questionnaire for school-age children with CF
- Presence of chronic respiratory infection with *Pseudomonas aeruginosa*,
  *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, or *Haemophilus*
  *influenzae*. Chronic infection was defined as three positive cultures over the
  previous six months, each at least one month apart.

Parent questionnaire for school-age children with CF
- Cough frequency over the week prior to testing, classified as never,
  intermittent (less than once daily) or every day.

Clinical examination in school-age children
- Presence of wheeze or crackles on chest auscultation was recorded.

Consultant questionnaire for preschool children with CF
- Whether the child had ever grown *Pseudomonas aeruginosa*,
  *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, or *Haemophilus*
  *influenzae*, on respiratory culture
- Whether the child was still considered to be infected with these organisms
  (with current bacterial infection defined as at least one positive culture of an
  organism from the three most recent samples prior to lung function testing)
- Whether the child had had a chest radiograph, chest computed tomogram,
  or ventilation scan previously, and whether the results had been normal or
  abnormal
- The consultant was asked to complete two visual analogue scores. The first
  was based upon their assessment of the child’s clinical course from birth to
  the time of assessment, with rating from zero (very mild disease) to 10
  (very severe disease). The second was based upon their assessment of the
  child’s respiratory status at the time of assessment, rated from zero (normal)
  to 10 (severely abnormal).
Parent questionnaire for preschool children with CF

- Cough frequency over the week prior to testing, classified as present or absent.

Clinical examination in preschool children

- Presence of wheeze or crackles on chest auscultation was recorded
- At conclusion of assessment, but prior to lung function testing, the author rated the child's clinical status on a visual analogue scale, rated from zero (mild lung disease) to 10 (severe lung disease). This assessment was based upon responses to the symptom questionnaire, and upon findings on clinical examination.

Consultant questionnaires were returned directly to a department secretary (Ms L Robinson), and were not seen by the author until November 2003, by which time all lung function parameters had been calculated.

Parents of control children also completed symptom questionnaires, and the children were examined by the author. The purpose of this assessment was to ensure that these children were healthy.

Clinical data from school-age children were transferred manually to a SPSS data file (Statistical Program for Social Sciences, SPSS Inc, Chicago, USA) by the author. Following all data entry, data were verified by selecting a sample of 10 cases and cross-checking values entered on the SPSS file against those recorded on the original questionnaires.

Clinical data from preschool children were entered into an Access relational database (Microsoft Corporation, Redmond, WA, USA) by C Saunders and W Kozlowska. Administration (i.e. appointments, chasing up consultant questionnaires, sending out results etc.) was performed by Ms L Robinson, following protocols written by the author (Appendices I). Results were sent to consultants and GPs, and also explained to parents (Appendices J and K).

5.3.5 Ethics

The study was approved by the North Thames Region Multicentre Research Ethics Committee, and by the Local Research Ethics Committees of each of the five collaborating centres.
5.4 Analysis

5.4.1 Outcome measures

FRC and LCI were calculated for each washout. For each parameter, the mean and SD from the three recordings was then calculated for each subject. Spirometry curves were visually inspected and passed or rejected (Chapter 3, Section 3.5.2). For school-age subjects, the highest \( \text{FEV}_1 \) recorded from any acceptable curve was reported for each child. The \( \text{MEF}_{25} \) was calculated from the curve that had the highest sum of FVC and \( \text{FEV}_1 \). MMEF was not reported in school-age subjects, as there are no published reference data for MMEF in healthy British schoolchildren. For preschool subjects, the highest \( \text{FEV}_1 \) and \( \text{FEV}_{0.5} \) recorded from any acceptable curve were reported from each child, and the MMEF was calculated from the curve with the highest sum of FVC and \( \text{FEV}_{0.75} \). For the analyses in preschool children, \( \text{FEV}_{0.5} \) was considered a primary outcome variable and \( \text{FEV}_1 \) a secondary outcome variable.

Height, weight, \( \text{FEV}_1 \), \( \text{MEF}_{25} \), \( \text{FEV}_{0.5} \), and MMEF results were converted into standard deviation scores (z-scores) using published reference data\(^{24,40,119,120} \).

5.4.2 Comparison with previously published data in school-age children

Results obtained from school-age children in this study were compared with data previously collected in Swedish school children\(^{27} \). These data were collected at the Department of Pediatrics, Central Hospital, Skövde, Sweden, using an identical MBW apparatus and analysis system to that employed at the Institute of Child Health. Data from the Swedish laboratory were obtained directly from the lead investigator of the Swedish research group: Dr P Gustafsson. The Swedish group tested healthy children and children with CF aged from three to 18 years, using a facemask apparatus for children aged six years and younger. For the current analysis, only data from Swedish children aged 16 or younger who had performed MBW using a mouthpiece apparatus were included.
5.4.3 Statistical analysis

Analyses were first performed separately for school-age and preschool populations. LCI and spirometry parameters were plotted against age, and against each other. LCI and spirometry z-scores for the CF and control groups were compared. Limits of Normality, defined as mean +/- 1.96 SD were calculated for LCI from the control data, and subjects with a LCI above the upper limit of normality were categorised as having an abnormal result. Subjects with a FEV$_1$, MEF$_{25}$, FEV$_{0.5}$, or MMEF z-score less than -1.96 were classified as having an abnormal result.

Group results are presented as mean (SD). Group comparisons were by t-test or Chi$^2$ test as appropriate. 95% CI for difference of means is presented for t-test results. A p-value below 0.05 was regarded as statistically significant.

Sensitivity and specificity were determined by cross tabulation, and by calculation of the area under Receiver-Operator Characteristic (ROC) curves. This was performed by calculating sensitivity and specificity for detecting the diagnosis of CF for all values of LCI, FEV$_1$ z-score, and MEF$_{25}$ z-score, plotting the sensitivity against 1 - specificity for each measure, and calculating the area under this curve (AUC). A measure that discriminates between two groups with perfect sensitivity and specificity will have an AUC of 1.0. A measure that does not discriminate at all will have an AUC of 0.5. For children with CF, lung function results were compared with clinical information. Finally LCI and FEV$_1$ results were compared for CF and control groups for preschool and school-age children together.

5.4.4 Power of study

a) School-age children: normal values for LCI are known to be age-independent in healthy school-age children (Chapter 4). Reference equations for FEV$_1$ and MEF$_{25}$, which allow correction for sex and height, are well established. Having corrected for age, sex and height, a sample size of 22 subjects in each group is sufficient to detect a difference of one SD in LCI, FEV$_1$ z-score, and MEF$_{25}$ z-score, between CF and control groups, or between children measured at the Institute of Child Health and those measured in Skövde, with 90% power at the 5% significance level.
b) For preschool children, a sample size of 50 subjects in each group was initially calculated to be sufficient to detect a difference of 0.5 SD in FEV_{0.5}, MMEF, and LCI between CF and control groups. Interim analysis performed in 2002^{114,123} indicated that the difference between populations for the primary outcome measures was likely to be greater than 0.5 SD, and the required sample size was therefore recalculated as 30 subjects per group.

5.5 Results in school-age children

5.5.1 Subjects
Details of the 55 children included in this analysis are presented in Chapter 4, section 4.5.1.

5.5.2 Comparison of Lung Function results for CF and control groups
Table 5.1 presents results for LCI, FEV₁ z-score, and MEF_{25} z-score, analysed by diagnosis.

| Table 5.1 Comparison of MBW and spirometry results for CF and control groups |
|-----------------------------|-----------------------------|-----------------------------|
|                             | CF                          | Controls                    | Mean difference |
|                             |                             |                             | (95% CI of difference) |
| LCI                         | 11.53 (2.86)                | 6.45 (0.49)                 | 5.08 (4.07, 6.10)*** |
| FEV₁ z-score                | -2.01 (1.45)                | 0.28 (0.86)                 | -2.29 (-2.92, -1.67)*** |
| MEF₁₂₅ z-score              | -1.90 (0.76)                | -0.06 (1.06)                | -1.83 (-2.36, -1.31)*** |

Legend: Group results presented as mean (SD) *** p<0.001. Difference calculated as CF – control.

For all parameters the difference between the two subject groups was highly significant. For LCI the upper limit of normality (ULN) was calculated as 7.41, and lower limit of normality (LLN) 5.49. Figure 5.1 shows the relationship between LCI and age for the control subjects and the children with CF. LCI was almost age
independent in healthy children, and rose with age in children with CF (r² 0.30, p=0.008).

Figure 5.1 LCI plotted against age, school-age children

Legend: Control children presented as open circles, and children with CF presented as closed circles.

Broken lines represent limits of normality for LCI. Note that normal values for LCI are age-independent.

5.5.3 Sensitivity and specificity of LCI and spirometry parameters

Receiver-Operator Characteristic curves (ROC curves) for LCI, FEV₁ z-score and MEF₂₅ z-score are presented in Figure 5.2.
Figure 5.2 Receiver-Operator Characteristic (ROC) curves for Lung Clearance Index, FEV₁ z-score, and MEF₂₅ z-score for school-age children

Legend: The outcome variable is the diagnosis of cystic fibrosis. ROC curve for LCI is presented in red, ROC curve for MEF₂₅ is presented in blue, and ROC curve for FEV₁ is presented in green.

The area under the Receiver-Operator Characteristic curve (AUC$_{ROC}$) for each parameter is presented in Table 5.2. All LCI, FEV₁ and MEF₂₅ results were classified as normal or abnormal, as described in Section 5.4.3. Sensitivity and specificity of each lung function parameter were calculated from this classification, and are also presented in Table 5.2. LCI was more sensitive at discriminating CF from control children than either FEV₁ or MEF₂₅, and had higher AUC$_{ROC}$ than either spirometry parameter.
Table 5.2 Sensitivity, Specificity, and area under the ROC curve for LCI, FEV₁, and MEF₂₅

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC&lt;sub&gt;ROC&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>95%</td>
<td>97%</td>
<td>0.997</td>
</tr>
<tr>
<td>FEV₁</td>
<td>50%</td>
<td>100%</td>
<td>0.910</td>
</tr>
<tr>
<td>MEF₂₅</td>
<td>45%</td>
<td>100%</td>
<td>0.937</td>
</tr>
</tbody>
</table>

Legend: The outcome variable is the diagnosis of cystic fibrosis.
Limits of normality for calculation of sensitivity and specificity are defined as 1.96 SD from mean.
AUC<sub>ROC</sub> = Area under the Receiver-Operator Characteristic curve.

5.5.4 Relationship between LCI and spirometry parameters

Figure 5.3 demonstrates the relationship between LCI and FEV₁ for both subject groups. In healthy children, there was no relationship between LCI and FEV₁. In children with CF, LCI and FEV₁ were negatively correlated ($r^2 0.63$, $p<0.001$).

Figure 5.3 also shows that 11 of 22 children with CF had a FEV₁ within the normal range, with some of these children having a FEV₁ z-score greater than zero. In comparison, only one of the 22 children with CF had a normal LCI.
**Figure 5.3 Lung Clearance Index plotted against FEV₁ z-score**

Legend: Control children presented as open circles, and children with CF presented as closed circles. Vertical broken line represents lower limit of normality for FEV₁ z-score. Horizontal broken line represents upper limit of normality for LCI. Note that many children with CF have FEV₁ z-score within normal range and LCI above normal range.

Figure 5.4 demonstrates the relationship between LCI and MEF₂₅ for both subject groups. In healthy children, there was no relationship between LCI and MEF₂₅. In children with CF, LCI and MEF₂₅ were negatively correlated ($r^2 0.46$, $p=0.001$). Figure 5.4 also shows that 12 of the 22 children with CF had a MEF₂₅ result in the normal range, though none had a MEF₂₅ z-score greater than zero.
Figure 5.4 Lung Clearance Index plotted against MEF\textsubscript{25} z-score

Legend: Control children presented as open circles, and children with CF presented as closed circles. Vertical broken line represents lower limit of normality for MEF\textsubscript{25}. Horizontal broken line represents upper limit of normality for LCI. Note that many children with CF have MEF\textsubscript{25} z-score within normal range and LCI above normal range.

5.5.5 Relationship between Lung Function Parameters and Clinical Information

Of the 22 children with CF, 10 had chronic respiratory infection with *Pseudomonas aeruginosa*, of whom one was also chronically infected with *Stenotrophomonas maltophilia*. Of the remaining 12 children, five were chronically infected with *Staphylococcus aureus*, one was chronically infected with *Haemophilus influenzae*, and two were chronically infected with both organisms. Four children had no evidence of chronic infection with any CF pathogen. The children infected with *Pseudomonas* were significantly older than the other children, with mean age difference 3.3 years (0.79, 5.9 years).
Children infected with *Pseudomonas aeruginosa* tended to have higher LCI, lower FEV₁ z-score, and lower MEF₂₅ z-score than those not infected with this organism, but none of these differences were statistically significant (Table 5.3).

**Table 5.3 Comparison of MBW and spirometry results for children with CF chronically infected and not chronically infected with *Pseudomonas aeruginosa***

<table>
<thead>
<tr>
<th></th>
<th>Pseudomonas positive (n=10)</th>
<th>Pseudomonas negative (n=12)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>12.8 (2.7)</td>
<td>10.5 (2.7)</td>
<td>2.3 (0.0, 4.7)φ</td>
</tr>
<tr>
<td>FEV₁ z-score</td>
<td>-2.40 (1.17)</td>
<td>-1.70 (1.63)</td>
<td>-0.70 (-1.99, 0.59)</td>
</tr>
<tr>
<td>MEF₂₅ z-score</td>
<td>-2.03 (0.70)</td>
<td>-1.79 (0.81)</td>
<td>-0.24 (-0.92, 0.44)</td>
</tr>
</tbody>
</table>

*Legend: Results are presented as mean (SD).*

*Difference is calculated as CF – control.*

φ  \( p = 0.053 \)

Of the 22 children with CF, 15 reported coughing at least once daily during the week prior to assessment, four reported no cough during this week, and three had coughed, but less frequently than once daily. Children who coughed at least once daily tended to have higher LCI, lower FEV₁ z-score, and lower MEF₂₅ z-score than those who coughed less frequently, but none of these differences were statistically significant (Table 5.4).
Table 5.4 Comparison of MBW and spirometry results for children with CF according to frequency of cough in the week prior to assessment

<table>
<thead>
<tr>
<th></th>
<th>Daily cough (n=15)</th>
<th>Cough less than once daily (n=7)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>12.2 (2.7)</td>
<td>10.1 (2.8)</td>
<td>2.1 (-0.5, 4.7)</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>-2.39 (1.43)</td>
<td>-1.20 (1.21)</td>
<td>-1.19 (-2.50, 0.11)</td>
</tr>
<tr>
<td>MEF$_{25}$ z-score</td>
<td>-2.01 (0.83)</td>
<td>-1.64 (0.52)</td>
<td>-0.37 (1.09, 0.35)</td>
</tr>
</tbody>
</table>

Legend: Results are presented as mean (SD). Difference is calculated as Daily cough – Cough less than once daily.

Of the 22 children with CF, nine had audible crackles on chest auscultation, whilst 13 had no abnormal chest sounds. Children who had audible crackles tended to have higher LCI, lower FEV$_1$ z-score, and lower MEF$_{25}$ z-score than those who had no audible crackles, but none of these differences were statistically significant (Table 5.5).

Table 5.5 Comparison of MBW and spirometry results for children with CF according to presence of audible crackles on chest auscultation

<table>
<thead>
<tr>
<th></th>
<th>Audible crackles (n=9)</th>
<th>No audible crackles (n=13)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>12.9 (3.2)</td>
<td>10.6 (2.2)</td>
<td>2.3 (-0.1, 4.8)</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>-2.38 (1.47)</td>
<td>-1.76 (1.44)</td>
<td>-0.62 (-1.94, -0.69)</td>
</tr>
<tr>
<td>MEF$_{25}$ z-score</td>
<td>-2.19 (0.84)</td>
<td>-1.69 (0.64)</td>
<td>-0.50 (-1.16, -0.16)</td>
</tr>
</tbody>
</table>

Legend: Results are presented as mean (SD). Difference is calculated as audible crackles – no audible crackles.
5.5.6 Comparison of results obtained at ICH with those previously reported from the Department of Pediatrics, Central Hospital, Skövde, Sweden

Data collected in school-age children tested in Skövde, Sweden were obtained from Dr P Gustafsson, as described in Section 5.4.2. LCI, \( \text{FEV}_1 \), and \( \text{MEF}_{25} \) data from 24 healthy children aged 7.6 to 15.7 years, and 31 children with CF, aged 7.3 to 16.9 years were available. When reporting these data Dr Gustafsson calculated predicted values for \( \text{FEV}_1 \) and \( \text{MEF}_{25} \) from prediction equations derived from healthy Swedish children, published in 1980 by Solymar et al\(^{121} \). Prior to comparing results obtained in Skövde with those obtained in the current study it was necessary to compare \( \text{FEV}_1 \) and \( \text{MEF}_{25} \) z-scores derived from the prediction equations of Solymar et al\(^{121} \), with those derived from the prediction equations of Rosenthal et al\(^{120} \), in the healthy Swedish children. These results are presented in Table 5.6.

### Table 5.6 \( \text{FEV}_1 \) and \( \text{MEF}_{25} \) z-scores for healthy children measured in Skövde, Sweden, calculated from Swedish and UK reference equations

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>95% CI of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{FEV}_1 ) z-score (Solymar)</td>
<td>-0.39 (0.90)</td>
<td>-0.77, -0.01*</td>
</tr>
<tr>
<td>( \text{FEV}_1 ) z-score (Rosenthal)</td>
<td>0.35 (0.88)</td>
<td>-0.02, 0.73</td>
</tr>
<tr>
<td>( \text{MEF}_{25} ) z-score (Solymar)</td>
<td>-0.80 (1.49)</td>
<td>-1.43, -0.17*</td>
</tr>
<tr>
<td>( \text{MEF}_{25} ) z-score (Rosenthal)</td>
<td>0.04 (1.07)</td>
<td>-0.41, 0.49</td>
</tr>
</tbody>
</table>

Legend: Z-scores calculated from reference equations derived from healthy Swedish children, published by Solymar et al\(^{121} \) (labelled Solymar), and from reference equations derived from healthy British children, published by Rosenthal et al\(^{120} \) (labelled Rosenthal). Analysis is by one-sided t-test, against zero. * \( p<0.05 \)

Mean z-scores for the Swedish control population should be zero, with a standard deviation of one, for all parameters, provided that equipment and methodology are similar to that used by previous groups; that the control population is similar to
those from whom the reference equations were derived; and that the equations describe the data accurately. The prediction equations were tested by one-sided $t$-test against zero.

Mean $z$-scores for $\text{FEV}_1$ and $\text{MEF}_{25}$ derived from the prediction equations of Solymar et al were significantly lower than zero. $Z$-scores for $\text{FEV}_1$ derived from the prediction equations of Rosenthal et al were higher than zero (though this difference was not significant), whilst $\text{MEF}_{25}$ $z$-scores derived from Rosenthal equations were not significantly different from zero. For all further analyses presented in this section $z$-scores for $\text{FEV}_1$ and $\text{MEF}_{25}$ derived from the prediction equations of Rosenthal et al were employed for both the Swedish and the British study populations.

Table 5.7 and Figure 5.5 present comparisons of results obtained in healthy children in the current study with those previously obtained in healthy children tested in Sweden.

**Table 5.7 Comparison of results obtained from healthy children at ICH with those previously obtained from healthy children tested in Sweden**

<table>
<thead>
<tr>
<th></th>
<th>ICH (n=33)</th>
<th>Skövde (n=24)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.3 (3.2)</td>
<td>11.4 (2.2)</td>
<td>-0.1 (-1.6, 1.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>41.1 (14.7)</td>
<td>40.3 (11.7)</td>
<td>0.8 (-6.5, 8.1)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.4 (18.3)</td>
<td>148.6 (13.0)</td>
<td>-1.2 (-10.0, 7.6)</td>
</tr>
<tr>
<td>LCI</td>
<td>6.4 (0.5)</td>
<td>6.3 (0.5)</td>
<td>0.1 (-0.1, 0.4)</td>
</tr>
<tr>
<td>$\text{FEV}_1$ $z$-score</td>
<td>0.28 (0.86)</td>
<td>0.35 (0.88)</td>
<td>-0.07 (-0.54, 0.39)</td>
</tr>
<tr>
<td>$\text{MEF}_{25}$ $z$-score</td>
<td>-0.06 (1.06)</td>
<td>0.04 (1.07)</td>
<td>-0.1 (-0.68, 0.48)</td>
</tr>
</tbody>
</table>

*Legend: Results presented as mean (SD) unless otherwise indicated. Comparison between groups is by $t$-test, difference calculated as ICH - Sweden.*
Control populations from the two centres were well matched for age, height and weight. Normal values for LCI, FEV₁ z-score, and MEF₂₅ z-score obtained in the two centres were identical.

Figure 5.5 Lung Clearance Index plotted against age for healthy children measured at ICH and in Sweden

Legend: Healthy children measured at ICH presented as closed circles, and healthy children measured in Sweden presented as open triangles. Broken lines represent limits of normality for LCI, calculated from ICH data.

Table 5.8 and Figure 5.6 present comparisons of results obtained in the current study with those previously obtained in school-age children with CF tested in Sweden. CF populations from the two centres were well matched for age. The population tested at ICH tended to be shorter and lighter than the population tested...
in Sweden. The population tested at ICH had significantly higher LCI, and significantly lower FEV1 and MEF25 z-scores than the population tested in Sweden.

Table 5.8 Comparison of results obtained from children with CF at ICH with those previously obtained from children with CF tested in Sweden

<table>
<thead>
<tr>
<th></th>
<th>ICH (n=22)</th>
<th>Gothenburg (n=31)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.5 (3.2)</td>
<td>11.7 (2.7)</td>
<td>-0.2 (-1.9, 1.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>36.9 (11.8)</td>
<td>41.2 (16.4)</td>
<td>-4.3 (-12.5, 3.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>142.5 (18.6)</td>
<td>148.6 (16.6)</td>
<td>-6.1 (-15.9, 3.7)</td>
</tr>
<tr>
<td>LCI</td>
<td>11.5 (2.9)</td>
<td>8.3 (2.9)</td>
<td>3.2 (1.6, 4.8) ***</td>
</tr>
<tr>
<td>FEV1 z-score</td>
<td>-2.01 (1.45)</td>
<td>-0.54 (1.73)</td>
<td>-1.47 (-2.38, -0.57) **</td>
</tr>
<tr>
<td>MEF25 z-score</td>
<td>-1.89 (0.75)</td>
<td>-0.87 (1.14)</td>
<td>-1.02 (-1.58, -0.46) **</td>
</tr>
</tbody>
</table>

Legend: Results presented as mean (SD) unless otherwise indicated. Comparison between groups is by t-test, difference calculated as ICH - Sweden.

** p<0.01  
*** p<0.001
Figure 5.6 Lung Clearance Index plotted against age for children with CF measured at ICH and in Sweden

Legend: Children with CF measured at ICH presented as closed circles, and children with CF measured in Sweden presented as open triangles. Broken lines represent limits of normality for LCI, calculated from ICH data.

Figure 5.7 demonstrates the relationship between LCI and FEV₁ for both healthy and control children tested at both centres. In healthy children, there was no relationship between LCI and FEV₁. In children with CF, LCI and FEV₁ were negatively correlated ($r^2 0.64, p<0.001$). LCI results were classified as normal or abnormal based upon an ULN of 7.41. Thirty-six of 53 children with CF had a FEV₁ within the normal range. In comparison, only 19 of the 53 children with CF had a normal LCI.
Figure 5.7 Lung Clearance Index plotted against FEV\textsubscript{1} z-score for healthy children and children with CF measured at ICH and in Sweden

Legend: Control children measured at ICH presented as open circles, control children measured in Sweden as open triangles, children with CF measured at ICH presented as closed circles, and children with CF measured in Sweden as closed triangles. Vertical broken line represents lower limit of normality for FEV\textsubscript{1} z-score. Horizontal broken line represents upper limit of normality for LCI, calculated from ICH data. Note that many children with CF have FEV\textsubscript{1} z-score within normal range and LCI above normal range.

Figure 5.8 demonstrates the relationship between LCI and MEF\textsubscript{25} for both healthy and control children tested at both centres. In healthy children, there was no relationship between LCI and MEF\textsubscript{25}. In children with CF, LCI and MEF\textsubscript{25} were negatively correlated ($r^2$ 0.50, $p<0.001$). Thirty-eight of the 53 children with CF had a MEF\textsubscript{25} result in the normal range.
Figure 5.8 Lung Clearance Index plotted against MEF$_{25}$ z-score for healthy children and children with CF measured at ICH and in Sweden

Legend: Control children measured at ICH presented as open circles, control children measured in Sweden as open triangles, children with CF measured at ICH presented as closed circles, and children with CF measured in Sweden as closed triangles. Vertical broken line represents lower limit of normality for MEF$_{25}$ z-score. Horizontal broken line represents upper limit of normality for LCI, calculated from ICH data. Note that many children with CF have MEF$_{25}$ z-score within normal range and LCI above normal range.

5.6 Results in preschool children

5.6.1 Subjects
Details of the preschool children included in this study are presented in Chapter 4, Section 4.5.1.
5.6.2 Comparison of Lung Function results for CF and control groups

As the first step in these analyses, z-scores for FEV\textsubscript{0.5}, MMEF, FVC, and FRC were calculated from data obtained in healthy preschool children. These scores were based upon regression of lung function parameters against height (Table 5.9).

### Table 5.9 Regression of lung function parameters against subject height

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Constant</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV\textsubscript{0.5} (L)</td>
<td>-0.671</td>
<td>0.0144</td>
<td>0.003</td>
<td>0.110</td>
</tr>
<tr>
<td>MMEF (L-sec\textsuperscript{-1})</td>
<td>-0.319</td>
<td>0.0151</td>
<td>0.009</td>
<td>0.380</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>-1.654</td>
<td>0.0257</td>
<td>0.003</td>
<td>0.125</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>-1.383</td>
<td>0.0186</td>
<td>0.003</td>
<td>0.116</td>
</tr>
</tbody>
</table>

Legend: \( SE (B) = \) standard error of the coefficient.
\( RSD = \) residual standard deviation for the regression.

This table allows calculation of z-scores for lung function parameters, as follows:

**Predicted value for parameter can be obtained from the equation**
\[
\text{Predicted value} = \text{Constant} + (\text{height}[cm] \cdot B)
\]

**Standard deviation score (Z score) can be calculated from the equation**
\[
Z\text{-score} = \frac{(\text{Observed value} - \text{Predicted value})}{RSD}
\]

Addition of subject sex and weight to each of these models had no significant effect upon model fit.

Addition of sex or weight to these models did not significantly affect model fit.

LCI was almost independent of height, weight, and sex in healthy children (Chapter 4), so z-scores were not calculated for this parameter.

Results for LCI, FRC z-scores, and spirometry z-scores, analysed by diagnosis, are presented in Table 5.10. Z-scores were calculated from local data (Table 5.9), and also from published reference equations.
Table 5.10 Lung function test results compared by diagnosis, preschool children.

<table>
<thead>
<tr>
<th></th>
<th>CF (n=30)</th>
<th>Healthy controls (n=30)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>9.61 (2.19)</td>
<td>6.89 (0.44)</td>
<td>2.72 (1.90, 3.54)***</td>
</tr>
<tr>
<td>FEV(_{0.5}) z-score (ICH)</td>
<td>-0.76 (1.31)</td>
<td>0.00 (1.00)</td>
<td>-0.76 (-1.37, -0.16)*</td>
</tr>
<tr>
<td>MMEF z-score (ICH)</td>
<td>-0.45 (1.15)</td>
<td>0.00 (1.00)</td>
<td>-0.45 (-1.00, 0.11)</td>
</tr>
<tr>
<td>FVC z-score (ICH)</td>
<td>-0.27 (1.6)</td>
<td>0.00 (1.00)</td>
<td>-0.27 (-0.97, 0.42)</td>
</tr>
<tr>
<td>FRC z-score (ICH)</td>
<td>-0.16 (0.80)</td>
<td>0.00 (1.00)</td>
<td>-0.16 (-0.64, 0.30)</td>
</tr>
<tr>
<td>FEV(_{0.5}) z-score(^{12})</td>
<td>-0.58 (1.0)</td>
<td>-0.09 (0.80)</td>
<td>-0.49 (-0.95, -0.03)*</td>
</tr>
<tr>
<td>MMEF z-score(^{9})</td>
<td>-0.97 (1.75)</td>
<td>-0.27 (1.61)</td>
<td>-0.70 (-1.57, 0.16)</td>
</tr>
<tr>
<td>FVC z-score(^{9})</td>
<td>-0.87 (1.75)</td>
<td>-0.68 (1.04)</td>
<td>-0.19 (-0.93, 0.56)</td>
</tr>
<tr>
<td>FRC z-score</td>
<td>-1.34 (0.53)</td>
<td>-1.14 (0.83)</td>
<td>0.19 (-0.17, 0.55)</td>
</tr>
</tbody>
</table>

Legend: Comparison between groups is by t-test, and difference is calculated as CF-control. Parameters labelled ICH represent z-scores calculated from the current control population.

* \(p<0.05\), *** \(p<0.001\)

Children with CF had significantly higher LCI than healthy children (\(p<0.001\)), and significantly lower FEV\(_{0.5}\) z-scores (\(p<0.05\)), irrespective of whether local controls or published reference equations were employed for calculation of z-scores.

Children with CF tended to have lower MMEF than control children, though this did not reach statistical significance. There was little difference in FVC or FRC results between the two groups. For LCI in preschool children the upper limit of normality (ULN) was calculated as 7.76, and lower limit of normality (LLN) 6.02.

It is also noted that for healthy children, z-scores for FVC calculated from Indianapolis reference data, and FRC z-scores calculated from French data are lower than zero. Possible reasons for these results are discussed in Section 5.7.
The relationship between LCI and age is presented in Figure 5.9. As for healthy school-age children, LCI was virtually age independent in healthy preschool children. In contrast with school-age children with CF, where a positive relationship between LCI and age was seen, there was no relationship between LCI and age in preschool children with CF.

Figure 5.9 Relationship between LCI and age in preschool children

Legend: Control children presented as open circles, and children with CF presented as closed circles. Broken lines represent limits of normality for LCI. Note that normal values for LCI are age-independent. There is no relationship between LCI and age in healthy children, or in children with CF.

The relationship between FEV$_{0.5}$ and age is presented in Figure 5.10. For this Figure, and for all further analyses presented in this chapter, the FEV$_{0.5}$ z-scores presented are those calculated from the control subjects tested for this study. Note that there is no relationship between FEV$_{0.5}$ z-score and age in children with CF.
Figure 5.10 Relationship between FEV$_{0.5}$ and age in preschool children

Legend: Control children presented as open circles, and children with CF presented as closed circles. Broken lines represent limits of normality for FEV$_{0.5}$ z-score, calculated as +/- 1.96 z-scores. Z-scores are corrected for height, so the lack of relationship between FEV$_{0.5}$ z-score and age in healthy children is expected. It should be noted that there is also no relationship between FEV$_{0.5}$ z-score and age in children with CF.

Figure 5.11 presents the relationship between MMEF z-scores and age. For this Figure, and for all further analyses presented in this chapter, the MMEF z-scores presented are those calculated from the control subjects tested for this study. Note that there is no relationship between MMEF z-score and age in children with CF.
Figure 5.11 Relationship between MMEF and age in preschool children

Legend: Control children presented as open circles, and children with CF presented as closed circles. Broken lines represent limits of normality for MMEF z-score, calculated as +/- 1.96 z-scores. Z-scores are corrected for height, so the lack of relationship between MMEF z-score and age in healthy children is expected. It should be noted that there is also no relationship between MMEF z-score and age in children with CF.

As there was little difference in FVC or FRC results between CF and control groups, the age relationships of these parameters are not presented here.

5.6.3 Sensitivity and specificity of LCI and spirometry parameters

The area under the Receiver-Operator Characteristic curve (AUC_{ROC}) for each parameter is presented in Figure 5.12 and Table 5.11.
Figure 5.12 Receiver-Operator Characteristic (ROC) curves for Lung Clearance Index, FEV$_{0.5}$ z-score, and MMEF z-score for preschool children

Legend: The outcome variable is the diagnosis of cystic fibrosis. ROC curve for LCI is presented in red, ROC curve for MEF$_{25}$ is presented in blue, and ROC curve for FEV$_1$ is presented in green.

All LCI, FEV$_{0.5}$ and MMEF results were classified as normal or abnormal, as described in Section 5.4.3. Sensitivity and specificity of each lung function parameter were calculated from this classification, and are also presented in Table 5.11. As for school-age children, LCI was more sensitive at discriminating CF from control children than spirometry parameters.
Table 5.11 Sensitivity and specificity of LCI, FEV\textsubscript{0.5}, and MMEF for distinguishing CF and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC\textsubscript{ROC}</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>77%</td>
<td>100%</td>
<td>0.95 (0.03)***</td>
</tr>
<tr>
<td>FEV\textsubscript{0.5}</td>
<td>7%</td>
<td>100%</td>
<td>0.66 (0.07)*</td>
</tr>
<tr>
<td>MMEF</td>
<td>27%</td>
<td>80%</td>
<td>0.62 (0.07)</td>
</tr>
</tbody>
</table>

Legend: Sensitivity and specificity defined by cut-offs of greater than 1.96 z-scores for LCI, and less than -1.96 z-scores for FEV\textsubscript{0.5} and MMEF. 

AUC\textsubscript{ROC} = Area under the Receiver-Operator Characteristic curve. 

AUC\textsubscript{ROC} presented as area (SE), where area of 1.0 represents perfect discrimination, and area of 0.5 represents no discrimination. 

*p<0.05, **p<0.001, where null hypothesis is that AUC\textsubscript{ROC} = 0.5.

5.6.4 Relationship between LCI and spirometry parameters

Twenty-two of the 30 children with CF had abnormal LCI. Two children with CF had an abnormal FEV\textsubscript{0.5} (Figure 5.13), and four had an abnormal MMEF (Figure 5.14), all of whom also had an abnormal LCI. None of the children with CF had abnormally raised FRC. 

In children with CF, weak, but statistically significant correlations between lung function parameters were noted. LCI was negatively correlated with FEV\textsubscript{0.5} ($r^2 = 0.21$, $p=0.01$), and MMEF ($r^2 = 0.28$, $p=0.003$). As for school-age children, there was no correlation between LCI and spirometry parameters in healthy children.
Figure 5.13 Lung Clearance Index plotted against FEV$_{0.5}$ z-score in preschool children

Legend: Open circles represent healthy children, and closed circles represent children with CF. Broken lines represent 95% limits of normality, calculated as -1.96 z-scores for FEV$_{0.5}$, and 6.89 + 1.96 · 0.44 for LCI.
Figure 5.14 Lung Clearance Index plotted against MMEF z-score in preschool children

Legend: Open circles represent healthy children, and closed circles represent children with CF. Broken lines represent 95% limits of normality, calculated as $-1.96 \times z$-scores for MMEF, and $6.89 + 1.96 \times 0.44$ for LCI.

5.6.5 Relationship between lung function parameters and clinical information in preschool children

Of the 30 children with CF, 22 were homozygous for the ΔF508 mutation, and eight had one ΔF508 mutation, plus one other. Of these eight children, three had an unidentified mutation, and one each had 1717-1G→A; G551D; 1898+1G→A; N1303K; and ΔI507. Fourteen of these children presented with respiratory symptoms, whilst the other 16 presented with gastrointestinal symptoms (failure to thrive, meconium ileus, or rectal prolapse). Children with respiratory presentations
were significantly taller, and tended to be heavier than children with gastrointestinal presentations, but there was no significant differences in lung function results between the two groups (Table 5.12).

Table 5.12 Lung function results in preschool children with CF, analysed by mode of presentation

<table>
<thead>
<tr>
<th></th>
<th>Resp (n=14)</th>
<th>GI (n=16)</th>
<th>Mean difference (95% Cl for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>9.63 (2.41)</td>
<td>9.58 (2.07)</td>
<td>0.05 (-1.63, 1.72)</td>
</tr>
<tr>
<td>FEV_{0.5} z-score</td>
<td>-0.79 (1.33)</td>
<td>-0.74 (1.33)</td>
<td>-0.06 (-1.05, 0.94)</td>
</tr>
<tr>
<td>MMEF z-score</td>
<td>-0.51 (1.26)</td>
<td>-0.39 (1.08)</td>
<td>-0.12 (-1.00, 0.75)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.29 (0.71)</td>
<td>4.56 (0.82)</td>
<td>-0.27 (-0.84, 0.31)</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.46 (1.22)</td>
<td>-0.17 (0.86)</td>
<td>0.63 (-0.15, 1.41)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>0.22 (1.21)</td>
<td>-0.62 (0.99)</td>
<td>0.84 (0.02, 1.67)*</td>
</tr>
</tbody>
</table>

Legend: Resp = respiratory presentation; GI = gastrointestinal presentation. Comparison between groups is by t-test, and difference is calculated as resp – GI. * p<0.05

Mothers of six of the 30 children had smoked tobacco during pregnancy. Four of these mothers reported still being smokers: whilst another four, who had not smoked during pregnancy, reported smoking now. There was no difference in lung function results when analysed by pregnancy smoking history. There was a trend for children whose mothers were current smokers to have higher LCI (10.34 versus 9.38, Z= -1.55, p=0.12). There was no difference in spirometry results for these two groups.

Nineteen of the children had been infected with *Staphylococcus aureus*; 15 had been infected with *Haemophilus influenzae*; and 25 had been infected with *Pseudomonas aeruginosa* on at least one occasion previously. Twelve children were currently infected with *P aeruginosa* at the time of lung function testing (Section...
These 12 children had significantly higher LCI, and tended to have lower FEV<sub>0.5</sub> and MMEF results than those children who were not currently infected with <i>P aeruginosa</i> (Table 5.13).

**Table 5.13 Lung function results in preschool children with CF, analysed by current infection with Pseudomonas aeruginosa**

<table>
<thead>
<tr>
<th></th>
<th>PA positive (n=12)</th>
<th>PA negative (n=18)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>10.77 (2.49)</td>
<td>8.83 (1.61)</td>
<td>1.94 (0.41, 3.45)*</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt; z-score</td>
<td>-1.30 (1.32)</td>
<td>0.41 (1.20)</td>
<td>-0.90 (-1.85, 0.06)</td>
</tr>
<tr>
<td>MMEF z-score</td>
<td>-0.89 (1.08)</td>
<td>-0.15 (1.13)</td>
<td>-0.74 (-1.59, 0.11)</td>
</tr>
</tbody>
</table>

Legend: PA positive = currently infected with <i>P aeruginosa</i>; PA negative = not currently infected with <i>P aeruginosa</i>. Comparison between groups is by t-test, and difference is calculated as PA positive – PA negative. *p<0.05

None of the children had undergone chest computerised tomography scanning, and only five had undergone radioisotope ventilation scanning. These results were not analysed further.

Fourteen of the children had a parental reported history of cough in the seven days prior to lung function testing. These children had significantly lower spirometry results and a trend to higher LCI than the children with no history of cough (Table 5.14).
Table 5.14 Lung function results in preschool children with CF, analysed by presence of parental reported cough in the seven days prior to lung function testing

<table>
<thead>
<tr>
<th></th>
<th>Cough (n=14)</th>
<th>No cough (n=16)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>10.35 (2.53)</td>
<td>8.95 (1.67)</td>
<td>1.39 (-0.19, 2.98)</td>
</tr>
<tr>
<td>FEV$_{0.5}$ z-score</td>
<td>-1.29 (1.24)</td>
<td>-0.30 (1.22)</td>
<td>-0.99 (-1.91, -0.07)*</td>
</tr>
<tr>
<td>MMEF z-score</td>
<td>-0.91 (1.20)</td>
<td>-0.04 (0.96)</td>
<td>-0.88 (-1.69, -0.07)*</td>
</tr>
</tbody>
</table>

Legend: Comparison between groups is by t-test, and difference is calculated as cough – no cough. *p<0.05

Only three children had abnormal chest signs (crackles or wheeze) on physical examination. These three children had significantly higher LCI (12.6 [0.38] versus 9.3 [2.06], Z = -2.45, p = 0.01) and lower MMEF z-score, (-1.88 [0.21] versus -0.29 [1.10], Z = -2.32, p = 0.02), and tended to have lower FEV$_{0.5}$ z-score (-2.20 [1.51] versus -0.60 [1.21], Z = -1.69, p = 0.09) than children without chest signs.

The investigator visual analogue score (VASi) was available for 29 of the 30 children, all completed by the author. In one child, another investigator conducted testing, and this score was not completed. There was no significant relationship between VASi and LCI ($r^2 = 0.11$, p = 0.09). There were weak, but statistically significant, negative relationships between VASi and FEV$_{0.5}$ z-score ($r^2 = 0.20$, p = 0.02), and MMEF z-score ($r^2 = 0.30$, p = 0.002).

Due to an administrative error, consultant questionnaires for nine of the 30 children were not completed contemporaneously. Information on genotype, mode of presentation, and microbiology should be unaffected by this error. However, the visual analogue scores on current status could not be reliably completed retrospectively, and the visual analogue scores on past medical history may be biased by knowledge of the lung function results. These consultant scores were not analysed for this reason.
5.7 Joint analysis of results in preschool and school-age children

A limited joint analysis of results obtained in the two age groups was performed, using LCI and FEV$_1$ as outcome measures. The rationale behind the choice of these measures, and the important cautions that must be applied to this analysis, are presented in Section 5.8.4.

LCI results were available in 63 healthy children, and in 52 children with CF. FEV$_1$ results were available in 50 healthy children, and 47 children with CF. Eighteen of the 60 preschool children were unable to produce an FEV$_1$. Children with CF had significantly higher LCI, and significantly lower FEV$_1$ z-score, than healthy children (Table 5.15).

Table 5.15 Comparison of LCI and FEV$_1$ results by diagnosis, all children

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Healthy controls</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>10.41 (2.65)</td>
<td>6.65 (0.52)</td>
<td>3.76 (3.09, 4.43)***</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>-1.18 (1.71)</td>
<td>0.27 (0.84)</td>
<td>-1.44 (-1.98, -0.91)***</td>
</tr>
</tbody>
</table>

Legend: LCI results were available in 63 healthy children, and in 52 children with CF. FEV$_1$ results were available in 50 healthy children, and 47 children with CF. Difference calculated as CF-control. *** p<0.001

As presented in Section 4.5.3, healthy preschool children had significantly higher LCI than healthy school-age children. There was no significant difference in FEV$_1$ z-score (Table 5.16, Figure 5.15). Preschool children with CF had significantly lower LCI, and significantly higher FEV$_1$ z-score than school-age children with CF (Table 5.17, Figure 5.15).
Table 5.16 Comparison of LCI and FEV₁ results by age group, in healthy children

<table>
<thead>
<tr>
<th></th>
<th>Preschool</th>
<th>School-age</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.88 (0.45)</td>
<td>6.45 (0.50)</td>
<td>0.43 (0.19, 0.67)**</td>
</tr>
<tr>
<td>FEV₁ z-score</td>
<td>0.21 (0.81)</td>
<td>0.30 (0.87)</td>
<td>-0.08 (-0.59, 0.42)</td>
</tr>
</tbody>
</table>

Legend: LCI results were available in 30 preschool children, and in 33 school-age children. FEV₁ results were available in 17 preschool children, and in 33 school-age children. Difference calculated as preschool-school-age.

**p<0.01

Table 5.17 Comparison of LCI and FEV₁ results by age group, in children with CF

<table>
<thead>
<tr>
<th></th>
<th>Preschool</th>
<th>School-age</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>9.61 (2.19)</td>
<td>11.53 (2.86)</td>
<td>-1.9 (-3.33, -0.52)**</td>
</tr>
<tr>
<td>FEV₁ z-score</td>
<td>-0.44 (1.59)</td>
<td>-2.01 (1.45)</td>
<td>1.57 (0.67, 2.47)**</td>
</tr>
</tbody>
</table>

Legend: LCI results were available in 30 preschool children, and in 22 school-age children. FEV₁ results were available in 25 preschool children, and in 22 school-age children. Difference calculated as preschool-school-age.

**p<0.01
Figure 5.15 LCI plotted against age, all subjects

Legend: Control children presented as open circles, and children with CF presented as closed circles. Limits of normality are not presented, as these differ for the preschool and school-age groups.

The relationship between FEV₁ and height is presented in Figure 5.16. This plot suggests that there is a curvilinear relationship between FEV₁ and height in healthy children. This relationship was not explored further, but is discussed in Section 5.8.
Figure 5.16 FEV\textsubscript{1} plotted against height, all children

Legend: Control children presented as open circles, and children with CF presented as closed circles. There appears to be a curvilinear relationship between FEV\textsubscript{1} and height in healthy children. This relationship is discussed in Section 5.8.

The relationship between FEV\textsubscript{1} z-score and age is presented in Figure 5.17. The majority of preschool children with CF have normal FEV\textsubscript{1} results, whilst half of the school-age children with CF have abnormal FEV\textsubscript{1} results (see also Section 5.5.3).
Figure 5.17 FEV₁ z-score plotted against age, all children

Legend: Control children presented as open circles, and children with CF presented as closed circles.

The relationship between FEV₁ z-score and LCI is presented in Figure 5.18. There was no relationship between the two variables for healthy children. In children with CF, there was a significant negative relationship ($r^2 = 0.49$, $p < 0.001$).
Figure 5.18 LCI plotted against FEV₁ z-score, all children

Legend: Control children presented as open circles, and children with CF presented as closed circles. Limits of normality are not presented, as these differ for LCI for the preschool and school-age groups.

Chrispin-Norman scores were available for 17 of the 26 preschool children with CF, and all 19 of the school-age children with CF. The remaining nine preschool children had their primary CF care at other London centres, and their chest radiographs were not scored. For the 36 children for whom results were available, the mean Chrispin-Norman score was 9.3 (5.3). This score was significantly higher in the school-age children (11.5 (5.7) versus 6.8 (3.4), mean difference school-age – preschool 4.7 (1.5, 7.9), p=0.005). There was a significant positive correlation between Chrispin-Norman score and LCI (r²=0.19, p=0.02), and a significant negative correlation between Chrispin-Norman score and FEV₁ z-score (r²=0.32, p=0.01)
5.8 Discussion

5.8.1 Summary

The aim of this study was to compare LCI with spirometry for children with CF and healthy children. Whilst group differences were seen with both techniques, analysis of individual results showed that more children with CF had abnormal MBW than had abnormal spirometry. These data support the hypothesis presented in Section 5.2.2.

5.8.2 Selection of outcome measures for spirometry

In school-age children, FEV₁ and MEF₂₅ were selected as outcome measures for spirometry. FEV₁ is the most commonly reported measure of pulmonary function in CF. It is employed in the clinic for tracking progress of individual patients, and is used as an outcome measure in clinical studies and survival studies\(^{18,19,122}\). In European centres, MEF₂₅ is the most commonly reported measure of airflow during forced expiration. Reference equations for MEF₂₅ in healthy British children are available\(^{120}\), and MEF₂₅ was an outcome measure in a recent report comparing spirometry and MBW results in school-age children measured in Sweden\(^{37}\). Data from this latter study are analysed further in this chapter.

It is not possible to use FEV₁ as the main outcome measure in preschool children. As described in Chapter 3, many preschool children complete forced expiration in less than one second, meaning that FEV₁ cannot be reported. Further, in those children who are able to exhale for one second, the FEV₁/FVC ratio is almost always greater than 90% (Table 3.5), implying that FEV₁ is of limited value in detecting mild to moderate airway obstruction in this age group. MMEF was selected ahead of MEF₂₅ as a measure of airflow in preschool children for practical reasons. There are no published data of MEF₂₅ in healthy preschool children. Although it would have been possible to calculate z-scores for MEF₂₅ from the current control group, it was considered preferable to select a measure that could be referenced to control data collected elsewhere. This difference in the choice of outcome measures limits the comparisons that can be made between spirometry results in preschool and school-age children, and this is discussed further below.
5.8.3 Relative sensitivity and specificity of spirometry and LCI

In school-age children, approximately half of the children with CF had normal spirometry results, whilst nearly all had abnormal ventilation distribution. In preschool children, LCI was abnormal in 22/30 children (73%), \( \text{FEV}_{0.5} \) was abnormal in two children (7%), and MMEF was abnormal in four (13%).

It was expected that MEF\(_{25}\) and MMEF would discriminate between CF and control populations better than \( \text{FEV}_1 \) and \( \text{FEV}_{0.5} \), but this was not demonstrated, suggesting that the inter-subject variability of MEF\(_{25}\) and MMEF is too high to reliably detect airway dysfunction. This may be technique-related, though it has already been demonstrated that MMEF results obtained in healthy children in this study are similar to those reported by other investigators (Chapter 3). This suggests that the variability of MMEF is a common problem between laboratories, rather than the result of poor technique in this study. Alternatively, the poor discriminative ability of MEF\(_{25}\) and MMEF may be because young children have larger airways relative to lung volume than older children, and in younger children expired volumes and flows are therefore determined more by lung capacity, elastic recoil, and muscle strength than by airway diameter. The relative merits of timed forced expired volumes versus airflows for detection of airway dysfunction at different ages is discussed further in Chapter 7.

5.8.4 Comparison with data collected in Sweden

There have been previous studies of gas mixing in CF using similar methods\(^{27-29,74,75}\). The most recent studied Swedish children using the same methods and equipment as now used in our laboratory\(^{27}\), and results obtained from the two centres have been compared in this chapter. This comparison was powered to detect a difference in LCI of 1 SD between healthy children measured in London and those measured in Sweden. As can be seen from Table 5.7, no significant difference was observed, with the mean difference in LCI between the two groups being 0.1. The upper limit of normality calculated from the two populations was similar, being 7.49 from the UK population, and 7.23 from the Swedish population. These differences between the healthy populations are minor compared to the differences between healthy and CF populations (mean difference in LCI in school-age children...
of 5.1 for the current study). The similarity of results in healthy children from the two centres indicates that the MBW technique can be transferred between laboratories, and offers the possibility of collating data for future analyses. The Swedish study also reported LCI from 43 of the 70 children attending the CF clinic in Gothenburg, most of who had FEV\textsubscript{1} and MEF\textsubscript{25} results within the normal range. The majority of these children were found to have abnormal LCI, although, in contrast to findings in the current study, there was only a weak correlation between ventilation indices and spirometry indices. In the current study only a small subgroup (22/199) of children attending the CF clinic at Great Ormond Street Hospital for Children were recruited. As no attempt was made to recruit all children attending the clinic, or to take a representative sample, it is likely that some selection bias operated in recruitment to this study, since children with more severe disease attend the clinic more frequently and were therefore more likely to be approached. Children with CF tested at ICH had significantly higher LCI, and significantly lower FEV\textsubscript{1} and MEF\textsubscript{25} z-score than children tested in Sweden (Table 5.8) However, meaningful comparison of CF populations from two centres would require recruitment of representative samples, and detailed information on treatment history and disease severity. These criteria were not met for the current analysis, but the consistency of LCI results in the healthy populations would allow future prospective studies, using LCI as an outcome measure. More detailed discussions of previous studies of MBW, and of previous studies of lung function in preschool children, are presented in Chapter 1, and - in the context of the current results - in Chapter 7.

5.8.5 Joint analysis of results from preschool and school-age children

A limited joint analysis of results obtained in both age-groups was performed. Before discussing the results, it is necessary to describe important weaknesses of this analysis. FEV\textsubscript{1} was selected as the outcome measure from spirometry, even though 18 of the 60 preschool children were unable to produce an FEV\textsubscript{1}. However, FEV\textsubscript{1} is the only spirometry outcome measure for which reference equations were available for both age groups. These reference equations differ for the preschool and school-age groups. The reference equation employed for preschool children is
derived from a study of children tested in Indianapolis, USA\textsuperscript{24}, whilst the reference equation employed for school-age children is derived from data collected in the UK\textsuperscript{120}. The data presented in Figure 5.17 demonstrate that most of the healthy children in this study have FEV\textsubscript{1} z-scores between +2 and -2, suggesting that these two equations are appropriate for our population. However, the analysis would be strengthened if a single reference equation could be applied. Data from healthy children presented in Figure 5.16 suggests that there is a curvilinear relationship between FEV\textsubscript{1} and height across the 2-16 year age range. It is of interest that a recent report from ICH suggests a similar curvilinear relationship between FEV\textsubscript{0.5} and height/length from birth to nine years. It should soon be possible to construct reference equations for spirometry variables that span infant, preschool, and school-age children. This important and exciting area of research is unfortunately beyond the scope of this thesis.

A second, related, caution is that FEV\textsubscript{1} may not measure the same aspects of airway function at different ages. In older school-age children and adults, the ratio of FEV\textsubscript{1} to FVC is approximately 70\%. As presented in Chapter 3, this FEV\textsubscript{1}/FVC ratio is typically greater than 90\% in preschool children. This topic is discussed further in Chapter 7.

As described in Chapter 4, healthy preschool children have significantly higher LCI than healthy school-age children. Possible reasons for this are discussed in Chapter 7.

The final caution to this analysis, already mentioned above, is that the school-age children with CF included in this study were recruited opportunistically, and selection bias may apply. Of the 30 preschool children with CF, 20 were recruited from the clinic at Great Ormond Street, representing all eligible children from this population (apart from those who refused consent), whilst the remaining 10 children were recruited opportunistically from the other centres participating in the LCCFS. With these cautions, the joint analysis demonstrates that school-age children with CF have higher LCI and lower FEV\textsubscript{1} z-score than preschool children with CF. It also shows that there is no relationship between LCI and FEV\textsubscript{1} z-score in healthy children, and that there is a moderate correlation between LCI and FEV\textsubscript{1} in children with CF.
5.8.6 Strengths and weaknesses of this study

All spirometry parameters are affected by subject height, which can confound interpretation of differences between populations. Reference equations for FEV\textsubscript{1} and MEF\textsubscript{25} in British school-age children are available\textsuperscript{120}, and were used here. Although reference equations for preschool children are now available\textsuperscript{24,40}, these have not yet been widely tested. The current control population was therefore used to calculate height-adjusted z-scores for FEV\textsubscript{0.5}, and MMEF for the primary analysis. It was not necessary to calculate z-scores for LCI as this parameter was almost independent of age and body size in healthy children.

The order of testing in this study was fixed as MBW first, and spirometry second for school-age children. For preschool children the order was MBW first, plethysmography second, and spirometry third. Direct comparison of relative feasibility for the two methods presented in this thesis is therefore not possible. This order of testing was selected because deep inspirations are known to affect airway mechanics during subsequent tidal breathing, and measurements of LCI (or sR\textsubscript{aw}) could have been altered if spirometry was performed earlier. As spirometry is technique and effort dependent, it is possible that fatigue towards the end of the testing session could have resulted in erroneous FEV\textsubscript{0.5} and MMEF results in some preschool children. However, data presented in Chapter 3 demonstrates that spirometry results obtained in healthy children undertaking the current study protocol are very similar to those reported by Eigen \textit{et al}\textsuperscript{24}.

Limited data on the clinical condition of the subjects with CF are presented. It would have been preferable to compare lung function results with measures of infection and inflammation obtained from bronchoalveolar lavage, or with structural changes measured on computed tomography. These investigations are not routinely performed in the clinic, and could not be justified for the purpose of this study. It was possible to compare results in preschool children by the presence of current \textit{Pseudomonas aeruginosa} infection, and demonstrate that infected children had significantly higher LCI than those who were not infected. It was also possible to analyse by the presence of cough, which showed that preschool children with cough had significantly worse spirometry results than those who did not cough. There was no significant difference in lung function results between preschool
children who presented with respiratory symptoms and those who presented with non-respiratory symptoms. This echoes the report of Ranganathan et al.¹⁴, who found no differences in lung function results in infants with CF, when analysed according to mode of presentation. In school-age children: those children who were infected with Pseudomonas aeruginosa; and those children with worse clinical symptoms or signs, tended to have more abnormal lung function results. However, none of these differences were statistically significant. All the above were secondary analyses, for which this study was not powered. In addition, there were methodological differences in how microbiological samples were processed in different laboratories, and parental/child report of cough is known to be sometimes unreliable¹²³. Results must therefore be interpreted with great caution.

Further discussion of the links between infection, inflammation and lung function in early CF lung disease, and of the clinical significance of these results, is presented in Chapter 7.

In conclusion, this study has demonstrated that abnormal ventilation distribution, as evidenced by an abnormally raised LCI, is found in many children with CF, including many preschool children with normal spirometry measurements. One interpretation of these findings is that destructive processes in the airways of children with CF start early in life, and that these changes are detectable by MBW, but not always by more commonly used measures of lung function. These issues are discussed in detail in Chapter 7.
Chapter 6: Results of phase III slope analysis

6.1 Introduction

It has already been demonstrated that the LCI, obtained from washout curve analysis of a MBW, is frequently abnormal in children with CF, including in those who record normal spirometry results. An abnormal LCI indicates increased inhomogeneity of ventilation distribution, or impaired gas mixing. This increased inhomogeneity can result from differences in specific ventilation between units subtended at branch points within conducting airways (convection dependent inhomogeneity [CDI]); or can be generated by size differences in units subtended at branch points within the diffusion-convection front, and by differences in airway calibre at these branch points (diffusion-convection dependent inhomogeneity [DCDI]). These concepts were explained in more detail in Chapter 1.

A number of studies in adult human subjects have described how indices representing CDI (conducting zone inhomogeneity [S_{cond}]) and DCDI (acinar zone inhomogeneity [S_{acin}]) can describe the relative contribution of these two mechanisms to overall inhomogeneity of ventilation distribution. The MBW studies from which these indices were derived were performed during controlled breathing, i.e. subjects were asked to breathe at a fixed respiratory rate and tidal volume. In contrast, the MBW studies in school-age and preschool children presented in Chapters 4 and 5 were performed during uncontrolled, spontaneous breathing. This chapter presents the results obtained from phase III slope analysis of the MBW studies presented in Chapter 5, applying the quality control criteria examined in Chapter 4.

Of the phase III slope parameters studied here, the first breath SnIII_{corr} is a measure of overall ventilation inhomogeneity, whilst S_{cond,corr} and S_{acin,corr} are measures of ventilation inhomogeneity generated by convective dependent mechanisms, and by mechanisms dependent on diffusion-convection interaction, respectively. It can therefore be predicted that the LCI, which is a measure of overall ventilation inhomogeneity, will be closely related to first breath SnIII_{corr}, and less closely related to S_{cond,corr} and S_{acin,corr}.
6.2 Aims and hypotheses

6.2.1 Hypotheses

- Children with CF with abnormal washout curve results will also have abnormal phase III slope results
- These children will have evidence of both acinar zone and conducting zone inhomogeneity
- Both measures of inhomogeneity will be greater in older children.

6.2.2 Aims and objectives

The primary aim of this study was:

- To investigate the relationships between parameters derived from MBW phase III slope analysis and age in a population of healthy British children and children with CF, aged from two to 16 years.

The secondary aims of this study were:

- To relate phase III slope parameters to those obtained from washout curve analysis (i.e. LCI) and forced expiration (i.e. FEV₁)
- To relate phase III slope parameters obtained in children with CF to clinical status, as measured by cough frequency, sputum microbiology, and chest radiograph findings.

6.3 Subjects and methods

6.3.1 Subject recruitment

This is described in Section 4.3.1 for school-age children, and Section 3.3.1 for preschool children.

6.3.2 Study design

This is described in Section 5.3.2. The same 115 children whose results are presented in Chapter 5 were entered into the analyses presented in this chapter.
6.3.3 Data collection

Collection and analysis of spirometry data, including criteria for accepting or discarding results, and derivation of the indices listed below, are described in detail in Chapters 2 and 3. Collection and analysis of MBW data are described in detail in Chapters 2 and 4.

6.3.4 Additional Clinical information and recording of clinical data

This is described in Section 5.3.4.

6.3.5 Ethics

The study was approved by the North Thames Region Multicentre Research Ethics Committee, and by the Local Research Ethics Committees of each of the five collaborating centres.

6.4 Analysis

6.4.1 Outcome measures

Calculation of FRC, LCI, and spirometry parameters is described in Section 5.4.1. The phase III slope of the first breath of the SF\textsubscript{6} washout (first breath SnIII) was calculated separately for each run - and the mean of the three results is presented. The difference between the first breath SF\textsubscript{6} SnIII and the first breath He SnIII (SnIII\textsubscript{SF6-He}) was calculated separately for each run, and the mean of the three results is presented. Conducting zone inhomogeneity (S\textsubscript{cond}) was calculated from an aggregated plot of SnIII versus lung volume turnover (TO) - derived from all three SF\textsubscript{6} washouts - as the slope of the regression over 1.5 to 6.0 TO, calculated after excluding SnIII results obtained from breaths of inadequate volume (see Chapters 2 and 4). Acinar zone inhomogeneity (S\textsubscript{acin}) was calculated by subtracting the S\textsubscript{cond} component from the mean first breath SnIII. This S\textsubscript{cond} component was calculated as:

\[ S_{cond} \cdot \text{first breath } V_T \cdot FRC^{-1} \]
where first breath $V_T$ is the mean expired volume of the first breath, and first breath $V_T$ and FRC were presented in the same units.

For all these calculations, first breath $V_T$, FRC, and TO were corrected for $V_{D_{app}}$.

It has already been demonstrated that there is a negative relationship between first breath SnIII and age in healthy children (Section 4.6) but that there is no relationship between age and the product of first breath SnIII and expired volume.

All phase III slope parameters were therefore corrected for expired volume:

- first breath SnIII; SnIII$_{SF6-He}$; and $S_{acin}$ were multiplied by the expired volume of the first breath
- $S_{cond}$ was multiplied by the mean expired volume from the entire washout.

In both cases, this expired volume was calculated as the mean from three runs, after correction for $V_{D_{app}}$. The resultant volume-corrected parameters (first breath SnIII$_{corr}$; SnIII$_{SF6-He,corr}$; $S_{cond,corr}$; and $S_{acin,corr}$) were the primary outcome variables for subsequent analyses.

### 6.4.2 Comparisons between groups, with other measures, and with clinical status

Analyses were performed jointly for preschool and school-age groups (see power calculation, below). Uncorrected phase III slope parameters were compared by diagnosis. Volume-corrected parameters were compared by diagnosis and age-group. The age relationships of the volume-corrected parameters were examined further by scatter plots and tested by regression analyses. The upper limits of normality for first breath SnIII$_{corr}$, SnIII$_{SF6-He,corr}$, $S_{cond,corr}$, and $S_{acin,corr}$ were calculated from the data obtained in healthy children as mean + 1.96SD, as previously described for LCI (Chapter 5). By these limits, children were defined as having normal or abnormal first breath SnIII$_{corr}$, SnIII$_{SF6-He,corr}$, $S_{cond,corr}$, or $S_{acin,corr}$ results. Volume-corrected parameters were compared with corresponding LCI and FEV$_1$ z-score results by scatterplot. Numbers of children categorised as normal or abnormal for each of these measures was compared. Finally, volume-corrected outcome measures in children with CF were compared by: presence or absence of chronic *Pseudomonas aeruginosa* infection (defined as in Section 5.3.4); by presence or absence of cough in the week prior to lung function testing (see Section 5.3.4); and by Chrispin-Norman score of the most recent chest radiograph.
6.4.3 Statistical analysis

Group comparisons were by t-test or Chi² test as appropriate. 95% CI for difference of means is presented for t-test results. A p-value equal to or below 0.05 was regarded as statistically significant.

6.4.4 Power of study

The analyses presented in this chapter are secondary to those presented in Chapter 5, and the number of subjects included is based upon the requirements of those primary analyses.

By post-hoc calculation, it was determined that a sample size of 50 subjects in each group (as for the joint preschool/school-age analyses) would be sufficient to detect a difference of 0.5 SD in first breath SnIII\text{corr}, SnIII\text{SF6-He,corr}, S\text{cond,corr}, and S\text{acin,corr} between CF and control groups. A sample size of 22 subjects in each group (as for separate preschool/school-age analyses) would be sufficient to detect a difference of one SD in these parameters between CF and control groups.
6.5 Results

6.5.1 Subjects

All 115 children in whom LCI and spirometry results were successfully obtained were entered into this analysis. This population has already been described in Table 4.5. $S_{\text{cond}}$ and $S_{\text{acin}}$ could be calculated in 47/60 preschool children, and in 47/55 school-age children (Table 6.1). There were no significant differences in success rates by age-group or diagnosis.

**Table 6.1 Children completing MBW in whom $S_{\text{cond}}$ and $S_{\text{acin}}$ could be calculated**

<table>
<thead>
<tr>
<th></th>
<th>Preschool children</th>
<th>School-age children</th>
<th>Mean difference (95% CI) preschool-school-age</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>26/30 (87%)</td>
<td>19/22 (86%)</td>
<td>1% (-19, 21)</td>
</tr>
<tr>
<td>Control</td>
<td>21/30 (70%)</td>
<td>28/33 (85%)</td>
<td>15% (-35, 6)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>17% (-2, 38)</td>
<td>2% (-19, 21)</td>
<td></td>
</tr>
</tbody>
</table>

Legend: results presented as n (%). Differences calculated from %. There were no significant differences in success by age-group or diagnosis.

Inadequate breath volume was by far the commonest reason for excluding breaths. For the 13 preschool children in whom results could not be calculated, one run was excluded in seven children, two runs in two children, and all three runs in four children. For the eight school-age children in whom results could not be calculated, one run was excluded in one child, two runs in three children, and three runs in four children. For these 42 excluded runs: 11 were excluded because the first breath was of inadequate volume for SnIII calculation; 17 because too many breaths had been excluded for the $S_{\text{cond}}$ regression to be calculated; and 14 because neither the first breath SnIII nor the $S_{\text{cond}}$ regression could be calculated.

For the 94 children in whom $S_{\text{cond}}$ and $S_{\text{acin}}$ could be calculated, children with CF had significantly lower height and weight z-scores than healthy children (Table 6.2).
Table 6.2 Details of final study population, analysed by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>CF (n=45)</th>
<th>Controls (n=49)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.32 (4.05)</td>
<td>8.00 (4.16)</td>
<td>-0.68 (-2.36, 1.01)</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.51 (1.19)</td>
<td>0.39 (0.99)</td>
<td>-0.80 (-1.32, -0.28)**</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>-0.22 (1.02)</td>
<td>0.54 (1.04)</td>
<td>-0.76 (-1.21, -0.31)**</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) *** p<0.001. Difference calculated as CF-control.

6.5.2 Analysis of raw phase III slope parameters

When raw results were analysed, a significant difference between CF and control groups was noted for all four measures (Table 6.3).

Table 6.3 Raw phase III slope parameters, analysed by diagnosis, for all children

<table>
<thead>
<tr>
<th></th>
<th>CF (n=45)</th>
<th>Controls (n=49)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath SnIII (L^{-1})</td>
<td>0.571 (0.330)</td>
<td>0.301 (0.243)</td>
<td>0.270 (0.152, 0.388)***</td>
</tr>
<tr>
<td>$S_{cond}$ (L^{-1})</td>
<td>0.297 (0.175)</td>
<td>0.067 (0.116)</td>
<td>0.230 (0.169, 0.292)***</td>
</tr>
<tr>
<td>$S_{acin}$ (L^{-1})</td>
<td>0.455 (0.319)</td>
<td>0.278 (0.238)</td>
<td>0.178 (0.063, 0.292)**</td>
</tr>
<tr>
<td>SnIII_{SF6-He} (L^{-1})</td>
<td>0.120 (0.115)</td>
<td>0.072 (0.086)</td>
<td>0.047 (0.006, 0.089)*</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) *** p<0.001, **p<0.01, *p<0.05. Difference calculated as CF-control.

In healthy children, there were significant negative relationships between age and first breath SnIII ($r^2=0.33$, p<0.001); age and $S_{cond}$ ($r^2=0.12$, p=0.02); and age and $S_{acin}$ ($r^2=0.27$, p<0.001). The negative relationship between age and SnIII_{SF6-He} was not significant ($r^2=0.07$, p=0.08). These relationships are presented in Figures 6.1 to 6.4.
Figure 6.1 First breath SnIII plotted against age, all children

Legend for Figures 6.1 to 6.4: Control children presented as open circles, and children with CF presented as closed circles. Note the significant negative relationships between: age and first breath SnIII; age and S\textsubscript{cond}; age and S\textsubscript{acin}; and age and SnIII\textsubscript{SFr-He,corr} in healthy children (see text for r\textsuperscript{2} and p-values).

Figure 6.2 S\textsubscript{cond} plotted against age
Figure 6.3 $S_{acim}$ plotted against age

Figure 6.4 first breath $Snill_{SF6:He}$ plotted against age, all children
6.5.3 Age relationships of volume-corrected phase III slope parameters

To facilitate comparison of results between CF and control groups, results for all four outcome variables were transformed as described in Section 6.4.1. The resultant volume-corrected outcome measures were virtually age independent in healthy children (Figures 6.5 to 6.8), with no significant differences noted between preschool and school-age groups (Table 6.4).

Table 6.4 Volume-corrected phase III slope parameters, analysed by age-group, healthy children

<table>
<thead>
<tr>
<th></th>
<th>Preschool (n=21)</th>
<th>School-age (n=28)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath SnIII_{corr}</td>
<td>0.087 (0.393)</td>
<td>0.075 (0.031)</td>
<td>0.012 (-0.009, 0.032)</td>
</tr>
<tr>
<td>S_{cond,corr}</td>
<td>0.021 (0.029)</td>
<td>0.011 (0.020)</td>
<td>0.010 (-0.004, 0.024)</td>
</tr>
<tr>
<td>S_{lacin,corr}</td>
<td>0.079 (0.042)</td>
<td>0.071 (0.031)</td>
<td>0.008 (-0.013, 0.029)</td>
</tr>
<tr>
<td>SnIII_{SF6-He,corr}</td>
<td>0.019 (0.022)</td>
<td>0.023 (0.021)</td>
<td>-0.004 (-0.016, 0.008)</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD). Difference calculated as preschool-school-age.
When all children were analysed together, significant differences in first breath $S_{nIII,corr}$, $S_{cond,corr}$, and $S_{acin,corr}$ were seen for the CF and control groups (Table 6.5). When these analyses were repeated separately for preschool and school-age children, a marked difference in results was noted. Whilst school age children with CF had significantly higher $S_{acin,corr}$ than healthy school-age children (Table 6.6), no difference in $S_{acin,corr}$ was seen in the preschool group (Table 6.7). In both age groups first breath $S_{nIII,corr}$ and $S_{cond,corr}$ were significantly higher in children with CF, whilst there was no difference by diagnosis in $S_{nIII,SF6-He,corr}$.

### Table 6.5 Volume-corrected phase III slope parameters, analysed by diagnosis, for all children

<table>
<thead>
<tr>
<th></th>
<th>CF (n=45)</th>
<th>Controls (n=49)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath $S_{nIII,corr}$</td>
<td>0.162 (0.096)</td>
<td>0.080 (0.035)</td>
<td>0.082 (0.052, 0.113)***</td>
</tr>
<tr>
<td>$S_{cond,corr}$</td>
<td>0.074 (0.036)</td>
<td>0.016 (0.024)</td>
<td>0.059 (0.046, 0.071)***</td>
</tr>
<tr>
<td>$S_{acin,corr}$</td>
<td>0.130 (0.096)</td>
<td>0.074 (0.036)</td>
<td>0.056 (0.025, 0.086)***</td>
</tr>
<tr>
<td>$S_{nIII,SF6-He,corr}$</td>
<td>0.032 (0.030)</td>
<td>0.021 (0.021)</td>
<td>0.010 (-0.001, 0.021)</td>
</tr>
</tbody>
</table>

**Legend:** Group results presented as mean (SD) *** $p<0.001$. Difference calculated as CF-control.
Table 6.6 Volume-corrected phase III slope parameters, analysed by diagnosis, school-age children

<table>
<thead>
<tr>
<th></th>
<th>CF (n=19)</th>
<th>Controls (n=28)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath SnIII corr</td>
<td>0.211 (0.107)</td>
<td>0.075 (0.031)</td>
<td>0.136 (0.084, 0.189)*****</td>
</tr>
<tr>
<td>Scond, corr</td>
<td>0.078 (0.040)</td>
<td>0.011 (0.020)</td>
<td>0.067 (0.046, 0.087)*****</td>
</tr>
<tr>
<td>Sacin, corr</td>
<td>0.177 (0.112)</td>
<td>0.071 (0.031)</td>
<td>0.107 (0.052, 0.162)*****</td>
</tr>
<tr>
<td>SnIII_{SF6-He, corr}</td>
<td>0.037 (0.032)</td>
<td>0.023 (0.021)</td>
<td>0.014 (-0.003, 0.031)</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) *** p<0.001. Difference calculated as CF-control. Note that children with CF have significantly higher first breath SnIII corr, Scond, corr, and Sacin, corr than healthy children.

Table 6.7 Volume-corrected phase III slope parameters, analysed by diagnosis, preschool children

<table>
<thead>
<tr>
<th></th>
<th>CF (n=26)</th>
<th>Controls (n=21)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath SnIII corr</td>
<td>0.126 (0.070)</td>
<td>0.086 (0.039)</td>
<td>0.040 (0.007, 0.072)*</td>
</tr>
<tr>
<td>Scond, corr</td>
<td>0.072 (0.033)</td>
<td>0.021 (0.029)</td>
<td>0.050 (0.032, 0.068)*****</td>
</tr>
<tr>
<td>Sacin, corr</td>
<td>0.095 (0.064)</td>
<td>0.079 (0.042)</td>
<td>0.017 (-0.015, 0.048)</td>
</tr>
<tr>
<td>SnIII_{SF6-He, corr}</td>
<td>0.028 (0.028)</td>
<td>0.019 (0.022)</td>
<td>0.008 (-0.006, 0.023)</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) *** p<0.001, *p<0.05. Difference calculated as CF-control. Note that preschool children with CF have significantly higher first breath SnIII corr and Scond, corr than healthy preschool children, but there is no difference in Scond, corr between the two groups. This contrasts with the results seen in school-age children (presented in Table 6.6).
The age relationships were explored further by scatter-plots. Figure 6.5 presents first breath $S_{III\text{corr}}$ plotted against age, for the CF and control groups. The upper limit of normality for first breath $S_{III\text{corr}}$ was calculated as 0.149. For the children with CF, there was a positive relationship between first breath $S_{III\text{corr}}$ and age ($r^2 = 0.41, p<0.001$). Nineteen of the children with CF (6/26 preschool children, and 13/19 school-age children) had first breath $S_{III\text{corr}}$ above the limit of normality.

**Figure 6.5 Volume-corrected first breath $S_{III}$ plotted against age, all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. The upper limit of normality for first breath $S_{III\text{corr}}$ (broken line) was calculated as 0.149. For the children with CF, there was a positive relationship between first breath $S_{III\text{corr}}$ and age ($r^2 = 0.41, p<0.001$). Nineteen of the children with CF (6/26 preschool children, and 13/19 school-age children) had first breath $S_{III\text{corr}}$ above the limit of normality.
Figure 6.6 presents $S_{\text{cond,corr}}$ plotted against age, for all children. The upper limit of normality was calculated as 0.063. For the children with CF, there was no relationship between $S_{\text{cond,corr}}$ and age ($r^2 = 0.02$, $p=0.3$). Thirty-one of the children with CF (16/26 preschool children, and 15/19 school-age children) had $S_{\text{cond,corr}}$ above the limit of normality. One healthy preschool child had a markedly raised $S_{\text{cond,corr}}$. This is addressed later in this chapter.

**Figure 6.6 Volume-corrected $S_{\text{cond}}$ plotted against age, all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. The upper limit of normality was calculated as 0.063 (broken line). For the children with CF, there was no relationship between $S_{\text{cond,corr}}$ and age ($r^2 = 0.02$, $p<0.3$). Thirty-one of the children with CF (16/26 preschool children, and 15/19 school-age children) had $S_{\text{cond,corr}}$ above the limit of normality.
The relationship between $S_{\text{acin,corr}}$ and age is presented in Figure 6.7. For this index, the upper limit of normality was calculated as 0.145. For the children with CF, there was a positive relationship between $S_{\text{acin,corr}}$ and age ($r^2 = 0.36, p < 0.001$). Only 16 of the children with CF (6/26 preschool children, and 10/19 school-age children) had $S_{\text{acin,corr}}$ above the limit of normality. Most children under the age of 10 years had normal, or near-normal $S_{\text{acin,corr}}$.

Figure 6.7 Volume-corrected $S_{\text{acin}}$ plotted against age, all children

Legend: Control children presented as open circles, and children with CF presented as closed circles. The upper limit of normality was calculated as 0.145 (broken line). For the children with CF, there was a positive relationship between $S_{\text{acin,corr}}$ and age ($r^2 = 0.36, p < 0.001$). Only 16 of the children with CF (6/26 preschool children, and 10/19 school-age children) had $S_{\text{acin,corr}}$ above the limit of normality. Note that most children under the age of 10 years had normal, or near-normal $S_{\text{acin,corr}}$. 

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Figure 6.8 demonstrates the relationship between SnIII$_{SF6-He,corr}$ and age. For this index, there was no significant difference between CF and control groups (Tables 6.5 – 6.7) and no age relationship in children with CF ($r^2 = 0.07$, $p = 0.08$). The upper limit of normality was 0.0622.

**Figure 6.8 Volume-corrected SnIII$_{SF6-He}$ plotted against age, all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. There was no significant difference between CF and control groups and no age relationship in children with CF. The upper limit of normality for SnIII$_{SF6-He,corr}$ was 0.0622 (broken line).
6.5.4 Relationships between phase III slope parameters and LCI

For the purpose of these analyses, the upper limit of normality for LCI is taken as 7.67 (calculated from measurements taken in all healthy children, Table 5.15). As there were no significant differences in $\text{SnIII}_{SFS-H\text{e,corr}}$ between CF and control groups, this measure is not analysed further. The relationship between first breath $\text{SnIII}_{\text{corr}}$ and LCI is presented in Figure 6.9.

**Figure 6.9 Lung Clearance Index plotted against volume-corrected first breath SnIII, all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. There was a positive relationship between the two indices in children with CF ($r^2 = 0.51$, $p<0.001$). Broken lines represent the upper limits of normality for the two indices. Note that more children with CF had abnormal LCI than had abnormal first breath $\text{SnIII}_{\text{corr}}$ (see text).
There was a positive relationship between the two indices in children with CF \( (r^2 = 0.51, p<0.001) \). Note that 21 children with CF had normal first breath SnIII\(_{corr}\) but abnormal LCI, whilst only one child with CF had abnormal first breath SnIII\(_{corr}\) but normal LCI. The relationship between \( S_{\text{cond,corr}} \) and LCI is presented in Figure 6.10.

**Figure 6.10 Lung Clearance Index plotted against volume-corrected \( S_{\text{cond}} \), all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. In children with CF, there was a weak positive relationship between the two indices \( (r^2=0.12, p=0.02) \). Broken lines represent the upper limits of normality for the two indices.

There was a weak positive relationship between the two indices in children with CF \( (r^2=0.12, p=0.02) \). As previously described, the majority of the children with CF had abnormal \( S_{\text{cond,corr}} \) (Figure 6.6). Only nine children with CF had normal \( S_{\text{cond,corr}} \) but abnormal LCI, whilst one child with CF had abnormal \( S_{\text{cond,corr}} \) but normal LCI.
Figure 6.11 presents $S_{a\text{cin,corr}}$ plotted against LCI.

Figure 6.11 Lung Clearance Index plotted against volume-corrected $S_{a\text{cin}}$, all children

![Graph showing relationship between $S_{a\text{cin,corr}}$ and Lung Clearance Index](image)

Legend: Control children presented as open circles, and children with CF presented as closed circles. In children with CF, there was a positive relationship between the two indices ($r^2 = 0.46, p<0.001$). Broken lines represent the upper limits of normality for the two indices. Note that more children with CF had abnormal LCI than had abnormal $S_{a\text{cin,corr}}$ (see text).

A positive relationship between the two indices was noted in children with CF ($r^2 = 0.46, p<0.001$). Twenty-four children with CF had normal $S_{a\text{cin,corr}}$ but abnormal LCI, whilst only one had abnormal $S_{a\text{cin,corr}}$ but normal LCI.
6.5.5 Relationships between phase III slope parameters and FEV₁

Figure 6.12 presents the relationship between first breath SnIII_{corr} and FEV₁.

**Figure 6.12** FEV₁ z-score plotted against volume-corrected first breath SnIII, all children

![Graph showing relationship between FEV₁ z-score and first breath SnIII_{corr} for all children.]

Legend: Control children presented as open circles, and children with CF presented as closed circles. In children with CF, there was a weak negative relationship between the two indices ($r^2 = 0.29$, $p<0.001$). Broken lines represent the limits of normality. Note that more children with CF had abnormal first breath SnIII_{corr} than had abnormal FEV₁ z-score (see text).

In children with CF, there was a weak negative relationship between the two indices ($r^2 = 0.29$, $p<0.001$). Seven children with CF had normal FEV₁ z-score but abnormal first breath SnIII_{corr}, whilst only two had had abnormal FEV₁ z-score but normal first breath SnIII_{corr}.
The relationship between $S_{\text{cond,corr}}$ and FEV$_1$ z-score is presented in Figure 6.13. There was no significant relationship between the two indices in children with CF ($r^2=0.06$, $p=0.14$).

**Figure 6.13 FEV$_1$ z-score plotted against volume-corrected $S_{\text{cond,corr}}$, all children**

Legend: Control children presented as open circles, and children with CF presented as closed circles. There was no significant relationship between the two indices in children with CF. Broken lines represent the limits of normality for the two indices. Note that more children with CF had abnormal $S_{\text{cond,corr}}$ than had abnormal FEV$_1$ z-score (see text).

Nineteen children with CF had normal FEV$_1$ z-score but abnormal $S_{\text{cond,corr}}$, whilst only two had abnormal FEV$_1$ z-score but normal $S_{\text{cond,corr}}$. 
The relationship between $S_{\text{acin,corr}}$ and FEV$_1$ z-score is presented in Figure 6.14. In children with CF, there was a weak negative relationship between the two variables ($r^2=0.22$, $p=0.002$).

**Figure 6.14** FEV$_1$ z-score plotted against volume-corrected $S_{\text{acin}}$, all children

Legend: Control children presented as open circles, and children with CF presented as closed circles. There was a weak negative relationship between the two indices in children with CF. Broken lines represent the limits of normality for the two indices. In contrast to the $S_{\text{cond,corr}}$ and FEV$_1$ relationship, almost as many children with CF had abnormal FEV$_1$ as had abnormal $S_{\text{acin,corr}}$ (see text).

In contrast to the $S_{\text{cond,corr}}$ and FEV$_1$ relationship, six children with CF had normal FEV$_1$ z-score but abnormal $S_{\text{acin,corr}}$, whilst four had abnormal FEV$_1$ z-score but normal $S_{\text{acin,corr}}$. All four of these children had abnormal $S_{\text{cond,corr}}$ and abnormal LCI.
6.5.6 Relationships between phase III slope parameters and clinical information in children with CF

Volume-corrected phase III slope outcome measures compared by presence of *Pseudomonas aeruginosa* infection are presented in Table 6.8. Children who were infected with *Pseudomonas aeruginosa* had significantly higher first breath $S_{\text{III,corr}}$ and $S_{\text{acin,corr}}$, but there was no difference in $S_{\text{cond,corr}}$.

Table 6.8 Volume-corrected phase III slope parameters, analysed by presence of *Pseudomonas aeruginosa* infection, all children with CF

<table>
<thead>
<tr>
<th></th>
<th>Pseudomonas positive (n=21)</th>
<th>Pseudomonas negative (n=24)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S_{\text{III,corr}}$</td>
<td>0.206 (0.112)</td>
<td>0.124 (0.059)</td>
<td>0.082 (0.030, 0.135)**</td>
</tr>
<tr>
<td>$S_{\text{cond,corr}}$</td>
<td>0.0836 (0.0343)</td>
<td>0.0656 (0.0352)</td>
<td>0.0180 (-0.0030, 0.0389)</td>
</tr>
<tr>
<td>$S_{\text{acin,corr}}$</td>
<td>0.171 (0.116)</td>
<td>0.094 (0.055)</td>
<td>0.077 (0.023, 0.130)**</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) ** $p<0.01$. Difference calculated as Pseudomonas positive – Pseudomonas negative.

These relationships were examined further by regression modelling. The presence of *Pseudomonas aeruginosa* infection explained 17% of the variability of first breath $S_{\text{III,corr}}$ ($p=0.003$), 14% of the variability of $S_{\text{acin,corr}}$ ($p=0.006$), but only 4% of the variability of $S_{\text{cond,corr}}$ ($p=0.09$). After correction for age, the presence of *Pseudomonas aeruginosa* infection was still a significant predictor of first breath $S_{\text{III,corr}}$ ($p=0.005$) and $S_{\text{acin,corr}}$ ($p=0.012$).

Results were also compared by presence of cough in the week prior to testing (Table 6.9). All three outcome measures were significantly higher in children who reported cough. By regression analysis, presence of cough only explained 9% of the variability of first breath $S_{\text{III,corr}}$ ($p=0.02$), 7% of the variability of $S_{\text{cond,corr}}$ ($p=0.05$), and 7% of the variability of $S_{\text{acin,corr}}$ ($p=0.05$). After correction for age, presence of cough was not a significant predictor of any of these variables.
### Table 6.9 Volume-corrected phase III slope parameters, analysed by presence of cough, all children with CF

<table>
<thead>
<tr>
<th></th>
<th>Cough (n=25)</th>
<th>No cough (n=20)</th>
<th>Mean difference (95% CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S_{n II corr}</td>
<td>0.191 (0.104)</td>
<td>0.126 (0.072)</td>
<td>0.065 (0.011, 0.119)*</td>
</tr>
<tr>
<td>S_{cond corr}</td>
<td>0.0833 (0.0348)</td>
<td>0.0624 (0.338)</td>
<td>0.0209 (0.0001, 0.0416)*</td>
</tr>
<tr>
<td>S_{acin corr}</td>
<td>0.155 (0.108)</td>
<td>0.098 (0.069)</td>
<td>0.057 (0.003, 0.110)*</td>
</tr>
</tbody>
</table>

Legend: Group results presented as mean (SD) * p<0.05. Difference calculated as cough – no cough.

For the 34 children for whom Chrispin-Norman scores were available, positive relationships were seen between the Chrispin-Norman score and first breath S_{n II corr} ($r^2=0.39$, p<0.001) and $S_{acin corr}$ ($r^2=0.40$, p<0.001). There was no relationship between the Chrispin-Norman score and $S_{cond corr}$ ($r^2=0.01$, p=0.6).
6.6 Discussion

6.6.1 Summary

The primary aims of this study were to compare parameters derived from phase III slope analysis with age in both preschool and school-age children. Volume-correction of phase III slope parameters created indices that were independent of age, allowing these relationships to be studied in more detail. First breath $S_{n, corr}$, $S_{cond, corr}$, and $S_{acin, corr}$ were all significantly higher in children with CF than in healthy children. Thirty-one of 45 children with CF had $S_{cond, corr}$ above the normal range, and no age relationship was seen. In marked contrast, only 16 children with CF had raised $S_{acin, corr}$, with higher values seen in older children. One explanation for these results is that lung disease in children with CF commences in the conducting airways and progresses distally. This and alternative explanations are discussed in detail in the next chapter.

A brief discussion of the main results follows. Detailed discussion of the physiological and pathophysiological implications of these results is presented in Chapter 7.

6.6.2 Volume correction of phase III slope parameters

The majority of lung function measures are dependent on age and body size in healthy subjects. Whilst the negative relationships between $S_{n, III}$ and age, and $S_{n, III}$ and lung volume, have been described previously, they have not been questioned. The rationale for volume correction of phase III indices is based upon the analyses presented in Chapter 4, and upon the observation that other measures of ventilation inhomogeneity, such as the LCI, are virtually age-independent, at least in the 2 to 16 year age range. Possible explanations for how volume correction eliminates age relationships for phase III parameters are presented in the next chapter. For now, it is noted that volume correction allows relationships with age and with other lung function parameters to be studied in children with CF, without the need for regression equations and calculation of z-scores. This has not been possible previously.
6.6.3 Limitations of this study

For analyses presented in this chapter, data collected in preschool children and data collected in school-age children are analysed together. Differences in recruitment methods and data collection methods between the two groups weaken this analysis. This is discussed further in Chapter 7.

In addition, there were three important weaknesses related to secondary outcome measures, which should be noted now. Both relate to the initial study design, which considered investigations in school-age children and those in preschool children as two separate studies.

First, although measurement of FEV₁ utilised identical equipment and similar measurement and analysis protocols, conversion of the raw FEV₁ data into z-scores required separate equations for the preschool and school-age groups. This has already been discussed in more detail in Chapter 5.

Second, the definition of chronic Pseudomonas aeruginosa infection in school-age children is slightly different from the definition of current Pseudomonas aeruginosa infection in preschool children. In the former case, chronic infection was defined as “at least three positive cultures within the previous six months”. In the latter, one positive culture within the previous three was defined as current infection. Also, for ten of these preschool children, CF care was delivered at one of the other centres participating in the London CF Collaboration, and sample collection and culture techniques may differ from those employed at Great Ormond Street Hospital.

Third, chest radiographs were not performed as part of this study. All children who attended Great Ormond Street Hospital for their clinical care had chest radiographs performed approximately once yearly for clinical indications. The radiograph closest in time to the lung function study (either before or after) was used for this secondary analysis.

6.6.4 Relationships between phase III slope parameters, LCI, and FEV₁

Of the phase III slope parameters studied here, the first breath S₁₁₁corr is a measure of overall ventilation inhomogeneity, whilst Scond,corr and Sacin,corr are measures of ventilation inhomogeneity generated by convective-dependent mechanisms, and by mechanisms dependent on diffusion-convection interaction, respectively. It can
therefore be predicted that the LCI, which is a measure of overall ventilation inhomogeneity, will be closely related to first breath $\text{SnIII}_{\text{corr}}$, and less closely related to $S_{\text{cond,corr}}$ and $S_{\text{acin,corr}}$. The results presented here confirm this prediction. Figure 6.8 demonstrates that almost all children with CF who had abnormal first breath $\text{SnIII}_{\text{corr}}$ also had abnormal LCI, but that the converse was not true, indicating that LCI is the more sensitive of the two indices for detecting abnormal lung function in children with CF.

Figure 6.9 demonstrates that all but eight of the children with CF with a raised LCI, and all but two of those with a LCI greater than 9, also have raised $S_{\text{cond,corr}}$. In contrast (Figure 6.10) the majority of children with raised LCI have normal $S_{\text{acin,corr}}$. This supports the conclusion that CF lung disease primarily results in ventilation inhomogeneity generated in the conducting airways, whilst only a minority of children with CF also have inhomogeneity generated more distally. It should be noted here that $S_{\text{cond,corr}}$ and $S_{\text{acin,corr}}$ are calculated by different means, and it is not possible to estimate overall ventilation inhomogeneity by calculating the sum of the two. Further analysis of these relationships is therefore not possible.

There were only weak relationships between phase III slope parameters and FEV$_1$ z-score. The reasons for this have already been discussed in Chapter 5.

**6.6.5 Relationship between phase III slope parameters and clinical information in children with CF**

It has already been demonstrated that preschool children with CF who are infected with *Pseudomonas aeruginosa* have significantly higher LCI than those who are not infected. Data presented in this chapter demonstrate that both infected and non-infected children have raised $S_{\text{cond}}$, but only infected children have significantly raised $S_{\text{acin}}$. Results presented in Table 6.9 demonstrate that there are small but significant relationships between the presence of cough and raised first breath $\text{SnIII}$, raised $S_{\text{cond}}$, and raised $S_{\text{acin}}$. Weak positive relationships between Chrispin-Norman score and first breath $\text{SnIII}$ and $S_{\text{acin}}$ were noted, whilst there was no relationship between Chrispin-Norman score and $S_{\text{cond}}$. It is also noted that mean Chrispin-Norman score in preschool children with CF was only 6.8, suggesting that this score is not a sensitive marker of mild CF lung disease.
These results are discussed further in the next chapter, but it should be noted again that these are all secondary analyses, for which this study is not powered, and results should be treated with caution.

6.6.6 Conclusion

In conclusion, this study has demonstrated that the $S_{\text{cond}}$ and $S_{\text{acin}}$ analysis is possible in preschool children, and that volume correction facilitates comparison of children with and without lung disease. Most children with CF, even young preschool children, have raised $S_{\text{cond}}$, though raised $S_{\text{acin}}$ is mainly seen in older school-age children.
Chapter 7: Discussion

7.1 Summary of most important results

The hypotheses for this thesis were originally presented in Chapter 1, as primary and preliminary hypotheses:

*Primary hypotheses:*

1. More children with CF will have abnormal washout results than will have abnormal spirometry results.
2. Children with CF with abnormal washout curve results will also have abnormal phase III slope results. These children will have evidence of inhomogeneity arising both from convective flow (i.e. $S_{cond}$), and from the interaction between diffusion and convection (i.e. $S_{acin}$). Both measures of inhomogeneity will be greater in older children.

*Preliminary hypotheses:*

A. Children aged two to five years are unable to meet some of the quality control criteria for spirometry, but alternative criteria may be feasible.

B. In healthy subjects, the LCI is independent of subject age, age group (i.e. preschool or school-age), sex, and body size. In all subjects, the LCI is independent of variations in respiratory rate, tidal volume, and functional residual capacity seen during spontaneous tidal breathing.

C. In healthy subjects, the phase III slope is negatively related to tidal volume, but otherwise independent of subject age, age group, sex, and body size. In all subjects, the phase III slope is negatively related to tidal volume, but is independent of variations in respiratory rate and functional residual capacity seen during spontaneous tidal breathing.

Results have been presented in four chapters. In chapter 3, preliminary hypothesis A was tested. It was demonstrated that 75% of preschool children are able to perform spirometry, but that most cannot meet the quality control criteria recommended for adults, and that some modifications to
these criteria may be appropriate. Related to this, FEV₁ is not an appropriate spirometry outcome measure for preschool children, and an alternative is required.

In chapter 4, preliminary hypotheses B and C were tested. It was demonstrated that almost 80% of preschool children can perform MBW at first attempt. It was also shown that LCI is largely independent of subject characteristics and breathing pattern during spontaneous breathing in healthy children; that there is a hyperbolic relationship between SnIII and the expired volume of that breath, and that SnIII as a product of expired volume is largely independent of subject characteristics. It was also demonstrated that exclusion of small breaths during quality control reduces the variability of the results without introducing bias, and that the plot of SnIII versus turnover is linear between 1.5 and 6.0 turnovers, even in preschool children.

In chapter 5, primary hypothesis 1 was tested. It was demonstrated that more children with CF have abnormal LCI than have abnormal spirometry; that there is a positive relationship between LCI and age; and a negative relationship between LCI and FEV₁. In a secondary analysis, it was demonstrated that preschool children with CF who are infected with *Pseudomonas aeruginosa* have higher LCI than those who are not infected.

In chapter 6, primary hypothesis 2 was tested. It was demonstrated that the Sncond and Snacin analysis is possible in preschool children, and that volume correction facilitates comparison of children of different ages. Most children with CF, even young preschool children, were noted to have raised Sncond, with no age relationship seen. In contrast, only a minority of children with CF had raised Snacin, and nearly all of these children were aged 10 years or older.

Interpretation and discussion is presented within the results chapters. The aim of this final chapter is to draw the information from the four results chapters together, and to discuss the implications of these results with regard to our understanding of the development of lung disease in preschool children with CF.
7.2 Strengths and weaknesses of study design

7.2.1 Overall study design
This study was performed in two stages. In the initial phase, the MBW technique was compared to spirometry in a population of children with CF and in a matched healthy population. In the second phase, a similar cross-sectional study was performed in matched populations of preschool children, with and without CF. The strengths and weaknesses of this design are discussed below.

7.2.2 Local control population
For well-established measures of lung function, reference equations for normal subjects are often available. These equations are derived from populations of healthy children, tested at one or more centres. A scatter of normal values is collated, and the values can be regressed against height, weight, sex, or sometimes a combination of predictor variables. The resultant regression equations can be applied to data obtained in subsequent children, with raw data converted into percent predicted, or z-scores.

It is possible to use these prediction equations as control data for observational studies. In other words, results obtained in children with disease are converted into z-scores, or percent predicted values, for interpretation. In effect, the researcher is using the healthy population from which the reference equations were derived as the control population for the study. The weakness of this method is that results may not be directly comparable, either because of differences in data collection methodology between centres, or because of differences in the test populations (e.g. different ethnic make-up, or differing body composition between countries or between eras). A strength of the current study is that a local, matched control population was employed. This allows direct comparison of lung function results between CF and control groups, and facilitates comparison of the relative sensitivity of different techniques. It is accepted that the original control group was not matched well for ethnicity, and further subjects had to be recruited. It is also true that the relatively small number of control subjects prevents detailed analyses of the effects of ethnicity, sex, maternal smoking history etc. upon lung function results in
this age group. However, the use of a local control group is still preferable to the
use of external controls.
Reference equations for spirometry parameters were available for both school-age
and preschool children, but these were employed to ensure that data collected at
ICH was similar to that previously reported from other centres. Encouragingly,
healthy children tested at ICH had normal z-scores for most parameters tested
(Chapters 3 and 5). Important exceptions were that healthy preschool children
tested at ICH had higher FEV\textsubscript{1} and FVC results than children previously tested in
Oslo\textsuperscript{40} (Table 3.6), with a smaller scatter, and that children tested at ICH had a
larger scatter in MMEF scores than children previously tested in Indianapolis\textsuperscript{24}.
One possible explanation for the discrepancy with the Oslo data is that some of the
FEV\textsubscript{1} and FVC results from Oslo may have been reported from expiratory efforts
that were terminated early. This would result in the Oslo group reporting results for
these parameters that were erroneously low, and with an erroneously large scatter.
An alternative explanation is that the Oslo group included some children with
respiratory disease. The authors of the Oslo study identified a number of children
with a history of respiratory symptoms, but claimed that this subpopulation had
identical spirometry results to those children with no respiratory symptoms. The
author has attempted to contact the Oslo group to discuss their data further, but has
been unable to do so. It is more difficult to explain the discrepancy with the
Indianapolis data. It may be that the high scatter seen at ICH is due to measurement
error at this laboratory, but this is contradicted by the good concordance in FVC and
FEV\textsubscript{1} results obtained in the two centres. On a related point, Table 5.6 presents an
analysis of spirometry z-scores in healthy Swedish children, calculated by Swedish
reference equations of Solymar et al\textsuperscript{121} and also by the equations of Rosenthal et
al\textsuperscript{120} which were employed for the school-age children tested at ICH. It was
demonstrated that results obtained from children tested in Sweden fit the equations
of Rosenthal et al more closely than they fit the Swedish equations. One possible
explanation for this discrepancy is that the Solymar reference equations are now
out-of date (they were published in 1980). Dr Per Gustafsson has been informed of
this finding, and will communicate it to Swedish paediatricians and researchers.
7.2.3 Quality control

There have been few previous reports of spirometry or MBW in the preschool age-group. In common with other lung function tests, results from these two techniques were predicted to be dependent on consistent protocols for data collection and analysis. Although such protocols are available for spirometry in adult subjects\textsuperscript{34,35}, one of the hypotheses of this study was that these quality control criteria would be inappropriate for the preschool age-group. Half of this thesis is therefore devoted to establishing quality control criteria for data collection in preschool children, and to examining the effects of subject and breath characteristics on the chosen outcome measures for both spirometry and MBW. Throughout the study, data collection methodology, data recording, and analysis and interpretation were regularly audited and cross-checked. Although the vast majority of the data were collected by the author with two co-researchers (Cara Oliver and Clare Saunders), other members of the Lung Function Research Team at the Institute of Child Health assisted with this quality control process. The experience and expertise of this team in performing similar quality control for infant lung function testing, and the availability of custom written software for data storage and retrieval, were major strengths of this study.

7.2.4 Selection of population

The primary aim of this study was to compare the relative sensitivity of two different lung function techniques for discriminating between children with CF and healthy children. It was therefore necessary to ensure that CF and healthy populations were well matched for age, sex, and ethnicity, but it was not essential to ensure that the CF sample was representative of the overall clinic population. It is likely that selection bias operated for both the school-age and preschool CF populations. The school-age CF population was recruited opportunistically from out-patient and ward attenders, and it is likely that children who had more severe disease, and who were therefore attending hospital more frequently, were more likely to be approached to participate in this study. For the preschool population, the families of all children with CF aged 2 to 5 years who were attending the CF clinic at Great Ormond Street Hospital were approached to take part in this study. As this
recruitment method did not yield an adequate number of subjects, the remaining
subjects were recruited opportunistically from the other centres participating in the
London CF collaboration. At commencement of this study, the primary aims were
to determine whether preschool children could perform these lung function tests,
and to compare the results obtained by different techniques. The study was more
successful in meeting the original aims than expected, and in retrospect it could be
argued that lack of rigour in subject recruitment has resulted in a lost opportunity to
answer further questions. Were the study to be designed again, an attempt should
have been made to recruit a representative sample of school age children with CF
from the GOS clinic. This would have allowed the results to be extrapolated to the
whole clinic population, and would also have allowed a comparison of outcomes
between the GOS clinic and the CF clinic in Gothenburg, Sweden.
Healthy school-age children were recruited largely as friends of children with CF.
Healthy preschool children were recruited largely from local schools and
playgroups. In all cases children (or their parents) were asked a series of health
questions to ensure that inclusion criteria were met. There is ongoing controversy as
to how control populations should be defined. In this study, children who had been
diagnosed with asthma or were on anti-asthma medication; children with cardiac,
neuromuscular or skeletal disease; children who were born small for gestational age
or preterm; children who had previously been hospitalised for a respiratory
condition; and children who had frequent respiratory symptoms over the previous
12 months were excluded from the control group. Children who had previously had
respiratory symptoms but had now outgrown these were not excluded. The rationale
for this is that respiratory symptoms are very common in infancy and early
preschool years, and that children with such a history but no ongoing symptoms
should still be considered part of the healthy population.
On a related point, it would have been preferable to fully record how many healthy
children were approached to participate in the study, how many responded, and how
the children included in the study compared to those who did not respond or refused
consent. This approach would have been consistent with recommendations for
reporting randomised controlled trials\textsuperscript{125}. Unfortunately, the hospital ethics
committee would not allow recording of any data on children whose parents or
guardians had not expressly consented to be included in the study. Instead, the recruitment method chosen for the study was to ask teachers to distribute recruitment letters and to await responses from parents. Although the number of recruitment letters sent to schools was recorded, there was no record of how many of these letters were actually distributed to families. In retrospect, this information should have been recorded and analysed.

7.2.5 Two studies rather than one

One clear weakness of this study design is that it is not possible to directly compare results between school-age and preschool children with CF. There are two reasons for this.

First, the data collection methodology for the two groups differs slightly, with the most important discrepancy being the subject-apparatus interface. In preschool children, a facemask apparatus with a putty seal was employed. School-age children wore nose-clips and breathed through a mouthpiece. The facemask system has a larger dead-space, and also allows the subject to breathe nasally. The effect of subject-apparatus interface upon MBW parameters has never been studied (though such a study is planned for the near future). It is possible that the discrepancy in LCI between healthy preschool and school-age children (Figure 4.4, Table 4.8) is related to the interface, or to the method used to correct LCI for apparatus dead-space.

Second, the method of recruitment for the preschool and school-age subjects differs, with school-age children being drawn exclusively from the clinic at Great Ormond Street, and preschool children drawn from all five tertiary CF centres in London. The analysis of school-age children was initially intended as a pilot study, with the primary objective of establishing the MBW methodology (Section 1.10). Comparison of results between preschool and school-age children was not a primary objective of this study. When data collection in school-age children first commenced (September 2000), the findings of Gustafsson et al. had not been fully analysed, let alone published. The finding that nearly all school-age children with CF have abnormal LCI, even when they have normal spirometry results, was unexpected. If this information had been available at the time the study was...
designed, then greater care would have been taken over recruitment methods for the school-age and preschool arms.

7.2.6 *Inclusion of body plethysmography in the original protocol.*

When this preschool study was first designed, the intention was to compare results obtained from MBW, body plethysmography, and spirometry. On interim analysis it became clear that $sR_{aw}$ results (from body plethysmography) obtained in healthy preschool children in the author's laboratory were not identical to those reported from other centres. A number of potential methodological reasons for this discrepancy were identified, which require investigation. Results from body plethysmography were therefore excluded from this thesis. However, it should be noted that all preschool children in this study performed MBW, body plethysmography, and spirometry, in that order, and were not considered to have completed the study protocol unless results were obtained from all three tests. This complicates comparison of success rates for the two methods reported in this thesis.

7.2.7 *Measures of inflammation and infection in preschool children with CF*

As described in Section 1.10, the ideal methodology for evaluating a lung function measure would be to compare it against a 'gold standard' method for the presence or absence of lung disease. For most lung diseases, and specifically for CF, no such gold standard exists.

Lung disease in CF is a progressive disease, caused by bacterial infection and an associated intense neutrophilic inflammatory response. This cycle of infection and inflammation results in excessive mucus production and airway narrowing. Products of neutrophils, such as neutrophil elastase\(^\text{126}\) account for much of the subsequent parenchymal destruction. It has been demonstrated that infants with CF have evidence of inflammation and infection in their lungs, even when they are asymptomatic, and that this inflammation is reduced by antibiotic treatment\(^\text{127}\).

Studies in infants, comparing presence of inflammation with lung function changes, have produced conflicting results. Dakin et al\(^\text{8}\) demonstrated significant relationships between specific respiratory system compliance and pathogen load, and with the number of neutrophils in BAL fluid. Nixon et al\(^\text{128}\) were unable to demonstrate a relationship between inflammation and reduced lung function (as
measured by the raised volume rapid thoracoabdominal compression technique [RVRTC]).

The aim of this study was to compare the sensitivity of different lung function techniques. Ideally, measures of inflammation and infection should have been obtained at the same time as the lung function measures and used to inform this analysis. This was not possible for practical reasons. For all the infant studies described above, inflammatory markers were measured from BAL. This is an invasive technique, which requires general anaesthesia or heavy sedation in young children. None of the CF centres involved in this study perform routine BAL as part of the clinical care of young children with CF, and the author did not believe that this procedure could be justified for the current study. This decision may be reviewed for future studies of MBW in young children.

Alternatively, less invasive measures of lung inflammation have been described recently. The three techniques generating the most interest are analysis of induced sputum, analysis of exhaled breath condensate (EBC), and measurement of exhaled nitric oxide (eNO). For all three of these techniques, methodology for data collection and interpretation have not yet been standardised for adults and older children. Modifications that allow EBC and eNO to be collected in infants have recently been described but this work is still in its infancy. It is not yet possible to employ these techniques in preschool children.

Information on chronic bacterial infection was available from sputum and cough swab cultures taken at the child's local hospital. These results suggested that preschool children infected with Pseudomonas aeruginosa had higher LCI than those who were not infected with this organism. However, culture from cough swab in young children may underestimate the presence of chronic infection when compared with culture from BAL.

7.2.8 Measures of structural change in preschool children with CF

High resolution computed tomograph (HRCT) has been proposed as a method for detection of early lung disease in CF. HRCT scans can be scored for presence of bronchiectasis, airway plugging, and small airways disease (detected by differences in signal density between corresponding inspiratory and expiratory images). Studies
using HRCT scoring have demonstrated that structural changes are already present in young children with CF and that these structural changes may be present in children with normal plain chest radiographs and normal spirometry results.

Ideally, the lung function results presented in this thesis should have been compared with scores obtained from inspiratory and expiratory HRCT scans. However, as for BAL, HRCT studies are not part of routine clinical care of children with CF attending the five CF centres in London, and the radiation exposure and expense of HRCT precluded these investigations being performed solely for comparison with lung function results.

Plain radiographs were performed regularly in the children studied here. Radiographs taken in children seen in the clinic at Great Ormond Street Hospital were scored according to the Chrispin-Norman scoring system, and these results were related to spirometry and MBW results. Weak correlations were seen.

7.2.9 Other measures of clinical status in preschool children with CF

Clinical assessments were made by the child’s consultant prior to lung function testing, and also by the author on the day of testing. Some of the questions asked were for the purpose of future analyses. Those data that were analysed here are discussed below:

- **Mode of presentation.** There was no significant relationship between mode of presentation (i.e. with respiratory symptoms or gastrointestinal symptoms) and lung function results. This finding echoes that of Ranganathan in his previous study of infants with CF from the London Collaborative study.

- **Maternal smoking.** There was no significant difference in lung function results between children of mothers who smoked, and those who did not.

- **Presence of cough.** Preschool children with cough had worse spirometry results, and tended to have worse LCI results.

- **Investigator visual analogue score.** There was no significant relationship between VASi and LCI, and only a weak negative relationship between VASi and spirometry parameters.
The mode of presentation was obtained from consultant questionnaire, and was confirmed by direct questioning of the parents. No discrepancies were noted, and this information is likely to be accurate.

The remaining three measures can be criticised. First, all secondary analyses are underpowered, and type two error is possible. History of maternal smoking was derived solely from parental report. It has previously been demonstrated that the prevalence of maternal smoking may be underestimated by this method\textsuperscript{145}, and this may apply to the data presented here. Ideally, salivary cotinine could have been measured from all preschool subjects participating in this study. This was not done because of a belief that the study protocol was already long and complicated, and that addition of a further investigation may have reduced success rates.

Presence of cough was defined by parental report. It has been demonstrated that parents do not describe wheeze in the same way as doctors\textsuperscript{146}, and the definition of asthma from parental reported symptoms alone is discredited. Cough is a more straightforward symptom, and the author has assumed that parents of children with CF define cough in the same way (as each other, and as their doctors would). It is known that children with CF do not quantify cough well\textsuperscript{123}, so for the current study, preschool children with CF were categorised simply by presence or absence of cough, rather than by cough frequency.

Investigator visual analogue scores are the most contentious of the clinical measures presented here. Visual analogue scores are not new, and are employed in a variety of research and clinical settings. However, they have not been validated in this setting, and intra- and inter-observer variability is unknown. All VASi scores were completed by the author, so inter-observer variability is not an issue, though within-observer, between occasion variability may still be a source of bias. Further, they were completed before lung function testing commenced, to minimise bias. However, it can still be argued that these scores are subjective.

In defence of this study, it is argued that there are no validated measures of clinical status for young children with CF. The most widely employed clinical scoring system in CF is the Shwachman-Kulczycki score. This scoring system can be heavily criticised, being complex and time consuming. More importantly, it dates from an earlier era of CF care, and has not been validated for the assessment of
mild lung disease in young children. The only other scoring systems available for
children with CF are designed for estimating prognosis of children with severe
lung disease, so that timing of lung transplantation can be optimised. These scores
would not have been appropriate for the current study.

7.3 Feasibility of spirometry and MBW in preschool children

Success rates for completion of spirometry and MBW in preschool children were
presented in Chapters 3 and 5. These data are combined in Table 7.1.
Younger children (those aged less than 4 years) were significantly less likely to
successfully produce FEV₀.₅ results than older children. In comparison, there was
no significant difference in success by age for LCI. These results compare
favourably with success rates reported in other studies of lung function in preschool
children 24;36;38,40;84,91.

Table 7.1 Number of preschool children successfully completing LCI
or FEV₀.₅ at first visit

<table>
<thead>
<tr>
<th>Age Group</th>
<th>All Children (n=77)</th>
<th>Cystic Fibrosis (n=40)</th>
<th>Healthy Controls (n=37)</th>
<th>2-&lt;3 years (n=6)</th>
<th>3-&lt;4 years (n=32)</th>
<th>4-&lt;5 years (n=24)</th>
<th>5-&lt;6 years (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>61 (79%)</td>
<td>30 (75%)</td>
<td>31 (84%)</td>
<td>3 (50%)</td>
<td>25 (78%)</td>
<td>20 (83%)</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>FEV₀.₅</td>
<td>59 (77%)</td>
<td>30 (75%)</td>
<td>29 (78%)</td>
<td>2 (33%)</td>
<td>23 (72%)</td>
<td>21 (88%)</td>
<td>13 (87%)</td>
</tr>
</tbody>
</table>

Legend: Results are presented as number (%). Success rates for MMEF were
identical to those for FEV₀.₅. Data are from Tables 3.2 and 4.3 (two subjects
included in Table 3.2 did not attempt MBW and are excluded here).
7.4 Quality control for spirometry in preschool children

7.4.1 Suggestions for spirometry quality control criteria in preschool subjects

There is now no doubt that spirometry in preschool children is feasible. Although some failure is inevitable, results can be obtained in 70 to 80% of these subjects. It remains to be proven whether measures obtained at this age are sensitive enough to influence clinical or research practice, but before these questions can be tested, it is essential that standards for quality control in the preschool age group are established. This process has recently been completed for infant lung function testing, and the European Respiratory Society and the American Thoracic Society have recently initiated a similar process for preschool lung function standardisation. Ideally, recommendations published by such working parties should be evidence based, but, as described in this thesis, such evidence is scarce in the preschool age group.

Data presented in Chapter 3 of this thesis may contribute to this standardisation effort for spirometry. At least, these data demonstrate that preschool children are unable to meet many of the quality control criteria stipulated for adult subjects. Based on the results presented in Chapter 3, the author is able to make the following suggestions:

a) All curves must be visually inspected, but this process could be facilitated by modifications to software
b) Start of test can be quantitatively assessed as in adults, but results greater than 80mL for VBE or 12.5% for VBE/FVC should be indications for visual re-inspection of the flow-volume trace, rather than automatic exclusion
c) FEV₁ should only be reported if FET for that expiration is greater than t
d) In all preschool children both FEV₀.₇₅ and FEV₀.₅ should be reported in addition to FEV₁
e) Repeatability can be assessed as for adults, but criteria of 100mL or 10% of best effort for ΔFVC and ΔFEV₁ may be more appropriate than the criteria applied to adults.
In addition to these recommendations, users should be cautioned that current spirometry software may result in erroneous reporting of FET and timed expired volumes. The author and his supervisor have contacted manufacturers with requests to amend software to allow quantitative assessment of end of test, and to allow recording and re-analysis of multiple manoeuvres, including those initially assessed as inadequate or borderline. These software amendments should allow data to be excluded from reports or summaries, whilst still being retained on databases to allow future re-analysis.

Before incorporating such recommendations into guidelines it will be necessary for other groups to assess these criteria in their own populations. Given the portability of spirometry equipment, these criteria should ideally be tested in both specialised laboratories and in general clinics or health centres. Accurate application and interpretation of spirometric assessment in the preschool age group has considerable potential benefit, both for clinical practice and research. This potential benefit justifies the effort required for standardisation.

7.4.2 Which timed volume, and why?

One of the key points of the above recommendations is that FEV₁ may not be a suitable spirometry outcome measure in preschool age-group. Table 3.5 presents the median (IQR) FEV₁/FVC ratio in the preschool population as 94% (91, 98%), with no difference between the CF and control populations. The FEV₀.₅/FVC ratio for the whole population was 81% (72, 87%), with a trend to lower results in children with CF compared with healthy children. The rationale for reporting FEV₁ in the adult population is that in healthy adults the FEV₁/FVC ratio is about 70%. In adults, therefore, the FEV₁ is completed relatively early in expiration, and is not affected by failure to maintain flow limitation at the end of expiration. Furthermore, moderate airway obstruction is predicted to cause prolongation of forced expiration, with reduced expiratory flow in mid-expiration. In adults, reduced mid-expiratory flow will reduce the FEV₁/FVC ratio further, meaning that both the FEV₁, and the FEV₁/FVC ratio, are more sensitive markers of obstructive lung disease than FVC alone. In preschool children, FEV₁ is very similar to FVC. In addition, respiratory muscle strength, essential for maintaining flow limitation at low lung volumes, is
less in young children than in adults. It can therefore be predicted that FEV₁ will be more variable than shorter timed expired volumes in this age group, and also that this index will be less able to discriminate subjects with airways disease than healthy subjects.

The observation that preschool children empty their lungs more rapidly than older children and adults is not unexpected. It has long been known that infants undergoing the RVRTC manoeuvre complete maximal forced expiration in less than one second. This phenomenon is attributed to large airway calibre relative to total lung volume in infants and younger children when compared to older children and adults. As a consequence, FEV₀.₄ or FEV₀.₅ are the preferred timed expired volumes reported from RVRTC in infants. On this point, a note of caution is appropriate. Many infant lung function laboratories in North America have adopted FEV₀.₅ as the preferred outcome measure from RVRTC. However, recent data by Ranganathan et al. suggest that FEV₀.₄ is the more discriminatory of the two measures. Some infant lung function centres are still recording and reporting both these timed volumes. Those who are not may subsequently discover that they are not able to fully interpret their data. For preschool children it can be concluded from the data presented in this thesis that either FEV₀.₇₅ or FEV₀.₅ would be suitable spirometry outcome measures. If the experience from infant lung function testing is a precedent, then it would be prudent for all preschool laboratories to record and store both these variables from all manoeuvres, at least until international standards are published.

7.5 Quality control and variability for MBW indices

7.5.1 How many MBW runs are needed?

Results presented in Figure 4.2 and Table 4.6 demonstrate that LCI calculated as a mean of two runs is similar to that calculated as a mean of three runs, with no difference in population mean LCI, and 95% limits of agreement of -0.54, 0.49. In Figure 4.3 and Table 4.7 it is demonstrated that LCI obtained from the first run only is similar to that calculated as the mean of three runs, with a mean difference of only 0.1, and 95% limits of agreement of -1.22, 1.01. Mean differences between CF
and control populations are far larger than these limits of agreement (being 5.08 in school-age children, and 2.72 in preschool children). It can be convincingly argued that calculating LCI from two, or even one MBW run will not reduce the ability of LCI to discriminate between CF and control populations. With such narrow limits of agreement, it is also likely that most children with CF who were identified as having abnormal LCI from the mean result of three runs, would have been labelled identically by LCI calculated as the mean of two runs. This analysis is beyond the scope of this thesis, but should be a priority for the future, as ability to report LCI from one or two MBW runs will reduce the duration and cost of the investigation.

Similar analyses could not be performed for S\textsubscript{cond} and S\textsubscript{acin}, as these indices were calculated from an aggregated plot of SnIII versus TO, taken from all three MBW runs. This method of analysis was selected following preliminary work performed in our collaborating laboratory in Gothenburg\textsuperscript{124}. It should be noted however that the best means of calculating S\textsubscript{cond} and S\textsubscript{acin} in spontaneously breathing children has not yet been determined (see below), and it may in future be possible to establish a method that does not require data from three runs. Chapter 4 presents a detailed analysis of the effect of subject and breath characteristics upon MBW indices.

### 7.5.2 How should LCI be corrected for dead-space?

The LCI, as presented in this thesis, is the simplest, and arguably the crudest method of describing the duration of a MBW run. Essentially, it describes how often the lungs have to recycle resident air in order to wash out a tracer gas. In Section 1.6.2 a number of other indices for describing washout curves were presented. The one that is most closely related to LCI is the mixing ratio (MR). The MR is presented as the ratio between the observed and the predicted number of breaths required to reduce the end tidal tracer concentration to 1/40\textsuperscript{th} of starting value, where the predicted number of breaths is that which would be needed to complete the washout in ideal mixing conditions\textsuperscript{47}. 

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Calculation of ideal number of breaths for Mixing Ratio:

\[
\text{Ideal number of breaths} = \frac{\ln(\text{end gas concentration} \cdot \text{starting gas concentration}^{-1})}{\ln (FRC \cdot (FRC + V_{T} - V_{Daw})^{-1})}
\]

For this calculation it is necessary to include a measure of airway dead-space \(V_{Daw}\), which is customarily estimated as \(2\text{mL} \cdot \text{kg body weight}\).

The ideal number of breaths is calculated from the ratio between the logarithm for the end-tidal SF6 at end-washout and the logarithm for the \(FRC \cdot (FRC + \text{alveolar } V_{T})^{-1}\) ratio. The alveolar tidal volume \(V_{T}\) is calculated as average \(V_{T}\) during the MBW minus the predicted airway dead space \(V_{Daw} = \text{b.w.} \cdot 2 \text{mL}\). In other words, there are only two differences between the MR and the LCI. First, the MR is presented as a ratio of observed versus predicted, whilst the LCI is presented as a raw number. Second, and more importantly, the MR includes a correction for airway dead-space, whilst the LCI does not.

Both LCI and MR are calculated in such a way that within-subject alterations in FRC and \(V_{T}\) are compensated. The LCI is calculated as the ratio of CEV to FRC. Any increase in FRC will increase the volume of tracer gas that needs to be washed out of the lung, and the CEV will also be increased. Any increase in \(V_{T}\) will reduce the number of breaths required for the subject to reach the end-point of the washout. However, as these breaths will be of larger volume, the CEV should not be affected. Apparatus dead-space is subtracted from any calculation of CEV, but airway dead-space is not. The mixing ratio (MR) is calculated as a ratio between the ‘actual’ and ‘ideal’ number of breaths taken to reach the end of the washout. The ideal number of breaths is calculated at the end of the washout, using a formula that takes both the size of FRC and \(V_{T}\) measured during that washout into consideration. From the formula above, it can be seen that as \(V_{T}\) increases, and as the FRC decreases, the calculated ideal number of breaths will decrease.
Edelman has argued that in a uniformly ventilated lung, a decrease in FRC (with no change in \( V_T \)) will produce an increase in LCI. The explanation for this is related to the effect of airway dead-space, and mirrors the argument that a fall in \( V_T \) will result in an increased LCI. Any subject who breathes at a very low tidal volume, such that the majority of ventilation is directed at airway dead-space may, theoretically, produce a high LCI. Likewise, at lower FRC the airway dead-space represents a larger proportion of the lung than at higher FRC. Edelman used these projections to justify the use of MR as an alternative index, as this index includes a correction for airway dead-space, and should therefore be less affected by changes in FRC and \( V_T \). However, recent studies reporting both LCI and MR have noted little difference between the two indices, and data presented in Chapter 4 of this thesis suggests that spontaneous changes in \( V_T \) and FRC during tidal breathing in children do not affect LCI, presumably because these changes in FRC and \( V_T \) were of insufficient magnitude. A preliminary analysis of data collected in school-age children (presented in the Appendix) demonstrated that the relationships between MR and \( V_T \) and FRC were of similar magnitude to those seen with LCI. This suggests that these relationships result from subtle changes in the physiology of gas mixing, rather than just the effect of dead-space correction (see Section 4.7.4).

### 7.5.3 Correction of SnIII for expired volume

Chapter 4 describes the relationships between MBW indices and subject and breath characteristics. In healthy children, LCI was noted to be largely independent of age, body size, and breath characteristics. In contrast, there was a negative relationship between first breath SnIII and age (Figure 4.16). Multivariate analyses suggest that this relationship is responsible for the apparent effect of subject characteristics upon SnIII. When subject characteristics were modelled against SnIII in healthy children, 38% of the variability of SnIII was explained. However, the product of SnIII and expired volume was independent of subject characteristics in healthy children (\( r^2 \) for multivariate model 2%, Figure 4.18).

The hyperbolic relationship between SnIII and expired volume could simply be explained mathematically. However, this requires two assumptions:
• First, the tracer gas concentration at the beginning of the alveolar phase for the first breath of an MBW is always the same for any individual, regardless of expired volume.

• Second, the tracer gas concentration at the end of the alveolar phase for the first breath of an MBW is always the same for any individual, regardless of expired volume.

SIII is calculated as the change in percentage gas concentration as a function of expired volume. The dimensions of SIII are therefore % \cdot \text{L}^{-1}. SIII is normalised by dividing by the mean gas concentration for the phase III interval, giving SnIII, the dimensions of which are \text{L}^{-1} (Figure 7.1). SnIII represents proportional change in gas concentration over the alveolar portion of the expired breath, expressed as a function of expired volume. If the volume of the expired breath is smaller, the volume of the alveolar phase will also be smaller. If it is assumed that the proportional change in gas concentration from beginning to end of the alveolar phase is the same, irrespective of the size of the expired breath, then a hyperbolic relationship between SnIII and expired volume is predicted. By the same logic the product of SnIII and expired volume (defined here as SnIII_{corr}) should be independent of expired volume.

Interestingly, SnIII_{corr} is dimensionless (as is LCI) and, like LCI, SnIII_{corr} is independent of age and body size in healthy children. This lends weight to the argument that the efficiency of gas mixing is constant throughout childhood, at least between the ages of two and 16 years.

The mathematical explanation presented is appealingly simple, but the two underlying assumptions are unlikely to be valid, as the tracer gas concentration at the first breath will be affected by the volume of the preceding inspiration, and by the FRC. For subsequent breaths the breathing pattern up to that point (including pauses and sighs) will affect gas concentrations at any given point in the MBW. It may be that the volume correction method proposed here does not hold for extremes of tidal volume, FRC or respiratory rate, and laboratory studies are necessary to investigate this.
Figure 7.1 Calculation of $S_{IIl_{corr}}$

Legend: $S_{III}$ is calculated as the change in gas concentration as a function of expired volume. The dimensions of $S_{III}$ are therefore $\% \cdot L^{-1}$. $S_{III}$ is normalised by dividing by the mean gas concentration for the phase III interval. The resultant normalised slope ($S_{III}$) has dimensions $L^{-1}$. It can be argued that if the size of the expired breath is smaller, the volume over which the alveolar phase develops will also be smaller, and the value of $S_{III}$ will be greater. Volume correction creates a new index ($S_{III_{corr}}$), which is dimensionless, and which expresses the proportional change in gas concentration from the beginning to end of the alveolar phase, irrespective of tracer gas concentration, and breath volume.

In Chapter 6, volume correction was applied to calculation of $S_{acin}$ and $S_{cond}$. $S_{acin_{corr}}$ was calculated as the product of $S_{acin}$ and first breath $V_{exp}$. $S_{cond_{corr}}$ was calculated as the product of $S_{cond}$ and mean $V_{exp}$. The resultant indices were age-independent in healthy subjects. The method of volume correction requires further investigation, as modifications could be proposed. In particular:

- Should breaths be corrected by the total expired volume (corrected for $V_{D_{app}}$) or by the volume of the alveolar phase only?
• Should $S_{\text{cond}}$ be calculated by the established method, and then volume-corrected, or should all breaths of an MBW be volume-corrected individually, and $S_{\text{cond,corr}}$ calculated from the resultant plot of $S_{\text{nIII,corr}}$ versus TO?

7.6 Clinical implication of results, and future research

7.6.1 Could MBW be used as a clinical tool in young children with CF?

At the beginning of this thesis, the starting aim was presented as "to investigate whether MBW could be employed as a measure of lung function in preschool children with CF". We know that this question cannot be fully answered by a cross-sectional observational study, but how much further have the data presented here taken us? What do we know now, and what remains to be answered? In this section we will discuss what MBW tells us about early lung disease that other lung function tests cannot, and also discuss priorities for future studies.

7.6.2 Why do some children with CF have normal spirometry and abnormal LCI?

An explanation for flow limitation during spirometry was presented in Section 1.4.1, with a description of wave-speed theory. As forced expiration proceeds, choke points multiply and cascade peripherally. Flow measured at the mouth represents the integrated output of all the airways, and therefore demonstrates the stepwise limitation of global sites of flow-limitation as these sites move from one level to the next. As upstream parallel sets of choke points are completed, these choke points 'jump' to the next level. McNamara et al. demonstrated interdependence of regional expiratory flows in excised canine lungs, showing that within a region of lung whose emptying is controlled by a regional choke point, more peripheral upstream airways may become flow limited asynchronously. As some of these airways contribute less to regional flow, other non flow-limited airways compensate by increasing flow. By this mechanism, non-uniformities in flow are masked. It can therefore be predicted that spirometry will not be a sensitive tool for detecting mild, non-uniform airway disease.
In contrast, LCI is predicted to be a sensitive marker of non-uniform airway disease, as it quantifies how quickly the resident gas of the lung is diluted by respiration. An increase in LCI can result from differences in specific ventilation between parallel lung units subtended at single branch points at any point within the conducting airways or within the diffusion-convection front of the lung. These differences in ventilation can be generated by more than one mechanism. Within the conducting zone of the lung they can be generated by differences in airway calibre, either because of inflammation, remodelling, changes in airway tethering secondary to parenchymal damage, or simply secondary to luminal mucus obstruction. In addition, inhomogenous changes in compliance between parallel units can be predicted to increase LCI. Alternatively, increased LCI can result from diffusion-convection interactions between lung units subtended more peripherally. With this mechanism, inhomogeneous ventilation distribution can result simply from differences in size and complexity of parallel units subtended at branch points within the diffusion-convection front. It is clear from the data presented in Chapter 5 that LCI is a more sensitive marker of abnormal lung function in children with CF than parameters derived from spirometry. But what, exactly, is MBW measuring? And do these abnormal results in very young children matter?

7.6.3 The origin and progression of CF lung disease

The relationship between inflammation and infection in young children with CF has been described in Section 7.2.6. The processes involved in the repair of airways affected by inflammation and infection are described as remodelling. The mechanisms involved are complex, and are not well understood in children with CF. In adults, increase in height of the respiratory epithelium has been described in lung explants obtained from CF patients undergoing transplantation. The same study demonstrated a reduction in the cartilaginous component of the outer airway wall. Lobectomy and biopsy specimens have demonstrated subepithelial fibrosis, with smooth muscle thickening and inner airway wall thickening, particularly in the peripheral airways. One study in children, only published as an abstract so far, has demonstrated increased reticular basement membrane thickness which was independent of neutrophil and interleukin-8 concentrations in BAL.
As inflammation and infection continue throughout childhood, increasingly abnormal lung function would be expected in later childhood, when compared with infancy. This does not appear to be the case. A number of studies have investigated lung function in infants with CF. These studies have demonstrated that symptomatic infants with CF have reduced compliance and conductance\textsuperscript{155,156}, increased FRC\textsubscript{He}, reduced V\textsubscript{max} FRC, and a reduced mixing index\textsuperscript{157}. Ranganathan\textsuperscript{144} measured airway function using the RTC and the RVRTC in infants with CF recruited through the LCCFS. The incidence of lung function abnormalities detected in infants with CF was high, with 31% having abnormal FEV\textsubscript{0.5}, and 12% having abnormal MMEF at first investigation. In contrast, in the current study, only 7% of preschool children have abnormal FEV\textsubscript{0.5}, whilst 27% of preschool children have abnormal MMEF (with the specificity of this parameter being only 80%) (Table 5.11). There are slight differences between the recruitment methods for Ranganathan’s study and the current study, but these differences are likely to result in children with more severe disease being over-represented in the preschool group, rather than the opposite.

The question of whether lung function in CF deteriorates or improves through early childhood is best answered by a longitudinal study, tracking changes in spirometric parameters from infancy to school-age. This is being undertaken for Ranganathan’s cohort, but the results are not yet available. In the meantime, Ranganathan noted the relatively poor lung function in infancy, compared with that seen in older children with CF, and proposed a number of possible explanations. In brief, these were:

a) Airways are more susceptible to closure or narrowing in infancy than later in childhood, and abnormalities are therefore more likely to be detected by spirometric techniques in infants

b) The RVRTC technique affects behaviour of airways (perhaps because of the deep inhalation required) so that it measures airway behaviour that conventional spirometry cannot detect

c) The volume parameters reported from infants and older children measure different things (although in the current thesis this problem has been addressed by reporting FEV\textsubscript{0.5} and FEV\textsubscript{0.75} as well as FEV\textsubscript{1})

d) Treatment improves lung function during early childhood
e) Data collected at school-age (or at preschool age) includes that of some subjects who were diagnosed late because they have a less severe CF phenotype.
f) Diminished airway function in infancy reflects poor nutrition as well as inflammatory damage.

All these possible explanations are contentious, with limited data to support them. Some could be tested by longitudinal studies, though the limitations of comparisons or RVRTC parameters with parameters obtained from standard spirometry remain. Data presented in the current thesis suggest an alternative approach.

7.6.4 Progression of CF lung disease, measured by MBW

It has already been described how LCI is more sensitive than spirometry for detecting abnormal lung function in children with CF. In addition, the volume correction of $S_{\text{cond}}$ and $S_{\text{acin}}$ allows us to compute these indices in children, and compare results in children of differing ages. The results presented in Chapter 6 are dramatic, and not entirely expected. Nearly all children with CF, including the youngest, have raised $S_{\text{cond,corr}}$, with no age relationship seen (Figure 6.5). In contrast, most children under the age of 10 years have normal $S_{\text{acin,corr}}$ (Figure 6.6), whilst this index is elevated in the majority of older school-age children. There are a number of possible explanations for these findings:

a) In children aged less than 10 years, CF affects airways within the conducting zone (by any of the mechanisms described in Section 7.7.1) but does not yet affect airways within the area of the diffusion-convection front, or cause structural damage to the acini. In children aged greater than 10 years, more peripheral airways are also involved, and/or parenchymal destruction results in altered relationships between peripheral lung units subtended at branch points within the diffusion-convection front.

b) The nature and location of CF airway disease at different ages is similar, but the location of the diffusion-convection front is more proximal in older children.

c) The ventilation inhomogeneity is the result of differences in compliance between parallel lung units, which are subtended at branch points within the
conducting airways in younger children, but also at branch points within the diffusion-convection front in older children.

These explanations are not mutually exclusive, but it is suggested that the first of them is the most consistent with our current understanding of CF lung disease.

In Section 1.7, previous studies of MBW in children with CF were presented. These studies described how indices of ventilation inhomogeneity are raised in CF subjects compared with healthy subjects, and how ventilation inhomogeneity is correlated to airway resistance and FEV1 in CF subjects. All of these studies were cross-sectional, and provided little information regarding the progression of CF lung disease. One month before this thesis was completed, the first longitudinal study of LCI in children with CF was published. This study, from the University of Berne, Switzerland, described longitudinal measurements of LCI (from nitrogen MBW), FRCpleth, SR_{eff}, and spirometry. Unfortunately the study duration and the number of investigations undertaken by each child are not clearly presented, but at least some of the subjects appear to have undergone repeated measurement from the ages of 6 to 20 years. The most important results from this study are: that LCI increases with age; that progressive rise in LCI is mirrored by progressive fall in FEV1 and MEF50; and that consistently abnormal lung function (defined as a result 2 SD from the mean, in two consecutive years) was seen earlier by LCI than by any other measure.

7.6.5 What about children with more severe lung disease?

The aim of this thesis was to investigate MBW in young children, most of who have relatively mild lung disease. Leading on from this, could MBW replace spirometry as an outcome measure in children (or adults) with more severe disease? The answer to this question is probably no. First, from a practical point, a MBW run in a teenager with CF will take far longer than in a younger subject, partly because of the low respiratory rate in older subjects, and partly because of poor gas mixing in subjects with more severe disease. By comparison, spirometry is a quicker and cheaper test. Second, FEV1 is known to predict survival in subjects with severe disease, not perfectly, but at least better than other objective measures. Extended longitudinal studies are necessary before MBW parameters could be demonstrated.
to provide the same prognostic information. Third, it can be predicted that some children with more severe lung disease may have an artificially low LCI, as some areas of lung that are severely diseased will be completely non-ventilating during tidal breathing, as airways leading to these regions are collapsed or completely obstructed by mucus. In this scenario LCI will only inform upon relatively healthy lung that is ventilated at rest, whilst severely damaged lung will be 'invisible'. In these children spirometry manoeuvres will be a better reflection of overall lung disease. The presence of non-ventilating regions could be investigated by comparing FRC measured from MBW (which will not include non-ventilating regions) with FRC measured plethysmographically (which will include such regions). Discrepancy between the two results will quantify the volume that is not ventilated during tidal breathing. Of course, this information is still useful, both to the physiologist, and to the clinician. However, it may mean that MBW is better suited to be an occasional tool in subjects with severe disease, rather than a primary monitoring tool.

7.6.6 What next?

Although it has now been demonstrated that many young children with CF have abnormal ventilation distribution, this does not necessarily mean that these children have clinically important lung disease, or that those with normal ventilation distribution do not. Comparison with a 'gold standard' is not possible, as there is no non-invasive gold standard for detecting CF lung disease. This question can only be answered by longitudinal studies, intervention studies, and studies relating lung function findings to other measures of lung disease.

a) Longitudinal studies: MBW studies in infants, employing basic washout curve indices, have already been described. In addition, it is possible that the $S_{acin}$ and $S_{cond}$ analysis outlined in this thesis could be employed to interpret data from infant MBW. This provides an unprecedented opportunity to track the early changes of CF lung diseases, avoiding some of the pitfalls of interpretation of spirometry at different ages. It is noted that other lung conditions could be investigated by similar methods. In order to determine whether MBW is a potential marker for early CF
lung disease, it will be necessary to perform MBW in a cohort of infants with CF, ideally recruited from a screened population, and to follow these children up at regular intervals. Ideally, this population should be followed into adulthood, so that survival can be used as the ultimate outcome measure. This would require very long study duration, and analysis will need to consider many confounding factors. In practice therefore this study could be continued into early school-age, with other measures of lung disease (e.g. spirometry results, or HRCT findings) as the outcome measure.

b) Intervention studies: Treatment of CF lung disease with anti-infective or anti-inflammatory agents is known to improve spirometry results and exercise test results in older subjects. At present there are no data on the effect of CF therapy upon MBW indices. The most straightforward intervention study would be to perform MBW before and after intravenous antibiotic therapy in children with acute infective pulmonary exacerbations. Interpreting data from such a study will not be straightforward. First, it will be necessary to quantify between occasion repeatability for MBW indices in this population. Second, it can be predicted that some children with more severe lung disease may have an increase in LCI following treatment, as areas of lung that are completely non-ventilating at the time of an exacerbation may ‘open up’ following treatment. If these areas of lung are more diseased than the rest of the lung, the result will be an increase in LCI. This pitfall can be overcome by restricting the study to subjects with mild disease, or by simultaneously measuring MBW and plethysmographic FRC before and after intervention. Following such a study, the effect of other interventions (e.g. long term anti-inflammatory therapy) could also be tested, with MBW measured alongside other outcomes.

c) Other measures of disease severity: Objective measurements of inflammation and structural lung damage have been discussed above. The majority of CF centres in the UK do not perform either routine BAL or routine HRCT in young children with CF. The cost of these procedures (both financial, and potential adverse effects) is considered to outweigh the potential benefit. However, with
increasing emphasis on detection of mild disease and early infection, this risk-benefit balance is changing, and a number of centres are considering these investigations for routine monitoring. The paediatric CF centre at the Royal Brompton Hospital now perform BAL upon diagnosis for all children with CF, though they do not perform regular repeat BAL, or HRCT, in children who are doing well (personal communication, A Bush, Royal Brompton Hospital, London). The CF clinic in Gothenburg performs HRCT on alternate years in all subjects (personal communication A Lindblad, Gothenburg). CF centres in Australasia have long performed regular surveillance BAL in all young children with CF\textsuperscript{158}, but the centres in Perth and Melbourne are now considering combining this investigation with limited slice HRCT, performed under the same anaesthetic if necessary, as part of a research protocol (personal communication S Ranganathan, Melbourne). The author is communicating with the Perth-Melbourne group about the possibility of introducing MBW into this research protocol.

7.7 Conclusion

The starting aim for this thesis was presented as: “to investigate whether the efficiency of gas mixing, measured by the inert gas multiple-breath washout technique, could be employed as a measure of lung function in preschool children with CF.” This broad aim led to multiple unanswered questions, which were clearly beyond the scope of a single thesis. A small number of specific hypotheses were therefore generated, and these have now been tested. It can now be concluded that preschool children are able to reliably perform both spirometry and MBW, provided that methodology for data collection and interpretation is modified. Furthermore, MBW is able to detect abnormal lung function in children with CF more frequently than spirometry, and modifications to MBW analysis may provide detailed information on the location and distribution of early CF lung disease. All of these findings support the hypothesis that MBW will have value as a clinical measure in this patient group, and suggest that further investigation is justified.
References


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Ref Type: Thesis/Dissertation


Ref Type: Thesis/Dissertation


Ref Type: Abstract


Appendix

Multiple-breath inert gas washout to detect inhomogeneity of ventilation distribution in pre-school children with cystic fibrosis

Appendices

Paul Aurora
University of London, 2005