This thesis has been submitted in fulfilment of the requirements for a postgraduate degree (e.g. PhD, MPhil, DClinPsychol) at the University of Edinburgh. Please note the following terms and conditions of use:

This work is protected by copyright and other intellectual property rights, which are retained by the thesis author, unless otherwise stated.
A copy can be downloaded for personal non-commercial research or study, without prior permission or charge.
This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author.
The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author.
When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given.
The Practical use of the Multiple Breath Washout Test in Children: Biological variability in health and disease.

Dr Helen Sheridan

Doctor of Medicine

University of Edinburgh

2017
Thesis Abstract

The Multiple Breath Washout (MBW) test is increasingly being recognised as a sensitive method of detecting early small airways lung disease. Indices of MBW include lung clearance index (LCI), \( S_{\text{cond}} \) and \( S_{\text{acin}} \). Factors that affect MBW variability have not been fully established. This thesis presents five studies which examine MBW repeatability in children with and without cystic fibrosis (CF) or asthma.

MBW was performed using 0.2% sulphur hexafluoride and the modified Innocor (Innovision). Testing was performed at the Clinical Research Facility of the Royal Hospital for Sick Children in Edinburgh.

(1) MBW and spirometry were performed in children with and without CF (n=20 in each group), initially while sitting and then 30 minutes after assuming a supine posture. LCI was found to significantly rise on lying supine in healthy children (p<0.01) and children with CF (p=0.03).

(2) Thirty two children with CF performed MBW and spirometry on four study visits, results were correlated with findings from high resolution chest computed tomography scans taken on the first visit. LCI showed the strongest correlation with extent and severity of bronchiectasis \( (r=0.66, \ p<0.01 \text{ and } r=0.69, \ p<0.01 \text{ respectively}) \). Variability of LCI was similar to FEV\(_1\) over the 4 visits.
(3) MBW and spirometry of 66 healthy children were compared to 63 children with stable asthma; lung function of asthmatic children was related to symptoms and medication use. LCI was higher in the asthmatic group (6.7 vs 6.3, p<0.01); within the asthmatic group LCI was significantly higher if asthma was less well controlled (p=0.02).

(4) Children with and without asthma (n=21 in each group) performed MBW and spirometry before and after exercise and again after salbutamol, symptom data was collected from asthmatic children. Baseline LCI was abnormal in the asthmatic group who had severe exercise induced bronchospasm during testing.

(5) Asthmatic children admitted to hospital due to exacerbation performed MBW and spirometry. Mean (SD) LCI was abnormally high at 8.5 (1.7) in the nine patients recruited and returned to normal 6.7 (0.6) in three patients who attended follow up.

I have presented evidence that LCI is repeatable and sensitive to early disease in CF and asthma. I have described for the first time the effects of exercise and exacerbation on MBW indices in asthmatic children. MBW is potentially a very useful tool in paediatrics; standardisation of testing and equipment may enable clinical use.
Lay Summary

The Multiple Breath Washout (MBW) test measures lung function and is easy for children of all ages to perform. It is proving to be a sensitive way of detecting early disease in conditions such as cystic fibrosis (CF). Its use had previously been limited as it required bulky and expensive equipment. More portable, less expensive equipment has now been developed and the test is growing in popularity.

Although there is evidence that the test could be very useful in a range of diseases we still have limited information regarding the factors which might affect test results. We need to know what affects MBW results so that changes can be interpreted meaningfully for the patient. In my thesis I have presented five studies in which different variables are examined.

The effect of body position on MBW in children with and without CF was examined. The variability in MBW results over several visits in children with CF was studied and related to disease identified by lung CT. MBW results were compared between healthy children and children with asthma. The effects of exercise in children with and without asthma was investigated. MBW was also performed in children admitted to hospital due to an exacerbation of asthma.

We found that change in posture does alter MBW test results. Results can reliably be repeated in children with CF over several visits. Children with asthma have different results compared to healthy children and asthmatic children with abnormal results are more likely to have exercise induced symptoms. MBW results are abnormal in children suffering an exacerbation of asthma.
Declaration

This thesis has been composed by myself. Either the work has been my own, or, where I have been a member of a research group, I have clearly indicated and made a substantial contribution. The work has not been submitted for any other degree or professional qualification. The included publication included a substantial contribution from myself and permission to include the paper has been granted by The Lancet Respiratory Medicine Journal Editor.
Dedication

This thesis is dedicated to the memory of my mother, Jenny Palmer.
Table of Contents

Thesis Abstract ......................................................................................................................... 2

Lay Summary ............................................................................................................................ 4

Declaration ................................................................................................................................. 5

1 Introduction ............................................................................................................................. 14

1.1 Small Airways Disease ................................................................................................. 14

1.2 Lung Function Testing In Children ............................................................................. 16

1.2.1 Spirometry ............................................................................................................. 18

1.2.2 The Multiple Breath Washout Test ........................................................................ 22

1.2.3 Equipment ............................................................................................................. 24

1.2.4 Performing MBW ............................................................................................... 30

1.2.5 Assays .................................................................................................................. 32

1.2.6 Single Breath Washouts ....................................................................................... 38

1.3 Variability of MBW derived indices in Children .......................................................... 40

1.3.1 Healthy population – Normal values .................................................................... 40

1.3.2 Evidence of repeatability and reproducibility in healthy children ...................... 43

1.3.3 Sources of possible variation: ................................................................................ 44

1.4 MBW in Disease ............................................................................................................. 51

1.4.1 Cystic Fibrosis ...................................................................................................... 52

1.4.2 Asthma .................................................................................................................. 71

1.5 Limitations of MBW ..................................................................................................... 90
1.6 The UK Cystic Fibrosis Gene Therapy Consortium ......................... 92
1.6.1 Previous CFGT Consortium Clinical Research Fellows............... 93
1.7 The Children’s Clinical Research Facility ........................................... 94

2 Projects ........................................................................................................... 95

2.1 Aims and Objectives of Projects .............................................................. 96

2.1.1 The effect of body position on MBW derived indices in healthy
children and children with cystic fibrosis ....................................................... 96

2.1.2 Longitudinal variability of MBW derived indices in children with
cystic fibrosis ..................................................................................................... 96

2.1.3 Assessment of MBW in children with stable asthma........................ 96

2.1.4 The effects of exercise on MBW derived indices in healthy children
and children with asthma .................................................................................. 97

2.1.5 Evaluation of MBW derived indices in asthmatic children during and
after exacerbation requiring oral corticosteroids...........................................(continued on the next page)

3 Common Methods .......................................................................................... 98

3.1 Multiple Breath Washout (MBW) ............................................................... 98

3.1.1 Equipment ............................................................................................. 98

3.1.2 Technique ............................................................................................ 101

3.1.3 Quality Control ..................................................................................... 104

3.1.4 Analysis .................................................................................................. 107

3.2 Spirometry .................................................................................................. 110
Project: The effect of body position on MBW derived indices in healthy children and children with cystic fibrosis

4.1 Introduction .......................................................................................... 112
4.2 Aims ..................................................................................................... 113
4.3 Methods .............................................................................................. 113
  4.3.1 Inclusion/Exclusion Criteria .......................................................... 113
  4.3.2 Recruitment .................................................................................. 114
  4.3.3 The Visit ....................................................................................... 115
  4.3.4 Analysis ....................................................................................... 116
  4.3.5 Ethical Approval .......................................................................... 116
  4.3.6 My Involvement .......................................................................... 116
4.4 Project Results ........................................................................................ 117
  4.4.1 Differences between CF and healthy groups ................................ 117
  4.4.2 Sitting Lung function .................................................................... 117
  4.4.3 Effects of change in posture .......................................................... 119
  4.4.4 Relationship between change in FRC and LCI with postural change 121
  4.4.5 Difference in posture related changes between CF and healthy cohorts .............................................................. 122
  4.4.6 Posture related changes compared to baseline lung function...... 123
4.5 Project Discussion ................................................................................................................. 125

5 Project: Longitudinal variability of MBW derived indices in children with cystic fibrosis .......................................................................................................................... 130

5.1 Introduction .......................................................................................................................... 130

5.2 Aims ....................................................................................................................................... 131

5.3 Methods ................................................................................................................................ 131

5.3.1 Inclusion/Exclusion Criteria ........................................................................................... 131

5.3.2 Recruitment ......................................................................................................................... 132

5.3.3 Study Visits ......................................................................................................................... 132

5.3.4 Analysis ............................................................................................................................... 133

5.3.5 Ethical Approval ................................................................................................................. 134

5.4 Results .................................................................................................................................... 135

5.4.1 Baseline Demographics .................................................................................................... 135

5.4.2 Correlation between FEV1, LCI, S_cond and S_acin ........................................................ 136

5.4.3 Intra-visit variability of MBW derived indices .................................................................... 142

5.4.4 CT scores ............................................................................................................................ 142

5.4.5 Variability of lung function over time ................................................................................ 145

5.5 Discussion ............................................................................................................................ 151

6 Project: Assessment of MBW in children with stable asthma ............................................. 157

6.1 Introduction ............................................................................................................................ 157

6.2 Aims ....................................................................................................................................... 158
6.3 Methods .................................................................................................................. 158

6.3.1 Paediatric Asthma Genes and Environment Study .................................. 158

6.3.2 Recruitment and Inclusion/Exclusion Criteria ...................................... 159

6.3.3 Study Visit ....................................................................................................... 159

6.3.4 Analysis ............................................................................................................. 161

6.3.5 Ethical Approval ........................................................................................... 161

6.4 Results ............................................................................................................. 162

6.4.1 Differences between asthmatic and healthy children ......................... 162

6.4.2 Correlation between lung function indices ........................................... 164

6.4.3 Asthma Severity ............................................................................................ 167

6.4.4 Asthma Control ............................................................................................ 167

6.4.5 Atopy and Allergy ....................................................................................... 168

6.5 Discussion ........................................................................................................... 170

7 Project: The effects of exercise on MBW derived indices in healthy children and children with asthma ....................................................... 175

7.1 Introduction ....................................................................................................... 175

7.2 Aims .................................................................................................................... 176

7.3 Methods: ............................................................................................................ 177

7.3.1 Inclusion/Exclusion Criteria ................................................................. 177

7.3.2 Recruitment .................................................................................................. 178

7.3.3 Study Visit .................................................................................................... 178
7.3.4 Analysis .............................................................................................. 181
7.3.5 Ethical Approval ................................................................................ 181

7.4 Results ....................................................................................................... 182
7.4.1 Demographics .................................................................................... 182
7.4.2 Baseline Lung Function ...................................................................... 185
7.4.3 Changes in lung function following exercise ..................................... 186
7.4.4 Changes in lung function following salbutamol ................................. 191
7.4.5 Symptoms following testing .............................................................. 194

7.5 Discussion ................................................................................................. 195

8 Project: Evaluation of MBW derived indices in asthmatic children during and after exacerbation requiring oral corticosteroids ................................. 200
8.1 Introduction ............................................................................................ 200
8.2 Aims ....................................................................................................... 201

8.3 Methods .................................................................................................... 202
8.3.1 Inclusion/Exclusion Criteria .............................................................. 202
8.3.2 Recruitment ....................................................................................... 202
8.3.3 Study Visits ...................................................................................... 203
8.3.4 Analysis ............................................................................................. 205
8.3.5 Ethical Approval ................................................................................. 205

8.4 Results .................................................................................................... 206
8.4.1 Demographics .................................................................................... 206
1 Introduction

1.1 Small Airways Disease

The lung has a surface area of over 100m$^2$, which is necessary for efficient gas exchange. To achieve this large surface area the human airway branches into 23 generations to supply more than 300 million alveoli. The first 16 airway generations are termed conducting airways and generations 17-23, intra-acinar airways. There are approximately 20-30,000 acini which consist of terminal respiratory bronchioles and alveolar ducts and sacs. Small airways are defined as those with a luminal diameter of less than 2mm, and these correspond to airway generations 8-23 (1).

Figure 1: Illustration of the airway tree including airway generations. Broken lines denote the total airway cross-sectional area which increases dramatically in the lung periphery (1).

Gas flows at high velocity through conducting airways by linear convection. As the airways divide, linear gas flow velocity declines. In the acinus gases mix by diffusion. The entry of the acinus marks the point at which the contribution of diffusion and convection to gas flow are equal, this has been termed the “diffusion convection front”.

Small airways disease is an important component of many serious paediatric respiratory conditions including cystic fibrosis (CF), asthma, bronchopulmonary dysplasia and obliterative bronchiolitis. Conventional lung function testing often has a low sensitivity for detecting early small airway disease in these conditions.
1.2 Lung Function Testing In Children

Lung function testing is an objective tool in the assessment of patients with respiratory disease. In the research setting, lung function testing is used to understand the natural history of disease and lung development and to measure outcome in clinical trials. Clinically, tests are used to assess disease severity, predict prognosis and aid treatment decisions. Testing in children differs from that in adults because of apparatus requirements, compliance with testing and in interpretation of results with growth.

To ensure reproducibility and repeatability of testing the American Thoracic Society (ATS) and European Respiratory Society (ERS) have produced joint guidelines for the standardisation of some lung function tests (including spirometry and the measurement of lung volumes) in adults and children (2-7). The reliability of a physiological test can depend on knowing what factors cause variability in its results. Standardisation of testing ensures that variance is minimised so that testing is comparable and can be used to identify deterioration or improvement in disease.

Most lung function tests including spirometry, the forced oscillation technique, the interrupter technique and plethysmography measure resistance to flow across airways. Resistance is raised in conditions which cause airways obstruction and is largely dependent on obstruction to medium and large airways. Airways of less than 2mm in diameter are estimated to contribute 10% of total airways resistance, despite a cross sectional area many magnitudes greater than that of larger airways (8). Measures of airways resistance may therefore be insensitive in detecting early peripheral airway disease.
Children with lung disease can often have normal conventional lung function. Many children with CF have normal FEV\(_1\) (9, 10); however some of these children will have structural lung damage detected using CT scanning (11, 12). Most children with chronic persistent asthma have normal FEV\(_1\) (13, 14) in whom symptoms correlate poorly with both FEV\(_1\) and FEF\(_{25-75}\) (13, 15). Disease in these groups may be missed because it exists within the small airways, a method of detecting this disease is required to improve understanding of pathogenesis, guide treatment and improve outcomes.
1.2.1 Spirometry

Spirometry is the most commonly used lung function test in paediatric respiratory clinics. Testing has been standardised by an ATS/ERS task force (3). Standardisation ensures that variability of results is minimised, accuracy is improved and abnormalities may be more easily detected.

Indices of lung function derived from spirometry need to be interpreted in the context of patient age, sex, height and race. The Global Lung Function Initiative (GLI) have compiled over 160,000 results from healthy non-smokers in 33 countries to produce continuous prediction equations for spirometric indices throughout growth (16). There are limitations within these equations, there are only 4 ethnic groups represented, Caucasian, African-American, North Asian and South East Asian; individuals who are not represented or who are of mixed race use a composite equation. The use of reference equations is dependent on accurate height and age, Quanjer et al demonstrated bias in results derived from inaccuracies in these variables, which is most pronounced in children (17).

In clinical practice spirometry is commonly presented as a percentage of predicted value. Interpretation of percentage of predicted values is difficult because the variability of spirometry changes with age. The ATS and ERS define the lower limit of normal for spirometry as the 5th centile of normal distribution or a z score of -1.64 (16). The GLI demonstrated variability in spirometry with age and showed that although a FEV\textsubscript{1} of 80% predicted may be abnormal in some children, in younger children it might fall within normal range (>5th centile), see figure 2.
Figure 2: Percentage of healthy male and female non-smokers (n=74,187) in whom FEV$_1$ is <80% predicted in the GLI cohort (16).

Spirometry is volitional and requires a coordinated effort from the patient. The participant must sit and wear a nose clip, inspire to total lung capacity (TLC) and exhale forcefully to residual volume (RV). Exhalation must continue until no longer possible; to terminate the test no change (<0.025L) in volume for ≥1 second must occur. Exhalation must last for ≥3 seconds if the child is <10 years old and ≥6 seconds if older than 10 years. There should be no leak, the patient must exhale continuously and any tests in which a cough occurs in the first second should be discarded. An adequate test requires a minimum of 3 acceptable manoeuvres, the difference between the largest and next largest FVC and FEV$_1$ should be ≤0.150L unless FVC is less than 1.0L in which case the difference should be ≤0.10L. Testing can be repeated if these criteria are not met within the first 3 tests but should generally not exceed 8 manoeuvres (3). Testing is generally felt to be possible from the age of 5-6 years. Spirometry in children as young as 2 years of age can be made possible using incentive spirometry but testing in pre-school children often does not fulfil ATS and ERS quality control criteria (18).
1.2.1.1 Forced Vital Capacity (FVC)

FVC is the maximal volume of air exhaled with maximally forced effort from a maximal inspiration expressed in litres, at body temperature and ambient pressure saturated with water vapour (BTPS). Volumes measured by spirometry are corrected for BTPS to reflect actual lung volumes.

1.2.1.2 Forced expiratory volume in 1 second (FEV₁)

FEV₁ is the maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration, expressed in litres at BTPS.
FEV\textsubscript{1} is a measure of airway obstruction and is widely used in clinical medicine as a diagnostic and monitoring tool in a wide range of respiratory diseases. It is accepted as a biological marker; a decline in FEV\textsubscript{1} is understood to directly indicate worsening disease. FEV\textsubscript{1} is also the most commonly used measure of lung function in research studies.

The largest FVC and FEV\textsubscript{1} are recorded even if they do not come from the same curve.

1.2.1.3  *Mean forced expiratory flow during the middle half of the FVC (FEF\textsubscript{25-75})*

Also known as the maximal mid-expiratory flow, FEF\textsubscript{25-75} is taken from the expiratory flow with the largest sum of FEV\textsubscript{1} and FVC. FEF\textsubscript{25-75} has been shown to be a more sensitive marker of small airway disease than FEV\textsubscript{1} (19). However, it is highly dependent on the validity of the FVC measurement and the level of expiratory effort. The GLI found the coefficient of variation (CV) of FEF\textsubscript{25-75} to be between 20 and 33% in healthy people aged <20 years (16). The very high CV limits its clinical utility; another more repeatable yet sensitive test of small airways disease is required.
1.2.2 The Multiple Breath Washout Test

The Multiple Breath Washout (MBW) test is a non-volitional lung function test that was first described in the 1950s, it is facing renewed interest because of its sensitivity in detecting small airways disease. MBW detects small airways disease by measuring efficiency of gas mixing within the lung by analysing the elimination of an inert gas during tidal breathing. Gas mixing is essential for effective respiration and requires efficient ventilation; inspired gas must reach areas of gas exchange and resident gas must be expired. Small airways disease disturbs ventilation to parts of the lung, due to inflammation, scarring, mucus or changes in airway tone or compliance. Obstructed alveoli can receive ventilation through collateral channels (8) but this consists of existing alveolar (not freshly inspired) air, thus reducing the efficiency of gas mixing.

Various MBW methods exist but all measure the efficiency of clearance of an inert marker gas from the lungs. The gas should be safe to inhale, not participate in gas exchange and not be absorbed or excreted by the body in significant amounts. Gases which have been used include nitrogen (N₂), argon, helium (He), methane and sulphur hexafluoride (SF₆). Different gases will convey information about different zones of the lung. Heavier gases such as SF₆ diffuse less readily than lighter gases and when used in MBW convey information from deep within the acini. Lighter gases such as helium diffuse more quickly and in MBW give information regarding more proximal regions. Comparison of the indices generated by simultaneously measurement of SF₆ and He clearance can confer additional information regarding the site of pathology.
In N₂ washouts resident N₂ is cleared from the lung with 100% oxygen (O₂). Advantages of N₂ washouts include the widespread availability of 100% O₂, lack of a wash-in phase (required with exogenous gases) and complete starting equilibrium of N₂ throughout the lung. N₂ is becoming the most commonly used gas in MBW because of newly developed commercially available washout systems which utilise N₂ and reduce the greenhouse burden of SF₆. A disadvantage of N₂ is that N₂ is excreted by the body in small amounts; the amount is not thought to significantly affect washouts in healthy subjects but in longer tests in diseased lungs may cause an over-estimation of functional residual capacity FRC (20, 21). 100% O₂ can affect breathing pattern in infants although it is not thought to significantly alter breathing pattern in older children (21).

SF₆ has been used in 4% and 0.2% concentrations for MBW. It is completely inert, not being absorbed or excreted by the body. There is some evidence that SF₆ in high concentrations (>56%) can cause temporary reductions in mental and psychomotor performance (22). In addition SF₆ is a potent greenhouse gas and is not internationally approved for use in MBW.
1.2.3 Equipment

The lack of a commercially available validated system for performing MBW has been a factor preventing its widespread use. Commercially available devices have now become available. The device must continuously measure and record inert gas concentrations and synchronise this with respiratory flow to determine inspired and expired inert gas volumes. Several different devices have been and are being used to perform MBW. Figure 4 shows a table detailing the characteristics of some current washout systems.

MBW has traditionally been performed using the respiratory mass spectrometer (RMS). It can measure multiple gases simultaneously, the sample flow is low so only a small sample is required which does not interfere with respiratory volumes and it has a short response time allowing accurate synchronisation with flow (23). However it is a large and highly expensive machine to buy and maintain.

More recently commercially available ultrasonic flowmeters (USFM) have been developed that measure SF$_6$ or N$_2$ indirectly. Indirect measurement is performed either through changes in molecular mass or using simultaneous oxygen and carbon dioxide measurement and Dalton’s law of partial pressures to calculate N$_2$ concentration (24). The mainstream USFM has been validated against the RMS in measuring FRC in infants (25) but a lack of validated BTPS correction algorithms have limited its use beyond this age group. USFM sidestream systems with the use of nafion tubing avoid problems with temperature and humidity and have been validated against the RMS in older children (21, 24), the dead space of sidestream USFMs precludes their use in infants. Indices of phase III slope analysis have not been reported from USFM molecular mass washouts but are possible with indirect nitrogen washouts using simultaneous oxygen and carbon dioxide analysis.
Figure 4: The characteristics of some currently available MBW systems - extracted from the 2013 ERS Consensus Statement on inert gas washout measurement (21).
Research performed at Edinburgh’s Royal Hospital for Sick Children and Western General Hospital has adapted the Innocor (Innovision, Denmark) device, a photoacoustic analyser originally designed to measure cardiac output, to perform MBW (26). The adaptions described below were made in consultation with Innovision and other centres performing MBW.

Figure 5: Innocor with adapted patient interface

The patient interface (or rebreathing valve unit) supplied with the Innocor is replaced (see figure 6). A mouthpiece fitted with a flowmeter and gas sampling port is connected to a detachable flow past tube which is used to supply tracer gas during the wash-in and is then removed at the start of washout. Pre sampling capillary equipment dead space is 36mls; ATS/ERS guidelines recommend a maximum apparatus dead space of 2ml/kg body weight (27) meaning this set up would be unacceptable for use in infants.
Nafion tubing is used to connect the sampling capillary to the Innocor. Nafion equilibrates the humidity of the sampled gas so that gas concentrations do not change on route to the analyser. The minimum length of nafion that equilibrates humidity is used (40cm) because longer lengths allow more spreading of the gas which causes a slower rise time within the analyser.

The Innocor uses photoacoustic spectroscopy to measure gases including SF$_6$ and carbon dioxide. The Innocor is a highly sensitive analyser and the signal: noise ratio is better than the RMS (944:53 vs 200:13 respectively) meaning that lower concentrations of inert gas can be accurately measured (26). In SF$_6$ Innocor washouts 0.2% SF$_6$ can be used as opposed to the 4% SF$_6$ required for the RMS, reducing the burden of this greenhouse gas on the environment.
The sample rate of the Innocor is higher than that of the RMS (100Hz vs 33Hz respectively) (21); a minimum of 100Hz has been recommended for use in infants to accurately detect changes in breathing pattern and reconstitute breathing pattern. The sample flow of the Innocor is higher than that of the RMS (120mL/min vs 20mL/min) but the sampling capillary is placed distal to the flowmeter to prevent volume recordings being affected.

Synchronisation of flow and gas concentration is integral to accurate volume calculation. Factors such as the delay between the transit of gas between sampling site and analyser and the analyser response time need to be considered and corrected for. Mainstream analysers reduce the impact of transit time, however mainstream analysers increase dead space which may affect ventilation, particularly in children. The length of analyser rise times are more important in faster breathing patterns, more often seen in young children. Changes to signal alignment in a mainstream N₂ ultrasonic MBW system have demonstrated significant alterations in calculated volumes and thereby MBW derived indices (28). A decrease in gas concentration delay time by 40ms lead to a mean increase in lung clearance index (LCI) of 12%, an increase in delay time by 40ms lead to a decrease in LCI of 9%.

The response time of the Innocor to a step change in SF₆ concentration is longer than the RMS (154 vs 64ms respectively). A response time of less than 100ms is recommended by the ERS/ATS expert group to prevent errors in calculation of gas volumes (21). To allow for the more prolonged response time, gas concentration and flow signals are offset by an additional 50ms during analysis. The accuracy of the modified Innocor system in integrating flow and gas signals with this offset has been validated using gas calibration syringes set to deliver different volumes of SF₆ (26). To improve the response time the oxygen analyser which is positioned in series, proximally to the photoacoustic analyser within the Innocor can be
bypassed. This prevents the spreading of gas within the oxygen analyser and improves the system rise time from 154ms to 97ms.

The Innocor is much less expensive and more portable than the RMS, and like the RMS it allows calculation of phase III slope indices. Its use is validated in school age children and adults (26).
1.2.4 Performing MBW

There are two phases in exogenous inert gas MBW, wash-in and washout. In the wash-in phase, the patient breaths in the inert gas through a mouth piece until it is evenly distributed throughout the lung (as determined by its equal concentration during inspiration and expiration). The patient wears a nose clip and the investigator must ensure that there are no leaks.

![Flow and SF6 concentration throughout a washout](Software courtesy University of Gothenburg)

The washout phase begins with the disconnection of the gas supply. This must occur during expiration so that the first breath of the washout does not contain an unknown volume of inert gas which subsequently makes calculation of MBW indices impossible. The patient can either be encouraged to achieve set tidal volumes throughout the washout or to breathe normally with the aid of some distraction (most commonly an age appropriate video). Flow and tracer gas concentration are displayed throughout the washout. The test is terminated when the maximum
end expiratory gas concentration falls to less than \(1/40^{th}\) of that at the end of the wash in phase. This is a historical threshold set due to the resolution of the gas analyser at the time.

The test is performed three times at each visit. Each test takes approximately 5-10 minutes depending on the degree of lung disease. There is slower clearance of gas in more severe disease meaning a longer testing duration.

MBW can be performed in preschool children and infants. As discussed, equipment needs to be suitable for use in small children with a small dead space and fast rise time. A face mask can be used instead of a mouthpiece; sedation may be necessary in infants.

Testing is operator dependant and adequate training is essential. The set-up of the equipment and observation of the patient throughout testing requires appreciation of the factors that might influence test results. I was trained by my predecessors within the UK Cystic Fibrosis Gene Therapy Consortium who adapted the Innocor for MBW and wrote a consortium wide standard operating procedure for its use.
1.2.5 Assays

Several indices of ventilation distribution inhomogeneity derived by performing MBW have been described. These include the Lung Clearance Index (LCI), which is a measure of overall ventilation inhomogeneity and indices of phase 3 slope analysis $S_{\text{cond}}$ and $S_{\text{acin}}$, which localise problems to either conducting airways or the acinar region respectively. Moment ratios are briefly discussed but have not been investigated within this thesis.

1.2.5.1 Functional Residual Capacity (FRC)

FRC is the volume of air present in the lungs at the end of passive expiration, it is mainly governed by the interaction between the elastic recoil of the chest and that of the lungs. FRC can be measured using plethysmography, helium dilution or MBW.

Figure 8: Static lung volumes and capacities based on a volume–time spirogram of an inspiratory vital capacity (IVC). IRV: inspiratory reserve volume; VT: tidal volume (TV); ERV: expiratory reserve volume; RV: residual volume; IC: inspiratory capacity; FRC: functional residual capacity; TLC: total lung capacity (4).
FRC is calculated during a washout by dividing the volume of expired inert gas ($V_{GAS}$) by the difference between end tidal gas concentrations at the start ($C_{INIT}$) and end ($C_{END}$) of washout.

$$FRC = \frac{V_{GAS}}{C_{INIT} - C_{END}}$$

FRC calculated using MBW or gas dilution excludes obstructed non ventilated regions of lung. In patients with severe airways obstruction FRC will be lower when calculated using MBW or helium dilution compared to plethysmography derived FRC (4).

1.2.5.2 Lung Clearance Index (LCI)

LCI reflects overall ventilation inhomogeneity. In most published MBW studies and this thesis it corresponds to the number of lung volume turnovers required to clear inert alveolar gas to $1/40^{th}$ of its starting concentration. Alveolar gas concentration is estimated using end tidal concentration ($C_{et}$); $C_{et}$ is dependent on accurate identification of the end of the breath. Recent studies have found that LCI calculated using the number of lung volumes required to clear inert gas to $1/20^{th}$ of initial concentration was repeatable in patients with and without CF disease and shortened duration of testing (29, 30). However there is some evidence that shortening the test may make it less sensitive to increasing disease severity in children with CF (31).

LCI is calculated by dividing the cumulative expired volume (CEV) required to clear the inert gas by the functional residual capacity (FRC). LCI will rise with delayed inert gas clearance from the lung due to ventilation heterogeneity. LCI will depend on the choice of inert gas; heavier gases such as $SF_6$ generate higher values compared to lighter gases such as He. $N_2$ MBW generates higher LCIs than $SF_6$, possibly because it is an endogenous gas (32).
LCI = CEV / FRC

LCI is reported as the mean of 2-3 technically acceptable manoeuvres in which the FRC differs by less than 10%.

1.2.5.3  Phase III slope Analysis

A tidal breath can be divided into three phases if inert gas concentration is plotted against expired volume, as illustrated in figure 9. During phase I the inspired gas sitting in anatomical dead space is expired. Phase II corresponds to a transition phase in which inspired gas mixes with alveolar gas. During phase III at typically 65-95% of expired volume, alveolar gas is expired and it is this phase III slope which can be analysed to assess ventilation heterogeneity. Individuals with diseased lungs and inhomogeneous gas mixing have steeper phase III slopes than those with healthy lungs. The first breath in a washout reflects gas flow from better ventilated parts of the lung. Subsequent breaths represent progressively more poorly ventilated areas.
In phase III slope analysis progressive change in the alveolar or phase III slope of each breath throughout the washout is examined. To allow comparison of progression each phase III slope (SIII) is normalised for mean tracer gas concentration over the slope (SnIII) and plotted against lung volume turn over (TO). SIII will depend on the tracer gas, higher SIII is seen with use of heavier gases in healthy people, but this may reverse in diseased lungs (21).

The site of ventilation inhomogeneity can be determined by the analysis of SnIII against TO (34). Abnormal compliance and resistance in the conducting airways are believed to cause convection dependent inhomogeneity (CDI), which causes a steady increase in SnIII throughout the washout and is measured by $S_{\text{cond}}$. $S_{\text{cond}}$ is calculated by determining the increase in SnIII between TO 1.5 and 6.0.

$$S_{\text{cond}} = \text{delta SnIII (1.5-6.0 TO)}$$
Structural asymmetries in the acini cause diffusion-convection dependent inhomogeneity (DCDI), and this is measured by subtracting the contribution of the conducting airways from the SnIII of the first breath to produce $S_{\text{acin}}$ (35).

$$S_{\text{acin}} = \text{first breath SnIII} - (\text{first breath TO} \times S_{\text{cond}})$$

The value of $S_{\text{cond}}$ or $S_{\text{acin}}$ increase as ventilation heterogeneity in the corresponding lung zone increases.

![Conceptual illustration of calculation of phase III slopes](image)

Figure 10: Conceptual illustration of calculation of phase III slopes. SnIII (O) for each breath plotted against corresponding TO value. Scond is calculated by the increase in SnIII between TO 1.5 and 6.0 (---). DCDI for each breath (△) = SnIII-CDI contribution. Sacin (▲) = first breath DCDI (1)

Fluctuating tidal volume affects SIII, therefore for optimum phase III slope analysis a fixed tidal volume and respiratory rate are set. However, set tidal volumes are impractical in some children and to attempt to correct for fluctuating tidal volumes and to allow comparison between individuals SnIII is multiplied by tidal volume. If tidal volumes fall below a certain minimum, identification of the phase III slope is impossible (35). For this reason Aurora’s
group stipulate a minimum expired breath volume of \((3.5 \times \text{body weight (kg)} + \text{precapillary dead space}) \times 2\) (ml). This assumes the SnIII slope starts at double the airway and instrument dead space. Breaths below this threshold are excluded from analysis (35). Breaths in which SIII is unidentifiable due to a breath hold or cough should also be excluded; washouts in which >3rd of breaths are excluded should not have phase III slope indices reported (21). I was unable to exclude breaths with the software I had to my disposal.

1.2.5.4 Moment Ratios

Moment Ratios (MDM) relate to the mean number of lung TOs that a molecule remains in the lung. Disease skews the washout curve to the right with slower peripheral release of inert gas and MDMs describe the degree of skewness. Concentration normalised end tidal inert gas concentration \((C_{et})\), \(C_{et}*\text{TO}\) and \(C_{et}*\text{TO}^2\) are plotted against TO and moments 0, 1 and 2 are calculated by determining the area under the each curve respectively. MDM₁ is the ratio between 1st and 0th moments of the washout and MDM₂ the ratio between the 2nd and 0th moments. Truncation of the washout at either 6 or 8 TOs is recommended to allow comparison between subjects. Moment ratios are useful in children with variable respiratory rate and tidal volume but healthy children may require longer washouts to achieve 6-8 turnovers and low gas concentrations require very accurate analysers (21). Moment ratios are measures of overall ventilation heterogeneity and are not thought to offer additional information to LCI, which is more widely reported in the literature (1).

1.2.5.5 Trapped gas volume \((V_{TG})\)

Gas trapping occurs due to airway closure in regional lung units and increases in conditions which cause peripheral airways obstruction. Gas trapping can be measured following
termination of standard MBW testing by asking the patient to take five inspiratory capacity breaths and measuring the volume of lung recruited. Only the regions which can be unobstructed by performing a large breath will be measured, regions which remain obstructed will not be measured.

1.2.6 Single Breath Washouts

Single breath washout (SBW) measures gas mixing within a single breath. The most commonly used SBW test uses N₂. The patient performs a vital capacity (VC) manoeuvre at constant low flow (400-500mL-s); following exhalation to residual volume (RV), the patient inhales 100% oxygen (O₂) to total lung capacity (TLC) and then exhales back to RV. The phase III slope of N₂ concentration over the mid portion of the exhalation from TLC to RV is measured (36). Other inert gases can be used and are washed in during inspiration between RV and TLC. The use of SBW in young children is limited because it requires a VC manoeuvre.
Peripheral airway contribution to VC SBW is unclear. VC SBW is thought to be more influenced by inter regional differences in gas distribution and airway closure during inspiration compared to tidal breathing techniques. The technique can be modified so that initial wash in occurs from FRC to TLC or a volume above FRC, which may better detect peripheral airway ventilation inhomogeneity (21). A SBW test using a single inert gas cannot localise the site of inhomogeneity. Recently, SBW with the simultaneous use of more than one inert gas with different molecular masses has made localisation of the site of inhomogeneity with SBW possible (37).
1.3 Variability of MBW derived indices in Children

1.3.1 Healthy population – Normal values

MBW testing is not standardised and reported healthy values vary between centres. Factors which are likely to affect normal values include the choice of inert gas, equipment and software.

Ranges of normality for MBW derived indices from infancy through to adulthood differ slightly (26, 35, 38, 39). MBW derived indices appear to decline through infancy and early childhood and then slowly increase in the elderly (40, 41). In pre-school children LCI is dependent on height but once children have obtained approximately 115cm in height at around 6 years of age height appears to become insignificant (41). In healthy people there are minimal gender differences in LCI (26, 41, 42). There have been no published studies regarding differences in normative values between ethnic groups.

In Edinburgh normative values for LCI using the modified Innocor and 0.2% SF₆ were derived in 48 healthy adults (aged 19-58 years) as 5.9 - 7.5 and from 12 healthy children (aged 6-16 years) as 5.3 - 7.3 (26). This was a small study but the range obtained was in concordance with the following published studies which used 4% SF₆. The upper limit of normal for LCI was reported as 7.23 in a UK study of 33 healthy controls aged 6-16 years, in whom testing was performed using a mass spectrometer (43). The normal range for LCI of pre-school children was determined from a group of 37 healthy children (mean age 4.2 years) in London using a
mass spectrometer as 6.0-7.8 (39). The upper limit of normal in a German/Austrian study using the ultrasonic flow sensor was 7.0 (44).

In healthy children LCI does not appear to correlate with FEV\(_1\) (43). As described earlier FEV\(_1\) is a measure of airway resistance and LCI a measure of ventilation heterogeneity of the small airways.

Reported healthy ranges for indices of phase III slope analysis vary; measurement has not been standardised and values appear dependant on the choice of inert gas, testing protocol and analysis. Verbanck et al performed N\(_2\) MBW on two groups of 10 adults (with no respiratory disease) with a targeted one litre tidal volume; they reported mean S\(_{\text{cond}}\) as 0.031-0.033L\(^{-1}\) and mean S\(_{\text{acin}}\) as 0.067-0.075L\(^{-1}\); the upper limit of normal for S\(_{\text{acin}}\) was described as 0.11-0.13 L\(^{-1}\) (45). The group performed a larger study in 63 healthy adults using the same technique and found mean (SD) S\(_{\text{cond}}\) was 0.028 (0.001) and S\(_{\text{acin}}\) was 0.072 (0.003) (46). In Downie et al’s study which included 17 healthy adults and used N\(_2\) MBW with a N\(_2\) analyser and set tidal volume of 1-1.3L, mean (SD) S\(_{\text{cond}}\) was 0.019L\(^{-1}\) (0.01), with an upper limit of normal of 0.037L\(^{-1}\), S\(_{\text{acin}}\) mean (SD) was 0.094L\(^{-1}\) (0.03) (47).

Gustafsson studied N\(_2\) RMS MBW in a group of 18 children (mean age 15 years) with relaxed tidal breathing and reported mean (SD) S\(_{\text{cond}}\) as 0.045L\(^{-1}\) (0.021) and S\(_{\text{acin}}\) as 0.090L\(^{-1}\) (0.022) (48). Sonnappa et al performed SF\(_6\) MBW with a RMS in 65 healthy pre-school children (aged 4-6 years) and mean (95% CI) S\(_{\text{cond}}\) was reported as 0.010L\(^{-1}\) (0.007-0.014), the upper limit of normal was 0.045L\(^{-1}\); mean S\(_{\text{acin}}\) was 0.042L\(^{-1}\) (95% CI 0.035-0.051) (49). In Edinburgh the modified Innocor, 0.2% SF\(_6\) and relaxed tidal breathing was used in a study of 29 healthy children, mean (SD) S\(_{\text{cond}}\) was 0.017L\(^{-1}\) (0.02) and Sacin was 0.12L\(^{-1}\) (0.06) (50). Phase III
slope indices appear dependent on testing protocol and seem to be different in children compared to adults thereby limiting comparison between studies.
1.3.2 Evidence of repeatability and reproducibility in healthy children

LCI has good intra and inter visit reproducibility in healthy volunteers (26). Forty nine healthy adults and 13 healthy children were studied in Edinburgh using the modified Innocor and 0.2% SF₆ in order to assess the intra and inter visit reproducibility of LCI. Participants were non-smokers with no history of significant respiratory disease. Tests were excluded if FRC varied by more than 10% from both the other 2 tests. The mean (SD) coefficient of variation (CV) for LCI was 3.6 (2.1) % for healthy adults and 5.4 (3.8) % for healthy children. In 16 participants testing was repeated after a mean of 36 days, the 95% limits of agreement for LCI were -0.78 to 0.46 (26).

A German and Austrian collaborative showed LCI to be highly repeatable with good short and long term reproducibility in healthy children and adolescents (44). Fuchs et al used the sidestream ultrasonic flow sensor to study 44 volunteers aged 5.3 to 20.3 years. All participants did the first set of 3 tests, 22 did a second set an hour later and 34 returned 6-15 months later for a 3rd set of testing. Mean (SD) LCI was 6.2 (0.4), 6.3 (0.4) and 6.0 (0.4) at the 1st, 2nd and 3rd set of tests respectively. The mean within set CV for LCI was 5.1%. Short term (set 1 to 2) repeatability, assessed using a mean intra individual CV was 4.2% with a mean difference of -0.13 (95% CI -0.35; 0.087) over the 2 sets. Long term (set 1 to 3) repeatability had a mean CV of 5.1% with a mean difference of 0.017 (95% CI -0.16; 0.348).

Age does not appear to affect the repeatability of testing for LCI. In studies of both pre-school and school aged children in London using a mass spectrometer the mean within subject intra-visit CV was 5.2% (39, 43).
LCI results from different countries appear to be very similar (43). Results from 33 children, aged 5.9-16.8 years in the UK were compared to those obtained in Sweden from 24 healthy children aged 7.6-15.7 years. The mean (SD) LCI in the UK was 6.45 (0.49) compared to 6.33 (0.46) in Sweden (43). In Fuchs et al’s study of the reproducibility and repeatability of LCI, subjects in Germany had a mean (SD) LCI of 6.13 (0.3) and in Austria mean (SD) LCI was 6.27 (0.5), there was no statistical difference between the two groups (44).

1.3.3 Sources of possible variation:

1.3.3.1 Age

It is possible to perform MBW in people of all ages. In preschool children a face mask can be used instead of the standard mouthpiece (39). Infants may require sedation (51, 52). LCI was initially thought to be independent of age in healthy children (42, 43), however there is increasing evidence that infants and preschool children have slightly higher ranges of normality (35, 41, 53).

MBW performed on the first day of life has demonstrated increased ventilation inhomogeneity which subsequently improves over the first day and week of life (51). This may be due to retention of fluid within the lung, or alveolar stabilisation and establishment of the FRC (51).

Infants have been shown to have slightly higher LCI than older children (35). There are several potential reasons for this: it could be an artefact due to the relatively larger apparatus dead space. It may alternatively be due to less negative end expiratory intra pleural pressure caused
by a more compliant chest wall resulting in a tendency for peripheral airway closure during tidal breathing. The difference nevertheless is small; the mean LCI of a group of 14 infants assessed by Aurora et al was 7.2, which lies within the healthy reference range for an adult (35).

Healthy preterm infants have been shown to have greater ventilation inhomogeneity than term babies (52). Gas mixing improves between 34 and 40 weeks post menstrual age but remains poorer than in babies born at term when tested at 40 weeks. It is postulated that this is because preterm birth impairs the formation of alveoli and decreases lung tissue elasticity.

Lum et al collected MBW data from 497 healthy subjects aged 2 weeks to 19 years from three centres using mass spectrometry and SF₆ (41). They found that age and height were independent predictors for FRC but that once height was excluded using multivariate analysis, age was no longer predictive for LCI. They found a small reduction in LCI throughout childhood but once children had attained a height of approximately 115cm (at around 6 years of age) changes were minimal. The higher LCI in younger children is postulated by the authors as being due to asymmetrical branching in the acinar region of lungs undergoing rapid alveolisation.

The range of normality for LCI is similar for healthy children and adults. Horsley et al. found a weak correlation between LCI and age in their study of healthy children and adults (Pearson $r^2=0.16, p<0.002$). However, the normal ranges for children ($\leq 16$ years) and adults overlapped markedly (5.3-7.3 and 5.9-7.5 respectively) (26).
Aurora et al reported that $S_{\text{cond}}$ and $S_{\text{acin}}$ were independent of age in children from birth to 16 years (35). Verbanck et al have more recently reported small increases in LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$ with age in adults aged 25-65 years (40).

Longitudinal studies of healthy individuals are required to definitively define the effect of age on MBW results. The evidence suggests that although the effects of age and height on MBW may be small they do need to be considered when interpreting results particularly when studying infants and pre-school children. The use of age and height matched control groups in pre-school studies could limit the influence of age and height on results. Reference equations to standardise LCI to z-scores in pre-school children have been published (41). However the patient cohort from which these equations were derived is limited (compared to cohorts used to determine reference equations for other tests including spirometry) by the number of subjects (497), testing centres (3) and ethnicity (80% white European). In addition testing was performed using a RMS and 4% SF$_6$ and equations would not be applicable to a different system. The European Cystic Fibrosis Society Clinical Trial Network Standardisation Committee have published LCI reference ranges for different equipment, gases, method of analysis, software and patient age (54).

1.3.3.2 Tidal Volume

Fluctuating tidal volumes throughout a washout may affect MBW indices. Large inspiratory breaths can release trapped gas, small inspiratory and expiratory breaths lead to steeper phase III slopes and expiration beyond FRC can lead to phase IV (21). Studies in adults have implemented set tidal volumes, often of 1 litre. However, this is not feasible in some children who may have difficulty achieving a set tidal volume throughout a washout.
Changes in breathing pattern can affect FRC and LCI. Yammine et al asked children with and without CF to perform MBW with normal tidal breathing and again with tidal volumes of 1 Litre. They found that FRC fell and LCI rose significantly with the fixed 1 litre breathing pattern in both groups (55). In children with CF, mean (SD) LCI increased from 11.0 (2.2) to 13.0 (2.6) (p=0.011) and in healthy children LCI rose from 6.8 (0.5) to 7.7 (1.4) (p=0.004).

Identification of the phase III slope can be impossible if the tidal volume falls too low, Aurora et al stipulated a minimum tidal volume of (3.5 x body weight (kg) + precapillary dead space) x2 (ml) (35). Fluctuating tidal volumes particularly in younger children who are especially susceptible to variable breathing patterns can affect indices of phase III slope analysis. The first breath is particularly important because its phase III slope is integral to \( S_{\text{acin}} \) calculation.

1.3.3.3 Posture

Change in posture is likely to affect ventilation heterogeneity. As described previously LCI is a function of the total volume of gas expired during a washout, divided by the functional residual capacity (FRC); LCI should logically be indirectly affected by variable FRC. The supine posture is associated with elevation of the diaphragm, pressure of the heart on pulmonary structures and increased sequestration of blood in the gravity dependent thorax, all of which cause a reduction in residual lung volumes in adults and children (56, 57). In adults, changes in posture have been shown to cause gravitational changes in ventilation distribution (58).
Imaging studies using MRI and CT have demonstrated atelectasis in children’s lungs when lying supine (59, 60); obstruction of small airways will affect gas mixing and MBW indices. However, these imaging studies were performed in sedated and ventilated children (as necessary for imaging) which may be responsible for some of this effect.

Gustafsson studied the effect of posture on ventilation inhomogeneity in asthmatic and healthy children using N\textsubscript{2} MBW (57). Ten asthmatic children (aged 10-17 years) and 12 healthy subjects (aged 11-18 years) were recruited. The participants all initially performed spirometry, followed by 2 MBW tests and then 3 measurements of inspiratory vital capacity (VC) while sitting. Participants then lay down and performed one MBW test at 0, 30 and 60 minutes following assumption of the supine position. VC was measured after each test. In both the asthmatic and control groups FRC fell immediately on lying by 21% and 24.9% respectively. There was no further change in FRC in the control group over the supine hour. In the asthmatic group FRC fell significantly further over 60 minutes to 24.7% less than sitting FRC. VC fell slightly in both groups. LCI did not significantly change in either group. This small study demonstrated an effect of posture on FRC in healthy and asthmatic children which was not translated into any change in LCI. MBW testing may have been affected by performing spirometry before MBW (the effects of forced manoeuvres on ventilation heterogeneity are unclear) and by only performing one MBW test at each time point as opposed to the standard three tests.

Gronkvist et al. studied 11 healthy men using a mass spectrometer. They found a rise in S\textsubscript{cond} in the supine position but no change in S\textsubscript{acin} or LCI (p<0.001) (58). Bouhuys et al studied 6 healthy men using nitrogen washout and found that their LCI increased in the supine position (61). In their analysis of MBW results from almost 500 children Lum et al found no difference
in LCI measurements made supine (in sedated children <2yrs) and older children studied sitting (CI -0.07, 0.34) when corrected for height (41). However, Lum’s study was comparing two different groups of children using different methodologies (the supine group were sedated and used a face mask).

In young children and infants it is necessary to perform MBW while the child is lying supine. In older children, however, the test is performed with the child sitting; for results to be comparable across age groups it is imperative that any effects posture may have on LCI are known. More research into the effects of posture on ventilation heterogeneity in healthy children and those with disease including CF and asthma is necessary.

1.3.3.4 Forced Airway Manoeuvres

Deep inspirations are thought to affect airway diameter during subsequent tidal breathing (39). Forced airway manoeuvres include activities that recruit lung volume by inspiring to total lung capacity, such as spirometry or some methods of physiotherapy. Physiotherapy in children with CF has been found to improve indices of phase III slope analysis measured by single breath washout (62). Performing physiotherapy or spirometry may transiently normalise inhomogeneity, by opening atelectatic regions (51, 63). For this reason MBW testing has not been performed directly after these manoeuvres in our centre.

However, in 2010 Fuchs et al published a study in which physiotherapy performed immediately before MBW in children with CF did not alter the repeatability of LCI (64). They studied 32 children, aged 5.7-15.9 years. MBW testing was performed twice with an interval
between testing of 90 minutes. Sixteen patients performed 30 minutes of physiotherapy between the tests, 11 did not. In the physiotherapy group intra individual coefficient of variation (CV) was 6.1% at the first test and 6.5% post physiotherapy. This was compared to the control group in which CV was 4.2% and 7.1% in the 2 tests. There was no consistent difference in LCI between the tests in either group. Reproducibility was assessed using the CV of LCI over the two tests for each patient. Mean inter-test CV was reported as 4.6% in the physiotherapy group and 2.6% in the control group. This study’s authors concluded that there are no consistent effects of physiotherapy on the variability of LCI; however children with CF had a mean initial LCI of only 7.76 suggesting mild disease and further study of more severely affected children may draw different conclusions. The study did not assess effects of physiotherapy on phase III slope analysis.
1.4 MBW in Disease

MBW can detect abnormalities in gas mixing in diseases including CF, asthma, COPD and bronchiolitis obliterans (65).

Ventilation inhomogeneity is also found in healthy preterm infants (52) and babies with respiratory distress syndrome. In ventilated infants with respiratory distress syndrome ventilation inhomogeneity improves after administration of surfactant (51). MBW is abnormal in chronic lung disease and can differentiate between mild, moderate and severe disease (51).

The degree of abnormality generally reflects the extent of disease. However, in patients with severe disease, variability in MBW derived indices may be worse due to intermittent obstruction of badly affected peripheral lung regions (66). Washout traces from patients with collapse of part of their lungs or widespread peripheral airway closure may be misleading. A normal LCI and a low FRC may be seen in these patients when the rest of the lung is ventilated evenly (35).

Reports of the within visit variability of MBW appear to be greater in patients with disease compared to healthy individuals (39, 43, 51). In Aurora et al’s study of school aged children with CF, the healthy control group had a within subject intra-visit LCI CV of 5.2% compared to 6.2% in those with CF (43). In their study of preschool children the mean within subject intra-visit CV of LCI was 5.2% in healthy children compared to 7.8% in children with CF (39). This was thought to be due to variable mucus plugging in the CF lung.
1.4.1 Cystic Fibrosis

CF is the most common inherited cause of early mortality. One in 2500 babies of Northern European descent are affected. The disease is a result of mutations in the gene that codes for the Cystic Fibrosis Transmembrane Regulator (CFTR) protein. CF is variable, depending on genetic and environmental factors. The pathology is characterised by collection of thick mucus in the lungs, sinuses, intestine, pancreas and reproductive organs.

Approximately 2000 mutations of the CFTR gene have been identified. There are 6 classes of mutation that either affect the quantity or function of the CFTR protein. Mutations that effect the quantity of CFTR include those that cause defective or reduced protein synthesis (classes I and V respectively), defects in folding and trafficking (class II) or reduced stability of the protein on the cell surface (class VI). Class III and IV mutations affect CFTR function by reducing channel opening (III) or ion transit (IV). F508del is the most common mutation with more than 91% of the UK CF population having at least one allele (CF Trust Registry Annual Data Report 2012). It causes severe CF disease by producing problems with CFTR trafficking, channel opening and stability.

Figure 12: Proposed 3D model of the CFTR protein (http://www.cfnametheraphy.org.uk)
CFTR is a cyclic adenosine monophosphate (cAMP) regulated chloride channel which is expressed in the apical surface of epithelium. The channel regulates chloride secretion but also other membrane proteins including the epithelial sodium channel (ENaC). CFTR and ENaC control the movement of water across the epithelium; faulty CFTR leads to fluid hyper-absorption and dehydration of the epithelial surface. This leads to abnormal thick mucus with altered biophysical properties.

CFTR is expressed in many tissues, including the lung, pancreas, intestine and reproductive tract. Defective mucus production in these tissues causes blocked ducts and impaired mucosal defence. CFTR is involved in the secretion of bicarbonate; lower pH due to reduced bicarbonate secretion has been shown to impede bacterial killing. CFTR malfunction is also thought to cause abnormal epithelial inflammation.

Lung disease is the most significant cause of morbidity and mortality in CF (67); lungs of infants appear largely normal shortly after birth, but pulmonary disease starts to develop within the first few months of life (68) and 90% of patients eventually die from respiratory complications. Pulmonary disease is characterised by persistent infection and exaggerated inflammation leading to chronic airways disease and progressive decline in lung function.

People with CF are living longer. A review of mortality in the US, between 1993 and 2002, found median survival for people with genotypes associated with little to no CFTR activity had increased to 36.3 years and for those with genotypes associated with residual CFTR activity it was 50 years (69). This has been achieved through more intensive monitoring and earlier treatment, delaying lung disease progression (70). Early lung disease is thought to
develop first in small peripheral airways (10, 12); more sensitive methods of detecting this are sought to detect earlier disease and delay progression (71, 72).

1.4.1.1 Lung function testing in CF

In CF, measurement of lung function is routinely performed in patients from the age of 4-5 years. This is typically done by performing spirometry which requires a coordinated expiratory manoeuvre on behalf of the patient. As discussed the feasibility of spirometry is limited in very young children due its volitional nature. It is well established that lung disease in CF can be present from as early as the first few months of life and an alternative method of detecting early disease is required (73).

FEV\textsubscript{1} is the most repeatable and referenced spirometric measure; it has proven to be a good predictor of outcome in patients with moderate to severe disease (74). However, FEV\textsubscript{1} is insensitive in detecting early CF pulmonary disease (12, 53) as it measures medium to large airways obstruction. CF lung disease is patchy and normal function in some regions may mask diseased regions. Many children with CF have normal FEV\textsubscript{1} (9, 10); however some of these children will have lung disease detected using CT scanning or MBW (10, 12, 39, 43). FEF\textsubscript{25-75} is more sensitive to small airways disease but is also highly variable, particularly in preschool children, which limits its use as a monitoring tool (39).
1.4.1.2 MBW in CF

CF lung disease is thought to exist before symptoms develop or spirometry is abnormal. In comparison with FEV\textsubscript{1}, LCI is more sensitive to early lung function abnormalities (10, 26, 35, 38, 39, 43, 75) and correlates better with airway damage seen on CT imaging (76). In children with CF, MBW derived indices correlate with FEV\textsubscript{1} (43, 77) and FEF\textsubscript{25-75} (39). However, MBW is often abnormal in children with normal spirometry and children are very unlikely to have normal MBW if spirometry is abnormal (10, 35, 39, 43, 75, 78). In addition LCI has been shown to be more sensitive in detecting abnormal lung function in pre-school children with CF than plethysmography (39). Ventilation heterogeneity has been detected using MBW in infants with CF (53, 79).

Children with CF who have chronic bacterial colonisation are known to have significantly worse lung function (MBW and spirometry) than non-colonised patients (10). Aurora et al found in their study of preschool children with CF, that children infected with pseudomonas aeruginosa had significantly higher LCI than uninfected patients despite non-significant differences in spirometry or plethysmography (39). Kraemer et al found LCI was higher in children (aged 6-16 years) with Allergic Bronchopulmonary Aspergillosis (ABPA) and that compared to indices of plethysmography and spirometry, LCI was the best discriminator between Aspergillus sensitisation and ABPA (80).

LCI has also been shown to predict pulmonary exacerbation in CF. A prospective study of 63 children (aged 5-19 years) over one year found the rate of exacerbations (requiring IV antibiotics) correlated with baseline LCI and that the time to first exacerbation decreased with increasing LCI (81). This effect was seen even in patients with normal FEV\textsubscript{1}. LCI was also
found to correlate with the respiratory domain of the standardised CF Questionnaire Revised (r=-0.43, p=0.04).

LCI is repeatable in children with CF. Aurora et al measured spirometry and MBW in school and preschool aged children with and without CF (22 children aged 6-16 years and 40 aged 2-5 years with CF and 33 healthy children aged 6-16 years and 37 aged 2-5 years). MBW was performed using 4% SF₆ and a RMS. They found that in school aged children the intra subject LCI CV was 6.2% (43) and in preschool children it was 7.8% (39). These CVs are higher than those of healthy children; school and preschool age LCI CVs were both 5.2% (39, 43). Poorer repeatability of MBW in children with CF may be due to variable mucus plugging.

Children with CF have been shown to have abnormal S_{cond} and S_{acin} suggesting disease of both conducting and acinar airways (48). In a Swedish study using N₂ MBW in 15 patients with CF, aged over 6 years, mean (SD) S_{cond} was 0.151(0.071)L⁻¹ compared to 0.045(0.021)L⁻¹ in healthy children (p<0.001) and S_{acin} was 0.307(0.207)L⁻¹ in CF patients and 0.090(0.022)L⁻¹ in healthy controls (p<0.01)(48). When compared to moderately severe asthmatic children, children with CF were shown to have significantly higher LCI and S_{acin}, but similar S_{cond} (48).

Horsley et al compared 0.2% SF₆ MBW derived indices (using the modified Innocor) and spirometry in 22 adults and 18 children with CF, 17 healthy adults and 29 healthy children (82). LCI was significantly higher in children with CF compared to healthy controls (7.3 vs 6.2, p=0.022) and in CF adults compared to controls (12.8 vs 6.7, p<0.0001). S_{acin} was significantly higher in children with CF compared to controls (0.192 L⁻¹ vs 0.117 L⁻¹, p=0.007) and in adults with CF compared to controls (0.366 L⁻¹ vs 0.112 L⁻¹, p <0.0001). S_{cond} was also significantly higher in children with CF (0.068 L⁻¹ vs 0.015 L⁻¹ p<0.0001) and adults with CF.
FEV₁ z score was significantly lower in adults with CF compared to controls (-3.03 vs 0.04, p<0.0001) but there was no difference between children with and without CF. S_acin and LCI were significantly higher in CF adults compared to CF children (p=0.0036 and p<0.0001) but there was no difference in S_cond between the age groups in CF. S_cond was elevated in several CF patients with normal LCI suggesting that it might be an early marker of disease. S_cond did not however rise with rising LCI or correlate with FEV₁ and therefore might not be a good indicator of severity. The authors postulate that S_cond has a ceiling value of 0.150L⁻¹ which is reached relatively early in CF. S_acin did correlate with LCI (r=0.86, p<0.0001) and FEV₁ (r=0.73, p=0.0002) but remained within the normal range until LCI exceeded 10.

1.4.1.3 CF MBW and Imaging

Computed tomography of the chest is the gold standard in identifying structural changes in CF. Disease can be seen in patients with apparently normal spirometry (12). However, the use of CT exposes the patient to radiation, thereby limiting its use as a monitoring tool. In addition CT does not provide information regarding the functional impact of disease. Structural lung disease, detected using CT, can exist in patients with normal spirometry (83, 84). As discussed in the following paragraphs LCI may be more sensitive than spirometry in predicting structural change seen on CT and may therefore serve as a useful monitoring tool to limit radiation exposure.

In a retrospective study by Gustafsson et al, HRCT scan scores, LCI and spirometry obtained from 44 CF patients (aged 5-19 years) over a 30 month period were compared (76). LCI was found to be more sensitive a predictor of abnormal structure seen on CT than FEV₁ or FEF25;
sensitivity for LCI was 85-94% compared to 19-26% for FEV\textsubscript{1} and 62-75% for FEF\textsubscript{75}. LCI also correlated better with HRCT scores (r=0.85) than FEV\textsubscript{1} (r=-0.62) or FEF\textsubscript{75} (r=-0.66) (p<0.001). Normal LCI made the presence of structural disease unlikely; abnormal structure was only found in 2 (15%) patients with a normal LCI. LCI was abnormal in some children with a normal CT; it was speculated that LCI may be more sensitive to disease than CT. Alternatively this may have been due to a HRCT protocol which limited radiation exposure reducing sensitivity compared to standard protocol.

Ellemunter et al investigated the accuracy of LCI in predicting CT diagnosed structural disease in a prospective study of CF patients with FEV\textsubscript{1} >80% (85). They recruited 34 patients aged 6-26 years, who underwent an ultra-low dose chest CT and then performed SF\textsubscript{6} USFM MBW. MBW and CT detected disease in almost 80% of the study population. LCI was abnormal (>7.0) in 76.5% of patients and correlated with CT findings in 82.3% (i.e. both either normal or abnormal). LCI had sensitivity for predicting the presence of structural lung changes of 88%. Only 3 patients had a normal LCI in the presence of disease on CT, indicating that patients with normal LCI are unlikely to have structural changes. However, these results may have been limited because patients did not have their CT and MBW performed on the same day; in fact some patients had up to almost 8 months between each test.

A prospective study performed by the London Cystic Fibrosis Collaboration found that LCI correlated better with CT than spirometry or plethysmography (78). The group studied 60 children with CF, aged 6-10 years; they had a HRCT (with a complete inspiratory volumetric dataset) and lung function testing on the same day. Eighty four percent of the children had abnormal LCI (>7.5), 58% abnormal plethysmographic lung volumes, 35% abnormal sRaw and 47% abnormal spirometry (FEV\textsubscript{1} or FEF\textsubscript{25-75}); CT scans were abnormal in 85%. Total CT
score correlated best with LCI \((r=0.77)\), compared to \(\text{FEV}_1\) \((r=-0.43)\), or indices of plethysmography. However, concordance between LCI and CT scores were not absolute, 5 out of the 9 children who had normal LCI had abnormalities on CT and 5/9 children with a normal CT had abnormal LCI. This small degree of discrepancy is possibly not surprising given the two tests identify different entities; CT defines structural disease whilst LCI demonstrates ventilation inhomogeneity.

More recently a German study by Stahl et al found that LCI correlated with MRI findings of airway wall abnormalities, mucus plugging and abnormal lung perfusion in infants \((p<0.05)\), toddlers \((p<0.001)\) and older children \((p<0.001)\) (86).

Correlation between MBW derived indices and structural changes on imaging have been demonstrated in cross sectional studies of patients with CF. More recent studies have investigated the progression of ventilation heterogeneity and its relationship with CF disease on imaging. These studies will be discussed in the next section.
1.4.1.4 Longitudinal change of MBW derived indices in CF

In Aurora et al’s study of school age children, LCI in children with CF correlated with age (43). This relationship is thought to be due to progressive lung disease seen with increasing age in CF. In the same group’s study of pre-school children however there was no correlation between LCI and age (39). To try to definitively determine the relationship between LCI and age in CF, longitudinal studies have been performed.

The London CF Collaboration undertook a study examining the changes in LCI, plethysmographic functional residual capacity (FRC\textsubscript{pleth}) and FEV\textsubscript{0.5} in infants with and without CF over the first year of life (79). Seventy two infants diagnosed with CF by new born screening and 44 controls were recruited. Testing was performed at 3 and 12 months of age. At 3 months infants with CF already had significantly worse lung function for all tests compared to healthy controls; the mean (95% CI) difference in LCI z-score was 0.5 (0.1 to 0.9), \( p=0.02 \) (87). LCI remained stable throughout the first year of life whereas FEV\textsubscript{0.5} improved. LCI at 3 months predicted poorer lung function at 1 year. LCI was significantly higher (0.6 to 2.1 z-scores) and FEV\textsubscript{0.5} significantly lower (-1.5 to -0.1) at 1 year in the group of infants who had had abnormal LCI at 3 months compared to the control group. However there was no difference in FEV0.5 or LCI at 1 year in infants who had normal LCI at 3 months compared to controls.

Kraemer et al assessed 142 children and adolescents with CF aged 6 – 20 years at annual intervals with spirometry, specific airway resistance, plethysmography, \( p \) aeruginosa infection status and LCI (using N\textsubscript{2} MBW) (75). They found that LCI was the first outcome to deteriorate, followed by FEF\textsubscript{50}, FVC and finally FEV\textsubscript{1}. The median age of onset for abnormal LCI was 6.4
years compared to 8.4 years for FEV$\text{I}$. LCI continued to increase, along with pulmonary hyperinflation and trapped gas volume, beyond the age of 12 years. Progression in female patients was significantly higher than in male patients. The mean (SE) annual change in LCI was 0.58 (0.06), p<0.0001 and FEV$\text{I}$ was -0.18 (0.01), p<0.0001. The slope of longitudinal progression of LCI was greatest in those chronically infected with pseudomonas aeruginosa (80). In this study changes in lung function over time were not related to structural changes on CT.

![LCI progression](image.png)

**Figure 13**: Progression of LCI in Kraemer’s longitudinal study of children with CF depending on ABPA, A fumigatus sensitisation and intermittent or chronic infection with pseudomonas aeruginosa. Regression lines of mean values of repeated annual measurements presented as z-scores (80).

In another study, by Aurora et al, preschool LCI was shown to predict school age lung function in children with CF (88). Forty five healthy children and 48 children with CF underwent MBW testing and spirometry on two visits. The first visit occurred when the children were aged 3-5 years and the second when the children were 6-10 years of age. The average interval between
tests was 3.7 years. LCI was abnormal in 35 out of 48 children with CF at the first visit; only 5 of these children had abnormal FEV₁. Thirty three out of the 35 children with early abnormal LCI also had abnormal LCI at school age, and 15 had abnormal FEV₁. Eleven out of 48 children had normal LCI at school age, nine of whom had normal LCI at pre-school testing; 32 had normal FEV₁ at school age, of which 12 had normal LCI at preschool. The positive predictive value for pre-school LCI predicting either abnormal LCI or FEV₁ was 94%, the negative predictive value was 62%. Preschool FEV₁ PPV was 100%, however NPV was only 25%. These results tell us that LCI can detect abnormalities earlier than FEV₁, early abnormalities in LCI persist and predict school age abnormalities in both LCI and FEV₁.

Fuchs et al prospectively tracked annual MBW, spirometry and ultra-low dose HRCT findings over a 3 year period in a group of 36 patients, aged 6-53 years with mild CF (89). Patients were included if their FEV₁ z-score was >-2; four data sets were recorded at times of clinical stability. MBW was performed with 4% SF₆ and a side-stream USFM. CTs were scored with a modified Bhalla score. At the start of the study all patients had an FEV₁ of ≥80% but most had abnormal CT scores and LCI results (29/36 and 30/36 patients respectively). LCI and CT scores correlated at baseline (r = -0.565, p<0.001) and at last visit (r = 0.547, p=0.001); FEV₁ did not correlate with CT score. CT scores and spirometry remained stable throughout the study. Mean LCI improved from 8.0 to 7.2 (p=0.001), 6 patients had normal LCI (<7.0) on their first visit which remained <7.0 throughout the remaining visits. Of the 22 patients with a baseline LCI of between 7.0 and 9.0, LCI improved in 77% and deteriorated in 23%. Eight patients had an initial LCI of >9.0 and LCI in all of these patients improved by a mean (SD) of 2.0 (0.54). Change in LCI did not correlate with change in CT score over the study. It was possibly surprising that LCI improved in CF patients over time, however this may be explained by the fact that regular hypertonic saline was commenced in the majority of patients during the study period. Possibly as a result of this there was a reduction in mucus plugging seen on
CT in some patients. Otherwise the general improvement may have been an effect of increased motivation to comply with therapy secondary to study involvement. It is possible the improvement seen was an artefact due to the small cohort coupled with variability of LCI, however this is contradictory to evidence of limited variation in LCI in stable patients with CF already presented (39, 43).

In Fuchs study, initial LCI correlated with CT scores 3 years later ($r = -0.554$, $p < 0.001$), FEV$_1$ did not (29/36 with normal initial FEV$_1$ had abnormalities on CT 3 years later) (89). 4 out of 6 patients with normal initial LCI had normal final CTs and 27/30 patients with abnormal baseline LCI had abnormal final CTs. The authors concluded that in 86% baseline LCI was indicative of the presence or absence of structural changes on final HRCTs. However as previously described, the correlation between CT findings and LCI was not absolute; 2/6 patients with normal initial LCI had mildly abnormal CTs and 3/30 who had abnormal initial LCI had normal CTs 3 years later. As previously discussed neither modality could replace the other as each examines different aspects of CF lung disease but routine use of MBW could reduce the frequency of CT and thereby radiation exposure. This study supports the findings of Aurora et al in that LCI does appear to be able to predict later disease.

LCI appears to correlate with age in cross sectional studies of patients with CF and is sensitive to, correlates with and predicts structural change found on CT. Results of longitudinal studies have shown conflicting results with regard to longitudinal change in LCI, possibly related to the introduction of new therapies. Larger studies are required to clarify this discrepancy.
MBW derived indices have been shown to deteriorate during CF exacerbation and improve following treatment, suggesting MBW is a useful clinical test. MBW has been used as an outcome measure in clinical trials but is not as yet used in routine clinical management.

Data from the UKCFGT Consortium have shown significant improvement in LCI (measured with the modified Innocor) between the start and end of intravenous (IV) antibiotics for CF exacerbation (90). Forty four patients over 10 years of age with CF who were treated for physician diagnosed exacerbations were assessed at the beginning and immediately after a course of IV antibiotics. Patients were assessed by MBW and 45 other measures including symptom scores, spirometry, CT and inflammatory markers. LCI fell in 69% of subjects, the mean fall in LCI was 0.8 (p=0.003). The patients in this study had significant lung disease, the lowest LCI post antibiotics was 9.4. The variable treatment response seen in LCI may have been due to opening up of previously obstructed severely diseased lung units. There were statistically significant improvements in FEV$_1$ (p<0.001); symptoms; CT scores for airway wall thickness, air trapping and large mucus plugs; serum CRP, IL-6 and calprotectin.
Robinson et al also showed a significant, but variable, improvement in LCI in children treated with IV antibiotics for a pulmonary exacerbation (91). Twenty eight children, aged 8-17 years, were recruited with an admission mean (range) FEV\textsubscript{1} of 61.4 (28-92)% predicted, LCI of 10.1 (6.87 - 14.83) and peak VO\textsubscript{2} of 31.2 (23.4 - 45.4)ml/kg/min. Patients were given 2 weeks of IV antibiotics. Mean LCI decreased by 3.8% (p=0.03) from admission to discharge, FEV\textsubscript{1} increased by 7% (p<0.01) and peak VO2 increased by 6.6% (p<0.01). LCI mean (SD) CV was 4.4(3.8%) in the children at admission and 4.9(3.8) % on discharge, which is higher than the mean observed improvement in LCI. In 14 out of 28 patients LCI improved by more than 5%, but in 5 out of 28 children LCI deteriorated by more than 5%. The deterioration in LCI in some children, which was also seen in the UKCFGT study, was thought to be due to recruitment through treatment of previously obstructed lung units and this was supported by a decrease in FRC seen in 3 out of these 5 patients. Change in LCI and peak VO\textsubscript{2} correlated with changes in a CF specific clinical severity score (CFCS) (r=0.480, p=0.01 and -0.50, p=0.02 respectively). Change in FEV\textsubscript{1} did not correlate with the CFCS.
A systemic literature review of studies examining LCI following treatment of CF pulmonary exacerbations with IV antibiotics was published in 2015 (92). Seven studies which included SF₆ and N₂ washout systems and over 176 pulmonary exacerbations were reviewed. Overall absolute LCI decreased by 0.4 (95% CI -0.6 - -0.19, p=0.004) or by 2.5% following treatment. The fall in N₂ and SF₆ generated LCI generally agreed apart from in one study in which SF₆ LCI fell more significantly. In one unpublished study in which patients performed both SF₆ and N₂ washouts before and after treatment there was no difference in the relative fall in LCI between the 2 systems. The degree of improvement in LCI following treatment was small but as described LCI response was heterogeneous and treatment may have opened obstructed airways. Higher LCI was associated with greater improvements in LCI suggesting that LCI could be useful in the study of severe disease.

Yammine et al examined the reasons behind the heterogeneous response of LCI to IV antibiotics in CF exacerbation using parallel plethysmography and MBW. By using multivariate regression analysis they found that improvement in LCI was partly explained by reduced gas trapping (as measured by change in FRC\textsubscript{MBW} minus residual volume derived from plethysmography) (93). Less gas trapping might occur secondary to less secretions and airway obstruction, better lung ventilation and less hyperinflation.

There is some evidence from single breath washout that ventilation heterogeneity improves during the treatment of an acute exacerbation (94). In a study of 58 patients with CF, improvement in phase 3 slope was more sensitive to patient improvement at discharge than the change in FEV\textsubscript{1}, FEF\textsubscript{2575}, VC, maximal voluntary ventilation or oxygen saturation (p=0.005) (94).
MBW has potential as an outcome measure in trials of treatments designed for use in early CF disease, in which FEV$_1$ is insensitive. Amin et al showed that LCI could detect improvement with hypertonic saline in children with CF and normal FEV$_1$ (95). Twenty children aged 6 to 18 years who had a baseline FEV$_1$ of $\geq 80\%$ were studied. The study was a crossover trial with two 4 week treatment periods of either hypertonic (7%) or isotonic (0.9%) saline and a 4 week washout phase. Treatment with hypertonic saline resulted in significantly lower mean (SD) LCI ($7.86(1.71)$) compared to isotonic saline ($8.89(2.1)$; $p=0.016$). Baseline LCI was no different between the treatment groups. There was no significant differences in FEV$_1$ or CFQ-R respiratory domain scores following treatment with either hypertonic or isotonic saline. The study demonstrated that LCI is both sensitive and responsive and can be used in interventional studies of early CF disease in children.

Ellemunter et al detected a long term treatment response with LCI to hypertonic saline in patients (aged 6 to 53 years) with CF (96). 34 patients with an FEV$_1$ of $\geq 70\%$ participated. Following a mean of 39.7 months after starting hypertonic saline, LCI had improved from mean (SD) 7.89 (1.35) to 6.96 (1.03), $p=0.0001$; FEV$_1$ did not improve.

In another study by Amin et al, LCI detected a treatment response following dornase alpha in children with CF and normal spirometry (97). Seventeen children aged 6-18 years with an FEV$_1 \geq 80\%$ predicted were recruited. They were given either dornase alfa or placebo for 4 weeks with a 4 week washout period and then the alternate treatment. Absolute LCI decreased by a mean (SD; 95%CI) of 0.71 (1.23; 0.12-1.3) in the dornase alfa group and 0.31(1.36; -0.33-0.95) in the placebo group. The relative improvement in LCI was 0.90 (1.44) with dornase alfa compared to placebo, $p=0.022$. FEF$_{25-75}$ also improved by 6.1(10.34) % with dornase alfa but there were no differences in FEV$_1$, FVC or the CFQ-R respiratory domain.
between the treated and placebo groups. Change with dornase alfa treatment in LCI correlated with change in FEF\textsubscript{25-75}\% predicted (r=-0.43, p=0.0001).

LCI has also been used as an outcome measure in infants and pre-school children with CF in an interventional trial (98). The Infant Study of Inhaled Saline trial used LCI to detect a difference in a multicentre randomised, controlled trial of hypertonic (7\%) versus isotonic (0.9\%) saline. Twenty seven children, aged 0.34-4.95 years, performed MBW before and after 48 weeks of twice daily nebulised treatment (hypertonic or isotonic saline). MBW was performed using 4\% SF\textsubscript{6} and a RMS. Twenty five out of twenty seven children had acceptable baseline and follow up LCI measurements (with at least 2 technically acceptable tests). LCI detected a significant treatment effect in the hypertonic saline group (p = 0.025).

LCI was used as a secondary outcome measure in the UKCFGT consortium’s recently published trial of gene therapy (99). The trial was a randomised double blind, placebo controlled trial of non-viral CFTR gene therapy. Patients were aged 12 years and older, had any combination of CFTR mutations and an FEV\textsubscript{1} of 50-90\%. They were randomised to either receive nebulised pGM169/GL67A gene-liposome complex or 0.9\% saline every 28 days for 1 year. The primary endpoint was the relative change in percent predicted FEV\textsubscript{1}. At 12 months there was a modest treatment effect in the gene therapy group versus placebo (3.7\%, 95\% CI 0.1-7.3; p=0.046). Response to treatment was heterogeneous, the FEV\textsubscript{1} treatment effect was seen due to stabilisation with gene therapy compared to deterioration in the placebo group. There was no significant difference in LCI between the groups at the end of the trial, the treatment effect was -0.28 (95\% CI -0.71-0.14), p=0.187. LCI may not have detected a difference due to the heterogeneity of the group, severely diseased lungs at baseline or varied response to treatment.
Studies have demonstrated that LCI can be used as a responsive outcome measure in studies involving patients with mild CF. There is less evidence regarding the use of LCI in interventional trials in more advanced disease. Evidence obtained from observational studies suggest that LCI response following treatment of exacerbation is variable, possibly due to the recruitment of previously obstructed lung units, however larger improvements do appear to correlate with worse baseline LCI and LCI may have a role in trials involving more severe disease. A minimal clinically important difference in LCI has not been established to guide interpretation and changes in management in an individual patient. Most studies have used non-commercially available equipment and standardisation to ensure reliability of MBW for use in a wider clinical setting is required.

1.4.1.6 Multi-centre MBW trials

In multi-centre trials, standardisation of MBW is important because normal ranges of results differ depending on set up and equipment. Comparable results also depend on consistent methodology across sites. An independent expert can be used to objectively determine site competency to qualify for study participation. As far as I am aware I was chosen as the first international verification expert for a multi-centre MBW trial because of my experience in performing MBW testing. Standardisation of testing for site verification has now become standard in both European and US CF trials (Saline Hypertonic in Preschoolers study, clinical trials.gov).

The multicentre trial in which I acted as the independent expert used LCI to detect a treatment response to ivacaftor in children with CF, a G551D-CFTR mutation and normal spirometry (100). The study was published in The Lancet Respiratory Medicine Journal and has been included with permission at the end of this thesis.
Prior to the initiation of the study I was chosen to assess and improve the quality of MBW testing in each centre. Equipment was standardised, 0.2% SF\(_6\) and the modified Innocor were used. Quality of testing was assessed through analysis of qualification MBW tests, performed on healthy volunteers. I identified, communicated and helped resolve problems with testing at each centre. I then qualified centres for inclusion into the study only once all technical issues had been resolved. During the study I was the blinded central investigator and analysed all study MBW tests.

The study was a multicentre, placebo-controlled, double blinded crossover study. Children were aged 6 years or older, with an FEV\(_1\) of >90% predicted, LCI of >7.4, and weighed at least 15kg at a screening visit. By the time of testing FEV\(_1\) had fallen <90% in some patients. There were two treatment phases each lasting 28 days, consisting of either 150mg ivacaftor (Vertex Pharmaceuticals, USA) or placebo twice daily. Patients were randomly allocated to receive either ivacaftor or placebo followed by a 28 day crossover period between treatment phases. The patients and all study personnel were blinded to treatment assignment. Twenty one patients were recruited and 17 of these completed the trial. Treatment with ivacaftor led to larger improvements in LCI compared to placebo. The average relative improvement in the treatment group compared to placebo group was -2.16 (95% CI -2.88 to -1.440, p<0.0001). FEV\(_1\) also showed improvement in the ivacaftor group compared to placebo, the average difference in mean FEV\(_1\) change was 8.67% (95% CI 2.36-14.97, p=0.0103). However, if patients in whom baseline FEV\(_1\) had fallen below 90% at baseline testing were excluded, there was no detectable difference in FEV\(_1\) between treatment groups, LCI still showed significant improvement. Post hoc analysis determined that LCI required a three to four times smaller sample size than FEV\(_1\) to achieve 80% power for between group comparisons.
Asthma is a chronic inflammatory condition of the lung which is common throughout childhood. In the UK 1.1 million children (1 in 11) have asthma (www.asthma.org.uk). The condition is heterogeneous; its natural history and clinical manifestations vary dramatically. Children may suffer daily symptoms or occasional exacerbations. Exacerbations may be life threatening, in the UK in 2015 21 children and young people under the age of 20 years died from asthma.

The aetiology of asthma is poorly understood but it is believed to be caused by a combination of environmental exposures with a background of genetic susceptibility. Inappropriate immune response to inhaled aeroallergens, viruses or pollutants cause inflammation, which leads to airway hyper-responsiveness and obstruction. There is evidence that inflammatory disease early in childhood affects the growth and differentiation of the lung leading to structural abnormalities.

Airflow obstruction is caused by smooth muscle constriction and airway inflammation leading to oedema, basement membrane thickening, sub epithelial collagen deposition, smooth muscle and mucous gland hypertrophy and mucus hypersecretion. Smooth muscle in asthmatic patients is hypersensitive; numerous provocation factors including aeroallergens, exercise, cold air, and pollutants can trigger bronchoconstriction.
Symptoms include intermittent dry cough and expiratory wheeze. There is often diurnal variation, symptoms are typically worse at night. Other symptoms include exercise limitation and fatigue.

Treatment involves reducing environmental exposure to stimuli, anti-inflammatory medications, treatment of co-morbid conditions including rhinitis, sinusitis and gastroesophageal reflux and management of acute exacerbations. The pharmacological management of chronic asthma involves a step wise approach depending on symptom severity. Assessing asthma control is difficult; reported symptoms often define levels of asthma control but symptom reporting is subjective. Objective measures of control are limited in children (13, 101). Medication requirements often define levels of asthma severity (102), but medication requirements are dependent on many factors including symptom perception and reporting, the physician and compliance with treatments. Identification of patients with severe disease who may be at risk of exacerbation is important so that preventative treatment can be optimised.

Asthma exacerbation treatment is dependent on severity as determined by the clinician. Children receive salbutamol and ipratropium multi dose inhalers (MDIs) via a spacer device or in more severe cases nebulised salbutamol, ipratropium and magnesium (at the time of my research children were not receiving nebulised magnesium in Edinburgh). Children tend to receive a short course of oral corticosteroids unless chronically very unstable in which case a longer course may be prescribed. If there is no improvement with inhaled bronchodilators children may also receive intravenous (IV) medications including hydrocortisone, magnesium, salbutamol or aminophylline. In Edinburgh if children require continuous IV medication they are admitted to the high dependency unit. In rare cases children may require intubation and ventilation while medications take effect.
1.4.2.1 Lung Function testing in Asthma

Currently spirometry is used in asthma clinics to assess asthma control. Spirometry is used to obtain maximal expiratory flow volume curve parameters such as FEV$_1$, FVC and FEF$_{25-75}$ and to measure reversibility following bronchodilator. Fractional exhaled Nitric Oxide (FeNO) is a biomarker which is thought to reflect airway and tissue eosinophilia and is measured in some tertiary centres.

![Flow Volume loops illustrating airways obstruction](http://www.morgansci.com).

**Figure 15:** Flow Volume loops illustrating airways obstruction (http://www.morgansci.com).

FEV$_1$ has been shown to be associated with past and future serious asthma exacerbations, symptoms and persistent wheeze in studies of children; these observations are most marked in patients with an FEV$_1$ of <80% (103-106). Better correlations between FEV$_1$ and symptoms are seen when the population FEV$_1$ is predominately abnormal (107). FEF$_{25-75}$ has been found
to be worse in children who report chronic persistent symptoms (108). FEF<sub>25-75</sub> is possibly more sensitive than FEV<sub>1</sub> in detecting reversible airway obstruction (19); however its high variability has limited its clinical use. High bronchodilator response (BDR) has been associated with poor asthma control, increased airway inflammation, bronchial hyper-responsiveness, good response to inhaled corticosteroids and worse future lung function in children (109).

Other evidence suggests that there is poor correlation between FEV<sub>1</sub> and symptoms in asthmatic children (13, 15, 110), possibly because the majority of children with chronic persistent asthma will have normal FEV<sub>1</sub> (13, 14). In studies in which correlations between FEV<sub>1</sub> and symptom scores are found, they are generally weak (111, 112).

Persistently high FeNO might suggest severe airways inflammation, poor treatment adherence, poor inhaler technique or ongoing allergen exposure. FeNO has been shown to correlate with markers of inflammation in asthma (110) but only with symptoms in some studies (112). Studies of FeNO in children have had variable results as the technique has not been standardised; the ERS have not recommended its use as a routine monitoring tool in asthma until further evidence is available (109).

Peak Expiratory Flow Rate (PEFR) measures the maximal expiratory flow following inhalation to vital capacity. It does not require expensive equipment and can be performed at home, regular PEFR monitoring may be advocated for patients with poor perception of symptoms. Evidence suggests however that using PEFR to guide treatment is no better than symptom guided management (109).
There is conflicting evidence concerning the utility of spirometry in the stable asthmatic child. Although abnormal FEV₁ is associated with poorer asthma outcomes it appears insensitive to disease in many children. In addition testing may not be feasible in pre-school children as many will have difficulty in achieving reproducible flow volume loops.
1.4.2.2 Asthma and the Small Airways

Small airway disease in asthma is increasingly being recognised as a major determinant of control and exacerbation. Studies of histopathology (113-115), High Resolution CT (116, 117) $^3$Helium weighted MRI(118) and PET (119) have all demonstrated small airways disease in asthma. The degree of small airways associated ventilation defect on $^3$He MRI has been shown to correlate with medication requirement, eosinophil count and symptom scores in asthmatic children (120). Spirometry can be insensitive in detecting early small airways disease; MBW is more sensitive whilst also being non-invasive, safe and inexpensive (45, 50, 121-123).

1.4.2.3 MBW testing in Asthma

MBW has been shown to identify abnormalities in ventilation heterogeneity in well controlled asthmatic patients with normal FEV$_1$ (50, 124, 125). The most common abnormality found is elevation of S$_{cond}$, localising disease to the small conducting airways (48, 126, 127). S$_{acini}$ has also been shown to be abnormal in some patients indicating the involvement of very peripheral airways (45, 127, 128). Ventilation heterogeneity appears to be associated with inflammation in some studies (125, 127). However abnormalities in MBW derived indices have been found to persist after inhaled corticosteroid treatment and despite normal FeNO, indicating the absence of inflammation (47, 50). Abnormalities have also been shown to persist despite salbutamol (48, 50, 126). Persistent abnormalities may be secondary to airway remodelling. Specimens taken at biopsy and autopsy from asthmatic children have shown marked changes in lung structure (113) and airway biopsy of even preschool children with wheeze have revealed thickened reticular basement membranes compared to healthy children (129).
In Edinburgh significant differences in LCI between healthy and asthmatic groups of children were demonstrated despite no difference in FEV\textsubscript{1} or FeNO (50). Thirty one children aged 5-15 years attending a tertiary asthma clinic and 29 healthy controls, aged 5-16 years participated in the study. Asthmatic patients were on different steps of the 2003 BTS treatment ladder; 6% were on step 2, 68% step 3 and 26% step 4; all patients were clinically stable at testing. MBW testing was performed using the modified Innocor and 0.2% SF6. Asthmatic patients attended 2 study visits, MBW and spirometry were performed before and 20 minutes after 200µg inhaled salbutamol or placebo, FeNO was measured before treatment on each occasion. Healthy controls attended one visit in which they performed MBW, spirometry and FeNO. Mean LCI in both groups was within the normal range but was significantly higher in the asthmatic group compared to the control group (6.69 versus 6.24, p = 0.02), despite no differences in FEV\textsubscript{1} or FeNO. There was a non-significant trend to higher S\textsubscript{cond} in the asthmatic group (0.026L\textsuperscript{-1}) versus the control group (0.017 L\textsuperscript{-1}), p = 0.06. There was no difference in S\textsubscript{acin} between the asthmatic and control groups (0.14 L\textsuperscript{-1} versus 0.12 L\textsuperscript{-1} respectively, p = 0.23). The CV of LCI in the asthmatic group was 4.2%. There was no significant change in LCI, S\textsubscript{cond} or S\textsubscript{acin} following salbutamol despite significant increases in FEV\textsubscript{1}. Abnormalities detected by LCI could not fully be explained by inflammation, as there was no correlation with FeNO, or bronchospasm as there was no improvement following salbutamol.

In Sigurs et al’s study of asthma and allergy patterns in childhood following RSV positive bronchiolitis LCI was significantly elevated in asthmatic children (n=21) compared to non-asthmatic children (n=117) at 18 years of age (125). However they found that LCI did correlate with FeNO ($r^2 = 0.29$, p=0.003) indicating inflammation may play a role in ventilation heterogeneity. LCI also correlated with airway hyper-responsiveness after dry air challenge ($r^2 = 0.26$, p=0.005). Asthmatic patients in this study were different from Macleod’s study;
asthmatic children were not necessarily attending a tertiary asthma clinic and had to have persistent episodes of physician diagnosed wheeze.

Gustafsson studied N\textsubscript{2} MBW in 15 asthmatic children (aged 12.3-18.6 years) and 11 healthy controls (48). The mean pre-bronchodilator FEV\textsubscript{1} % predicted of the asthmatic group was 77% indicating relatively severe disease. LCI and S\textsubscript{cond} were significantly abnormal compared to healthy controls but there was no difference in S\textsubscript{acin}. LCI fell following bronchodilator so that there was no difference with the control group. Post bronchodilator S\textsubscript{cond} did fall but remained significantly abnormal compared to the control group.

Sonnappa et al compared the repeatability of LCI, S\textsubscript{cond} and S\textsubscript{acin} in 62 children with stable recurrent wheeze (mean (SD) age 5.4 (0.6) years) to 28 healthy controls (6.1 (0.7) years old) (130). Children attended 2 visits, on one visit children repeated 2 sets of MBW (performed with 4% SF\textsubscript{6} and a RMS) with a 20 minute interval without intervention and on another occasion they were given 200µg salbutamol after the first set of MBW. Visits occurred a median of 5.3 months apart in the wheezy children and 9 months in the healthy children. Details of symptom frequency and severity were not reported and spirometry was not performed, applicability of results to other populations may therefore be limited. There were no significant differences in the short term (within visit) variability of LCI, S\textsubscript{cond} or S\textsubscript{acin} between the healthy and wheezy groups at baseline. S\textsubscript{cond} and S\textsubscript{acin} had larger baseline variability than LCI; higher variability reduces the ability to discriminate between health and disease and to determine significant change. There were no changes in any outcome over the 2 visits and the variability of LCI and S\textsubscript{acin} were similar in both groups. S\textsubscript{cond} was significantly more variable over the 2 visits in the group with recurrent wheeze compared to the healthy group but did detect a bronchodilator response, which was not detected by LCI.
Verbanck et al detected ventilation inhomogeneity in adults with mild asthma; they also found that abnormalities in $S_{\text{cond}}$ representing ventilation heterogeneity in the small conducting airways were not completely reversible with salbutamol (126). The same group found that $S_{\text{acin}}$ was higher in stable asthmatic patients receiving $\geq 500\mu g$ beclomethasone dipropionate equivalent compared to patients receiving smaller doses and that $S_{\text{acin}}$ correlated with alveolar nitric oxide ($r=0.61$, $p=0.015$) (128). Patients with abnormal $S_{\text{acin}}$ were also shown to have lower FEV$_1$ compared to patients with normal $S_{\text{acin}}$ (131). Verbanck’s group studied adults in whom asthma pathophysiology may differ from children. Unlike in Macleod and Gustafsson’s studies, Verbanck asked patients to achieve a set tidal volume, which may have aided detection of abnormalities in $S_{\text{acin}}$.

Farah et al also found that $S_{\text{acin}}$ and $S_{\text{cond}}$ were both abnormal in stable asthmatic adults and that both indices were associated with asthma control assessed using the Asthma Control Questionnaire - 5 (ACQ-5) (132). MBW was performed in 105 asthmatic adults using N$_2$ and a set tidal volume protocol. Patients were categorised as poorly controlled if their ACQ-5 score was $\geq 1.5$ and well controlled if their score was $\leq 0.75$. Poorly controlled patients had worse FEV$_1$, FeNO, $S_{\text{cond}}$ and $S_{\text{acin}}$ values than the well-controlled group. However $S_{\text{cond}}$ and $S_{\text{acin}}$ were not independent predictors of ACQ-5 scores, unlike FEV$_1$ and FeNO. Baseline FEV$_1$ was low in both the well and poorly controlled groups (83 and 73% predicted respectively) which indicates a group of adults with relatively severe disease.

In Keen et al’s study of asthmatic children LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$ were all significantly elevated in asthmatic patients compared to controls, but $S_{\text{cond}}$ was the most common abnormality in asthmatic children (abnormal in 31/47 children); in contrast only 7/47 children had abnormal LCI (127). Children who had uncontrolled asthma (based on an asthma control score of $>20$)
had significantly higher LCI (p=0.04) and S\text{cond} (p=0.04); there was no significant difference in FEV$_1$ between groups with uncontrolled and controlled asthma. LCI and S\text{cond} were found to correlate with FeNO and alveolar NO, whereas S\text{acin} only correlated with alveolar NO. Airway hyperresponsiveness was associated with higher S\text{cond} but not S\text{acin}. 47 atopic asthmatic children and 74 healthy controls were studied using 4% SF6 and a RMS, airway hyperresponsiveness was assessed using a dry air hyperventilation challenge. Quality control criteria required for reporting of S\text{cond} and S\text{acin} were regular tidal breathing with a tidal volume of 10-15ml/kg. Only 36/74 healthy children performed MBW well enough to fulfil these quality criteria, all the asthmatic patients managed. The correlations between S\text{cond} and S\text{acin} with FeNO and alveolar NO suggest that small airways disease in asthmatic children is associated with inflammation. LCI appeared less sensitive than S\text{cond} in detecting disease in stable asthmatic patients, in whom the small conducting airways appear to be the major site of the disease process. However very peripheral airways may also be abnormal, reflected in the elevation of S\text{acin} in some children.

Indices of MBW have been shown to correlate with evidence of small airways disease in asthma detected using different imaging techniques. Baseline S\text{cond} in 14 asthmatic adults correlated with airway closure secondary to methacholine challenge, detected using combined SPECT and HRCT scans (133). In 37 asthmatic adults S\text{acin} correlated with abnormalities in acinar diffusion identified during $^3$He MRI scanning (134).

Evidence suggests that MBW can detect small airways disease in asthma and could be used to further our understanding of the disease. However its clinical utility in monitoring asthma still has to be established. Indices are often abnormal in more severe disease but in these children FEV$_1$ may be sufficiently sensitive. In mild disease results have been higher than in control
groups but have fallen within normal ranges rendering their use as a marker for an individual less clear. MBW may be more useful in detecting bronchial hyper-responsiveness in symptomatic individuals when FEV$_1$ is not sufficiently sensitive.
1.4.2.4 Asthma and Exercise

The clinical presentation of exercise induced asthma (EIA) includes cough, wheezing, shortness of breath and/or chest tightness. The reported prevalence varies; it affects approximately 80-90% of people with asthma and 5-20% of the general population (135, 136). Asthma medications can prevent EIA; however in some patients, symptoms persist despite medication.

Exercise induced bronchoconstriction (EIB) can occur during or up to 30 minutes after cessation of exercise (137, 138). The mechanism by which EIB is caused is unclear and may vary between individuals. Many experts speculate that EIB is due to changes in airway physiology, including increased airway osmolarity, caused by hyperventilation (136, 138, 139). There is an inflammatory response associated with EIB, which is thought to be caused by this change in airway osmolarity (136, 138). Inflammatory mediators interact with smooth muscle cells to cause bronchoconstriction. In addition airway cooling during hyperventilation stimulates cholinergic receptors to increase airway tone and secretions. Inhalation of cold air also causes pulmonary vasoconstriction, then when exercise stops, secondary reactive hyperaemia occurs; this causes vascular bronchial congestion, oedema and further airway narrowing (136). Rapid rewarming is not thought to contribute to EIB in children (140).

An exercise challenge test is used to diagnose EIA in patients with symptoms during or after exercise. Standard practice is to perform spirometry before and after 4-6 minutes of vigorous exercise, a deterioration in FEV₁ of at least 10% is diagnostic of EIA (136, 138). Correlation between reported symptoms and clinical testing is poor; many children who experience exercise related symptoms do not demonstrate a fall in FEV₁ on exercise testing (135).
Bronchial provocation studies, examining airway hyper-reactivity have involved examination of ventilation heterogeneity. Indices of ventilation heterogeneity obtained through SBW have been shown to deteriorate with cold dry-air hyperventilation challenge in children and adults with asthma (121, 122). SBW and MBW indices have been shown to correlate with bronchial hyper-responsiveness measured following methacholine (47) and cold dry-air hyperventilation challenges (47, 122, 127).

Exercise induced symptoms represent a more 'natural' phenomenon compared to events precipitated by pharmaceutical agents. Studies of hyperpolarised helium MRI have demonstrated heterogeneous ventilation in asthmatics following exercise testing (141). Indices of MBW may offer an alternative method of detecting exercise induced ventilation inhomogeneity.
1.4.2.5 Asthma Exacerbation

Asthmatic exacerbations increase steroid burden, reduce school attendance, cause hospitalisation and at worst can be fatal. The risk of exacerbation is increased in children with poor adherence to treatment, poor asthma control, frequent exacerbations and elevated FeNO (109). In exacerbation of asthma, hyper-responsive airways constrict due to inflammation, bronchospasm and mucus plugging, causing breathlessness. There is evidence that much of the dyspnoea is caused by constriction of small terminal airways (119).

Venegas et al used positron emission tomography (PET) to show that bronchoconstriction induced by methacholine leads to patchiness in ventilation in asthmatic patients (119). Ventilation heterogeneity occurred due to clustering of constricted terminal bronchioles and was independent of large airway disease. The group then used a computational model to explain how even minimal heterogeneity in ventilation disrupts the symmetry of the bronchial tree due to short and long range feedback mechanisms to cause large clusters of poorly ventilated lung units.

Zeidler et al performed HRCT imaging, spirometry and closing volumes in 10 patients with cat induced asthma before and 6 and 23 hours post exposure to cat allergen (117). All patients had an acute drop in FEV₁ of ≥20% but by 6 hours FEV₁ had returned to baseline, there was no further change in FEV₁ at 23 hours. FEF₂₅-₇₅ was abnormal at 6 hours but returned to baseline by 23 hours. End expiratory HRCT scans and closing volumes demonstrated increased air trapping at 6 and 23 hours following exposure, indicating the persistence of small airways disease once larger airways obstruction had resolved. At 23 hours post exposure, HRCT image analysis revealed increased small airways hyper-responsiveness to methacholine.
challenge compared to before allergen exposure. The implications of this study were that small airways may be significantly involved in late asthmatic responses to allergens and small airway associated bronchial hyper responsiveness may persist long after exposure.

In Zeidler’s study small airways disease during asthmatic exacerbation was detected using multiple HRCT scans. As previously discussed ventilation inhomogeneity detected using washout techniques deteriorates following bronchial provocation challenges and can predict bronchial hyper-responsiveness (47, 121, 122). MBW may offer a way of detecting small airways disease during asthmatic exacerbation without exposure to radiation.

Thompson et al studied the effects of asthmatic exacerbation on indices of phase III slope analysis in 18 adults (142). The group used N₂ MBW with a fixed tidal volume protocol and compared results to those of 19 stable asthmatic patients. Patients were included if their PEFR was ≤50% of their personal best on admission, testing occurred within 48 hours of admission but a minimum of 4 hours after salbutamol. Predicted values of $S_{cond}$ and $S_{acin}$ were derived from a previous study of 180 healthy adults (40). In patients suffering exacerbation, the median FEV₁ was 59% of predicted value, $S_{cond}$ 185% and $S_{acin}$ 225%. FEV₁ correlated with $S_{acin}$ ($r=-0.67, p=0.006$) but not $S_{cond}$. $S_{acin}$ also correlated with the level of pre admission treatment ($r=0.59, p=0.016$). Median $S_{cond}$ and $S_{acin}$ normalised within the 11 patients who attended follow up at 4 weeks post discharge. LCI was not presented. A fixed breathing pattern was adopted to aid measurement of phase III slopes, but a fixed tidal volume is not feasible in many children and severely breathless adults. The authors concluded that acinar disease was an important determinant of airways obstruction as measured by FEV₁. However this was an observational study and causation could not be established.
MBW can detect asthmatic exacerbation in adults and may offer a method of detecting persistent abnormalities following normalisation of FEV1. Persistent small airways disease may indicate ongoing bronchial hyper responsiveness. There are no published studies regarding the effects of asthmatic exacerbation on MBW indices in children.
1.4.2.6 Asthma Medications and MBW

The small airways have been shown to be involved in the pathophysiology of asthma, the effects of some asthma treatments on small airways have been investigated with imaging and MBW. Studies using MBW have shown that salbutamol does not completely normalise ventilation inhomogeneity in asthma (48, 50, 126). The incomplete resolution of changes seen on MBW testing after salbutamol inhalation has been hypothesised to be due to structural changes within the small conducting airways and mechanical obstruction of inhaled therapies. Inhaled therapies may not reach the most diseased areas of the lung and settle instead in well ventilated areas exacerbating ventilation inhomogeneity. This could explain why intravenous therapies are required in severe asthmatic exacerbations.

There is evidence of improvement in ventilation inhomogeneity following inhaled corticosteroids (IHC). Goldin et al used HRCT to demonstrate improvements in gas trapping following a 4 week double blind, randomised trial of extra fine IHC versus standard IHC (143). The group treated with extra fine IHC had better improvement in gas trapping and less air trapping on methacholine challenge after treatment than the standard IHC group.

MBW derived indices have been shown to predict and correlate with symptomatic improvement of asthmatic adults following inhaled corticosteroids. Farah et al studied 105 asthmatic adults before and 3 months after high dose ICS treatment and correlated changes in symptom control questionnaire with $S_{\text{cond}}$ ($r=0.32$, $p=0.02$) and $S_{\text{acin}}$ ($r=0.41$, $p<0.01$) (132). The same group found that baseline $S_{\text{cond}}$ correlated with improvement in patient symptom scores following upwards titration of ICS treatment after 8 weeks ($r=-0.64$, $p<0.01$) (144).
Moreover, S_{acin} correlated with deterioration in patient symptom score following downwards titration of ICS treatment (r=0.40, p<0.01) (144).

Downie et al studied 18 sub-optimally controlled asthmatic adults who were given a 3 month course of inhaled beclomethasone dipropionate (47). Ventilation heterogeneity, assessed using N_{2} MBW, improved following inhaled corticosteroid treatment. LCI improved from 8.54 to 7.78, S_{cond} from 0.067 to 0.052 and S_{acin} from 0.159 to 0.142 (p=0.001, 0.009 and 0.035 respectively). FEV_{1} also improved from 80.1 to 85.9% predicted (p<0.001). After treatment abnormal S_{cond} (>0.037 L^{-1}) had a high sensitivity and specificity for the presence of persistent airway hyper responsiveness (90.0% and 87.5% respectively). Airway hyper-responsiveness was assessed using a methacholine dose response ratio (DDR), percentage fall in FEV_{1}/methacholine dose. In a multiple linear regression model following IHC treatment S_{cond} was the only predictor of log DRR (r^{2}=0.64, p=0.004).

A study by Zeilder et al. examined the effect of montelukast on gas trapping on HRCT as a measure of small airways disease in 16 mild-to-moderate steroid-naive asthmatics (116). The study was a double-blind crossover study comparing the effect of montelukast versus placebo after 4 weeks of treatment; HRCT was performed at residual volume, before and after methacholine at the end of each study period. Montelukast resulted in significantly less regional air-trapping on HRCT on the pre-methacholine images when compared with placebo, as well as improvement in total quality of life scores and symptom sub-scores. This difference was not detected with spirometry. However, montelukast treatment had no effect on increases in regional air-trapping on HRCT in response to methacholine.
The effects of treatments on small airways disease in asthma needs further evaluation. If small airways disease persists despite current treatments new therapies which target this disease should be sought and could improve asthma care.
1.5 **Limitations of MBW**

Many of the limitations of the MBW test have been discussed throughout this introduction. Primarily the minimal clinically meaningful differences in indices of MBW have not been determined and are required so that MBW can be used in a clinical setting.

As discussed in depth testing has not been standardised and this is necessary to produce valid comparable results. A variety of different analysers, gases and software systems have been used, which have an effect on indices. A commercially available device produced with a view to optimise accurate concentration and flow alignment which would be acceptable for use across all age ranges and allow calculation of phase III slope indices is required. This would allow studies to produce and collate data to produce universal reference equations.

The value of LCI is becoming well established in interventional trials in mild lung disease. However in more marked disease there are concerns that washout traces from patients with partial lung collapse or widespread peripheral airway closure secondary to atelectasis may be misleading.

Indices of phase III slope appear dependent on tidal volume and in the paediatric population achieving set tidal volumes is more challenging. In addition phase III slope indices are time consuming to produce and dependent on the skill of the investigator in their measurement.
Lastly MBW is more time consuming than performing spirometry, particularly in patients with more severe disease and this might not be appropriate in the clinical setting. In addition although most children tolerate the test very well some young children may become restless.
1.6 The UK Cystic Fibrosis Gene Therapy Consortium

The UK Cystic Fibrosis Gene Therapy Consortium (UKCFGT) was formed in 2001 from the UK’s three leading CF gene therapy groups at Edinburgh and Oxford University and Imperial College London (www.cfgenetherapy.org.uk). The consortium’s aim is to develop gene therapy for CF into a clinical reality. The consortium is funded by the CF Trust (www.cysticfibrosis.org.uk), Flutterby Fundraisers, Just Gene Therapy and private donations.

![Figure 16: UK Cystic Fibrosis Gene Therapy Consortium](image)

The consortium has recently published a multi-dose gene therapy study in which patients in Edinburgh and London participated, the results are reported earlier in this thesis and show some promising results (99).
Prior to the clinical trial the consortium ran a “tracking study” and a “run-in” study to select the best assays for detecting change in CF disease with treatment. The tracking study identified assays that could detect improvement following treatment of CF exacerbation with IV antibiotics (90). The run-in study was designed to identify which assays could sensitively detect CF disease progression. Some patients who participated in the run-in study were selected for gene therapy trial. The run-in study involved approximately 200 children and adults who attended visits in London and Edinburgh. Methods of assessing lung disease included lung function tests (LCI and spirometry), imaging, laboratory tests, quality of life measures and exercise testing.

Once the gene therapy product had been developed and its safety assessed in animal models, a pilot study was performed to assess safety and efficacy (145). The vector used in the aforementioned multi dose gene therapy study was GL67A/pGM169, a combination of cationic liposome (GL67A) and plasmid DNA expressing CFTR (pGM169). The consortium is now working towards developing a lentiviral vector for gene therapy (146).

1.6.1 Previous CFGT Consortium Clinical Research Fellows

The development of the MBW test in Edinburgh was well established prior to my post because of the work of previous CFGT Consortium Research Fellows. Dr Alex Horsley and Dr Kenneth Macleod adapted the Innocor to perform MBW and validated its use in clinical studies (26). With Dr Nick Bell they produced a standard operating procedure for performing the test and a spreadsheet for determining indices of phase III slopes. Dr Macleod and Dr Bell taught
me how to perform the test and analyse the data on software we had courtesy of Professor Per Gustafsson (Department of Paediatrics, University of Gothenburg, Sweden).

1.7 The Children’s Clinical Research Facility

The Children’s Clinical Research facility is part of Edinburgh’s Clinical Research Facility based in the city’s Royal Hospital for Sick Children. It is part of both NHS Lothian and Edinburgh University and provides a child friendly environment in which to conduct paediatric studies. All of my patient visits were performed in this department.
2 Projects

Within this thesis I report 5 projects that were achieved during my period of research:

1. The effect of body position on MBW derived indices in healthy children and children with cystic fibrosis (chapter 4)
2. Longitudinal variability of MBW derived indices in children with cystic fibrosis (chapter 5)
3. Assessment of MBW in children with stable asthma (chapter 6)
4. The effects of exercise on MBW derived indices in healthy children and children with asthma (chapter 7)
5. Evaluation of MBW derived indices in asthmatic children during and after exacerbation requiring corticosteroids (chapter 8)

The projects had many common methodologies, these will be described together, with additional project specific methods provided within relevant sections.
2.1 Aims and Objectives of Projects

2.1.1 The effect of body position on MBW derived indices in healthy children and children with cystic fibrosis

To investigate the effect of posture (sitting vs. lying) on MBW derived indices in healthy children.

To investigate the effect of posture (sitting vs. lying) on MBW derived indices in children with CF.

To compare the effects of posture on MBW indices and spirometry in children with and without CF.

2.1.2 Longitudinal variability of MBW derived indices in children with cystic fibrosis

To assess the longitudinal variation in MBW derived indices in children with CF.

To investigate the correlation between MBW derived indices, spirometry and CT findings in children with CF.

2.1.3 Assessment of MBW in children with stable asthma

To assess whether indices of MBW are abnormal in children with stable asthma.

To assess whether indices of MBW related to asthma control.

To compare whether indices of MBW were better able to identify asthma control than spirometry.
2.1.4 The effects of exercise on MBW derived indices in healthy children and children with asthma

To investigate changes in indices of MBW in asthmatic individuals following exercise.

To relate the changes in MBW derived indices to changes in spirometry and reported exercise induced symptoms.

To determine whether baseline indices of MBW can predict exercise induced lung function responses.

To investigate the effects of salbutamol on MBW derived indices following exercise in asthmatic children.

2.1.5 Evaluation of MBW derived indices in asthmatic children during and after exacerbation requiring oral corticosteroids.

To determine whether MBW derived indices are abnormal during an exacerbation of asthma.

To investigate the effects of salbutamol multi-dosing on MBW indices during an exacerbation.

To measure indices of MBW in children 4-6 weeks after discharge from hospital.

To relate persistent abnormalities in MBW to symptoms, characteristics of the child and severity of exacerbation.
3 Common Methods

3.1 Multiple Breath Washout (MBW)

3.1.1 Equipment

3.1.1.1 Innocor (Innovision, Denmark)

The Innocor is a device designed originally to measure cardiac output. It uses photoacoustic spectroscopy technology to measure the concentration of specific gases in exhaled breath. The patient interface that comes with the Innocor was not used and was replaced with a custom made set up with a flow meter, gas sampling line, bacterial filter and mouthpiece.

![Multiple Breath washout interface: T-piece, Flowmeter with sampling line, filter and mouthpiece](image)

*Figure 17: Multiple Breath washout interface: T-piece, Flowmeter with sampling line, filter and mouthpiece*
3.1.1.2 Pneumotach/Flowmeter (Hans Rudolph USA)

The pneumotach was used to measure flow. It has a small dead space to prevent rebreathing of exhaled air. The dead space of the mouth piece, pneumotach and bacterial filter was 36mls.

A gas sampling line consisting of a 20G needle attached to nafion tubing was inserted into the distal part of the flow meter and connected to the Innocor.

3.1.1.3 Mouthpiece (Hans Rudolph, USA), nose clip and bacterial filter (Air Safety, UK)

A mouthpiece was used to deliver gases directly to the mouth. A nose clip was essential in preventing escape of gas. An inspiratory leak during wash-in phase prevents equilibration of the inert gas and an expiratory leak during washout will cause inaccuracies in expired volumes. A bacterial filter was necessary for infection control.

3.1.1.4 Interface Positioning Stand

The mouthpiece and flow meter were positioned in front of the patient using a goose necked microphone stand. The flow meter was positioned so that the gas sampling needle and flow transducer lines were not pointing downwards in case moisture should collect and travel down the lines. These lines were positioned between 9 and 3 o’clock.
3.1.1.5 Sulphur Hexafluoride (SF₆) (BOC Ltd)

SF₆ was the inert gas that was used throughout MBW testing during my research. Being completely inert it is neither absorbed nor excreted by the body. It is however a potent greenhouse gas. It can be supplied at different concentrations. I used 0.2% (2000ppm) SF₆ concentration in medical quality dry air, supplied by BOC Ltd.

3.1.1.6 Flow past Circuit

The SF₆ canister was connected to a rebreathing bag (to maximise availability of SF₆ during inspiration) which was connected to approximately 0.5m of tubing. The tubing connected to a T-piece, on the other end of which was another length of tubing, approximately 1-2m long. This longer section of tubing functioned as the exhaust. The T-piece was attached to the flow meter during the wash-in phase and then removed during washout.

Figure 18: Flow-Past Circuit (UKCFGT consortium SOP)
3.1.1.7 Data Analysis Software

The raw flow and gas concentration data was required to be converted into volume and end-tidal gas concentration for each breath of the washout. Custom software was available for data analysis, courtesy University of Gothenburg, Dept. Paediatric Physiology, Gothenburg, Sweden. A spreadsheet designed by Dr Alex Horsley (previous Research Fellow with the UKCFGT consortium) was used to calculate phase III slope indices.

I also had the use of a second more automated software package “Simple Washout” designed by Dr Nick Bell (previous Research Fellow with the UKCFGT consortium), however it could only be used to calculate LCI and not phase III slope indices.

3.1.2 Technique

As described MBW testing throughout this thesis was performed using the modified Innocor and exogenous 0.2% SF$_6$. In exogenous gas MBW there are 2 phases, wash-in and washout. Throughout testing the patient was encouraged to breathe regularly, through a mouthpiece, with a nose clip on. The test was performed with the patient sitting and watching television (as a distraction). A pneumotach attached to the mouthpiece measured respiratory flow and gas samples were taken from the pneumotach at 10ms intervals for analysis of concentration by the Innocor gas analyser. The Innocor displayed online SF$_6$ concentration with an approximate 1.5 second delay. Displayed numerical concentration values were only updated every second so actual peak values may have differed. Data were extracted after testing for offline integration of flow and concentration signals and generation of MBW indices.
The set-up of equipment and testing room were standardised to maximise reliability. The room in which testing was performed was large and well ventilated to minimise SF₆ rebreathing by the subject during the washout. In addition exhaled SF₆ was cleared from the immediate area surrounding the subject by a fan. Ambient temperature and humidity were recorded each day testing occurred.

Testing was explained to participants. Patients were shown the equipment and the disconnection of gas supply was demonstrated. Patient positioning was adjusted to avoid slouching and crossing of legs was discouraged. Patients were given time to get used to the mouthpiece and nose clip and any potential leaks were corrected before testing began. Patients were asked to breathe regularly and avoid breaking the mouth piece or nose clip seal. Patients were warned that they might get a dry mouth and that water would be available after each test.

During the wash-in phase, the patient breathed in 0.2% SF₆ until it was evenly distributed throughout the lung. The gas flow was set at between 10-15L/minute depending on the size of the patient and a reservoir bag was placed in line with the tubing to maximise the volume of SF₆ available during inhalation. Wash-in was determined as complete when the maximum and minimum concentrations of SF₆ displayed on the Innocor were within 0.003% for 3 successive breaths.

Following equilibrium of gas concentration the gas supply was disconnected during an exhaled breath, by removal of the T piece, and the washout phase began. Identification of exhalation was done by watching the chest rise and fall and was difficult in some children. The T piece was covered immediately after removal to prevent escape of SF₆ into the immediate vicinity of the patient and the SF₆ supply was turned off. The patient continued to breathe regularly.
through the mouthpiece until maximum SF$_6$ concentration fell to less than 0.003% for 3 successive breaths. MBW derived indices are derived after end tidal SF$_6$ concentrations ($C_{et}$) falls to $1/40^{th}$ of that at the end of the wash-in phase ($C_{init}$). The cut off of 0.003% is less than $1/40^{th}$ of 0.2% but it allowed a margin of error to prevent premature termination of tests being discovered on subsequent offline analysis.

![Figure 19: Innocor online signal concentration display](image)

Each test took approximately 5-10 minutes, with slower wash-in and washout phases in more diseased lungs. MBW derived indices are reported as the mean of 2-3 acceptable tests. Testing was performed a minimum of three times, but was repeated more often if tests were technically unacceptable as discussed in the next section.
3.1.3 Quality Control

Calibration of the Innocor with the flowmeter and calculation of the Innocor’s flow-gas delay occurred before testing and was repeated at 4 hourly intervals. If the flowmeter was cleaned or replaced the ability of the Innocor to interpret pressure changes to linear flow at the flow meter was calibrated and corrected through a process of linearization. Calibration was performed with a bacterial filter in place as a filter can affect the flow through the flowmeter. In addition, the Innocor was serviced annually by Innovision.

Flowmeter calibration and linearization were performed using a 3 litre calibration syringe at room temperature. Linearization involved filling and emptying the calibration syringe into the flowmeter at a range of different rates to simulate different respiratory flow rates. This was continued until the error in each manoeuvre across the range of flow rates was less than 1%. The daily flowmeter calibration involved a more limited number of filling and emptying manoeuvres at different rates to ensure that accuracy was within 2%. If accuracy was out with 2%, equipment was checked and linearization repeated.

To calibrate flow gas delay, eleven slow exhaled breaths followed by sharp inspirations were performed into the flowmeter via a filter. This was done to obtain a sudden transition from expiration to inspiration and a sharp fall in CO₂ at the flowmeter. The Innocor calculated the delay from change in flow to drop in CO₂ concentration within the analyser. Flow-gas delay was recorded for later use in the analysis of raw data. During analysis an additional 50ms was added to the calculated flow gas delay to correct for the Innocor’s response time to changes in SF₆ concentration as discussed previously. Flow gas delay variation was within 20-40mls on testing days.
Only technically acceptable tests were accepted for analysis. SF₆ was required to be adequately washed in, disconnection had to occur during expiration and washouts had to be complete to be analysed. Children were distracted throughout testing with a DVD and encouraged to maintain a relaxed regular breathing pattern. Tests were not excluded on the occasion that a child exhibited erratic breathing patterns so long as other quality control criteria were met.

Care was taken to ensure that 0.2% SF₆ was adequately washed in. This was checked during testing by ensuring that the difference between inhaled and exhaled SF₆ concentration was <0.003% for 3 successive breaths. During analysis this difference was checked and tests in which wash-in was >0.003% were discarded. Insufficient wash-in reduces the volume required to wash out the lungs, hence invalidating results. Failure of SF₆ to equilibrate during wash-in may have been caused by insufficient wash-in time (in more severe disease), insufficient gas flow in the flow past circuit (causing rebreathing of exhaled air) or a leak within the patient interface (at the mouth or nose).

Disconnection of SF₆ at the start of the washout phase had to occur during expiration. If disconnection occurred during inspiration an unquantifiable volume of additional inhaled SF₆ would be added to the exhaled SF₆ volume of the first breath and would invalidate the washout. In a correctly disconnected test, SF₆ concentration fell to (almost) zero on the first inspiration post disconnection. Tests in which SF₆ disconnection occurred during inspiration were easily identified as SF₆ concentration did not fall to zero and were excluded from analysis.
The Innocor reset every 300 seconds or 5 minutes to maintain accuracy. During resets there was a one second loss of flow data during which the Innocor recorded flow as -100L/s. In offline analysis resets were flattened by using the flow immediately prior to the reset, however this may have slightly distorted gas volumes. Resets were particularly important to avoid in the first 10 breaths of the washout when the largest SF$_6$ volumes were being expired. If the test was approaching 5 minutes at the end of wash-in, disconnection was delayed until after the reset.

Only tests in which SF$_6$ was sufficiently washed out were analysed. To terminate a test maximum end tidal SF$_6$ concentration ($C_{et}$) had to be less than one 40th of end tidal concentration at the end of wash-in ($C_{init}$). As discussed during testing the maximum SF$_6$ concentration displayed on the Innocor had to be less than 0.003% for 3 successive breaths before the test was terminated. On offline analysis, tests were termed completed and therefore
acceptable if \( C_e \) fell below the termination concentration (\( 1/40^{th} C_{in} \)), the breath volume was larger than a stipulated minimum termination volume (MTV) and that breath was followed by another breath which met both criteria. MTV was set to avoid terminating washouts on the basis of an aberrantly small breath in which end tidal concentration was not reflective of peripheral gas concentration. MTV (ml) was calculated by doubling the predicted airway dead space for a given height \( (2.9 \times \text{Height (in cm)} - 215) \).

3.1.4 Analysis

Gas concentration and flow data for each test was recorded by the Innocor and could be accessed through a Microsoft Windows program. Tests were accessed through Windows, saved on a memory stick and analysed offline using software provided courtesy of University of Gothenburg. Software incorporated the flow and gas concentration data using the flow gas delay and produced graphic illustrations of SF\(_6\) concentration versus expired volume for each breath.

Analysis involved determining when disconnection of SF\(_6\) occurred, checking to ensure each breath and end tidal concentration were correctly identified and determining when the washout terminated. Functions on the software allowed manual override to correct errors. Identification of phase III slope was set for between 65 and 95% of expired breath volume but required manual correction in most washouts.
Figure 21: SF$_6$ expirogram: phase III slope identified in red by altering breath volume percentage at which phase III slope starts and finishes.

Analysis using University of Gothenburg software produced LCI and FRC values. Indices of phase III slopes were determined following input of analysed washouts into a UKCFGT consortium spreadsheet. UKCFGT consortium software did not allow exclusion of breaths in which there was no clear SIII (due to a cough or small tidal volume).

The calculation of MBW indices has been discussed in detail. The following calculations were used within this thesis.

Lung Clearance Index (LCI) was calculated by dividing the total amount of expired gas during a washout (CEV) by the functional residual capacity (FRC).

$$\text{LCI} = \frac{\text{CEV}}{\text{FRC}}$$
FRC was calculated during a washout by dividing the volume of expired inert gas ($V_{gas}$) by the difference between end tidal gas concentrations at the start ($C_{init}$) and end ($C_{end}$) of washout.

$$FRC = \frac{V_{gas}}{C_{init} - C_{end}}$$

LCI in this thesis is reported as the mean of at least 2 tests in which LCI differed by no more than 20% and FRC by less than 10%.

In phase III slope analysis SIII was divided by mean slope $S_{F6}$ concentration to produce a normalised slope ($SnIII$). $SnIII$ was multiplied by expired breath volume to correct for the effects of variable breath volume.

$S_{cond}$ was calculated by determining the slope of the graph of $SnIII$ vs number of lung turnovers (TO) from TO=1.5 to TO=6. (TO at each point in washout calculated by dividing the cumulative expired volume at that time by FRC).

$$S_{cond} = \text{delta } SnIII \ (1.5-6.0 \ TO)$$

$S_{acin}$ was calculated by subtracting $S_{cond}$ multiplied by the number of lung turnovers at the first breath from $SnIII$ at the first breath.

$$S_{acin} = SnIII\ \text{breath 1} \ - \ (S_{cond} \times TO\text{breath 1})$$

$S_{cond}$ and $S_{acin}$ were reported in this thesis as the mean of at least 2 tests not excluded for excess variability in either LCI (>20%) or FRC (>10%).
3.2 **Spirometry**

3.2.1 **Equipment**

The EasyOne™ Spirometer uses digital ultrasonic flow measurement technology to measure spirometric volumes. Ultrasonic spirometers do not cause resistance to air flow and have no moving parts which may be easily broken. However ultrasonic spirometers may record slightly lower volumes than heated wire spirometers (147).

The EasyOne™ is portable and has inbuilt software to calculate whether technically acceptable tests have been achieved. Tests can be stored on the meter and downloaded after testing. The Easyone is calibrated with a 3 litre syringe and has been shown to remain accurate for prolonged periods of time (148, 149). The patient blows into a disposable spirette which is changed between patients for infection control.

![EasyOne portable spirometer](http://www.nddmed.com)

*Figure 22: Easyone portable spirometer (http://www.nddmed.com)*

3.2.2 **Technique**

Spirometry required the child to inhale to total lung capacity and then to perform a full forced expiratory manoeuvre. Testing complied with ATS/ERS standards(3), which were discussed
in the introduction. If the child had no experience of spirometry then they were shown what to do and allowed to practice. Unless otherwise stated children stood during testing and wore nose clips. The child was asked to repeat testing to try to achieve 3 technically acceptable tests but testing did not exceed 8 manoeuvres. The difference between the largest and next largest FVC and FEV₁ had to be ≤0.150L unless FVC was less than 1.0L in which case the difference had to be ≤0.10L. The best FEV₁ and FVC and the FEF<sub>25-75</sub> of the manoeuvre with the best combined FEV₁ and FVC were recorded.

Indices generated by spirometry including FVC, FEV₁ and FEF<sub>25-75</sub> have already been discussed. Standard deviation scores and percent predicted FEV₁, FVC and FEF<sub>25-75</sub> were calculated for each patient using GLI equations (16).
4 Project: The effect of body position on MBW derived indices in healthy children and children with cystic fibrosis

4.1 Introduction

Standardisation of MBW testing is important in order to produce valid results. For effective standardisation it is important to establish the factors that affect MBW results within an individual over time. One factor that theoretically might affect MBW derived indices is posture, which is significant because infants undergo MBW testing in a supine position, whereas in older children measurements are made in a sitting position. It is therefore important to understand whether changes in posture influence measures of MBW and if so the degree of effect, and the difference in effect in health and disease.

There is evidence that posture affects lung volumes (56, 57) and ventilation distribution (58) and imaging has demonstrated atelectasis in the lungs of supine children (59, 60). However studies assessing the effects of posture on indices derived from MBW have been contradictory. One small study has shown no change in LCI in asthmatic and healthy children on lying supine (57). Other small studies of healthy men have demonstrated rises in $S_{cond}$ (58) and LCI in the supine position (61). We sought to clarify how posture affects MBW derived indices in healthy children and children with CF.
4.2 **Aims**

To investigate the effect of posture (sitting vs. lying) on MBW derived indices in healthy children.

To investigate the effect of posture (sitting vs. lying) on MBW derived indices in children with CF.

To compare the effects of posture on MBW indices and spirometry in children with and without CF.

4.3 **Methods**

4.3.1 **Inclusion/Exclusion Criteria**

Patients with cystic fibrosis (CF) and healthy volunteers were studied. Participants were aged 5–16 years. Healthy volunteers had no significant medical history, no history of respiratory disease and did not smoke. Patients had a confirmed diagnosis of CF by either sweat or genetic testing, were clinically stable (no exacerbations requiring antibiotics in the previous 4 weeks) and had an FEV\(_1\) of greater than 40% predicted.

Healthy volunteers were excluded if they had a past history of recurrent wheezing episodes, pneumonia, cystic fibrosis, pertussis or tuberculosis; a previous diagnosis of asthma or were taking asthma medication; or a previous hospitalisation for respiratory infection. Patients with CF were excluded from the study if their FEV\(_1\) was less than 40% of predicted or if they had had an exacerbation requiring antibiotics in the previous four weeks. Volunteers from both groups were excluded if they were born before 34 weeks gestation; if they had any neuromuscular weakness or bone disease likely to affect respiration; or if they had any congenital cardiac defects requiring treatment.
4.3.2 Recruitment

The group of healthy volunteers had either participated in a previous MBW study, were family members of hospital staff or siblings of patients. Initial notification of the pending study was sent out by email with an invitation to contact the research team. Patient information sheets were then sent out to interested families, followed by telephone calls to arrange testing.

Patients with CF were first approached at out-patient clinic appointments. Information sheets were given to interested families, who were contacted by telephone at least 24 hours later to ascertain whether the patient was willing to volunteer and if so to arrange testing.
Testing was performed in the Children’s Clinical Research Facility (CCRF) of the RHSC, Edinburgh. Consent was obtained from parents and children prior to testing. Children were asked screening questions to ensure they complied with entry criteria and they were well enough to participate. Subsequently their weight and height were measured. They then underwent three MBW tests followed by standard spirometry (as described earlier). Children were then asked to lie down for 30 minutes after which testing was repeated in the same order in the supine position.
4.3.4 **Analysis**

MBW raw data was extracted from the Innocor and analysed as described earlier to produce values for LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$. Z-scores and percent of predicted values for FEV$_1$, FVC and FEF$_{25-75}$ were calculated.

Data were analysed using Minitab Version 17 statistical software (Minitab, USA). Data are presented as mean with standard deviation (SD) or median with interquartile range (IQ). Demographic and lung function results were compared between CF and healthy groups with either 2 sample t tests or Mann-Whitney tests depending on distribution. Changes in lung function on lying flat were analysed using paired t or Wilcoxon signed rank tests. Pearson or Spearman correlation coefficients were used to assess relationships between sitting lung function and change after lying. Significance was assumed at $p=0.05$.

4.3.5 **Ethical Approval**

The study was approved by the Lothian Research Ethics Committee. Written consent was obtained from parents and capable children, verbal assent was obtained from all children.

4.3.6 **My Involvement**

This study was designed and started by my predecessor Dr Kenneth Macleod. I was involved with recruiting and testing patients. I have analysed of all the washouts collected in the study and presented my interpretation of the results.
4.4 Project Results

4.4.1 Differences between CF and healthy groups

20 children with CF and 20 healthy controls were recruited between August 2008 and May 2011. Controls were significantly, older, taller and heavier than patients with CF (table 1).

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>CF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>12 M: 8 F</td>
<td>8 M: 12 F</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8(3.7)</td>
<td>8.5 (2.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>145.5(21.6)</td>
<td>127.6 (17.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>40.3 (15.4)</td>
<td>29.5 (12.3)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Table 1: Demographics of healthy and CF groups. Mean (standard deviation SD). 2 sample t tests used to compare age, height and weight.*

4.4.2 Sitting Lung function

Table 2 shows the lung function results of the children while in the sitting position. Data were normally distributed for FEV$_1$, FVC, FEF$_{25-75}$ and S$_{cond}$, and so are presented as mean (SD); LCI, FRC and S$_{acin}$ were not normally distributed and are therefore presented as median (IQ).
Healthy children had normal lung function with a mean (SD) FEV₁ percent predicted of 97.6% (11.7), a FVC of 99.5% (10.5) and FEF₂₅-₇₅ of 93.8% (20.5); LCI was also normal, median (IQ) 6.1 (5.8, 6.3).

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>CF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ % predicted</td>
<td>97.6 (11.7)</td>
<td>81.8 (17.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FEV₁ z-score</td>
<td>-0.2 (1.0)</td>
<td>-1.4 (1.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>99.5 (10.5)</td>
<td>90.8 (16.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-0.1 (0.9)</td>
<td>-0.8 (1.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ % predicted</td>
<td>93.8 (20.5)</td>
<td>68.1 (24.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ z-score</td>
<td>-0.3 (1.0)</td>
<td>-1.6 (1.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LCI</td>
<td>6.1 (5.8, 6.3)</td>
<td>7.6 (6.6, 8.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.7 (1.3, 2.2)</td>
<td>1.0 (0.9, 1.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>0.02 (0.02)</td>
<td>0.06 (0.02)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sacin (L⁻¹)</td>
<td>0.10 (0.09, 0.13)</td>
<td>0.13 (0.10, 0.25)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 2: Sitting lung function in healthy and CF groups. Mean (SD) displayed and 2 sample t tests used for FEV₁, FVC, FEF₂₅-₇₅ and Scond. Median (IQ) displayed and Mann-Whitney test used for LCI, FRC and Sacin.

The CF group had significantly lower lung function compared to controls demonstrated in all indices except FVC z-score. Mean (SD) FEV₁ was 81.8% (17.7), FEF₂₅-₇₅ 68.1% (24.6), and median (IQ) LCI 7.6 (6.6, 8.7). Comparison between the 2 groups was done using 2 sample t or Mann-Whitney tests.
4.4.3 Effects of change in posture

In the healthy group, FEV₁, FVC and FEF_{25-75} % predicted and z-score significantly fell on retesting in the supine position. LCI and FRC also deteriorated; there was a significant rise in LCI from 6.1 to 6.4 (p<0.01) and a fall in FRC from 1.7 to 1.1 (p<0.01). S_{cond} and S_{acin} did not change. Variability in MBW derived indices were similar in both positions. LCI showed good repeatability, the mean coefficient of variation (CV) for LCI was 5.3% sitting and 4.5% supine. Phase III slope variation was much higher, mean CV for S_{cond} was 188% sitting and 142% supine and for S_{acin} it was 34% sitting and 34% supine.

<table>
<thead>
<tr>
<th>Posture</th>
<th>Mean change (95% CI), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>Supine</td>
</tr>
<tr>
<td>FEV₁ % pred</td>
<td>97.6 (11.7)</td>
</tr>
<tr>
<td>FEV₁ SDS</td>
<td>-0.2 (1.0)</td>
</tr>
<tr>
<td>FVC % pred</td>
<td>99.5 (10.5)</td>
</tr>
<tr>
<td>FVC SDS</td>
<td>-0.1 (0.9)</td>
</tr>
<tr>
<td>FEF_{25-75} % pred</td>
<td>93.8 (20.5)</td>
</tr>
<tr>
<td>FEF_{25-75} SDS</td>
<td>-0.3 (1.0)</td>
</tr>
<tr>
<td>LCI</td>
<td>6.1 (5.8, 6.3)</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.7 (1.3, 2.2)</td>
</tr>
<tr>
<td>S_{cond} (L⁻¹)</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>S_{acin} (L⁻¹)</td>
<td>0.10 (0.09, 0.13)</td>
</tr>
</tbody>
</table>

Table 3: Healthy children’s lung function sitting and lying. Mean (SD) FEV₁, FVC, FEF_{25-75} and S_{cond} and median (IQ) LCI, FRC and S_{acin}. Change in lung function presented as a mean with 95% CIs and P value. Paired t or Wilcoxon Signed rank test used.
In the CF group, FEV₁ and FVC % predicted and z-score significantly fell on retesting in the supine position, the change was not significant for FEF₂₅₋₇₅. LCI rose significantly from 7.6 to 7.8 (p<0.03) and FRC fell from 1.0 to 0.8 (p<0.01). In the CF group S_{cond} and S_{acin} did not significantly change. See table 4. The repeatability of LCI in the CF group was good in both positions, mean CV for LCI was 5.2% while sitting and 5.7% supine. Phase III slope variability was high, S_{cond} CV was 51.4% sitting and 51% supine and for S_{acin} CV was 28% sitting and 40% supine.

<table>
<thead>
<tr>
<th>Posture</th>
<th>Mean change (95% CI), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sitting</td>
</tr>
<tr>
<td>FEV₁ %pred</td>
<td>81.8 (17.7)</td>
</tr>
<tr>
<td>FEV₁ SDS</td>
<td>-1.4 (1.5)</td>
</tr>
<tr>
<td>FVC %pred</td>
<td>90.8 (16.1)</td>
</tr>
<tr>
<td>FVC SDS</td>
<td>-0.8 (1.3)</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅ %pred</td>
<td>68.1 (24.6)</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅ SDS</td>
<td>-1.6 (1.3)</td>
</tr>
<tr>
<td>LCI</td>
<td>7.6 (6.6, 8.7)</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.0 (0.9, 1.3)</td>
</tr>
<tr>
<td>S_{cond} (L⁻¹)</td>
<td>0.06 (0.02)</td>
</tr>
<tr>
<td>S_{acin} (L⁻¹)</td>
<td>0.13 (0.10, 0.25)</td>
</tr>
</tbody>
</table>

*Table 4: CF children's lung function sitting and lying. Mean (SD) FEV₁, FVC, FEF₂₅₋₇₅ and S_{cond} and median (IQ) LCI, FRC and S_{acin}. Change in lung function presented as a mean with 95% CIs and P value. Paired t or Wilcoxon Signed Rank tests used.*
4.4.4 Relationship between change in FRC and LCI with postural change

There was no relationship between change in FRC and change in LCI in either group. The spearman correlation coefficients comparing change in FRC and LCI in the heathy group was $r = -0.104$, $p=0.68$ and CF group was $r=-0.007$, $p=0.98$.

Figure 23: Scatterplot of change in LCI from sitting to supine versus change in FRC from sitting to supine for both groups with regression lines.
4.4.5 Difference in posture related changes between CF and healthy cohorts

The mean group change in each test are presented for the healthy and CF cohorts in table 5. Two sample t tests were used to compare the changes between groups. The only significant difference between the two groups was seen in a greater fall in FRC in the healthy group compared to the CF group (mean change -0.6 vs -0.3, p<0.01).

<table>
<thead>
<tr>
<th></th>
<th>Sitting to lying mean change (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>CF</td>
</tr>
<tr>
<td>FEV1 %pred</td>
<td>-8.4 (-12.3, -4.4)</td>
<td>-6.8 (-12.2, -1.4)</td>
</tr>
<tr>
<td>FEV1 SDS</td>
<td>-0.7 (-0.9, -0.4)</td>
<td>-0.5 (-0.9, -0.1)</td>
</tr>
<tr>
<td>FVC %pred</td>
<td>-5.0 (-8.4, -1.6)</td>
<td>-7.0 (-11.1, -3.0)</td>
</tr>
<tr>
<td>FVC SDS</td>
<td>-0.4 (-0.7, -0.2)</td>
<td>-0.5 (-0.8, -0.2)</td>
</tr>
<tr>
<td>FEF25-75 %pred</td>
<td>-13.8 (-19.6, -7.9)</td>
<td>-6.8 (-14.1, 0.5)</td>
</tr>
<tr>
<td>FEF25-75 SDS</td>
<td>-0.6 (-0.9, -0.3)</td>
<td>-0.3 (-0.7, 0.1)</td>
</tr>
<tr>
<td>LCI</td>
<td>0.3 (0.1, 0.4)</td>
<td>0.5 (0.05, 0.9)</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>-0.6 (-0.7, -0.4)</td>
<td>-0.3 (-0.4, -0.2)</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>&lt;-0.01 (-0.01, 0.01)</td>
<td>0.01 (-0.01, 0.03)</td>
</tr>
<tr>
<td>Sasin (L⁻¹)</td>
<td>&lt;-0.01 (-0.02, 0.04)</td>
<td>&lt;-0.01 (-0.04, 0.02)</td>
</tr>
</tbody>
</table>

Table 5: Mean change in lung function from sitting to lying in healthy and CF groups with 95% confidence intervals. 2 sample t test used to compare changes in healthy and CF groups.
4.4.6 Posture related changes compared to baseline lung function

Baseline LCI did not correlate with the change in LCI on lying supine. In the CF group in particular, lying had a very variable effect on LCI, which could not be predicted from baseline testing. See Figure 24, which shows a scatterplot of individual baseline LCI against the change on adopting the supine position.

![Scatterplot of change in LCI versus baseline sitting LCI](image)

*Figure 24: Change in LCI on lying versus baseline sitting LCI*
Baseline FEV\textsubscript{1} % predicted for both groups did correlate with change in FEV\textsubscript{1} % predicted on lying (Pearson correlation coefficient -0.49, p<0.01). Patients with better FEV\textsubscript{1} had larger drops in their FEV\textsubscript{1} on lying supine. See Figure 25.

Figure 25: Change in FEV\textsubscript{1} % predicted on lying versus baseline sitting FEV\textsubscript{1} % predicted.
4.5 Project Discussion

This study has shown that MBW derived indices are abnormal in children with CF compared to healthy controls and that supine posture affects FEV₁, FVC, LCI and FRC in both groups. FRC was more affected by posture in healthy individuals compared to CF patients. LCI deteriorated equally in CF patients and controls despite a smaller fall in FRC in the CF group demonstrating that increased ventilation inhomogeneity was not entirely due to reduction in FRC. A postural difference in S\textsubscript{cond} and S\textsubscript{acin} could not be detected, possibly because of high variability.

Children with CF in this study had significantly reduced baseline lung function when compared to a healthy group with no respiratory disease. All spirometry and MBW derived indices with the exception of FVC z-score were significantly worse in the CF group. Healthy children’s lung function fell within the normal range. Most but not all of the children with CF had abnormal lung function. The variability of lung function within the group with CF was larger than the healthy group as reflected by the higher standard deviations for % predicted FEV₁ (11.7 in healthy group compared to 17.7 in the CF group) and interquartile ranges for LCI (5.8, 6.3 in healthy children compared to 6.6, 8.7 in children with CF). This suggests that the group of children with CF had varying degrees of lung disease which was not surprising given that only severely diseased children with an FEV₁ of less than 40% were excluded.

Supine posture caused a reduction in FEV₁, FVC and FRC and a small rise in LCI in healthy children and children with CF. It is well established that posture effects residual lung volumes (56). Bouhuys et al had previously found that LCI increased on lying flat in a small study of healthy adults (61). A rise in LCI was predicted on lying supine because FRC was expected to
fall and LCI is calculated using FRC to divide the total volume of gas expired during the washout. However in our study change in LCI in individual patients did not correlate with change in FRC suggesting that the rise in LCI was due to other factors. The rise in LCI may have been due to increased ventilation heterogeneity secondary to atelectasis or the effects of gravity on ventilation distribution. Atelectasis has been demonstrated in children’s lungs on lying flat using MRI and CT imaging (59, 60). The results of our study that was performed without anaesthetic suggest that not all of the atelectasis seen on imaging studies was likely to be due to the use of anaesthetic and artificial ventilation. Gravity dependent changes in ventilation distribution which shift ventilation to more diseased regions of the lung on lying supine may also increase ventilation inhomogeneity.

The results of this study contrast to the findings of some published studies of posture and MBW. Gustafsson et al found that LCI did not change in the supine position in healthy children (57). Differences in methodology may be to blame for the contrasting results as in this Swedish study spirometry was performed before MBW and only one MBW test was performed at each time point (0, 30 and 60 minutes following assuming the supine position). Lum et al also found no difference in LCI measurements in erect and supine positions, however they compared LCI between two very different groups of patients (a group of <2 year olds with a group aged ≥3 years) (41). In addition the two groups Lum et al studied performed MBW differently, the infants were sedated and used a face mask and the older children were not sedated and used a mouthpiece.

The effects of posture are different in CF lungs compared to healthy lungs. FRC fell significantly more in the healthy group compared to the CF group (mean change -0.6L versus -0.3L respectively (p<0.01)). Despite the smaller drop in FRC, the rise in LCI appeared larger
in the CF group, with a mean increase in LCI of 0.5, compared to 0.3 in the healthy group, however this difference was not significant. The variation in change of LCI was much more marked in the CF group; the 95% CIs for change in the CF group were 0.05 - 0.9 compared to 0.1 - 0.4 in the healthy group. The effect of posture could not be explained by baseline LCI because change on moving supine did not correlate with initial LCI. In fact several patients with abnormal initial LCI had an improvement in their LCI on lying supine. Improvement in LCI in these patients could have been caused by complete obstruction of peripheral regions of diseased lung on lying supine. CF patients with normal LCI appeared to follow a similar pattern to the healthy children with regard to their change in LCI on lying. These observations need clarification in larger groups of patients with categorised disease severity.

Indices of phase III slope were almost identical in sitting and supine postures, suggesting that they may be useful when comparing the lung function of patients in different positions. However the very high variability, reflected in the CV, in $S_{cond}$ and $S_{acin}$ may be the reason a difference could not be detected. In Gronkvist’s study of 11 healthy men there was a rise in $S_{cond}$ in the supine position (no change in $S_{acin}$) (58). The methodology in this study differed from our own, helium was used as well as SF$_6$ which will have altered the density of gas being inhaled; subjects were also asked to breath to a set tidal volume, which will have affected FRC and also reduced variability in phase III slopes.

Adoption of the supine posture reduced FEV$_1$ and FVC in healthy and CF groups, drops appeared greater in the healthy group but the differences were not significant. There was a significant correlation between baseline FEV$_1$ %predicted and change in FEV$_1$% predicted on lying supine, $p<0.01$. Patients with better initial FEV$_1$ had larger falls in FEV$_1$ on lying supine.
This effect means that it is not possible to compare spirometry across different postures. Standardised spirometry guidance has been published to ensure consistency (3).

The results of this study have been combined with a similar study performed by Horsley et al in Sheffield in which another 15 children with CF and 8 healthy controls were recruited. In this group MBW was repeated as soon as the patient was supine and the equipment was ready, unlike in our study in which we waited 30 minutes after the patient adopted the supine position before testing. The results were similar with a larger fall in FRC in healthy children compared to controls (28.4% vs 24.0% respectively, p<0.001). LCI rose from 6.25 to 6.47 in the healthy group and from 7.72 to 8.22 in the group with CF. Combined results of these studies have been submitted for publication.

This study was limited by a number of factors. A larger more diverse group of healthy children across all ages would be required for results to be applicable to all healthy children. The group of children with CF was limited and had varying disease severity, more information may be discerned from studying children in categories of disease severity. Assumptions with regard to why ventilation inhomogeneity increases on lying supine have been made. Incorporating imaging with MRI or CT within the study protocol could have allowed us to compare changes in functional ventilation heterogeneity with structural changes. This comparison would have allowed us to determine more definitively why children with CF had such a variable response to lying supine. In our study derived indices of phase III slope analysis were highly variable, this may have been due to not having a set tidal volume protocol during testing which is difficult for many children. However the software I had to my disposal meant that I could not exclude breaths in which the phase III slopes were indiscernible and this may have increased the variability of my results.
This study demonstrates that LCI is affected by posture and for comparison of results posture should be standardised. In addition the study demonstrated that the effects of a variable on MBW derived indices can be different in diseased lungs compared to healthy lungs.
5 Project: Longitudinal variability of MBW derived indices in children with cystic fibrosis

5.1 Introduction

It is well established that cystic fibrosis (CF) is a progressive disease, most evident for changes in lung function with age. There is however limited data on longitudinal measures in larger cohorts that not only provide evidence for individual measures of disease progression, but also the inter-relationship of these measures for anatomical, physiological and inflammatory markers. This work was developed within the UK CF gene therapy consortium as part of the requirement to be able to adequately measure response to new therapies that may be more sensitive to change than the current standard (accepted by the FDA and EMA) FEV$_1$.

FEV$_1$ is understood to decline with progressive disease severity (74), but has been shown to be insensitive in detecting early disease (12, 83, 84). LCI appears to correlate with age in cross sectional studies of patients with CF (43) and may be more sensitive to early disease than FEV$_1$ (76, 85). There is evidence that LCI correlates with structural changes found on CT (78, 89) suggesting that LCI may be a marker of severity, however studies have shown conflicting results with regard to longitudinal change in LCI (75, 89). If indices of MBW relate to disease severity a decline over time would be expected, determining the rate of decline might allow assessment of the efficacy of new therapies in the future. This could be especially helpful in patients with mild disease to which FEV$_1$ is insensitive. However if MBW indices vary significantly over repeated testing visits the ability of MBW to detect a meaningful change in lung function will be limited. This study was performed to examine the variability of MBW derived indices in children with CF over multiple visits and determine whether MBW could detect change in lung function over time.
5.2 **Aims**

To assess the longitudinal variation in MBW derived indices in children with CF

To investigate the correlation between MBW derived indices, spirometry and CT findings in children with CF.

5.3 **Methods**

A “Run-In” study was performed by the UK CF gene therapy consortium. It was designed to assess the stability and repeatability of a wide range of clinical and laboratory assays over time in patients with Cystic Fibrosis, at times of clinical stability. The study was a precursor to a multi dose gene therapy trial (99) in which some participants of this study subsequently volunteered. One of the methods that was studied was MBW.

Adults and children with CF were recruited from across Scotland and London. My work in the study involved running the clinical aspects of the Scottish children’s visits; I will present only the data from their visits. I started work with the study midway through the children’s third visit but have analysed and presented data from all 4 visits.

5.3.1 **Inclusion/Exclusion Criteria**

Patients were eligible to take part in the paediatric group if they were aged 10-16 years, with a diagnosis of CF on sweat or genetic testing. They were excluded if they had an FEV$_1$ below 40%, a chronic day time oxygen requirement, were awaiting, referred for or post lung transplant, had a history of previous spontaneous pneumothorax (unless subsequent pleurodesis), recurrent severe haemoptysis, colonisation with MRSA or *B. cepacia complex*, advanced liver disease (varices or significant, sustained elevation of transaminases: ALT/
AST > 100 IU/l), significant renal impairment (serum creatinine > 150 μmol/l), significant coagulopathy, requirement for immunosuppressive agents (methotrexate, cyclosporine or intravenous immunoglobulin), were taking part in another interventional trial, pregnant or were a current smoker.

5.3.2 Recruitment
Children, aged 10-16 years were recruited from Edinburgh, Glasgow, Dundee and Stirling. Meetings were held to present and discuss the study at each site. Suitable children were identified by local clinical teams. Patients were sent information about the study, some were approached by a CF nurse and others attended a parent education event. This was followed up with telephone calls. Full information sheets were then distributed to interested families.

5.3.3 Study Visits
At the time I completed my research fellowship all patients had completed four study visits spaced at 4-6 monthly intervals. Patients were asked to attend at times of clinical stability and appointments were rescheduled if symptoms indicated exacerbation, the patient had been prescribed antibiotics in the past 2 weeks or their regular CF medications had changed over the preceding 4 weeks.

At each visit patient’s height and weight were measured, baseline observations were taken, including temperature, respiratory rate, heart rate, blood pressure and oxygen saturations. A physical examination of the chest was performed. Patients and their families then completed a quality of life questionnaire. Subsequently the children performed MBW testing (as detailed earlier) followed by spirometry using the EasyOne spirometer. Patients performed exhaled
breath condensate testing and had blood, urine and sputum taken for multiple laboratory assays. Finally patients performed a shuttle walk test.

In between each visit, patients were asked to keep a diary of their symptoms, perform weekly forced expiratory manoeuvres into an electronic peak flow meter and wear a pedometer for a week.

At their first visit patients underwent a low dose high resolution CT chest scan using a Siemens 64 slice scanner. Each examination exposed the patient to an effective radiation dose of 1.4 mili Sieverts. The scan included an inspiratory volumetric and an expiratory limited interspaced high resolution CT. The scans were anonymised and then assessed by two independent radiologists (at the Royal Brompton Hospital, London). Scans were scored using a previously reported scoring system (150). Each lobe was scored for extent of bronchiectasis (0-3), severity of bronchiectasis (0-4), bronchial wall thickness (0-4), presence of small and large airway plugs (0-2), consolidation (0-4) and gas trapping (percentage basis). A mean of the scored lobes was then recorded for each feature.

5.3.4 Analysis

MBW raw data were extracted from the Innocor and analysed as described earlier to produce values for LCI, $S_{cond}$ and $S_{acin}$. Z-scores and percent of predicted values for FEV$_1$, FVC and FEF$_{25-75}$ were calculated.

Data were analysed using Minitab Version 17 statistical software (Minitab, USA). Data are presented as mean with standard deviation (SD). Pearson correlation coefficients were used to
examine relationships between MBW and spirometric indices and between these indices and CT scores. Significance was assumed at \( p = 0.05 \). Intra and inter visit coefficient of variation (CV) was used to describe variability of tests.

5.3.5 Ethical Approval

The study was approved by the National Research Ethics Service. Written consent was obtained from parents and capable children, verbal assent was obtained from all children.
5.4 **Results**

5.4.1 **Baseline Demographics**

Thirty four children (12 girls and 22 boys) with CF made up the subset within the CFGT Consortium Run-In study who attended the Royal Hospital for Sick Children. All children attended the first visit at which time the group’s mean age was 13.0 years (range from 10.1 to 17.5 years) and mean (SD) height was 152.6 (10.8) cm. The group comprised children with varied genotypes and disease severity.

Lung function varied within the group, mean (SD) and the range of values obtained are displayed in table 6. The group’s mean LCI and $S_{cond}$ were abnormal whereas mean $FEV_1$, $FVC$, $FEF_{25-75}$ and $S_{acin}$ fell within 1.64 standard deviations of healthy mean values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FEV_1$ % predicted</td>
<td>81.0 (15.3)</td>
<td>39.1</td>
<td>105.3</td>
</tr>
<tr>
<td>$FEV_1$ SDS</td>
<td>-1.6 (1.3)</td>
<td>-5.2</td>
<td>0.4</td>
</tr>
<tr>
<td>$FVC$ % predicted</td>
<td>87.5 (11.2)</td>
<td>58.8</td>
<td>104.8</td>
</tr>
<tr>
<td>$FVC$ SDS</td>
<td>-1.1 (1.0)</td>
<td>-3.8</td>
<td>0.4</td>
</tr>
<tr>
<td>$FEF_{25-75}$ % predicted</td>
<td>69.3 (25.7)</td>
<td>15.3</td>
<td>107.7</td>
</tr>
<tr>
<td>$FEF_{25-75}$ SDS</td>
<td>-1.6 (1.5)</td>
<td>-5.3</td>
<td>0.3</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.9 (0.5)</td>
<td>1.2</td>
<td>3.4</td>
</tr>
<tr>
<td>LCI</td>
<td>9.0 (1.8)</td>
<td>6.7</td>
<td>14.2</td>
</tr>
<tr>
<td>$S_{cond}$ (L$^{-1}$)</td>
<td>0.08 (0.03)</td>
<td>0.02</td>
<td>0.15</td>
</tr>
<tr>
<td>$S_{acin}$ (L$^{-1}$)</td>
<td>0.19 (0.09)</td>
<td>0.06</td>
<td>0.52</td>
</tr>
</tbody>
</table>

*Table 6: Run-In Study Visit 1 lung function*
Age at first visit correlated with FEV\(_1\) and FVC z-scores (r = -0.41 (p = 0.02) and r = -0.51 (p < 0.01) respectively) and mean LCI and FRC (r = 0.34 (p = 0.048) and r = 0.39 (p = 0.02) respectively). Age did not correlate with FEF\(_{25-75}\), S\(_{cond}\) or S\(_{acin}\).

5.4.2 Correlation between FEV\(_1\), LCI, S\(_{cond}\) and S\(_{acin}\)

Figures 26 and 27 (scatterplots of visit 1 LCI versus FEV\(_1\) percent predicted and z-score respectively) demonstrate the range of FEV\(_1\) and LCI within the group. Reference lines indicate the 1.64 standard deviation limits of healthy children, FEV\(_1\) values below the horizontal line and LCI values to the right of the vertical line are out with 95% of healthy values. The limit for LCI of 7.01 was taken from the mean (6.24) plus 1.64 standard deviations (1.64 x 0.47) of LCI obtained in a previously published study of MBW in 29 healthy children in Edinburgh using the same equipment and methodology (50). FEV\(_1\) and LCI correlated within individual patients (Pearson r = 0.64, P<0.01), patients with CF lung disease are expected to develop abnormal lung function. However, LCI was abnormal (>7.01) in the majority of patients (71%) with a normal FEV\(_1\) (>80% or >-1.64 SDS) possibly indicating that LCI is more sensitive to early disease. There were no patients with a normal LCI (< 7.01) despite an abnormal FEV\(_1\) (<80%).
Figure 26: Visit 1 FEV1 % predicted vs LCI. Reference lines at 80% predicted FEV1 and LCI = 7.01 (mean + 1.64 SD in healthy children).

Figure 27: Visit 1 FEV1 z-score vs LCI. Reference lines from mean +/- 1.64 SD in healthy children (LCI = 7.01)
Figure 28 shows a scatterplot of individual FEV$_1$ z-score versus S$_{cond}$. The reference lines show the 1.64 standard deviation limits for FEV$_1$ and S$_{cond}$. Values below the FEV$_1$ limit and to the right of the S$_{cond}$ limit are out with 95% of values in healthy children. The 1.64 standard deviation limit for S$_{cond}$ was taken from the mean (0.017 L$^{-1}$) and standard deviation (0.02) of S$_{cond}$ in a previous study of 29 healthy children in Edinburgh using the same equipment and methodology (50). 28 out of 33 children had a S$_{cond}$ above 1.64 standard deviations of the mean. Only 13 of these patients also had FEV$_1$ below 1.64 standard deviations of the healthy mean. Sixteen patients had a normal FEV$_1$ (>1.64 SDS) but abnormal S$_{cond}$ (>1.64 SDS), suggesting that S$_{cond}$ may also be a sensitive marker of early lung disease in CF. S$_{cond}$ did not correlate with FEV$_1$ z-score. There was one patient with an abnormal FEV$_1$ and a S$_{cond}$ within the 1.64 standard deviation limit.

![Figure 28: Visit 1 FEV1 z-score versus S$_{cond}$. Reference lines from mean + 1.64 SD in healthy children (S$_{cond}$ = 0.05L$^{-1}$)](image)
Figure 29 shows a scatterplot of individual FEV\textsubscript{1} z-score versus S\textsubscript{acin} and 1.64 standard deviation limits. The 1.64 standard deviation limit for S\textsubscript{acin} was taken from the mean (0.12 L\textsuperscript{-1}) and standard deviation (0.06) of S\textsubscript{acin} in healthy children using the same methodology (50). S\textsubscript{acin} did weakly correlate with FEV\textsubscript{1} (Pearson r= -0.34, p=0.049). However there were seven patients with abnormal FEV\textsubscript{1} and a normal S\textsubscript{acin} and seven patients with normal FEV\textsubscript{1} an abnormal S\textsubscript{acin} suggesting that neither test is more sensitive to early disease and that the tests possibly identify different processes.

Figure 29: Visit 1 FEV\textsubscript{1} z-score versus S\textsubscript{acin}. Reference lines from mean + 1.64 SD in healthy children (S\textsubscript{acin} = 0.22L\textsuperscript{-1})
Figures 30 and 31 show scatterplots of individual $S_{\text{cond}}$ and $S_{\text{acin}}$ against LCI with reference lines marking the previously described 1.64 standard deviation limits. LCI values to the right of the vertical reference line and $S_{\text{cond}}$ or $S_{\text{acin}}$ values above the horizontal reference line are out with 95% of values previously obtained in healthy children. $S_{\text{cond}}$ did not correlate with LCI. Only 5 patients had a $S_{\text{cond}}$ within 1.64 standard deviations of the healthy mean, three of these patients had a normal LCI and the other two had $S_{\text{cond}}$ values that were approaching the cut-off value. Six patients had normal LCI and three of these patients had abnormal $S_{\text{cond}}$, suggesting that $S_{\text{cond}}$ may be even more sensitive to early disease than LCI.

*Figure 30: Visit 1 $S_{\text{cond}}$ vs LCI. Reference lines from mean + 1.64 SD in healthy children ($S_{\text{cond}} = 0.05 \text{L}^{-1}$ and LCI = 7.01)*
$S_{acin}$ correlated with LCI (Pearson $r=0.68$, $p<0.01$) and FEV$_1$, suggesting that $S_{acin}$ may be a marker of disease severity. However there were 16 patients with a normal $S_{acin}$ but abnormal LCI suggesting that $S_{acin}$ may not be as sensitive to early disease as LCI.

![Figure 31: Visit 1 $S_{acin}$ vs LCI. Reference lines from mean + 1.64 SD in healthy children ($S_{acin} = 0.22L^{-1}$ and LCI = 7.01)](image-url)
5.4.3 Intra-visit variability of MBW derived indices

Intra visit coefficients of variation (CV) for MBW indices at visit 1 are detailed in table 7. LCI and FRC have CVs of 5.4 and 3.5% respectively but $S_{\text{cond}}$ and $S_{\text{acin}}$ have CVs of greater than 25%.

<table>
<thead>
<tr>
<th></th>
<th>Mean CV (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>5.4% (3.2)</td>
</tr>
<tr>
<td>FRC</td>
<td>3.5% (2.3)</td>
</tr>
<tr>
<td>$S_{\text{cond}}$</td>
<td>29.8% (22.6)</td>
</tr>
<tr>
<td>$S_{\text{acin}}$</td>
<td>25.4% (22.0)</td>
</tr>
</tbody>
</table>

Table 7: Intra visit coefficient of variation for LCI, FRC, $S_{\text{cond}}$ and $S_{\text{acin}}$ at Visit 1

5.4.4 CT scores

Every child who attended visit 1 had a CT on the same day. CTs were scored as described in the methodology by two independent radiologists. Each lobe was scored for extent of bronchiectasis (0-4), severity of bronchiectasis (0-4), bronchial wall thickness (0-4), presence of small and large airway plugs (0-2), consolidation (0-4) and air trapping (percentage basis). A mean of the scored lobes was then recorded for each feature. The results are shown in figures 32 and 33. The majority of children had some bronchiectasis, only 5 children had consolidation.
Figure 32: CT scores at visit 1

Figure 33: Air trapping on CT at visit 1
FEV\(_1\), FVC and LCI correlated with the extent and severity of bronchiectasis, wall thickness, small and large plugs and air trapping. \(S_{\text{acin}}\) correlated with extent and severity of bronchiectasis, large plugs and airway trapping. \(S_{\text{cond}}\) only showed moderate correlation with wall thickness \((r=0.34, p=0.05)\) and large plugs \((0.38, p=0.03)\). No lung function measure correlated with consolidation. LCI correlated most strongly (compared to the other lung function indices) with extent and severity of bronchiectasis and wall thickness \((r=0.66, 0.69 \text{ and } 0.58, p<0.01)\). Of the six patients with normal LCI \((<7.01)\) five (83%) had no bronchiectasis on CT, of the 28 patients with abnormal LCI, 27 (96%) had bronchiectasis on CT. FEV\(_1\) correlated best with large plugs and air trapping \((r=0.62 \text{ and } 0.45, p<0.01)\). FVC showed the strongest correlation with small plugs \((r=0.51, p<0.01)\).

<table>
<thead>
<tr>
<th></th>
<th>Extent Bronchiectasis</th>
<th>Severity Bronchiectasis</th>
<th>Wall Thickness</th>
<th>Small Plugs</th>
<th>Large Plugs</th>
<th>Air Trapping</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_1)</td>
<td>-0.44 ((p&lt;0.01))</td>
<td>-0.49 ((p&lt;0.01))</td>
<td>-0.52 ((p&lt;0.01))</td>
<td>-0.43 ((p=0.01))</td>
<td><strong>-0.62 ((p&lt;0.01))</strong></td>
<td><strong>-0.45 ((p&lt;0.01))</strong></td>
</tr>
<tr>
<td>FVC</td>
<td>-0.41 ((p=0.02))</td>
<td>-0.45 ((p&lt;0.01))</td>
<td>-0.45 ((p&lt;0.01))</td>
<td><strong>-0.51 ((p&lt;0.01))</strong></td>
<td>-0.57 ((p&lt;0.01))</td>
<td>-0.37 ((p=0.03))</td>
</tr>
<tr>
<td>FEF(_{25-75})</td>
<td>-0.31 ((p=0.07))</td>
<td>-0.37 ((p=0.03))</td>
<td>-0.48 ((p&lt;0.01))</td>
<td>-0.19 ((p=0.29))</td>
<td>-0.50 ((p&lt;0.01))</td>
<td>-0.42 ((p=0.01))</td>
</tr>
<tr>
<td>LCI</td>
<td><strong>0.66 ((p&lt;0.01))</strong></td>
<td><strong>0.69 ((p&lt;0.01))</strong></td>
<td><strong>0.58 ((p&lt;0.01))</strong></td>
<td>(0.47 ((p&lt;0.01)))</td>
<td>(0.55 ((p&lt;0.01)))</td>
<td>(0.42 ((p=0.01)))</td>
</tr>
<tr>
<td>(S_{\text{cond}})</td>
<td>0.28 ((p=0.11))</td>
<td>0.25 ((p=0.15))</td>
<td>0.34 ((p=0.05))</td>
<td>0.18 ((p=0.30))</td>
<td>0.38 ((p=0.03))</td>
<td>0.29 ((p=0.09))</td>
</tr>
<tr>
<td>(S_{\text{acin}})</td>
<td>0.45 ((p&lt;0.01))</td>
<td>0.39 ((p=0.02))</td>
<td>0.23 ((p=0.19))</td>
<td>0.18 ((p=0.32))</td>
<td>0.50 ((p&lt;0.01))</td>
<td>0.35 ((p=0.05))</td>
</tr>
</tbody>
</table>

*Table 8: Correlation between lung function and CT scores. Pearson correlation coefficients and \(p\) values displayed. Strongest correlation for each CT feature in bold.*
5.4.5 Variability of lung function over time

Thirty two children subsequently attended 3 further visits, one withdrew after the first visit due to *Burkholderia cepacia complex*, and another after the third visit for personal reasons. The visits occurred at intervals of 3 to 10 months, over a period of 19 months. The median gaps (and IQ ranges) between visits 1 and 2, 2 and 3 and 3 and 4 were 3.9 (3.7-4.3) months, 3.5 (3.2-4.2) months and 5.9 (5.6-7.0) months respectively.

There were no consistent trends in lung function for the whole group over time. Table 9 shows the mean (SD) for each measure of lung function of the group at each visit.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1 (n=34)</th>
<th>Visit 2 (n=33)</th>
<th>Visit 3 (n=33)</th>
<th>Visit 4 (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ % predicted</td>
<td>81.0 (15.3)</td>
<td>82.3 (17.1)</td>
<td>79.5 (20.0)</td>
<td>79.3 (15.6)</td>
</tr>
<tr>
<td>FEV₁ SDS</td>
<td>-1.6 (1.3)</td>
<td>-1.5 (1.4)</td>
<td>-1.8 (1.7)</td>
<td>-1.8 (1.3)</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>87.5 (11.2)</td>
<td>89.3 (13.6)</td>
<td>86.5 (16.4)</td>
<td>86.7 (13.7)</td>
</tr>
<tr>
<td>FVC SDS</td>
<td>-1.1 (1.0)</td>
<td>-1.0 (1.2)</td>
<td>-1.2 (1.5)</td>
<td>-1.2 (1.2)</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ % predicted</td>
<td>69.3 (25.7)</td>
<td>70.9 (27.4)</td>
<td>68.8 (32.2)</td>
<td>67.6 (26.0)</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ SDS</td>
<td>-1.6 (1.5)</td>
<td>-1.5 (1.6)</td>
<td>-1.7 (1.8)</td>
<td>-1.7 (1.5)</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.9 (0.5)</td>
<td>1.8 (0.5)</td>
<td>1.9 (0.6)</td>
<td>2.0 (0.6)</td>
</tr>
<tr>
<td>LCI</td>
<td>9.0 (1.8)</td>
<td>9.3 (2.4)</td>
<td>9.7 (2.1)</td>
<td>9.2 (2.1)</td>
</tr>
<tr>
<td>Scond (L⁻¹)</td>
<td>0.08 (0.03)</td>
<td>0.07 (0.03)</td>
<td>0.08 (0.03)</td>
<td>0.07 (0.03)</td>
</tr>
<tr>
<td>Scin (L⁻¹)</td>
<td>0.19 (0.09)</td>
<td>0.19 (0.11)</td>
<td>0.21 (0.13)</td>
<td>0.20 (0.09)</td>
</tr>
</tbody>
</table>

*Table 9: Lung function across all visits. Mean (SD) displayed.*
There was no group difference over time in any mean lung function result. Between visits 1 and 4 LCI rose in 21 patients by a mean (SD) of 0.95 (0.86) but fell in 11 patients by a mean (SD) of -1.14 (0.98). FEV₁ z-score fell in 21 patients by a mean of -0.76 (0.60) and rose in 11 patients by a mean (SD) of 0.65 (0.44). The change in LCI between visits 1 and 4 correlated with change in FEV₁ z-score (Pearson r = -0.421, p=0.017). Changes in S_{cond} and S_{acin} did not correlate with either FEV₁ or LCI.

![Figure 34: Scatterplot of change in FEV₁ z-score against change in LCI from Visit 1 to Visit 4.](image)
Lung function varied over time for each patient. FVC varied the least with the lowest inter-visit CV across the 4 visits of 6.8%. FEV₁ had a CV of 8.1% and LCI 9.1%. FEF₂₅₋₇₅% predicted had a CV of 19.0%, S_{acin} varied by 29.5% but S_{cond} varied the most with a CV of 31.2%. See table 10.

<table>
<thead>
<tr>
<th>Variable</th>
<th>V1-4 Mean</th>
<th>V1-4 Inter-Visit CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ % predicted</td>
<td>80.0 (16.0)</td>
<td>8.1%</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>87.4 (12.3)</td>
<td>6.8%</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅% predicted</td>
<td>68.0 (25.4)</td>
<td>19.0%</td>
</tr>
<tr>
<td>FRC (L)</td>
<td>1.9 (0.5)</td>
<td>9.5%</td>
</tr>
<tr>
<td>LCI</td>
<td>9.3 (1.9)</td>
<td>9.1%</td>
</tr>
<tr>
<td>S_{cond} (L⁻¹)</td>
<td>0.07 (0.02)</td>
<td>31.2%</td>
</tr>
<tr>
<td>S_{acin} (L⁻¹)</td>
<td>0.20 (0.09)</td>
<td>29.5%</td>
</tr>
</tbody>
</table>

*Table 10: Mean (SD) lung function over 4 visits plus mean inter-visit CV.*
Figures 35-37 demonstrate how patients’ LCI varied over time. The patients are split depending on LCI at first visit. Figure 33 shows patients with a normal LCI (<7.01) at visit 1, figure 34 shows patients with an LCI of between 7.01 and 9.5 and figure 35 shows patients with an initial LCI of greater than 9.5. Variability of LCI appeared greater if baseline LCI was above 9.5. Mean inter-visit CV was 7.7 if initial LCI was <7.01, 8.2 if initial LCI was 7.01-9.5 and 10.7 if LCI was >9.5.

Figure 35: Individual variation in LCI over visits 1-4 in patients with an initial LCI of <7.01
Figure 36: Individual variation in LCI over visits 1-4 in patients with an initial LCI of 7.01-9.5

Figure 37: Individual variation in LCI over visits 1-4 in patients with an initial LCI of >9.5
Figures 38 and 39 show how FEV\textsubscript{1} percent predicted varied across the visits. Patients were split depending on whether their initial FEV\textsubscript{1} z-score was less than -1.64.

**Figure 38:** Line plot of individual FEV\textsubscript{1}\% predicted over the 4 visits when initial FEV\textsubscript{1} was >-1.64

**Figure 39:** Line plot of individual FEV\textsubscript{1}\% predicted over the 4 visits when initial FEV\textsubscript{1} was <-1.64
5.5 Discussion

This study detected no significant deterioration in LCI, \( S_{\text{cond}} \), \( S_{\text{acin}} \) or FEV\(_1\) over a period of 19 months in children with CF. LCI and \( S_{\text{cond}} \) were abnormal in several children in whom FEV\(_1\) was normal suggesting LCI and \( S_{\text{cond}} \) may be more sensitive to early disease than FEV\(_1\). There were significant correlations between LCI and structural changes on CT scanning, LCI showed the strongest correlation of any lung function parameter with severity and extent of bronchiectasis. The repeatability of LCI over multiple visits was similar to FEV\(_1\) but \( S_{\text{cond}} \) and \( S_{\text{acin}} \) were highly variable over multiple test visits.

The ages of the children in this study ranged from 10.1 to 17.5 years, age positively correlated with worse FEV\(_1\), FVC and LCI. Aurora et al published similar findings regarding correlation between age and LCI in CF in school age children (43). This relationship may be due to the progressive nature of CF lung disease. Correlations were not strong but it is well established that there may be multiple factors that determine lung disease severity in a group of children with different CF genotypes. Interestingly \( \text{FEF}_{25-75} \) and indices of phase III slopes did not correlate with age, this may be due to the intrinsic variability of the indices themselves and a larger group may be necessary to measure a correlation.

Twenty one out of the 34 children in this study had normal FEV\(_1\) (>80% predicted). Sly et al found that lung disease was detectable in 80% of infants with CF using CT within the first few months of life (68). In addition bronchiectasis has been demonstrated with CT in children with CF who have normal FEV\(_1\) (72). Given that CF lung disease is known to be present so early in life and deteriorate with age it is unlikely that the children in our study had normal lungs. It may be more likely that FEV\(_1\) is insensitive in detecting early CF lung disease.
FEV₁ and LCI correlated within individual patients (Pearson r=0.64, p<0.01). FEV₁ is known to decline with deteriorating CF disease severity (74) and correlation with LCI suggests that LCI may also be useful as a marker of disease progression. In addition, LCI was abnormal (>7.01) in 15 of the 21 patients with normal FEV₁ (>1.64 SDS), in keeping with multiple published studies in which LCI detected abnormalities in CF when FEV₁ did not (10, 35, 39, 43, 75, 78) suggesting that LCI may be more useful in early disease than FEV₁. Especially as abnormal LCI in childhood has been shown to persist through early childhood and even predict those who will develop abnormal FEV₁ (88).

Scond was abnormal in 29 of the 34 children in this study and did not correlate with FEV₁ or LCI. Of the five patients with normal Scond four had normal FEV₁ and three had normal LCI. The patients with normal Scond and abnormal FEV₁ (n=1) and abnormal LCI (n=2) had a Scond just within the normal range. The normal range was formulated on the basis of a previous study in the same centre using identical equipment in 29 healthy children and may have been too small to accurately identify the normal range (which may be below the cut off used in this study (0.05L⁻¹)). Three patients had abnormal Scond but normal LCI. Theoretically Scond will detect change in small conducting airways and be the first indicator of early disease. Horsley et al previously found that Scond was highly sensitive to early disease but that once a ceiling value of 0.150L⁻¹ was reached it could no longer discriminate more severe disease (82). Gustafsson found that although Scond was abnormal in children with CF and asthma it could not discriminate between the groups (48). The use of Scond in CF may be limited to detecting the first signs of disease.

Sacin was abnormal in 14 of the 34 children and correlated with both FEV₁ and LCI (r=-0.34 and 0.68 respectively) suggesting Sacin may be a marker of disease severity. The correlation
was much stronger for LCI which is logical as both indices are measures of ventilation inhomogeneity compared to FEV$_1$ which is primarily a measure of larger airway obstruction. Horsley et al have previously demonstrated correlations between $S_{acin}$, FEV$_1$, and R$_{aw}$(0.5) in adults with CF and postulated that these correlations resulted from proximal displacement of the diffusion convection front secondary to obstruction of small conducting airways (82). Sixteen patients had a normal $S_{acin}$ but an abnormal LCI suggesting that $S_{acin}$ may be less sensitive to early disease than LCI.

This study suggests that LCI appears to be repeatable within children with CF. The intra visit variability of LCI across the three MBW tests performed at visit one were similar to previously published figures in healthy children. The mean coefficient of variation for LCI in this study was 5.4% compared to reported CVs from groups of healthy children in Edinburgh, London and a German and Austrian collaboration which were 5.4% (26), 5.2% (43) and 5.1% (44) respectively.

Intra-visit variability of $S_{cond}$ and $S_{acin}$ were high with mean CVs of 29.8% and 25.4% respectively. In our study MBW was performed without targeted tidal volumes. During analysis tidal volumes were corrected however it is unclear exactly how tidal volumes effect phase III slopes. By not targeting tidal volume, phase III slopes may have been more variable. In addition I was unable to exclude breaths in which phase III slopes were indiscernible. Without targeted tidal volumes and the ability to exclude aberrant breaths it does not appear that measurement of phase III slopes in children with CF is repeatable.

This study supports previous findings that LCI is highly sensitive to the presence of CF disease on CT (85) and correlates better with CF disease on CT than FEV$_1$ (76, 78, 89). Both LCI and
FEV₁ correlated with every aspect of the CT score but LCI correlated better than FEV₁ with extent and severity of bronchiectasis and bronchial wall thickening. Eighty three percent of patients with normal LCI had no bronchiectasis on CT. Ninety six percent of patients with an abnormal LCI had bronchiectasis on CT scanning and unfortunately Fuchs et al showed that abnormalities on CT scanning in children with CF persist (89). FEV₁ correlated better than LCI with large plugs and air trapping, illustrating that if regions of lung are obstructed they might not contribute to indices of ventilation heterogeneity.

S_{cond} only correlated weakly with bronchial wall thickness (r=0.34) and large plugs (r=0.38), it did not correlate with extent or severity of bronchiectasis, small plugs or air trapping. S_{cond} was abnormal in our group of children with CF compared to our healthy controls but as with Horsley at al’s group it did not correlate with severity of disease (82). S_{acin} did correlate with extent and severity of bronchiectasis, large plugs and air trapping but correlations were weaker than for LCI.

There were no overall statistically significant differences in any lung function parameter between the first and last visit. This differed from Kraemer et al who showed a deterioration in LCI and FEV₁ over a much more prolonged period of time (75) and Fuchs et al who showed an improvement in LCI over a 3 year period (89). Our study group was a motivated group of children with varied genotypes and lung disease, who had families who were willing to attend regular prolonged study visits. Within this group there were some individuals in whom FEV₁ and LCI deteriorated and the change in these parameters correlated. No differences in overall FEV₁ or LCI were seen probably due to the size, motivation and variation of the group in combination with the short time frame.
There was some variation in all lung function parameters over the four visits despite being performed at times of clinical stability. Mean inter-visit CV for individuals across the 4 visits was lowest for FVC at 6.8%, followed by FEV$_1$ at 8.1% and then LCI at 9.1%. LCI was almost as reproducible as FEV$_1$ despite the group including some severely diseased individuals, in whom CV was slightly higher, possibly because of intermittent obstruction of diseased lung sections secondary to mucus plugging. Low variability in LCI has meant that LCI has already been used to detect a treatment effect in clinical trials of hypertonic saline, dornase alpha, gene therapy and ivacaftor (95, 97, 99, 100). The inter-visit CV for $S_{\text{cond}}$ was 31.2%, $S_{\text{acin}}$ was 29.5% and $\text{FEF}_{25-75}$ was 19%. This was despite no difference in lung function from the start to the end of test visits. The inherent variability of $S_{\text{cond}}$, $S_{\text{acin}}$ and $\text{FEF}_{25-75}$ makes detecting a treatment effect during clinical trials or interpreting change in an individual patient very difficult.

The study was limited by several factors. The relatively short time frame may have prevented significant change in lung function from being detected. Change may have been detected had the group been larger. A larger group may have also allowed categorisation of disease severity to assess the utility of MBW in detecting change in different stages of CF. Repetition of a HRCT at the end of the study period would have assessed whether there was deterioration in structural disease that was not detected using lung function. The variability of phase III indices might have been reduced with the use of a fixed tidal breathing protocol however this could have been impractical in some of our children. In addition analysis of phase III slopes would have been improved with the use of software allowing exclusion of breaths in which the phase III slope was difficult to determine.
Results from this study suggest that LCI is a more sensitive test of early CF lung disease than FEV$_1$. LCI appears to be a repeatable and meaningful clinical test in CF, variability over time is limited and correlation with structural disease on CT is strong. Although $S_{cond}$ was abnormal in most children with CF, it did not correlate with FEV$_1$, LCI or degree and extent of bronchiectasis. Although $S_{acin}$ did correlate with markers of disease severity it did not appear as sensitive or correlate as well with CT changes as LCI. In addition $S_{cond}$ and $S_{acin}$ were both extremely variable over multiple test visits limiting their utility.
6 Project: Assessment of MBW in children with stable asthma

6.1 Introduction

The assessment of control in stable asthmatic children and decisions regarding changes in treatment are often made on the basis of subjective symptom reporting. Objective tools are limited (13, 101). Spirometry is the most commonly used lung function test in outpatient clinics, abnormal FEV$_1$ has been shown to relate to symptoms and risk of exacerbation (103-106), but most children with stable asthma have normal FEV$_1$ (13, 14). FEF$_{25-75}$ is possibly more sensitive than FEV$_1$ in detecting reversible airways obstruction (19); however its high variability has limited its clinical use. FeNO, which is believed to be a marker of airway inflammation, does not appear to consistently correlate with symptoms (112). In addition spirometry and FeNO may not be achievable in pre-school children.

MBW might offer an alternative option in the assessment of stable asthmatic children as it can be performed in children of all ages and can detect early small airways disease. Small airways disease has been shown to correlate with measures of asthma control (120) and MBW can detect abnormalities in well controlled asthmatic patients with normal FEV$_1$ (50, 124, 125). This study sought to determine whether MBW derived indices are abnormal in asthmatic children and whether abnormalities relate to asthmatic control, in order to establish whether MBW could be used as a clinical tool to guide treatments and improve outcomes.
6.2 **Aims**

To assess whether indices of MBW are abnormal in children with stable asthma.

To assess whether indices of MBW related to asthma control.

To compare whether indices of MBW were better able to identify asthma control than spirometry.

6.3 **Methods**

6.3.1 **Paediatric Asthma Genes and Environment Study**

The study population was a cohort of children (aged 5-16 years inclusive) within the Paediatric Asthma Genes and Environment Study (PAGES) attending the Royal Hospital for Sick Children, Edinburgh, UK. PAGES was designed to examine relationships between genetic variations and environmental factors in asthmatic children across Scotland (151). The Chief Investigator was Dr Stephen Turner based at the University of Aberdeen. Fifteen Scottish centres were involved, Edinburgh was the only centre to perform MBW. In Edinburgh recruitment and study visits were run by Debbie Miller, a senior research nurse at the Children’s Clinical Research Facility.

My involvement was in the analysis of the MBW tests. I examined relationships between abnormalities in MBW with conventional lung function, asthma severity, exacerbating factors and medication.
6.3.2 Recruitment and Inclusion/Exclusion Criteria

Between October 2008 and November 2010 children with consultant paediatrician diagnosed asthma attending the RHSC Asthma Clinic were invited to participate in the study. Exclusion criteria were any coexisting respiratory conditions, including cystic fibrosis or bronchopulmonary dysplasia (BPD), cerebral palsy, Down’s syndrome, gastro-oesophageal reflux (prescribed medications) and marked developmental delay.

Children acting as controls were previously recruited for other studies from families of hospital staff and local schools and had no history of respiratory disease, prematurity (<34 weeks), congenital cardiovascular disease, neuromuscular or bone disease likely to affect respiration or viral symptoms at the time of testing.

6.3.3 Study Visit

Children with asthma were asked to attend on a day when they were not taking oral corticosteroids to treat an acute exacerbation. At the study visit assessments were performed in the following order; MBW, exhaled nitric oxide, spirometry, skin prick reactivity, collection of salivary samples and bronchodilator response.

Parents were asked to complete questionnaires relating to their child’s asthma and environment either before or at the study visit. Questionnaires included an asthma questionnaire (available at [http://www.asthma-pages.com/participants/what/](http://www.asthma-pages.com/participants/what/)); this included respiratory questions validated in the BREATHE study (152), asthma control questions (the Child Asthma Control Test®, used with permission) and details of environmental exposure (from Biobank). Parents were also asked to complete the Paediatric Asthma Quality of Life Questionnaire
Patients performed three MBW as described in the common methods section.

Spirometry was performed in accordance with European Respiratory Society / American Thoracic Society guidelines using the portable ML3500 MicroLab Viasys spirometer. Bronchodilator response was measured by the change in FEV₁ 15 minutes after inhalation of 200 micrograms salbutamol, delivered from a pressurised metered dose inhaler via a large volume spacer device (Volumatic, Glaxo Smith Kline, UK). Parents and children were asked to withhold short acting beta agonists for 6 hours and long acting beta agonists for 12 hours prior to testing.

Exhaled NO was measured using a validated (153) portable NO analyser (NIOX MINO, Aerocrine, Solna Sweden). Measurements were performed in accordance with internationally recommended guidelines (154).

Salivary samples were collected for cotinine with a sterile absorbent cotton wool swab (Salivette, Sarstedt Ltd, Leicester, UK). Samples were processed using ELISA (ABS laboratories, Welwyn Garden City, UK). Levels below the limit of detection were assigned a value of 0.1 ng/ml.
Skin prick testing to assess sensitivity to Dermatophagoides pteronyssinus, cat dander, dog dander, whole egg, Alternaria alternans, Aspergillus fumigatus, peanut and grass was performed using standard methodology (155) (ALK, Northampton). Histamine 10mg/ml was used as a positive control and 0.9% saline as a negative control. Testing was withheld if a patient had taken antihistamines within 72 hours and peanut testing was withheld in patients with a history of peanut anaphylaxis.

6.3.4 Analysis

MBW raw data were extracted from the Innocor and analysed as described earlier to produce values for LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$. Z-scores and percent of predicted values for FEV$_1$, FVC and FEF$_{25-75}$ were calculated.

Minitab Version 17 statistical software (Minitab, USA) was used for data analysis. Data are presented as mean with standard deviation (SD). Two sample T-tests were used to compare between groups. Pearson and Spearman correlation coefficients were used to compare variables. Significance was assumed at $p=0.05$.

6.3.5 Ethical Approval

The study was approved by the Cornwall and Plymouth Research Ethics Committee. Written consent was obtained from parents at completion of questionnaire and verbal consent was sought from the child at the study visit.
6.4 Results

6.4.1 Differences between asthmatic and healthy children

Sixty three children (51% male) with asthma aged 5.1-16.5 years (mean 10.3 years) were recruited. The control group comprised 66 children (63% male) aged 5.0-16.1 years (mean 11.2 years). The criteria for recruitment was inclusive of all types and severities of asthma. The cohort included patients on all levels of the BTS treatment ladder (BTS/SIGN Asthma guideline 2009) including two patients who were only receiving salbutamol as required and two patients who required maintenance oral prednisolone at the time of testing, see table 11.

<table>
<thead>
<tr>
<th>BTS Step</th>
<th>Number of patients</th>
<th>Percentage of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>24%</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>52%</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>17%</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3%</td>
</tr>
</tbody>
</table>

*Table 11: Patients on each step of the BTS ladder*

Sixteen patients had had at least one admission to hospital in the year and eight patients were hospitalised in the six months prior to testing. Thirty four patients had had at least one course of prednisolone in the year and 25 patients had had oral steroids in the six months prior to testing.
<table>
<thead>
<tr>
<th>Severity Indicator</th>
<th>Percentage of asthmatic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital admission in past year</td>
<td>25%</td>
</tr>
<tr>
<td>Hospital admission in past 6 months</td>
<td>13%</td>
</tr>
<tr>
<td>Prednisolone course in past year</td>
<td>54%</td>
</tr>
<tr>
<td>Prednisolone course in past 6 months</td>
<td>40%</td>
</tr>
<tr>
<td>BTS therapy ≥ 4</td>
<td>21%</td>
</tr>
<tr>
<td>Continuous oral prednisolone</td>
<td>3%</td>
</tr>
<tr>
<td>Wheeze only with colds</td>
<td>14%</td>
</tr>
</tbody>
</table>

Table 12: Proportion of asthmatic group reporting severity indicator.

Significant differences were observed between children with asthma and healthy controls for LCI and FEF<sub>2575</sub>, but not FEV<sub>1</sub> (table 13). There were no significant differences in S<sub>cond</sub> or S<sub>acin</sub> between the groups. Mean values for all lung function indices in the asthmatic group fell within healthy ranges.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic</th>
<th>Control</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.7 (0.9)</td>
<td>6.3 (0.5)</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.03 (0.03)</td>
<td>0.03(0.02)</td>
<td>P=0.21</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.15 (0.07)</td>
<td>0.14 (0.07)</td>
<td>P=0.60</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; z score</td>
<td>-0.65 (0.8)</td>
<td>-0.41 (1.0)</td>
<td>P=0.16</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;2575&lt;/sub&gt; z score</td>
<td>-1.20 (0.9)</td>
<td>-0.51 (1.0)</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table 13: Mean (SD) results for asthmatic and control groups with significance levels for comparisons using 2 sample t-tests.
6.4.2 Correlation between lung function indices

In children with asthma significant correlations were identified between LCI and \( S_{\text{cond}} \) (Pearson \( r=0.37, P<0.01 \)), \( S_{\text{acin}} \) (Pearson \( r=0.53, p<0.0001 \)), \( \text{FEF}_{25-75} \) (Pearson \( r=-0.31, p=0.04 \)), bronchodilator response (Pearson \( r=0.33, p=0.03 \)) but not \( \text{FEV}_1 \). 1.64 SDS values were calculated using values obtained in the control group; the 1.64 SDS for LCI was 7.12, for \( S_{\text{cond}} \) it was 0.06L\(^{-1}\) and for \( S_{\text{acin}} \) it was 0.25L\(^{-1}\). Figures 40-43 show scatterplots illustrating the correlation between different indices of lung function with reference lines at 1.64 SDSs. Values to the right of the vertical line show LCI values greater than 95% of normal values. Values below the horizontal line in the \( \text{FEF}_{25-75} \) graph and above the horizontal line in the \( S_{\text{cond}} \) and \( S_{\text{acin}} \) graphs show values out with 95% of healthy values.

![Figure 40: Scatterplot of \( \text{FEV}_{25-75} \) z-score versus mean LCI in asthmatic patients. Reference lines at 1.64 SDS.](image)

164
Figure 41: Scatterplot of $S_{cond}$ versus mean LCI in asthmatic patients. Reference lines at 1.64 SDS.

Figure 42: Scatterplot of $S_{acin}$ versus mean LCI in asthmatic patients. Reference lines at 1.64 SDS.
FEV₁ and FEF₂₅-₇₅ z-scores and S\textsubscript{cond} correlated with exhaled nitric oxide, r=−0.39, (p=0.007), r=−0.33, (p=0.02) and r=0.29, (p=0.02) respectively (Pearson correlation coefficients). LCI and S\textsubscript{acin} and did not.
6.4.3 **Asthma Severity**

LCI, $S_{acin}$, FEV$_1$ and FEF$_{25-75}$ did not correlate with number of hospital admissions, or number of courses of steroids over the previous six months or year. $S_{cond}$ correlated with courses of prednisolone over the previous year (Spearman $r=0.27$, $P=0.035$) but not courses over the previous six months.

$S_{cond}$ correlated with BTS score (Pearson $r=0.30$, $p=0.02$), patients on step 3, 4 or 5 had significantly higher $S_{cond}$ (0.034 L$^{-1}$), than patients on steps 1 or 2 (0.021 L$^{-1}$), $p=0.04$. LCI, $S_{acin}$, FEV$_1$ and FEF$_{25-75}$ did not correlate with BTS score.

6.4.4 **Asthma Control**

In the asthma questionnaire parents were asked to report on how well they considered their child’s asthma to be controlled; options were “Not controlled at all”, “Poorly controlled”, “Somewhat controlled”, “Well controlled” or “Completely controlled”. Patients whose parents reported that their child’s asthma was either completely or well controlled had significantly lower LCI (6.4) than those whose parents described their asthma as somewhat controlled or worse (6.9, $p=0.02$). There were no differences in FEV$_1$ or FEF$_{25-75}$ between these groups.

Parents were also asked to grade the frequency of their child’s symptoms and their interference with daily life. LCI correlated with time that asthma affected school work, salbutamol use and perceived control (see table 14). FEV$_1$ did not correlate with any parent reported factor. FEF$_{25-75}$ z-score correlated with perceived control.
Table 14: Spearman correlation r values (and p values) between lung function and reported symptoms.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Asthma days</th>
<th>Wheeze days</th>
<th>Night waking</th>
<th>School work</th>
<th>SOB</th>
<th>Early waking</th>
<th>Blue Inhaler</th>
<th>Asthma Control</th>
<th>Blue 6Mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>0.17 (0.25)</td>
<td>0.28 (0.058)</td>
<td>0.08 (0.59)</td>
<td><strong>0.36 (0.02)</strong></td>
<td>0.29 (0.055)</td>
<td>0.10 (0.50)</td>
<td><strong>0.33 (0.03)</strong></td>
<td><strong>0.35 (0.02)</strong></td>
<td>0.20 (0.12)</td>
</tr>
<tr>
<td>S$_{\text{cond}}$ (L$^{-1}$)</td>
<td>-0.10 (0.52)</td>
<td>-0.00 (0.96)</td>
<td>-0.04 (0.96)</td>
<td>0.08 (0.58)</td>
<td>0.14 (0.34)</td>
<td>0.05 (0.77)</td>
<td>0.25 (0.10)</td>
<td><strong>0.30 (0.05)</strong></td>
<td>0.13 (0.31)</td>
</tr>
<tr>
<td>FEV$_1$ZS</td>
<td>0.12 (0.50)</td>
<td>-0.22 (0.20)</td>
<td>-0.16 (0.37)</td>
<td>-0.15 (0.37)</td>
<td>-0.11 (0.52)</td>
<td>-0.09 (0.58)</td>
<td>0.08 (0.64)</td>
<td>-0.17 (0.31)</td>
<td>0.01 (0.92)</td>
</tr>
<tr>
<td>FEF$_{2575}$ZS</td>
<td>0.03 (0.88)</td>
<td>-0.25 (0.15)</td>
<td>-0.12 (0.49)</td>
<td>-0.30 (0.07)</td>
<td>-0.31 (0.06)</td>
<td>-0.30 (0.07)</td>
<td>-0.11 (0.51)</td>
<td><strong>-0.35 (0.03)</strong></td>
<td>-0.06 (0.69)</td>
</tr>
</tbody>
</table>

6.4.5 Atopy and Allergy

Thirty six patients had eczema, 26 had rhinitis and 36 had food allergies at the time of testing. Twenty five patients had skin prick testing; only three patients were not reactive to any antigen. Two children were sensitive to Alternaria alternans, 17 to cat dander, 10 to dog dander, 17 to grass, 18 to house dust mite and three to peanut; no patients were sensitive to whole egg or Aspergillus fumigatus.

No differences in LCI, S$_{\text{cond}}$, S$_{\text{acin}}$ or FEV$_1$ corresponded to parent reported triggers including exercise or pets. Patients who reported dust as a trigger factor had a worse FEF$_{2575}$ z score (-1.46 vs -0.9, P=0.03).
There were no differences in LCI, $S_{cond}$, $S_{acin}$ or FEV$_1$ between groups of children in whom salivary cotinine was either detected or not. Children in whom salivary cotinine was detected had significantly lower FEF$_{25-75}$ z-score compared to those in whom cotinine was not detected (-1.77 versus -1.06 respectively, p=0.01).
6.5 **Discussion**

In this study LCI in stable asthmatic children was significantly higher than in healthy children. LCI was significantly higher in patients whose parents reported poorer asthma control, something not evident for FEV$_1$ or FEF$_{25-75}$. There was no difference in $S_{\text{cond}}$ or $S_{\text{acin}}$ between asthmatic and healthy groups but within the asthmatic group $S_{\text{cond}}$ correlated with regular medications and courses of prednisolone over the preceding six months.

The cohort of asthmatic children had a wide range of disease severity; current control was not a precondition of inclusion, however mean FEV$_1$ and FEF$_{25-75}$ z-scores fell within normal ranges. This is in keeping with evidence suggesting that most children with stable asthma have normal FEV$_1$ (13, 14). No difference in FEV$_1$ between the healthy and control groups could be detected. Mean FEF$_{25-75}$ z-score was significantly lower in the asthmatic group compared to the control group. FEF$_{25-75}$ has previously been shown to be worse in children reporting chronic persistent symptoms (108) and our group included some children who reported poor asthma control.

Mean LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$ in the group of asthmatic children fell within previously reported healthy ranges (50, 54, 82) and within a standard deviation of the study control group. Mean LCI of in the asthmatic group was, however, significantly higher than in the control group. There was no difference in $S_{\text{cond}}$ or $S_{\text{acin}}$ between the groups. Previous studies have demonstrated abnormalities in $S_{\text{cond}}$ and $S_{\text{acin}}$ in asthmatic patients (45, 48, 126-128). Differences in $S_{\text{cond}}$ and $S_{\text{acin}}$ were not found in this study, despite differences in LCI and FEF$_{25-75}$, possibly because the size of the study it was not possible to detect differences in these highly variable parameters.
Reported healthy values for $S_{\text{cond}}$ and $S_{\text{acin}}$ in children vary, possibly due to methodology, choice of inert gas and equipment. In this and previous studies using the modified Innocor, 0.2% SF$_6$ and without a fixed tidal breathing protocol, reported values of phase III slopes in healthy children and adults are different than in other centres (1, 50, 82). For example, Verbanck described the upper limit of normal of $S_{\text{acin}}$ as 0.11-0.13 L$^{-1}$ (45) which is below the healthy mean reported in this study. However, Verbanck used fixed tidal volumes and N$_2$. N$_2$ is lighter than SF$_6$ and might theoretically decrease $S_{\text{acin}}$ due to faster diffusion (58). In addition tidal volume is known to affect the phase III slope (21), fixed tidal volumes are likely to therefore have an effect on indices of phase III slope analysis. In studies in which tidal volume is not fixed, tidal volume is corrected but this adjustment has not been validated.

In asthmatic children LCI correlated with $S_{\text{cond}}$, $S_{\text{acin}}$ and FEF$_{25-75}$. However, eleven children had abnormal LCI and normal $S_{\text{cond}}$, only two children had abnormal $S_{\text{cond}}$ and normal LCI. Ten children had abnormal LCI and normal $S_{\text{acin}}$ and only two had abnormal $S_{\text{acin}}$ and normal LCI. This might suggest that LCI is more sensitive to asthmatic disease than $S_{\text{cond}}$ or $S_{\text{acin}}$. Keen et al have previously published MBW data from asthmatic children in whom $S_{\text{cond}}$ was the commonest MBW abnormality (127). Keen et al required children to breath between 10-15ml/kg, we did not set limits but encouraged children to breathe regularly. The limits of normality in both studies were calculated on the basis of the mean and standard deviation of the respective control groups. The variability within $S_{\text{cond}}$ and $S_{\text{acin}}$ were much larger in our study compared to Keen et al’s (reflected by a larger standard deviation) and the limits of normality in our study were therefore greater. Sonappa et al also found higher variability in $S_{\text{cond}}$ and $S_{\text{acin}}$ than in LCI (130). The higher variation in indices of phase III slopes in our study may have limited the ability to detect differences between groups.
In this study FEV$_1$ and FEF$_{2575}$ z-scores and S$_{cond}$ correlated weakly with exhaled nitric oxide ($r=-0.39$, $p=0.007$, $r=-0.33$, $p=0.02$ and $r=0.29$, $p=0.02$), LCI and S$_{acin}$ and did not. Previous studies have been contradictory, some studies finding no correlation between FeNO and MBW indices (47, 50). In Keen et al.’s study a correlation was found between S$_{cond}$ and FeNO, however LCI also correlated with FeNO (127). LCI was also shown to correlate with FeNO in a study investigating the effects of early severe bronchiolitis, in patients with asthma (125). In addition, correlations between S$_{acin}$ and exhaled alveolar nitric oxide have also been found (127, 128). Airway remodelling in the small airways of asthmatic children is known to exist (113); and may be the reason why no correlation was found between LCI and exhaled NO, despite elevated LCI in the asthmatic group.

LCI and S$_{acin}$ did not correlate with recent courses of prednisolone or admissions to hospital. Since these factors indicate exacerbation, it appears that LCI and S$_{acin}$ may not be higher in patients having frequent exacerbation. Or it may be that patients who experienced numerous recent exacerbations had increases in their daily medication and as a result were better controlled at the time of study testing. Neither LCI nor S$_{acin}$ correlated with BTS score either but this is complicated because MBW indices have been shown to improve with inhaled corticosteroids (47). An earlier study of 31 asthmatic children within our department also found that LCI did not correlate with BTS step (50). However Verbanck found that S$_{acin}$ was higher in adults receiving 500µg or more of beclomethasone dipropionate than those on smaller doses (128). Relationships between lung function indices and treatments appear complex, asthma is a heterogeneous condition and a larger study or more specified cohort may lead to better understanding.
S_{cond} was significantly higher in patients on step three, four or five of the BTS ladder, than in patients on steps one or two. S_{cond} also correlated with the number of courses of prednisolone over the year prior to testing. This could imply that S_{cond} is an indicator of severity. However there were no significant correlations between S_{cond} and reported recent hospital admissions or courses of prednisolone over the 6 months prior to testing. Although the asthmatic group as a whole did not have significantly higher S_{cond} than the control group when the group with a BTS score of 3 or more was compared to the control group there was a significant difference (p=0.028). Other studies have found differences in S_{cond} between healthy and asthmatic groups, we may not have in the whole asthmatic group because of the inclusion of mild asthmatic patients.

Children who’s asthma was described as either completely or well controlled had significantly lower LCI (6.4) than those whose parents described their asthma as somewhat controlled or worse (6.9, p=0.02). LCI also correlated with time that asthma affected school work and salbutamol use (see table 14). S_{cond} and FEF_{2575} also correlated with perceived control but did not correlate with any other parent reported factor, FEV_{1} did not correlate with reported symptoms. LCI and S_{cond} have previously been shown to be significantly higher in patients with uncontrolled compared to controlled asthma (127, 132). This has implications for the routine monitoring of asthmatic patients. As symptom perception and reporting varies between individuals and families LCI may be of use in objectively assessing patients who might benefit from an increase in medication. This study was, however, subject to the same biases of symptom perception and reporting.

This study was limited because it was not designed to examine relationships between MBW indices and asthma control and severity. The analysis has been performed on a group of
patients who were participating in a wider study regarding genetic and environmental influences in asthma. Multiple statistical tests were performed on the data to identify correlations between variables, some of which may have been coincidental. It would be interesting to further investigate some of the associations identified in a prospectively designed study with predefined hypotheses. In addition recruitment of asthmatic children did not discriminate between phenotypes and in such a heterogeneous condition different abnormalities in lung function may be possible in different groups. More specified groups might detect more significant results. Data regarding asthma control was subject to bias because it relied on retrospective patient reporting, this could possibly have been reduced with symptom diaries. Lastly as described in previous studies the variability in phase III slopes may have been reduced with software allowing the exclusion of breaths in which phase III slopes were difficult to identify.

Asthma is a heterogeneous disease and therefore lung function may be best interpreted by reference to change within the individual. LCI may be considered a better surrogate of symptom control than FEV₁ and presents an alternative option for the objective assessment of the stable asthmatic child. Despite correlations with FeNO and regular treatments, S_cond appeared too variable to discriminate between healthy and asthmatic patients. Further work to define the effects of different phenotypes of asthma on LCI and the benefits of LCI guided treatment is warranted.
7 Project: The effects of exercise on MBW derived indices in healthy children and children with asthma

7.1 Introduction

Many children with asthma suffer from exercise induced symptoms. However the sensitivity of detecting abnormalities in symptomatic children using conventional exercise challenge testing with spirometry is poor (135). MBW has been shown to detect abnormalities in asthmatic children with normal FEV$_1$ (50, 124, 125), and may therefore also be more sensitive to exercise induced changes in lung function. MBW has not previously been performed following exercise in asthmatic children, but studies of hyperpolarised helium MRI have demonstrated heterogeneous ventilation in asthmatics following exercise (141). MBW indices have also been shown to correlate with bronchial hyper-responsiveness (47), and therefore MBW might be able to predict children who develop exercise induced bronchoconstriction. The effects of salbutamol on MBW indices following exercise are unknown and are important to establish. Evidence suggests that salbutamol does not completely normalise ventilation inhomogeneity in asthma (48, 50, 126) and alternative treatments might reduce symptoms.

This study was performed to determine the effects of exercise on MBW in asthmatic children in order to assess whether MBW could be a more sensitive way of detecting exercise induced asthma (EIA) than FEV$_1$. This would be important in improving our understanding of EIA pathophysiology, detecting more children with exercise induced asthma and guiding clinical decision making in these children.
7.2 Aims

To investigate changes in indices of MBW in asthmatic individuals following exercise.

To relate the changes in MBW derived indices to changes in spirometry and reported exercise induced symptoms.

To determine whether baseline indices of MBW can predict exercise induced lung function responses.

To investigate the effects of salbutamol on MBW derived indices following exercise in asthmatic children.
7.3 Methods:

7.3.1 Inclusion/Exclusion Criteria

Children with respiratory physician diagnosed asthma and healthy controls recruited from local schools were invited to participate. Participants were aged 5-16 years.

Asthmatic children had respiratory paediatrician diagnosed asthma and were currently or previously under the care of the respiratory physicians at the RHSC. They were clinically stable, with no exacerbations requiring oral corticosteroids or increased bronchodilator use in the two weeks prior to testing.

Healthy children were excluded if they had any significant respiratory history including asthma or regular anti-asthma medications, previous hospitalisation for a respiratory infection, recurrent wheezing episodes, exercise related respiratory symptoms, pneumonia, cystic fibrosis, pertussis or tuberculosis. In addition they were excluded if they were born before 34 weeks gestation, had neuromuscular weakness or bone disease likely to affect respiratory function, congenital cardiac defects requiring treatment or a history of atopy (including eczema, hay fever or allergic rhinitis).

No participant had any symptoms of viral upper respiratory tract infection in the week prior to testing.
7.3.2 Recruitment

Asthmatic children were identified through the RHSC asthma out-patient clinic. Letters were sent to families inviting them to participate. Letters were followed up by telephone calls for discussion and to assess willingness to participate.

Healthy volunteers were recruited by letters sent to parents through local schools. Families who replied were contacted by phone to confirm interest and provide further information regarding the study.

7.3.3 Study Visit

Each child attended one study visit at the RHSC. Participants followed the order of testing as illustrated in figure 44. Children were asked not to take bronchodilators for six hours prior to the study visit, all other medications were given as normal.

Consent for study participation was taken on arrival and then children and their parents completed a symptom questionnaire. The questionnaires used in the study were designed using the International study of Asthma and Allergies in Childhood (ISAAC) study core questionnaire as a framework (156).
Figure 44: Exercise in Asthma Study Flowchart
Baseline exhaled NO (FeNO) testing using the NioxMino (Aerocrine)/Niox was performed. Patients were required to breathe out through a mouthpiece against partial pressure to obtain a steady state FeNO signal. Visual incentives were displayed for encouragement.

Patients then performed a set of three MBW tests, followed by spirometry (in accordance with ERS/ATS guidelines using a CareFusion spirometer).

Children subsequently performed an exercise challenge on a treadmill in accordance with ATS guidelines (157). A target heart rate was set for each child at 80% of their calculated maximum heart rate (220-age in years). Once this was achieved children under the age of 12 years ran for a further 4 minutes and those over 12, ran for a further 6 minutes.

Following completion of the exercise challenge the child was asked to perform one forced expiratory manoeuvre within a minute, the aim of which was to record \( \text{FEV}_1 \). This was followed by a single MBW test. This series was repeated to achieve a total of three \( \text{FEV}_1 \) measurements and three MBW tests. The child then performed a further full set of spirometry.

Following post exercise testing, 400 micrograms of salbutamol was administered to both healthy and asthmatic children. This was delivered from a pressurised metered dose inhaler via a large volume spacer device (Volumatic, GlaxoSmith Kline, UK). After ten minutes a further set of three MBW tests and then spirometry were repeated.
If at any time a child’s FEV₁ fell to below 20% of their baseline or if the child experienced significant discomfort, testing was stopped. Inhaled salbutamol was given if appropriate and the child was assessed 10 minutes later with spirometry.

Asthmatic children were followed up by telephone 12-16 days after their visit. The call was made by a research nurse in which questions designed to assess post visit symptom control were asked.

7.3.4 Analysis
MBW raw data was extracted from the Innocor and analysed as described earlier to produce values for LCI, S_{cond} and S_{acin}. Z-scores and percent of predicted values for FEV₁, FVC and FEF_{25-75} were calculated. Exercise induced changes in lung function were calculated by dividing change by baseline value.

Data were analysed using Minitab Version 17 statistical software (Minitab, USA). Two sample t tests were used to compare data between groups and paired t tests within groups before and after exercise or salbutamol. Correlations were assessed using Pearson correlation coefficients. Significance was assumed at p=0.05.

7.3.5 Ethical Approval
Ethical Approval was granted by the Lothian Research Ethics committee. Parents and where possible children provided written informed consent.
7.4 Results

7.4.1 Demographics

Twenty one asthmatic patients (eight girls, 13 boys), aged 7.8-15.9 years (mean 11.5 years) and 21 healthy controls (nine girls and 12 boys) aged 7.6-15.4 years (mean 12.1) were recruited. One healthy girl was excluded before testing as she was incidentally found to have complete heart block.

All of the asthmatic patients were attending the RHSC asthma clinic. Nine children reported one admission to hospital over the preceding 2 years, twelve children reported no admissions. Most children had had courses of oral corticosteroids over the preceding 2 years, the numbers of children requiring multiple courses are illustrated in figure 45. Six patients had had at least one oral corticosteroid course in the 6 months prior to the study. Only two children had had an increase in their regular medications in the 6 months before their study visit.
Eleven children had a history of food allergy, 16 allergy to animals, 15 children had a history of eczema, 16 reported hay fever and 17 reported rhinitis. Seven patients reported nocturnal symptoms and six reported early morning symptoms in the two weeks preceding their study visit. The number of 100mcg doses of inhaled salbutamol each child reported in the seven days preceding their study visit are shown in figure 46.
The amount and type of regular exercise children reported varied but all children described at least one hour of exercise per week. Nine children reported prophylactically using salbutamol prior to exercise. Thirteen children reported using salbutamol during exercise, eight of whom said they needed salbutamol every time they exercised. Sixteen children said they experienced wheeze or difficulty breathing when they exercised, seven of whom said symptoms occurred every time they exercised. Only two children felt that their asthma prevented them from doing as much exercise as they would like.
7.4.2 Baseline Lung Function

The initial mean (SD) LCI of the asthmatic group was 7.2 (0.7) which was significantly higher than the control group (6.8 (0.4)), \( p = 0.02 \). Initial FEV\(_1\) and FEF\(_{25-75}\) z scores and FeNO were also significantly worse in the asthmatic group (\( p = 0.01, p < 0.01 \) and \( p = 0.05 \) respectively).

There were no significant differences in \( S_{\text{cond}} \), \( S_{\text{acin}} \) or FVC (see table 15).

<table>
<thead>
<tr>
<th></th>
<th>Healthy Children</th>
<th>Asthmatic Children</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.8 (0.4)</td>
<td>7.2 (0.7)</td>
<td>( p = 0.02 )</td>
</tr>
<tr>
<td>( S_{\text{cond}} ) (L(^{-1}))</td>
<td>0.02 (0.03)</td>
<td>0.04 (0.02)</td>
<td>( p = 0.07 )</td>
</tr>
<tr>
<td>( S_{\text{acin}} ) (L(^{-1}))</td>
<td>0.18 (0.06)</td>
<td>0.17 (0.06)</td>
<td>( p = 0.40 )</td>
</tr>
<tr>
<td>FEV(_1) z-score</td>
<td>0.08 (0.73)</td>
<td>-0.58 (0.85)</td>
<td>( p = 0.01 )</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.18 (0.85)</td>
<td>0.36 (0.90)</td>
<td>( p = 0.53 )</td>
</tr>
<tr>
<td>FEF(_{25-75}) z-score</td>
<td>-0.33 (0.91)</td>
<td>-1.43 (1.07)</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td>FeNO</td>
<td>19.7 (21.7)</td>
<td>37.7 (33.6)</td>
<td>( p = 0.05 )</td>
</tr>
</tbody>
</table>

Table 15: Baseline mean (SD) lung function. 2 sample t tests used to compare healthy and asthmatic groups.

In asthmatic patients LCI correlated with \( S_{\text{acin}} \) (Pearson \( r = 0.54, p = 0.02 \)), but not \( S_{\text{cond}} \), FEV\(_1\) z-score or FEF\(_{25-75}\) z-score. Neither \( S_{\text{cond}} \) nor \( S_{\text{acin}} \) correlated with FEV\(_1\) or FEF\(_{25-75}\) z-score. No lung function parameter correlated with FeNO.

FEF\(_{25-75}\) z-score correlated with the number of courses of oral corticosteroids children had had in the preceding two years (Spearman \( r = -0.57, p <0.01 \)). No other lung function parameter correlated with number of oral corticosteroid courses or hospital admissions in the preceding two years. There were no correlations between baseline lung function and reported symptom frequency or salbutamol use in the period immediately preceding testing.
7.4.3 Changes in lung function following exercise

Following exercise FEV$_1$ fell by more than 20% in three of the asthmatic children. These children had abnormal baseline lung function with a mean (SD) LCI of 7.6 (0.8), FEV$_1$ z-score of -1.7 (1.2) and FEF$_{25-75}$ z-score of -2.7 (0.6). However FEF$_{25-75}$ z-score was the only parameter to be significantly worse when compared to the group who did not experience a drop in FEV$_1$ of more than 20% (p = 0.03). Baseline FVC z-score was normal (0.16 (1.1)).

S$_{cond}$ 0.06 L$^{-1}$ (0.02), S$_{acin}$ 0.16 L$^{-1}$ (0.03) were also within 1.64 standard deviations of healthy mean values. There are limited data on these three children following exercise as only one patient managed to perform MBW following exercise. These patients have had to be excluded from the following analysis.

Following exercise, FEF$_{25-75}$ was still significantly worse in the asthmatic group compared to the healthy group (p = 0.004). In addition S$_{cond}$ was now significantly elevated in the asthmatic group (p = 0.001). FEV$_1$ and LCI were no longer significantly worse in the asthmatic group compared to the healthy volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthmatic</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.9 (0.4)</td>
<td>7.3 (1.0)</td>
<td>p = 0.08</td>
</tr>
<tr>
<td>S$_{cond}$ (L$^{-1}$)</td>
<td>0.01 (0.01)</td>
<td>0.034 (0.026)</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>S$_{acin}$ (L$^{-1}$)</td>
<td>0.21 (0.09)</td>
<td>0.19 (0.06)</td>
<td>p = 0.36</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>0.07 (0.71)</td>
<td>-0.39 (0.69)</td>
<td>p = 0.051</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.03 (0.89)</td>
<td>0.19 (0.97)</td>
<td>p = 0.61</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>-0.10 (0.75)</td>
<td>-0.98 (0.97)</td>
<td>p = 0.004</td>
</tr>
</tbody>
</table>

*Table 16: Post exercise lung function. 2 sample t tests used to compare groups*
Within the healthy children there were no significant changes in lung function following exercise. Table 17 shows baseline and post exercise lung function in the healthy group.

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Post exercise</th>
<th>95% CI for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.8 (0.4)</td>
<td>6.9 (0.4)</td>
<td>-0.09, 0.26 (p = 0.31)</td>
</tr>
<tr>
<td>S_{cond} (L^{-1})</td>
<td>0.03 (0.03)</td>
<td>0.01 (0.01)</td>
<td>-0.03, 0.0004 (p = 0.06)</td>
</tr>
<tr>
<td>S_{acin} (L^{-1})</td>
<td>0.18 (0.07)</td>
<td>0.21 (0.09)</td>
<td>-0.01, 0.07 (p = 0.15)</td>
</tr>
<tr>
<td>FEV_1 z-score</td>
<td>0.08 (0.73)</td>
<td>0.07 (0.71)</td>
<td>-0.11, 0.10 (p = 0.90)</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.15 (0.84)</td>
<td>0.03 (0.89)</td>
<td>-0.30, 0.07 (p = 0.21)</td>
</tr>
<tr>
<td>FEF_25-75 z-score</td>
<td>-0.25 (0.93)</td>
<td>-0.10 (0.75)</td>
<td>-0.04, 0.35 (p = 0.11)</td>
</tr>
</tbody>
</table>

Table 17: Initial and post exercise lung function Mean (SD) in healthy children. Change determined using paired t tests.

In the asthmatic patients there were significant changes in S_{acin}, FVC and FEF\_25-75 z-score following exercise. S_{acin} and FVC deteriorated (p = 0.02 and 0.004), but FEF\_25-75 actually improved following exercise (p = 0.001). LCI trended upwards but this was not significant (p = 0.14).

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Post exercise</th>
<th>95% CI for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>7.1 (0.6)</td>
<td>7.4 (1.1)</td>
<td>-0.11, 0.66 (p = 0.14)</td>
</tr>
<tr>
<td>S_{cond} (L^{-1})</td>
<td>0.04 (0.02)</td>
<td>0.04 (0.03)</td>
<td>-0.02, 0.02 (p = 0.86)</td>
</tr>
<tr>
<td>S_{acin} (L^{-1})</td>
<td>0.17 (0.06)</td>
<td>0.19 (0.05)</td>
<td>0.001, 0.045 (p = 0.04)</td>
</tr>
<tr>
<td>FEV_1 z-score</td>
<td>-0.39 (0.66)</td>
<td>-0.39 (0.69)</td>
<td>-0.12, 0.12 (p = 0.99)</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.39 (0.88)</td>
<td>0.19 (0.97)</td>
<td>-0.32, -0.07 (p = 0.004)</td>
</tr>
<tr>
<td>FEF_25-75 z-score</td>
<td>-1.23 (0.99)</td>
<td>-0.98 (0.97)</td>
<td>0.11, 0.38 (p = 0.001)</td>
</tr>
</tbody>
</table>

Table 18: Initial and post exercise lung function Mean (SD) in asthmatic children. Change determined using paired t tests.
Children were asked if their exercise was limited by their asthma. Nine children said that their asthma did not affect how much exercise they did. Four children said that they could exercise as long as they took salbutamol. Only two children said that their asthma stopped them exercising even if they took salbutamol. One patient did not answer the question. If the children were divided into two groups depending on if they reported needing salbutamol (n=6) or not (n=9) the change in LCI following exercise appeared higher in the group requiring salbutamol. In the group who reported requiring salbutamol LCI increased by a mean of 6.6% compared to 2.7% in the group who did not, this trend was not statistically significant (p = 0.50). Table 19 shows the percentage change in the other lung function parameters depending on whether children reported requiring salbutamol or if they were not limited by their asthma at all. There appeared to be a more marked deterioration in FEV\textsubscript{1} in the group who reported requiring salbutamol to exercise, but again this was not significant.

<table>
<thead>
<tr>
<th></th>
<th>No reported exercise limitation</th>
<th>Exercise limitation without salbutamol</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>2.7 (8.5)%</td>
<td>6.6 (11.7)%</td>
<td>p = 0.50</td>
</tr>
<tr>
<td>S\textsubscript{cond}</td>
<td>25 (111)%</td>
<td>-11.5 (43.2)%</td>
<td>p = 0.40</td>
</tr>
<tr>
<td>S\textsubscript{acin}</td>
<td>29.9 (45.2)%</td>
<td>14.2 (29.1)%</td>
<td>p = 0.43</td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>0.26 (3.9)%</td>
<td>-0.78 (2.4)%</td>
<td>p = 0.51</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75}</td>
<td>5.6 (9.0)%</td>
<td>10.14 (8.1)%</td>
<td>p = 0.31</td>
</tr>
</tbody>
</table>

Table 19: Mean (SD) percentage change in lung function following exercise in groups that reported no exercise limitation and requirement for salbutamol. Two sample t tests used for comparison.

In the asthmatic group change in lung function parameters following exercise did not correlate with each other. In addition there were no correlations between change in any lung function parameter and baseline FeNO, number of oral corticosteroid courses or number of hospital
admissions over the preceding two years or symptoms and salbutamol use in the weeks preceding testing.

Figures 47 and 48 are line plots of individual LCI and FEV₁ before and after exercise, the patients who demonstrated a fall in FEV₁ of greater than 20% have been included (Patients 1, 17 and 20). The graph of LCI illustrates that in many individuals there is an immediate increase in LCI with the first MBW test that tends to fall by the second or third test. FEV₁ stayed relatively stable throughout post exercise testing (except in the three patients who were excluded due to dramatic falls in FEV₁).

Figure 47: Line plot of individual LCI from mean baseline, at each test post exercise and mean following salbutamol
Figure 48: Line plot of individual FEV1 from best at baseline, at each blow post exercise and best following salbutamol
7.4.4 Changes in lung function following salbutamol

Following salbutamol the only significant differences between healthy and asthmatic groups were in $S_{\text{cond}}$ and $\text{FEF}_{25-75}$ z-score ($p = 0.02$ and 0.003 respectively). Mean LCI and $\text{FEV}_1$ z-score were not significantly different between groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthmatic</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>7.1 (0.4)</td>
<td>7.4 (0.6)</td>
<td>$p = 0.09$</td>
</tr>
<tr>
<td>$S_{\text{cond}}$ (L$^{-3}$)</td>
<td>0.01 (0.03)</td>
<td>0.03 (0.02)</td>
<td>$p = 0.02$</td>
</tr>
<tr>
<td>$S_{\text{acin}}$ (L$^{-1}$)</td>
<td>0.25 (0.18)</td>
<td>0.17 (0.06)</td>
<td>$p = 0.08$</td>
</tr>
<tr>
<td>$\text{FEV}_1$ z-score</td>
<td>0.23 (0.70)</td>
<td>-0.10 (0.88)</td>
<td>$p = 0.18$</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.03 (0.81)</td>
<td>0.25 (0.84)</td>
<td>$p = 0.40$</td>
</tr>
<tr>
<td>$\text{FEF}_{25-75}$ z-score</td>
<td>0.15 (0.75)</td>
<td>-0.69 (0.94)</td>
<td>$p = 0.003$</td>
</tr>
</tbody>
</table>

*Table 20: Post salbutamol lung function. 2 sample t tests used to compare groups.*

Administration of salbutamol to healthy children caused an improvement in $\text{FEV}_1$ z-score from 0.07 to 0.23 ($p=0.02$) and $\text{FEF}_{25-75}$ z-score from -0.10 to 0.23 ($p=0.001$). Paradoxically there was a deterioration in mean LCI from 6.9 to 7.1 ($p=0.01$).

<table>
<thead>
<tr>
<th></th>
<th>Post exercise</th>
<th>Post salbutamol</th>
<th>95% CI for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>6.9 (0.4)</td>
<td>7.1 (0.4)</td>
<td>0.05, 0.38 ($p = 0.01$)</td>
</tr>
<tr>
<td>$S_{\text{cond}}$ (L$^{-1}$)</td>
<td>0.01 (0.01)</td>
<td>0.01 (0.03)</td>
<td>-0.01, 0.014 ($p = 0.95$)</td>
</tr>
<tr>
<td>$S_{\text{acin}}$ (L$^{-1}$)</td>
<td>0.21 (0.09)</td>
<td>0.26 (0.18)</td>
<td>-0.04, 0.13 ($p = 0.27$)</td>
</tr>
<tr>
<td>$\text{FEV}_1$ z-score</td>
<td>0.07 (0.71)</td>
<td>0.23 (0.70)</td>
<td>0.04, 0.30 ($p = 0.02$)</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.03 (0.89)</td>
<td>0.00 (0.83)</td>
<td>-0.15, 0.09 ($p = 0.59$)</td>
</tr>
<tr>
<td>$\text{FEF}_{25-75}$ z-score</td>
<td>-0.10 (0.75)</td>
<td>0.23 (0.75)</td>
<td>0.15, 0.50 ($p = 0.001$)</td>
</tr>
</tbody>
</table>

*Table 21: Healthy children changes in lung function following salbutamol (paired t tests used)*
In the asthmatic group administration of salbutamol improved both FEV\textsubscript{1} z-score from -0.39 to -0.06 (P=0.006) and FEF\textsubscript{25-75} z-score from -0.98 to -0.64 (P=0.03). There were no other significant changes.

<table>
<thead>
<tr>
<th></th>
<th>Post exercise</th>
<th>Post salbutamol</th>
<th>95% CI for Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>7.5 (1.2)</td>
<td>7.3 (0.6)</td>
<td>-0.62, 0.22 (p = 0.33)</td>
</tr>
<tr>
<td>S\textsubscript{cond} (L\textsuperscript{-1})</td>
<td>0.04 (0.03)</td>
<td>0.03 (0.02)</td>
<td>-0.03, 0.00 (p = 0.10)</td>
</tr>
<tr>
<td>S\textsubscript{acin} (L\textsuperscript{-1})</td>
<td>0.19 (0.05)</td>
<td>0.18 (0.06)</td>
<td>-0.05, 0.01 (p = 0.20)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} z-score</td>
<td>-0.39 (0.69)</td>
<td>-0.06 (0.72)</td>
<td>0.11, 0.56 (p = 0.006)</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>0.19 (0.97)</td>
<td>0.25 (0.83)</td>
<td>-0.20, 0.32 (p = 0.63)</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75} z-score</td>
<td>-0.98 (0.97)</td>
<td>-0.64 (0.87)</td>
<td>0.04, 0.65 (p = 0.03)</td>
</tr>
</tbody>
</table>

Table 22: Asthmatic children changes in lung function following salbutamol (paired t tests used)
Figures 49 and 50 illustrate the changes in LCI and FEV$_1$ from baseline, following exercise and after salbutamol in both groups of children.

**Figure 49:** Interval plot of baseline, post exercise and post salbutamol mean (plus 95% CI) LCI in the asthmatic and healthy (HV) groups.

**Figure 50:** Interval plot of baseline, post exercise and post salbutamol mean (plus 95% CI) FEV$_1$ z-score in the asthmatic and healthy (HV) groups.
7.4.5 Symptoms following testing

Twelve to 16 days after each visit families were called and asked whether they thought their child’s asthma was better, the same or worse. No family reported improvement in symptoms, 13 said symptoms had been similar and four stated that symptoms had been worse than usual. There were no significant differences in exercise induced changes in any lung function parameter between the group with similar and the group who reported worse symptoms.

<table>
<thead>
<tr>
<th></th>
<th>No change in symptoms</th>
<th>Worse Symptoms</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>3.5 (11.1)%</td>
<td>3.53 (6.76)%</td>
<td>p = 0.98</td>
</tr>
<tr>
<td>S-cond</td>
<td>31.0 (100)%</td>
<td>-20.1 (40.2)%</td>
<td>p = 0.20</td>
</tr>
<tr>
<td>S-acin</td>
<td>26.0 (43.1)%</td>
<td>8.2 (30.5)%</td>
<td>p = 0.40</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.6 (2.9)%</td>
<td>-0.1 (2.2)%</td>
<td>p = 0.65</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt;</td>
<td>7.2 (6.4)%</td>
<td>13.2 (8.1)%</td>
<td>p = 0.25</td>
</tr>
</tbody>
</table>

*Table 23: Mean (SD) percentage change in lung function following exercise in groups with similar and worse symptoms post visit. Two sample t tests used for comparison.*
The group of asthmatic children in our study had significantly worse LCI compared to our healthy group. Baseline LCI was abnormal in children who experienced large falls in FEV\textsubscript{1} following exercise. There were trends towards an increase in LCI following exercise in the asthmatic group and a higher increase in the group who reported requiring salbutamol to exercise.

The group of asthmatic children were varied and included a large proportion of patients who were asymptomatic in the period preceding testing. Twelve out of 21 children reported no symptoms in the previous two weeks, and nine children reported not using salbutamol for one week before their study visit. Eleven children had had fewer than two courses of oral corticosteroids in the previous two years and twelve children had not been admitted to hospital during this time. Four children denied ever suffering from exercise induced wheeze or breathlessness.

The asthmatic group had significantly higher FEV\textsubscript{1} and FEF\textsubscript{25-75} values than the healthy group. However the mean values of each of these indices were within normal ranges. Studies have found that most children with stable asthma have normal FEV\textsubscript{1} (13, 14) but that patients with low FEV\textsubscript{1} may be more symptomatic and at greater risk of exacerbation (103). In addition FEF\textsubscript{25-75} has previously been found to be lower in chronic persistent asthma compared to well controlled asthma (108) and in our study baseline FEF\textsubscript{25-75} z-score correlated with the number of courses of oral corticosteroids received in the two years prior to testing. The phenotypes of children in our group were probably mixed.
The asthmatic group had significantly higher LCI than the healthy group but the asthmatic group mean fell within 1.64 standard deviations of the healthy mean (7.46). Five patients had an LCI of greater than 7.46. There were no differences between the healthy and asthmatic groups in $S_{\text{cond}}$ or $S_{\text{acin}}$. $S_{\text{cond}}$ has previously been shown to be the most commonly abnormality of MBW in asthma (48, 126, 127) but in this study only two patients had a $S_{\text{cond}}$ out with 1.64 standard deviations of the healthy mean. $S_{\text{acin}}$ was only outside 1.64 standard deviations of the healthy mean in one asthmatic patient but did correlate with LCI. There were no other correlations between lung function parameters possibly because so many children had normal results. Indices of MBW did not correlate with FeNO, in keeping with previous studies suggesting that ventilation heterogeneity can be independent of airway inflammation (47, 50).

In the children in whom FEV$_1$ fell by more than 20% after exercise mean baseline FEV$_1$, FEF$_{25-75}$ and LCI were abnormal. Baseline ventilation heterogeneity has been shown previously to correlate with airway hyper-responsiveness triggered by methacholine or cold-dry air hyperventilation challenges (47, 122, 127). In this study elevated LCI appeared to be associated with exercise induced bronchospasm (EIB) however the difference in baseline LCI between the groups who did and did not have large drops in FEV$_1$ was not significant. The trend may have been significant in a group in which more patients experienced EIB.

Following exercise mean LCI in the asthmatic children rose from 7.1 to 7.4 but the 95% CIs for change were -0.11, 0.66 ($p = 0.14$). The trend in rise in LCI following exercise occurred despite the group including children who did not report exercise induced symptoms and with the exclusion of children who experienced significant EIB. There was almost no difference in FEV$_1$, (CIs for change -0.12, 0.12). Ventilation heterogeneity in asthmatics following exercise has been demonstrated by hyperpolarised helium MRI (141), the ability of this study to
significantly demonstrate increased heterogeneity using LCI may have been limited by its size. $S_{\text{cond}}$ which appeared significantly worse in the asthmatic compared to healthy groups following exercise did not actually change in the asthmatic group following exercise. The difference between the healthy and asthmatic groups is likely due to a slight fall in $S_{\text{cond}}$ with exercise in the healthy group.

$S_{\text{acin}}$ and FVC were the only assays that significantly deteriorated following exercise. $S_{\text{acin}}$ has previously been shown to correlate strongly with FEV$_1$ during exacerbation suggesting that it may be a major determinant of airflow obstruction (142); $S_{\text{acin}}$ might therefore be used to detect EIB. However, despite the deterioration in $S_{\text{acin}}$ following exercise, $S_{\text{acin}}$ in the asthmatic group following exercise appeared lower than the healthy group (0.19 L$^{-1}$ versus 0.21 L$^{-1}$ respectively) meaning that it could not discriminate between the two groups. It is possible that both the changes in FVC and $S_{\text{acin}}$ relate to variation in breathing pattern as testing began immediately after exercise. Inhalation to full vital capacity may have been limited due to breathlessness, thereby reducing FVC. The change in $S_{\text{acin}}$ may have been related to alteration of tidal volume and breathing rate, which are known to effect the phase III slope (21), as opposed to exercise induced peripheral ventilation heterogeneity. FEF$_{25-75}$ z-score paradoxically improved with exercise in the asthmatic group (95% CI 0.11, 0.38 p < 0.01). FEF$_{25-75}$ is more sensitive to small airways disease than FEV$_1$ but is reliant on the validity of FVC and the level of expiratory effort (19). The apparent improvement in FEF$_{25-75}$ following exercise is possibly a result of the change in FVC asthmatic patients had following exercise.

Asthmatic children who reported exercise limitation or requirement for salbutamol to prevent limitation appeared to have greater increases in LCI than children who stated that their asthma did not limit their exercise (6.6% compared to 2.7%). The difference was not significant (p =
0.50) but the group was small and only six children stated that their exercise was limited or that they needed salbutamol to exercise. No significant difference in the fall in FEV$_1$ z-score was observed either. Correlation between fall in FEV$_1$ on exercise testing and exercise related symptoms is known to be poor (135). There are no previous studies regarding the effects of exercise on MBW derived indices or correlation with symptoms, a larger study of children with asthma is required to determine this relationship.

There were no correlations between change in lung function and baseline FeNO. FeNO has previously been shown to be associated with bronchial hyper responsiveness (127). This relationship was not demonstrated in this group possibly because baseline FeNO was relatively low or because the group did not show significant deterioration in lung function with exercise.

Post exercise lung function testing lasted up to 27 minutes following cessation of exercise. The individual line plot of LCI over the three MBW tests (figure 47) illustrates that in several children LCI deteriorated in the first test after exercise only to normalise on tests two and three. Analysis of the change in MBW derived indices used a mean of the three post exercise tests but heterogeneity may increase immediately following exercise and improve with time.

Administration of salbutamol to the asthmatic group following completion of their post exercise testing caused significant improvements in FEV$_1$ (95% CI 0.11, 0.56, p < 0.01) and FEF$_{25-75}$ (95% CI 0.04, 0.65, p = 0.03). There were no significant changes in LCI, S$_{cond}$ or S$_{acin}$. Salbutamol has previously been shown to partially but not completely reverse abnormalities in MBW in asthmatic patients (48, 50, 126). The lack of effect seen in this study may have been due to poor peripheral deposition of inhaled salbutamol or the relative normality of LCI and S$_{cond}$ in the group.
This study was limited by size, patient selection and possibly subjective symptom reporting. Asthma is a heterogeneous condition, as demonstrated in the demographics of the cohort not all children with asthma have exercise induced symptoms. Exercise may have different effects on MBW derived indices in children with exercise induced symptoms than those without and results of either group may not be applicable to the other. This study did seek to determine whether there were different effects in groups categorised depending on symptoms but numbers were small and although trends were observed differences were not significant. Categorising children depending on reported symptoms depends on accurate reporting and awareness of symptoms. Although the majority of children said that their exercise was not limited by their asthma, some of these children still took salbutamol before or during exercise. Children in this study were not asked to keep a diary in the weeks prior to their visit, which may have improved the accuracy of symptom reporting.

Mean LCI was higher in asthmatic children compared to healthy controls but LCI could not detect change following exercise in asthmatic children in whom FEV₁ fell by less than 20%. The effects of exercise on MBW in asthmatic children who experienced large falls in FEV₁ could not be assessed as immediate treatment was the priority. However, the study was not designed to assess the effects of MBW derived indices in children with large falls in FEV₁ but instead assess whether MBW could be more sensitive in detecting exercise induced changes in asthmatic children in whom there is no change in FEV₁. It appeared that more symptomatic children had larger increases in LCI following exercise and either a larger study of asthmatic children or a study of children who all suffer from exercise induced symptoms is necessary to clarify these preliminary findings.
8 Project: Evaluation of MBW derived indices in asthmatic children during and after exacerbation requiring oral corticosteroids

8.1 Introduction

There is evidence to suggest that there is significant ventilation heterogeneity during exacerbation of asthma (119). Indices of phase III slope analysis have demonstrated abnormalities during exacerbation in adults (142) but there have been no studies of MBW in paediatric exacerbation. There have therefore been no studies assessing the effects of salbutamol on MBW derived indices in children during exacerbation. This is important because evidence suggests that salbutamol does not completely normalise ventilation inhomogeneity in asthma (48, 50, 126) and alternative treatments targeting the small airways may be beneficial. The natural history of ventilation heterogeneity following exacerbation is unknown. Following provoked exacerbation of asthma in adults, small airways disease was shown to persist after normalisation of FEV$_1$ and correlated with bronchial hyper-responsiveness (117). If ventilation heterogeneity persists following exacerbation and predisposes children to bronchial hyper-responsiveness, medication should possibly be altered in the period following exacerbation.

This study sought for the first time to determine the effects of exacerbation and salbutamol multi-dosing on MBW derived indices in asthmatic children.
8.2 **Aims**

To determine whether MBW derived indices are abnormal during an exacerbation of asthma.

To investigate the effects of salbutamol multi-dosing on MBW indices during an exacerbation.

To measure indices of MBW in children 4-6 weeks after discharge from hospital.

To relate persistent abnormalities in MBW to symptoms, characteristics of the child and severity of exacerbation.
8.3 Methods

8.3.1 Inclusion/Exclusion Criteria

Children aged 5-16 years with previously diagnosed asthma (either attending primary care or hospital clinic) who were admitted to the RHSC with an exacerbation requiring corticosteroids were recruited. Children had to be well enough to attend the research facility within the hospital and at the time of testing did not require salbutamol more frequently than at two hourly intervals. Children who could not leave their bed as they were too unwell or required daytime oxygen or salbutamol more frequently than at two hourly intervals were not included until they had improved.

8.3.2 Recruitment

Patients were identified through either the hospital’s general medical or respiratory teams. The family were approached and the study described; written information sheets were left if interest was shown. Consent was taken once the family had at least one hour to consider participation. Admission details were extracted from medical notes. The child was given an asthma severity score corresponding to the child’s pre admission BTS treatment step. The treatments prescribed during admission and the length of time between salbutamol administration and testing were recorded. The child was given a maximal therapy grade depending on treatments required for the exacerbation (mechanical ventilation = 5; intravenous (IV) therapy = 4; High Dependency Unit (HDU) care = 3; any nebulised bronchodilators = 2; multi dose inhaled bronchodilators (MDIs) only (+oral corticosteroids) = 1).
8.3.3 Study Visits

Testing was performed in the RHSC Children’s Clinical Research Facility (CCRF). The child’s first visit occurred as soon as practically possible after admission; when the patient no longer required salbutamol more frequently than at 2 hourly intervals. Testing was undertaken at least one hour after the patient’s last dose of salbutamol.

At the first visit height and weight were measured. Baseline observations including heart rate, respiratory rate and oxygen saturations were recorded. The family were asked to complete a short questionnaire relating to the child’s asthma, including details such as normal medications, exacerbating factors, frequency of exacerbation and symptoms preceding admission. The patient underwent MBW and spirometry shortly before and 15 minutes after salbutamol (1000 micrograms via spacer). Timing of salbutamol coincided with the clinical team’s prescription.

A second visit was scheduled for four to six weeks after discharge to coincide with a clinic visit where possible. MBW testing and spirometry were repeated at this time.
Patient admitted with exacerbation of asthma

Discussion of study and dissemination of Patient Information Sheets

Consent obtained

Patient spaced to 2 hourly salbutamol via Metered Dose Inhaler (MDI)

Review of treatment required during admission

Visit 1
Height and weight/Observations
Questionnaire 1
3 x MBW & Spirometry

Patient discharged from hospital
4-6 weeks later

Visit 3
Height and weight / Observations
Questionnaire 2
3 x MBW & Spirometry
8.3.4 Analysis

MBW raw data were extracted from the Innocor and analysed as described earlier to produce values for LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$. Z-scores and percent of predicted values for FEV$_1$, FVC and FEF$_{25-75}$ were calculated.

Data were analysed using Minitab Version 17 statistical software (Minitab, USA). Paired t-tests were used to assess change in baseline FEV$_1$, LCI, $S_{\text{cond}}$ and $S_{\text{acin}}$ following bronchodilator and after recovery. Pearson correlation coefficients were used to examine relationships between lung function indices. Significance was assumed at $p = 0.05$.

8.3.5 Ethical Approval

Ethical Approval was granted by the Lothian Research Ethics committee. Parents and where possible children provided written informed consent.
8.4 **Results**

8.4.1 **Demographics**

Nine children (six female) aged 6.4-13.6 years (median 9.0 years) were recruited. Children’s heights ranged from 132-163cm. Children all had a prior diagnosis of asthma before presenting to hospital. All children reported multiple triggers for their symptoms including viral upper respiratory tract infections (URTI), pets, dust, exercise, cigarette smoke, weather, emotional upset, hay fever and paint fumes.

The group’s regular medications prior to exacerbation varied greatly; one child was on step 1 of the BTS treatment ladder, two were on step 2, three were on step 3, two were on step 4 and one was on step 5 (BTS/SIGN Asthma guideline 2009). Three patients said that on average they used salbutamol more than once a day, four said they used salbutamol daily and only one patient said they used salbutamol occasionally. The number of courses of oral corticosteroids and hospital admissions children had over the previous 12 months are illustrated in figures 51 and 52. Days of school absence due to asthma over the previous year varied from 0 - 50.
Figure 51: Frequency of Hospital Admission over the 12 months prior to study participation.

Figure 52: Frequency of oral corticosteroid courses over the 12 months prior to study participation.
Exacerbation was reported as having started 17 to 72 hours prior to testing. Six patients reported they knew the trigger for their current exacerbation, triggers included URTI, weather, emotional upset, dust, hay fever and animals.

The severity of exacerbation varied. All children received oral corticosteroids and inhaled bronchodilators; eight of the nine children also required nebulisers and three required high dependency care with intravenous therapy. None of the children required intubation or mechanical ventilation.
8.4.2 Baseline Lung function

Children were tested at a mean (SD) of 36.6 (17.0) hours following admission. Seven of the children were receiving four hourly inhaled salbutamol by this time; the remaining two were at three hourly intervals. Testing began at a mean (SD) of 3.5(0.5) hours post last inhaled salbutamol.

Baseline mean (SD) LCI was 8.5 (1.7), $S_{cond}$ was 0.06 L\(^{-1}\) (0.03), $S_{acin}$ 0.19L\(^{-1}\) (0.10), FEV\(_1\) z-score -3.4 (1.6) and FEF\(_{25-75}\) z-score was -3.0 (1.5). The upper limits of normal based on the mean plus 1.64 standard deviations of 66 healthy children involved in a previous study (page 156) were an LCI of 7.12, $S_{cond}$ of 0.06L\(^{-1}\) and a $S_{acin}$ of 0.25L\(^{-1}\). Intra-visit CV for LCI was 4.4%, $S_{cond}$ was 15.2% and $S_{acin}$ was 22.2%.

Pre bronchodilator FEV\(_1\) z-score correlated strongly with LCI ($r = -0.81$, $p = 0.008$) and $S_{acin}$ ($r = -0.91$, $p = 0.001$). FEF\(_{25-75}\) z-score also strongly correlated with LCI and $S_{acin}$ ($r = -0.92$, $p = 0.001$ and $r = -0.84$, $p = 0.004$ respectively). LCI correlated with $S_{acin}$ ($r = 0.85$, $p = 0.004$). There were no correlations between $S_{cond}$ and any other parameter.
Figure 53: Baseline FEV$_1$ z-score vs mean LCI in asthmatic patients presenting with exacerbation.

Figure 54: Baseline FEV$_1$ z-score vs mean $S_{acin}$ in asthmatic patients presenting with exacerbation.
8.4.3 Pre Admission Asthma Control

Children were divided into groups depending on the level of treatment they were receiving prior to hospital admission (BTS/SIGN Asthma guideline 2009). The mean (SD) lung function parameters for each group are displayed in table 24.

<table>
<thead>
<tr>
<th>BTS Step</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LCI</td>
<td>9.3</td>
<td>7.8 (0.3)</td>
<td>9.8 (2.1)</td>
<td>6.7 (0.7)</td>
<td>8.6</td>
</tr>
<tr>
<td>$S_{\text{cond}}$ (L$^{-1}$)</td>
<td>0.04</td>
<td>0.07 (0.02)</td>
<td>0.08 (0.02)</td>
<td>0.04 (0.05)</td>
<td>0.04</td>
</tr>
<tr>
<td>$S_{\text{acin}}$ (L$^{-1}$)</td>
<td>0.19</td>
<td>0.16 (0.09)</td>
<td>0.26 (0.11)</td>
<td>0.10 (0.01)</td>
<td>0.25</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>-3.8</td>
<td>-2.6 (0.4)</td>
<td>-4.3 (1.5)</td>
<td>-1.5 (0.6)</td>
<td>-5.3</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-2.4</td>
<td>-1.9 (0.3)</td>
<td>-3.4 (1.4)</td>
<td>-1.0 (0.2)</td>
<td>-5.4</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>-4.3</td>
<td>-2.6 (1.2)</td>
<td>-3.6 (1.9)</td>
<td>-1.5 (1.3)</td>
<td>-3.2</td>
</tr>
</tbody>
</table>

Table 24: Baseline mean (SD) lung function in groups divided by preadmission BTS treatment score (BTS/SIGN Asthma guideline 2009).

Pre admission control was also assessed using symptom questionnaires, (although one patient did not complete a questionnaire). Patients were asked to report how frequently they used their salbutamol inhaler (0=never, 1=occasionally, 2=daily, 3=more than once a day), their responses related to their lung function are detailed in table 25. $S_{\text{cond}}$ tended to worse and FEV$_1$, FVC and FEF$_{25-75}$ tender to be better in patients taking more salbutamol.
<table>
<thead>
<tr>
<th>Salbutamol Frequency</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>LCI</td>
<td>9.3</td>
<td>8.3 (2.4)</td>
<td>8.4 (1.7)</td>
<td></td>
</tr>
<tr>
<td>S_{cond} (L^{-1})</td>
<td>0.04</td>
<td>0.05 (0.02)</td>
<td>0.09 (0.01)</td>
<td></td>
</tr>
<tr>
<td>S_{acin} (L^{-1})</td>
<td>0.19</td>
<td>0.22 (0.12)</td>
<td>0.14 (0.06)</td>
<td></td>
</tr>
<tr>
<td>FEV1 z-score</td>
<td>-3.8</td>
<td>-2.8 (1.4)</td>
<td>-1.9 (1.0)</td>
<td></td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-2.9</td>
<td>-2.0 (1.1)</td>
<td>-1.3 (0.5)</td>
<td></td>
</tr>
<tr>
<td>FEF25-75 z-score</td>
<td>-3.8</td>
<td>-2.7 (1.6)</td>
<td>-1.8 (1.1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 25: Baseline mean (SD) lung function in groups divided by pre admission salbutamol use.

Patients also reported how many courses of prednisolone they had used in the 6 months preceding admission. The results and corresponding lung function for each group are reported in table 26.

<table>
<thead>
<tr>
<th>Prednisolone (6 months)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LCI</td>
<td>10.3</td>
<td>7.8 (0.3)</td>
<td>9.4 (2.1)</td>
<td>6.0</td>
<td>7.6</td>
</tr>
<tr>
<td>S_{cond} (L^{-1})</td>
<td>0.10</td>
<td>0.07 (0.02)</td>
<td>0.07 (0.03)</td>
<td>0.0001</td>
<td>0.06</td>
</tr>
<tr>
<td>S_{acin} (L^{-1})</td>
<td>0.21</td>
<td>0.16 (0.09)</td>
<td>0.23 (0.14)</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>FEV1 z-score</td>
<td>-3.0</td>
<td>-1.6 (0.03)</td>
<td>-3.1 (1.8)</td>
<td>-1.6</td>
<td>-3.6</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-1.8</td>
<td>-1.1 (0.2)</td>
<td>-2.2 (1.3)</td>
<td>-1.1</td>
<td>-2.8</td>
</tr>
<tr>
<td>FEF25-75 z-score</td>
<td>-3.1</td>
<td>-1.5 (0.3)</td>
<td>-3.3 (2.0)</td>
<td>-1.4</td>
<td>-2.7</td>
</tr>
</tbody>
</table>

Table 26: Baseline mean (SD) lung function in groups divided by reported courses of prednisolone over preceding 6 months

Patients also described their usual triggers, hospital admissions and school absences. There were no correlations between these indicators and lung function during exacerbation.
8.4.4 Exacerbation Severity

Children were divided into groups depending on the maximal therapy they required during their exacerbation. Children were graded 1 if they only required MDIs and oral corticosteroids, 2 if they were given nebulisers, 3 if they required IV medication, 4 if they were admitted to HDU and 5 if they were mechanically ventilated. Only one child admitted to hospital only needed MDIs and corticosteroids, 5 were given nebulisers in addition and 3 children were admitted to HDU. No children were given IV medications and not admitted to HDU and none were mechanically ventilated during this admission. The mean lung function of each group is displayed in table 27.

<table>
<thead>
<tr>
<th>Maximal therapy grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>LCI</td>
<td>7.4</td>
<td>8.6 (2.1)</td>
<td>8.6 (1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S_{cond} (L^{-1})</td>
<td>0.07</td>
<td>0.05 (0.04)</td>
<td>0.07 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S_{scin} (L^{-1})</td>
<td>0.11</td>
<td>0.21 (0.12)</td>
<td>0.20 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1} z-score</td>
<td>-1.9</td>
<td>-3.7 (2.0)</td>
<td>-3.2 (0.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-1.1</td>
<td>-3.1 (1.9)</td>
<td>-2.3 (0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF\textsubscript{25-75} z-score</td>
<td>-2.4</td>
<td>-3.1 (1.9)</td>
<td>-2.9 (1.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 27: Baseline mean (SD) lung function in groups divided by maximal therapy required for admission.

When the children were divided into two groups depending on whether or not they were admitted to HDU there were no differences in lung function. Mean (SD) LCI in the group admitted to HDU was 8.6 (1.5) compared to 8.4 (1.9) in the group who were not (p = 0.86); mean (SD) FEV\textsubscript{1} z-score in the HDU group was -3.2 (0.3) compared to -3.4 (2.0) in the non HDU group (p = 0.85).
Two out of the nine children were on three hourly and seven of nine were on four hourly salbutamol MDIs by the time of testing. There were no differences in any assay between these two groups at the time of testing.

8.4.5 Change in lung function following bronchodilator

Post bronchodilator mean (SD) LCI was 8.3 (1.4), $S_{cond}$ was 0.06 L$^{-1}$ (0.03), $S_{acim}$ 0.17 L$^{-1}$ (0.06), FEV$_1$ z-score -2.8 (1.4) and FEF$_{25-75}$ z-score was -2.5 (1.3). Post salbutamol LCI correlated with post salbutamol FEV$_1$ z-score (Pearson r=-0.83, P=0.005).

![Figure 55: Scatterplot of Post bronchodilator FEV1 z-score versus mean LCI](image)

Figure 55: Scatterplot of Post bronchodilator FEV1 z-score versus mean LCI
The change in each lung function parameter following bronchodilator is shown in table 28. The only significant difference following salbutamol was seen in the change in FEV<sub>1</sub> z-score (P=0.04). No other significant changes were seen.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post salbutamol</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>8.5 (1.7)</td>
<td>8.3 (1.4)</td>
<td>P=0.41</td>
</tr>
<tr>
<td>S&lt;sub&gt;cond&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.06 (0.03)</td>
<td>0.06 (0.03)</td>
<td>P=0.75</td>
</tr>
<tr>
<td>S&lt;sub&gt;acin&lt;/sub&gt; (L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.19 (0.10)</td>
<td>0.17 (0.06)</td>
<td>P=0.28</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; z-score</td>
<td>-3.4 (1.6)</td>
<td>-2.8 (1.4)</td>
<td>P=0.04</td>
</tr>
<tr>
<td>FVC z-score</td>
<td>-2.6 (1.6)</td>
<td>-2.2 (1.4)</td>
<td>P=0.08</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt; z-score</td>
<td>-3.0 (1.5)</td>
<td>-2.5 (1.3)</td>
<td>P=0.14</td>
</tr>
</tbody>
</table>

Table 28: Baseline and post salbutamol mean (SD) lung function in children admitted for exacerbation of asthma. Pre and post values compared using paired t tests.
In some children administration of salbutamol caused a deterioration (an increase) in LCI, see figure 56. Larger improvements in LCI appeared to occur if baseline LCI was worse but the correlation between initial LCI and change following salbutamol was not significant (Pearson $r = -0.61$, $p = 0.08$). Initial FEV$_1$ z-score significantly correlated with bronchodilator change (Pearson $r = -0.71$, $p = 0.03$).

*Figure 56: Scatterplot of percentage change in LCI following salbutamol and initial mean LCI.*
Change in LCI significantly correlated with change in FEV₁ in individual patients (Pearson r = -0.71, p = 0.03). There were no significant correlations between changes in LCI and FEV₁ or FEF₂₅-₇₅ with S-cond or S-acin.

Figure 57: Percentage change in LCI following salbutamol versus percentage change in FEV₁ in individual patients.
8.4.6 Follow up after discharge

Three patients returned 5-10 weeks after discharge. All three patients said that their asthma was either well or completely controlled at the time of follow up. The group had normal mean lung function with a mean (SD) LCI of 6.7 (0.6), $S_{\text{cond}}$ of 0.03 L$^{-1}$ (0.02), $S_{\text{acin}}$ of 0.13 L$^{-1}$ (0.06), $\text{FEV}_1$ z-score of -0.6 (0.6) and a $\text{FEF}_{25-75}$ z-score of -1.2 (0.6). One patient (patient 5) had persistently abnormal LCI (7.36) and $\text{FEF}_{25-75}$ z-score (-1.74), this patient had higher $S_{\text{acin}}$ and lower $\text{FEV}_1$ compared to the other two patients but the values were within 1.64 standard deviations of the healthy mean. Figure 58 illustrates individual LCI at baseline, post salbutamol and at follow up (in the 3 patients who attended).

![Figure 58: Individual line plot of LCI at baseline, post salbutamol and at follow up.](image-url)
Patients 1 and 3 only required nebulised treatment during their hospital stay, patient 5 required HDU admission. All three patients were on four hourly salbutamol MDIs when they were initially tested. Patient 5 had been using salbutamol more than once daily prior to admission but was only on step 3 of the BTS treatment ladder and had not had any courses of oral corticosteroids or days of school absence in the preceding year.
8.5 **Discussion**

This study has demonstrated for the first time that LCI is abnormal and repeatable in children during an acute exacerbation of asthma. During exacerbation LCI correlated with FEV$_1$, but despite significant bronchodilator response in FEV$_1$, salbutamol had a variable effect on LCI.

LCI was abnormal (>7.12) in eight out of nine asthmatic children during exacerbation. The mean (SD) baseline LCI of the group was 8.5(1.7). The CV of LCI at baseline testing was low (4.4%) suggesting that LCI is repeatable during asthmatic exacerbations. There are no other studies detailing the effects of asthmatic exacerbation on LCI, however small airways disease has been detected using HRCT scanning in provoked exacerbation of asthmatic adults (117).

Thompson et al reported abnormalities in $S_{\text{cond}}$ and $S_{\text{acin}}$ during asthmatic exacerbation in adults (142). In our study mean $S_{\text{cond}}$ and $S_{\text{acin}}$ were within 1.64 standard deviations of the healthy mean determined in our centre (using the same equipment and methodology). Throughout studies in this thesis, variability of $S_{\text{cond}}$ and $S_{\text{acin}}$ have been high, in this study the CV of $S_{\text{cond}}$ was 15.2% and $S_{\text{acin}}$ was 22.2%. The high variability in indices of phase III slope analysis has limited their ability to differentiate disease. The variability of phase III slopes in Thompson’s study was not specified, percentage of predicted values (derived from a study by Verbanck et al (40)) were presented but the limits of normality were not quoted.

During asthmatic exacerbation FEV$_1$ and FEF$_{25-75}$ z-score strongly correlated with LCI and $S_{\text{acin}}$. There were no correlations between $S_{\text{cond}}$ and other lung function indices. Thompson et al showed similar correlations between $S_{\text{acin}}$ and FEV$_1$ with a lack of association between $S_{\text{cond}}$ and FEV$_1$ during exacerbation (142). Verbanck had previously shown that patients with
abnormal $S_{\text{acin}}$ had lower FEV$_1$ z-scores (131). The correlations between $S_{\text{acin}}$, FEV$_1$ and LCI might suggest that the acinar zone is a major determinant of ventilation heterogeneity and airflow obstruction in exacerbation of asthma. However a causal relationship has not been established, alternatively FEV$_1$ may be a major determinant of acinar ventilation heterogeneity.

There were no significant differences in LCI, $S_{\text{acin}}$, $S_{\text{cond}}$, FEV$_1$ or FEF$_{25-75}$ between groups of children depending on pre admission BTS treatment step. In Thompson’s study of asthmatic adults they found a correlation between $S_{\text{acin}}$ and pre admission treatment levels which they suggested proved a relationship between disease in the acinar region and asthma severity (142). Verbanck et al had previously found that $S_{\text{acin}}$ was higher in patients receiving higher doses of inhaled corticosteroids (128) and Farah et al found that $S_{\text{acin}}$ was associated with deterioration in symptoms following downwards titration of medication (144). The lack of correlation between MBW indices and pre admission treatment level in this study does not disprove a relationship between asthma severity and ventilation heterogeneity because treatment levels can be a poor measure of asthma severity (102), especially during exacerbation when pre admission treatment levels may be an underestimate of short term severity.

The study group had a high rate of oral corticosteroid courses, school absence and salbutamol use indicating poor control in the period before admission to hospital. There appeared to be a trend of worse $S_{\text{cond}}$ but better FEV$_1$, FVC and FEF$_{25-75}$ in patients taking more salbutamol. Farah et al found worse FEV$_1$, $S_{\text{cond}}$ and $S_{\text{acin}}$ in patients with poorly controlled asthma, assessed on the basis of an asthma control questionnaire (132). My study numbers were too small to make any conclusions.
There were no differences between lung function parameters in relation to maximal grade of treatment received during exacerbation. Maximal therapy was intended to be used to indicate exacerbation severity. At the time of testing patients were almost all receiving the same treatment, 3 – 4 hourly salbutamol MDIs. I had hypothesised that abnormalities in ventilation inhomogeneity might persist because of obstruction preventing peripheral deposition of inhaled therapies. Abnormalities in indices of MBW have been show to persist despite inhaled salbutamol (48, 50, 126). However patients on higher maximal therapy grades received IV medication which will have bypassed airways obstruction. Persistent small airways disease despite salbutamol has been found and suggested to be due to small airways remodelling (113). However abnormalities of MBW indices found in stable asthmatics possibly reflecting remodelling have been small (47, 50) and probably masked by the large changes we found during exacerbations. There is evidence of persistent small airways disease demonstrated by HRCT scanning following normalisation of FEV$_1$ and FEF$_{25-75}$ in allergen induced exacerbation in which patients were not given medication (117). Persistence in small airways disease was postulated as being due to the inhalation of fine particulate antigens that were deposited in the lung periphery. The patients in my study were admitted with exacerbations caused by a variety of triggers and patients were given medications.

Following salbutamol multi-dosing the only significant improvement in lung function was in FEV$_1$ ($p=0.04$). There was no difference in LCI, $S_{cond}$ or $S_{acin}$ but change in FEV$_1$ correlated with change in LCI within individual patients ($r = -0.71$, $p = 0.03$). Children with higher LCIs tended to show more improvement following salbutamol, but this trend was not significant ($r = -0.61$, $p = 0.08$). Administration of salbutamol has previously been shown to at least partially reverse ventilation inhomogeneity in asthma (48, 50, 126). Children in this study were receiving very regular salbutamol and tolerance to its effect may have developed, however effect was still seen in FEV$_1$. The lack of effect may have been due to obstruction preventing
peripheral deposition of salbutamol. In this study salbutamol administration was associated with an increase in LCI in some children. Increases in LCI may have been caused by increased inhomogeneity following deposition of salbutamol in well ventilated regions of lung. The lack of significant effect from inhaled salbutamol on MBW derived indices suggests that outcomes in asthmatic exacerbation could be improved with alternative therapies targeting the small airways.

In patients who attended follow up mean LCI fell to within healthy range, indicating that asthmatic exacerbation was the likely factor causing elevation in LCI. Only three children attended follow up but this group included the two patients who had had the most severely abnormal LCI at initial testing. One patient had persistently abnormal LCI and FEF$_{25-75}$ and this child was the only patient out of the three who attended follow up who had required HDU care and IV medication. The abnormality in LCI in this patient may have been a persistent effect of the exacerbation, however the patient did not report worse symptoms than the other two patients following discharge. Abnormal LCI may have predated the exacerbation. There is evidence that suggests abnormal ventilation heterogeneity is associated with bronchial hyper responsiveness (47, 122, 127) and possibly this patient may have had a more severe exacerbation because of pre-existing ventilation heterogeneity. To prove this association a much larger prospective study would be required.

This study had several limitations, partly associated with the difficulties of testing children during an acute illness. MBW testing could not be performed at the most severe stage of asthmatic exacerbation. Children could not undergo testing until they were stable and not requiring acute medical intervention. MBW testing could not be performed on a ward or in the Accident and Emergency department and could not be undertaken by a child receiving
supplemental oxygen. It was necessary for there to be at least an hour following administration of salbutamol before testing and therefore the interval between salbutamol doses had to be of a minimum duration of two hours. Testing was undertaken a mean of 36 hours following admission by which time seven of the nine children were only receiving four hourly inhaled salbutamol. The initial design of the study included testing patients twice during their admission, once as soon as they were clinically stable and again on the day of discharge. I found however that this was not possible because the patient was usually discharged on the same day they became stable enough to have their first study visit.

Only a small number of children were recruited to this study because of practicalities and time constraints. Families and children were generally very happy to participate but involvement was limited by the availability of the research facility and the necessity to perform testing during standard working hours (due to staffing). The number of children may have limited detection of significant correlations between lung function with pre admission control and treatment, exacerbation severity and persistent abnormalities of lung function at follow up. Testing at follow up also proved to be difficult as follow up appointments either did not coincide with availability of myself or the clinical research facility, patients were not followed up in the hospital or families did not wish to miss further school because of an additional hospital visit.

There was high within testing variability in asthmatic children’s $S_{\text{cond}}$ and $S_{\text{acin}}$ results. High variability suggests poor repeatability and reduces the power of an assay to detect a change within an individual. In addition there was high variability of mean $S_{\text{cond}}$ and $S_{\text{acin}}$ results across the control group of healthy children used for comparison. High variability across the control group caused a wide normal range and may have prevented these assays from differentiating
between healthy and diseased groups. As discussed in previous studies within this thesis, many published studies investigating $S_{\text{cond}}$ and $S_{\text{acina}}$ have occurred in adults and have used a fixed tidal volume breathing protocol to reduce variability in the phase III slope. Detection of the phase III slope can be difficult in children with variable tidal volumes and I did not have the ability with the software to my disposal to exclude breaths with indeterminable phase III slopes, which will probably have increased variability.

The study design could have been improved. FeNO was not tested, but may have allowed evaluation of the relationship between inflammation and ventilation heterogeneity during exacerbation and following recovery. FeNO may have helped to determine whether persistent abnormalities in lung function were secondary to ongoing inflammation or pre-existing small airways remodelling. Symptom data was collected by questionnaire and was subject to reporter error, symptom diaries following discharge may have been more useful. Attempts at relating lung function to pre admission treatments were made but there was no evaluation of compliance, this could have been attempted with review of repeat prescription records.

LCI can detect increased ventilation heterogeneity during asthmatic exacerbation, it is repeatable during exacerbation and improves following recovery. No significant improvement in LCI was seen following administration of inhaled salbutamol during exacerbation, suggesting that treatments targeting the small airways could improve outcomes in asthmatic exacerbation. A larger prospective study is required to assess whether pre-existing abnormalities in LCI can predict exacerbation severity.
9 Conclusions

MBW is a safe non-invasive method of detecting small airways disease through assessment of ventilation heterogeneity. It has been shown to be sensitive to disease in cystic fibrosis and asthma. However there was limited evidence regarding its variability in health and disease and my objective was to further define this. I used the modified Innocor to perform SF$_6$ washouts in healthy children, children with CF and children with asthma in five different studies.

Throughout, LCI was higher in children with disease compared to those without. In children with CF, LCI correlated with the extent and severity of bronchiectasis on CT scanning and in asthma LCI appeared to deteriorate during exacerbation. Children with poorer perceived asthma control had higher LCI, and we showed that children who had severe EIB had abnormal baseline LCI. LCI correlated with FEV$_1$ in children with CF and in children experiencing an exacerbation of asthma, suggesting that LCI relates to disease severity. In addition, in CF and asthma, LCI appeared more sensitive to early disease than FEV$_1$. FEV$_1$ could not discriminate between and healthy and asthmatic groups and correlated less well with bronchiectasis on CT than LCI.

LCI was found to be repeatable in healthy children and children with CF and asthma, with consistently low variation in intra-visit testing. The longitudinal study of children with CF demonstrated some variability in LCI over multiple visits despite no significant overall change, however this inter-visit variability was very similar to that of FEV$_1$ suggesting it is unlikely to limit utility. Inter-visit variability of LCI was higher in children with more severe CF possibly reducing the sensitivity of LCI for detecting change in these patients. In order to
compare LCI results, between tests or individuals, the posture study demonstrated that posture must be standardised during MBW testing.

In contrast, indices of phase III slope analysis were highly variable both within and between visits. This high variability may have limited the ability of $S_{\text{cond}}$ and $S_{\text{acin}}$ to differentiate between healthy and diseased groups and to detect postural or longitudinal change in CF and exercise or exacerbation induced changes in asthma. As described the variability of phase III slope indices in my studies may have been increased compared to other centres as I did not use a fixed volume tidal breathing protocol and could not exclude small breaths during analysis.

There were some common limitations to the studies described in this thesis. Several studies were limited by sample size and subject selection. Possibly due to limited size and subject recruitment no change in LCI over time in patients with CF and no significant rise in LCI following exercise in asthmatic patients were detected. However, the statistical analysis throughout this thesis was performed without a professional statistician whom may have been able to define significant changes. In addition, multiple statistical tests were performed on some of the data, particularly in relation to asthma control and therefore some findings may have been coincidental.

LCI has the potential to be clinically useful in the management of individual patients once testing and equipment are standardised. In CF LCI is sensitive to early disease, correlates with markers of severity and improves with treatments. However in patients with more severe CF lung disease, variability appears higher and further studies establishing the variability and treatment effects are required. In asthma, LCI may relate to asthma control and might predict
those who develop EIB and more severe exacerbation. There are variable effects of salbutamol on LCI and the effects of many other treatments are unknown. A prospective longitudinal study is required to assess the relationship between LCI, symptoms, exacerbation and treatments to assess whether LCI could be used to guide asthma management in the future.
10 Acknowledgments

The funding for my post as a clinical research fellow came from the UKCFGT consortium to whom I am very grateful. The consortium conducted a great deal of research in preparation for the multi-dose gene therapy study and I was employed to help in the clinical aspects of this work.

At the time I was a Clinical Research Fellow, the Children’s Clinical Research facility in the RHSC was run by Lead Research Nurse Kay Riding who together with Senior Research Nurse Debbie Miller helped with all of the study visits. In particular, they recruited patients for the study of stable asthmatic children and the exercise in asthma study and performed the lung function testing in many of these children.

Dr Kenneth Macleod was my immediate predecessor and had started two of the studies described in this thesis (assessing the effects of posture and longitudinal variation on MBW derived indices in CF). Dr Macleod and Dr Nick Bell (a UKCFGT research fellow in the Western General Hospital) taught me how to perform and analyse MBW tests.

My supervisor Professor Steve Cunningham supported me throughout my research and has given me invaluable guidance.

Last but not least, I’d like to thank my husband David for all his support and for looking after our two girls, Eva and Zoe, during the writing of this thesis.
11 Reference List

References


