The involvement of the lung periphery in cystic fibrosis: an exploration using multiple-breath nitrogen washout and helium-3 diffusion magnetic resonance

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

by

Noor Ali Al-Khathlan

Department of Infection, Immunity and Inflammation, Division of Child health

University of Leicester

September, 2014
The involvement of the lung periphery in cystic fibrosis: an exploration using multiple-breed nitrogen washout and helium-3 diffusion magnetic resonance

Abstract ................................................................. ii

Acknowledgments ......................................................... iii

Publications arising from this thesis ................................ iv

List of abbreviations ..................................................... vi

Table of Contents ........................................................ viii

List of Figures .......................................................... xiii

List of Tables ........................................................... xviii
The involvement of the lung periphery in cystic fibrosis: an exploration using multiple-breath nitrogen washout and helium-3 diffusion magnetic resonance

Noor Ali Al-Khathlan

Abstract

Background: The assessment of cystic fibrosis (CF) lung disease requires sensitive, repeatable and safe markers of early involvement of peripheral airways. Multiple-breath washout (MBW) and hyperpolarised $^3$He magnetic resonance ($^3$HeMR) have been demonstrated to be more sensitive to early changes than spirometry. Limited research has explored longitudinal changes in lung clearance index (LCI), and none has looked at phase III slope indices, markers of ventilatory inhomogeneity derived from MBW. The dimensions of lung microstructure using $^3$HeMR in children with CF have also not been investigated.

Aims: To monitor longitudinal changes in LCI and phase III slope indices in comparison with conventional lung function measures in children with CF and to estimate the dimensions of the lung periphery using $^3$HeMR.

Methods: Serial measurements of MBW indices, spirometry and plethysmography were obtained from 27 children with CF over a 4-year period. Single $^3$HeMR measurements were obtained from 18 patients.

Results: LCI showed the highest change over time and was the earliest to deteriorate with age, being elevated in all children at seven years. Conversely, the preliminary results of the association between VI arising from the conducting and acinar airways ($S_{\text{cond}}, S_{\text{acin}}$) with age have shown that $S_{\text{cond}}$ reached an asymptote with a maximum of 0.10 L$^{-1}$ and did not increase further with increasing disease severity. This limits the ability to follow these indices longitudinally. $^3$HeMR showed that the apparent diffusion coefficient, a marker of alveolar size, was significantly lower in CF patients than controls with no difference in alveolar sleeve depth or radius.

Conclusion: These findings highlight the significance of LCI in the early detection of functional changes in CF. The unexpected outcomes from $^3$He MR may be attributable to physiological or technical factors. Alternatively, they may suggest that CF does not cause structural damage to the acini in the early stages of the disease, but instead that it predominantly affects airways within the conducting zone.
Acknowledgments

I would like to express my greatest gratitude to the people who have helped and supported me throughout my study project.

I would like to express my very great appreciation to my supervisors Dr. Caroline Beardsmore and Dr. Erol Gaillard for their valuable and constructive suggestions during the planning and development of this research work. Their willingness to give their time so generously is very much appreciated.

Special thanks go to my colleagues in the Respiratory investigation Centre at Leicester Royal Infirmary: Prakash Patel and Teresa McNally for their valuable support and help with the collection of my data. My special thanks are also extended to Dr. Kathryn Staley, Dr. Gemma Fisher, Jackie Philips, Vicki Scales and Lucy Marshall for their assistance with the recruitment of healthy controls. Assistance provided by the staff at CF clinics at LRI and Glenfield Hospital is also greatly appreciated.

I wish also to acknowledge the help provided by Professor John Owers-Bradley, Dr. Iain Ball and Steven Hardy at Nottingham University, Department of Physics and Astronomy in the collection and analysis of $^3$He diffusion MR data.

Statistical advice given by Dr. Maria Viskaduraki, Dr. Richard Matthews, and Dr. Stephanie Hubbard has been a great help in analysing my data and was very much appreciated.

I would like as well to thank the Saudi Arabian Cultural Bureau in London and University of Dammam in Saudi Arabia for their financial sponsorship of this course of study. Their support, guidance and motivation were very much appreciated.

Thanks also go to my parents for their prayers, support and encouragement without whom I would have been unable to complete my project.

I want also to thank my husband Dr. Mohammed AL-Mohaithef for his infinite support and motivation throughout my study. We have been together all the way through this extended education journey with hope to have brighter future. Our son, Yazen, was a great and inspiring gift from God throughout this period.

Finally, I would like to thank my God who made all things possible.
Publications arising from this thesis

Work included in this thesis has been presented at national and international conferences as both poster and spoken presentations.


2. The role of ventilation inhomogeneity indices compared to spirometry in interventional studies in children with Cystic Fibrosis. **Al-Khathlan, N.**; Gaillard, E.; Beardsmore, C. European Respiratory Journal, 2013; 42: Suppl. 57, P3609


10. What is the role of $^3$He pulmonary MRI in children with cystic fibrosis?
   Gaillard, E.; Ball, I.; Panesar, K.; Narayanan, M.; Al-Khathlan, N.; Beardsmore, C.; Owers-Bradley, J. UK Pulmonary MRI meeting; University of Sheffield, Sheffield, 2011.

**Awards and Honours:**

1. Distinguished Saudi Scholar 2014 - Royal Embassy of Saudi Arabia (London, United Kingdom)

2. Distinguished Saudi Scholar 2012 - Royal Embassy of Saudi Arabia (London, United Kingdom)

Statement of work performed

I designed or co-designed all the studies in this thesis. Children with CF included in this thesis were part of CF pediatric clinic at Leicester Royal Infirmary (LRI) who enrolled in the annual review that began in January 2010. They children and their parents were all consented by me or the Pediatric Respiratory registrar during a visit to the routine CF clinic. For young adults with CF, I personally recruited all of them from adult CF clinic at Gleinfeld hospital and all were consented at their visits to the Respiratory Laboratory prior to testing. Healthy school-age children included in this thesis were recruited as part of a separate study and consented by a research nurse or other research personnel. They were recruited either from the Leicester Respiratory Cohorts by sending postal invitation letters, or by displaying posters in out-patient respiratory clinics (siblings of patients), or personally approached by study personnel from general and orthopaedic surgical wards. I have also used previously collected data from a sample of healthy children and young adults tested in our laboratory as part of different study (Narayanan, Owers-Bradley et al. 2012). These subjects were recruited from the Leicester Respiratory Cohorts and the Community Health Services Database i.e. a random population-based cohort. However, I have re-analysed their data to eliminate any observer bias in data analysis which might affect its comparability to our own data.

Physiological Measurements: The majority of lung function measurements (Spirometry, Plethysmography and N\textsubscript{2}MBW) were performed and analysed by me, with the reminder being performed by a small number of other experienced investigators. I was also responsible for all data entry and statistical analysis thereafter. Induced sputum was performed and processed by an experienced member of staff at LRI.

\textsuperscript{3}HeMR Scanning: Children and young adults who attended \textsuperscript{3}HeMR scanning at Nottingham University, Department of Physics and Astronomy, were accompined by me and one other investigator. I was responsible for all the coordination of the work performed at the collaborating facilities. I personally performed spirometry for all participants prior to lung scan whereas \textsuperscript{3}HeMR scanning and data analysis was perfomed by an experienced member of staff at Nottingham University.
**Database:** I designed the database and was solely responsible for all data entry. Finally, I was responsible for all the statistical analysis of the data and preparation and presentation of all the abstracts at national and international meetings. The thesis is all my own work.
List of abbreviations

ADC  The apparent diffusion coefficient
AUC_{ROC}  The area under the receiver-operator characteristic curve
CDI  Convection dependent inhomogeneity
CEV  Cumulative expired volume
CF  Cystic fibrosis
CO₂  Carbon dioxide
CV%  Coefficient of variation
DCDI  Diffusion-convection dependent inhomogeneity
FEV₁  Forced expiratory volume in first second
FEF₂₅₋₇₅  Mean expiratory flow between 25% and 75% of forced vital capacity
FEF₇₅  Flow measured after 75% of forced vital capacity has been exhaled
FRC  Functional residual capacity
FRC\textsubscript{N₂}  Functional residual capacity derived from N₂MBW
FRC\textsubscript{pleth}  Functional residual capacity derived from plethysmography
FVC  Forced vital capacity
h  Alveolar sleeve depth
He  Helium gas
\textsuperscript{3}He  Hyperpolarized helium-3 gas
\textsuperscript{3}HeMR  Hyperpolarized helium-3 Magnetic Resonance
\textsuperscript{4}He  Hyperpolarized helium-4 gas
LCI  Lung Clearance Index
MBW  Multiple-breath washout
MM  Molecular mass
MRI  Magnetic resonane imaging
\textsuperscript{N₂}  Nitrogen
N₂MBW  Nitrogen multiple-breath washout test
O₂   Oxygen
R    Alveolar radius
ROC  Receiver-operator characteristic curve
RR   Respiratory rate
RV   Residual volume
$S_{\text{acin}}$  Ventilation inhomogeneity at acinar airways
$S_{\text{acin,corr}}$  Volume corrected $S_{\text{acin}}$
SBW  Single-breath washout
$S_{\text{cond}}$  Ventilation inhomogeneity at conductive airways
$S_{\text{cond,corr}}$  Volume corrected $S_{\text{cond}}$
SD   Standard deviation
SE   Standard error
SF₆  Sulphur hexafluoride
SIII Phase III slope
$S_a$III Normalised phase III slope
TLC  Total lung capacity
TO   Lung volume turnover (calculated as number of FRCs)
$V_D$  Dead-space volume
$V_t$  Tidal volume
VI   Ventilation inhomogeneity
$^{129}$Xe  Xenon-129
z-score Standard deviation score
Table of Contents

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW .......................... 1

1.1 PHYSIOLOGY OF SMALL AIRWAYS .......................................................... 2

1.2 PHYSIOLOGY AND PATHOPHYSIOLOGY OF LUNG DISEASE IN CYSTIC FIBROSIS ...... 4
   1.2.1 Cystic fibrosis: ......................................................................................... 4
   1.2.2 Pathophysiology of lung disease in CF ...................................................... 4
   1.2.3 Physiology and pathology of small airways in CF: ..................................... 5

1.3 ASSESSMENT AND MONITORING OF CF LUNG DISEASE: .......................... 6
   1.3.1 Physiological and functional tests .............................................................. 6
   1.3.2 Imaging techniques and scans ................................................................. 9

1.4 MULTIPLE-BREATH NITROGEN WASHOUT TEST (N₂MBW) ......................... 10
   1.4.1 Principle of the test: .................................................................................. 10
   1.4.2 The shift from SBW to MBW ................................................................. 12
   1.4.3 The Significance of MBW in CF .............................................................. 14
   1.4.4 The Lung Clearance Index (LCI) derived from N₂MBW .......................... 15
   1.4.5 Phase III slope indices derived from N₂MBW ......................................... 21

1.5 HYPERPOLARISED HELIUM-3 MAGNETIC RESONANCE (³He MR) .................. 31
   1.5.1 Principle of MRI: ...................................................................................... 31
   1.5.2 The development of hyperpolarized noble gases MRI: ............................... 33
   1.5.3 Hyperpolarized ³He diffusion MR: ......................................................... 34
   1.5.4 The apparent diffusion coefficient (ADC) derived from hyperpolarized ³He diffusion MR ............................................................................................... 35
   1.5.5 The development of Yablonskiy acinar model to measure peripheral airways dimensions ........................................................................................................ 38
   1.5.6 Studies of Hyperpolarized ³He MRI in subjects with CF: .......................... 41
   1.5.7 Studies comparing ³He MRI and MBW: ...................................................... 41

CHAPTER 2 THESIS AIMS AND HYPOTHESES ................................................. 43

2.1 WHERE ARE WE? ......................................................................................... 43

2.2 WHERE IS THE GAP IN KNOWLEDGE? .................................................... 43
   2.2.1 N₂MBW: ................................................................................................. 43
   2.2.2 ³He diffusion MR: .................................................................................... 44

2.3 THESIS AIMS: .............................................................................................. 45
2.4 Thesis Hypotheses: ................................................................. 45
2.5 Structure of Thesis ................................................................. 46

CHAPTER 3 METHODS ...................................................................... 47

3.1 Summary of Study Design ........................................................... 47
3.2 Subjects ...................................................................................... 47
  3.2.1 Subjects with CF ................................................................. 47
  3.2.2 Healthy Controls ................................................................. 49
  3.2.3 Ethical Approval ................................................................. 51
3.3 Test Procedure ............................................................................ 51
3.4 Spirometry and Body Plethysmography: ....................................... 52
  3.4.1 Equipment and calibration ................................................... 52
  3.4.2 Data collection and reporting ................................................. 52
3.5 Multiple-breath Nitrogen Washout Test (N2MBW) ......................... 55
  3.5.1 N2MBW using a modified Medgraphics Profiler ..................... 55
  3.5.2 N2MBW using Exhalyzer D .................................................. 71
3.6 Induced Sputum ........................................................................... 80
3.7 Hyperpolarised Helium-3 Diffusion MR (3He MR) ............................ 81
  3.7.1 3He MR equipment: ............................................................ 81
  3.7.2 3He diffusion MR: Data collection ......................................... 82
  3.7.3 3He MR: Data acquisition and analysis techniques ................ 84
3.8 The reasons for using two N2MBW devices for data collection ......... 88
3.9 Statistical Analysis ....................................................................... 89

CHAPTER 4 STANDARDISATION THE METHODOLOGY OF N2MBW AND INVESTIGATION INTO SOURCES OF VARIATIONS ................................. 90

4.1 Introduction: ............................................................................... 90
4.2 Aims: ......................................................................................... 91
4.3 Hypotheses: .............................................................................. 91
4.4 Power Calculations ..................................................................... 91
4.5 Study 1: The effect of subject characteristics upon N2MBW indices in school-age children ......................................................... 92
  4.5.1 Rationale: ........................................................................... 92
  4.5.2 Material and Methods: .......................................................... 92
4.5.3 Results.................................................................................................................. 93
4.5.4 Discussion:.......................................................................................................... 105

4.6 STUDY 2: MULTIPLE-BREATH $\text{N}_2$ WASHOUT: A COMPARATIVE STUDY BETWEEN
TWO DIFFERENT INSTRUMENTS USING TWO MEASUREMENT TECHNIQUES .......... 108

4.6.1 Rationale:.............................................................................................................. 108
4.6.2 Material and Methods: ...................................................................................... 108
4.6.3 Results:.................................................................................................................. 109
4.6.4 Discussion:.......................................................................................................... 126

CHAPTER 5 LONGITUDINAL CHANGES IN LCI: A COMPARISON WITH
STANDARD LUNG FUNCTION TESTS IN SCHOOL-AGE CHILDREN WITH
CF ........................................................................................................................................ 129

5.1 RATIONALE:............................................................................................................ 129
5.2 AIMS: ...................................................................................................................... 130
5.3 HYPOTHESES: ...................................................................................................... 130
5.4 SUBJECTS AND METHODS: .................................................................................. 131

5.4.1 Subjects and study design: ................................................................................ 131
5.4.2 Data collection and analysis: ............................................................................. 132
5.4.3 Statistical analysis: ............................................................................................ 133

5.5 RESULTS ................................................................................................................. 135
5.5.1 Longitudinal study results: ............................................................................... 135
5.5.2 Interventional cross-sectional study results: .................................................... 164

5.6 DISCUSSION: ........................................................................................................... 169

5.6.1 Between group comparison, repeatability and sensitivity of LCI at baseline
visit: ............................................................................................................................ 169
5.6.2 Relationship between LCI and conventional lung function measurements at
baseline visit: .............................................................................................................. 169
5.6.3 Longitudinal changes in LCI and conventional lung function measurements
over a 4 year period: ................................................................................................. 170
5.6.4 The association between changes in lung function measurements with age,
$P. \text{aeruginosa}$ infection and CFTR genotypes: ....................................................... 173
5.6.5 Association between LCI and $FEV_1$ over time: .............................................. 177
5.6.6 Changes in LCI, spirometry and plethysmography parameters in response
to intravenous antibiotics ....................................................................................... 177
CHAPTER 6 COMPARISON OF HYPERPOLARIZED \textsuperscript{3}He DIFFUSION MAGNETIC RESONANCE (\textsuperscript{3}He MR) WITH N\textsubscript{2}MBW ........................................... 181

6.1 RATIONALE: .................................................................................................................. 181
6.2 AIMS: ................................................................................................................................ 182
6.3 HYPOTHESES: .................................................................................................................. 182
6.4 SUBJECTS AND METHODS: .............................................................................................. 183
  6.4.1 Subjects and study design: ............................................................................................. 183
  6.4.2 Data collection and analysis: ......................................................................................... 183
  6.4.3 Statistical analysis: ........................................................................................................ 184
6.5 RESULTS: ........................................................................................................................... 185
  6.5.1 Subject characteristics: ............................................................................................... 185
  6.5.2 Lung function results: ................................................................................................... 186
  6.5.3 \textsuperscript{3}He diffusion MR results: ................................................................................. 187
  6.5.4 Relationships between \textsuperscript{3}He diffusion MR parameters with FEV\textsubscript{1} and small airway markers: .................................................................................................................. 189
  6.5.5 Relationships between \textsuperscript{3}He diffusion MR parameters and LCI: ......................... 198
6.6 DISCUSSION: ...................................................................................................................... 201
  6.6.1 \textsuperscript{3}He diffusion MR in CF and healthy controls ................................................. 201
  6.6.2 Relationships between \textsuperscript{3}He MR parameters with FEV\textsubscript{1} and small airway markers: ................................................................................................................................. 204
  6.6.3 Relationships between \textsuperscript{3}He MR parameters and LCI: ........................................ 205

CHAPTER 7 CONCLUSION AND FUTURE WORK: ................................................................. 207

7.1 SUMMARY OF MOST IMPORTANT RESULTS ................................................................. 207
7.2 CLINICAL IMPLICATION OF RESULTS AND FUTURE RESEARCH: .............................. 209
7.3 CONCLUSION: .................................................................................................................... 212

APPENDICES ......................................................................................................................... 213

APPENDIX A: ETHICAL APPROVAL, PIS AND CONSENT FORM FOR LONGITUDINAL STUDY .................................................................................................................. 213

APPENDIX B: ETHICAL APPROVAL, PIS, CONSENT FORM AND QUESTIONNAIRES FOR ASTHMA STUDY IN WHICH HEALTHY CONTROLS RECRUITED ........................................ 226
APPENDIX C: ETHICAL APPROVAL, PIS, CONSENT FORM AND QUESTIONNAIRES FOR MEASURING LUNG DEVELOPMENT USING $^{3}$HE MR STUDY FROM WHICH DATA FROM HEALTHY CONTROLS (CHILDREN AND YOUNG ADULTS) WERE USED .......................... 256

APPENDIX D: ETHICAL APPROVAL, PIS AND CONSENT FORM FOR HELIUM-3 MR STUDY FOR CHILDREN AND YOUNG ADULTS WITH CF .......................................................... 284

APPENDIX F: RESULTS OF CHAPTER 4 ............................................................................. 318

POWER CALCULATION FOR STUDY 1: ................................................................. 318

POWER CALCULATION FOR STUDY 2: ................................................................. 319

APPENDIX G: RESULTS OF CHAPTER 5 ............................................................................. 321

APPENDIX H: PERSONAL DOCUMENTS: HONORARY CONTRACT, CERTIFICATES FOR GCP TRAINING AND CONSENT FOR RESEARCH TRAINING .................................................. 328

REFERENCES .................................................................................................................. 333
List of Figures:

FIGURE 1.1 SCHEMATIC STRUCTURE OF TRACHEOBRONCHIAL TREE ................................................. 3
FIGURE 1.2 PROPOSED MECHANISM FOR THE DEVELOPMENT OF CHRONIC AIRWAY INFECTION ON CF ................................................................................................................................. 5
FIGURE 1.3 A PLOT OF MULTIPLE-BREATH N₂ WASHOUT ................................................................. 11
FIGURE 1.4 A TYPICAL PLOT OF SINGLE-BREATH N₂ WASHOUT (ROBINSON ET AL. 2009 THEORETICAL BACKGROUND) ........................................................................................................... 14
FIGURE 1.5 GAS TRANSPORT THROUGH AIRWAY TREE ................................................................... 22
FIGURE 1.6 NORMALIZED PHASE III SLOPE (S₃III) ANALYSIS .......................................................... 24
FIGURE 1.7 THE PRINCIPLE OF MRI .................................................................................................. 32
FIGURE 1.8 SCHEMATIC STRUCTURE OF AN ACINAR AIRWAY ......................................................... 39
FIGURE 3.1 SCHOOL-AGE CHILDREN WITH CF INCLUDED IN THE LONGITUDINAL STUDY .......... 48
FIGURE 3.2 HEALTHY SCHOOL-AGE CHILDREN INCLUDED IN THIS THESIS .................................... 50
FIGURE 3.3 SCHOOL-AGE CHILD PERFORMING PLETHYSMOGRAPHY (WITH PERMISSION) .... 54
FIGURE 3.4 THE COMPLETE SET-UP OF THE MODIFIED MEDGRAPHICS PROFILER ..................... 56
FIGURE 3.5 COMPONENT OF THE MODIFIED MEDGRAPHICS PROFILER BREATHING APPARATUS .................................................................................................................................... 57
FIGURE 3.6 CHILD PERFORMING N₂ MBW USING MODIFIED MEDGRAPHICS PROFILER (WITH PERMISSION) .................................................................................................................. 58
FIGURE 3.7 MATLAB (R2011b) MAIN PROGRAM WINDOW ............................................................. 59
FIGURE 3.8 THE WASHOUT CURVE ANALYSIS WINDOW ................................................................... 60
FIGURE 3.9 REPRESENTATION OF CORRECTION OF VOLUME DELAY ............................................... 61
FIGURE 3.10 PHASE III SLOPE (S₃III) ANALYSIS WINDOW ............................................................... 63
FIGURE 3.11 THE DETERMINATION OF CONCENTRATION-NORMALIZED PHASE III SLOPE (S₃III) INDICES ........................................................................................................................................ 65
FIGURE 3.12 PROFILER EXAMPLES OF ACCEPTABLE AND UNACCEPTABLE N₂ MBW BREATHS ............................................................................................................................................... 67
FIGURE 3.13 PROFILER EXAMPLES OF ACCEPTABLE AND UNACCEPTABLE N₂ MBW RECORDINGS .......................................................................................................................................... 68
FIGURE 3.14 THE COMPLETE SETUP OF EXHALYZER D ..................................................................... 71
FIGURE 3.15 COMPONENTS OF EXHALYZER D APPARATUS .................................................................. 73
FIGURE 3.16 N₂ MBW INCENTIVE ANIMATION FROM EXHALYZER D AND SPIWARE 3.1
.......................................................................................................................................................... 74
FIGURE 3.17 Spiroware 3.1.6 (N₂MBW test screen) .............................................. 75
FIGURE 3.18 Spiroware 3.1.6 (Analysis screen) .................................................. 76
FIGURE 3.19 Exhalyzer D and Spiroware 3.1.6 examples of acceptable and unacceptably washout breaths ................................................................. 77
FIGURE 3.20 Exhalyzer D and Spiroware 3.1.6 examples of unacceptable
N₂MBW recording .............................................................................................. 78
FIGURE 3.21 T-permanent magnetic system .......................................................... 81
FIGURE 3.22 Child lying in the scanner to perform the lung scan (with
permission) ........................................................................................................ 83
FIGURE 3.23 The calculation of ADC value ......................................................... 84
FIGURE 3.24 ADC for each of the 3 diffusion weighted scans performed on a
subject ............................................................................................................... 85
FIGURE 3.25 Sequence diagram for the diffusion sensitizing signal of the Q-
space technique ............................................................................................ 86
FIGURE 3.25 The calculation of dimensions of lung microstructure .......... 87
FIGURE 4.1 The relationship between LCI with age, height, and weight in
healthy children and children with CF .......................................................... 95
FIGURE 4.2 Sex difference of LCI in healthy children and children with CF .... 96
FIGURE 4.3 The relationship between S_Acin with age, height, and weight in
healthy children and children with CF .......................................................... 99
FIGURE 4.4 Sex difference of S_Acin in healthy children and children with CF 100
FIGURE 4.5 The relationship between S_Cond with age, height, and weight in
healthy children and children with CF .......................................................... 103
FIGURE 4.6 Sex difference of S_Cond in healthy children and children with CF 104
FIGURE 4.15 Bland-Altman plot of the agreement between LCI obtained from
the Exhalyzer and that obtained from the Profiler in A) healthy
controls and B) children with CF ................................................................. 112
FIGURE 4.16 Bland-Altman plot of the agreement between FRC obtained from
the Exhalyzer and that obtained from the Profiler in A) healthy
controls and B) children with CF ................................................................. 114
FIGURE 4.17 Bland-Altman plot of the agreement between S_Acin obtained from
the Exhalyzer and that obtained from the Profiler in A) healthy
controls and B) children with CF ................................................................. 116
FIGURE 4.18 BLAND-ALTMAN PLOT OF THE AGREEMENT BETWEEN SCOND OBTAINED FROM
THE EXHALYZER AND THAT OBTAINED FROM THE PROFILER IN A) HEALTHY
CONTROLS AND B) CHILDREN WITH CF ................................................................. 118
FIGURE 4.19 ROC CURVE FOR LCI OBTAINED FROM THE PROFILER ............................... 120
FIGURE 4.20 ROC CURVE FOR LCI OBTAINED FROM THE EXHALYZER .............................. 121
FIGURE 4.21 ROC CURVE FOR SACIN OBTAINED FROM THE PROFILER ............................. 122
FIGURE 4.22 ROC CURVE FOR SACIN OBTAINED FROM THE EXHALYZER ........................... 123
FIGURE 4.23 ROC CURVE FOR SCOND OBTAINED FROM THE PROFILER .......................... 124
FIGURE 4.24 ROC CURVE FOR SCOND OBTAINED FROM THE EXHALYZER .......................... 125
FIGURE 5.1 RECEIVER-OPERATOR CHARACTERISTIC (ROC) CURVE FOR LCI FOR SCHOOL-
AGE CHILDREN ........................................................................................................... 141
FIGURE 5.2 THE RELATIONSHIPS BETWEEN LCI AND FEV1, FEV25-75, FEF25 AND FEF75 Z-
SCORES IN HEALTHY CHILDREN AND CHILDREN WITH CF AT BASELINE VISIT ................. 143
FIGURE 5.3 THE RELATIONSHIPS BETWEEN LCI AND RV/TLC Z-SCORE AND FRCPLETH Z-
SCORE IN HEALTHY CHILDREN AND CHILDREN WITH CF AT BASELINE VISIT ................. 145
FIGURE 5.4 COMPARISON OF LUNG FUNCTION RESULTS OBTAINED FROM 37 CHILDREN
WITH CF CLASSIFIED ACCORDING TO THEIR P. AERUGINOSA INFECTION STATUS IN THE
PRECEDING 12 MONTH TO THEIR BASELINE LUNG FUNCTION VISIT .................................. 149
FIGURE 5.5 CHANGES IN LCI AND CONVENTIONAL LUNG FUNCTION MEASUREMENTS OVER
A 4 YEAR PERIOD ........................................................................................................... 155
FIGURE 5.11 CHANGES IN FRCPLETH Z-SCORE OVER TIME IN CHILDREN WITH CF INFECTED
AND NON-INFECTED WITH P. AERUGINOSA .................................................................. 159
FIGURE 5.7 CHANGES IN LUNG FUNCTION PARAMETERS WITH AGE IN 27 CHILDREN WITH
CF ..................................................................................................................................... 161
FIGURE 5.8 ASSOCIATION BETWEEN LCI AND FEV1 Z-SCORES OVER TIME IN SCHOOL-AGE
CHILDREN WITH CF ...................................................................................................... 162
FIGURE 5.9 NORMAL AND ABNORMAL MEASUREMENTS DETECTED BY FEV1 ....................... 163
FIGURE 5.10 CHANGES IN LCI, SPIROMETRY AND PLETHYSMOGRAPHY PARAMETERS IN
RESPONSE TO INTRAVENOUS ANTIBIOTIC TREATMENT IN SCHOOL-AGE CHILDREN
WITH CF ......................................................................................................................... 166
FIGURE 6.1 COMPARISON OF GLOBAL ADC VALUES BETWEEN HEALTHY SUBJECTS AND
SUBJECTS WITH CF ....................................................................................................... 188
FIGURE 6.2 THE RELATIONSHIP BETWEEN GLOBAL ADC WITH FEV1 AND SMALL AIRWAY
MARKERS IN HEALTHY SUBJECTS AND SUBJECTS WITH CF ......................................... 190
Figure 6.3 The relationship between R (alveolar radius) with FEV\textsubscript{1} and small airway markers in healthy subjects and subjects with CF .................... 193

Figure 6.4 The relationship between h (alveolar sleeve depth) with FEV\textsubscript{1} and small airway markers in healthy subjects and subjects with CF............ 196

Figure 6.5 The relationship between global ADC with LCI in healthy subjects and subjects with CF .......................................................... 198

Figure 6.6 The relationship between R (alveolar radius) with LCI in healthy subjects and subjects with CF.............................................................. 199

Figure 6.7 The relationship between h (alveolar sleeve depth) with LCI in healthy subjects and subjects with CF ....................................................... 200
List of Tables:

TABLE 4.1 CHARACTERISTICS OF STUDY POPULATIONS ...................................................... 94
TABLE 4.2 CORRELATION BETWEEN LCI AND SUBJECT CHARACTERISTICS IN HEALTHY
    CHILDREN AND CHILDREN WITH CF ........................................................................... 94
TABLE 4.3 RESULTS OF MULTIVARIATE REGRESSION OF SUBJECT CHARACTERISTICS
    AGAINST LCI IN HEALTHY CHILDREN ........................................................................... 97
TABLE 4.4 CORRELATION BETWEEN $S_{ACIN}$ AND SUBJECT CHARACTERISTICS IN HEALTHY
    CHILDREN AND CHILDREN WITH CF ........................................................................... 98
TABLE 4.5 RESULTS OF MULTIVARIATE REGRESSION OF SUBJECT CHARACTERISTICS
    AGAINST $S_{ACIN}$ IN HEALTHY CHILDREN ................................................................... 101
TABLE 4.6 CORRELATION BETWEEN $S_{COND}$ AND SUBJECT CHARACTERISTICS IN HEALTHY
    CHILDREN AND CHILDREN WITH CF ........................................................................... 102
TABLE 4.7 RESULTS OF MULTIVARIATE REGRESSION OF SUBJECT CHARACTERISTICS
    AGAINST $S_{COND}$ IN HEALTHY CHILDREN ................................................................... 105
TABLE 4.12 CHARACTERISTICS OF THE STUDY POPULATION ........................................... 109
TABLE 4.13 REPEATABILITY OF FRC AND $N_2MBW$ INDICES IN SCHOOL-AGE CHILDREN
    ................................................................................................................................. 110
TABLE 5.1 CHARACTERISTICS OF CHILDREN WITH CF AT THE POINT WHERE THEY WERE
    ENROLLED INTO THE STUDY .......................................................................................... 136
TABLE 5.2 DEMOGRAPHICS OF HEALTHY CONTROLS IN COMPARISON TO CHILDREN WITH
    CF .................................................................................................................................. 137
TABLE 5.3 COMPARISON OF SPIROMETRIC RESULTS IN HEALTHY CHILDREN AND CHILDREN
    WITH CF AT BASELINE VISIT ....................................................................................... 138
TABLE 5.4 COMPARISON BETWEEN PLETHYSMOGRAPHIC RESULTS IN HEALTHY CHILDREN
    AND CHILDREN WITH CF AT BASELINE VISIT .............................................................. 138
TABLE 5.5 WITHIN-OCCASION REPEATABILITY OF $N_2MBW$ INDICES IN HEALTHY
    CHILDREN AND CHILDREN WITH CF AT BASELINE VISIT .......................................... 139
TABLE 5.6 COMPARISON BETWEEN $N_2MBW$ RESULTS IN HEALTHY CHILDREN AND
    CHILDREN WITH CF AT BASELINE VISIT ...................................................................... 140
TABLE 5.7 SENSITIVITY, SPECIFICITY AND AREA UNDER THE ROC CURVE FOR LCI ... 141
TABLE 5.8 CORRELATION BETWEEN LCI AND SPIROMETRY PARAMETERS ....................... 142
TABLE 5.9 CORRELATION BETWEEN LCI AND PLETHYSMOGRAPHY PARAMETERS ........... 144
TABLE 5.10 Comparison of lung function results obtained at baseline visit from 37 children with CF classified according to their *P. aeruginosa* infection status in the preceding 12 months ................................................................. 147

TABLE 5.11 Clinical characteristics of study population .................................................. 152

TABLE 5.12 Regression equations for VI indices .................................................................. 153

TABLE 5.13 Changes in lung function over time in school-age children with CF ............................ 154

TABLE 5.14 Associations between changes in lung function parameters with age, the status of *P. aeruginosa* infection during study period and CFTR genotypes .............................................................................................. 158

TABLE 5.15 Characteristics of study subjects ........................................................................ 164

TABLE 5.16 Lung function measurements in children with CF pre- and post-short-term intravenous antibiotic treatment ................................................................. 165

TABLE 6.1 Characteristics of study population........................................................................ 185

TABLE 6.2 Lung function results for healthy subjects and subjects with CF 186

TABLE 6.3 ³He diffusion MR results for healthy subjects and subjects with CF ................................. 187

TABLE 6.4 Correlation between global ADC with FEV₁ z-score and small airway markers in healthy subjects and subjects with CF ................................................................. 189

TABLE 6.5 Correlation between R (alveolar radius) and small airway markers in healthy subjects and subjects with CF ................................................................. 192

TABLE 6.6 Correlation between H (alveolar sleeve depth) and small airway markers in healthy subjects and subjects with CF ................................................................. 195

TABLE 6.7 Correlation between global ADC and LCI in healthy subjects and subjects with CF .................................................................................................................... 198

TABLE 6.8 Correlation between R and LCI in healthy subjects and subjects with CF ................................................................. 199

TABLE 6.9 Correlation between H and LCI in healthy subjects and subjects with CF ................................. 200

xx
Chapter 1 Introduction and literature review

Overview

The main function of the lung is gas exchange which occurs at peripheral airways where the blood-gas interface exists. This function depends on the structure, integrity, and functioning of the airways which either promote or disturb the evenness of ventilation (Yablonskiy, Sukstanskii et al. 2009, Robinson, Latzin et al. 2013). Uneven ventilation distribution is an important factor contributing to impaired gas exchange, and occurs in patients with lung diseases such as cystic fibrosis (CF) (West 2011). CF lung disease is characterized by recurrent infection and inflammation that leads to peripheral airways destruction and progressive lung damage from early life, impairing the distribution of ventilation (Gustafsson, De Jong et al. 2008, Hamutcu 2002). Spirometry and chest X-ray are the primary tools used to serially evaluate subjects with CF for disease progression, but are unable to assess gas mixing, are relatively insensitive to early disease and fail to reflect changes in peripheral airways (Koumellis, van Beek et al. 2005, Osmanagic, Sukstanskii et al. 2010).

The multiple-breath washout test (MBW), is an alternative technique that has grown in prominence to assess the efficiency of ventilation distribution. Lung clearance index (LCI), a measure of overall ventilation inhomogeneity, is the most sensitive marker of early CF lung disease (Horsley 2009, Aurora 2011). It has been shown to be altered in subjects with CF and to correlate with structural abnormalities detected by high resolution CT scan (HRCT) (Gustafsson, De Jong et al. 2008, Owens, Aurora et al. 2011). Other derived measures of ventilation inhomogeneity at the level of acinar and conductive airways (S_{acin} and S_{cond}, respectively) may provide more information about the location of the underlying disease process (Verbanck, Schuermans et al. 1997).

In parallel, a recently developed technique of lung morphometry using hyperpolarized ^3He diffusion magnetic resonance (^3HeMR) permits in vivo study of lung microstructure at the alveolar level (van Beek, Hill et al. 2007, Osmanagic, Sukstanskii et al. 2010). The apparent diffusion coefficient (ADC), a surrogate measure of alveolar size, obtained from ^3HeMR has been shown to be more sensitive for measuring short-term changes in the CF lung than PFT measurements (Kirby, Svenningsen et al. 2013).
The first part of this introduction concerns the physiology of small airways, the physiology and pathophysiology of lung disease in CF and assessment and monitoring of CF lung disease. The second part concerns N₂MBW as a sensitive test to assess small airway disease in CF. The principle of the washout tests, their applications, factors affecting derived indices and their significance in subjects with CF will also be covered. The final part of the introduction describes hyperpolarized ³HeMR as a novel technique in probing lung microstructure changes and considers the current literature in this method.

1.1 Physiology of small airways

Small airways are the non-cartilaginous airways with less than 2 mm internal diameter. They include the terminal bronchus, bronchioles, alveolar ducts, and the alveolar sacs which represents generations 8 and beyond of the tracheobronchial tree where air movement, occurs by a combination of convection and diffusion air flow (Weibel, Sapoval et al. 2005, Usmani, Barnes 2012) (Figure 1.1). Compared to the large airways, the walls of small airways are much thinner with a higher proportion of smooth muscles and greater cross-sectional surface area which will maximize gas mixings within the lung for efficient gas exchange (Weibel, Sapoval et al. 2005, Usmani, Barnes 2012). However, they contribute only a small proportion of the total airway resistance to flow. This was investigated over 40 years ago by Brown and colleagues who examined the effects of obstruction in the small and large airways (Brown, Woolcock et al. 1969). In their study they artificially obstructed the airways of excised lobes of dog and pig lungs with large and small beads. In both species, pulmonary resistance increased by approximately 10% when small airways were obstructed (Brown, Woolcock et al. 1969). In pigs, which lack collateral ventilation, the vital capacity was reduced by 50%, whereas in dogs, which have collateral channels, small airway obstruction had no measureable effect on vital capacity. This was attributed to air entering into the air spaces beyond the occluded airways via collateral channels in dogs (Brown, Woolcock et al. 1969). Consequently, in species which have collateral ventilation such as humans, extensive disease can be present with little effect on spirometry (Tiddens 2002, Usmani, Barnes 2012). Therefore, the region of the lung containing the small airways is known as the “silent zone”.
Subsequent experimental and clinical studies have looked at the effects of small airways obstruction on different physiological measures other than conventional indices of expiratory volume and flows (Hogg, Brunton et al. 1972, Macklem 1998, Verbanck 2003, Verbanck, Schuermans et al. 2004). Findings from these studies showed that small airways obstruction, whilst having little impact on spirometry, has a major effect on ventilation distribution measured using nitrogen washout tests (Hogg, Brunton et al. 1972, Macklem 1998, Verbanck 2003, Verbanck, Schuermans et al. 2004). Small airways obstruction also has an effect on the development of pulmonary hyperinflation and air trapping measured by body plethysmography (Kraemer, Blum et al. 2005, Usmani, Barnes 2012). This suggests that measures of ventilatory inhomogeneity and hyperinflation are more capable of detecting early physiological changes within small airways than conventional expiratory volume and flows in subjects with small airways disease (Usmani, Barnes 2012).

Figure 1.1 Schematic structure of tracheobronchial tree

Legend: Small airways represent generations 8 and beyond of the tracheobronchial tree where air movement occurs by a combination of convection and diffusion air flow. Within the first 14-16 generations of the tracheobronchial tree, convective airflow predominates. This followed by a transitional area where the diffusion convection front occurs. Within the last few generations diffusive airflow becomes dominant (Adopted from (Usmani, Barnes 2012) with permission.
1.2 Physiology and pathophysiology of lung disease in cystic fibrosis

1.2.1 Cystic fibrosis:

Cystic fibrosis (CF) is the most prevalent hereditary disease in the Caucasian population, affecting approximately 1 in 2,500 newborns (Davis 2007, Horsley 2009). Currently, over 10,000 people in the United Kingdom have this complex multisystem disease (The UK Cystic Fibrosis Registry 2014). CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Many mutations of the CFTR gene that have been described; the deletion of phenylalanine at codon 508 (ΔF508) is the commonest mutation and occurs in about 70% of CF patients (Davis 2007). CFTR protein is expressed in the apical plasma membrane of epithelial cells in the upper and lower airways, pancreas, gastrointestinal tract, reproductive tracts and other tissues where it functions as an ion channel that regulates fluids through chloride (Cl\(^-\)) secretion and inhibition of sodium (Na\(^{+2}\)) absorption (Southern 1997, Davis 2007). Defective CFTR protein, therefore, will affect the function of the organs where the receptor is expressed; however, the most prominent pathological effects are manifest in the lungs.

1.2.2 Pathophysiology of lung disease in CF

It is assumed that the dysfunction of the CFTR protein alters fluid transport across the airways due to a reduction in Cl\(^-\) secretion into the airways and active absorption of Na\(^{+2}\) which dehydrate the airway surface causing failure of mucociliary clearance (Jiang 1993, Verkman 2003, Zhang, Button et al. 2009) (Figure 1.2). It is also proposed that defective CFTR gene leads to increase the viscosity of secretions from exocrine glands which foster chronic airway infection (Tiddens 2002). Together with the excessive inflammatory response to pathogens observed in CF, both can lead to structural changes in the airways in terms of airway wall thickening and extensive epithelial damage that further impairs mucociliary clearance and allows bacterial colonisation and mucosal infection (Tiddens 2002, Sly, Brennan 2004). Eventually, irreversible airway damage occurs due to endobronchial infection, initially from *Staphylococcus aureus* and *Pseudomonas aeruginosa* leading to respiratory failure which is the primary cause of death in subjects with CF (Armstrong, Grimwood et al. 1997, Tiddens 2002, Horsley 2009).
Figure 1.2 Proposed mechanism for the development of chronic airway infection on CF

Legend: In the left panel: normal airways are shown where hydration is controlled by Na+ absorption & Cl- secretion. In the right panel: CF airways is shown where the dysfunction of CFTR leads to reduce Cl- secretion, excessive Na+ absorption and associated dehydration of the airway surface causing mucociliary impairment. Mucus becomes adherent in plaques which allow bacterial colonisation (Adopted from (Zhang, Button et al. 2009) with permission.)

1.2.3 Physiology and pathology of small airways in CF:

Bronchiectasis and bronchiolectasis (dilation of small airways) together with mucus plugging of airways appear to be the most important pathological manifestations and the predominant abnormalities in children and younger teenagers with CF (Sobonya, Taussig 1986, Hamutcu 2002, Tiddens 2010). This was evident by increased volume of bronchial tissue, increased mean small airways diameter and decreased percentage of smallest small airways (≤ 0.35 mm) in children with CF compared to controls as reported by two pathological studies in subjects with CF aged 2 to 27 years old (Sobonya, Taussig 1986, Hamutcu 2002). Sobonya and Taussing also showed mild alveolar enlargements in most children from 6 to 17 years old without histologic evidence of destructive emphysema (Sobonya, Taussig 1986). These pathological studies provide evidence of the presence of small airways disease in children with CF (Sobonya, Taussig 1986, Hamutcu 2002). Further evidence from physiological
and imaging studies (Castile, Hayes et al. 2000, de Jong 2004, Bannier 2010) have also shown that lung disease starts early in life in most children with CF and more markedly in the small airways, but eventually lead to the destruction of the larger airways. This could be explained by the high levels of CFTR protein expression throughout the distal airways, mainly the respiratory bronchioles and alveoli compared to the bronchial epithelium in human lung as documented by histopathological studies (Engelhardt 1994, Fang, Song et al. 2006, Regnier, Dannhoffer et al. 2008). Although the role of CFTR protein at alveolar level is still unclear with respect to the CF lung disease (Regnier, Dannhoffer et al. 2008), further investigations of the alveolar region using non-invasive methods is required to improve our understanding of the disease.

1.3 Assessment and monitoring of CF lung disease:

Regular monitoring of CF lung disease from an early stage is essential to assess the progression of the disease and avoid the decline in lung functions. Routinely, a number of physiological and imaging techniques are used as part of clinical monitoring of patients with CF lung disease. Recent imaging studies have shown that HRCT scan are more sensitive in detecting early structural abnormalities in subjects with CF and provides a more precise scoring than chest X-ray (Demirkazık, Arıyürek et al. 2001, Tiddens 2002, Brody 2005, Tiddens 2010). Therefore, in this section standard lung function tests, high-resolution computed tomography (HRCT) scans, and comparative studies between them will be presented.

1.3.1 Physiological and functional tests

1.3.1.1 Spirometry:
The forced expired volume in one second (FEV₁) obtained from spirometry has been regarded for many years as the gold standard to monitor disease progression in both adults and children with CF (Kraemer, Blum et al. 2005, Aurora 2010). Although it is widely used and is well established in both clinical and research settings as a good predictor of prognosis in subjects with moderate to severe CF lung disease, there is mounting evidence in the literature showing that FEV₁ as an outcome measure is insensitive to early CF lung disease (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Horsley 2009, Aurora 2010). This has been attributed to the potential commencement of
CF lung disease in the peripheral airways and the little contribution of peripheral airways to total airways resistance and thus relatively little effect on FEV\(_1\) (Aurora 2005, Beydon 2007, Horsley 2009).

Other measures that can be obtained from spirometry are the forced expiratory flows at low lung volumes (FEF). The reduction in the instantaneous flow measured after 75% of the forced vital capacity (FVC) has been exhaled (FEF\(_{75}\)) and/or the reduction in mean expiratory flow between 25% and 75% of FVC have been considered as an early signs of small airways obstruction and peripheral lung damage even before FEV\(_1\) become abnormal (Horsley 2009, Tiddens 2010). Different clinical studies in subjects with CF have looked at FEF\(_{25-75}\) and FEF\(_{75}\) and reported a significant reduction in these two parameters in adults and school-age children compared to healthy controls (Desmond, Coates et al. 1986, Aurora, Gustafsson et al. 2004, Gustafsson, De Jong et al. 2008, Horsley 2009, Singer, Kieninger et al. 2013). Furthermore, cross-sectional data from the European Epidemiologic Registry of Cystic Fibrosis have shown that FEF\(_{25-75}\) is the first spirometric parameter to decline with age in patients with CF (Tiddens 2002). However, these measures of FEF can only be obtained reliably from 6 years of age, in which many children are able to perform flow/volume tests in a reproducible fashion (Tiddens 2002). This, in turn, may affect the sensitivity of these measures in younger children and limit the use of these measures in clinical settings for early detection proposes. Aurora and colleagues looked FEF\(_{25-75}\) in pre-school children and found no significant differences between children with CF and healthy controls (Aurora 2005). They suggested that the inability of FEF\(_{25-75}\) to detect airway dysfunction in this age-group might be technique-related as they reported a high inter-subject variability of FEF\(_{25-75}\) (Aurora 2005).

Furthermore, the latest recommendation from the American Thoracic Society and the European Respiratory Society (ATS/ERS) do not support the use of FEF\(_{25-75}\) and FEF\(_{75}\) for determining small airways changes and they stated that “abnormalities in these mid-range flow measurements during a forced exhalation are not specific for small airway disease in individual patients” (Pellegrino, Viegi et al. 2005)(p.953). This is because these parameters may be affected by airflows and volume changes in the larger airways, highly dependent on an accurate FVC manoeuvre, have a very high variability and poor
correlation with measures of air trapping (Usmani, Barnes 2012, Quanjer, Stanojevic et al. 2012).

1.3.1.2 Plethysmography:

Lung volumes including the functional residual capacity (FRC) and the ratio of residual volume to total lung capacity (RV/TLC) are derived from plethysmography. An increase in these indices, which is indicative of gas trapping and/or hyperinflation due to airway obstruction, has been shown to be present in subjects with moderate to severe CF lung disease as documented by a number of cross sectional and longitudinal studies (Desmond, Coates et al. 1986, Bonnel, Song et al. 2004, Kraemer, Blum et al. 2005, Kraemer, Baldwin et al. 2006, Horsley, Macleod et al. 2008). However, in children with mild CF lung disease no significant differences were found in RV/TLC compared to healthy controls (Gustafsson 2003, Bonnel, Song et al. 2004). Although fewer abnormalities were detected by the measures of hyperinflation in children with mild CF lung disease, it has been shown to be strongly correlated with abnormalities detected by HRCT (Goris, Zhu et al. 2003, Owens, Aurora et al. 2011). It was also found that both total CT score and bronchiectasis sub-score were always abnormal in the presence of hyperinflation detected by plethysmography (Owens, Aurora et al. 2011).

Although the assessment of airway obstruction alterations alone may be inadequate for following disease progression in CF (Kraemer, Baldwin et al. 2006), the importance of involving lung volume measurements in monitoring the progression of CF lung disease is still uncertain due to the lack of supporting data. Recent studies by Kraemer and colleagues have acknowledged that the relationships between lung function parameters may provide important information about disease progression in CF (Kraemer, Blum et al. 2005, Kraemer, Baldwin et al. 2006). They found in a cohort of subjects with CF followed over a substantial life span of 6 to 20 years that the highest progression (i.e. deterioration) among lung function measurements was for FRC derived from plethysmography (FRC_{pleth}) (Kraemer, Blum et al. 2005). They also observed that the rate of progression of functional abnormality was accelerated within the group of patients having pulmonary hyperinflation and trapped gas which makes FRC_{pleth} among the strongest indicators of disease progression (Kraemer, Blum et al. 2005, Kraemer, Baldwin et al. 2006). Therefore, they concluded that early assessment of pulmonary hyperinflation and gas trapping in parallel with the assessment of the degree of airway
obstruction may provide a means for monitoring functional progression in CF disease (Kraemer, Baldwin et al. 2006).

1.3.2 Imaging techniques and scans

With growing the interest toward using lung imaging to detect and assess the progression of lung disease as complementary to the information provided by lung function tests, a number of techniques have been developed that enable scanning the lung in a great detail providing structural and functional information (Brody 2005, Plotkowiak, Burrowes et al. 2009). Among these techniques, High Resolution Computed Tomography (HRCT) scans are considered the gold standard and the most commonly used to demonstrate early structural lung abnormalities in CF, particularly in the small airways (Demirkazık, Arıyürek et al. 2001, McMahon, Dodd et al. 2006, Gustafsson, De Jong et al. 2008, Aurora 2010, Owens, Aurora et al. 2011). However, its use is limited by exposure to radiation, which makes it unsuitable in longitudinal studies (Brody, Klein et al. 2004, de Jong 2004, Aurora 2010, Gustafsson, De Jong et al. 2008). HRCT scans have shown repeatedly to be more sensitive than conventional lung measurements in children and adults with CF (de Jong 2004, Judge, Dodd et al. 2006, Gustafsson, De Jong et al. 2008, Owens, Aurora et al. 2011). In two large studies performed in school-age children with mild to moderate CF lung disease to detect and monitor progression lung damage using HRCT in combination with pulmonary function tests, the authors showed that the rate of decline in HRCT appearance was more significant than the rate of decline in spirometry results (Brody, Klein et al. 2004, de Jong 2004). Furthermore, among HRCT abnormalities found in children with CF, de Jong et al. showed that the severity, extent and peripheral extension of bronchiectasis was the abnormality that was most significantly worsened (de Jong 2004). In contrast, Brody et al. found that air trapping was the most common abnormality occurring in the cohort studied, followed by bronchiectasis and mucus plugging which suggests that air trapping may be useful feature in assessing children with mild CF lung disease (Brody, Klein et al. 2004). This was supported by a cross-sectional study in children with mild CF lung disease where authors found that the size of air trapping defects detected using HRCT was the best discriminator between patients and healthy subjects (Goris, Zhu et al. 2003). They also documented a lack of association between air trapping and spirometry results, except for RV/TLC (Goris, Zhu et al. 2003). These findings have also been confirmed by others (Bonnel, Song et al. 2004).
Airway wall thickening is also another abnormality that has been shown by CT studies to be present in subjects with CF (Castile, Hayes et al. 2000, de Jong, Nakano et al. 2005, Long, Williams et al. 2004). In a study over 2 year interval in 23 children with CF, progressive airway wall thickening was observed and most markedly in the peripheral airways (de Jong, Nakano et al. 2005). They also reported an association between the progression of airway wall thickening and changes in mid expiratory flows (de Jong, Nakano et al. 2005).

However, although HRCT can detect structural changes and air trapping during exhalation scans, it is questionable whether it can be considered a sensitive test to monitor small airways while it lacks the ability to detect geometric alterations in airways smaller than 2mm in diameter (Bonnel, Song et al. 2004, Gustafsson, De Jong et al. 2008, Tiddens 2010).

1.4 Multiple-breath nitrogen washout test (N₂MBW)

1.4.1 Principle of the test:

Inert gas washouts tests have been described since 1940’s as an open circuit method to assess ventilation distribution and gas mixing efficiency (Darling, Courmand et al. 1940, Courmand, Baldwin et al. 1941). They can be performed over a single vital capacity breath or over a series of tidal breaths, designated single or multiple breath washout (SBW and MBW, respectively) (Robinson, Goldman et al. 2009). A number of inert marker gases, which have a relatively low solubility in blood and that do not participate in gas exchange, such as the endogenous gases nitrogen (N₂) and argon or exogenous gases helium (He), and sulphur hexafluoride (SF₆), can be used during washout tests. The exogenous inert gases are first “washed in” to the lungs until equilibrium is achieved, and then washed out of the lung using normal air. The principle of MBW test is based on the measurement of the inert gas before and after its distribution through alveolar spaces during quiet breathing (Courmand, Baldwin et al. 1941).

However, the most widely used is the N₂ washout test where pure oxygen (100% O₂) which is readily available is delivered to the subject during tidal breathing over sequential breaths to wash the resident N₂ out of the lungs. Throughout the test, the expired volume and N₂ concentration are recorded and measured. The resulting data (Figure 1.3) are then analysed and used to determine the resting lung volume (FRC) and
thereafter provide an index of pulmonary emptying named the lung clearance index (LCI) which is useful as a measure of effectiveness of ventilation (Darling, Cournand et al. 1940). The LCI represents the number of lung turnover (TO=a unit of volume equivalent to FRC) required to reduce the end tidal N₂ concentration to 1/40th of starting concentration. It can be calculated as the ratio of cumulative expired volume (CEV) to FRC, after subtracting the apparatus dead-space from any calculation of CEV (Gustafsson 2003).

In healthy individuals, the normal range of LCI is between 6 and 7 TO, but increases in disease which indicates uneven ventilation distribution. This can occur as a result of either partial airway obstruction which will increase the airway resistance to flow, or small airway enlargement which may result in incomplete diffusion along a terminal lung unit (West 2011). In both cases poorly ventilated regions emptied last which prolongs the rate of emptying and increases LCI.

**Figure 1.3 A plot of multiple-breath N₂ washout**

Legend: A plot of multiple-breath N₂ washout test where N₂ concentration (%/10) and tidal volume (L) are plotted against the washout time (sec). The red arrow indicates the start of N₂ washout by inspiring pure oxygen. FRC can be calculated by dividing the cumulative expired volume (CEV) which is the sum of all expiratory Vt over the washout by the difference between the initial and final end-tidal N₂ concentrations (e-t N₂start and e-t N₂end, represented as black dotted lines). LCI is calculated as the ratio of CEV to FRC. This figure was derived from a multiple breath washout of a child with CF, and is also shown in more detail in Figure 3.8.
Although this method of assessing the effectiveness of ventilation distribution has been described since the 1940s after the introduction of the respiratory mass spectrometer, it has been used infrequently due to the lack of carefully validated robust commercial washout setups and the complexity of data analysis (Newth 1997, Aurora 2006, Robinson, Latzin et al. 2013, Horsley 2009). In recent years with several developments, in particular the improvements in technology, testing equipment and data processing and analytical techniques, the interest in this field has re-emerged (Aurora 2006, Robinson, Goldman et al. 2009). This in parallel has led to the development of new indices derived from phase III slopes (SIII) of consecutive breaths of the MBW test which are used to separate conductive and acinar components of ventilation heterogeneity (details in section 1.4.3.2) (Verbanck 1998). As a result, numerous studies have been conducted to assess the effectiveness of MBW measurements in detecting early airways changes in different diseases including CF (Aurora, Gustafsson et al. 2004, Gustafsson 2003, Horsley, Macleod et al. 2008). This has been concurrent with the recognition that conventional spirometry may not detect early changes until lung disease is well-established (Aurora 2006).

### 1.4.2 The shift from SBW to MBW

SBW test is a useful tool that has been widely used in research and clinical trials (Verbanck, Schuermans et al. 1997). However, increasing the interest towards MBW test in paediatric settings particularly was driven by the feasibility of performing MBW in children from any age group with a minimum of cooperation during relaxed tidal breathing (Robinson et al. 2013, Beydon 2007). The principle of single-breath nitrogen washout (N₂SBW) is based on washing out the N₂ from the lungs by having the subject breathe 100% oxygen from FRC to TLC followed by a slow expiration (Wanger, Clausen et al. 2005). As a result, a plot of N₂ concentration against expired volume can be obtained (Figure 1.4). Conventionally, the resulting plot is divided into four phases (Rupple 2009, West 2011). In phase I, pure O₂ is exhaled from the upper airways containing zero N₂ concentration. In the second phase, the transitional phase, the N₂ concentration increases rapidly as the anatomical dead space washed out by alveolar gas. Phase III which is known as the alveolar plateau, represents the N₂ released from the alveoli. In normal subjects, this phase is nearly flat with a small upward slope whereas in patients with different lung diseases including CF, where uneven ventilation distribution present, SIII becomes steeper (Horsley 2009, West 2011). In phase IV
which is known as the closing volume, a sharp rise in N\textsubscript{2} concentration occurs in some subjects and it is proposed to be due to the closure of the airways in the lower lung regions towards residual volume (Robinson, Goldman et al. 2009, Horsley 2009).

The sloping alveolar plateau as a result of non-uniformity of gas mixing was recognised over 60 years ago and thereafter it has been considered as a measure of ventilation inhomogeneity (VI) over different regions in the lung (Fowler 1949, West 2011, Robinson, Goldman et al. 2009). Increasing VI will result in a steeper SIII and this has been shown to correlate significantly with the pathological scores of small airways abnormalities in smokers (Cosio, Ghezzo et al. 1978). However, in 1990’s it has been identified that SIII of SBW is significantly affected by gravity and airway closure below FRC and not merely the changes in small airways which makes it unsuitable to monitor the small airways (Guy, Prisk et al. 1994). With this respect, SIII slopes derived from consecutive breaths of the MBW have an advantage of being less influenced by gravity and airway closure; therefore they are more suitable to reflect intrinsic airway structure and elastic properties of the lung (Prisk, Guy et al. 1995, Verbanck, Schuermans et al. 1997, Verbanck, Schuermans et al. 2004). Also clinical studies have showed that analysing SIII slopes over consecutive breaths of the washout can help to distinguish the contribution of conductive and peripheral airways to the VI (Verbanck 1998, Verbanck, Schuermans et al. 2004, Horsley 2009). An additional advantage of MBW over SBW is that it is easier to perform and more reproducible (Newth 1997, Horsley 2009).
Figure 1.4 A typical plot of single-breath N₂ washout (Robinson et al. 2009 theoretical background)

Legend: This plot was derived from SBW of normal subject. Phase I represents the upper airway and apparatus dead space, Phase II is the transitional phase, Phase III corresponds to the alveolar gas and Phase IV to gas expired below the closing volume (Adopted from (Robinson, Goldman et al. 2009) with permission.

1.4.3 The Significance of MBW in CF

The long-standing belief that CF lung disease commences early in the peripheral airways has highlight the need for sensitive and non-invasive measures of early changes in peripheral airways as the conventional spirometry are known to be insensitive (Lamarre, Reilly et al. 1972, Gustafsson 2003, Tiddens 2010). The measures of VI, derived from MBW, have grown in prominence as an alternative and have been shown by a number of cross-sectional and longitudinal studies to be sensitive to early changes in peripheral airways in different lung disease and to be altered in subjects with CF (Verbanck 1998, Verbanck 1999, Gustafsson 2003, Aurora 2004, Gustafsson 2007, Kraemer, Blum et al. 2005). The LCI, a measure of overall VI, is the main index derived from MBW. Other indices that can be derived from MBW are the SIII indices; \( S_{\text{cond}} \) and \( S_{\text{acin}} \). It has been shown that these two indices can distinguish the contribution of conductive and peripheral airways to the VI, respectively (Verbanck 1998). Increasing these indices indicate inhomogeneity of ventilation distribution or gas mixing inefficiency.
1.4.4 The Lung Clearance Index (LCI) derived from $N_2$MBW

1.4.4.1 Evidence of the sensitivity of LCI to early changes in the lung in CF:

LCI has become increasingly a measurement of choice in the evaluation and monitoring of early changes in the lung in CF (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Gustafsson, De Jong et al. 2008, Owens, Aurora et al. 2011). The recognition of the significance of LCI has come from large body of evidence showing that it is more sensitive than spirometry and plethysmography in detecting early involvement of peripheral airways in children and adolescents with CF (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Aurora 2004, Kraemer, Blum et al. 2005, Kieninger, Singer et al. 2011, Singer, Kieninger et al. 2013). Gustafsson and colleagues studied 43 children with CF (aged 3 to 18 yrs.) and reported an elevation in LCI in half to two thirds of those with normal spirometry or RV/TLC (Gustafsson 2003). This study was supported by subsequent cross-sectional studies in pre-school and school-age children with CF, all of which have demonstrated that children with CF have an elevated LCI even with normal FEV$_1$ (Aurora, Gustafsson et al. 2004, Aurora 2005, Horsley, Macleod et al. 2008, Singer, Kieninger et al. 2013). It was also shown that LCI is more sensitive than the conventional measures of small airway obstruction (FEF$_{25-75}$ and FEF$_{75}$) (Aurora 2004, Aurora, Gustafsson et al. 2004).

Interventional studies have also provided evidence of the sensitivity of LCI as an assessment tool in gauging response to intravenous antibiotics in acute pulmonary exacerbation (Robinson 2009, Horsley, Davies et al. 2013). These studies showed significant changes in LCI after two weeks course of intravenous therapy in more than 60% of children and adults with CF treated for acute pulmonary exacerbation though there was considerable heterogeneity of response (Robinson 2009, Horsley, Davies et al. 2013). This lack of uniform response to treatment in LCI has been suggested to be due to recruitment of lung units previously not contributing to ventilation (Robinson 2009, Horsley, Davies et al. 2013). It should be noted that subjects involved in these investigations had spirometry that was consistent with moderately severe impairment and patients with mild airways disease were not well represented (Robinson 2009, Horsley, Davies et al. 2013).
Further evidence of the importance of LCI as a sensitive marker of early CF lung disease has come from longitudinal studies evaluated the progression of lung disease based on serial lung function measurements including LCI between infancy to school-age and between school-age to adolescent in CF subjects (Kraemer, Blum et al. 2005, Kieninger, Singer et al. 2011, Aurora 2011). Two of these studies were performed in a modest number of subjects tested on two occasions and their findings support the clinical usefulness of LCI as an early predictor of lung disease and subsequent lung function in children with CF (Aurora 2011, Kieninger, Singer et al. 2011). The study of Kraemer and colleagues was based on serial lung function measurements performed in 142 children with CF followed over a substantial life span of 6 to 20 years (Kraemer, Blum et al. 2005). They showed that the progression of lung disease is detected earliest by LCI and that it represents much better functional progression than FEV₁ (Kraemer, Blum et al. 2005).

HRCT studies have also reinforced the significance of LCI in detecting early changes in the lungs at an earlier stage than spirometry (Gustafsson, De Jong et al. 2008, Ellemunter, Fuchs et al. 2010, Owens, Aurora et al. 2011). It has been found that VI as reflected by LCI is significantly associated with structural abnormalities detected by HRCT (Gustafsson, De Jong et al. 2008, Ellemunter, Fuchs et al. 2010, Owens, Aurora et al. 2011). Owen and colleagues found concordance between abnormal LCI and CT results in 39/53 children with mild CF lung disease (Owens, Aurora et al. 2011). The sensitivity of LCI to detect structural lung abnormalities including bronchiectasis and air trapping in these studies reached 85–94% (Gustafsson, De Jong et al. 2008, Ellemunter, Fuchs et al. 2010).

1.4.4.2 Repeatability and reproducibility of LCI:

The recent increasing acceptance of LCI has also been driven by its being highly repeatable and reproducible in health and disease. The repeatability and reproducibility of LCI has been assessed in school-age children in different studies using the coefficient of variation (CV) which was calculated as the standard deviation (SD)/mean expressed as a percentage (Aurora, Gustafsson et al. 2004, Fuchs 2009, Amin, Subbarao et al. 2010, Robinson, Stocks et al. 2012, Singer, Kieninger et al. 2013). All these studies were used SF₆MBW to derive LCI except Singer et al. who used N₂MBW and all demonstrated the reliability of this index and of the MBW test in general. Fuchs et al. found that within-test repeatability of LCI derived from three technically acceptable
MBW measured in 44 healthy children and adolescents aged 5-20 years was 5.1% which is similar to that reported elsewhere (Aurora, Gustafsson et al. 2004, Fuchs 2009, Singer, Kieninger et al. 2013). They also reported the short term reproducibility after 1 hour in 22 out of 44 volunteers and it was 4.2% and the long term reproducibility after 6 to 15 months in 34 out of 44 volunteers was 5.1% (Fuchs 2009). For school-age children with CF, the within-test repeatability of LCI was between 4.9-7.38% (Aurora, Gustafsson et al. 2004, Amin, Subbarao et al. 2010, Robinson, Stocks et al. 2012, Singer, Kieninger et al. 2013) and between visits repeatability (8 weeks apart) was 9.2% (Amin, Subbarao et al. 2010).

1.4.4.3 Age and height dependence of LCI:
LCI was found to be independent of age and height in school-age children and adolescents (Aurora 2004, Fuchs 2009). This makes its reference values very similar across the age range from infancy to adolescence and has led to the recognition that LCI can be considered as an ideal index for longitudinal screening of paediatrics with lung diseases (Aurora, Gustafsson et al. 2004, Gustafsson 2005, Fuchs 2009). However, recent study has shown that in the first 5 years of life (i.e. infant and pre-school children) LCI appears to be dependent on the body size and to be decreased as height increased (Lum, Stocks et al. 2013). A sex difference was also found for LCI as reported by a longitudinal study in school-age children with CF (Kraemer, Blum et al. 2005). They found that the progression (i.e. deterioration) of the LCI was significantly higher in females than in males and they suggested that to be due to the differences in the breathing pattern especially due to higher FRC$\text{N}_2$ in females (Kraemer, Blum et al. 2005). In another study in adult subjects, age was found to explain 36% of the variability in LCI, in which a steady worsening of VI increased with age (Verbanck, Thompson et al. 2012). Therefore, age, height and gender may need to be adjusted when using the MBW indices in studies involving pre-school children or adults.

1.4.4.4 Impact of choice of inert gas on LCI measurements:
The use of different marker gases to perform MBW test has also an effect on LCI (Robinson, Latzin et al. 2013). The majority of studies conducted in school-age children have used exogenous SF$_6$ as the tracer gas (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Horsley, Macleod et al. 2008, Fuchs 2008, Gustafsson, De Jong et al. 2008, Fuchs 2009, Amin, Subbarao et al. 2010, Owens, Aurora et al. 2011, Fuchs, Ellemunter et al. 2012). The reported LCI on these studies was between 7.3–11.5 in children with CF and
between 6.19-6.89 in healthy children. Studies using N₂ MBW reported higher LCI values between 11.29-12 for children with CF, and comparable values in health (Gustafsson 2007, Jensen, Stanojevic et al. 2013, Singer, Kieninger et al. 2013). These differences in LCI could be explained by the different populations studied and/or different marker gases used (Singer, Kieninger et al. 2013). The N₂ and SF₆ have different physiological properties and different molecular mass (MM) which may affect the gas diffusion rate at lung periphery (Grönkvist, Bergsten et al. 2002, Robinson, Latzin et al. 2013, Jensen, Stanojevic et al. 2013). The N₂ has an advantage of having a small MM of 14 g.mol⁻¹ and is resident within all lung units including the slowly ventilated units (Robinson, Latzin et al. 2013, Jensen, Stanojevic et al. 2013, Singer, Kieninger et al. 2013) whereas SF₆ is heavier with a MM of 146 g.mol⁻¹ and needs to be washed-in to the lungs until equilibrium is reached, which may not be achieved in the presence of extremely slowly ventilated regions (Robinson, Latzin et al. 2013, Jensen, Stanojevic et al. 2013). Consequently, CEV₆ will be reduced and LCI underestimated. This was supported by a study comparing the results obtained from a N₂ washout system to that obtained from SF₆ based system in children with CF, which showed a closer agreement between FRCₚлег and FRCₙ₂ than between FRCₚлег and FRC₆ (Jensen, Stanojevic et al. 2013). They suggested that the lower FRC₆ values may be a result of not capturing the volume contribution of the slowly ventilated regions during MBW₆ (Jensen, Stanojevic et al. 2013).

Therefore, LCIₙ₂ may be able to more accurately reflect the degree of VI than LCI₆ especially in subjects with greater disease severity (Robinson, Latzin et al. 2013, Jensen, Stanojevic et al. 2013). However, in those subjects N₂ MBW appears to take substantially longer time than SF₆ MBW which limits the feasibility of performing this test routinely in the clinical settings. Recent studies have looked at the feasibility of shortening the washout time and/or reducing the number of trials required to obtain reproducible results (Aurora 2005, Yammine, Singer et al. 2012, Robinson, Stocks et al. 2012). These studies compared mean LCI measured from 3 runs to that measured from the first two runs and they concluded that LCI based on two successful runs is as robust as LCI based on 3 runs and sensitive enough to detect abnormal peripheral airway function in CF (Aurora 2005, Yammine, Singer et al. 2012, Robinson, Stocks et al. 2012). They also reported that mean LCI from two runs may detect differences between any two populations more readily than LCI calculated from three runs, if the difference
in LCI between these populations is greater than 0.54 (Aurora 2005). Furthermore, Yammine et al. provided a preliminary evidence for the possibility of reducing the test duration in children with CF without compromising the sensitivity of N₂MBW (Yammine, Singer et al. 2012). They suggested an earlier cut-off concentrations in the washout at 5% N₂ (LCI₅), which is equivalent to 1/20th of N₂ starting concentration as they found it the earliest point to meet all quality criteria (Yammine, Singer et al. 2012). In contrast, a recent study by Verbanck et al. recommended not shortening the washout in subjects with severe CF lung disease for better quantification of VI at the level of the conductive airways (Verbanck, Paiva et al. 2013).

1.4.4.5 Impact of different apparatus for MBW on LCI measurements:
The use of different setups for MBW measurements has also had an influence on the measures of VI (Singer, Kieninger et al. 2013). Most of the evidence for LCI in school-age children has come from studies using mass spectrometry system (MS) that uses SF₆ (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Gustafsson, De Jong et al. 2008, Horsley, Macleod et al. 2008, Owens, Aurora et al. 2011). MS has an advantage of online analysis of multiple tracer gases, allowing simultaneous measurement of gases of differing diffusivities, though it is limited by being expensive, immobile and requiring considerable technical skills (Fuchs 2006, Jensen, Stanojevic et al. 2013). Therefore, new systems use different flow meters, gas analysers and software to determine inspired and expired inert gas volumes, by continuously measuring inert gas concentrations synchronised with respiratory flow have been developed recently to allow widespread application of MBW in clinical research and clinical practice (Fuchs 2006, Horsley 2009, Singer, Houltz et al. 2012). These new devices have been tested against the current gold standard MS and found to be a possible alternative (Fuchs 2006, Horsley 2009). However, in a study by Fuchs et al, the authors found that FRC and LCI values were slightly higher when using the MS system compared to ultrasonic flow sensor prototype system (NDD) while using the same tracer gas (SF₆) and this was statistically significant for FRC (Fuchs 2006). Similarly, Jensen et al. found that LCI derived from N₂ washout system (Exhalyzer D) cannot be used interchangeably with LCI derived from MS system although they have similar discriminative power and both are repeatable (Jensen, Stanojevic et al. 2013). In addition, Horsley found a difference in SF₆ concentration operating range between MS and Innocor gas analyser which interfere with the direct comparison between these devices. Therefore, he conducted in
vitro technical comparison between both systems and concluded that Innocor can accurately assess VI in adults and older children, but does not meet the recommendation for MBW apparatus in pre-school children (Horsley 2009).

1.4.4.6 Impact of different breathing protocols on LCI measurements:

Other factors that have an impact on LCI and maybe the cause for the differences between paediatric studies are adopting different breathing protocols (free breathing vs. one litre tidal volumes) (Singer, Kieninger et al. 2013). It has been shown by a small number of studies in adults that breathing with varying tidal volumes (Vt) and respiratory rate (RR) has an effect on LCI (BOUHUYS 1961, Young, Martin et al. 1968, Crawford 1986, Grönkvist, Bergsten et al. 2002). Therefore, a one litre Vt has been adopted as a fixed breathing protocol in adults and adolescents for many years to improve comparability of results between subjects breathing at different Vt and to ensure sufficient SIII for the identification of S_acin and S_cond (Crawford 1985, Verbanck, Schuermans et al. 1997). However, the latest ERS/ATS consensus suggested that breathing protocols of 1 L Vt may not be feasible in all age groups (Robinson, Latzin et al. 2013). This is because children have a wide range of Vt related to their body size and they may find it difficult to maintain stable breathing pattern throughout the washout (Beydon 2007, Robinson, Goldman et al. 2009). Little is known about the impact of fixed breathing protocol on VI indices in children. Currently, there are different approaches employed in children that varying from entirely free breathing while watching video in younger subjects<10 yrs. or watching the Vt trace on a computer screen in older subjects, to breathing using incentives with a given Vt between 8-15ml/kg, or breathing with a fixed protocol using 0.5 L in younger and 1 L in older subjects (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Gustafsson 2007, Horsley, Macleod et al. 2008, Singer, Kieninger et al. 2013). A recent paediatric study in 35 children (20 with CF) have compared VI indices derived from free breathing N2MBW to that derived from a fixed 1 L Vt (Yammine, Singer et al. 2014). They found that different MBW protocols strongly influence measures of VI and that 1 L Vt MBW protocol leads to overestimation in LCI and S_cond while S_acin did not change significantly (Yammine, Singer et al. 2014). However, they have not looked at the influence of different breathing rates on MBW indices in children.
1.4.5 Phase III slope indices derived from N\textsubscript{2}MBW

1.4.5.1 The basis of SIII analysis:
Over the last two decades, a sophisticated analysis technique of MBW has been developed to enable evaluating the site and mechanisms underlying the inhomogeneity of gas mixing and ventilation distribution within the lung compartments (Paiva, Engel 1981, Paiva, Engel 1984, Crawford 1985, Verbanck, Schuermans et al. 1997). This analytical technique emerged as a result of theoretical work using mathematical modelling and simulation of gas mixing in two-trumpet and multi-branch point models by Paiva and Engel which was subsequently applied experimentally by Crawford et al. in healthy adult subjects (Paiva 1975, Paiva, Engel 1981, Paiva, Engel 1984, Crawford 1985). The principle behind the development of the MBW analysis technique was based on considering each expiration as a SBW and determining the slope of alveolar plateau breath by breath (Paiva 1975, Crawford 1985). This slope is then divided by the mean expiratory gas concentration to give a concentration-normalized phase III slope (S\textsubscript{nIII}) in order to account for the dilution of the gas. This is to allow the comparison between S\textsubscript{nIII} slopes of subsequent breaths. Then by studying the progression of the S\textsubscript{nIII} slopes over subsequent breaths of the washout, the behaviour of the slope reveals the mechanisms by which it is generated (Crawford 1985). Two mechanisms have been proposed to be responsible for VI and give rise to the alveolar SIII; the convection-dependent inhomogeneity (CDI) and the diffusion and convection dependent inhomogeneity (DCDI) (Paiva 1975, Crawford 1985). The CDI, originates from concentration differences and flow sequencing between parallel lung units larger than acini (i.e. between unequally ventilated large units subtended by centrally located branch points) where gas transport occurs by convection (Crawford 1985). DCDI, results from concentration inequalities generated from asymmetrical small units subtended by relatively peripheral branch points or from inequality in airway cross sections at the peripheral branch points (Crawford 1985). As the total cross-sectional area increases with increasing airway generation towards the lung periphery, the convective velocity of the gas falls and diffusion become the dominant mechanism of gas transport in the most distal regions of the lung (Crawford 1985) (Figure 1.5). The interactions between diffusion and convection in the region of the diffusion-convection front will give rise to DCDI.
Figure 1.5 Gas transport through airway tree

Legend: This schematic structure of the tracheobronchial tree shows the predominant gas transport throughout airway generations. Broken lines represent the total airway cross-sectional area which increases dramatically in the lung periphery. The convection gas transport predominates in the conducting airway zone which represents generation 0 till 16. At the transitional area (i.e. at the entry of the acinus), relative contributions of convection and diffusion to gas mixing become similar, forming a diffusion-convection front. Whereas within the intra-acinar airways (generation 17-23), diffusion is the predominant mechanism of air flow. Airway pathology that alters gas concentration and flow sequencing between lung units or changes the dimensions of small airways will affect the distribution of ventilation among different parallel pathways and give rise to the alveolar phase III slope. Adopted from (Robinson, Goldman et al. 2009) with permission.
Based on the model analyses, both mechanisms of VI were found to contribute to the increase in $S_{nIII}$ of the initial 4-6 breaths of the washout and, beyond that, the contribution of DCDI stays constant (reaching asymptote) and any further increase in $S_{nIII}$ is diffusion independent and reflect only the CDI contribution. Findings from Crawford experimental study in 4 normal subjects using simultaneous MBW of He and SF$_6$ were consistent with model analyses (Crawford 1985). They observed increasing the difference between $S_{nIII}$ values for He and SF$_6$ which have different diffusivity during the first five breaths of the washout with a fixed difference thereafter despite a progressive increase in $S_{nIII}$ (Crawford 1985). Further adaptation of the analysis technique by Sylvia Verbanck has allowed the separation of the contribution of the convective and acinar airways to the VI through two indices $S_{cond}$ and $S_{acin}$, respectively (Verbanck, Schuermans et al. 1997). These two indices have been given designations based upon the supposed anatomical location of the gas mixing process therefore increasing $S_{cond}$ and $S_{acin}$ indicates increasing VI at conductive and acinar level, respectively (Verbanck, Schuermans et al. 1997).

Verbanck modified the SIII analysis based on the analysis method of Crawford et al., however, there were some methodological differences in the computation of CDI and DCDI which were thereafter called $S_{cond}$ and $S_{acin}$, respectively (Crawford 1985, Verbanck, Schuermans et al. 1997). Verbanck and colleagues derived $S_{cond}$ and $S_{acin}$ values from the plot of $S_{nIII}$ vs. lung TO (Verbanck, Schuermans et al. 1997). They used lung TO as opposed to the breath number used by Crawford to allow for better comparison of subjects with different lung volumes and dilution (Verbanck, Schuermans et al. 1997). Therefore, they determined CDI or $S_{cond}$ by linear regression between TO=1.5 and TO=6 (Figure 1.6) as opposed to a single exponential curve fit after the fourth breath applied by Crawford et al. (Crawford 1985, Verbanck, Schuermans et al. 1997). Whereas $S_{acin}$ is calculated in the same way by subtracting the part contributed to the conductive airways from the slope of the first breath (i.e. $S_{acin} =$ the first breath $S_{nIII}$ value minus $S_{cond}$ multiplied by TO of the first breath) (Verbanck, Schuermans et al. 1997). The calculation of $S_{nIII}$ indices up to TO=6 has been chosen because after 6 turnovers $S_{nIII}$ values become more variable due to the poor resolution of gas analysers in these concentration ranges (Robinson, Goldman et al. 2009).
Figure 1.6 Normalized phase III slope ($S_{nIII}$) analysis

Legend: This shows $S_{nIII}$ plotted against lung TO, from where phase III indices can be derived. Ideally, $S_{nIII}$ measured values (circles) shows progressive increase throughout the washout because the least-ventilated unit (with largest $N_2$ concentration) empties predominantly late in the expiration. If this not the case, the quality of the recording should be closely examined. $S_{cond}$ (straight line) which is an index reflecting convection-dependent inhomogeneity (CDI) can be calculated as the increase in $S_{nIII}$ between $TO=1.5$ to $TO=6$ (i.e. $S_{nIII}$ difference per unit $TO$ between 1.5 and 6 $TO$ where only conductive airways are known to contribute to the rate of rise of normalized slope). While $S_{acin}$ (solid triangle), which represents the diffusion and convection dependent inhomogeneity (DCDI) can be calculated by subtracting the contribution of CDI to $S_{nIII}$ of the first breath. In this plot the contribution of DCDI to the $S_{nIII}$ for each breath (unfilled triangles) is also illustrated to show that the contribution of the DCDI mechanism reaches asymptote beyond $TO$ 1.5 (Adopted from (Robinson, Latzin et al. 2013) with permission.)
1.4.5.2 The application of SIII analysis in clinical studies:
Verbanck and colleagues have applied their modified analysis technique to a number of clinical studies in different patient groups. The findings from these studies have emphasised the clinical effectiveness of $S_{cond}$ and $S_{acin}$ in separating the contribution of different lung zones to VI (Verbanck, Schuermans et al. 1997, Verbanck 1998, Verbanck 1999, Verbanck 2003, Verbanck, Schuermans et al. 2004, Gustafsson 2007, Horsley, Macleod et al. 2008). In hyperresponsive asymptomatic subjects, $S_{cond}$ was shown to be affected by histamine provocation with no significant change in $S_{acin}$ (Verbanck, Schuermans et al. 1997). In contrast, in a group of patients with chronic obstructive pulmonary disease (COPD), both $S_{cond}$ and $S_{acin}$ were become abnormal (Verbanck 1998) with absence of significant changes in both indices after bronchodilator (Verbanck 1999). This indicates that there are independent alterations at conductive and acinar lung zones that are responsible for airways obstruction in COPD and these are irreversible (Verbanck 1998, Verbanck 1999). Similar patterns of parallel increase of SIII measures were seen in smokers with COPD, but with a further increase in $S_{acin}$ in the emphysematous group which reflects a further destruction of the peripheral airspaces (Verbanck, Schuermans et al. 2004). In asthmatics, baseline measurements of $S_{cond}$ and $S_{acin}$ were found to be abnormal and improved significantly after salbutamol administration (Verbanck 1999). Despite the improvement their values had not returned to normal values except for $S_{acin}$ in subjects with mild asthma (Verbanck 1999, Verbanck 2003, Gustafsson 2007). In a comparative study between COPD and asthma subjects, $S_{acin}$ appeared the most impaired in COPD whereas $S_{cond}$ value was not significantly different from that found for the asthma group (Verbanck 1999). The impairment in conductive VI in both groups, however, should be due to different mechanisms because it was partly reversed in the asthma group (Verbanck 1999).

1.4.5.3 The application of SIII analysis in studies involving subjects with CF and the repeatability of the derived indices:
It has been suggested that the developed analysis techniques of the washout curve may provide more information about the site of early CF lung disease, and to the nature of its progression (Verbanck, Schuermans et al. 1997, Gustafsson 2003, Beydon 2007). Also that the relationships between indices derived from MBW test may help localize the site of structural changes (Verbanck, Paiva et al. 2012, Robinson, Latzin et al. 2013).
However, limited research has been done in this area due to limited number of repeatability data for \( S_{nIII} \) indices and much of the attention has been focused on LCI (Gustafsson 2007, Horsley, MacLeod et al. 2008).

Few studies have examined the reproducibility and repeatability of \( S_{nIII} \) indices in health and disease, all of which were in adult subjects (Downie, Salome et al. 2007, Biddiscombe, Verbanck et al. 2009, Gonem, Ball et al. 2011, Gonem, Natarajan et al. 2012, Horsley 2009). Biddiscombe and colleagues assessed the same-day and day-to-day reproducibility of \( S_{cond} \) and \( S_{acin} \) in 24 adult healthy volunteers and 12 patients with mild to moderate asthma (Biddiscombe, Verbanck et al. 2009). They performed two \( N_2MBW \) runs (each consists of 3 tests) for each subject each day on three separate days and showed acceptable same-day and day-to-day reproducibility of \( S_{cond} \) and \( S_{acin} \) in both populations (Biddiscombe, Verbanck et al. 2009). For healthy volunteers, same-day and day-to-day coefficient of variation (CV %) was the same for \( S_{acin} \) (17%), but increased for \( S_{cond} \) from 8% to 21%, respectively. In asthmatics, day-to-day variability was consistently greater than same-day variability in both indices (Biddiscombe, Verbanck et al. 2009). Downie et al. also looked at day-to-day reproducibility of \( S_{cond} \) and \( S_{acin} \) in 11 healthy adult subjects and 10 with asthma and showed a good repeatability with an intra-class correlation coefficient (ICC) of 0.80 for \( S_{cond} \) and 0.78 for \( S_{acin} \) in healthy subjects and 0.84 for \( S_{cond} \) and 0.95 for \( S_{acin} \) in asthmatics (Downie, Salome et al. 2007). In different study, Gonem and co-worker looked at the repeatability of \( S_{acin} \) in 29 patients with asthma and showed excellent repeatability of \( S_{acin} \) (Gonem, Ball et al. 2011). The reported interclass correlation coefficient (ICC, a measure of repeatability) for \( S_{acin} \) was 0.914, 0.897 and 0.879 for within-visit, 2 week and 3 month respectively (Gonem, Ball et al. 2011). Another study by the same group in 71 patients with asthma and 18 healthy control subjects, the authors found that the first breath \( S_{nIII} \) and \( S_{acin} \) were the most repeatable parameters after LCI (Gonem, Natarajan et al. 2012). Conflicting results were reported by Horsley who found large CV% of intra-subject repeats for \( S_{cond} \) and \( S_{acin} \) in adults with CF and healthy subjects (Horsley 2009). The variability of \( S_{cond} \) was higher than that of \( S_{acin} \) in both groups with the highest CV being for \( S_{cond} \) in healthy subjects (Horsley 2009). The author suggested that the poor reproducibility of \( S_{cond} \) may be due to the very low values of this index which largely impact on measures of reproducibility with any small variations in slope (Horsley 2009).
One of the limited published studies that looked at the relationships between VI indices was the study by Gustafsson in 11 children with CF, 15 with asthma and 18 healthy controls before and after bronchodilator (Gustafsson 2007). Findings from this study show that baseline LCI and S_{acin} were both markedly abnormal in CF than in asthma, whereas the increases in S_{cond} in both conditions were of a similar degree (0.15 (0.071) vs. 0.127 (0.041)) (Gustafsson 2007). Following bronchodilator, there was an improvement in S_{acin} (though not to normal values), with LCI and S_{cond} remaining elevated in subjects with CF. Whereas all indexes improved in asthma after treatment and only S_{cond} stayed abnormally elevated (Gustafsson 2007). This suggests that peripheral airways close to or in the gas exchange region are more involved in CF, in addition to the contribution of the more proximal conducting airways (Gustafsson 2007). A subsequent study by Horsley et al in 40 adults and children with CF found that S_{cond} derived from SF_{6}MBW became elevated at an earlier stage than S_{acin} in patients in whom LCI was within the normal range (Horsley, Macleod et al. 2008). The study also showed that S_{cond} reached an asymptote and did not increase further with increasing disease severity (as reflected by increasing LCI) whereas S_{acin} does in adults with CF with LCI greater than 10 (Horsley, Macleod et al. 2008).

The findings from preceding two studies however violated one of the basic assumptions distinguishing CDI from DCDI in which DCDI assumes to generate a horizontal asymptote after 4-6 breaths of the washout, while CDI steadily increases as the washout progresses within lung turnover range between 1.5-6 TO (Crawford 1985, Verbanck, Schuermans et al. 1997, Verbanck, Paiva et al. 2013). They have also been criticised by Verbanck and colleagues in the way of determination of S_{cond} in the sub-group of subjects with advanced CF lung disease which may have been invalidated by the severity of the ventilation heterogeneity in these patients (Verbanck, Paiva et al. 2013). Therefore, Verbanck and co-workers proposed an alternative S_{cond} computation method in patients with severe VI (leading to alternate parameter S_{cond*}) based on lowering the upper end of the TO range from 6 to 3 and computing the regression slope in the range TO = 0-3, with excluding the first breath S_{nIII} to minimize acinar effects (Verbanck, Paiva et al. 2013). This method has been validated by a conceptual model and experimental study in 45 subjects with CF and 25 sex and age matched healthy controls and shown to provide a more accurate reflection of S_{cond} in advanced CF lung disease (Verbanck, Paiva et al. 2013).
1.4.5.4 Factors affecting $S_{nIII}$ indices and the introduction of volume correction method:

Although SIII indices have been shown to be useful for characterization of VI arising from different lung zones, they have been shown to be affected by age, gender, inert marker gas diffusivity, using different setups and changes in Vt (Crawford 1985, Crawford 1986, Crawford 1989, Verbanck, Thompson et al. 2012, Grönkvist, Bergsten et al. 2002, Gustafsson 2003, Ljungberg, Gustafsson 2003). In healthy adults, age was found to explain 7-16% of the variability in $S_{cond}$ and $S_{acin}$ and gender has a small but significant effect on $S_{acin}$ only (Verbanck, Thompson et al. 2012). $S_{acin}$ was found to be slightly greater in men than in women and this has been suggested to be due to the more symmetric intra-acinar bifurcation, which is a major determinant of acinar VI, in men (Verbanck, Thompson et al. 2012). In a different study in preschool and school-age healthy children, the authors found that 43% of the variability of $S_{nIII}$ was explained by subject characteristics such as age, height and gender in multivariate regression analysis model. Moreover, they noticed a strong relationship between first breath $S_{nIII}$ and expired volume (Aurora 2005, Aurora, Kozlowska et al. 2005). When they multiplied first breath $S_{nIII}$ by expired volume of that breath in litre and plotted against age, the dependence of $S_{nIII}$ on expired volume was reduced and the association between $S_{nIII}$ and age abolished (Aurora 2005, Aurora, Kozlowska et al. 2005). Therefore, Aurora and colleagues proposed the earlier method which they termed as “volume correction method” when calculating $S_{nIII}$ indices to reduce the dependence of $S_{nIII}$ on expired volume (Aurora 2005, Aurora, Kozlowska et al. 2005). Studies on healthy children and adults have confirmed the independence of volume corrected $S_{nIII}$ indices on age (Horsley, Macleod et al. 2008, Horsley 2009). They also suggested that the application of volume correction method may help to reduce the variability associated with breaths of different sizes, and will allow the comparison of calculated $S_{nIII}$ indices between different age groups and with different studies (Horsley, Macleod et al. 2008, Horsley 2009).

1.4.5.5 Impact of different marker gases on $S_{nIII}$ indices:

The diffusivity of different inert marker gases has also an effect on the location of changes in corresponding lung structures and thereafter on the derived indices from the washout test (Ljungberg, Gustafsson 2003, Robinson, Latzin et al. 2013). He and SF$_6$ are the most commonly used inert marker gases to perform MBW tests in the literature.
The diffusion rate of these marker gases is inversely affected by their molecular masses which are widely different (Ljungberg, Gustafsson 2003, Robinson, Latzin et al. 2013). $S_{\text{cond}}$ or CDI which is diffusion-independent index was found to be unaffected when using gases of different diffusivity (Ljungberg, Gustafsson 2003). However, $S_{\text{acin}}$ or DCDI is dependent on the site the diffusion front for a specific gas molecule (Robinson, Latzin et al. 2013, Ljungberg, Gustafsson 2003). Engel and colleagues found that in normal healthy subjects under normal gravity, $S_{n,\text{III}}$ of SF$_6$ is greater than $S_{n,\text{III}}$ for He and they suggested it was due to diffusion-dependent concentration differences among lung units ventilated in parallel (Engel, Paiva et al. 1979). This was supported by the observation of Crawford et al. in healthy subjects, in which they reported increasing the difference between $S_{n,\text{III}}$ values for He and SF$_6$ during the first five breaths of the washout (Crawford 1985).

Furthermore, in 2 studies in subjects with asthma using a double-tracer gases He and SF$_6$ SBW test to estimate the location of response in the lung periphery to a challenge test, authors found a fall in FEV$_1$ of at least 20% after the challenge test with a greater increase in the He vs. SF$_6$ $S_{n,\text{III}}$ (Gustafsson 2003, Ljungberg, Gustafsson 2003). This suggests that the diffusion-convection front for the relatively more diffusible He gas molecule is more proximal to the entrance of the acinus as opposed to SF$_6$ which heavier in its MM and diffuses nearly six times more slowly (Gustafsson 2003, Ljungberg, Gustafsson 2003). This finding, therefore, indicates the occurrence of peripheral airway obstruction in asthma at the acinar entrance (Gustafsson 2003, Ljungberg, Gustafsson 2003).

### 1.4.5.6 Impact of changing Vt on $S_{n,\text{III}}$ indices:

Breathing at higher lung volumes has also an effect on $S_{n,\text{III}}$ indices (Crawford 1989, Robinson, Latzin et al. 2013). Crawford and co-workers examined the effect of pre-inspiratory lung volume on VI indices derived from N$_2$MBW test in seven healthy subjects and found that distribution becomes progressively more inhomogeneous at higher lung volumes over a range of volumes above closing capacity (Crawford 1989). Changing Vt was similarly found to influence the results of $S_{n,\text{III}}$ indices (Crawford 1986, Grönkvist, Bergsten et al. 2002, Yammine, Singer et al. 2014). In two studies examining the influence of increased Vt on $S_{n,\text{III}}$ indices obtained from MBW in healthy
adults, authors found that increasing Vt was associated with reduced $S_{acin}$, but greater $S_{cond}$ (Crawford 1986, Grönkvist, Bergsten et al. 2002). Therefore, a one litre Vt has been adopted as a component of a fixed breathing protocol in adults and adolescents to improve comparability of results between subjects breathing at different Vt and to ensure sufficient SIII for the identification of $S_{acin}$ and $S_{cond}$ (Crawford 1985, Verbanck, Schuermans et al. 1997). However, using 1 L Vt MBW protocol in paediatrics was found to overestimate $S_{cond}$, with no significant effect $S_{acin}$ (Yammine, Singer et al. 2014). This was attributed to be due to the increase in airway volume dead space with increasing Vt, and therefore, increasing the ratio of $V_D/Vt$ which has been shown to correlate significantly with $S_{cond}$ (Horsley 2009). Until now, there is no standardized MBW breathing protocol for children, but there is emphasis in ensuring sufficient breath size for adequate SIII identification (Robinson, Latzin et al. 2013). Therefore, different approaches have been employed that vary from entirely free breathing to breathing using incentives with a given Vt or breathing with a fixed protocol using 0.5 L in younger and 1 L in older subjects (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Gustafsson 2007, Horsley, Macleod et al. 2008).
1.5 Hyperpolarised helium-3 magnetic resonance (³He MR)

1.5.1 Principle of MRI:

MRI relies on the magnetic properties of the proton, the positively charged spinning nucleus of hydrogen atoms (Figure 1.7A) that are abundant in the human body in tissues containing water, proteins, and lipids (Edelman, Warach 1993, Ball 2011). In the presence of a magnetic field, the proton can align in two directions, either with or against the direction of the magnetic field (Figure 1.7B) (Edelman, Warach 1993, Ball 2011). By applying radiofrequency energy, the protons aligned with the magnetic field absorb the energy and reverse their direction and subsequently release the absorbed energy and relax back to the original alignment (Figure 1.7C) (Edelman, Warach 1993). During the relaxation process, the protons produce MR signal in a radio frequency receiver called a body coil that surrounds the patient or in a specialized small coil used to optimize the image quality over localized regions of the body (Edelman, Warach 1993, Ball 2011). The stronger the magnetic field, the stronger the signal and subsequently faster imaging, thinner slices and better resolution of images can be obtained (Zapke 2006). However, the application of this traditional proton MRI for lung imaging provides low quality images due to the low density of water molecules in lung tissue and the inhomogeneous magnetic field inside the thorax (van Beek, Wild 2005, Ball 2011).
Figure 1.7 The principle of MRI

Legend: These figures illustrate the interaction between the nuclear spin and the magnetic field, which is the basis of MRI. A. atoms which have protons in their nucleus spin. B. in the presence of magnetic field, spinning nuclei aligns themselves with or against the field. C. by applying radiofrequency energy, the nucleus aligned with the magnetic field absorb the energy and reverse their direction and subsequently release the absorbed energy (MRI signals) and relax back to the original alignment. Adopted from (Gibbs 2013).
1.5.2 The development of hyperpolarized noble gases MRI:

In 1990’s, a number of animals and human studies showed that the non-radioactive noble gases such as 129-xenon ($^{129}$Xe) and He-3 ($^{3}$He) can provide high-resolution MRI of the lungs when the nuclear spin polarisation of noble gases is increased by a technique called laser optical pumping (Albert, Cates et al. 1994, Ebert, Grossmann et al. 1996, Wagshul, Button et al. 1996, Bachert, Schad et al. 1996). This technique, which involves direct optical pumping from polarised laser light to the nuclei of the noble gases, produce hyperpolarised $^{129}$Xe and $^{3}$He in which the nuclear polarisation (i.e. alignment to one direction) is enhanced and is about $10^5$ times greater than the polarization of water protons used in conventional MRI (Albert, Cates et al. 1994, Bachert, Schad et al. 1996). This increased polarisation easily overcomes the loss in signal due to the lower gas density (Ebert, Grossmann et al. 1996, Middleton, Black et al. 1995). Albert and colleagues were the first to demonstrate the possibility of obtaining faster and higher resolution images of the lung by introducing hyperpolarized $^{129}$Xe in to mouse lungs (Albert, Cates et al. 1994). In a subsequent study by the same group, authors were able to obtain high quality images of the lungs of a guinea pig using hyperpolarized $^{3}$He as the source of the MR signal (Middleton, Black et al. 1995). The use of $^{3}$He MRI was then reported in human beings by Ebert et al. in 1996 when they imaged the lungs of a 27 years old healthy volunteer who inhaled hyperpolarised $^{3}$He in the supine position (Ebert, Grossmann et al. 1996). Their findings support the ability of $^{3}$He MRI to image air spaces and to investigate regional differences in ventilation distribution as they found that different regions of the lungs reflect different signal intensity, which is proportional to the concentration of $^{3}$He gas in these regions (Ebert, Grossmann et al. 1996). In their study also they proposed that the subject inhale two breaths of pure $^{4}$He before the inhalation of hyperpolarized $^{3}$He to reduce the concentration of oxygen in the airways because the oxygen has a paramagnetic effect that destroys the polarization of $^{3}$He and may decrease the relaxation time of the hyperpolarized $^{3}$He nuclei, thus decreasing image resolution (Ebert, Grossmann et al. 1996, Ball 2011, van Beek, Wild 2005).
1.5.3 Hyperpolarized $^3$He diffusion MR:

Following the pioneering work of hyperpolarized $^3$He MRI in human lungs, several clinical studies have been conducted, the majority using hyperpolarized $^3$He to visualize air spaces and assess patients with lung diseases (van Beek 2004, Kauczor, Ebert et al. 1997, de Lange, Mugler III et al. 1999). The increasing use of $^3$He in the diagnosis testing in human was due to its higher polarization levels, longer relaxation times, higher signal-to-noise ratio, lack of anaesthetic properties and the ability to be polarized in larger amounts (Wagshul, Button et al. 1996, van Beek 2004, van Beek, Wild 2005). $^3$He has also an advantage of high diffusion coefficient and low solubility in blood (van Beek, Wild 2005, Ball 2011).

One of the applications of $^3$He MRI is lung morphometry which relies on studying $^3$He gas diffusion in lungs (Saam, Yablonskiy et al. 2000, Yablonskiy, Sukstanskii et al. 2002, Ball 2011). It has been suggested that measurement of $^3$He gas diffusivity in the lung air spaces has potential for identifying changes in lung microstructure (Yablonskiy, Sukstanskii et al. 2002, Saam, Yablonskiy et al. 2000, Salerno, de Lange et al. 2002). Theoretical developments in the area of gas diffusion have linked diffusion-attenuated MR signals and lung microstructure (Yablonskiy, Sukstanskii et al. 2002, Sukstanskii, Yablonskiy 2008, Yablonskiy, Sukstanskii et al. 2009, Osmanagic, Sukstanskii et al. 2010). Since gas diffusion in normal lungs is mostly restricted at acinar level by alveolar walls, the measured diffusivity of $^3$He gas and the resultant MR signal decay were described as a marker of alveolar size called the apparent diffusion coefficient (ADC) (Yablonskiy, Sukstanskii et al. 2002). The ADC derived from $^3$He MRI provides a quantitative measure that reflects the relative difficulty with which $^3$He diffuses within a restricted structure such as the lungs or in other words reflects the restricted diffusion of gas molecule from the small length scales of the lung (Mada 2009, van Beek, Wild 2005). It can be obtained for whole lung (global ADC or WL ADC) by a single measurement over short-period of several milliseconds (ADC$_{msec}$), which is the most frequently reported in human experiments, or over a longer period of several seconds (ADC$_{sec}$) (Verbanck, Paiva 2007, Mada 2009). A diffusion map can also be produced showing the differences in diffusion over different lung regions (Mada 2009).
1.5.4 The apparent diffusion coefficient (ADC) derived from hyperpolarized $^3$He diffusion MR

Changes in the lung microstructure altered ADC measurements (Emami, Stephen et al. 2009). In healthy lungs where the alveolar walls act as barriers and restrict the diffusion of $^3$He, ADC as measured with MR imaging will be reduced. In contrast, in diseased lungs, where peripheral airway walls are destroyed, the diffusion is less restricted and the ADC will be increased (Mada 2009, Diaz, Casselbrant et al. 2009, Osmanagic, Sukstanskii et al. 2010). This has been confirmed by a number of clinical studies where the ADCs of patients with emphysema were found to be significantly larger than those of healthy subjects due to the expansion of alveoli and airways and destruction of tissue, which resulted in increased diffusivity of gas molecule (Saam, Yablonskiy et al. 2000, Salerno, de Lange et al. 2002, Yablonskiy, Sukstanskii et al. 2002, Diaz, Casselbrant et al. 2008). The reported normative values of ADC in these studies were comparatively low (about 0.2 cm$^2$/sec), however, measurements in patients with emphysema had a wide distribution, with an average of 0.55 cm$^2$/sec (Saam, Yablonskiy et al. 2000, Salerno, de Lange et al. 2002, Yablonskiy, Sukstanskii et al. 2002, Diaz, Casselbrant et al. 2008). This was proposed to be due to the varying severity of the disease in each study group which will impact on the underlying length scales, surface to volume ratio and the morphology of the distal air spaces of the lung (Salerno, de Lange et al. 2002). This explanation was supported by Bink and co-workers where they reported a statistically significant difference in mean ADC values among healthy, transplanted, emphysematous and fibrotic lungs with the highest values being for the fibrotic and emphysematous lung tissues (Bink, Hanisch et al. 2007).

1.5.4.1 Factors affecting ADC measurements

Although ADC measurements derived from $^3$He diffusion MR are relatively new, it has been shown by a number of in vivo studies to be affected by several factors (Osmanagic, Sukstanskii et al. 2010, Parra-Robles, Wild 2012, Fain, Altes et al. 2005, Waters, Owers-Bradley et al. 2006). In a multi-centre study on 44 healthy adult never-smokers aged 18-69 years old, ADC was found to be dependent on age and this was suggested to be due increasing alveolar volume during the aging process (Fain, Altes et al. 2005). This was supported further by a study conducted on 29 healthy subjects over a wide age-range from pre-school to adulthood where authors reported an increase of
ADC with age (Altes, Mata et al. 2006). More recently, a study by Narayanan and colleagues has shown that dimensions of alveoli increased significantly with age, height and lung size during childhood and adolescence (Narayanan, Owers-Bradley et al. 2012).

ADC measurements have also been shown to rely substantially on the details of the technique used in MR experiment such as diffusion time and the volume of inhaled gas prior to measurements (Osmanagic, Sukstanskii et al. 2010, Parra-Robles, Wild 2012, Diaz, Casselbrant et al. 2008, Waters, Owers-Bradley et al. 2006). Several studies have suggested that ADC obtained over a period of several seconds maybe more sensitive than ADC obtained over a shorter-period of several milliseconds especially in subjects with airflow obstruction (Wang, Altes et al. 2008, Gonem, Ball et al. 2011, Gonem, Hardy et al. 2014). Wang and co-workers demonstrated significant elevations in ADC values obtained over a period of 1.5 sec in patients with asthma compared to healthy subjects, as opposed to ADC measurements over a shorter-period of 1 ms (Wang, Altes et al. 2008). Gonem et al. have also reported similar findings in 37 subjects with asthma and 17 healthy controls (Gonem, Hardy et al. 2014). This may be attributed to the diffusive molecular transport which can cover only limited peripheral air spaces as much as one or two alveolated airways over a period of milliseconds (Verbanck, Paiva 2007). Over periods of seconds, molecular diffusion may cover an entire gas exchanging unit such as an acinus, which is thought to be the site of airflow obstruction in patients with asthma (Verbanck, Paiva 2007, Verbanck, Paiva 2010).

Furthermore, ADC was found to be dependent on the volume of inhaled gas prior to $^3$He diffusion MRI (Diaz, Casselbrant et al. 2008, Waters, Owers-Bradley et al. 2006). Diaz et al. have observed a minor but significant increase in mean ADC in healthy subjects and patients with emphysema with increased inhaled hyperpolarized $^3$He volume prior to imaging (Diaz, Casselbrant et al. 2008). Their study subjects were imaged on three separate days over a seven-day period and received two different volumes (6% and 15% of TLC) of hyperpolarized $^3$He each day prior to MRI (Diaz, Casselbrant et al. 2008). Similarly, Waters et al. found a simple linear relationship between change in the volume of gas in the lungs and the corresponding change in ADC, relative to their values at FRC (Waters, Owers-Bradley et al. 2006).
1.5.4.2 The effects of inhomogeneous ventilation distribution within the lung on $^3$HeMRI reliability, and calculation of the ADC:

On the MR images, well ventilated areas of the lung receive more helium gas and thus appear brighter than poorly ventilated areas of the lung as it produce higher signal intensity (Ball 2011). Investigators found that in normal healthy subjects with almost complete and homogeneous ventilation distribution, $^3$He gas was uniformly distributed throughout the lung parenchyma on the ventilation MR images and the diffusion-weighted ADC images were also homogeneous (Salerno et al. 2002; Kauczor et al. 2001). Ventilation distribution inhomogeneity can be assessed by measuring the distribution widths from the ADC histograms of each lung that are derived from ADC images (Ball 2011). The mean ADC values and the SD values for the individual subjects were determined by performing a weighted average (weighted by the amount of gas in those regions) of the corresponding values from all sections (Ball 2011). In a study conducted by Salerno et al., the ADC histograms obtained from 16 healthy volunteers demonstrated mean values that were low compared with those for 11 patients with COPD and were narrow, corresponding to low SD values (Salerno et al. 2002). This was postulated to be due to the presence of many small evenly sized air spaces in healthy lung (Salerno et al. 2002). In contrast, the marked variation in the ADC data obtained from COPD patients was suggested to be a result of substantial differences in size, morphology, or both, of the air spaces within the lung (Salerno et al. 2002). These findings were confirmed by Parraga et al. 2007 (Parraga, Ouriadov et al. 2007).

The reliability of calculated ADC was supported further by a study in subjects with CF in which the author found significantly wider right lung ADC histograms as compared to those of healthy controls (Ball 2011). This observation correlates well with the radiologic finding that pulmonary changes in CF were in the right upper lobe in teenagers and young adults (Reinig et al. 1985).

1.5.4.3 Reproducibility of ADC measurements

More recently, a number of clinical studies have examined the reproducibility of ADC in both health and disease (Morbach, Gast et al. 2005, Parraga, Ouriadov et al. 2007, Diaz, Casselbrant et al. 2008). Diaz et al. demonstrated good reproducibility of ADC in both healthy volunteers and patients with emphysema over several days (Diaz,
Another study demonstrated low intra-subject variability in mean ADC between two measurements obtained within 20 minutes without repositioning the subjects (Morbach, Gast et al. 2005). The reported variability was 5.1% and 6.1% for healthy controls and patients with emphysema, respectively (Morbach, Gast et al. 2005). These findings where supported by a subsequent study were authors reported a high reproducibility of ADC for same-day scan-rescan within 10 minutes and 7-day rescan in 6 healthy and COPD subjects (Parraga, Ouriadov et al. 2007).

1.5.5 The development of Yablonskiy acinar model to measure peripheral airways dimensions

Although restricted diffusion of gas in lungs represented by ADC is used as a marker of alveolar size, it is still unclear which structural changes at the peripheral airways are probed by gas ADC measurements (Yablonskiy, Sukstanskii et al. 2002). It is has been argued that during the several-millisecond time duration of the MR diffusion measurement, $^3$He gas can diffuse out of alveoli and across the airways (Yablonskiy, Sukstanskii et al. 2002). This has led to the development of a new mathematical model of $^3$He gas diffusion in lung acinar airways where the acinar airways (ducts and sacs) considered rather than alveoli as the elementary geometrical units (Yablonskiy, Sukstanskii et al. 2002). The mathematical model was based on the Haefeli-Bleuer and Weibel model of human acinar airways, in which all airways distal to a terminal bronchiole in the acinus are considered as cylinders covered with alveolar sleeves ($h$) with internal airway radius ($r$) that falls between acinar generations and a constant outer radius ($R$, including the sleeve of alveoli) (Haefeli-Bleuer, Weibel 1988, Yablonskiy, Sukstanskii et al. 2002)(Figure 1.8).
Figure 1.8 Schematic structure of an acinar airway

Legend: This diagram shows an acinar airway with 8 alveoli distributed along the annular ring. Each airway (duct or sac) can be considered geometrically as a cylindrical object consisting of an alveolar sleeve. The diagram defines inner radius \( r \) and outer radius \( R \) along with the distance between alveolar walls \( L \) and alveolar sleeve depth \( h \). Adopted from (Yablonskiy, Sukstanskii et al. 2009)

Using this geometrical model, the reduction in \(^3\)He diffusion over relatively short diffusion times and the resultant decay in MRI signals can be described in terms of two diffusion coefficients; longitudinal and transverse with respect to the individual acinar airway axis (Yablonskiy, Sukstanskii et al. 2002). The reduction in longitudinal diffusion were proposed to be due two main structures along acinar airways; these are the alveolar sleeves and in alveolar sacs that are open only from one side to connect to alveolar ducts and respiratory bronchioles (Yablonskiy, Sukstanskii et al. 2002). A smaller internal radius \( r \) may also result in a smaller longitudinal diffusivity (Sukstanskii, Yablonskiy 2008). Transverse diffusion, is proposed to be restricted by the external boundaries (airway walls) and by the internal boundaries (alveolar sleeves) (Sukstanskii, Yablonskiy 2008). By applying the mathematical model, Yablonskiy et al. linked the reduction in longitudinal diffusion to ADC and reduction in the transverse diffusion to airway radius \( R \) and tested that on two healthy controls and four patients with severe emphysema (Yablonskiy, Sukstanskii et al. 2002). Their findings demonstrate substantial elevation in \( R \) (0.61mm vs 0.35mm) and ADC (0.46 cm\(^2\)/sec vs
0.20 cm$^2$/sec) in patients with emphysema compared to healthy subjects (Yablonskiy, Sukstanskii et al. 2002). In a subsequent study by the same group, authors validated their mathematical model by demonstrating very good agreement between MRI measurements and direct morphometric measurements on the same lung specimens in which substantial differences in ADC, R and $h$ were found between normal and emphysematous lungs (Yablonskiy, Sukstanskii et al. 2009). They also demonstrated a rapidly growing longitudinal diffusivity (ADC) at initial stages of emphysema that approached the limit of free diffusion coefficient (~0.88 cm$^2$/sec) in severe cases as well as a substantially increased transverse diffusivity due to enlarged radius R and reduced alveolar sleeve depth $h$ with emphysema progression (Yablonskiy, Sukstanskii et al. 2009). A more recent study by Quirk et al. using the Yablonskiy model of gas diffusion to detect early emphysematous changes in 30 smokers has shown a significant reduction in alveolar depth (0.07 mm vs 0.13 mm) and enlarged acinar ducts (0.36 mm vs 0.30 mm) in smokers with mild pulmonary disease (i.e. reduced FEV$_1$/FVC) compared with those with no pulmonary disease (i.e. normal FEV$_1$/FVC) (Quirk, Lutey et al. 2011). They also showed that the mean alveolar geometry measurements in the healthiest subjects with normal FEV$_1$/FVC were in excellent agreement with the previously published normative values obtained by using invasive techniques (R=0.30 mm and $h$=0.14 mm) (Quirk, Lutey et al. 2011). However, data from 29 subjects with moderate to severe asthma have shown no differences in ADC, R and $h$ when compared to healthy controls and patients with asthma or between asthmatic patients with and without evidence of acinar airspace disease as measured by the washout test (Gonem, Ball et al. 2011).

Although earlier studies in healthy and emphysematous lungs have validated the derived geometrical parameters of acinar airways (R and $h$) and showed their sensitivity to detect structural changes at alveolar level, very few attempts have been made to quantify those measures in different disease groups (Yablonskiy, Sukstanskii et al. 2009). This may be due to difficulty in obtaining these measures as compared to other standard parameters (Yablonskiy, Sukstanskii et al. 2009). However, knowing these measures will permit estimation of different physiological characteristics of acinar airways such as alveolar volume which may be altered by different diseases as a result of compression or displacement of airspaces by fluids or abnormal tissue or inflammatory interstitial processes (Bancalari, Clausen 1998, Yablonskiy, Sukstanskii et al. 2009).
1.5.6 Studies of Hyperpolarized $^3$He MRI in subjects with CF:

The majority of studies using $^3$HeMR imaging conducted in subjects with CF used it to evaluate ventilation defects, localize airways obstruction in the early stages of CF and assess the response to bronchodilator and chest physiotherapy (Donnelly, MacFall et al. 1999, van Beek, Hill et al. 2007, Koumellis, van Beek et al. 2005, Bannier 2010, Mentore, Froh et al. 2005, Woodhouse, Wild et al. 2009).

More recently, a pre-liminary study by Kirby et al. used $^3$He diffusion MR to assess the changes in the dimensions of the lung microstructure based on quantitative measure of alveolar size (i.e. ADC) in patients with CF (Kirby, Villemaire et al. 2013). They evaluated ADC on two occasions 7±2 days apart in 11 CF patients and found statistically significant differences between scan and rescan for both WL ADC and ADC maps with no differences in all conventional lung function test measurements (Kirby, Villemaire et al. 2013). The regional postural differences in ADC which was calculated as ADC anterior-posterior difference at scan and rescan were also found to be significantly changed and correlated significantly with the difference in WL ADC and the difference in FEV$_1$ between the scan–rescan (Kirby, Villemaire et al. 2013). This suggests that ADC measurements may be more sensitive for measuring short term changes in CF lung due to the movement of mucus plugs and the subsequent alterations in gas trapping than standard lung function measurements (Kirby, Villemaire et al. 2013).

1.5.7 Studies comparing $^3$He MRI and MBW:

Hyperpolarized $^3$He MRI has been shown to provide high resolution images of lung ventilation and to probe lung microstructure via different measures of peripheral airspace dimensions (ADC, R and $h$) that are more sensitive than spirometry to detect early changes in the lungs CF. Similarly, MBW test provides measures of ventilation inhomogeneity (LCI, $S_{cond}$ and $S_{acin}$) which are also sensitive to early changes in the lungs in CF. Few studies have looked at the association between measures obtained from both techniques (Gonem, Ball et al. 2011, Gonem, Hardy et al. 2013, Marshall, Horsley et al. 2013). Gonem et al. reported significant positive correlations between $S_{acin}$ and ADC measured at short and long time scales of 14 ms, 1.5 sec, and 3 sec in 29 subjects with asthma (Gonem, Hardy et al. 2013). However, in a previous study by the same group in 17 patients with moderate asthma and 12 with severe asthma using both
techniques to localise the major site of airway obstruction, $S_{\text{acin}}$ was the only index that was significantly raised in patients with asthma compared to healthy controls (Gonem, Ball et al. 2011). Moving into subjects with CF, preliminary findings from Marshall et al. suggest that hyperpolarized $^3$He MRI may be more sensitive to early ventilation changes in CF than LCI (Marshall, Horsley et al. 2013). In their study, they observed ventilation abnormalities in all $^3$He MR images obtained from 4 children with CF, whereas LCI was abnormal in 2 of the 4 (Marshall, Horsley et al. 2013).
Chapter 2 Thesis aims and hypotheses

2.1 Where are we?

Evidence from pathological, physiological and imaging studies have showed that lung disease starts early in life in subjects with CF and more markedly in the peripheral airways (Sobonya, Taussig 1986, Hamutcu 2002, Gustafsson, De Jong et al. 2008, Horsley, Macleod et al. 2008, Castile, Hayes et al. 2000, de Jong 2004, Bannier 2010). Therefore, sensitive, repeatable and safe markers of early involvement of peripheral airways are needed that are feasible over a wide age range. Standard lung function tests, chest radiograph and HRCT were either unable to detect early changes in the peripheral airways, or safety considerations preclude their use in longitudinal monitoring in children (Gustafsson 2003, Owens, Aurora et al. 2011, Gustafsson, De Jong et al. 2008, Brody, Klein et al. 2004). As an alternative, recently developed techniques of N$_2$MBW and hyperpolarised $^3$He diffusion MR have been shown to be more sensitive than spirometry and chest radiography and comparable to HRCT in detecting early functional and structural changes within lung periphery (Owens, Aurora et al. 2011, Aurora, Gustafsson et al. 2004, Gustafsson, De Jong et al. 2008, van Beek, Hill et al. 2007, Kirby, Villemaire et al. 2013, McMahon, Dodd et al. 2006). They have also been shown to be feasible in children and safe for repeated measurements needed to assess the progression of disease (Kraemer, Blum et al. 2005, Woodhouse, Wild et al. 2009, Aurora 2011, Narayanan, Owers-Bradley et al. 2012).

2.2 Where is the gap in knowledge?

2.2.1 N$_2$MBW:

Few studies have looked at longitudinal changes in VI indices derived from N$_2$MBW in comparison the standard lung function tests in school-children with CF (Kraemer, Blum et al. 2005, Kieninger, Singer et al. 2011, Aurora 2011). These studies have not looked at the changes in S$_n$III indices over time due to lack of repeatability data of these measures in subjects with CF and the time consuming protocol of 3 technically successful tests. In addition, much of the published work on the development of SIII
analysis has been done on adult subjects (Crawford 1985, Verbanck, Schuermans et al. 1997).

Furthermore, the potential of N₂MBW indices to identify sites of abnormality in the lung periphery based on their relationships to each other has only been explored in two cross-sectional studies (Gustafsson 2007, Horsley 2008). The assessment of short-term responses to treatment in children with mild CF lung disease has also not been fully explored (Gustafsson 2007, Horsley, Macleod et al. 2008, Robinson 2009, Horsley, Davies et al. 2013).

2.2.2 ³He diffusion MR:

Until recently, only one preliminary study looked at the dimensions of lung microstructure in 11 adult subjects with CF (Kirby, Villemaire et al. 2013). No studies look at peripheral airways dimensions (R and h) in subjects with CF. Few preliminary studies have looked at the association between ADC R and h, VI indices and standard lung function measurements, all of which involved subjects with asthma (Gonem, Ball et al. 2011, Gonem, Hardy et al. 2013).
2.3 Thesis aims:

1. To prospectively follow a cohort of school aged children with CF in order to monitor changes in VI indices in comparison to conventional lung function measures.
2. To assess the ability of LCI to detect the short-term response to intravenous antibiotics as compared to the gold standard FEV$_1$ in children with mild to moderate CF lung disease.
3. To estimate the dimensions of the lung peripheral microstructure in subjects with CF lung disease and to compare this to age-matched healthy controls using $^3$He diffusion MR. In addition, to investigate the relationships between $^3$He diffusion MR parameters with (i) LCI derived from N$_2$MBW and (ii) FEV$_1$ as well as with conventional markers of small airways obstruction.

2.4 Thesis Hypotheses:

1. In school-age children with CF, N$_2$MBW indices will be more sensitive to changes in the lung periphery over time than conventional lung function measurements. N$_2$MBW indices will show earlier deterioration with age than conventional lung function.
2. In children with mild to moderate CF lung disease admitted for short term IV antibiotic treatment, LCI will improve prior to FEV$_1$.
3. In subjects with CF, there will be an increase in ADC and R, and a decrease in $h$ compared to healthy controls. If indices from $^3$He diffusion MR and LCI both reflect changes in the peripheral airways, there will be an association between these indices. There will also be a relationship between $^3$He MR parameters and conventional markers of small airway obstruction, but not with FEV$_1$ z-score.
Prior to undertaking these investigations, it is necessary to undertake a series of methodological studies for N₂MBW to explore the sources of variations:

**Preliminary hypotheses:**
1. In healthy children, LCI, $S_{acin}$ and $S_{cond}$ are independent of age, height, weight and sex.
2. In all children, results obtained from the two different N₂MBW systems will be comparable.

### 2.5 Structure of thesis

This thesis is presented in seven chapters. Introduction and literature review were presented in Chapter 1, and aims and hypotheses are presented here in Chapter 2. The remaining 5 chapters will cover:

i. Materials and Methods, including data processing

ii. Standardisation the methodology of N₂MBW and investigation in to sources of variations

iii. Longitudinal changes in LCI: A comparison to standard lung function tests in school-age children with CF

iv. Comparison of $^3$He diffusion MR with N₂MBW

v. Conclusion chapter with a summary of most important findings, clinical implication of results and future research

Other than the description of equipment and data processing, each results chapter is self-contained, with a detailed description of aims and hypotheses, a description of the study population and statistical methods, results, and an interpretation of these results.
Chapter 3 Methods

3.1 Summary of study design

This chapter describes the equipment and data processing methods employed throughout my research where I aimed to collect:

- Longitudinal lung function data including spirometry, plethysmography and multiple-breath nitrogen washout (N\textsubscript{2}MBW) from school-age children with CF,
- Cross-sectional lung function data from age-matched healthy children,
- Cross-sectional helium-3 diffusion magnetic resonance data from children and young adults with CF and age-matched healthy controls.

3.2 Subjects

3.2.1 Subjects with CF

In this prospective longitudinal cohort study, school-age children (aged 6 to 17 years) with a diagnosis of cystic fibrosis confirmed by sweat testing and CF genotype, who attended outpatient CF paediatric clinic at Leicester Royal Infirmary (LRI) and enrolled in the annual review that began January 2010 were included and tested in the lung function laboratory. These children were studied annually during their annual review visits over a 4-year period at times of clinical stability (Figure 3.1). They were also reviewed by paediatric respiratory consultant every 2 months.

A subset of those school-age children with CF were also recruited during the study period to attend \textsuperscript{3}He MRI scanning at Nottingham University. I also recruited young adults (aged 18 to 37 years) with a confirmed diagnosis of cystic fibrosis from the adult CF clinic at Leicester Glenfield hospital between January and April 2013. These patients attended for lung function testing at LRI and \textsuperscript{3}He MRI scanning at Nottingham University.
Legend: Of the 37 children, 34 (92%) attended on two consecutive study visits. Of those, 30 children completed all lung function tests including $N_2$MBW (26 on profiler; 4 on Exhalyzer). The washout test was not available for 2 subjects on the day of testing and 2 performed poor quality washouts. On the third study visit, 28 (76%) children attended. Of those, 25 children were able to complete all lung function including $N_2$MBW (13 on profiler; 12 on Exhalyzer). The washout test was not available for 2 subjects, and one child refused to do the test. On the final study visit, 15 (41%) children were attended and 14 completed all lung function tests including $N_2$MBW (6 on profiler; 8 on Exhalyzer). One child refused to perform the test.
3.2.2 Healthy Controls
Healthy school-age children included in this thesis were recruited as part of a separate study from either the Leicester Respiratory Cohorts by sending postal invitation letters, or displaying posters in out-patient respiratory clinics (siblings of patients), or personally approached by study personnel from general and orthopaedic surgical wards (Appendix B). Exclusion criteria included any previous hospital admission for a respiratory condition; the presence of current or previous (in the last 12 months) wheeze, cough or shortness of breath; prematurity i.e. less than 36 weeks gestation at birth; the presence of surgical lesions in chest e.g. congenital diaphragmatic hernia; or physician diagnosis of chronic airways disease such as asthma at any time in the past.

We have also used previously collected data from a sample of 66 healthy children and 5 young adults tested in our laboratory as part of different study (Narayanan, Owers-Bradley et al. 2012). They were recruited from the Leicester Respiratory Cohorts and the Community Health Services Database i.e. a random population-based cohort (Appendix C). However, we have re-analysed their data to eliminate any observer bias in data analysis which might affect its comparability to our own data. $^3$He MR data for 24 of these subjects (19 children and 5 adults) provided an age-matched comparison group to our CF population. Figure 3.2 shows healthy school-age children included in this thesis.
Figure 3.2 Healthy School-age children included in this thesis

Legend: Of the 64 children recruited, 52 completed N₂MBW (9 on profiler; 28 on Exhalyzer and 15 on both machines). Of those, 13 completed all lung function tests. The washout test was not available for 6 subjects on the day of testing and six children produced unacceptable results. The lung function data from 66 children obtained from previous work were collected in our laboratory using the same equipment. Of those, 19 children were age-matched with children with CF who underwent $^3$He MR scanning.
3.2.3 *Ethical Approval*

Research Ethics Committee approval was obtained for all studies included in this thesis and written informed consent was obtained for all enrolled subjects. R&D approval, subjects and parents information sheets, consent form were provided for:

- The longitudinal study which involved **children with CF** in Appendix A
- **Healthy children** recruited to the asthma study in Appendix B
- Measuring lung development using $^3$He MR study where **healthy children and young adults were** recruited in Appendix C
- Measuring lung development using $^3$He MR study where **children and young adults with CF were** recruited in Appendix D, and
- Measuring lung development using $^3$He MR study where **healthy adults aged > 22 yrs.** were recruited in Appendix E.

Written informed consent was obtained from all children with CF and their parents during a visit to the routine CF clinic. Young adults with CF gave written informed consent in the respiratory laboratory, prior to testing. All healthy children and their parents gave written consent at the time of their laboratory visits. Their eligibility was confirmed with a brief respiratory questionnaire (Appendices B and C).

3.3 *Test Procedure*

During visits of subjects to the laboratory, all procedures were explained and demonstrated to them and their parents when appropriate. For each subject, baseline characteristics were taken at each visit. Standing height without shoes was measured in cm to 1 decimal place by a height measure scale (Invicta Plastics LTD, Leicester, UK). Weight was measured in kg to 1 decimal place with indoor clothing without shoes by calibrated electronic scale (Tanita HD-305, Tanita Corporation, Tokyo, Japan). Subjects then performed spirometry, body plethysmography, and $N_2$MBW, followed by induced sputum where this formed part of the specific study protocol. Details of equipment and data collection for spirometry, plethysmography, $N_2$MBW, induced sputum and $^3$He MR are presented below.
3.4 Spirometry and Body Plethysmography:

3.4.1 Equipment and calibration

Spirometry and plethysmography were performed with a Jaeger MasterScreen Body Plethysmography (Care Fusion GmbH, Leibnizstrasse, Germany). It is a ‘constant-volume, pressure-change’ whole-body plethysmograph with Jaeger pneumotachograph to measure the flow with a range between 0 to 20 L/sec and accuracy of 0.2 to 12L/sec. Volume is identified by digital integration with a range of ± 20L and accuracy of ± 3%. The box pressure transducer is piezo resistive with a range of ± 10.2 cmH₂O and accuracy of ±2% while the mouth pressure transducer has a range of ±204.5 cmH₂O and similar accuracy.

Calibration was performed once daily in two steps, according to manufacturers’ instructions after assessing and recording temperature, barometric pressure, and humidity. Step one is the pneumotachograph calibration and it is performed using a 2L calibration syringe that is attached to the mouthpiece and pumped at a high, medium and low flow to assess changes in flow and volume through the pneumotachograph. Step two is the box pressure calibration and it is performed automatically to assess the background leak and calibration factor, with the door closed and cabin empty. Ideally, the background leak should have a half-life of 4 to 7 seconds to avoid any source of error in measurements.

3.4.2 Data collection and reporting

All measurements were performed with the subject wearing a nose-clip, sitting upright and reaching the mouthpiece without having to flex or extend the neck (Figure 3.3). Disposable microbial filters (MicroGard, Care Fusion GmbH, Leibnizstrasse, Germany) were used for all subjects.

Spirometry was performed by asking the subject to start breathing quietly then take maximal inspiration and exhale forcefully. They were encouraged by the operator to continue the exhalation until the end of the test. For younger children, prolonged expiration was encouraged using a Jaeger incentive program. To check the adequacy of child’s effort during expiration, the flow-volume curve was monitored on the screen. The test was repeated up to eight times or until at least three acceptable loops and two repeatable results within 5% were obtained, with no cough, variable efforts, glottis closure, delay in rise to peak flow or abrupt end of flow in accordance with ATS/ERS
recommendations (Miller, Crapo et al. 2005). Unacceptable manoeuvres were excluded, but saved for possible future re-analysis and inspection. The highest value for FEV$_1$ and FVC were reported and the recording with the highest sum of FVC and FEV$_1$ was used to obtain FEF$_{25-75}$ and FEF$_{75}$. Predicted values and z-scores for spirometry parameters were based on all-age spirometry reference values (Stanojevic, Wade et al. 2008). A z-score is the deviation of an individual’s value from the mean value of a reference population, divided by the standard deviation of the reference population (Quanjer, Stanojevic et al. 2012, Quanjer, Weiner 2014). FEV$_1$ in this thesis is expressed as z-score and not % predicted because z-score is independent of age, height, sex, and ethnic group, unlike the use of % predicted, and is recommended by ATS/ERS (Pellegrino, Viegi et al. 2005, Beydon 2007).

Plethysmography was performed with the plethysmograph door closed. After allowing 2 minutes for thermal equilibrium, the child was instructed to breathe quietly with hands on cheeks until a stable end-expiratory level was achieved. Airflow occlusion was then performed by closing the shutter for 2-3 seconds while the child was breathing in order to measure thoracic gas volume (TGV or FRC$_{pleth}$). This was followed by a slow vital capacity. The test was repeated until we obtained at least three technically acceptable FRC$_{pleth}$ measurements (i.e. loops are closed, crossed the zero pressure line without thermal drift or excessive efforts) and three FRC$_{pleth}$ values repeatable within 5% were obtained, according to ATS/ERS recommendation (Wanger, Clausen et al. 2005). The mean value for FRC$_{pleth}$ was reported and used with the mean inspiratory capacity (IC) to work out the total lung capacity (TLC). Then the highest vital capacity (VC) was subtracted from TLC to obtain residual volume (RV). Z-scores for plethysmography were based on Rosenthal reference values (Rosenthal, Bain et al. 1993). At the end of the test sessions, flow-volume curves and FRC$_{pleth}$ traces were visually inspected by the author and by at least one other investigator (Dr C Beardsmore or P Patel).
Figure 3.3 School-age child performing plethysmography (with permission)

Legend: Note that the pneumotachometer is connected to the mouthpiece via a bacterial filter to prevent cross-infection. The child is sitting inside the box with the door closed and placing hands on cheeks and breathing regularly until a stable end-expiratory level is achieved. Then the shutter is closed for 2-3 seconds while the child is making breathing efforts in order to measure thoracic gas volume (TGV or FRC<sub>pleth</sub>). This was followed by a slow vital capacity.
3.5 Multiple-breath nitrogen washout test (N\textsubscript{2}MBW)

MBNW was performed using two different devices; modified medgraphics profiler (Medical Product Service GmbH, Germany) and Exhalyzer D (Eco Medics, Switzerland).

3.5.1 \textit{N\textsubscript{2}MBW using a modified Medgraphics Profiler}

3.5.1.1 Profiler N\textsubscript{2}MBW: Equipment and calibration

The Medgraphics profiler is a complete pulmonary function testing system that can be used to measure functional residual capacity (FRC) by N\textsubscript{2} washout (Figure 3.4). It utilizes Breeze Suite™ software for testing and the patented bi-directional differential pressure Pitot tube pneumotach (MedGraphics preVent Pneumotach, Medical Product Service GmbH, Germany) for measuring the flow and deriving tidal volumes. The pneumotach is linearized to provide accurate (±3%) flow determinations of ±18 L/sec (Figure 2.4). It also uses a nitrogen analyser (N\textsubscript{2}), a vacuum pump, and 100% O\textsubscript{2}. The N\textsubscript{2} analyser’s measuring technique is photo-spectrometry and it is linearized to give accurate measurements from 0% to 100% N\textsubscript{2}, with response time < 20msec, flow rate <5ml/min and change in N\textsubscript{2} reading < 0.1% at 100% saturation, 37 C. The vacuum pump draws a sample into the analyser. It is then ionized with a high voltage source, filtered, and the detected with a photodiode. This sample is then aligned with the corresponding (patient’s) breath throughout the entire test. The data acquisition rate using this system is 100Hz.

The Medgraphics profiler was modified by; (1) incorporating an auditory signal (a bell sound) to guide the tidal volume and help subjects to maintain the required tidal volume while performing the washout test; (2) replacing the demand valve by the Y-shape non-rebreathing valve (model 1420A; Hans Rudolph, inc., USA) because it has lower resistance, is quiet and easy to breathe through; and (3) introducing an O\textsubscript{2} reservoir at ambient pressure.

The Profiler was calibrated once daily, after 30 minutes warm-up time in accordance with manufacturers’ instructions. It was a two-point calibration; flow calibration using 3L syringe and N\textsubscript{2} sensor calibration using a certified concentration calibration gas mixture containing 0.3%CO, 0.5%Neon, 21%O\textsubscript{2} and balance N\textsubscript{2}. 

55
3.5.1.2 Profiler N$_2$MBW: Patient circuit

All school-age children and adults used a mouthpiece and nose clip apparatus with bacterial filter (MicroGard, Care Fusion GmbH, Leibnizstrasse, Germany) to prevent cross-infection. The mouthpiece was connected to the pneumotachometer from one end with N$_2$ analyser clip connected to the capillary port (Figure 3.5). From the other end, the pneumotachometer was connected via custom-made connectors (labelled connectors 1) to two-way non-rebreathing Y-shape valve (model 1420A; Hans Rudolph, inc., USA) that is attached via custom-made connectors (labelled connectors 2) to the one-way valve and oxygen balloon (Rubber Latex Balloons (KCI-10), Kaymont, New York). For this setup, dead space was measured by water displacement and separated in two parts; pre-capillary dead space (DS1) and post-capillary dead space (DS2). The bacterial filter, which was used for all subjects, had 50 ml dead space and this was added to DS1. Accordingly DS1 (between the child’s lips and the capillary line) was 113 ml. The DS2 (between the capillary line and the one-way valve) was 29 ml. Although using a bacterial filter significantly increased DS1, we decided to use it as the test equipment must adhere to infection control guidelines (Robinson, Latzin et al. 2013).
3.5.1.3 **Profiler N₂MBW: Data collection**

Washout tests were performed for all subjects in the sitting position and with the use of a nose clip (Figure 3.6). At the start of the test, subjects were encouraged to breathe normally (room air) through the mouthpiece with an inspiratory effort that reaches a pre-set tidal volume (Vt). For school-age children, Vt was set at approximately 1/3 of FRC determined by plethysmography, while for adults a one litre Vt breathing protocol was used (Verbanck, Thompson et al. 2012). The modified Medigraphics Profiler provided an auditory signal to guide the tidal volume. During expiration, the operator switched the breathing valve and 100% oxygen from the inspiratory balloon was delivered to the subject while s/he was breathing quietly. The subject was encouraged to continue breathing to washout the N₂ from the lungs gradually until the end-tidal N₂ concentration was fallen to 1/40th of the initial concentration. At the end of the test, the subject was asked to take a slow vital capacity (VC) breath. For all subjects, the washout time lasted between 1 to 5 minutes. This was dependent on the subject’s breathing pattern and/or lung pathology (Verbanck, Schuermans et al. 1997, Horsley 2009). The washout was repeated up to four times or until at least three technically satisfactory washouts were obtained. There was a time interval between any two
subsequent MBNW tests for at least the washout time plus two minutes to allow N₂ concentration to return to baseline values (Robinson, Latzin et al. 2013).

**Figure 3.6** Child performing N₂MBW using modified Medgraphics Profiler (with permission)

*Legend: Note that the child is breathing through bacterial filter (to prevent cross-infection) that is connected to the pneumotachometer where the N₂ analyser is attached.*

### 3.5.1.4 Profiler N₂MBW: Data analysis

Analysis was performed using custom-built software (MATLAB (R2011b)) developed by Ruslan Garipov (Figure 3.7). This software allows washout curve analysis where FRC₉₂ and the lung clearance index (LCI) are calculated automatically after manually adjusting the dead space values and the delay between volume and flow signals (Figure 3.8, 3.9).

It also allows SIII analysis where the indices of VI at conductive and acinar airways (S₉₉ and S₉₉ acin respectively) are determined automatically by displaying breath-by-breath auto-fit S₉₃III and allowing for any manual adjustment of the slopes if required (Figure 3.10).
Legend: On this main window we can choose from the list on the top left either (MBW5.m) to analyse raw data text files or (MBW_analyzer.m) to combine and create global report for pre-analysed files for certain subject.
Legend: The large graph at the top of the figure displays the concentration of nitrogen (in blue) and respiratory tidal volume (in red) as a function of time, as shown on the profiler display. The red arrow indicates the start of inspiriting pure oxygen. The graph in the bottom left shows $N_2$ concentration plotted against volume for all breaths, aligned at the right-hand side (end expiration). On this window we can check and amend (if needed) dead space values (DS1 and DS2), the start and the end $N_2$ concentration (black dotted lines) and the alignment of volume and nitrogen traces by magnifying first breath of the washout (as shown in the next figure). We can also choose between two different options for fitting straight lines through the phase 3 slopes in preparation for the next window. If the expired volume breath-to-breath variability is <70% it is best to select option1 (volume-fraction) and if it is >70%, select (volume value) fit option2.
Figure 3.9 Representation of correction of volume delay

Legend: This figure shows the nitrogen trace (blue graph) and volume trace (red and blue graph) of the first breath of the washout. To align volume and nitrogen traces, the first breath of the washout curve is magnified (as shown on this figure) and cursors are used to shift the blue dotted line to halfway down the fall of the nitrogen trace. Then the red dotted line is shifted to the left of the blue dotted line with delta V being as close to DS2 value as possible (in the example above DS2 is 0.029L (29ml) so the red dotted line was shifted until delta V value became 29ml). This step is done because during inspiration, a volume equivalent to DS2 has to pass through the umbilical before a fall of nitrogen concentration is detected.
Through the washout curve analysis window we can determine $\text{FRC}_{N_2}$ and the Lung Clearance Index (LCI). The determination of both parameters was in accordance with the recent guidelines for $N_2$MBW in school-age children (Robinson, Latzin et al. 2013), in which $\text{FRC}_{N_2}$ is calculated at airway opening after subtracting DS1 (i.e. between subject mouth and sample point). This is performed by dividing the cumulative expired volume (CEV), which is the sum of all expiratory Vt (after subtracting the cumulative re-inspired volume i.e. volume of DS2 between sample line and the one-way valve) over the washout, by the difference between the initial and final end-tidal $N_2$ concentrations (e-t $N_2$start and e-t $N_2$end) as follows:

$$\text{FRC}_{N_2} = \sum (N_2^{\text{exp} \cdot X \Delta V^{\text{exp}}}) - \sum (N_2^{\text{re-insp} \cdot X \Delta V^{\text{re-insp}}}) - \text{DS1}$$

e-t $N_2$start and e-t $N_2$end

LCI is calculated as the ratio of CEV to $\text{FRC}_{N_2}$. It is defined as the number of FRC turnovers (TO) required to reduce end tidal gas concentration to 1/40th of starting value.
Figure 3.10 Phase III slope (SIII) analysis window

Legend: This window consists of 5 graphs. The graph in the top left shows individual expirations in turn with SIII automatically-fitted between 50% and 90% of the exhaled volume (green line). This SIII fit can be manually adjusted if distorted. The large graph in the bottom left show SIII (the green lines) for all breaths. The graph in the top right shows normalised phase 3 slope ($S_{\text{III}}$) vs. lung turnover (which is a unit of volume equivalent to FRC). The black dotted lines represent the turnover range (1.5 to 6 turnovers) for the $S_{\text{cond}}$ regression line and each red dot represent $S_{n,\text{III}}$ for a breath. The other two graphs ($\log_{10} N_2$ vs. Turnover) and (Volume vs. breath number) add extra information about exhaled and inhaled Vt, $FRC_{N_2}$ and dead space for each breath throughout the washout.
From the SIII analysis window we can calculate the indices of VI at conductive and acinar airways (S_{cond} and S_{acin} respectively). Both can be identified by studying the progression of the concentration-normalized phase III slope (S_{nIII}) over subsequent breaths of the washout (Verbanck, Schuermans et al. 1997). For each breath, SIII can be obtained by fitting a linear regression over 50% and 90% of the expired Vt (Figure 3.11). It is then normalised for the mean gas concentration over SIII to allow the comparison between SIII slopes of subsequent breaths. S_{nIII} slopes are then plotted against lung TO and the linear regression between 1.5 and 6 TO represents S_{cond}. S_{acin} is the first breath S_{nIII} value minus S_{cond} multiplied by the TO of the first breath (Verbanck, Schuermans et al. 1997). The calculated S_{cond} and S_{acin} were then corrected for the expired Vt using the volume correction method proposed by Aurora et al. (Aurora 2005) where:

\[ S_{\text{cond,corr}} = S_{\text{cond}} \times \text{the mean expired Vt from the entire washout} \]

\[ S_{\text{acin,corr}} = S_{\text{acin}} \times \text{the expired Vt of the first breath} \]
Figure 3.11 The determination of concentration-normalized phase III slope ($S_{III}$) indices

Legend: $S_{III}$ for each breath is determined by fitting linear regression over the expired $V_t$. There are two different options for fitting the regression line through $S_{III}$. 1. If breath-to-breath variability in the expired volumes during the washout varied by less than 70% which occurs in most cases, the regression line is fitted between 50% and 90% of expiration trace as shown in breath #3 in the example above. 2. If the variation in the expired tidal volume is greater than 70% which means high breath-to-breath variability in expired volumes, the regression line is fitted by choosing an appropriate expired volume which will give a phase 3 plateau. Normalized $S_{III}$ slopes for all breaths are then plotted against lung TO and the software automatically fits a straight line through the breaths points between 1.5 and 6 TO to calculate $S_{cond}$ and $S_{acin}$. 
Throughout data analysis, washout recordings and individual breaths were visually inspected by the author and one other investigator (Dr C Beardsmore). Improper fitting of regression line over SIII of any breath were adjusted manually. Breaths with too small or too big volume or with signal noise were excluded (Figure 3.12). Unacceptable recordings were also excluded, but saved for future re-analysis and inspection (Figure 3.13).

Acceptability criteria of washout recordings are

- The initial N₂ concentration before washout takes place should be between 70% and 80%.
- Good starting of the first inspiration of pure O₂ (i.e. rapid fall of N₂ concentration to zero without delay or hesitating).
- Regular and stable breathing pattern throughout the washout.
- Continue the washout until the alveolar N₂ concentration fall below 2% without evidence of leak, or volume drift.
Figure 3.12 Profiler examples of acceptable and unacceptable N\textsubscript{2}MBW breaths

Legend: (A) acceptable breath size (total expired volume 800 ml) with good fitting of regression line over SIII, (B) Breath with too small volume (total expired volume 470 ml) where SIII cannot be identified, and (C) Breath with signal noise and poor resolution at low nitrogen concentration.
Figure 3.13 Profiler examples of acceptable and unacceptable N\textsubscript{2}MBW recordings
\[ \Delta t = 0.13 \text{ s}; \Delta V = 25 \text{ mL} \]

\[ \Delta t = 0.07 \text{ s}; \Delta V = 25 \text{ mL} \]
Legend: (A) Acceptable $N_2$ washout recording (stable breathing pattern, good start of the washout test without delay, any leak or cough or volume drift. (B), (C), (D) and (E) unacceptable washout recordings. (B) Leak detected on breath #9, (C) Apparent volume drift throughout the washout, (D) Fast breathing pattern with less stable baseline and (E) Breathing with large tidal volume (equivalent to FRC) which led to reduction in $N_2$ concentration below 2% very quickly and therefore affected the calculation of $FRC_{N2}$ and LCI.

3.5.1.5 Profiler $N_2$MBW: Data reporting

The mean values for $FRC_{N2}$ and LCI from a minimum of two washouts, in which $FRC_{N2}$ differs less than 10% (in relation to the lower) were reported according to ATS/ERS recommendation (Beydon 2007). If $FRC_{N2}$ or LCI variability exceeded 10% without an apparent artefact we still used the data. However, tests where $FRC_{N2}$ differs by >25% from the median $FRC_{N2}$ value across the three tests were automatically rejected in accordance with latest ERS/ATS consensus statement (Robinson, Latzin et al. 2013). For $S_{acin}$ and $S_{cond}$ data were reported from at least 2 washout tests and were multiplied by the expired volume in litres to correct for variability in expired tidal volume (Aurora 2005).
3.5.2 \( N_2 \)MBW using Exhalyzer D

3.5.2.1 Exhalyzer D \( N_2 \)MBW: Equipment and calibration

Exhalyzer D and Spiroware 3.1.6 are an open-circuit \( N_2 \)MBW hardware and software package (Eco Medics, Switzerland) (Figure 3.14). This new nitrogen-washout device measures the \( N_2 \) fraction (\( F_N^2 \)) indirectly from main-stream carbon dioxide (\( CO_2 \)) and side-stream oxygen (\( O_2 \)) signals based on Dalton’s law of partial pressures:

\[
F_{CN^2} = 1 - F_{CO_2} - F_{CO_2} - F_{Argon}
\]

Where: \( F_{cx} \): Fractional Concentration of Gas \( X \), \( F_{Argon} = 0.93\%

The \( F_{Argon} 0.93\% \) is treated as a fixed proportion of the \( F_N^2 \) assuming similar washout during \( N_2 \)MBW. The principle of this test has been validated in-vivo and in-vitro and shown to provide higher accuracy than \( N_2 \) analyser and reliably measure lung volumes and LCI (Singer, Houltz et al. 2012).

![Figure 3.14 The complete setup of Exhalyzer D](image)

- Computer with Spiroware 3.1 software
- Air and \( O_2 \) source
- \( O_2 \) analyser
- \( CO_2 \) analyser
- Ultrasonic flowmeter
3.5.2.2 Exhalyzer D N₂MBW: Apparatus

The apparatus consists of a main stream ultrasonic flowmeter to measure the flow and derive tidal volumes. Gas concentration was measured by a side-stream laser O₂ sensor (Oxigraf, Inc, Mountain View, CA, USA) and a main-stream infra-red CO₂ sensor (CapnostaH 5, Respironics Novametrix LLC, Wallingford, CT, USA) (Figure 3.15). The CO₂ sensor has a faster response time (<60 ms) than the O₂ sensor (140 ms), but the device align their signals automatically to reach an accuracy of 1ml/sec by applying a speeding algorithm to the O₂ signal to reduce its response to 110 ms (Singer, Houltz et al. 2012). The system also provides continuous bypass flow to reduce any additional work of breathing and data acquisition rate of 200Hz.

Between gas sensors and the flowmeter we used post-capillary dead space reducers (DSR) (set 3 with subjects weight>35kg) or (set 2 with subject weight 15-35kg) and hygienic inserts (Spirette) provided by the manufacturer (Eco Medics, Switzerland). Accordingly, apparatus post-capillary dead space (volume between CO₂/O₂ sampling points and bypass) was 26.9 ml and 16 ml respectively as measured by water displacement. Pre-capillary dead space (volume between subject’s mouth and CO₂/O₂ sampling point) along with bacterial filter which had 30 ml dead space (air eco slimline, Vickers Ind Est, LA, UK) was 37.9ml.

The Exhalyzer D was calibrated as following; daily flow calibration and verification with three litre calibration syringe (if Set3 DSR is used) or with 100ml syringe (if Set2 DSR is used), weekly O₂ channel calibration and zero calibration of the CO₂ sensor. The sensors require only 2 minutes warm up time to reached full performance.
**3.5.2.3 Exhalyzer D N₂MBW: Data collection**

N₂MBW was performed in the sitting position using a nose clip and a mouthpiece. The Exhalyzer D starts automatically to deliver continuous air flow as the subject breathes through the circuit. When a stable breathing pattern is maintained, 100% O₂ is delivered to the subject through continuous bypass flow system and the washout starts. Subjects were encouraged to breathe normally throughout the washout with Vt equivalent to 1/3 of FRC determined by plethysmography (if child) or 1 litre (if adult). They were guided by graphical animation provided by the software (Figure 3.16). The washout trace was monitored on the screen to check its acceptability. If a leak was detected, the test was terminated immediately by the operator. The washout ends after three consecutive breaths with N₂ concentration less than 1/40th of the initial concentration to avoid early test termination due to small breaths. The washout time on Exhalyzer D was noticed to be longer than the washout time on Profiler (1 to 7 min vs. 1 to 4 min) especially for subjects with advanced lung disease. For all, the washout was repeated up to four times or until at least three technically satisfactory washouts were recorded. There was a time interval between any two subsequent N₂MBW tests for at least the washout time plus two minutes.
Legend: The software provides only one animation which is the ‘smiley face’ incentive to guide the subject to breathe within pre-set Vt range. The face becomes smiley and green when subjects breathe within the pre-set Vt limits, and becomes red and sad when subjects breathe below or above Vt limits.

3.5.2.4 Exhalyzer D N₂MBW: Data analysis and reporting

Data were analysed using Spiroware 3.1.6 software (Figure 3.17 and 3.18). LCI was estimated automatically by logarithmic extrapolation. SIII identified by auto-fitting regression line over 65% to 90% of exhaled Vt. $S_{scin}$ calculated from S₃III of the first breath after subtracting the contribution of convective airflow and $S_{cond}$ calculated between 1.5 and 6 lung turnovers. Washout recordings and individual breaths were visually inspected by the author and one other investigator (Dr C Beardsmore). Breaths with too small or too big volume or improper fitting of regression line over SIII were excluded (Figure 3.19). Unacceptable recordings with evidence of leak, irregular breathing pattern, cough or bad starting of the washout were excluded, but saved for future re-analysis and inspection (Figure 3.20).
Figure 3.17 Spiroware 3.1.6 (N₂MBW test screen)

Legend: The top trace of the screen (black) displays the flow as measured by Ultrasonic flow sensor, the (red) trace below displays N₂ concentration and the actual washout, the (blue) trace displays O₂ concentration as measured by the side stream O₂ sensor and the (green) trace displays CO₂ concentration as measured by Capnography. The three graphs on left of the screen represent flow, CO₂ concentration and N₂ concentration plotted against volume. These traces help the operator to check the acceptability of each breath during the washout in terms of their size and shape.
Legend: The first and second graphs on the left represent normalised $N_2$ concentration and $SnIII$ versus lung turnover. The third graph represents normalised $N_2$ concentration versus the volume of each breath. From this graph operator can check closely the acceptance of regression line fitting over $SIII$ of each breath. The table on the top shows mean values, standard deviation, predicted, %predicted, z-score and coefficient of variations of $N_2$MBW indices from all acceptable runs. The Table in the middle shows $N_2$MBW indices values for individual run. From this table the operator can exclude unacceptable runs. The last table shows the washout breaths (breath-by-breath) where the operator can exclude any improper breaths.
Figure 3.19 Exhalyzer D and Spiroware 3.1.6 examples of acceptable and unacceptable washout breaths

Legend: (A) Good breath size (total expired volume 450 ml) with good fitting of regression line over SIII (B) Breath with too big volume (total expired volume 1250 ml) which flattened the auto-fitting of regression line over SIII (C) Breath with too small volume (total expired volume 100 ml) where SIII cannot be identified (D) Good breath size, but regression line auto-fitted over phase 4 instead of phase 3.
Figure 3.20 Exhalyzer D and Spiroware 3.1.6 examples of unacceptable N₂MBW recording
Legend: Three unacceptable N₂MBW recordings; (A) Evidence of leak on breath #10. (B) Inappropriate start of the washout, and (C) Irregular breathing pattern.
3.6 Induced Sputum

Induced sputum was obtained from children with CF every year during their annual review in order to identify the presence of infective organisms that is relevant for the longitudinal study reported in Chapter 5. It was performed by an experienced member of staff (PP) using ultrasonic nebulizer (Omron UltraAir U17, Omron, The Netherlands) with increasing concentrations (3, 4 and 5%) of hypertonic saline in a controlled environment. The procedure was performed as described by Pin et al. and in accordance with ERS Task Force recommendations (Pin, Gibson et al. 1992, Djukanović, Sterk et al. 2002).

Sputum samples were also collected from children who could readily expectorate every 2 months at follow-up visit to CF clinic. For children who could not expectorate, cough swabs were obtained. For children who expectorated rarely we encourage their parents to collect sputum samples at home. All samples were processed and cultured for various bacterial species including *P. aeruginosa* by experienced staff at Leicester Royal Infirmary according to the UK Cystic Fibrosis Trust Standards (The UK Cystic Fibrosis Trust Microbiology Laboratory Standards Working Group 2010). Patients were categorized at each annual review visit according to their *P. aeruginosa* status over the previous 12 months on the following basis (Lee, Brownlee et al. 2003); (1) Chronic infection, when >50% of months, when samples had been taken, were *P. aeruginosa* culture positive, (2) Intermittent infection, when 50% or less of months, when samples had been collected, were *P. aeruginosa* culture positive, (3) Free of infection, no growth of *P. aeruginosa* during the previous 12 months, having previously been *P. aeruginosa* culture positive, (4) Never *P. aeruginosa* never cultured from sputum or cough swab.
3.7 Hyperpolarised helium-3 diffusion MR ($^3$He MR)

3.7.1 $^3$He MR equipment:

$^3$He diffusion MR was undertaken in a 0.15 T permanent magnet system (Inter-magnetics General Corporation, New York) with a Surrey Medical Imaging Systems console (Surrey, UK) (Figure 3.21). The $^3$He gas was hyperpolarised via optical pumping in a custom made polarisation system. It was then stored, mixed with a buffer of $^4$He to a particular volume and transferred to the subject via 1 litre disposable one-way valve Tedlar bag (SKC Limited, Blandford Forum, UK) (Ball 2011).

Figure 3.21 T-Permanent magnetic system
3.7.2 $^3$He diffusion MR: Data collection

**Pre-scan:** subjects were instructed to remove anything metal that might interfere with measurements e.g. belts with metal buckles, wrist watches and took off their shoes. The test is also contraindicated in the presence of any metals in the body such as pins, plates or cochlear implants. Prior to testing, all subjects had a practice at breathing from the Tedlar bag and holding the breath.

**During-scan:** data was collected when the subject was lying supine in the scanner with a receiver coil of appropriate size around the chest. Subjects were instructed to inhale helium a gas mixture containing 10 ml hyperpolarized $^3$He from FRC from a Tedlar bag and breath-hold between 5-10 seconds (Ball 2011) (Figure 3.22). There were two types of measurements; short-breath hold scan for 5 sec and long-breath hold scan for 10 seconds. Short-scans were performed three times over about 1 hour in order to calculate ADC and long-scans were performed twice in order to calculate peripheral airways dimensions. The amount of helium gas mixture for each measurement was varied between ~350ml to ~550ml depending on subject size.

**Post-scan:** after each test session, data were visually inspected by at least two physicists (I Ball or S Hardy and J Owers-Bradley) from Department of Physics and Astronomy, University of Nottingham where data acquisition took place. It was then analysed and the measured values of ADC, R, and $h$ were corrected for the effects of different concentration of helium and lung inflation (Ball 2011).
Figure 3.22 Child lying in the scanner to perform the lung scan (with permission)

Legend: The receiver coil around the child chest is used to receive radiofrequency signals.
3.7.3 \textsuperscript{3}He MR: Data acquisition and analysis techniques

Data were acquired and analysed using two techniques.

1) Rapid acquisition with refocused echoes (RARE) sequence to determine the apparent diffusion coefficient (ADC) (Ball 2011). It is the most common sequence used for capturing diffusion data. This technique uses multiple pulses to refocus spins into echoes. Echo heights which represent signals size were plotted against the acquisition time. As the helium diffuses through airways, the echo peak heights decay as the diffusion process leads to signal dephasing. The signal loss due to diffusion is given by

\[ S = S_0 e^{-bADC} \]

where \( S_0 \) is the echo signal of the previous echo in the train and \( b \) is a function of the applied magnetic field gradient and the echo time. For this research, we have used 64 data acquisition time points over short time scale of 14 milliseconds and fixed gradient strength, giving \( b=0.3 \text{ s.cm}^{-2} \) (Figure 3.23) (Ball 2011).

\textbf{Figure 3.23 The calculation of ADC value}

\begin{center}
\includegraphics[width=0.5\textwidth]{echo_decay.png}
\end{center}

Legend: The graph represents 64-Echo sequence with decaying amplitude. \textsuperscript{3}He diffusion induced signal decay as a function of time in milliseconds. The ADC value is calculated by taking the peak of each of the 64 exponentially decaying echoes (taken from (Ball 2011))
ADC was calculated by taking the peak value of each of the 64 exponentially decaying echoes $S$ and fitting them to the equation above (Ball 2011). The mean value of the 64 individual ADC measurements was a measure of the global ADC. The standard deviation of these values was an estimate of the uniformity of ADC within the lung (Ball 2011) which is small for healthy lungs giving rise to straight lines on a log plot as shown on Figure 3.24. For each subject, a minimum of three RARE diffusion weighted scans were acquired. Natural logarithms were then performed to give a straight line of which the slope is proportional to ADC (Ball 2011).

**Figure 3.24 ADC for each of the 3 diffusion weighted scans performed on a subject**

![Log Signal Decay Curve](image)

*Legend: The output of the analysis software. The slope to determine ADC was found by applying a least squares linear fit to the data of which the range of fitting could be controlled by the user (taken from (Ball 2011))*

At least three technically satisfactory ADC values were obtained from each subject. The mean value was taken as the uncorrected ADC. ADC values were then corrected for the effects of differences in lung inflation between subjects (Narayanan, Owers-Bradley et al. 2012).
2) Q-space technique where global pulsed field gradient sequence is used to determine the geometrical parameters $R$ and $h$. In this sequence diffusion weighted free induction decay (FID) signals are acquired following the application of a radiofrequency (RF) pulse that is interrupted by a bipolar magnetic field gradient pulse of variable amplitude (Ball 2011). Bipolar gradient pulses were used to sensitize the MR signal to $^3$He gas molecular diffusion, and thus will impact on nuclear spins and result in decreasing spin signal amplitude (Figure 3.25) (Yablonskiy, Sukstanskii et al. 2009, Ball 2011). The diffusion sensitization of the nuclei ($b$-value) were varied for each acquisition ($b=0$ to 15 s.cm$^{-2}$ with diffusion time $\Delta=5$ ms). For each scan, a total of 50 FIDs were acquired of which 40 diffusion weighted FIDs and 10 have no diffusion gradients, $b=0$ s.cm$^{-2}$. The sequence is segmented into blocks with each block consisting of 4 diffusion weighted acquisitions followed by a single non-diffusion weighted acquisition to allow measurements to be corrected for attenuation due to RF depletion (Ball 2011).

**Figure 3.25 Sequence diagram for the diffusion sensitizing signal of the q-space technique**

[Sequence Diagram]

*Legend: Pulse gradient waveform used to sensitize the MR signal to $^3$He gas molecular diffusion. Characteristic parameters of the waveform are the diffusion time $\Delta$, the pulse width $\delta$, and the ramp time $\tau$ (taken from (Ball 2011)).*
The acquired 50 FIDs were post processed and the resultant q-space data were then fitted to Yablonskiy’s acinar model to determine the geometrical parameters $R$ (mean alveolar duct diameter, including alveolar sleeve), and $h$ (mean alveolar sleeve depth) (Ball 2011). In the Yablonskiy model the diffusion induced signal decay is characterised by longitudinal and transverse diffusion coefficients with respect to the individual acinar airway axis (Yablonskiy, Sukstanskii et al. 2009) (described in detail in Chapter 1, section 1.5.5). Therefore, by applying the acinar model of Yablonskiy to the q-space data we can determine $R$ and $h$ from the values of the longitudinal and transverse diffusion coefficients (Yablonskiy, Sukstanskii et al. 2009) (Figure 3.25).

**Figure 3.26 The calculation of dimensions of lung microstructure**

![Signal Decay Curve](image)

Legend: Diffusion induced signal decay with Yablonskiy model fitted over data. The derived values for the geometric parameters $R$ and $h$ are shown (taken from (Ball 2011)).
3.8 The reasons for using two N₂MBW devices for data collection:

In this project, N₂MBW data were collected primarily using the modified Profiler which was the only device available at LRI at the start of my PhD studies. This device had been used extensively during previous and ongoing research (Narayanan, Owers-Bradley et al. 2012). The earliest recordings from patients with CF that formed part of the longitudinal study (Chapter 5) had already been made with this device shortly before I joined the Department. The Profiler had the advantage of using a nitrogen analyser, thereby allowing direct recording of nitrogen concentration and the Phase III slopes. It was known that the Profiler was becoming obsolete, since the manufacturer was abandoning the use of a direct nitrogen analyser and replacing it with a device relying on measuring O₂ and CO₂. However, the replacement equipment from Medgraphics (the Ultima) was on trial in Leicester and compared with the Profiler and found to be unsuitable as the response times of O₂ and CO₂ sensors were very different. This meant that the Phase III slopes could not be accurately derived.

During my data collection, the Profiler became less reliable. The Exhalyzer D (Ecomedics, Switzerland) became available and this has software that compensates for the different response times of the different analysers. Hence it is possible to get a phase III slope from the Exhalyzer D, and we acquired it to continue my data collection. As a first step after acquiring the new device we compared N₂MBW data obtained from it to that obtained from the Profiler (Chapter 4, Section 4.8, Study 5). However, we found that data obtained from both machines was not strictly comparable and cannot therefore be used interchangeably. This affects the longitudinal study where occasions on which comparable nitrogen washout data were obtained were fewer than originally planned. The smaller number of occasions with comparable N₂MBW measurements may compromise the robustness of my observations.

In the following chapters majority of the studies were conducted using the Profiler, except two studies where we used the Exhalyzer D. These are (i) the comparative study between the Profiler and the Exhalyzer D (Chapter 4, Section 4.8, Study 5), and (ii) a study to assess the ability of VI indices to detect the short-term response to intravenous antibiotics (Chapter 5, Section 5.5.2.1). Appropriate independent normative data to define the upper limit of normality for each system were used throughout these studies.
3.9 Statistical analysis

Each results chapter in this thesis is self-contained, with a detailed description of the study population and statistical methods. The statistical analyses throughout this thesis were performed using the Statistical Package for the Social Sciences version 18 (SPSS Inc., Chicago, IL, USA) and Prism (GraphPad Software Inc, CA, USA).
Chapter 4 Standardisation the Methodology of N₂MBW and Investigation in to Sources of Variations

4.1 Introduction:

The multiple breath washout test (MBW) has been the subject of great interest recently as a sensitive test for detection of early lung damage and assessment of small airway disease such as in cystic fibrosis (CF) (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Owens, Aurora et al. 2011). Different indices can be obtained from the washout test and the most commonly reported is the lung clearance index (LCI), a measure of overall ventilation inhomogeneity (VI) (Aurora, Gustafsson et al. 2004). Other indices that can be derived from phase III slope analysis of sequential breaths of the washout are $S_{\text{cond}}$ and $S_{\text{acin}}$, which are said to reflect VI arising from the convective and acinar airways, respectively (Crawford 1985, Verbanck, Schuermans et al. 1997). Increases in these indices indicate inhomogeneity of ventilation distribution or gas mixing inefficiency. These indices have the advantage of being independent of age and height in healthy school-age children (Aurora 2005, Horsley 2009, Lum, Stocks et al. 2013). However, over a wide age range from pre-school to adulthood, significant association were found between VI indices and subject characteristics (Horsley 2009, Verbanck, Thompson et al. 2012, Lum, Stocks et al. 2013).

Current evidence supporting the clinical significance of MBW and VI indices has come from studies using different systems, the majority with SF₆ as a tracer gas; however, N₂MBW may be better suited in clinical settings (Jensen, Stanojevic et al. 2013). N₂MBW is only a washout of lung resident N₂ with 100% O₂ which is much cheaper, safe and readily available in all hospitals (Singer, Kieninger et al. 2013). Moreover, it has been reported that $\text{LCI}_{\text{N₂}}$ may be more sensitive at detecting peripheral airway disease compared to $\text{LCI}_{\text{SF₆}}$ especially in subjects with greater disease severity (Robinson, Latzin et al. 2013, Jensen, Stanojevic et al. 2013). Additionally, all principal studies on intra-pulmonary gas mixings, and the theoretical work, mathematical modelling, and supporting experiments of $S_{\text{III}}$ analysis techniques were performed using N₂MBW (Darling, Courmand et al. 1940, Paiva, Engel 1984, Crawford 1985, Verbanck, Schuermans et al. 1997). Currently, the commercially available N₂MBW systems use two different techniques to determine N₂ concentration; either directly with
N\textsubscript{2} analyser such as that used in modified Medgraphics Profiler or indirectly by measuring CO\textsubscript{2} and O\textsubscript{2} during testing and then calculated N\textsubscript{2} based on Dalton’s law of partial pressures. The latter technique is used in Exhalyzer D. The impact of different N\textsubscript{2}MBW systems on washout indices remains unclear although this is important for longitudinal studies and normative values.

4.2 Aims:
The aim of this chapter was to examine the effect of various analysis methods and different N\textsubscript{2}MBW systems upon MBW indices. The specific objectives of this chapter were:

I. To investigate whether age, height, weight and sex influence LCI and phase III indices in school-age children.
II. To compare the results obtained from modified Medgraphics Profiler to those generated by Exhalyzer D in healthy children and children with CF and whether there is an agreement between results obtained from both systems.

4.3 Hypotheses:
1. In healthy children, LCI, S\textsubscript{acin} and S\textsubscript{con}d are independent of subject age, height, weight and sex.
2. In all children, results obtained from the two different N\textsubscript{2}MBW systems will be comparable.

4.4 Power calculations
For study 1 that looks at the effect of subject characteristics upon N\textsubscript{2}MBW indices in school-age children, a sample size of 82 subjects was predicted to be sufficient to detect significant correlations with a power of 80% at a two sided 5% significance level. For study 2, our estimate of the sample size was based on LCI values from 90 school-age healthy children with mean ± SD LCI of 6.4 ± 0.7. To detect a minimum difference of one SD in LCI (between two N\textsubscript{2}MBW systems) with a power of 95% at a two sided 5% significance level, 16 children would be needed (Screenshouts of G*Power calculations are provided in Appendix F).
4.5 Study1: The effect of subject characteristics upon N₂MBW indices in school-age children

4.5.1 Rationale:
Previous studies in healthy school-age children and adults have shown no relationship between age and height with either LCI or phase III slope indices (Aurora 2005, Horsley 2009, Lum, Stocks et al. 2013). However, over a wide age range including pre-school, school-age and adult subjects, significant association were found between VI indices and subject characteristics (Horsley 2009, Verbanck, Thompson et al. 2012, Lum, Stocks et al. 2013). One would expect increased ventilation inhomogeneity with increasing age from infancy to adulthood. Horsley reported no significant association between LCI and age in healthy adult subjects, but when children younger than 16 years were included in the analysis, a weak but statistically significant positive correlation of LCI with age over the range 5 to 58 years appeared (Horsley 2009). In contrast, Lum et al. demonstrated a decrease in LCI with increasing age and height in 497 subjects (aged 2 weeks to 19 years old), but when the analysis was limited to children older than 6 years, LCI was independent of both age and height (Lum, Stocks et al. 2013). Furthermore, Verbanck et al. found that all VI indices increased consistently with age in adult subjects (Verbanck, Thompson et al. 2012). These contradictory findings need further investigation into the effect of subject characteristics upon N₂MBW indices.

Therefore, prior to tracking changes in LCI and phase III slope indices over time in our CF population, it is necessary to examine the effect of subject characteristics upon VI indice

4.5.2 Material and Methods:

4.5.2.1 Test subjects, equipment and procedure:
A retrospective analysis was performed of N₂MBW data collected in our laboratory from both healthy children and children with CF. Children with CF were tested as part of their annual review and healthy controls were recruited from the Leicester Respiratory cohorts for different research studies, each of which had been conducted with ethical approval from local and regional ethics committees (details provided in Chapter 3, Section 3.2.3). All children performed N₂MBW test in triplicate using nitrogen washout hardware (Modified Medgraphics Profiler, Medical Product Service...
GmbH, Germany) with a pre-set target Vt for each child, which was approximately 1/3 of the plethysmographic FRC, and a minimum target of 500mL. An auditory signal indicated when the child had inspired the pre-set Vt. N₂MBW data were analysed with custom-built software (MATLAB, R2011b, UK). Both hardware and software are described in detail in Chapter3.

4.5.2.2 Data Presentation and Statistical Analysis:

Subject characteristics (age, height, weight and sex) were compared between healthy children and children with CF using a Mann-Whitney test. For each subject, we reported the mean values of LCI, S_acin, S_cond from at least two washout tests. For each population, the relationships between N₂MBW indices and subjects characteristics were examined using Pearson or Spearman correlation coefficient (r). If correlation existed, linear regression analysis was performed and the square of the correlation coefficient (r²) was reported. This was followed by multivariate regression analysis with all predictor variables against each of the dependent variables. For each model, the constant, coefficient (B), standard error (SE) and the residual standard deviation (RSD) was reported. A p-value below 0.05 was regarded as statistically significant. Limits of normality for VI indices were defined as mean +/- 1.96 SD and calculated from the control data.

4.5.3 Results

One-hundred and twenty-seven school-age children were included in this study. Ninety were healthy controls and 37 were children with CF. Of the 37 school-age children with CF, 18(49%) subjects were homozygotes (∆F508), 12(32%) were heterozygotes (∆F508/other), 4(11%) were mixed genotype (other/other) and 3(8%) unknown. Thirty-four (92%) were pancreatic insufficient. Characteristics of study populations are presented in Table 4.1. CF and controls were well matched for gender, but children with CF were significantly younger, lighter and shorter than healthy controls.
Table 4.1 Characteristics of study populations

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=90)</th>
<th>CF (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>45%</td>
<td>51%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14.3 (7.8-17.1)</td>
<td>9.7 (5.9-17.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.0 (122.6-190.1)</td>
<td>134.5 (112.5-177.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.7 (22.5-93.0)</td>
<td>30.9 (20.4-71.0)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>20.2 (14.4-31.3)</td>
<td>17.6 (13.8-24.8)</td>
</tr>
</tbody>
</table>

Data are presented as median and ranges except for sex.

4.5.3.1 Effect of subject characteristics on N\(_2\)MBW indices:

LCI with age, height, and weight:

The relationships between LCI and age, height, and weight for healthy children and children with CF are shown in Table 4.2 and Figure 4.1. In children with CF, LCI was age, height, and weight-independent. However, in healthy children, there was a weak negative relationship between LCI and age (r\(^2\)=0.212, r= - 0.34, p<0.0001), LCI and weight (r\(^2\)=0.121, r= - 0.33, p<0.01), and a moderate negative relationship between LCI and height (r\(^2\)=0.291, r= - 0.555, p<0.0001).

Table 4.2 Correlation between LCI and subject characteristics in healthy children and children with CF

<table>
<thead>
<tr>
<th>LCI</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>- 0.34</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>- 0.55</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>- 0.33</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

r= correlation coefficient. *Statistically significant correlation.
Figure 4.1 The relationship between LCI with age, height, and weight in healthy children and children with CF

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for LCI. Note that normal values for LCI are age, height and weight dependent (see text for $r^2$, $r$ and $p$-values).
LCI with sex:

There was no significant sex difference for LCI in healthy children (median (ranges) 6.3 (5.21 -8.31) male vs. 5.99 (5.1-8.3) female), p=0.053) and children with CF (median (ranges) 11.06 (8.6 -16.2) male vs. 11.05 (8.09 -15.5) female, p=0.456) (Figure 4.2).

Figure 4.2 Sex difference of LCI in healthy children and children with CF

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for LCI. Note that values for LCI in both groups are sex-independent.
Results of multivariate regression analysis of LCI against subject characteristics for healthy children are presented in Table 4.3. For children with CF, none of subject characteristics were significantly correlated to LCI therefore no further analysis was performed.

Table 4.3 Results of multivariate regression of subject characteristics against LCI in healthy children

<table>
<thead>
<tr>
<th>LCI</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>11.409</td>
<td>0.942</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.026</td>
<td>0.048</td>
<td>0.584</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.037</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.019</td>
<td>0.007</td>
<td>0.012</td>
</tr>
<tr>
<td>Sex</td>
<td>0.014</td>
<td>0.143</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. After accounting for all variables, height and weight make a significant contribution to the model fit, but the greatest contribution is made by the constant. For the model as a whole, the RSD is 0.594, and the $r^2$ is 0.35, indicating that 35% of the variability of LCI in healthy children is explained by this regression model.
**S_{acin} with age, height, and weight:**

The relationship between $S_{acin}$ and subject characteristics for healthy children and children with CF are shown in Table 4.4 and Figure 4.3. In healthy children, there was significant positive relationships between $S_{acin}$ and age ($r^2=0.078$, $r=0.263$, $p=0.012$), height ($r^2=0.032$, $r=0.274$, $p<0.01$), weight ($r^2=0.045$, $r=0.246$, $p=0.019$).

In children with CF, there was significant positive relationships between $S_{acin}$ and age ($r^2=0.283$, $r=0.461$, $p<0.01$), height ($r^2=0.25$, $r=0.5$, $p<0.01$), and weight ($r^2=0.212$, $r=0.51$, $p<0.01$).

**Table 4.4 Correlation between $S_{acin}$ and subject characteristics in healthy children and children with CF**

<table>
<thead>
<tr>
<th>$S_{acin}$</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>0.263</td>
<td>0.012*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.274</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.246</td>
<td>0.019*</td>
</tr>
</tbody>
</table>

$r =$ correlation coefficient. *Statistically significant correlation.
Figure 4.3 The relationship between $S_{acin}$ with age, height, and weight in healthy children and children with CF

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for $S_{acin}$. Note the significant positive relationships between $S_{acin}$ and age, height, and weight in children with CF. There were also significant positive relationships between $S_{acin}$ and age, height, and weight in healthy children (see text for $r^2$, $r$ and $p$-values). Note different scales on X-axes.
$S_{\text{acin}}$ with sex:

There was no significant sex difference for $S_{\text{acin}}$ in healthy children (median (ranges) 0.079 (0.009, 0.234) male vs. 0.067 (-0.008, 0.149) female, p=0.555) and children with CF (median (ranges) 0.231 (0.059, 0.595) male vs. 0.104 (0.029, 0.463) female, p=0.091) (Figure 4.4).

**Figure 4.4 Sex difference of $S_{\text{acin}}$ in healthy children and children with CF**

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for $S_{\text{acin}}$. No significant sex difference for $S_{\text{acin}}$ in healthy children (see text for p-value).
Results of multivariate regression analysis of $S_{acini}$ against subject characteristics for healthy children are shown in Table 4.5 and for children with CF are presented in Appendix F, Table 1.

Table 4.5 Results of multivariate regression of subject characteristics against $S_{acini}$ in healthy children

<table>
<thead>
<tr>
<th>$S_{acini}$</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.023</td>
<td>0.06</td>
<td>0.706</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.005</td>
<td>0.003</td>
<td>0.079</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.000</td>
<td>0.001</td>
<td>0.839</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.74</td>
</tr>
<tr>
<td>Sex</td>
<td>0.014</td>
<td>0.009</td>
<td>0.128</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. None of the variables make a significant contribution to the model fit. For the model as a whole, the RSD is 0.0380, and the $r^2$ is 0.105, indicating that 10.5% of the variability of $S_{acini}$ in healthy children is explained by this regression model.
**S_{cond} with age, height, and weight:**

The relationship between $S_{cond}$ and subject characteristics for the control subjects and the children with CF are shown in Table 4.6 and Figure 4.5. In healthy children, $S_{cond}$ was negatively related to age ($r^2=0.054$, $r=-0.335$, $p<0.01$), and height ($r^2=0.021$, $r=-0.23$, $p=0.033$). Normal values for $S_{cond}$ were and weight-independent.

In children with CF, $S_{cond}$ was positively related to age ($r^2=0.408$, $r=0.709$, $p<0.0001$), height ($r^2=0.455$, $r=0.703$, $p<0.0001$), and weight ($r^2=0.33$, $r=0.59$, $p<0.0001$).

**Table 4.6 Correlation between $S_{cond}$ and subject characteristics in healthy children and children with CF**

<table>
<thead>
<tr>
<th>$S_{cond}$</th>
<th>Healthy controls</th>
<th></th>
<th>Subjects with CF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>-0.335</td>
<td>p&lt;0.01*</td>
<td>0.709</td>
<td>p&lt;0.0001*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.23</td>
<td>0.033*</td>
<td>0.703</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.18</td>
<td>0.077</td>
<td>0.59</td>
<td>p&lt;0.0001*</td>
</tr>
</tbody>
</table>

$r$ = correlation coefficient. *Statistically significant correlation.
Figure 4.5 The relationship between $S_{\text{cond}}$ with age, height, and weight in healthy children and children with CF

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for $S_{\text{cond}}$. Note the significant positive relationships between $S_{\text{cond}}$ and age, height, and weight in children with CF. In contrast, negative relationship was found between $S_{\text{cond}}$ and age, height and weight in healthy children (see text for $r^2$, $r$ and $p$-values). Note different scales on Y-axes.
$S_{\text{cond}}$ with sex:

There was also no significant sex difference for $S_{\text{cond}}$ children with CF (0.073 (0.006, 0.107) male vs. 0.052 (0.011, 0.111) female, $p=0.839$) (Figure 4.6). However, there was a significant sex difference for $S_{\text{cond}}$ in healthy children. $S_{\text{cond}}$ was significantly higher in males (0.029 (0.004, 0.089) than in females 0.021 (0.002, -0.07), $p=0.025$).

**Figure 4.6** Sex difference of $S_{\text{cond}}$ in healthy children and children with CF

Legend: Control children presented as green triangles, and children with CF presented as red circles. Broken lines represent limits of normality for $S_{\text{cond}}$. Note the significant sex difference for $S_{\text{cond}}$ in healthy children (see text for $p$-value).
Results of multivariate regression analysis of $S_{\text{cond}}$ against subject characteristics for healthy children are presented in Table 4.7 and for children with CF are presented in Appendix F, Table 2.

**Table 4.7 Results of multivariate regression of subject characteristics against $S_{\text{cond}}$ in healthy children**

<table>
<thead>
<tr>
<th>$S_{\text{cond}}$</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.039</td>
<td>0.024</td>
<td>0.108</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.002</td>
<td>0.001</td>
<td>0.209</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.006</td>
<td>0.000</td>
<td>0.99</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.005</td>
<td>0.000</td>
<td>0.612</td>
</tr>
<tr>
<td>Sex</td>
<td>0.006</td>
<td>0.004</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. None of the variables make a significant contribution to the model fit. For the model as a whole, the RSD is 0.0152, and the $r^2$ is 0.096, indicating that 9.6% of the variability of $S_{\text{cond}}$ in healthy children is explained by this regression model.

**4.5.4 Discussion:**

**4.5.4.1 Summary of results:**

In this study, the relationships between subject characteristics and $N_2$MBW indices were studied in healthy school-age children and children with CF. Our findings demonstrated that LCI is largely independent of subject characteristics. In healthy school-age children, only 35% of the variability of LCI is explained by subject age, sex, height and weight (Table 4.6). This was consistent with results reported by Aurora in healthy children aged 2 to 16 years (Aurora 2005). We have also showed that phase III slope indices ($S_{\text{acin}}$ and $S_{\text{cond}}$) are independent of subject characteristics. In healthy school-age children, only 10.5% of the variability in $S_{\text{acin}}$ and 9.6% of variability in $S_{\text{cond}}$ are explained by subject age, sex, height and weight.
### 4.5.4.2 Relationship between subject characteristics and N₂MBW indices:

In the present study, we found a weak negative relationship between LCI and both age weight and a moderate negative relationship between LCI and height in healthy children. However, variations in subject characteristics accounted only for 35% of variability in LCI. The effect of subject characteristics on VI indices have been reported previously by different studies in healthy adults, school-age and pre-school children, but these have produced conflicting results (Aurora 2005, Horsley 2009, Verbanck, Thompson et al. 2012, Lum, Stocks et al. 2013). Whereas Aurora and Horsley found no relationship between age and either LCI, $S_{\text{acin}}$ or $S_{\text{cond}}$ in healthy children and adults respectively, Lum et al. demonstrated a nonlinear decrease in LCI with increasing age and height in children aged 2 weeks to 19 yrs. old with no significant association with sex (Aurora 2005, Horsley 2009, Lum, Stocks et al. 2013). When the analysis was limited to children older than 6 years, however, LCI was independent of both age and height (Lum, Stocks et al. 2013). It should be noted that the study of Lum et al. was the largest study of its kind with MBW data collated from 3 centres from a large sample size of 497 subjects, which strengthens their findings. Furthermore, Verbanck et al. found that all three MBW indices increased consistently with age between 25-65 years (Verbanck, Thompson et al. 2012). They also reported a small but significant gender difference for $S_{\text{acin}}$ in healthy adult subjects (Verbanck, Thompson et al. 2012). $S_{\text{acin}}$ was found to be slightly greater in men than in women (Verbanck, Thompson et al. 2012). Kraemer et al. reported a strong sex difference for rate of deterioration in LCI in school-age children with CF with females having a significantly higher rate compared to males (Kraemer, Blum et al. 2005). They suggested that it was due to the differences between genders in the breathing pattern particularly the higher $FRC_{N2}$ in females (Kraemer, Blum et al. 2005). In our healthy population we found a weak positive association between $S_{\text{acin}}$ and both age and height, and a weak negative association between $S_{\text{cond}}$ with age and height. A significant sex difference for $S_{\text{cond}}$ was also noted in our healthy children, being slightly higher in males. However, when variations in subject characteristics were entered into the regression models, they accounted for only 9.6% of variability in $S_{\text{cond}}$ and 10.5% of variability in $S_{\text{acin}}$. 
We have also noticed from the relationship between $S_{\text{cond}}$ and age in children with CF (Figure 4.5) that the increase in $S_{\text{cond}}$ with age reached an asymptote, with a maximum value of 0.10 L$^{-1}$ and did not increase further with increasing disease severity. These findings confirm previous observations of $S_{\text{cond}}$ reaching a maximum as inhomogeneity becomes more severe in older subjects (Horsley, Macleod et al. 2008). More recently, Verbanck et al. criticised the determination of $S_{\text{cond}}$ between 1.5 and 6 lung TO in patients with more advanced CF lung disease as it may have been invalidated by the severity of the ventilation heterogeneity (Verbanck, Paiva et al. 2013). Instead, they proposed an alternative $S_{\text{cond}}$ computation method, that provides a more accurate reflection of $S_{\text{cond}}$ in advanced CF lung disease, based on lowering the upper end of the TO range from 6 to 3 (Verbanck, Paiva et al. 2013). However, our analysis programme is based on the determination of $S_{\text{cond}}$ between 1.5 and 6 lung TO. This, therefore, will limit the ability to follow the changes in $S_{\text{cond}}$ and $S_{\text{acin}}$ over years in children with CF.

In conclusion, findings from this study showed that there were some statistically significant effects of physical characteristics on indices of VI in healthy children. However, the effects were trivial when regression analysis was used to determine the size of the effects. After accounting for all variables, the greatest contribution for all indices was made by the constant. Therefore, the use of the ULNs for the assessments of VI indices on single occasions during school-age will be appropriate as the variables explored here may not have any significant clinical impact on interpretation. In contrast, expressing the results as z-scores, using prediction equations reflecting the developmental changes occurring across childhood, will be needed when serial measurements are being undertaken at any age.
4.6 Study 2: Multiple-breath N₂ washout: A comparative study between two different instruments using two measurement techniques

4.6.1 Rationale:
The data in this thesis has been collected using two different N₂MBW systems that employed different techniques to measure N₂ concentration, and different systems for data collection and analysis. These are the modified Medgraphics Profiler (Medical Product Service GmbH, Germany) and Exhalyzer D (Eco Medics, Switzerland). The Profiler determines N₂ concentration directly with a N₂ analyser using photospectrometry. The Exhalyzer D determines N₂ concentration indirectly by measuring carbon dioxide (CO₂) and O₂ during testing and then calculating N₂ based on Dalton’s law of partial pressures. Thus this may lead to differences in the VI measurements obtained using these two different N₂MBW systems. Recent MBW recommendations stated that data collection method may impact upon LCI measurements (Beydon 2007, Robinson, Latzin et al. 2013). Moreover, a previous study compared different MBW systems based on SF₆ as the tracer gas and demonstrated similar LCI values, but slightly significantly different FRC values (Fuchs 2006). Therefore, the aim of this study was to determine whether the results obtained using these two N₂MBW systems can be used interchangeably in both healthy children and children with CF as this important for our longitudinal study and normative values. In addition, we aimed to quantify the discriminatory power of each system to differentiate health and disease.

4.6.2 Material and Methods:

4.6.2.1 Test subjects, equipment and procedure:

This is a prospective cross-sectional study conducted on school-age children. Children with CF were tested as part of their annual review and healthy controls were recruited from the Leicester Respiratory cohort as part of a separate project (Appendix B). All children performed N₂MBW on two systems (Profiler and Exhalyzer D) in triplicate in random order on the same day and the data obtained analysed with its corresponding software (Mat lab and Spiroware, respectively). Informed written consent was obtained from all the parents of healthy children and children with CF. The description of Modified Medgraphics Profiler (Medical Product Service GmbH, Germany) and
Exhalyzer D (Eco Medics, Switzerland), data collection and analysis techniques were described in detail in Chapter 3.

4.6.2.2 Data Presentation and Statistical Analysis:

For each outcome measure, the intra-subject coefficients of variation (CV%) were calculated for children with CF and healthy controls; for each device separately. The agreement between Profiler and Exhalyzer D was then assessed using Bland-Altman plots. The mean difference (i.e. bias) and limits of agreements (95% LA) were calculated as recommended by Bland and Altman (Martin Bland, Altman 1986). To test whether there is a significant difference between VI indices obtained from the two different N₂MBW systems, paired t-test or Wilcoxon matched-pairs signed ranked test were used for parametric and non-parametric data respectively. Additional analysis using receiver operating characteristics (ROC) curves were used to determine the ability of each piece of equipment to discriminate between health and disease. We have reported the area under the ROC curve, the standard error (SE) and 95% CI.

4.6.3 Results:

Thirty-three school-age children (18 with CF and 15 healthy controls) had paired measurements on both systems and included in the analysis. Each subject completed at least two acceptable N₂MBW tests. Overall, healthy controls were taller and heavier than children with CF (Table 4.12).

<table>
<thead>
<tr>
<th>Table 4.8 Characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls (n=15)</td>
</tr>
<tr>
<td><strong>M:F</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
</tr>
<tr>
<td><strong>FEV₁z-score</strong></td>
</tr>
</tbody>
</table>

*Data presented as median (ranges), unless otherwise indicated*
4.6.3.1 Repeatability of FRC and \( N_2 \)MBW indices in both systems:

The within-test variability of FRC and LCI were good in health and disease in both \( N_2 \)MBW systems (Table 4.13). The within-test CV% of FRC and LCI was slightly higher in children with CF compared to healthy controls in both systems. It was also higher in Profiler compared to Exhalyzer D, but remain <10%. Our repeatability data for LCI derived from Exhalyzer D were consistent with previously published repeatability data using the same system (Singer, Kieninger et al. 2013).

The within-test variability of \( S_{acin} \) derived from both systems was acceptable in both health and disease, but it was slightly higher in Profiler compared to Exhalyzer D. In contrast, the variability of \( S_{cond} \) was high in healthy controls in both systems and in children with CF when derived from Profiler. The variability of \( S_{cond} \) derived from Exhalyzer D in children with CF was reasonable.

Table 4.9 Repeatability of FRC and \( N_2 \)MBW indices in school-age children

<table>
<thead>
<tr>
<th></th>
<th>Exhalyzer D</th>
<th></th>
<th>Profiler</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD) of CV%</td>
<td></td>
<td>Mean (SD) of CV%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HC (n=15)</td>
<td>CF (n=18)</td>
<td>HC (n=15)</td>
<td>CF (n=18)</td>
</tr>
<tr>
<td>FRC</td>
<td>2.5 (1.4)</td>
<td>4.0 (2.3)</td>
<td>6.6 (3.1)</td>
<td>6.0 (4.5)</td>
</tr>
<tr>
<td>LCI</td>
<td>2.7 (2.1)</td>
<td>4.6 (3.3)</td>
<td>5.8 (3.6)</td>
<td>8.1 (5.4)</td>
</tr>
<tr>
<td>( S_{acin} (L^{-1}) )</td>
<td>26.8 (20.6)</td>
<td>25.8 (33.7)</td>
<td>29.5 (21.7)</td>
<td>37.6 (54.9)</td>
</tr>
<tr>
<td>( S_{cond} (L^{-1}) )</td>
<td>92.7 (43.8)</td>
<td>24.1 (22.9)</td>
<td>48.6 (41.2)</td>
<td>41.3 (33.3)</td>
</tr>
</tbody>
</table>

Data are given as mean (SD) of CV%. Extreme outliers (n=4, 1 HC: 3CF) with variations>300 in \( S_{acin} \) and \( S_{cond} \) were excluded from the final calculation of CV%.
4.6.3.2 LCI comparison between \textit{N}_2\text{MBW} systems:

Difference in mean LCI between both systems was calculated as mean LCI obtained from the Exhalyzer minus mean LCI obtained from the Profiler. There was statistically significant difference in LCI for the group as a whole, and for healthy children and those with CF when considered separately. The median (ranges) for differences in LCI was 1.3 (-0.62-7.43, P<0.0001) for the whole cohort, 0.85 (-0.53-1.56, P<0.0001) for healthy controls, and 2.4 (-0.62-7.43, P<0.0001) for CF subjects.

In healthy children, the Exhalyzer generated higher values of LCI (mean difference (LCI_{Exhalyzer}-LCI_{profiler}) = 0.902, with wide limits of agreement (-0.178 to 1.98), but there was uniform scatter of points around the mean difference on Bland-Altman plot (Figure 4.15A).

In children with CF, the mean difference between systems (LCI_{Exhalyzer}-LCI_{profiler}) was almost three times that in health (2.56) with a clear bias such that LCI_{Exhalyzer} was disproportionately higher than LCI_{profiler} as the average LCI values increased (Figure 4.15B). Also the limits of agreement were wide, going from -1.35 to 6.47. This large mean of the difference indicates that the two systems are systematically producing different results.
Figure 4.7 Bland-Altman plot of the agreement between LCI obtained from the Exhalyzer and that obtained from the Profiler in A) healthy controls and B) children with CF

Legend: The solid horizontal lines represent the mean difference, and the dashed lines represent the limits of agreement (calculated as mean difference+/− 1.96SD). Note we used different scales on x and y axes. In health (A), the mean difference was 0.902, with wide limits of agreement (-0.178 to 1.98), but with uniform scatter of points around the mean difference. In contrast, in CF (B) there was an obvious bias (mean difference 2.56 (LA -1.35 to 6.47)) such that LCI_{exhalyzer} increased disproportionately to LCI_{profiler} as mean LCI increased which indicates that the two systems are systematically producing different results.
4.6.3.3 **FRC comparison between N₂MBW systems:**

The difference in mean FRC between both systems was calculated as mean FRC obtained from Exhalyzer minus mean FRC obtained from Profiler. There was no significant difference in FRC for the group as a whole, for healthy controls and children with CF. Mean (SD) difference for the group as a whole is -0.048 (0.25) (95%CI -0.042 to 0.138, \(P=0.286\)).

In healthy children, the Profiler generated non-significantly higher values of FRC (mean (SD) difference \((\text{FRC}_{\text{Exhalyzer}}-\text{FRC}_{\text{Profiler}}) = -0.133 (0.28)\) (95% CI -0.291 to 0.0257, \(P=0.094\)), but no bias was observed between systems and there was uniform scatter around the mean difference. Though limits of agreements were wide (-0.693 to 0.428) (Figure 4.16A).

In children with CF, FRC obtained from the Exhalyzer was slightly higher than that from the Profiler, though the difference was non-significant. The mean (SD) difference between systems \((\text{FRC}_{\text{Exhalyzer}}-\text{FRC}_{\text{Profiler}})\) was smaller than that on healthy controls 0.022 (0.21) (95%CI -0.082 to 0.126, \(P=0.66\)), limits of agreement (-0.390 to 0.434). However, on Bland-Altman plot there was a trend towards increasing the difference between systems as mean FRC increased (Figure 4.16B).
Figure 4.8 Bland-Altman plot of the agreement between FRC obtained from the Exhalyzer and that obtained from the Profiler in A) healthy controls and B) children with CF

Legend: The solid horizontal lines represent the mean difference, and the dashed lines represent the limits of agreement (calculated as mean difference +/- 1.96SD). In health (A), the Profiler produced higher values of FRC; the mean difference was -0.133 (LA -0.693 to 0.428), with no bias observed between systems. In CF (B), the mean difference between systems was smaller than that of healthy controls 0.022 (LA -0.39 to 0.434), but becoming disproportionately greater with higher mean FRC.
4.6.3.4 $S_{acin}$ comparison between N2MBW systems:

The difference in mean $S_{acin}$ between both systems was calculated as mean $S_{acin}$ obtained from Exhalyzer minus mean $S_{acin}$ obtained from Profiler. There was a statistically significant difference in $S_{acin}$ for the group as a whole, for healthy children and those with CF. The median (ranges) for differences in $S_{acin}$ was -0.034 (-0.108-0.019, $P<0.0001$) for the whole cohort, -0.027 (-0.096 - (-0.003), $P<0.0001$) for healthy controls, and -0.0407 (-0.108-0.019, $P<0.0001$) for CF subjects

In healthy children, the Profiler generated higher values of $S_{acin}$ (mean difference ($S_{acinExhalyzer} - S_{acinProfiler}$) = -0.031. This was apparent on a Bland-Altman plot as the difference tended to increase with increasing the mean. The wider limits of agreement between systems -0.08 to 0.017 may be due to the outlier (Figure 4.17A).

In children with CF, $S_{acin}$ obtained from the Profiler was also higher than that from the Exhalyzer. The mean difference between systems ($S_{acinExhalyzer} - S_{acinProfiler}$) (-0.046), was greater than in health, with wider limits of agreement (-0.109 to 0.018). On a Bland-Altman plot, there was a trend towards a more marked difference as the mean increased (Figure 4.17B).
Figure 4.9 Bland-Altman plot of the agreement between $S_{acin}$ obtained from the Exhalyzer and that obtained from the Profiler in A) healthy controls and B) children with CF.

Legend: The solid horizontal lines represent the mean difference, and the dashed lines represent the limits of agreement (calculated as mean difference +/- 1.96SD). In health (A), the Profiler generated higher values of $S_{acin}$; the mean difference was -0.031 (LA -0.08 to 0.017) and there was a trend towards more marked difference as the mean increased. In CF (B), the mean difference between systems was greater than in health (-0.046) and the limits of agreement were much wider (-0.109 to 0.018). Also the difference between systems was disproportionately greater with higher mean $S_{acin}$.
4.6.3.5 $S_{cond}$ comparison between $N_2$MBW systems:

Difference in mean $S_{cond}$ between both systems was calculated as mean $S_{cond}$ obtained from Exhalyzer minus mean $S_{cond}$ obtained from Profiler. There was a statistically significant difference in $S_{cond}$ for the group as a whole, for healthy children and children with CF. The median (ranges) for $S_{cond}$ was -0.009 (-0.077-0.074, $P=0.004$) for the whole cohort, -0.005 (-0.040-0.054, $P=0.035$) for healthy controls, and -0.014 (-0.077-0.074, $P=0.02$) for CF subjects.

In healthy children, the Profiler generated higher values of $S_{cond}$ (mean difference ($S_{condExhalyzer} - S_{condProfiler}$) = -0.005, but no obvious bias was observed between systems with uniform scatter around the mean difference. The limits of agreements were wide (LA -0.04 to 0.03) (Figure 4.18A).

In children with CF, $S_{cond}$ obtained from the Profiler was also higher than that from the Exhalyzer. The mean difference between systems ($S_{condExhalyzer} - S_{condProfiler}$) was greater than in health -0.014 and limits of agreement between systems were wider (LA -0.077 to 0.049). On a Bland-Altman plot, there was a trend towards more marked difference as the mean increased (Figure 4.18B).
Figure 4.10 Bland-Altman plot of the agreement between $S_{\text{cond}}$ obtained from the Exhalyzer and that obtained from the Profiler in A) healthy controls and B) children with CF.

Legend: The solid horizontal lines represent the mean difference, and the dashed lines represent the limits of agreement (calculated as mean difference +/- 1.96SD). In health (A), Profiler generated higher $S_{\text{cond}}$ values, the mean difference (-0.005 (LA -0.04 to 0.03)), with no bias observed between systems. In CF (B), the mean difference between systems was greater than in health (-0.014) with wider limits of agreement (-0.077 to 0.049). Also the difference between systems was disproportionately greater with higher mean $S_{\text{cond}}$. 
4.6.3.6 The sensitivity of \( \text{N}_2 \text{MBW} \) systems:

As we found that the Exhalyzer D on average generated higher LCI values and lower \( S_{\text{acin}} \) and \( S_{\text{cond}} \) values than the Profiler, the interpretation of parameters measured by these different \( \text{N}_2 \text{MBW} \) systems will require independent normative values to define an appropriate upper limit of normal. Therefore, we have used independent normative values to define the upper limit of normal for each system to enable us to study the sensitivity and specificity of parameters obtained from them.

For the Profiler, the ULN was calculated from data obtained from 90 healthy school-age children whereas for the Exhalyzer D, the ULN was calculated from data obtained from 43 healthy school-age children. Both healthy populations were tested in our laboratory at Leicester Royal Infirmary (details of healthy population provided in Chapter 3, section 3.2.2).

4.6.3.7 The sensitivity of lung clearance index on both \( \text{N}_2 \text{MBW} \) systems:

Using ROC analysis to assess the sensitivity of LCI, the area under the ROC curve for LCI obtained from the Profiler was 0.967 (SE 0.0262, CI 0.915 to 1.018) (Figure 4.19). When we used the ULN (LCI=7.73) obtained from 90 healthy controls on the same machine in our laboratory as the cut-off value for abnormality, the sensitivity of LCI derived from the Profiler to detect the disease was 61.1% and specificity was 100%.
Legend: The sensitivity and specificity of LCI derived from the Profiler to discriminate between health and disease was represented by the area under the ROC curve which was 0.967. When we set the ULN of LCI (7.73) as the cut-off point, the sensitivity of LCI to detect the disease was 61.1% and the specificity to exclude the disease was 100%.

The area under the ROC curve for LCI obtained from the Exhalyzer was 0.988 (SE 0.0135, CI 0.963 to 1.015) (Figure 4.20). When we used the ULN (LCI=7.75) obtained from 43 healthy controls on the same machine in our laboratory as the cut-off value for abnormality, the sensitivity of LCI derived from the Exhalyzer to detect the disease was 88.89% and specificity was 93.3%.
Figure 4.12 ROC curve for LCI obtained from the Exhalyzer

Legend: The sensitivity and specificity of LCI derived from the Exhalyzer to discriminate between health and disease was represented by the area under the ROC curve which was 0.988. When we set the ULN of LCI (7.75) as the cut-off point, the sensitivity of LCI to detect the disease was 88.89% and the specificity to exclude the disease was 93.3%.
4.6.3.7. The sensitivity of ventilation inhomogeneity at acinar airways on both N2MBW systems:

Using ROC analysis to assess the sensitivity of $S_{\text{acin}}$, the area under the ROC curve for $S_{\text{acin}}$ obtained from Profiler was 0.691 (SE 0.095, CI 0.505 to 0.088) (Figure 4.21). When we used the ULN ($S_{\text{acin}}=0.153$) obtained from 90 healthy controls who performed the washout test on the same machine in our laboratory as the cut-off value, the sensitivity of $S_{\text{acin}}$ derived from the Profiler to detect the disease was 16.7% and specificity was 93.3%.

**Figure 4.13 ROC curve for $S_{\text{acin}}$ obtained from the Profiler**

Legend: The sensitivity and specificity of $S_{\text{acin}}$ derived from the Profiler to discriminate between health and disease was represented by the area under the ROC curve which was 0.691. When we set the ULN of $S_{\text{acin}}$ (0.152) as the cut-off point, the sensitivity of $S_{\text{acin}}$ to detect the disease was 16.7% and the specificity to exclude the disease was 93.3%.
The area under the ROC curve for $S_{acin}$ obtained from the Exhalyzer was 0.600 (SE 0.103, CI 0.398 to 0.801) (Figure 4.22). When we used the ULN ($S_{acin}=0.065$) obtained from 43 healthy controls who performed the washout test on the same machine on our laboratory as the cut-off point for abnormality, the sensitivity of $S_{acin}$ derived from the Exhalyzer was 38.89% and specificity was 93.33%.

**Figure 4.14 ROC curve for $S_{acin}$ obtained from the Exhalyzer**

![ROC curve](image)

Legend: The sensitivity and specificity of $S_{acin}$ derived from the Exhalyzer to discriminate between health and disease was represented by the area under the ROC curve which was 0.600. When we set the ULN of $S_{acin}$ (0.065) as the cut-off point, the sensitivity of $S_{acin}$ to detect the disease was 38.89% and the specificity to exclude the disease was 93.3%. 

123
4.6.3.8 The sensitivity of ventilation inhomogeneity at the conductive airways on both N2MBW systems:

Using ROC analysis to assess the sensitivity of $S_{\text{cond}}$, the area under the ROC curve for $S_{\text{cond}}$ obtained from the Profiler was 0.919 (SE 0.0548, CI 0.81 to 1.02) (Figure 4.23). When we used the ULN ($S_{\text{cond}}=0.058$) obtained from 90 healthy controls who performed the washout test on the same machine on our laboratory as the cut-off point, the sensitivity of $S_{\text{acin}}$ derived from the Profiler was 55.7% and specificity was 100%.

Figure 4.15 ROC curve for $S_{\text{cond}}$ obtained from the Profiler

Legend: The sensitivity and specificity of $S_{\text{cond}}$ derived from the Profiler to discriminate between health and disease was represented by the area under the ROC curve which was 0.919. When we set the ULN of $S_{\text{cond}}$ (0.058) as the cut-off point, the sensitivity of $S_{\text{cond}}$ to detect the disease was 55.7% and the specificity to exclude the disease was 100%.
The area under the ROC curve for $S_{\text{cond}}$ obtained from the Exhalyzer was 0.982 (SE 0.0176, CI 0.947 to 1.016) (Figure 4.24). When we used the ULN ($S_{\text{cond}}=0.022$) obtained from 43 healthy controls who performed the washout test on the same machine on our laboratory as the cut-off point for abnormality, the sensitivity of $S_{\text{cond}}$ derived from the Exhalyzer was 83.33% and specificity was 100%.

**Figure 4.16 ROC curve for $S_{\text{cond}}$ obtained from the Exhalyzer**

Legend: The sensitivity and specificity of $S_{\text{cond}}$ derived from the Exhalyzer to discriminate between health and disease was represented by the area under the ROC curve which was 0.982. When we set the ULN of $S_{\text{cond}}$ (0.022) as the cut-off point, the sensitivity of $S_{\text{cond}}$ to detect the disease was 83.33% and the specificity to exclude the disease was 100%.
4.6.4 Discussion:
To the best of our knowledge, no other study has compared VI indices derived from two N\textsubscript{2}MBW systems in healthy children and children with CF. Our findings demonstrated that VI indices derived from different N\textsubscript{2}MBW systems that incorporated different software for data analysis are not comparable in health and disease. This has important implications for longitudinal studies and normative values in general and specifically for our longitudinal study. We found that the Exhalyzer D had better discriminative power and intra-session repeatability than the Profiler. On average the Exhalyzer D generated higher LCI values and lower S\textsubscript{acin} and S\textsubscript{cond} values than the Profiler. However, for lung volume measurements (FRC), there were no significant difference between systems in health and disease. As such, interpretation of parameters measured by different N\textsubscript{2}MBW systems will require independent normative values to define an appropriate upper limit of normal and this is what we did to enable us to study the sensitivity and specificity of such systems.

The feasibility of N\textsubscript{2}MBW in school-age children has been described previously using the Exhalyzer D as the device for lung function measurements (Singer, Kieninger et al. 2013). However, authors of that study did not include comparison between Exhalyzer D and other N\textsubscript{2}MBW systems. A recent study by Jensen et al. has compared results obtained from the Exhalyzer D with those obtained from SF\textsubscript{6} based MBW system in healthy children and children with CF (Jensen, Stanojevic et al. 2013). Therefore, results from the Jensen et al. study are not directly comparable to our study.

The observed differences in VI indices between N\textsubscript{2}MBW systems in our study in healthy children and children with CF clearly demonstrate that the two systems cannot be used interchangeably. These differences could be potentially explained by a variety of factors including the measurement techniques for N\textsubscript{2} concentration and flow. However, the lack of significant differences between two systems in calculating FRC means that both can accurately measure the expired volume and the N\textsubscript{2} concentration.

Therefore, the differences between the measurements of ventilation distribution derived from these N\textsubscript{2}MBW systems may be attributed to other factors related to equipment (i.e. the valve system and apparatus dead space), procedure (i.e. breathing protocols) or analysis methods (i.e. software algorithms used for calculation of indices). The last factor that may lead to variation in measured indices between systems will not be
discussed here as it mainly relates to computer programming, which is beyond the scope of this thesis.

The equipment used for the Profiler was different from that of the Exhalyzer D (both described in details in Chapter 3). In the Profiler, subjects breathe through a non-rebreathing valve whereas in the Exhalyzer D a bias flow system is used. The earlier approach may lead to increased work of breathing especially in subjects with lung disease such as CF. Accordingly, the breathing pattern may be altered and become more rapid and shallow, which may affect ventilation distribution and reduce the time for efficient gas mixing especially at peripheral airways (Roos, Dahlstrom et al. 1955, Grönkvist, Bergsten et al. 2002). It may also reduce the cumulative expired volume needed to complete the washout. This may explain the lower LCI values and higher $S_{acin}$ and $S_{cond}$ values obtained from Profiler as compared to that obtained from Exhalyzer D in children with CF in our study, with the greatest differences in children with more severe disease. Furthermore, the reported differences in mean LCI, $S_{acin}$ and $S_{cond}$ obtained from the two N$_2$MBW systems were greater in children with CF than healthy children which may support this explanation.

Furthermore, we have noticed that the washout time on Exhalyzer D was longer than the washout time on Profiler (1 to 7 min vs. 1 to 4 min, respectively) especially for subjects with advanced lung disease. In practice this will increase the number of breaths required to complete the washout and the resultant cumulative expired volume. Therefore, LCI values obtained from Exhalyzer will be disproportionately higher than those obtained from Profiler. Our data demonstrate these differences in healthy children and children with CF, but progressively much more pronounced in children with greater disease severity. This suggests that the Exhalyzer D may be more accurate in reflecting the degree of VI than the Profiler and this was supported by our sensitivity analysis. However, this conclusion should be taken with caution because recent evidence has shown that during long washouts seen in subjects with significant VI an increasing amount of N$_2$ dissolved in the tissue will diffuse from blood into the alveoli, which may then impact on the washout progression (Jensen, Stanojevic et al. 2013, Robinson, Latzin et al. 2013). In a lung model representing a CF child, the excreted N$_2$ has been shown to increase the end of washout N$_2$ concentration by 24-49% (Horsley et al. 2013).
The pre-inspired dead space was also different between N2MBW systems used in the current study. The Profiler pre-capillary dead space (volume between the child’s mouth and sampling point) was larger than that used in the Exhalyzer D (113 ml vs 37.9 ml, respectively) and this may help to explain the differences between VI measurements obtained. A study in lung model representing a CF child has shown that increasing dead space caused an increase in LCI measurement error by 6-13% (Horsley et al. 2013). However, a recently published paediatric study in 97 healthy controls and 93 with CF have shown that LCI is minimally affected by equipment dead space (Haidopoulou, Lum et al. 2012). They draw their conclusion from the way in which LCI is calculated by dividing the cumulative expired volume (CEV) by FRC after correcting for equipment dead space (Haidopoulou, Lum et al. 2012). The impact of the apparatus dead space upon phase III slope indices though has not been examined yet in children (Aurora, Kozlowska et al. 2005). Therefore, it is possible that the higher values of S_{acin} and S_{cond} derived from the Profiler as compared those obtained from the Exhalyzer D in both health and disease in our population may be explained in part by increasing the apparatus dead space in Profiler.

In conclusion, we demonstrated that VI indices derived from different N2MBW systems (Exhalyzer D vs. Profiler) incorporating different software for data analyses are not comparable in health and disease. This has important implications on longitudinal studies and normative values in general and on our longitudinal study in particular. We have also demonstrated that the Exhalyzer D had better discriminative power and intra-session repeatability than the Profiler, which favours its use in clinical settings and future clinical studies. However, the longer washout time especially in subjects with significant VI may limit the feasibility of MBW in the clinical settings. Preliminary evidence suggested the possibility of shortening the washout time by choosing a higher cut-off concentration earlier in the washout that does not compromise the sensitivity of N2MBW (Yammine, Singer et al. 2012). In contrast, another recent study recommended not shortening the washout in subjects with severe CF lung disease for better quantification of ventilation inhomogeneity at the conductive airways (Verbanck, Paiva et al. 2013).
Chapter 5 Longitudinal changes in LCI: A comparison with standard lung function tests in school-age children with CF

5.1 Rationale:

Evidence from pathological, physiological and imaging studies have shown that lung disease starts early in life in subjects with CF and early changes are more marked in the peripheral airways (Sobonya, Taussig 1986, Hamutcu 2002, Gustafsson, De Jong et al. 2008, Horsley, Macleod et al. 2008, Castile, Hayes et al. 2000, de Jong 2004, Bannier 2010). Sobonya and Taussig have demonstrated mucus plugs and dilation in the small airways in children and young teenagers, but there was stenosis of the small airways in older subjects (Sobonya, Taussig 1986). Therefore, sensitive, feasible, repeatable and safe markers of early involvement of peripheral airways are needed to slow the decline in lung function from early age. Standard lung function tests, chest radiographs and HRCT were either unable to detect early changes in the peripheral airways, or safety considerations preclude their use in longitudinal monitoring in children (Gustafsson 2003, Brody, Klein et al. 2004, Aurora, Kozlowska et al. 2005, Owens, Aurora et al. 2011, Gustafsson, De Jong et al. 2008). As an alternative, an index from multiple-breath washout tests, the lung clearance index (LCI), has been shown to be more sensitive than spirometry in detecting early functional changes within lung periphery and to be associated with structural abnormalities detected by HRCT (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Aurora 2004, Gustafsson, De Jong et al. 2008, Owens, Aurora et al. 2011). Washout tests have also been shown to be feasible in children and safe for repeated measurements needed to assess the progression of disease (Kraemer, Blum et al. 2005, Kieninger, Singer et al. 2011, Aurora 2011).

However, most of the evidence relating to MBW comes from cross-sectional studies while longitudinal studies, which generally have more statistical power and are more robust, are limited (Edwards 2000). Kraemer and colleagues were the first and the only group who evaluated the progression of lung disease in CF based on lung function parameters including LCI over a span of 6 to 20 years (Kraemer, Blum et al. 2005). They concluded that the progression of lung disease in CF is detected earliest by LCI. Two recent studies have suggested that there might be tracking of LCI from infancy and
preschool to school-age in some children with CF tested on two occasions (Kieninger, Singer et al. 2011, Aurora 2011).

Moreover, cross-sectional studies in children with CF have also shown that chronic infection with *Pseudomonas aeruginosa* (*P. aeruginosa*) is associated with a lower $FEV_1$, higher LCI and faster decline in lung function (Lee, Brownlee et al. 2003, Aurora 2005, Singer, Kieninger et al. 2013). However, there is a need for longitudinal follow-up of children both affected and unaffected with *P. aeruginosa* for validation of *P. aeruginosa* infection as a correlate of disease severity.

Lastly, LCI has also been shown to be sensitive as an assessment tool in gauging response to intravenous antibiotics by two studies performed in children with moderately severe impairments of lung function (Robinson 2009, Horsley, Davies et al. 2013). However, patients with mild airways disease were not well represented in these studies. Therefore, the significance of $N_2$MBW indices in assessing the response to IV antibiotic treatment in children with mild CF lung disease needs further investigation.

### 5.2 Aims:

1. To prospectively follow a cohort of school aged children with CF in order to monitor changes in LCI in comparison with conventional lung function measures, over a 4 year period.
2. To assess the associations over time between changes in lung function measurements with age, and potential risk factors such as *P. aeruginosa* infection and CFTR genotypes.
3. To assess the association over time between LCI and $FEV_1$, the gold standard measure for assessment of CF progression.
4. To assess the ability of LCI to detect the short-term response to intravenous antibiotics as compared to the gold standard $FEV_1$ in children with mild to moderate CF lung disease.

### 5.3 Hypotheses:

1. In school-age children with CF, LCI will be more sensitive to changes in the lung periphery over time than conventional lung function measurements.
2. There will be relationships between changes in lung function parameters with age and LCI will show earlier deterioration with age than conventional lung
function tests. Furthermore, the infection with *P. aeruginosa* will be associated with deterioration in lung function parameters over time whereas CF genotypes will not be associated with changes in lung function measurements.

3. In children with CF, changes in LCI will precede changes in FEV₁ over time.

4. In children with mild to moderate CF lung disease admitted for intravenous antibiotics treatment, LCI will improve prior to FEV₁.

Prior to tracking changes in LCI and conventional lung function measurements, and examining their relationships to each other in children with CF over time, it is necessary to look at the children’s baseline lung function. Before the longitudinal comparison, the cross-sectional association between LCI and conventional lung function parameters in health and disease needs to be explored. Furthermore, it is important to assess the repeatability and sensitivity of LCI in health and disease. We also need to investigate the effect of *P. aeruginosa* infection on lung function, initially and on subsequent visits.

5.4 Subjects and Methods:

5.4.1 Subjects and study design:

This is a prospective, longitudinal cohort study performed in school-aged children with mild to moderate CF lung disease to assess changes in ventilation distribution, flow limitation and lung volume over a 4 year period. Children with CF were tested when they were clinically stable as part of their annual review at Leicester Royal Infirmary (LRI) between the periods from January 2010 to December 2013. Healthy school-aged children were recruited from the Leicester Respiratory Cohorts and a Community Health Services Database as part of different research studies (details in Chapter 3, Section 3.2.2). The treatment protocol for children with CF did not change during the study period and children were prescribed standard aggressive treatment including airway clearance techniques and oral, nebulized or intravenous antibiotics depending on the nature and frequency of the bacterial isolate and the deterioration in lung function. Nebulized DNase was offered for most children once they reached 5 years of age. Prophylactic antibiotics were routinely prescribed for all children colonised with *P. aeruginosa* and whenever they had a run of respiratory infections. The classification of CF lung disease severity was based on baseline FEV₁ %predicted.
As part of this study, we assessed the ability of LCI in comparison to FEV\textsubscript{1} to detect the short-term response to IV antibiotics in a subset of children with CF admitted to the LRI between July 2012 and June 2013 for 2 to 3 weeks.

5.4.2 Data collection and analysis:

5.4.2.1 Longitudinal study:

Children with CF performed N\textsubscript{2}MBW tests annually over 4 years using the modified Medgraphics Profiler (Medical Product Service GmbH, Germany). Data from at least 2 washout tests were analysed using custom-built software developed by Ruslan Garipov based on (MATLAB (R2011b)) and reported according to the latest consensus statement for inert gas washout measurement (Robinson, Latzin et al. 2013). Spirometry and plethysmography were also performed annually in all children with CF with a Jaeger MasterScreen Body Plethysmography (Care Fusion GmbH, Leibnizstrasse, Germany). Data from both tests were reported according to the latest ATS/ERS recommendations (Miller, Crapo et al. 2005, Wanger, Clausen et al. 2005). Also, one set of all lung function data was obtained from school-age healthy children and used to define the upper limit of normality. Detailed description of subjects included, data collection and analysis were provided in Chapter 3.

For this study the specific targets were (1) the lung clearance index (LCI), (2) the indices of flow limitation (FEV\textsubscript{1}, FEF\textsubscript{25-75}, FEF\textsubscript{50} and FEF\textsubscript{75} z-scores), and (3) indices of lung volumes determined by whole-body plethysmography and N\textsubscript{2}MBW (RV/TLC, FRC\textsubscript{pleth} and FRC\textsubscript{N2} z-scores).

Induced sputum was also obtained from children with CF annually during their annual review. Sputum samples were also collected from children who could expectorate every 2 months at follow-up visit to CF clinic. For children who cannot expectorate cough swabs were obtained, and for children who expectorate rarely we encourage their parents to collect sputum samples at home. All samples were processed and cultured for various bacterial species including \textit{P. aeruginosa} at LRI according to the UK Cystic Fibrosis Trust Standards (The UK Cystic Fibrosis Trust Microbiology Laboratory Standards Working Group 2010). Patients were categorized at each annual review visit according to their \textit{P. aeruginosa} status over the previous 12 months on the basis of previously validated method (Lee et al. 2003)
5.4.2.2 Interventional cross-sectional study:

Children with CF admitted for intravenous antibiotics completed N₂MBW using the Exhalyzer D (Eco medics, Switzerland), and spirometry and plethysmography using the Jaeger MasterScreen. Measurements were made in triplicate within 24 hours of admission and two to three weeks later at the end of treatment. N₂MBW data were analysed using Spiroware 3.1.6. A detailed description of data collection and analysis was provided in Chapter 3.

For this cross-sectional study, changes in LCI and FEV₁ z-score before and after treatment were the primary outcome measures. Secondary outcome measures included differences in airflow limitation parameters (FEF₂₅₋₇₅ and FEF₇₅ z-scores) and plethysmography parameters (FRC₉₀pleth and RV/TLC z-scores) and FRC₉₀N₂ z-score.

The choice of intravenous antibiotics was made by the primary physician, based on sputum sensitivities and previous history of clinical improvement with particular antibiotic regimens. Each patient was reviewed by a specialist CF nurse and dietician on admission, and received physiotherapy daily during their hospital admission.

5.4.3 Statistical analysis:

5.4.3.1 Longitudinal Study:

Spirometry and plethysmography parameter z-scores were calculated from published reference equations (Stanojevic, Wade et al. 2008, Rosenthal, Bain et al. 1993). FRC₉₀N₂ z-scores were calculated from data obtained from 90 healthy school-age children and based upon regression of FRC₉₀N₂ against height (Appendix G, Table 1). Subjects with FEV₁, FEF₂₅₋₇₅ FEF₅₀ and FEF₇₅ z-scores less than -2 or FRC₉₀N₂, FRC₉₀pleth and RC/TLC z-scores more than +2 were classified as having an abnormal result.

LCI z-scores were calculated from data obtained from 90 healthy school-age children (section 5.5.1.12.1) and used for the longitudinal analysis only. This is because the use of fixed ULNs for assessments on single occasions in children >6 years of age may not have any significant clinical impact on interpretation as we shown in Chapter 4, and the evident from previously reported data in healthy school-age children (Lum, Stocks et al. 2013, Aurora 2005). Limits of Normality, defined as mean +/- 1.96 SD, were calculated for LCI from the control data, and subjects with values above the ULN were categorised as having an abnormal result.
Group means were compared by unpaired t-tests for parametric data and Mann-Whitney tests for non-parametric data. For these comparisons, a sample size of 22 subjects in each group was calculated to be sufficient to detect a difference of 1 SD in LCI and FEV₁ z-score between both populations with 90% power at the 5% significance level.

Within-test repeatability for LCI was determined by calculating the coefficients of variation (CV% = SD/mean x100). Sensitivity and specificity of VI indices for CF lung disease were determined by calculation of the area under Receiver-Operator Characteristic (ROC) curves. A measure that discriminates between two groups with perfect sensitivity and specificity will have an AUC of 1.0. A measure that does not discriminate at all will have an AUC of 0.5. The ULN for LCI derived from 90 healthy school-age children was used as a cut-off point to discriminate between health and disease.

We assessed the relationship between LCI and conventional lung function measures in both healthy children and children with CF using both correlation (Pearson or Spearman (r)) and linear regression models (r²). A p-value below 0.05 was regarded as statistically significant.

We compared LCI and conventional lung function results obtained at the baseline visit from children with CF, either infected or uninfected with *P. aeruginosa* in the preceding 12 months, using unpaired t-tests for parametric data and Mann-Whitney tests for non-parametric data.

To assess longitudinal changes in lung function over time and to assess the association with age, *P. aeruginosa* and CFTR genotypes, advanced statistical approaches were used. These were univariate and multivariate linear mixed-model (LMM) regression analyses. These statistical tests can deal with data obtained at irregular time intervals and with incomplete data sets due to missing scheduled visits (Edwards 2000).

Finally, the strength of agreement between LCI and FEV₁ over time was assessed using Kappa agreement test. If there are agreements between parameters, Kappa value will be 1, or 100%. If there are no agreements, the observed value will be zero (Viera, Garrett 2005). A p-value of <0.05 was accepted as statistically significant. The statistical
analyses were performed using the Statistical Package for the Social Sciences version 18 (SPSS Inc., Chicago, IL, USA) and Prism5.

5.4.3.2 Interventional cross-sectional study:

Differences in lung function parameters before and after intravenous antibiotics were compared using paired t-tests or Wilcoxon signed rank tests. Clinically meaningful thresholds of 7% and 10% change in LCI and FEV\textsubscript{1} were generated using repeatability data from our CF population and currently available published data (Cooper, Robertson et al. 1990, Gozal, Bailey et al. 1993, Robinson 2009, Fuchs, Ellemunter et al. 2012).

Our estimate of the sample size for this study was based on LCI values from our healthy population with mean (SD) LCI of 6.3 (0.7). Therefore, to detect a minimum difference of one SD in LCI pre and post IV therapy with a power of 80% at a two sided 5% significance level, 16 children would be needed to complete the study. The mean value of LCI derived from at least two baseline N\textsubscript{2}MBW and two N\textsubscript{2}MBW tests after treatment was used for analysis.

5.5 Results

5.5.1 Longitudinal study results:

5.5.1.1 Subject characteristics:

Children with CF:

Thirty-seven school-age children with CF were included in our cohort study; 18(49%) subjects were homozygotes (ΔF508), 12(32%) were heterozygotes (ΔF508/other), 4(11%) were mixed genotype (other/other) and 3(8%) unknown. Thirty-four (92%) were pancreatic insufficient.

Of the 37 children, 34 (92%) attended on two consecutive study visits and of those 30 (88%) completed all lung function tests including N\textsubscript{2}MBW (26 on the profiler; 4 on the Exhalyzer D). The washout test was not available for 2 subjects on the day of testing and 2 subjects performed poor quality washouts. The main reasons for failure to complete N\textsubscript{2}MBW tests were unstable breathing patterns with leaks.

On the third study visit, 28 (76%) children attended. Of those 25 (89%) children were able to complete all lung function including N\textsubscript{2}MBW (14 on the profiler; 11 on the
Exhalyzer D). The washout test was not available for 2 subjects and one child refused to do the test.

On the final study visit, 14 (38%) children attended and 13 (93%) completed all lung function tests including N₂MBW (5 on the profiler; 8 on the Exhalyzer D). One child refused to do the N₂MBW test. A detailed diagram of subjects included and excluded from the longitudinal study was presented in Chapter 3, Section 3.2.1.

Over the whole study period, 27 children were tested using the same N₂MBW equipment (modified Medgraphics profiler) on two or more test occasions. Therefore, they have been included in the final analysis. Characteristics of the study population at the start of the study are summarized in Table 5.1.

<table>
<thead>
<tr>
<th></th>
<th>Whole CF population (n=37)</th>
<th>Children tested on&gt;2 occasions (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>51%</td>
<td>44%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8 (3.6)</td>
<td>10.4 (3.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139 (17.8)</td>
<td>137.3 (15.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>36.7 (14.5)</td>
<td>34.2 (11.6)</td>
</tr>
<tr>
<td>BMI</td>
<td>18.2 (2.8)</td>
<td>17.6 (2.3)</td>
</tr>
</tbody>
</table>

_Data are presented as mean (SD) unless otherwise stated._

**Healthy children:**

Ninety healthy school-age children were included in this study. Of those 24 children were recruited during the study period (between 2010 and 2013) and data from 66 children were available from previous work conducted in our laboratory (between 2007 and 2010) (Narayanan, Owers-Bradley et al. 2012). We have re-analysed the data collected previously to eliminate any observer bias in data analysis which might affects comparability to our own data. A statistical comparison between the two sets of healthy controls data is presented in Appendix G, Table 2.

Table 5.2 shows the demographics of healthy controls included in the study in comparison to children with CF. CF and controls were well matched for gender.
However, children with CF were significantly younger, lighter and shorter than healthy controls.

Table 5.2 Demographics of healthy controls in comparison to children with CF

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=90)</th>
<th>CF (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>45%</td>
<td>51%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14.3 (7.8, 17.1)</td>
<td>9.7 (5.9, 17.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (122.6, 190.1)</td>
<td>134.5 (112.5, 177.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.7 (22.5, 93.0)</td>
<td>30.9 (20.4, 71.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.2 (14.4, 31.3)</td>
<td>17.6 (13.8, 24.8)</td>
</tr>
</tbody>
</table>

Data are presented as median and ranges unless otherwise stated.

5.5.1.2 Comparison of conventional lung function results for healthy children and children with CF:

Spirometry:

All children with CF and healthy controls successfully completed spirometry measurements. A comparison of conventional lung function measurements for healthy children and children with CF at the baseline visit is presented in Table 5.3. There were statistically significant differences in all spirometric measurements between healthy children and children with CF (P<0.001). Spirometry measurements were reduced in children with CF compared to healthy children.
Table 5.3 Comparison of spirometric results in healthy children and children with CF at baseline visit

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=90)</th>
<th>CF (n=37)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FEV_1$ z-score</td>
<td>-0.02 (1.01)</td>
<td>-1.07 (1.5)</td>
<td>-1.1 (-1.6 to -0.5)*</td>
</tr>
<tr>
<td>$FEV_1$ % predicted</td>
<td>99.6 (11.9)</td>
<td>87.1 (18.1)</td>
<td>-12.5 (-19.0 to -6.1)*</td>
</tr>
<tr>
<td>$FEV_1/FVC$ %</td>
<td>86.6 (5.4)</td>
<td>78.2 (8.5)</td>
<td>-8.3 (-11.4 to -5.3)*</td>
</tr>
<tr>
<td>$FEF_{25-75}$ z-score</td>
<td>-0.47 (1.09)</td>
<td>-1.84 (1.47)</td>
<td>-1.4 (-1.8 to -0.9)*</td>
</tr>
<tr>
<td>$FEF_{50}$ z-score</td>
<td>-0.49 (0.9)</td>
<td>-1.04 (1.13)</td>
<td>0.5 (0.2 to 0.9)*</td>
</tr>
<tr>
<td>$FEF_{75}$ z-score</td>
<td>-0.23 (0.97)</td>
<td>-1.44 (1.6)</td>
<td>-1.2 (-1.8 to -0.6)*</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) and mean difference 95% confidence interval. *P-value<0.001.

Plethysmography:

All children with CF successfully completed plethysmography measurements. In healthy controls, we performed plethysmography measurements successfully for 76/90 (84%) subjects whereas the test was not available for 14 subjects. A comparison of plethysmographic measurements for healthy children and children with CF at the baseline visit is presented in Table 5.4. There were statistically significant differences in $FRC_{pleth}$ z-score, and $RV/TLC_z$-score between children with CF and healthy controls (p<0.0001).

Table 5.4 Comparison between plethysmographic results in healthy children and children with CF at baseline visit

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=76)</th>
<th>CF (n=37)</th>
<th>Mean difference (95% CI for difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$FRC_{pleth}$ z-score</td>
<td>-0.42 (0.98)</td>
<td>0.39 (1.13)</td>
<td>0.8 (0.39 to 1.21)*</td>
</tr>
<tr>
<td>$RV/TLC$ z-score</td>
<td>0.49 (1.21)</td>
<td>1.94 (1.52)</td>
<td>1.45 (0.92 to 1.92)*</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD) and mean difference 95% confidence interval. *P-value<0.0001.
5.5.1.3 \textit{N}_2\textit{MBW success rate and within occasion repeatability in healthy children and children with CF at baseline visit:}

All children with CF successfully completed \textit{N}_2\textit{MBW} measurements \textbf{on the first laboratory visit}. Twenty-eight (76\%) children completed 3 technically acceptable washout tests and the remaining 9 (24\%) children completed only 2 technically acceptable washout tests.

All healthy children successfully completed \textit{N}_2\textit{MBW} measurements, 71/90 (78\%) completed 3 technically acceptable washout tests. Nineteen children (21\%) had only two technically acceptable measurements.

For all subjects, the main reasons for inability to complete 3 technically acceptable washout tests, thereby necessitating the exclusion of at least one test, were either volume drift during the washout, irregular breathing pattern with leaks, a premature end to the test, or the resultant FRC differed by >25\% from the median FRC value across the three tests.

The repeatability of FRC and LCI was good in healthy children and children with CF (Table 5.5). In children with CF, only six (16\%) subjects had a coefficient of variability in FRC and LCI>10\%.

\textbf{Table 5.5 Within-occasion repeatability of \textit{N}_2\textit{MBW} indices in healthy children and children with CF at baseline visit}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & \textbf{Healthy controls} & \textbf{CF (n=37)} \\
 & (n=90) & \\
\hline
\textbf{FRC}_{\textit{N}_2} (CV \%) & 7.9 (5.5) & 7.3 (3.8) \\
 & 6.6 (3.7,10.9) & 6.8 (5.1, 9.3) \\
\hline
\textbf{LCI} (CV \%) & 6.7 (4.1) & 7.1 (4.8) \\
 & 6.6 (4.0, 8.7) & 7.15 (3.1, 8.9) \\
\hline
\end{tabular}
\end{table}

\textit{Data are presented as mean (SD) of CV\% and median (25\%-75\% Interquartile range).}
### 5.5.1.4 Comparison between N₂MBW results in healthy children and children with CF at baseline visit:

There were statistically significant differences in LCI between healthy children and children with CF. Children with CF had significantly higher values compared to healthy children (Table 5.6). Based on data from healthy children, the upper limit of normality (ULN) for LCI was calculated as 7.73 (based on mean LCI + 1.96 SD from control subjects), and the lower limit of normality (LLN) was 4.84.

**Table 5.6 Comparison between N₂MBW results in healthy children and children with CF at baseline visit**

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=90)</th>
<th>CF (n=37)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRC N₂z-score</td>
<td>0.13 (1.0)</td>
<td>-0.14 (0.89)</td>
<td>0.152</td>
</tr>
<tr>
<td>LCI</td>
<td>6.2 (5.1, 8.3)</td>
<td>11.1 (8.1, 16.2)</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD) or median (ranges). * Statistically significant difference.
5.5.1.5 Sensitivity and specificity of LCI for CF lung disease:

The Receiver-Operator Characteristic (ROC) curve for LCI is presented in Figure 5.1.

Figure 5.1 Receiver-Operator Characteristic (ROC) curve for LCI for school-age children

Legend: The outcome variable is the diagnosis of cystic fibrosis. It appears that LCI is a good discriminator between health and CF lung disease with high sensitivity and area under the ROC curve.

The area under ROC curve (AUC \( \text{ROC} \)) for LCI is presented in Table 5.7. The upper limit of abnormality for LCI (7.73) derived from 90 healthy school-age children was used as a cut-off point to discriminate between health and disease. Sensitivity and specificity for LCI was calculated based on this cut-off point and is presented in Table 5.7. LCI was sensitive at discriminating CF from control children and had large AUC \( \text{ROC} \).

Table 5.7 Sensitivity, specificity and area under the ROC curve for LCI

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC ( \text{ROC} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>100%</td>
<td>94.5%</td>
<td>0.999</td>
</tr>
</tbody>
</table>

The outcome variable is the diagnosis of cystic fibrosis. The upper limit of normality for LCI was used for calculation of sensitivity and specificity. \( AUC_{\text{ROC}} = \text{Area under the Receiver-Operator Characteristic curve} \).
5.5.1.6 Relationship between LCI and spirometry parameters in healthy children and children with CF at baseline visit:

The relationship between LCI and FEV$_1$, FEV$_{25-75}$ and FEF$_{75}$ z-scores:

The relationships between LCI and spirometry parameters for healthy children and children with CF are demonstrated in Table 5.8. In children with CF, there was a statistically significant negative relationship between LCI and FEV$_1$z-score ($r^2=0.138$, $r=-0.37$, $p=0.023$). No significant relationships were found between LCI and FEV$_{25-75}$, FEF$_{50}$ and FEF$_{75}$ z-scores. In healthy children, there was a borderline significant negative relationship between LCI and FEV$_{75}$z-score only ($r^2=0.03$, $r=-0.211$, $p=0.045$).

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>-0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>-0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>FEF$_{50}$ z-score</td>
<td>-0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>FEF$_{75}$ z-score</td>
<td>-0.21</td>
<td>0.045*</td>
</tr>
</tbody>
</table>

$r=\text{correlation coefficient. *Statistically significant correlation.}$

Table 5.8 Correlation between LCI and spirometry parameters

Figure 5.2 shows that 29 of 37 (78%) children with CF had a FEV$_1$z-score within the normal range, 19 (51%) had normal FEV$_{25-75}$z-scores, 32 (86%) had normal FEF$_{50}$ z-scores and 26 (70%) had normal FEV$_{75}$z-scores. In comparison, all children with CF had abnormal LCI.
Figure 5.2 The relationships between LCI and FEV₁, FEV₂₅₋₇₅, FEF₅₀ and FEF₇₅ z-scores in healthy children and children with CF at baseline visit.
Legend: Control children presented as triangles, and children with CF presented as circles. Vertical broken line represents lower limit of normality for spirometry parameters. Horizontal broken line represents upper limit of normality for LCI. Note the significant negative relationship between LCI and FEV\textsubscript{1z}-score in children with CF and the significant relationship between LCI and FEF\textsubscript{75 z-score} in healthy children (see text for r\textsuperscript{2}, r and p-values).

5.5.1.7 Relationship between LCI and plethysmography parameters at baseline visit:

The relationships between LCI and plethysmography parameters in healthy children and children with CF are shown in Table 5.9. In children with CF, there was a significant positive relationship between LCI and RV/TLC z-score (r\textsuperscript{2}=0.195, r=0.44, p<0.01), whereas no significant association was found between LCI and FRC\textsubscript{pleth} z-score in either group.

<table>
<thead>
<tr>
<th>Table 5.9 Correlation between LCI and plethysmography parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy controls</strong></td>
</tr>
<tr>
<td><strong>Subjects with CF</strong></td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
</tr>
<tr>
<td>FRC\textsubscript{pleth} z-score</td>
</tr>
</tbody>
</table>

r = correlation coefficient. *Statistically significant correlation.
Air trapping as represented by increasing FRC_{pleth} z-score >2 SD was present in 3 (8%) children with CF whereas pulmonary hyperinflation represented as increasing RV/TLC z-score by >2 SD was present in 15 (41%) children with CF. All children with airtrapping or pulmonary hyperinflation had LCI \geq 10 (Figure 5.3).

**Figure 5.3** The relationships between LCI and RV/TLC z-score and FRC_{pleth} z-score in healthy children and children with CF at baseline visit

Legend: Control children presented as triangles, and children with CF presented as circles. Horizontal broken lines represent upper limit of normality for LCI and vertical broken lines represents upper limits of normality for plethysmography parameters. Note the significant positive relationships between LCI and RV/TLC z-score in children with CF (see text for r^2, r and p-values). Note different scales on X-axes.
5.5.1.8 Comparison of \( \text{N}_2 \text{MBW} \) and conventional lung function results for children with CF infected and non-infected with \( P. \text{aeruginosa} \) at baseline visit:

All children with CF had cough swabs and/or sputum samples taken every 2 months at regular clinic visits as well as during their annual reviews. A minimum of 6 samples were available for each child annually. During the study period, \( P. \text{aeruginosa} \) was never cultured in 6 (16%) children with CF whereas 31 (84%) children were infected at different times.

The prevalence of \( P. \text{aeruginosa} \) infection in the 31 children with CF was defined on each annual lung function measurements visits as either ‘chronic’ when more than 50% of the preceding 12 months were \( P. \text{aeruginosa} \) culture positive, ‘intermittent’ when \( \leq 50\% \) of the preceding 12 months were \( P. \text{aeruginosa} \) culture positive, or ‘free of \( P. \text{aeruginosa} \)’, if there was no growth of \( P. \text{aeruginosa} \) during the previous 12 months.

On the baseline visit, 6 children with CF were never infected with \( P. \text{aeruginosa} \), 17 children were free of infection, 12 had intermittent infection and 2 were chronically infected. A comparison of lung function results by the presence and prevalence of \( P. \text{aeruginosa} \) infection is presented in Table 5.10.
Table 5.10 Comparison of lung function results obtained at baseline visit from 37 children with CF classified according to their *P. aeruginosa* infection status in the preceding 12 months

<table>
<thead>
<tr>
<th>Baseline visit</th>
<th>Not-infected with <em>P. aeruginosa</em></th>
<th>Infected with <em>P. aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never (n=6)</td>
<td>Free (n=17)</td>
</tr>
<tr>
<td>Sex (% Male)</td>
<td>33%</td>
<td>47%</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>6.9 (5.8, 9.9)</td>
<td>10.2 (6.4, 17.5)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>122.8 (112.5,130.1)</td>
<td>133.9 (113.4,177.0)</td>
</tr>
<tr>
<td>FEV₁z-score</td>
<td>-0.49 (-1.79,0.05)</td>
<td>-0.47 (-4.3-1.7)</td>
</tr>
<tr>
<td>FEF₂₅-₇₅ z-score</td>
<td>-1.51 (-2.25,-0.76)</td>
<td>-0.84 (-5.1,1.2)</td>
</tr>
<tr>
<td>FEF₅₀ z-score</td>
<td>-1.39 (-1.68, -0.06)</td>
<td>-0.64 (-3.1, 1.84)</td>
</tr>
<tr>
<td>FEF₇₅ z-score</td>
<td>-1.35 (-1.77, -0.78)</td>
<td>-0.56 (-4.33,4.04)</td>
</tr>
<tr>
<td>FRCplethz-score</td>
<td>-0.49 (-1.5-0.68)</td>
<td>0.12 (-2.14,2.58)</td>
</tr>
<tr>
<td>FRCN₂z-score</td>
<td>-0.52 (-0.07, -0.19)</td>
<td>-0.38 (-1.07,-0.07)</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>1.78 (0.05-3.88)</td>
<td>1.47 (-1.22-5.37)</td>
</tr>
<tr>
<td>LCI</td>
<td>11.04 (9.5-12.5)</td>
<td>10.9 (8.6-11.9)</td>
</tr>
</tbody>
</table>

Data presented as median (ranges). Bold values indicate statistically significant differences between groups.
The numbers of children never infected with *P. aeruginosa* and chronically infected with *P. aeruginosa* were too small to perform comparison between all four groups and draw firm conclusions, therefore, we compared lung function results between the non-infected group with *P. aeruginosa* (never infected children + children free of infection, n=23) and the group infected with *P. aeruginosa* (chronically and intermittently infected children, n=14).

No age or height differences were found between the two groups, but the proportion of boys in the infected group with *P. aeruginosa* was higher compared to non-infected group. Children infected with *P. aeruginosa* in the previous 12 months had a significantly lower FEF\textsubscript{25-75} z-score (mean difference (95%CI) = 0.97, 0.11 to 1.8, p=0.028), FEF\textsubscript{50} z-score (mean difference= 0.82, 0.17 to 1.5, p=0.015) and FEF\textsubscript{75} z-score (mean difference (95%CI) = 1.29, 0.24 to 2.33, p=0.018), and significantly higher LCI (mean difference (95%CI) = -1.26, -2.5 to -0.01, p=0.048), than those not infected with this organism (Figure 5.4).
Figure 5.4 Comparison of lung function results obtained from 37 children with CF classified according to their *P. aeruginosa* infection status in the preceding 12 month to their baseline lung function visit.
Legend: Median and ranges of LCI, $\text{FEF}_{25\text{-}75}$ z-score, $\text{FEF}_{50}$ z-score and $\text{FEF}_{75}$ z-score obtained at baseline visit from 37 children with CF. Children infected with Pseudomonas aeruginosa in the preceding 12 months of their lung function visit have significantly lower $\text{FEF}_{25\text{-}75}$ z-score, $\text{FEF}_{50}$ z-score and $\text{FEF}_{75}$ z-score, and significantly higher LCI than those not infected with this organism.
5.5.1.9 Longitudinal changes in LCI and conventional lung function measurements over a 4 year period:

Over the study period, ventilation inhomogeneity measurements which are the primary outcome measures were obtained using the same N₂MBW system (modified Medgraphics Profiler) from 27/37 (73%) children with CF on two or more study visits.

Ventilation inhomogeneity measurements were obtained from 27 children on the 1<sup>st</sup> visit, 26 children on the 2<sup>nd</sup> visit, 14 children on the 3<sup>rd</sup> visit and from 5 children on the 4<sup>th</sup> study visit. A total of 72 VI measurements were obtained from all children over the study period.

Conventional lung function measurements were obtained from all 27 children with CF on the 1<sup>st</sup> and 2<sup>nd</sup> visits whereas on the 3<sup>rd</sup> visit 21/27 attended (one child did not attend, 4 transferred to adult clinic and one child moved to a different city). On the 4<sup>th</sup> visit, conventional lung function measurements were obtained from 13/27 (5 transferred to adult clinic, one child moved to a different city and 8 children are due to be tested end of 2014). A total of 88 conventional lung function measurements were obtained from all 27 children over the study period.

The average interval between visits was 14.2 (3.9) months between 1<sup>st</sup> and 2<sup>nd</sup> visit, 12.8 (2.5) months between 2<sup>nd</sup> and 3<sup>rd</sup> visit, and 11.8 (2.1) months between 3<sup>rd</sup> and 4<sup>th</sup> visit. Clinical characteristics of children with CF included in this study are summarized in Table 5.11.
Table 5.11 Clinical characteristics of study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children with CF tested on &gt;1 study visit (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender (% M)</td>
</tr>
<tr>
<td></td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>CF genotype:∆F508/∆F508</td>
</tr>
<tr>
<td></td>
<td>11 (40%)</td>
</tr>
<tr>
<td></td>
<td>∆F508/other</td>
</tr>
<tr>
<td></td>
<td>11 (40%)</td>
</tr>
<tr>
<td></td>
<td>Other/other</td>
</tr>
<tr>
<td></td>
<td>5 (19%)</td>
</tr>
<tr>
<td></td>
<td>Lung function visit no. (number of children included)</td>
</tr>
<tr>
<td></td>
<td>Visit1 (n=27)</td>
</tr>
<tr>
<td></td>
<td>Visit2 (n=27)</td>
</tr>
<tr>
<td></td>
<td>Visit3 (n=21)</td>
</tr>
<tr>
<td></td>
<td>Visit4 (n=13)</td>
</tr>
<tr>
<td></td>
<td>Age (years)</td>
</tr>
<tr>
<td></td>
<td>10.4 (3.3)</td>
</tr>
<tr>
<td></td>
<td>11.6 (3.3)</td>
</tr>
<tr>
<td></td>
<td>11.7 (2.3)</td>
</tr>
<tr>
<td></td>
<td>12.8 (2.6)</td>
</tr>
<tr>
<td></td>
<td>Weight (kg)</td>
</tr>
<tr>
<td></td>
<td>34.3 (11.6)</td>
</tr>
<tr>
<td></td>
<td>38.5 (12.3)</td>
</tr>
<tr>
<td></td>
<td>40.3 (12.4)</td>
</tr>
<tr>
<td></td>
<td>43.5 (10.6)</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
</tr>
<tr>
<td></td>
<td>137.5 (16.1)</td>
</tr>
<tr>
<td></td>
<td>143.1 (14.6)</td>
</tr>
<tr>
<td></td>
<td>147.8 (13.8)</td>
</tr>
<tr>
<td></td>
<td>152.3 (11.2)</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
</tr>
<tr>
<td></td>
<td>17.6 (2.3)</td>
</tr>
<tr>
<td></td>
<td>18.3 (2.8)</td>
</tr>
<tr>
<td></td>
<td>18.1 (2.7)</td>
</tr>
<tr>
<td></td>
<td>18.5 (2.3)</td>
</tr>
<tr>
<td></td>
<td>CF pathogen, n (%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10 (37%)</td>
</tr>
<tr>
<td></td>
<td>12 (44%)</td>
</tr>
<tr>
<td></td>
<td>10 (37%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>13 (48%)</td>
</tr>
<tr>
<td></td>
<td>15 (55%)</td>
</tr>
<tr>
<td></td>
<td>9 (43%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>8 (38%)</td>
</tr>
<tr>
<td></td>
<td>10 (48%)</td>
</tr>
<tr>
<td></td>
<td>1 (5%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mycobacterium abscessus</td>
<td>9 (69%)</td>
</tr>
<tr>
<td></td>
<td>3 (23%)</td>
</tr>
<tr>
<td></td>
<td>3 (23%)</td>
</tr>
<tr>
<td></td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>3 (11%)</td>
</tr>
<tr>
<td></td>
<td>3 (11%)</td>
</tr>
<tr>
<td></td>
<td>1 (5%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Achromobacter</td>
<td>2 (7%)</td>
</tr>
<tr>
<td></td>
<td>2 (7%)</td>
</tr>
<tr>
<td></td>
<td>1 (5%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>2 (7%)</td>
</tr>
<tr>
<td></td>
<td>2 (7%)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Haemophilus influenzae type B</td>
<td>9 (33%)</td>
</tr>
<tr>
<td></td>
<td>5 (19%)</td>
</tr>
<tr>
<td></td>
<td>2 (10%)</td>
</tr>
<tr>
<td></td>
<td>2 (15%)</td>
</tr>
</tbody>
</table>

Data presented as mean (SD) or number (percent) unless otherwise noted. CF pathogen data were obtained during the preceding 12 months of each annual lung function measurement visits.
5.5.1.9.1 Changes in LCI and conventional lung function measurements over time:

As the first step in these analyses, z-scores for LCI were calculated from data obtained from 90 healthy school-age children (Appendix G). They were based upon regression of LCI against height and weight (Table 5.12).

Table 5.12 Regression equations for VI indices

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>11.609</td>
<td>Ht. -0.04, Wt. 0.018</td>
<td>0.007</td>
<td>0.596</td>
</tr>
</tbody>
</table>

Legend: SE (B) = standard error of the coefficient.

RSD = residual standard deviation for the regression.

This table allows calculation of LCI z-scores as follows:

*Predicted value for LCI can be obtained from the equation = Constant + (height (cm)*B) + (weight (kg)*B)*

*Standard deviation score (z-scores) for LCI can be calculated from the equation = (observed value – Predicted value)/ RSD*

*Addition of subject sex and age to LCI model had no significant effect upon model fit.*
Changes in LCI and conventional lung function measurements over time:

Linear mixed model analysis (LMM) was used to provide reliable estimates of both changes in each lung function measure over time and the association with age, *P. aeruginosa infection* and CFTR genotypes. It was preferred over repeated measures ANOVA because of its advantage to deal with missing values, and data obtained at irregular time intervals.

As shown in Table 5.13, the highest change over time was found for LCI (F=30.1, p<0.0001), and FRC\textsubscript{N2} (18.5, p<0.0001). This was followed by FEF\textsubscript{50} z-score, RV/TLC z-score, FEF\textsubscript{25-75} z-score and FEV\textsubscript{1} z-score respectively. Apart from spirometry parameters; LCI and RV/TLC showed improvement over time. FRC\textsubscript{N2} increased over time, but remained within normal limits in majority of the children. Changes in lung function parameters over time are shown in Figure 5.5.

**Table 5.13: Changes in lung function over time in school-age children with CF**

<table>
<thead>
<tr>
<th>Univariate LMM analysis</th>
<th>F-value</th>
<th>Estimate of annual changes</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV\textsubscript{1}z-score</td>
<td>4.6</td>
<td>-0.14</td>
<td>0.046*</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75} z-score</td>
<td>5.6</td>
<td>-0.16</td>
<td>0.03*</td>
</tr>
<tr>
<td>FEF\textsubscript{50} z-score</td>
<td>7.4</td>
<td>-0.14</td>
<td>0.01*</td>
</tr>
<tr>
<td>FEF\textsubscript{75} z-score</td>
<td>3.3</td>
<td>-0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>FRC\textsubscript{pleth}z-score</td>
<td>0.91</td>
<td>-0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>FRC\textsubscript{N2}z-score</td>
<td>18.5</td>
<td>0.49</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>RV/TLCz-score</td>
<td>6.0</td>
<td>-0.29</td>
<td>0.017*</td>
</tr>
<tr>
<td>LCI z-score</td>
<td>30.1</td>
<td>-1.42</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

In this model, each lung function parameter was considered as dependent variable and visit number as a covariate with fixed and random effects. This model assumed that each lung function measure follows a linear regression versus time for each child with random child-specific slope and intercept, and then provided estimates of the mean intercept and slope for all children over time. Bold values indicate statistically significant change over time in the lung function parameter.
Figure 5.5 Changes in LCI and conventional lung function measurements over a 4 year period
Legend: Changes in VI indices and conventional lung function parameters over a 4 year period in school-age children with CF. Horizontal broken lines represent the lower limits of normality for spirometry parameters and the upper limits of normality for plethysmography and LCI. Note significant changes in all parameters over time except for FEF$_{75}$ and FRC$_{pleth}$z-scores (see table for $p$-values). Note different scales on Y-axes.
The association between changes in lung function measurements with age, *P. aeruginosa* infection and CFTR genotypes:

During the study period, 19 children (70%) were infected with an increased *P. aeruginosa* intermittently. Associations of lung function measurements with age, *P. aeruginosa* infection status and CFTR genotypes have been evaluated by multivariate LMM analysis and given in Table 5.14. We found that changes in some lung function parameters were associated with age and *P. aeruginosa* infection, but not with CFTR genotypes.

Table 5.14 Associations between changes in lung function parameters with age, the status of *P. aeruginosa* infection during study period and CFTR genotypes

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>Associations with</th>
<th>Time</th>
<th>Age</th>
<th><em>P. aeruginosa</em> infection status over study period</th>
<th>CFTR genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Significance (p-value)</td>
<td>Significance (p-value)</td>
<td>Significance (p-value)</td>
<td>Significance (p-value)</td>
</tr>
<tr>
<td>FEV(_1) z-score</td>
<td>2.6 0.11</td>
<td>18.4 0.000</td>
<td>0.84 0.36</td>
<td>1.2 0.29</td>
<td></td>
</tr>
<tr>
<td>FEF(_{25-75}) z-score</td>
<td>1.2 0.31</td>
<td>13.3 0.001</td>
<td>0.44 0.51</td>
<td>1.8 0.19</td>
<td></td>
</tr>
<tr>
<td>FEF(_{50}) z-score</td>
<td>0.08 0.78</td>
<td>8.4 0.007</td>
<td>0.79 0.38</td>
<td>1.9 0.17</td>
<td></td>
</tr>
<tr>
<td>FEF(_{75}) z-score</td>
<td>2.3 0.14</td>
<td>15.8 0.000</td>
<td>0.47 0.49</td>
<td>1.8 0.19</td>
<td></td>
</tr>
<tr>
<td>FRC(_{\text{pleth}}) z-score</td>
<td>5.6 0.024</td>
<td>3.7 0.065</td>
<td>9.1 0.004</td>
<td>0.09 0.76</td>
<td></td>
</tr>
<tr>
<td>FRC(_{\text{N2}}) z-score</td>
<td>18.7 0.000</td>
<td>2.0 0.166</td>
<td>0.001 0.97</td>
<td>0.73 0.40</td>
<td></td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>10.4 0.001</td>
<td>10.7 0.002</td>
<td>1.2 0.28</td>
<td>0.27 0.61</td>
<td></td>
</tr>
<tr>
<td>LCI z-score</td>
<td>43.0 0.000</td>
<td>6.5 0.017</td>
<td>2.4 0.135</td>
<td>0.8 0.38</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant values are highlighted in Bold. Using multivariate LMM analysis, each lung function parameters was entered as the dependent variable. Visit number and age were covariates. *Pseudomonas aeruginosa* infection status during study period (zero=no infection, 1=infected) and genotypes (zero= homozygous genotype (ΔF508), 1=heterozygous genotypes) were entered as factors.
Association between changes in lung function parameters with *P. aeruginosa*:

We found that intermittent infection with *P. aeruginosa* is associated with an increased FRC<sub>pleth</sub> z-score (F=9.1, p<0.01). Over the study period, children infected with *P. aeruginosa* showed statistically higher FRC<sub>pleth</sub> z-score (P<0.01) compared to non-infected children (Figure 5.11).

**Figure 5.6 Changes in FRC<sub>pleth</sub>z-score over time in children with CF infected and non-infected with *P. aeruginosa***

![Graph showing changes in FRC<sub>pleth</sub> z-score over time](image)

Legend: Over study period, a significant association was found between FRC<sub>pleth</sub> z-score and intermittent infection with *P. aeruginosa* (p<0.01). Children infected with *P. aeruginosa* showed higher FRC<sub>pleth</sub> z-scores compared to non-infected children.
Association between lung function parameters with age:

Results from multivariate LMM analysis showed significant association between age and all spirometry parameters, RV/TLC z-score and LCI in children with CF. The highest F value was found for FEV$_1$ z-score (F=18.4, P<0.0001). However, no associations were found between age and FRC$_{pleth}$ and FRC$_{N2}$ z-scores in children with CF.

Looking further at the age of occurrence of abnormal lung function, defined as the age when lung function data of two consecutive years were outside the normal predicted range of two standard deviations of the z-score, we found that all the above lung function parameters started to deteriorate and become abnormal at the age of ≥10 years, except LCI z-score (Figure 5.7). LCI z-score was abnormal in all children by the time of their 7$^{th}$ birthday. It was followed by FEF$_{25\text{-}75}$ z-score and RV/TLC z-score (mean age of occurrence 11 years). FEF$_{75}$ started to deteriorate after the age of 12 years, whereas for FEV$_1$ and FEF$_{50}$ z-scores, deterioration began after the age of 13 years.
Figure 5.7 Changes in lung function parameters with age in 27 children with CF

Legend: The means ±SEM of repeated annual lung function measurements from 27 children with CF plotted against age. Horizontal broken lines represent lower limits of normality for spirometry parameters, and the upper limits of normality for plethysmography and LCI. Note different scales on Y-axes.
5.5.1.9.3 The association between LCI and FEV\textsubscript{1} over time:

Our regression model showed that FEV\textsubscript{1} is one of the best predictors of progression in CF; in comparison LCI was the earliest to show abnormal changes. In our population, there was a statistically significant negative relationship between LCI and FEV\textsubscript{1} z-score over time ($r^2=0.25$, $r=-0.49$, $p<0.0001$). However, the agreement between FEV\textsubscript{1} and LCI (co-normal and co-abnormal) was found in only 30 (42\%) of the 72 complete lung function measurements obtained from 27 school-age children with CF over the study period (Figure 5.8). When FEV\textsubscript{1} was normal (within 2 z-scores), LCI detected abnormal lung function (undetected by FEV\textsubscript{1}) in 58\%; when FEV\textsubscript{1} was abnormal (> -2 z-scores), there were no cases undetected by LCI.

Using the Kappa agreement test, the strength of agreement between LCI and FEV\textsubscript{1} was poor with Kappa of 0.098 (SE 0.038, 95\%CI: 0.024 to 0.17).

**Figure 5.8** Association between LCI and FEV\textsubscript{1} z-scores over time in school-age children with CF

Legend: This figure demonstrate 72 complete lung function measurements obtained from 27 school-age children with CF over a 4 year period. The horizontal broken line represents the ULN for LCI and vertical line represents the LLN for FEV\textsubscript{1} z-score. There was a negative relationship between LCI and FEV\textsubscript{1}. The linear regression of LCI vs. FEV\textsubscript{1} z-score was ($r^2=0.25$, $p<0.0001$) and is represented by the diagonal line. The agreement between FEV\textsubscript{1} and LCI (co-normal and co-abnormal) was found in only 42\% of the measurements obtained from children with CF over study period.
Looking further at the data, we found that 15/22 (68%) of the measurements detected by FEV$_1$ as abnormal were for children aged 13 and above. The majority of abnormalities undetected by FEV$_1$ were found in early school-age children between 6 and 11 years old (Figure 5.9).

**Figure 5.9 Normal and abnormal measurements detected by FEV$_1$**

Legend: Horizontal broken line represents the ULN for LCI and vertical line represents the LLN for FEV$_1$ z-score. Children aged 13 and above presented as green quadrangles. Majority of measurements detected by FEV$_1$ as abnormal were for children aged 13 and above.
5.5.2 Interventional cross-sectional study results:

5.5.2.1 Subjects characteristics:

Seventeen children with CF (10 male, 7 female) admitted for intravenous antibiotics completed spirometry, plethysmography and N₂MBW in triplicate within 24 hours of admission. Of those, 13 children were tested two to three weeks later upon completion of IV treatment. The remaining 4 children were either missed or refused to be tested before discharge. Two children were admitted twice during the study period; therefore, 15 sets of data were included in the final analysis. The mean duration of antibiotic treatment was 14 days. Eleven subjects (84%) received 2 weeks of treatment and the other 2 subjects received 3 weeks. Five of the admissions were routine scheduled admissions for IV antibiotics.

In the group studied, baseline spirometry was consistent with mild impairment, with a mean (SD) %predicted FEV₁ of 73.4% (18.9). Five were homozygous for the ΔF508, 6 were heterozygous for the ΔF508, and one child was a compound heterozygote for other mutations (N1303K/621+1G>T). All but one subject were pancreatic insufficient. Six subjects were infected with *P. aeruginosa* and 3 had isolate of atypical mycobacterium. The characteristics of subjects included are shown in Table 5.15.

<table>
<thead>
<tr>
<th>Table 5.15 Characteristics of study subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children with CF</strong></td>
</tr>
<tr>
<td>(n=13)</td>
</tr>
<tr>
<td><strong>Sex (%male)</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td><strong>ΔF508 homozygous (%)</strong></td>
</tr>
<tr>
<td><strong>P. aeruginosa colonized (%)</strong></td>
</tr>
<tr>
<td><strong>Pancreatic insufficient (%)</strong></td>
</tr>
</tbody>
</table>

*Data are presented as median (ranges), unless otherwise stated.*
5.5.2.2 Changes in $N_2$MBW indices, spirometry and plethysmography parameters in response to intravenous antibiotics

All 13 subjects completed spirometry, plethysmography and $N_2$MBW successfully pre- and post-treatment. The intra-visit repeatability of LCI on admission and discharge was good; mean (SD) coefficient of variation (CV%) LCI was 5.3 (2.9) % and 4.9 (3.4) %, respectively. Changes in lung function measurements in response to intravenous antibiotic treatment are shown in Table 5.16.

Table 5.16 Lung function measurements in children with CF pre- and post- short-term intravenous antibiotic treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-IV antibiotics</th>
<th>Post-IV antibiotics</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$z-score</td>
<td>-2.23 (1.56)</td>
<td>-2.17 (1.43)</td>
<td>-0.06 (-0.34, 0.22)</td>
<td>0.66</td>
</tr>
<tr>
<td>FEV$_1$%predicted</td>
<td>73.4 (18.9)</td>
<td>74.2 (17.2)</td>
<td>-0.8 (-4.3, 2.7)</td>
<td>0.65</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>-2.43 (1.54)</td>
<td>-2.46 (1.59)</td>
<td>0.03 (-0.37, 0.42)</td>
<td>0.90</td>
</tr>
<tr>
<td>FEF$_{75}$ z-score</td>
<td>-2.16 (1.55)</td>
<td>-2.09 (1.69)</td>
<td>-0.08 (-0.6, 0.45)</td>
<td>0.77</td>
</tr>
<tr>
<td>FRC$_{\text{pleth}}$ z-score</td>
<td>0.09 (1.2)</td>
<td>-0.005 (1.3)</td>
<td>0.095 (-0.33, 0.41)</td>
<td>0.83</td>
</tr>
<tr>
<td>FRC$_{N2}$ z-score</td>
<td>0.45 (0.62)</td>
<td>0.44 (0.73)</td>
<td>-0.007 (-0.20, 0.18)</td>
<td>0.95</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>3.3 (2.4)</td>
<td>2.8 (2.09)</td>
<td>0.5 (-0.52, 0.52)</td>
<td>0.99</td>
</tr>
<tr>
<td>LCI</td>
<td>13.06 (4.6)</td>
<td>12.3 (4.01)</td>
<td>0.76 (-0.13, 1.6)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Data presented as mean (SD), mean difference and 95% confidence interval.

No significant difference was found following intravenous antibiotics treatment in any of lung function parameters (Figure 5.10). However, there was a clinically significant change in FEV$_1$ by >10% in one subject. There was also a clinically significant change in LCI by >7% in 7 (46%) of the 15 tests with a decrease (improvement) in LCI in 5 and an increase (deterioration) in LCI in 2 subjects. For the group as a whole, the mean change in LCI and FEV$_1$ were 0.76 turnovers and -0.06 z-score, respectively.
Figure 5.10 Changes in LCI, spirometry and plethysmography parameters in response to intravenous antibiotic treatment in school-age children with CF
Legend: Changes in spirometry, plethysmography and LCI with intravenous antibiotic treatment. Each set of points joined by a single line represents a single patient. Horizontal lines represent group means. Note the lack of statistically significant differences between admission and discharge for all lung function parameters. Note different scales on Y-axes.
5.6 Discussion:

In this chapter we attempted to monitor changes in the LCI in comparison to conventional lung function measures in school-age children with CF based on repeated measurements of airway obstruction, lung volume and ventilation distribution over a 4 year period. In addition, we assessed the association over time between changes in lung function with age, *P. aeruginosa* infection and CFTR genotypes. Lastly, the ability of LCI to detect the short-term response to intravenous antibiotic treatment in comparison the gold standard FEV$_1$ in children with mild to moderate CF lung disease was investigated cross-sectionally.

5.6.1 Between group comparison, repeatability and sensitivity of LCI at baseline visit:

In our study population of school-age children, we found that LCI, flow limitation and lung volume parameters were significantly different between children with CF and healthy controls. Children with CF had higher ventilation inhomogeneity, more air trapping and more hyperinflation than healthy subjects. They had also significantly reduced forced expiratory volume and flows compared to healthy controls. These findings were consistent with previously reported results in school-age children (Horsley, Macleod et al. 2008, Aurora, Gustafsson et al. 2004, Gustafsson 2003, Singer, Kieninger et al. 2013).

We also showed a good within-test repeatability of LCI in both health (CV 6.7%) and disease (CV 7.1%). Other studies in school-age children reported a similar CV ranging between 4-8% in CF, and 4-5% in healthy controls (Aurora 2005, Fuchs, Ellemunter et al. 2012, Singer, Kieninger et al. 2013). LCI has also showed a high sensitivity (100%) at discriminating CF lung disease with AUC$_{ROC}$ of 0.999.

5.6.2 Relationship between LCI and conventional lung function measurements at baseline visit:

Relationship between LCI and conventional lung function measurements

Our findings showed a moderate negative correlation between LCI with FEV$_1$ in children with CF and a weak negative association with FEF$_{75}$ in healthy controls. However, approximately two-thirds of the children with CF who had abnormal overall ventilation distribution had normal spirometric results. Furthermore, almost all children
with abnormal forced expiratory volumes and flows had abnormal ventilation distribution. These findings are consistent with previous findings from ventilation distribution studies in subjects with CF (Gustafsson 2003, Gustafsson, De Jong et al. 2008, Aurora 2004). This suggests that LCI might be more sensitive in identifying small airway dysfunction than any other parameters of lung function.

We also found a strong relationship between increasing ventilation inhomogeneity represented by LCI and the level of hyperinflation represented by RV/TLC in children with CF. An agreement between these two measurements was found in 41% of our population with mild to moderate CF lung disease. However, changes in LCI appeared to precede an increase in RV/TLC in the detection of peripheral airways abnormalities as a majority of the children had abnormal LCI before hyperinflation developed. This corresponds to two physiological studies involving children with more severe CF lung disease and adult subjects with CF (Desmond, Coates et al. 1986, Horsley 2009). Although fewer abnormalities were detected by RV/TLC than when using the LCI, imaging studies using HRCT have shown that CT score, air trapping defects and bronchiectasis sub-score were always abnormal in the presence of hyperinflation detected by plethysmography, but more closely related to LCI (Goris, Zhu et al. 2003, Owens, Aurora et al. 2011). This result is not unexpected; parenchymal changes and small airway destruction due to recurrent infection and inflammation in CF may lead to progressive lung hyperinflation and at the same time they may delay or alter the distribution of ventilation between lung units. Though air trapping alone is less likely to alter gas mixing efficiency because the regions of trapped air are not in communication with the inspired gas, this might explained the lack of association between LCI and FRC_{pleth} z-score in the present study and in an earlier study conducted by Desmond et al. (Desmond, Coates et al. 1986).

5.6.3 **Longitudinal changes in LCI and conventional lung function measurements over a 4 year period:**

In the current study, changes in airway obstruction, lung volume and ventilation distribution measurements in 27 school-age children with CF were assessed through the use of serial lung function tests over a 4 year period. Our findings demonstrate significant improvement in LCI and RV/TLC z-score over time. Despite improving lung function in our population over time, there was deterioration in airflow limitation
parameters. \( \text{FRC}\text{N}_2 \) increased, but remained within normal limit in majority of the children. These conflicting results may be attributed to several factors that will be discussed in turn.

First of all, longitudinal changes in lung function during childhood may be influenced by the marked developmental changes that occur during the first 10 years of life (Merkus, Tiddens et al. 2002, Lum, Stocks 2010, Aurora 2011). For instance, younger children have larger airway calibre relative to lung volume and consequently are able to empty their lungs more quickly than older subjects during forced expirations (Aurora 2011). This may lead to relatively higher spirometry measurements in younger children and affect the comparability with subsequent measurements over time. In our population, 16 (59%) children with CF started their annual review lung function measurements under the age 10 years.

On the other hand, our data collection method may have an impact upon \( \text{FRC}\text{N}_2 \) and LCI measurements. We have used in our equipment set-up a large dead space (DS) of 113 ml due to the inclusion of bacterial filters; this DS is greater than the upper limit of 70 mL that is recommended for adults including bacterial filters (Robinson, Latzin et al. 2013). It had been anticipated that increases the apparatus dead-space may provide a further compartment in which incomplete gas mixing may occur (Aurora 2005). Furthermore, in a study using a \( \text{N}_2 \text{MBW} \) lung model to assess the impact of varying degree of DS on LCI, increasing DS caused an increase in LCI by 6-13% in the CF child model (Horsley et al. 2013). Therefore, the large DS we used may possibly impact on our measurements by underestimating \( \text{FRC}\text{N}_2 \) and overestimating LCI especially in younger children (≤10 years) and this was apparent when we looked at the progression of LCI with age in our population (Figure 5.7). Mean LCI at 6 years old was about 11.8 and as children grew-up LCI decreased to 9.1 and then after 11 years old LCI started to increase again.

When we looked at the difference between \( \text{FRC}_{\text{pleth}} \) and \( \text{FRC}\text{N}_2 \text{z-scores} \), we found that the difference decreased over time (mean (SD): 0.54 (1.34) at baseline visit vs. -0.36 (0.85) z-scores at last visit). This implies that either there is an improvement in air trapping in children with CF over time or they may be an error in FRC measurements. However, Robinson et al. criticized the use of this approach (i.e. the difference between \( \text{FRC}_{\text{pleth}} \) and \( \text{FRC}\text{N}_2 \text{z-scores} \)) to assess trapped gas in children because its sensitivity is limited by
the variability in FRC measurements using plethysmography and N\textsubscript{2}MBW (Robinson, Goldman et al. 2009). For reliable detection of trapped gas using this approach, trapped gas volume greater than 500 ml needs to be present within the lungs of an adult subject (Robinson, Goldman et al. 2009).

Another explanation for increasing $\text{FRC}_{\text{N}_2}$ and decreasing LCI over time is the increase in N\textsubscript{2} excreted from the tissue particularly during long washouts in children with significant VI. Nitrogen dissolved in the tissues diffuses from the blood into the alveoli mostly in the 1st phase of the washout and contributes to exhaled N\textsubscript{2} later in the washout (Wanger, Clausen et al. 2005, Robinson, Latzin et al. 2013). It has been estimated that excreted nitrogen increased the final nitrogen concentration by 24-49% in a paediatric N\textsubscript{2}MBW lung model (Horsley et al. 2013). Potentially, this will introduce greater error in longer tests in terms of overestimating FRC measurements (Robinson, Latzin et al. 2013). However, it was suggested that unless lung disease is severe the tissue nitrogen contribution will be relatively low (Jensen, Stanojevic et al. 2013). Therefore we postulate that the sometimes lengthy tests in the older children may have resulted in higher measured values of $\text{FRC}_{\text{N}_2}$ than would have been apparent with shorter test times.

The improvement in LCI over time in our CF population could be attributed to genuine improvement, rather than the result of methodological inaccuracy. Children with CF enrolled in the annual review were prescribed standard aggressive treatment including intravenous antibiotics when required. The precise antibiotic treatment depended on the nature and frequency of the bacterial isolate and the deterioration in lung function. This may explain the reported improvement in LCI, as well as in $S_{\text{acin}}$ and RV/TLC z-score over time in our CF population. Evidence of statistically significant improvement in LCI and RV/TLC with short-term IV treatment intervention has been reported previously by two cross-sectional studies in children and young adults with CF (Robinson 2009, Horsley, Davies et al. 2013). Furthermore, it has been reported that in children with mild CF lung disease effective aggressive treatment may slow the rate of decline in lung function or even improve it (de Gracia, Mata et al. 2005).
5.6.4 The association between changes in lung function measurements with age, *P. aeruginosa* infection and CFTR genotypes:

**Association between changes in lung function measurements and age:**

In our cohort of school-age children with CF, we tested the association between changes in lung function measurements with age over a 4 year period. In addition we looked at the age of occurrence of abnormal lung function. Our findings showed that except for FRC<sub>pleth</sub> and FRC<sub>N2</sub> z-scores, changes in lung function parameters were strongly associated with age, with FEV<sub>1</sub> z-score presenting the highest association with age.

When we looked at the age of occurrence of abnormal lung function, we found that LCI was the earliest lung function index to deteriorate with age. It was elevated above the limit of normal in all children with CF as early as the 7<sup>th</sup> year of life. In a longitudinal study by Kraemer and colleagues, the authors found that LCI started to deteriorate in children with CF at median age of 6.4 years, in parallel with our observation (Kraemer et al., 2005).

Findings from our study have also shown that the deterioration in FEF<sub>25-75</sub> and RV/TLC z-scores occurred at a mean age of 11 years and was followed by FEF<sub>75</sub> z-score which started to deteriorate after the age of 12 years. FEV<sub>1</sub> and FEF<sub>50</sub> z-score started to deteriorate later in the progression of CF lung disease after the age of 13 years. Data from long-term evaluation of 64 patients with CF observed over a time span of 16 years demonstrated that FEF<sub>25-75</sub> and FEF<sub>75</sub> % predicted were generally within normal limits until 10-14 years of age, which was in line with our findings (Farrell, Li et al. 2003). Furthermore, the CF Foundation Registry in 2001 showed that obstruction as measured by FEV<sub>1</sub> occurs relatively late in the progression of lung disease in CF after 13 years of age (Kraemer, Blum et al. 2005). The earlier deterioration in forced expiratory flows (apart from FEF<sub>50</sub>) with age in the present study as opposed to the late deterioration in FEV<sub>1</sub> was also in agreement with previously reported findings (Tiddens 2002).

In contrast to our findings, Kraemer et al. reported an earlier onset of abnormal FEF<sub>50</sub> and FEV<sub>1</sub> at median age 7.2 and 8.6 years in a cohort of CF patients followed over a substantial span of 6 to 20 years (Kraemer, Blum et al. 2005). However, their study was retrospective and only 3 indices of flow limitation were considered as outcome measures (FEV<sub>1</sub>, FVC and FEF<sub>50</sub>). They did not look at forced expiratory flows
between 25% to 75% and at 75% of FVC which may explain the discrepancy between our findings (Kraemer, Blum et al. 2005). Another reason may relate to the use of different reference equations to derive predicted values for spirometry parameters and thereafter z-scores. The advantage of their study over the present study is that they followed a larger sample size over a longer period of time (Kraemer, Blum et al. 2005). However, it must be taken in consideration when interpreting the findings that treatments for CF are continually being improved and those prevalent at the start of this extended report by Kraemer and colleagues would be different from those at the end.

In a different study by the same group, the authors identified a progressive increase in pulmonary hyperinflation as represented by FRC\textsubscript{pleth} z-score in more than one third of cases as early as age 6 to 8 years (Kraemer, Baldwin et al. 2006). In our study, FRC\textsubscript{pleth} z-score showed no significant association with age. This may be explained by the different populations studied, in which our population mainly had mild to moderate CF lung disease. In contrast, RV/TLC showed a progressive increase with age and became abnormal at a mean age of 11 years. Supporting evidence from longitudinal evaluation of patients with CF over a time span of 16 years by Farrell et al. have revealed that the RV/TLC ratio values are generally normal until at least 10 years of age (Farrell, Li et al. 2003).

Association between changes in lung function parameters and \textit{P. aeruginosa} infection:

We evaluated the associations between \textit{P. aeruginosa} infection in children with CF and subsequent lung function measurements cross-sectionally and over time. Cross-sectional data showed that children infected with \textit{P. aeruginosa} in the 12 months previous to their lung function visit have significantly lower FEF\textsubscript{25-75} z-score, FEF\textsubscript{50} z-score and FEF\textsubscript{75} z-score, and significantly higher LCI than those not infected with this organism. We also noted a trend towards increasing ventilation inhomogeneity as infection category in the previous 12 months increased. Singer et al. looked at the correlation between LCI and \textit{P. aeruginosa} infection in 73 children with CF; 15 of them were never infected, 23 were free of infection, 19 were intermittently infected and 16 were chronically infected (Singer, Kieninger et al. 2013). After adjusting for age in a multivariable model, they found that LCI was strongly correlated with \textit{P. aeruginosa} with an increase in LCI by 2.1 units per \textit{P. aeruginosa} category increase (Singer, Kieninger et al. 2013). Our
findings was further supported by another study where children with CF infected with *P. aeruginosa* tended to have higher LCI, lower FEV₁ z-score, and lower FEF₇₅ z-score than those not infected with this organism, though none of these parameters reached statistical significant (Aurora 2005). In an autopsy study to localize *P. aeruginosa* in patients with CF, the authors demonstrated the presence of *P. aeruginosa* at the small bronchioles (mainly <1mm) (Baltimore, Christie et al. 1989). It would be expected that lower respiratory tract infections with *P. aeruginosa* will impact upon measures of small airway obstruction, degree of air trapping and ventilation inhomogeneity arising from peripheral airways. However, in the absence of infection these measures will not be affected until the disease progresses.

Another study by Aurora et al. in pre-school children with CF demonstrated significantly higher LCI in infected children with *P. aeruginosa* compared to non-infected children, but with no significant difference in other lung function measures (Aurora 2004). More recently the same group conducted a longitudinal study to track lung function results from the preschool years to early school age in children with CF and they demonstrated no significant difference in any lung function outcome either at preschool or early school age between infected and never-infected (Aurora 2011). However, they claimed that their study was not designed or powered to specifically detect such relationships (Aurora 2011). In their study, they examined 40 infected children vs. 8 children who had never been infected with *P. aeruginosa* by pre-school age.

On the other hand, a different longitudinal study by Kraemer et al. in school-age children with CF showed significant associations between the onset of chronic *P. aeruginosa* infection and all lung function parameters, except FRC₇₅ (Kraemer, Blum et al. 2005). Therefore, they concluded that the onset of chronic *P. aeruginosa* infection can be used as an important marker of disease progression in CF (Kraemer, Blum et al. 2005). Findings from our longitudinal study showed a strong association between *P. aeruginosa* infection and changes in FRCₑₚₑₜₗ z-scores (Table 5.18, Figure 5.11). Over the study period, children infected with *P. aeruginosa* tended to have higher FRCₑₚₑₜₗ z-score compared to non-infected children. This result was expected as it has been shown that the presence of *P. aeruginosa* in the lower airways is related to the disease of the surrounding tissue (Baltimore, Christie et al. 1989). Additionally, a longitudinal study in children with CF has shown that pulmonary hyperinflation and the development of
trapped gas are closely associated with different types of chronic bronchial infection, especially *P. aeruginosa* (Kraemer, Baldwin et al. 2006). A strong correlation between pathogens in the lower airways and the degree of air trapping has also been reported in a study conducted on infants and young children with CF (Dakin, Numa et al. 2002). The lack of association between *P. aeruginosa* and other lung function parameters longitudinally in the current study may be attributable to our small sample size that resulted from equipment breakdown during the data collection period especially with regards to the measurements of ventilation inhomogeneity.

**Associations between changes in lung function parameters and CFTR genotypes:**

As part of a secondary analysis in our longitudinal study we evaluated the association between lung function measurements and CFTR genotypes. Our findings showed no significant association between CFTR genotypes and any of lung function parameters in children with CF (Table 5.14). An earlier paediatric study in 119 patients with CF has reported the lack of association between disease severity and genotype (Lester, Kraut et al. 1994). Further supporting data from The Cystic Fibrosis Genotype-Phenotype Consortium showed no associations between pulmonary disease and the genotype in 399 patients with CF (The Cystic Fibrosis Genotype-Phenotype Consortium 1993). However, other investigators demonstrated that patients with heterozygous-genotype and genotype groups other than ∆F508 had milder disease and better lung function (Kerem, Corey et al. 1990). Patients with ∆F508 homozygous genotype had faster decline in lung function (Gan, Heijerman et al. 1994). Furthermore, findings from the study of Kraemer et al. suggested that the CFTR genotype plays an important role in determining the longitudinal functional progression of lung disease in CF as it showed a strong association with observed changes in FVC and FEV₁ (Kraemer, Blum et al. 2005). It should be noted that 58.5% of children with CF in the study of Kraemer et al. had the ∆F508 homozygous genotype (Kraemer, Blum et al. 2005).

The lack of correlation between genotype and lung function in the present study was consistent with the previous paediatric studies (The Cystic Fibrosis Genotype-Phenotype Consortium 1993, Lester, Kraut et al. 1994). The discrepancy reported may be due to our small sample size as we examined 11 children with homozygous genotype vs. 16 children with heterozygous genotype, whereas ideally this type of study needs larger sample sizes in each genotype group. Another reason may be that we carried out
our study in children with relatively mild lung disease in which effective treatment may slow the progression of lung damage whereas all previous studies that showed correlation between CFTR genotypes and lung function were conducted on patients with more severe lung disease and from a wide age ranges including adult subjects (de Gracia, Mata et al. 2005).

5.6.5 Association between LCI and FEV$_1$ over time:

In the current study, we looked at the association between the gold standard lung function measure in the assessment of CF lung disease (FEV$_1$) and the current most sensitive index of abnormal lung function. Our findings showed a statistically significant negative relationship between LCI and FEV$_1$ z-score over time (Figure 5.13). However, poor agreement was noted between these two parameters in discriminating CF lung disease. In our CF population, LCI detected more than 50% of the cases not detected by FEV$_1$. The ability of LCI to detect CF lung disease at an earlier stage than FEV$_1$ has been reported by a number of cross-sectional studies over a wide age range (Lum 2007, Aurora 2004, Aurora, Gustafsson et al. 2004, Gustafsson 2003, Horsley, Gustafsson et al. 2008), all of which have proposed that LCI may reflect distal heterogeneous changes in airway function that FEV$_1$ as a measure of larger airways cannot detect. This can be supported further by the poor ability of FEV$_1$ to detect abnormalities in younger children aged 6 to 11 years in our population.

In the present study changes in LCI with age have also been shown to precede changes in FEV$_1$ z-score which started to deteriorate after the age of 13 years in children with CF, though FEV$_1$ showed a stronger association with age.

5.6.6 Changes in LCI, spirometry and plethysmography parameters in response to intravenous antibiotics

In this cross-sectional study, we assessed the ability of LCI to detect the short-term response to intravenous antibiotics as compared to the gold standard FEV$_1$ in children with mild to moderate CF lung disease. Our findings demonstrate a clinically significant improvement in LCI (>7% change) with intravenous antibiotic treatment in 1/3 of children with mild to moderate CF lung disease, but this did not reach statistical significant. Evidence of improvement in LCI by 3.8% to 5.9% with short-term intravenous treatment intervention has been reported previously by two studies in children and young adults with CF (Robinson 2009, Horsley, Davies et al. 2013).
Robinson et al. demonstrated a statistically significant improvement in LCI of 0.48 lung turnovers with intravenous antibiotic treatment for acute pulmonary exacerbation in 75% of the subjects with moderate CF lung disease (Robinson 2009). They also reported clinically significant changes in LCI (>5% change), but with heterogeneous response to treatment. Whereas 2/3 of the children had an improvement in LCI, 5 had a deterioration and increase in LCI (Robinson 2009). Horsley et al. also reported a significant improvement in LCI with treatment of 0.8 lung turnovers in 69% of children and young adults with CF (Horsley, Davies et al. 2013). However, as previously described by Robinson et al., there was considerable heterogeneity of LCI response. In the current study we also found a lack of uniform response of LCI to treatment in children with mild CF lung disease. This has been suggested to be due to recruitment of lung units previously not contributing to ventilation as mucus is cleared, and thus increasing VI overall (Robinson 2009, Horsley, Davies et al. 2013). If this were the case we would expect a significant fall in air trapping as represented by the difference between FRC_{pleth} and FRC_{N2} or RV/TLC; however this was not observed neither in the whole group, nor in patients with increase in LCI. Horsley et al. demonstrated significant improvement in air trapping on CT with treatment, but no significant change in FRC_{N2} in subjects with CF whereas Robinson et al found a statistically significant reduction in RV/TLC by a mean of 1.7% post-treatment (Robinson 2009, Horsley, Davies et al. 2013). However, a study by Fuchs et al. to assess the short-term effect of chest physiotherapy on LCI in CF paediatric patients has shown that physiotherapy did not appear to have any significant effect on LCI and suggested that this possibly reflected variable mucus plugging, and, thus, variable trapped air in patients with CF (Fuchs, Toussaint et al. 2010). Therefore, we proposed that the heterogeneous response of LCI to treatment in our population may be a result of the heterogeneous nature and severity of CF lung disease.

Furthermore, in the present study we found no significant improvement in FEV$_1$ post-IV antibiotic treatment, but there was a significant clinical improvement >10% in one subject. Previous studies showed significant improvements in FEV$_1$ with average change range between 0.11 L-0.32L, but with no statistically significant correlations between change in FEV$_1$ and LCI (Robinson 2009, Horsley, Davies et al. 2013). As opposed to our study, subjects with mild airways disease were not well represented in the earlier two studies and this may explain the lack of a statistically significant
improvement in FEV\textsubscript{1} in our cohort. Surprisingly, a more recent study by Welsh et al. did not support the use LCI or FEV\textsubscript{1} to gauge the short term clinical response to intravenous antibiotic therapy in school-age children with CF (Welsh, Nesci et al. 2014). In their study, they reported an improvement in LCI in 55% of patients treated with intravenous antibiotics for acute exacerbation compared with 67% improvement in FEV\textsubscript{1} (Welsh, Nesci et al. 2014).

In the present study, we have also been unable to show any statistically significant changes in any other lung function parameters. It should be noted here that our sample size estimation was based on LCI and not on the secondary outcomes measurements which may be more variable and this might explain the lack of any detectable changes.

5.6.7 Methodological issues and study limitations

The statistical concept of primary outcome and thus of power calculation was not appropriate for the longitudinal study because the design of the study did not permit such an analysis to be undertaken. Therefore, the sample size was opportunistic and not based on a power calculation and we included all children attending the paediatric CF clinic at LRI with a diagnosis of CF confirmed by sweat testing and CF genotype. Ideally, this type of study requires a large sample size followed over a substantial span of time and this is what the principal investigator aimed for when planning the study. What is covered in this thesis is the first four years of the follow-up. Our original intention was to complete repeated measurements in 37 children with CF. Ultimately we were able to test 27 children with CF on more than one test occasion. The reasons for the inability to follow-up all children included at the start of the study over a 4 years period were that some of those children moved to the adult clinic or different cities. In addition, the equipment in use at the start (the modified Medgraphics Profiler) became obsolete, and sometimes failed to function when needed. But to overcome these difficulties we used an advanced statistical test to analyse our data that takes into consideration data obtained from individuals in irregularly spaced serial measurements and can deal with incomplete data set due to missing scheduled visits (Edwards 2000). All of the above factors may have an effect upon the robustness of our findings although they were consistent with results from previous cross-sectional studies. However, it should be noted that longitudinal analyses of lung function data using the LMM
regression approach give a better statistical assessment in contrast to cross-sectional investigations (Kraemer, Blum et al. 2005).

Furthermore, our data collection commenced from 6 years of age and we did not include a control group that followed over the same time-span, which limits interpretation as to what constitutes a clinically significant change over time. Including control subjects allows for a correction to be made for the normal variability of the outcome measures over the study period (Lum, Stocks 2010, Aurora 2011). However, the workload would have required resources that were unavailable at the time, and we had access to a large body of data from a healthy population of children and young people, studied immediately before the commencement of my study. We did recruit and study 64 healthy controls during the time course of the work presented here.

Also it would be very valuable to compare changes in lung function parameters over time with structural lung changes as measured by HRCT scan, which has been proposed as a method for detection of early lung disease in CF. However, HRCT studies are not part of routine clinical care of children with CF attending CF clinic at LRI and the radiation exposure and expense of HRCT precluded these investigations being performed solely for comparison with lung function results.

For the interventional cross-sectional study, we based our sample size calculation on LCI and not on the secondary outcomes measurements which may explain the lack of statistically significant differences post-treatment. Also, we have not included a cohort of healthy children to assess normal variability in lung function parameters over a 2 week period.

To sum up, previous studies looking at the potential role of N₂MBW have had their limitations, including small sample sizes, paucity of data regarding either short-or longer-term repeatability of LCI, differences in measurement procedures, equipment and analysis software. These, together with the different populations studied, have made it difficult to establish the role of N₂MBW in clinical settings. The reference values appear to be equipment and/or software-specific and based mainly cross-sectional samples. The contribution of the work described in this chapter has been to confirm the importance of LCI as the first sign of deterioration of lung function in CF.
Chapter 6 Comparison of hyperpolarized He-3 diffusion magnetic resonance ($^3$He MR) with N$_2$MBW

6.1 Rationale:

Physiological and imaging studies have shown that lung disease starts early in life in most children with CF. It begins more markedly in the small airways, but eventually leads to the destruction of the larger airways (Gustafsson, De Jong et al. 2008, Horsley, Macleod et al. 2008, Castile, Hayes et al. 2000, de Jong 2004, Bannier 2010). A number of cross-sectional and longitudinal studies have shown that the measures of ventilation inhomogeneity (VI), derived from MBW, are sensitive to early changes in small airways in different lung diseases, as well as being shown to be altered in subjects with CF (Verbanck 1998, Verbanck 1999, Gustafsson 2003, Aurora 2004, Gustafsson 2007, Kraemer, Blum et al. 2005). The LCI, a measure of overall VI, is the main index derived from MBW.

Both the development of sensitive lung function measures and their quantitative validation can be significantly enhanced by means of functional imaging techniques (Plotkowiak, Burrowes et al. 2009). Furthermore, it has been suggested that ventilation imaging in combination with LCI measurement may be able to identify the scale of relevant ventilation inhomogeneity and to the extent to which ventilation inhomogeneity detected by LCI is peripheral in any given disease (Verbanck, Paiva et al. 2012).

Hyperpolarised $^3$He diffusion magnetic resonance ($^3$He MR) is a recently developed method that provides functional and structural information about the lung without the danger of side effects from ionizing radiation that are typically associated with HRCT, which is currently the gold standard in lung morphology assessment (Saam, Yablonskiy et al. 2000, Yablonskiy, Sukstanskii et al. 2002, Diaz, Casselbrant et al. 2009, Osmanagic, Sukstanskii et al. 2010). The application of $^3$He MR enables the measurements of the apparent diffusion coefficients of $^3$He gas and subsequent investigatory probing into lung microstructure and the airspace size within the lung (Yablonskiy, Sukstanskii et al. 2002, Saam, Yablonskiy et al. 2000, Salerno, de Lange et al. 2002). It is a well-tolerated technique that requires minimal subject cooperation with breath-hold of up to 10 sec following inhalation of hyperpolarised $^3$He gas mixture. This makes it applicable for use with school-age children and as well as for application
in longitudinal studies to monitor disease progression (Plotkowiak, Burrowes et al. 2009, Ball 2011).

The apparent diffusion coefficient (ADC), a surrogate measure of alveolar size, obtained from $^3$He MR has been shown to be more sensitive than standard lung function tests in the measurements of short-term changes in the CF lung (van Beek, Hill et al. 2007, Kirby, Svenningsen et al. 2013). This measure has also been seen to be elevated in subjects with chronic obstructive pulmonary disease (COPD). In subjects with asthma, the reported ADC values were not significantly different from those of healthy subjects, but they were significantly lower than the active smokers with COPD (Fain, Altes et al. 2006).

Furthermore, the development of a new mathematical model of $^3$He gas diffusion in lung acinar airways by Yablonsky et al. has enable the quantitative measurement of lung air spaces dimensions at the alveolar level (Yablonskiy, Sukstanskii et al. 2009). By applying this model, the reduction in $^3$He diffusion over relatively short diffusion times has been shown to be linked to the alveolar radius (R) and the alveolar sleeve depth ($h$).

6.2 Aims:

The aims of this study were:

1. To estimate the dimensions of the lung peripheral microstructure in subjects with CF lung disease and to compare this to age-matched healthy controls using $^3$He diffusion MR.
2. To investigate the relationship between the parameters derived from $^3$He diffusion MR and (i) LCI derived from N$_2$MBW and (ii) FEV$_1$ z-score, as well as with conventional markers of small airway obstruction.

6.3 Hypotheses:

1. In subjects with CF, there will be an increase in ADC and R and a decrease in $h$ compared to healthy controls.
2. If indices from $^3$He diffusion MR and N$_2$MBW both reflect changes in the peripheral airways, there will be an association between these indices. There will also be a relationship between $^3$He MR parameters and conventional markers of small airway obstruction, but not with FEV$_1$ z-score.
6.4 Subjects and methods:

6.4.1 Subjects and study design:
This is a cross-sectional study performed in school-age children and adults with CF lung disease, compared against age-matched healthy controls, in order to measure the dimensions of the lung peripheral microstructure using \(^3\)He MR and to compare this with lung function measures. Children with CF were invited with their parents to attend \(^3\)He MR scanning sessions at Nottingham University during their annual review visits at Leicester Royal Infirmary (LRI), whereas adults with CF were recruited from the adult CF clinic at Glenfield hospital. Healthy subjects were recruited from the Leicester Respiratory cohorts, Community Health Services Database and LRI staff members.

6.4.2 Data collection and analysis:
Prior to \(^3\)He MRI scanning, all subjects performed \(N_2\)MBW tests using modified a Medgraphics Profiler (Medical Product Service GmbH, Germany), then spirometry and plethysmography using a Jaeger MasterScreen Body Plethysmography (Care Fusion GmbH, Leibnizstrasse, Germany) at LRI.

Spirometry and plethysmography parameter z-scores were calculated from published reference equations (Stanojevic, Wade et al. 2008, Rosenthal, Bain et al. 1993, Kraemer, Zehnder et al. 1986). Subjects with a \(FEV_1\), \(FEF_{25-75}\) and \(FEF_{75}\) z-scores less than -2 or \(FRC_{N_2}\), \(FRC_{pleth}\) and \(RV/TLC\) z-scores more than +2 were classified as having an abnormal result.

LCI has been found to be virtually independent of height, weight, and sex in healthy children (Chapter 4); therefore ULN was used for this assessment. The limits of normality, defined as mean +/- 1.96 SD, were calculated for LCI from the control data, and subjects with values above the ULN were categorised as having an abnormal result.

The \(^3\)He diffusion MR was then undertaken at the Department of Physics and Astronomy, University of Nottingham in a 0.15 T permanent magnet system (Intermagnetics General Corporation, New York) with a Surrey Medical Imaging Systems console (Surrey, UK). Five scans were performed for each subject, after they had inhaled a helium gas mixture containing 10 ml hyperpolarized \(^3\)He from a Tedlar bag. Two types of measurements were taken: a short-breath hold scan for 5 sec to calculate ADC and a long-breath hold scan for 10 seconds to calculate R and \(h\). At least three
technically satisfactory ADC values were obtained for all subjects and the mean was taken as the uncorrected ADC. Global ADC (the apparent diffusion constant of the entire lung) was corrected for the effects of differences in lung inflation between subjects (Waters, Owers-Bradley et al. 2006). The equation used for the correction was 

\[ D = ADC + (0.36 \times ADC \times \frac{\text{volume (He)}}{\text{volume (FRC)}}) \]

The resultant global volume weighted ADC values were used in the final analysis. Data acquired were also fitted to the mathematical acinar model developed by Yablonskiy to derive values for mean alveolar duct diameter, R (including alveolar sleeve), and mean alveolar sleeve depth, h (Yablonskiy, Sukstanskii et al. 2009). Data were obtained and analysed by two physicists from the University of Nottingham. A detailed description of the subjects included, data collection and data analysis can be seen in Chapter 3.

**6.4.3 Statistical analysis:**

We used an unpaired t-test or a Mann-Whitney test to compare lung function parameters and the dimensions of peripheral microstructure between healthy subjects and subjects with CF. The relationships between indices derived from $^3$He MR and lung function parameters were assessed using both correlation (Pearson or Spearman (r)) and linear regression models ($r^2$). A p-value of <0.05 was accepted as being statistically significant. For this study, a sample size of 24 subjects in each group was deemed sufficient to detect significant differences between healthy subjects and subjects with CF, with a power of 80% at a two sided 5% significance level.
6.5 Results:

6.5.1 Subject characteristics:

Subjects with CF:

Although a sufficient number of subjects with CF were recruited, only 18 subjects (14 school-age children and 4 adults) completed all of the tests. The remaining patients were either unwell, admitted for IV antibiotics, or withdrew on the day of testing. Of the subjects included, 8 (44%) were homozygotes (ΔF508), 8 (44%) were heterozygotes (ΔF508/other) and 2 subjects (11%) has mixed genotype (other/other). Sixteen (88%) subjects were pancreatic insufficient.

Healthy subjects:

Nineteen age-matched children and 8 healthy adults were included in this study. All children and 5/8 adults were recruited and tested as part of a previous study (Narayanan et al. 2012). The N₂MBW and ³He MR data obtained from those subjects were re-analysed to eliminate any observer bias in the data analysis. Three more healthy adults, age-matched to our CF adults, were recruited from hospital staff. The characteristics of the entire study population are summarized in Table 6.1. No statistically significant difference was found between the two populations, in any of the physical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=27)</th>
<th>Subjects with CF (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F</td>
<td>14:13</td>
<td>8:10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8 (7.8-37)</td>
<td>10.1 (5.9-37.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>148.1 (122.6-193.3)</td>
<td>138.2 (112.5-180)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>48.2 (22.5-98)</td>
<td>34.5 (21.1-79.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>21 (14.6-35.6)</td>
<td>20.2 (15-25.1)</td>
</tr>
</tbody>
</table>

Data are presented as median (ranges).
6.5.2 Lung function results:

All subjects with CF successfully completed spirometry, plethysmography and N₂MBW in triplicate, except in the case of one adult, for whom N₂MBW was unavailable on the day of testing. All healthy subjects performed spirometry and all but one adult successfully performed plethysmography. N₂MBW was successfully performed by 24 healthy subjects in triplicate, but was not available for 3 adults on the day of testing. Comparisons of lung function results between both populations are presented in Table 6.2. Statistically significant differences were found between subjects with CF and healthy controls in spirometry measurements, RV/TLC z-score, and LCI. Subjects with CF have higher RV/TLC z-score, and LCI values and lower FEV₁, FEF₂₅₋₇₅ z-scores compared to healthy subjects whereas no differences were found between groups in FRCₚleth z-score or FRCₐₗ₂ z-score.

Table 6.2 Lung function results for healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=27)</th>
<th>Subjects with CF (n=18)</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁z-score</td>
<td>0.03 (-1.24,2.7)</td>
<td>-1.27 (-4.9,0.7)</td>
<td>U=98, p&lt;0.01*</td>
</tr>
<tr>
<td>FEF₇₅z-score</td>
<td>-0.04 (-2.01,1.4)</td>
<td>-1.98 (-4.2,0.15)</td>
<td>U=55, p&lt;0.0001*</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅z-score</td>
<td>-0.32 (-1.98,1.38)</td>
<td>-2.35 (-4.6,0.47)</td>
<td>U=68, p&lt;0.0001*</td>
</tr>
<tr>
<td>FRCₚleth-z-score</td>
<td>-0.02 (-1.6,1.5)</td>
<td>1.6 (-1.5,5.4)</td>
<td>U=188, p=0.272</td>
</tr>
<tr>
<td>RV/TLCz-score</td>
<td>0.4 (-0.87,4.08)</td>
<td>1.9 (-2.2,6.6)</td>
<td>U=82,p&lt;0.0001*</td>
</tr>
<tr>
<td>FRCₐₗ₂z-score</td>
<td>-0.17 (-0.93,0.71), n=24</td>
<td>-0.34 (-0.7, 1.4), n=17</td>
<td>U=0.172, p=0.589</td>
</tr>
<tr>
<td>LCI</td>
<td>6.7 (5.2,8.3), n=24</td>
<td>11.3 (9.1,14.5), n=17</td>
<td>U=0.00, p&lt;0.0001*</td>
</tr>
</tbody>
</table>

Data are presented as median (ranges). * Statistically significant difference.
6.5.3 ${}^3$He diffusion MR results:

Comparison of global ADC, R and $h$ between healthy subjects and subjects with CF:

${}^3$He MR scanning was performed successfully for all subjects with CF and acceptable ADC values were obtained from all participants. However, acceptable R and $h$ values were only available from 14 of the 18 subjects. The data collected from three children and one adult had signals that were either too low or inconsistent, therefore the extracted R and $h$ values were excluded from final analysis. ${}^3$He MR scanning was also performed successfully for all healthy subjects and acceptable ADC, R and $h$ values were obtained from all participants. However, V.W. ADC was not calculated in one healthy adult, because of the lack of FRC data. The comparisons of ${}^3$He diffusion MR results between both populations are presented in Table 6.3. Global ADC values were found to be significantly lower in subjects with CF in comparison to those of healthy controls. However, no statistically significant difference was found in R and $h$ between both populations (Figure 6.1).

| Table 6.3 $^3$He diffusion MR results for healthy subjects and subjects with CF |
|-------------------------------|-------------------|-------------------|--------------------|
|                               | Healthy controls (n=27) | Subjects with CF (n=18) | Level of Significance |
| Global ADC (cm$^2$/sec)       | 0.144 (0.019), n=26    | 0.127 (0.022)        | $t=2.6$, $p=0.011^*$ |
| R ($\mu$m) (the radius of the alveoli) | 446 (397, 600)         | 430 (376, 476), n=14  | U=131, $p=0.115$    |
| $h$ ($\mu$m) (the alveolar sleeve depth) | 243 (132, 282)         | 245 (205, 294), n=14  | U=186, $p=0.946$    |

Data are presented as mean (SD) or median (ranges). * Statistically significant difference.
Figure 6.1 Comparison of global ADC values between healthy subjects and subjects with CF

Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Lines represent group means ± SD for ADC, but median and range for R and h. Note the significant difference in global ADC values between both populations (p=0.011).
6.5.4 Relationships between $^3$He diffusion MR parameters with FEV$_1$ and small airway markers:

Global ADC with FEV$_1$ z-score and small airway markers:

The correlations between global ADC measurements with FEV$_1$ z-score and small airway markers are shown in Table 6.4 and Figure 6.2. No statistically significant relationships were observed between global ADC and FEV$_1$ z-score or small airway markers in both healthy subjects and subjects with CF.

Table 6.4 Correlation between global ADC with FEV$_1$ z-score and small airway markers in healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th>Global ADC</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>0.36</td>
<td>0.069</td>
</tr>
<tr>
<td>FEF$_{75}$ z-score</td>
<td>0.16</td>
<td>0.41</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>-0.11</td>
<td>0.604</td>
</tr>
<tr>
<td>FRC$_{pleth}$ z-score</td>
<td>-0.19</td>
<td>0.33</td>
</tr>
</tbody>
</table>

$r =$ correlation coefficient.
Figure 6.2 The relationship between global ADC with FEV₁ and small airway markers in healthy subjects and subjects with CF
Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of small airway markers. No significant relationships were found between global ADC and small airway markers in both healthy subjects and subjects with CF.
Alveolar radius (R) with small airway markers:

The correlations between R measurements and small airway markers are shown in Table 6.5 and Figure 6.3. There was a statistically significant negative association between R and FEF\textsubscript{25-75} z-score ($r^2=0.35$, $r=-0.59$, $p=0.03$), R and FEF\textsubscript{75} z-score ($r^2=0.36$, $r=-0.59$, $p=0.03$) in subjects with CF. A statistically significant positive relationship was also found between R and RV/TLC z-score in subjects with CF ($r^2=0.445$, $r=0.62$, $p=0.02$). However, no significant associations were found between R and any of the small airway markers in healthy subjects.

Table 6.5 Correlation between R (alveolar radius) and small airway markers in healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th>R (alveolar radius)</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>FEV\textsubscript{1} z-score</td>
<td>-0.123</td>
<td>0.54</td>
</tr>
<tr>
<td>FEF\textsubscript{75} z-score</td>
<td>-0.002</td>
<td>0.99</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75} z-score</td>
<td>-0.11</td>
<td>0.59</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>0.11</td>
<td>0.59</td>
</tr>
<tr>
<td>FRCpleth z-score</td>
<td>0.12</td>
<td>0.60</td>
</tr>
</tbody>
</table>

$r=\text{correlation coefficient. } ^*\text{Statistically significant correlation.}$
Figure 6.3 The relationship between R (alveolar radius) with FEV\(_1\) and small airway markers in healthy subjects and subjects with CF.
Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of small airway markers. Note the significant negative relationships between R and FEF$_{25-75}$ z-score and FEF$_{75}$ z-score and the significant positive relationship between R and RV/TLC z-score in subjects with CF (see text for $r^2$, $r$ and p-values).
Alveolar sleeve depth ($h$) with small airway markers:

The correlations between $h$ measurements and small airway markers are shown in Table 6.6 and Figure 6.4. There was a statistically significant positive association between $h$ and FEV$_1$ z-score ($r^2=0.28$, $r=0.59$, p=0.027), FEF$_{25-75}$ z-score ($r^2=0.41$, $r=0.72$, p<0.01), FEF$_{75}$ z-score ($r^2=0.42$, $r=0.72$, p<0.01). Statistically significant negative relationships were also found between $h$ and RV/TLC z-score ($r^2=0.53$, $r=-0.73$, p<0.01). However, there were no statistically significant relationships between $h$ and small airway markers in healthy subjects.

Table 6.6 Correlation between $h$ (alveolar sleeve depth) and small airway markers in healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th>$h$ (alveolar sleeve depth)</th>
<th>Healthy controls</th>
<th></th>
<th>Subjects with CF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>FEV$_1$ z-score</td>
<td>0.104</td>
<td>0.604</td>
<td>0.59</td>
<td>0.027*</td>
</tr>
<tr>
<td>FEF$_{75}$ z-score</td>
<td>-0.077</td>
<td>0.702</td>
<td>0.72</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>FEF$_{25-75}$ z-score</td>
<td>0.019</td>
<td>0.92</td>
<td>0.72</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>RV/TLC z-score</td>
<td>-0.014</td>
<td>0.94</td>
<td>-0.73</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>FRC$_{pleth}$ z-score</td>
<td>-0.137</td>
<td>0.50</td>
<td>-0.41</td>
<td>0.14</td>
</tr>
</tbody>
</table>

$r$ = correlation coefficient. *Statistically significant correlation.
Figure 6.4 The relationship between $h$ (alveolar sleeve depth) with FEV$_1$ and small airway markers in healthy subjects and subjects with CF.
Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of small airway markers. Note the significant positive relationships between h and FEV$_1$ z-score, FEF$_{25-75}$ z-score, and FEF$_{75}$ z-score in subjects with CF (see text for $r^2$, r and p-values). There was also a significant negative relationship between h and RV/TLC z-score in subjects with CF.
6.5.5  Relationships between $^3$He diffusion MR parameters and LCI:

Global ADC with LCI:

The correlations between global ADC measurements and LCI are shown in Table 6.7 and Figure 6.5. There was no statistical significant association between global ADC and LCI in both healthy subjects and subjects with CF.

Table 6.7 Correlation between global ADC and LCI in healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th>Global ADC</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCI</td>
<td>$r$ 0.37 $p$-value 0.079</td>
<td>$r$ -0.18 $p$-value 0.49</td>
</tr>
</tbody>
</table>

$r= correlation coefficient.$

Figure 6.5 The relationship between global ADC with LCI in healthy subjects and subjects with CF

Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of LCI. No significant relationships were found between global ADC and LCI in healthy subjects and subjects with CF (see table for $r$ and $p$-values).
Alveolar radius (R) with LCI:

The correlations between R measurements and LCI are shown in Table 6.8 and Figure 6.6. There was no statistical significant association between R and LCI in healthy subjects and subjects with CF.

Table 6.8 Correlation between R and LCI in healthy subjects and subjects with CF

<table>
<thead>
<tr>
<th>R (alveolar radius)</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>LCI</td>
<td>0.113</td>
<td>0.59</td>
</tr>
</tbody>
</table>

$r$ = correlation coefficient.

Figure 6.6 The relationship between R (alveolar radius) with LCI in healthy subjects and subjects with CF

Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of LCI. No significant relationships were found between R and LCI in healthy subjects and subjects with CF (see text for $r^2$, $r$ and p-values).
**h (Alveolar sleeve depth) with LCI:**

The correlations between $h$ measurements and LCI are illustrated in Table 6.9 and Figure 6.7. There was no statistical significant association between $h$ and LCI in healthy subjects and subjects with CF.

**Table 6.9 Correlation between $h$ and LCI in healthy subjects and subjects with CF**

<table>
<thead>
<tr>
<th>$h$ (alveolar sleeve depth)</th>
<th>Healthy controls</th>
<th>Subjects with CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R$</td>
<td>p-value</td>
</tr>
<tr>
<td>LCI</td>
<td>0.059</td>
<td>0.78</td>
</tr>
</tbody>
</table>

$r =$ correlation coefficient.

**Figure 6.7 The relationship between $h$ (alveolar sleeve depth) with LCI in healthy subjects and subjects with CF**

*Legend: Healthy subjects presented as green triangles, and subjects with CF presented as red circles. Vertical broken lines represent limits of normality of LCI. No significant relationships were found between $h$ and LCI in healthy subjects and subjects with CF (see text for $r^2$, $r$ and p-values).*
6.6 Discussion:
This cross-sectional study sought to estimate the size of peripheral microstructure measured using $^3$He diffusion MR in school-age children and young adults with CF lung disease, in comparison with aged-matched healthy subjects. In addition, this study aimed to investigate the relationship between parameters derived from $^3$He diffusion MR and (i) LCI derived from N$_2$MBW and (ii) FEV$_1$ z-score and with conventional markers of small airway obstruction.

To our knowledge, this study is the first to describe non-invasive in vivo observation of alveolar size in children with CF.

6.6.1 $^3$He diffusion MR in CF and healthy controls

ADC:
Although evidence has been provided by pathological studies that mild alveolar enlargement occurs in most children with CF aged 6 to 17 years old, and our corresponding expectations of an increased ADC (Sobonya, Taussig 1986, Hamutcu 2002), our findings show the reverse. Global ADC values were found to be significantly lower in subjects with CF compared to healthy controls. There are a number of possible explanations for the observed reduction in alveolar size in the CF population. Theoretically, ADC is reduced in the presence of barriers that restrict the diffusion of $^3$He (Mada 2009, Diaz, Casselbrant et al. 2009, Osmanagic, Sukstanskii et al. 2010). One of the most important pathological manifestations and the predominant abnormality in children and younger teenagers with CF, is the plugging of airways by mucus (Sobonya, Taussig 1986, Konstan, Berger 1997, Hamutcu 2002, Tiddens 2010). It has been shown that obstruction of small airways will prevent helium entering the distal airspaces, thereby preventing the calculation of ADC from this region (Bink, Hanisch et al. 2007). One of the reported limitations of ADC measurements is that it can only be effectively obtained from sufficiently ventilated areas with hyperpolarised $^3$He, whereas regions with airflow obstruction will produce a lower signal intensity which may be insufficient to properly determine the ADC (Ball 2011). Therefore, we propose that the significant reduction in ADC values seen in subjects with CF compared to healthy controls occurred as a result of small airway obstruction with mucus, as this will act as barrier and restrict the diffusion of $^3$He. This was evident from the significant difference between both populations in the markers of small airways obstruction. Subjects with CF
have significantly higher RV/TLC z-scores and significantly lower FEF\textsubscript{25-75} and FEF\textsubscript{75} z-scores than healthy controls. The presence of a trend towards an association between ADC and small airways markers in our CF population would also support this explanation, although this did not reach statistical significance. More recently, a study to evaluate changes in ADC over short periods of time in 11 young adults with CF found statistically significant reductions in ADC between scan and rescan. The study concluded that these changes may arise due to the movement of mucus plugs and the subsequent alterations in gas trapping (Kirby, Villemaire et al. 2013).

Another possible explanation for the reported ADC results in our CF and healthy control populations is related to issues of technique. ADC measurements are extremely dependent on the details of the technique used in the MR experiment (Osmanagic, Sukstanskii et al. 2010). In other words, the diffusion signal from which quantitative information is extracted about lung microstructure dependent on a number of factors, including diffusion time, magnet strength and the complex branching acinar geometry (Parra-Robles, Wild 2012). In the current study, ADC measurements were obtained over a diffusion time of 14 milliseconds (ms). The distance that \(^3\)He gas diffused over this diffusion time was estimated to reach a couple of alveoli and a bit of duct (Ball 2011). However, Parra-Robles and Wild have criticized the use of long diffusion times (>5 ms) to measure ADC, because the gas sampling length scales in the long diffusion times far exceed the alveolar duct and may inaccurately estimate the alveolar size (Parra-Robles, Wild 2014). Even though we may not measure the size of an alveolus accurately, however, our ADC measurements should still indicate abnormalities in the lung at the duct and sac level, otherwise the ADC would be the same as free gas 0.9 cm\(^2\)/sec. Therefore, our findings suggest that CF lung disease may not induce major changes at the lung periphery (i.e. at acinus or alveolar level) in early stages, instead suggesting that damage begins in slightly larger airways and progressed distally.

Furthermore, Parra-Robles and Wild report that the increase in airway dimensions with lung expansion may change the relative contribution of the different structural sizes (e.g., alveolar size vs. duct length) to the measured ADC, meaning that under certain conditions an increase of alveolar size and duct length (i.e., airway enlargement) may even produce a slight decrease in the ADC (Parra-Robles, Wild 2014). However, it should be noted that our ADC measurements in healthy controls were found to be in
agreement with the previously reported normative data, which supports the accuracy of our approach (Bink, Hanisch et al. 2007).

On the other hand, recent studies have shown that ADC obtained over a long period of several seconds is more sensitive than ADC obtained over short periods of several milliseconds, especially in subjects with an airflow obstruction (Wang, Altes et al. 2008, Gonem, Ball et al. 2011, Gonem, Hardy et al. 2014). It has been suggested that this is due to the diffusive molecular transport which can cover only limited peripheral air spaces, as little as one or two alveolated airways, over a short period of milliseconds (Verbanck, Paiva 2007). In contrast, over long periods of seconds, molecular diffusion may cover an entire gas exchanging unit, such as an acinus (Verbanck, Paiva 2007, Verbanck, Paiva 2010). Gonem et al. found that ADC measurements obtained at 1 sec were significantly higher in patients with asthma compared to healthy controls, but ADC at 13 ms did not differ significantly between the groups (Gonem, Hardy et al. 2014). Wang and colleagues have also demonstrated significant elevations in ADC values obtained over long periods of 1.5 sec in patients with asthma compared to healthy subjects, as opposed to ADC measurements over short periods of 1 ms (Wang, Altes et al. 2008). Furthermore, another study in subjects with asthma has shown a lower mean ADC value obtained over a short period of ms in asthmatics when compared to healthy subjects, although this did not reach statistical significant (Fain, Altes et al. 2006).

**Peripheral airways dimension (R and h):**

Our $^3$He MR finding shows no significant difference in R and h between healthy subjects and subjects with CF. However, in patients with CF, it would be expected that the inflammatory processes could thicken the alveolar walls and effectively reduce the alveolar depth (Yablonskiy, Sukstanskii et al. 2009, Quirk, Lutey et al. 2011). Based on pathological studies in children with CF we would expect an increase in the alveolar duct radius due to the presence of mild alveolar enlargement (Sobonya, Taussig 1986).

It should be noted that the calculation of the dimension of peripheral airways in the present study was based on the acinar model (Yablonskiy model) which assumes that estimates of airway dimensions are independent of diffusion time (Yablonskiy, Sukstanskii et al. 2009). However, theoretical work and *in-vivo* experiments in healthy
volunteers have shown that the estimated airways dimensions \((R\) and \(h)\) increase linearly with increasing diffusion time (Parra-Robles, Wild 2012). These authors criticize the acinar model that we used to derive \(R\) and \(h\), stating that the acinar model is not valid at diffusion times above approximately 2 ms, as it overestimates airway dimensions (Parra-Robles, Wild 2012). This study has used a diffusion time of 5 ms to estimate the dimension of peripheral airways and found that our normative data for the acinar duct radius \((R)\) was 446 mm, while for the alveolar sleeve depth \((h)\) it was 243 mm. These measurements were higher than those reported previously in healthy subjects (Quirk, Lutey et al. 2011). Using a diffusion time of 1.8 ms, the acinar duct radius\((R)\) and alveolar depth \((h)\) they reported were 300 µm and 140 µm respectively; these values were consistent with previously published normative values obtained using invasive techniques (Haefeli-Bleuer, Weibel 1988, Quirk, Lutey et al. 2011). However, it has been argued by Narayanan and colleagues that for longer diffusion times (i.e. 5 ms) the root-mean-square displacement of \(^3\text{He}\) atoms is only 1.58 times larger than that obtained with shorter diffusion times (i.e. 2 ms) (Narayanan, Owers-Bradley et al. 2014). This justifies our measurements in that they are sensitive to alveolar dimensions. Obviously, further work is needed to resolve the controversy arising with regards to obtaining these measurements.

6.6.2 Relationships between \(^3\text{He}\) MR parameters with \(\text{FEV}_1\) and small airway markers:

Our study shows no statistically significant relationships between global ADC and \(\text{FEV}_1\), \(z\)-score or small airway markers in both healthy subjects and subjects with CF. Previous studies in healthy subjects and patients with asthma have reported a similar lack of correlation between ADC with spirometry (Wang, Altes et al. 2008, Ball 2011). The absence of association between ADC and \(\text{FEV}_1\) is to be expected, especially in healthy subjects and subjects with mild lung disease, because each of these two indices reflects different sites in the tracheo-bronchial tree. However, a recent study in adult subjects with CF has illustrated a significant correlation between the scan–rescan change in ADC and \(\text{FEV}_1\), attributed this to the movement of mucus plugs and the subsequent alterations in gas trapping (Kirby, Villedarne et al. 2013).

In contrast, we would assume an association between ADC and the markers of small airway obstruction mainly in subjects with CF. This assumption is based on the existing evidence of the presence of mild alveolar enlargement and small airways disease from
early in the life of most children with CF (Sobonya, Taussig 1986, Hamutcu 2002, Gustafsson, De Jong et al. 2008, Horsley, Macleod et al. 2008, Castile, Hayes et al. 2000, de Jong 2004, Bannier 2010). In our CF population, there was a trend towards such association between these measures, but none of these reached statistical significance. This might be explained by the fact that the majority of our CF population only had mild to moderate CF lung disease. A study in subjects with severe asthma has shown an increasing association between ADC and air trapping as asthma severity increased (Ball 2011). They have also shown an association between R and h with the ratio of RV/TLC, but not with spirometry (Ball 2011).

The findings of the current study show a statistically significant positive relationship between R and RV/TLC z-score and statistically significant negative relationships between h and RV/TLC z-score in subjects with CF. It can be assumed that the increase of R and the decrease of h with increasing air trapping in subjects with CF reflects an increase in the number of constricted peripheral airways as the disease progresses. This can be supported further by the statistically significant negative association discovered between R and both small airways markers (FEF25-75 and FEF75 z-scores), in addition to the statistically significant positive association between h with FEV1, FEF25-75, and FEF75 z-scores in our CF population.

6.6.3 Relationships between $^3$He MR parameters and LCI:

Our findings show no statistically significant relationships between $^3$He MR parameters and LCI in both health and disease. In the present study, subjects with CF displayed evidence of large and small airways obstruction and air trapping, with reduced FEV1, FEF75, FEF25-75 and increased RV/TLC z-scores, compared to healthy controls. This is in parallel with greater overall VI. However, ADC was found to be within normal limits in all subjects with CF in our population, with no correlation with any small airway marker. This suggests that the alveoli are not a major site of involvement in CF or that our ADC measurements were biased by the short-diffusion times of 14 ms.

Recently, there has been growing interest towards investigation of the association between $^3$He MR parameters and N2MBW indices due to the need to identify the extent to which ventilation heterogeneity is sited mainly in the lung periphery in any given disease (Verbanck, Paiva et al. 2012). However, the majority of the studies examining this association were conducted on subjects with asthma (Ball 2011, Gonem, Ball et al.
Two of these studies reported significant association between ADC and $S_{\text{acin}}$ in subjects with moderate to severe asthma, one reported a lack of association between $S_{\text{acin}}$ and ADC and one found significant association between ADC, R and $h$ with LCI in 10 severe asthmatics (Ball et al. 2011, Gonem et al. 2013, Gonem et al. 2014). The only study that focused on this association in subjects with CF have suggested that hyperpolarized $^3$He MRI may be more sensitive to early ventilation changes in CF than LCI derived from $N_2$MBW (Marshall et al. 2013). Their study looked at abnormalities at $^3$He MR images obtained from 4 children with CF, rather than the apparent diffusion coefficients. They observed ventilation abnormalities in all $^3$He MR images whereas LCI was abnormal in only 2 of the 4 children (Marshall et al. 2013). The discrepancy reported between findings from previous studies, as well as findings from the present study might be technique related, as described earlier (section 6.6.1). Therefore, a complete understanding of how different measurement techniques impact on the data is essential if we are to draw firm conclusions and compare findings between different centres.

In conclusion, hyperpolarised $^3$He diffusion MR is a well tolerated and safe technique that can be performed in children from school-age with comparatively little cooperation. It is a sensitive and non-invasive test with which it is possible to estimate acinar geometry, but this is currently hindered by the use of different techniques in different centres.

We hypothesised that ADC and R would be larger and $h$ smaller in CF, when compared to healthy controls. Our findings did not support this hypothesis, as ADC was lower compared to healthy controls, with no differences in R and $h$. This leads us to speculate that CF lung disease may not initially become evident in the extreme periphery, but commence in slightly larger airways and then progress distally. The strong correlation shown between peripheral airway dimension parameters with the severity of the disease as determined by small airways markers ($\text{FEF}_{25-75}$, $\text{FEF}_{75}$ and RV/TLC $z$-scores) supported our second hypothesis. This indicates the potential of this technique to improve our understanding of the site and severity of CF lung disease, as well as to target this disease more effectively from an early age. Larger sample sizes including subjects with different severity of CF lung disease are required to extend the findings from this study.
Chapter 7 Conclusion and Future work:

7.1 Summary of most important results

Prior to the studies presented in this thesis, LCI was already recognised as being a more sensitive measure of airways dysfunction in children, with a normal range that was stable throughout childhood (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Gustafsson, De Jong et al. 2008, Owens, Aurora et al. 2011). However, the majority of the assessments looking at LCI were based on cross-sectional data (Gustafsson 2003, Aurora, Gustafsson et al. 2004, Aurora 2004, Kraemer, Blum et al. 2005, Kieninger, Singer et al. 2011, Singer, Kieninger et al. 2013).

The first part of this thesis deals with standardizing the methodology of N2MBW test (Chapter 4). The results of this chapter demonstrate that physical characteristics have a trivial effect on the indices of VI in healthy children, so ULNs can be used for the assessment of VI indices on single occasions during school-age as they may not have any significant clinical impact on interpretation. Moreover, the findings from this chapter confirm previous observations of $S_{\text{cond}}$ reaching a maximum as inhomogeneity becomes more severe in older subjects (Horsley, Macleod et al. 2008). As a result, this has hindered the ability to follow the changes in $S_{\text{cond}}$ and $S_{\text{acin}}$ over years in children with CF and highlights the need to develop a new analysis technique for the determination of phase III indices in subjects with severe VI. Finally, I have showed that VI indices derived from two different N2MBW systems using different techniques to measure N2 concentration, as well as from different data collection and analysis methods, are incomparable in health and disease. This has important implications for longitudinal studies and normative values.

The following section of the thesis (Chapter 5) deals with the longitudinal follow-up of 27 school-age children with CF to monitor changes in LCI in comparison to conventional lung function measures. In addition, we studied the association between changes in lung function over time with age, *P. aeruginosa* infection and CFTR genotypes. The findings from this study were not entirely as expected. More than half of children with CF showed improvement in LCI and RV/TLC over time, but with deterioration in airflow limitation parameters. This may indicate a true improvement in ventilation distribution and pulmonary hyperinflation throughout the study period, which began at the time when new treatment protocols and improved annual review
procedures were introduced to the clinical care of children with CF. Alternatively, it is possible that LCI and lung volume measurements might be influenced by methodological and technical factors.

Nevertheless, the statistical model in the current study has shown that LCI presented the earliest index to change over time, followed by $\text{FRC}_{\text{N2}}$. Apart from $\text{FRC}_{\text{pleth}}$ and $\text{FRC}_{\text{N2}}$, changes in lung function parameters were found to be strongly associated with age, with $\text{FEV}_{1}$ z-score presenting the highest association with age. The results here also revealed the age of occurrence of abnormal lung function for all parameters that showed significant association with age. LCI was found to be the earliest index to deteriorate with age, being elevated in all children with CF by the time of their 7th birthday. On the other hand, $\text{FRC}_{\text{pleth}}$ was the only parameter to be associated with $P. \text{aeruginosa}$ infection in children with CF over time. Over a 4 year period, children infected with $P. \text{aeruginosa}$ tended to have a higher $\text{FRC}_{\text{pleth}}$ than non-infected children. My cross-sectional data from 37 children with CF demonstrated that children infected with $P. \text{aeruginosa}$ in the previous 12 months of their lung function visit have significantly lower forced expiratory flows, as well as significantly higher VI than those not infected with this organism. No significant association between CFTR genotypes and any of the lung function parameters in children with CF were found in our study. This non-significant result may be attributable to our small sample size, as we examined 11 children with homozygous genotype vs. 16 children with heterozygous genotype, while this type of study is generally considered to need larger sample sizes in each genotype-group.

Another study described in chapter 5 involved a subgroup of children with mild to moderate CF lung disease in whom the ability of LCI to detect the short-term response to intravenous antibiotics was compared to the gold standard, $\text{FEV}_{1}$. The findings demonstrated a clinically significant improvement in LCI (>7% change) with IV antibiotic treatment in 1/3 of children, but this did not reach statistical significance. No significant improvement in $\text{FEV}_{1}$, or any other lung function measurements were reported post IV treatment. It should be noted that our sample size estimation for this study was based on LCI and not on the secondary outcomes measurements, which may be more variable. This might explain the lack of any detectable changes.
The last section of this thesis (Chapter 6) deals with the estimation of the dimensions of the lung peripheral microstructure in subjects with CF on a single occasion in comparison to age-matched healthy controls. Data from $^3$He diffusion MR have shown that the apparent diffusion coefficient (ADC), a surrogate marker of alveolar size, was significantly lower in 18 subjects with CF compared to 27 healthy controls. No significant difference was found in the alveolar sleeve depth (h) and alveolar radius (R) between both populations. These unexpected results suggest that CF lung disease may not induce very large changes in most of the microstructure in early stages i.e. acinus or alveolar level. Alternatively, the significant reduction in ADC values might be technique-related or may be attributable to small airway obstruction with mucus, as this will act as barrier and restrict the diffusion of $^3$He, thereby interfering with the accurate determination of ADC.

7.2 Clinical implication of results and future research:

Interpretation and discussion of our results is presented within the relevant results chapters. This chapter will consolidate the information from the three results chapters and provide a cogent discussion of the implications of these results with regards to the assessment of early CF lung disease and our understanding of the progression of CF lung disease in school-age children.

Methodological aspects:

The findings from the standardization N$_2$MBW methodology chapter (Chapter 4) highlight the need to adopt a single piece of equipment and a unified analysis technique to derive VI indices as this has important implications for longitudinal studies and normative values. In addition to the need to develop a new analysis technique for the determination of phase III indices in subjects with severe VI.

What is the role of LCI in comparison to conventional lung function measures in the assessment of CF lung disease?

In chapter 5, it was found that the changes in LCI preceded changes in conventional lung function measurements over time. As the disease progressed, lung volume measurements showed a closer relation to LCI than spirometry in terms of the direction of change. This suggests that these measures are sensitive to early changes in the lung periphery in which spirometry measures are insensitive. These findings partly prove our
first hypothesis in that LCI will be more sensitive to changes in the lung periphery over time than conventional lung function measurements. For this reason, we suggest that the $N_2$MBW test should be used as a complementary technique to conventional measures in the assessment of early CF lung disease.

The findings from the interventional cross-sectional study demonstrate the validity of the application of LCI as an outcome measure to detect the response to treatment in children with mild lung disease. Clinically significant improvement was demonstrated in LCI (>7% change) with IV antibiotic treatment in 1/3 of children, though this did not reach statistical significance. This trend supports our third hypothesis in that LCI will improve prior to FEV₁ in response to IV treatment in subjects with mild to moderate CF lung disease.

**What about children with more severe lung disease?**

This study postulates that the significance of LCI and thus their clinical use is most useful in the early stages of the disease. This is because children with severe CF lung disease have been noted to experience a prolonged washout test, with LCI increasing until it reached an asymptote of 16 TO. Therefore, single breath washout rather than MBW may be sufficient to monitor those subjects at a more advanced stage of the disease, in parallel with spirometry.

**What is the role of $^3$He MR?**

In chapter 6, a significant reduction was demonstrated in the ADC in subjects with CF compared to age-matched healthy controls. I cannot explain these results and postulate that they might be related to either physiological (i.e. obstruction with mucus plugs) or technical factors. No changes were found in the peripheral airways dimension ($R$ and $h$) between both populations. An alternative explanation is that CF does not affect airways within the area of the diffusion-convection front, or cause structural damage to the acini in early stages of the disease, but that it instead affects airways within the conducting zone. This corresponds with our understanding regarding the site of early pathology in CF. These limited data do not support the fourth hypothesis in which it was postulated that ADC and $R$ will increase in CF, whereas $h$ would decrease. In order to draw firm conclusions with regards to the role of this technique in early CF lung disease, a larger study is required, involving subjects with a range of disease severity.
What next?

Although the importance of LCI and its role in the detection of early changes in CF lung disease has been shown, there is still a need for further studies that involve:

a) Other measures of disease severity: A study that incorporates other clinical markers and techniques such as pulmonary and systematic inflammatory markers and structural changes on CT into a larger longitudinal study would likely be of great importance. Imaging clearly plays an important role in the study of small airways, but because the radiation exposure associated means that it is unlikely to be used in a routine clinical setting. In addition, the inclusion of control subjects will allow correction to be made for the normal variability of the outcome measures over the study period.

c) Interventional studies: There is a need to assess the capacity of MBW indices to detect long-term response to IV antibiotics as well as to clinically assess improvement in large numbers of subjects with different severity of CF lung disease. In addition, there is a need to assess normal variability in lung function parameters over a period equivalent to the treatment period.

d) Comparison between different MBW systems, and analysis methods: A comparison is needed between data obtained from two MBW systems, but using a single analysis software and vice versa in order to determine the source of variation between measurements and whether it is machine specific, equipment or software specific. I have an attempt to collect data using one machine (Exhalyzer D) and analyse results using different software (Matlab 2011 vs. Spiroware 3.1), but statistically and clinically significant differences were found between them. The population studied and N₂ MBW data collection as part of the previous comparative study occurred at a different centre; therefore, the findings were not included in this thesis.

e) Further $^3$He MR studies: There is a need for repeatability data to be gathered for $^3$He diffusion MR parameters in subjects with CF. In addition, there is a need for a larger combined study of lung $^3$He diffusion MR with physiological assessment to identify and monitor ventilation inhomogeneity in early CF lung disease and to assess response to new treatments. The comparison of results from these studies with CT, the current imaging gold standard will be of great importance.
7.3 Conclusion:

Based on studies conducted in this thesis, it can be concluded that LCI derived from MBW test is currently the best and the most sensitive measurement to assess the progression of early CF lung disease and to detect functional changes over time. The adoption of a single piece of equipment and standard analysis technique would facilitate the transition from research into clinical practice.

The findings from the \(^3\)He MR, although it was unexpected, it highlights the potential of this technique to help improving our understanding of the site and severity of CF lung disease and to target this disease effectively from early age. Further studies utilising a larger sample size, including subjects with different severity of CF lung disease, are required to extend the findings from this study.
Appendices

Appendix A: Ethical approval, PIS and consent form for longitudinal study
24 September 2012

Dr Erol A Galliard
Senior Lecturer in Child Health
University of Leicester
Dept of Infection, Immunity &
Inflammation, University of
Leicester, RKCSB, LRI, Leicester
LE2 7LX

Dear Dr Galliard

Study title: Longitudinal study of microbial diversity, viruses, fungi
and biomarkers in sputum and blood of patients with
cystic fibrosis.

IRAS project reference: 68332
REC reference: 12/WM/0286

The Research Ethics Committee reviewed the above application at the meeting held on 12 September 2012. Thank you for attending to discuss the study.

Ethical opinion

1. The Committee asked how long potential participants would have to decide whether or
not to participate in the study, you stated that parents will be given the information
sheets and can take as long as they want before deciding whether to participate.

2. The Committee queried the collection of sputum from younger participants via
broncoscopy, you informed the committee that a lot of young children who have a
cough will be treated with antibiotics; if two courses of antibiotics are not successful in
treating the cough then a broncoscopy will be performed if it is felt the patient requires
it on clinical grounds. You went on to inform the committee that there has been an
Australian study which performs routine annual broncoscopies on children however
you do not feel this is useful and this study will only perform broncoscopies when it is
clinically indicated.

3. You were asked for further information on the blood samples which will be collected.
You informed the committee that participants will have an annual comprehensive blood
profile which will review all elements of the blood. The committee went on to ask how
the extra blood will be used, you stated that inflammatory biomarkers will be looked at
and if accurate then it may be a way to manage participants rather than doing scans
etc.

4. It was highlighted to you that the participant information sheet should have the relevant:
PALS contact number added to the participant information sheet.
The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

**Ethical review of research sites**

**NHS Sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

**Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

**Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.**

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.rdforum.nhs.uk](http://www.rdforum.nhs.uk).

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

1. The Participant Information Sheet should contain the relevant PALS contact number.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

**Approved documents**

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering Letter</td>
<td></td>
<td>16 August 2012</td>
</tr>
<tr>
<td>Evidence of insurance or indemnity</td>
<td></td>
<td>24 August 2012</td>
</tr>
<tr>
<td>Investigator CV</td>
<td></td>
<td>01 May 2012</td>
</tr>
<tr>
<td>Other: Unfavourable Opinion Letter</td>
<td></td>
<td>21 June 2012</td>
</tr>
<tr>
<td>Participant Consent Form: Parent/Guardian Children with</td>
<td>2</td>
<td>23 July 2012</td>
</tr>
</tbody>
</table>
Cystic Fibrosis 5 years Old and Younger
Participant Consent Form: Parent/Guardian of Child with Cystic Fibrosis
2 23 July 2012
Participant Consent Form: Adult Patients 17 Years and Older with Cystic Fibrosis
2 23 July 2012
Participant Consent Form: Parent/Guardian of Child with Cystic Fibrosis’s Request to use Existing Sputum Samples
1 23 July 2012
Participant Consent Form: Adult Patients 17 Years and Older with Cystic Fibrosis’s Request to use Existing Sputum Samples
1 23 July 2012
Participant Information Sheet: Parent/Guardian Children with Cystic Fibrosis 5 years Old and Younger
2 23 July 2012
Participant Information Sheet: Parent/Guardian Children with Cystic Fibrosis 6-16 Years Old
2 23 July 2012
Participant Information Sheet: Young Children with Cystic Fibrosis 6-10 Years
2 23 July 2012
Participant Information Sheet: Adult Patients 17 Years and Older with Cystic Fibrosis
2 23 July 2012
Participant Information Sheet: Parent/Guardian Children with Cystic Fibrosis’s Request to use Existing Sputum Samples
1 23 July 2012
Participant Information Sheet: Young Children with Cystic Fibrosis 6-10 Years Request to use Existing Sputum Samples
1 23 July 2012
Participant Information Sheet: Children with Cystic Fibrosis 11-16 Years Request to use Existing Sputum Samples
1 23 July 2012
Participant Information Sheet: Adult Patients 17 Years and Older with Cystic Fibrosis’s Request to use Existing Sputum Samples
1 23 July 2012
Protocol
2 23 July 2012
REC application
B6332/354677/1/139 16 August 2012
Referees or other scientific critique report
Dr Kenneth Bruce 07 August 2012

Membership of the Committee
The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance
The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review
Reporting requirements
The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study
The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

| 12/WM/0265 | Please quote this number on all correspondence |

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr Rex J Poisson
Chair

Email: lisa.gregory@nottsuct.nhs.uk

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments

‘After ethical review – guidance for researchers’ [SL-AP2]

Copy to: Ms Wendy Gamble
Mrs Carolyn Maloney, University Hospitals of Leicester NHS Trust
24/10/2012

Dr Erol A Gaillard  
University of Leicester  
Dept of Infection, Immunity & Inflammation  
RKGSE, Leicester Royal Infirmary  
Leicester  
LE2 7LX

Dear Dr Erol A Gaillard

Ref: UHL 11204

Title: Longitudinal study of microbial diversity, viruses, fungi and biomarkers in sputum and blood of patients with cystic fibrosis.

Project Status: Project Approved  
End Date: 01/10/2017

I am pleased to confirm that with effect from the date of this letter, the above study has Trust Research & Development permission to commence at University Hospitals of Leicester NHS Trust. The research must be conducted in line with the Protocol and fulfil any contractual obligations agreed with the Sponsor. If you identify any issues during the course of your research that are likely to affect these obligations you must contact the R&D Office.

In order for the UHL Trust to comply with targets set by the Department of Health through the 'Plan for Growth', there is an expectation that the first patient will be recruited within 30 days of the date of this letter. If there is likely to be a problem achieving this target, please contact the office as soon as possible. You will be asked to provide the date of the first patient recruited in due course. In addition, the Title, REC Reference number, local target recruitment and actual recruitment for this study will be published on a quarterly basis on the UHL Trust external website.

All documents received by this office have been reviewed and form part of the approval. The documents received and approved are as follows:

<table>
<thead>
<tr>
<th>Documents</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering Letter</td>
<td>15.08.12</td>
<td></td>
</tr>
<tr>
<td>Evidence of insurance or indemnity</td>
<td>24.08.12</td>
<td></td>
</tr>
<tr>
<td>Other: Unfavourable Opinion Letter</td>
<td>21.08.12</td>
<td></td>
</tr>
<tr>
<td>PCH: Parent/Guardian Children with Cystic Fibrosis 5 years old and Younger</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PCF: Parent / Guardian of Child with Cystic Fibrosis</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PCF: Adult Patients 17 Years and Older with Cystic Fibrosis</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PCF: Parent / Guardian of Child with Cystic Fibrosis Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PCF: Adult Patients 17 Years and Older with Cystic Fibrosis Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Parent / Guardian Children with Cystic Fibrosis 5 Years Old and Younger</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Parent / Guardian Children with Cystic Fibrosis 6 – 16 Years Old</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Young Children with Cystic Fibrosis 8-16 Years</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Adult Patients 17 Years and Older with Cystic Fibrosis</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Parent / Guardian Children with Cystic Fibrosis Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Young Children with Cystic Fibrosis 8-16 Years Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Children with Cystic Fibrosis 11-16 Years Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>PIS: Adult Patients 17 Years and Older with Cystic Fibrosis Request to use Existing Sputum Samples</td>
<td>1</td>
<td>23.07.12</td>
</tr>
<tr>
<td>Protocol</td>
<td>2</td>
<td>23.07.12</td>
</tr>
<tr>
<td>REC Application</td>
<td>07.08.12</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** As per my email dated 24/10/12 the only personnel approved to work on the study are Dr Erol Gaillard, Dr Chandra Chri, Dr Stuart Wilkinson and Dr Simon Range. Everyone else will have to be added onto the study at a later date as a minor amendment.

*Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.*

Undertaking research in the NHS comes with a range of regulatory responsibilities. Please ensure that you and your research team are familiar with, and understand the roles and responsibilities both collectively and individually.

Documents listing the roles and responsibilities for all individuals involved in research can be found on the R&D pages of the Public Website. It is important that you familiarise yourself with the Standard Operating Procedures, Policies and all other relevant documents which can be located by visiting www.leicestershospitals.nhs.uk/aboutus/education-and-research

The R&D Office is keen to support and facilitate research wherever possible. If you have any questions regarding this or other research you wish to undertake in the Trust, please contact this office. Our contact details are provided on the attached sheet.

We wish you every success with your research.

Yours sincerely,

David Helmski
Assistant Director of R&D

Encs: R&D Office Contact Information
INFORMATION SHEET FOR PARENT(S)/GUARDIAN(S)
Children with Cystic Fibrosis (6-16 years old)

Study title: Longitudinal study of microbial diversity, viruses, fungi and biomarkers in sputum and blood of patients with cystic fibrosis
Principal Investigator: Dr Erol Gaillard

We are inviting your child to take part in a research study. Before deciding, it is important for your child and your family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask the study team if there is anything that is not clear or if you would like more information. Our contact details are at the end of this information leaflet. Take time to decide whether or not you wish your child to take part.

What is the purpose of the study?
In cystic fibrosis (CF), chest infections are the main problem affecting the lung. Using standard tests we can only identify very few bacteria. Over recent years, the development of new techniques has allowed a much more detailed analysis of the bacteria, viruses and fungi present in the airways. Not surprisingly, the number and diversity of organisms revealed using these techniques is much larger than previously thought.

This is a new field of research and it is unknown how the type and numbers of organisms in the lungs of people with CF change during acute respiratory infections, and over time with the use of antibiotics. Furthermore, the link between infections and lung inflammation is not known. Answers to these questions may alter the way we look at cystic fibrosis lung disease and may influence medical treatment in the future.

Why has my child been chosen?
We have selected your child because he/she has cystic fibrosis.

Does my child have to take part?
It is up to you and your child to decide whether or not to take part. If your child decides to take part you will be given this information sheet to keep and be asked to sign a consent form. If your child decides to take part they can withdraw at any time, without giving a reason. A decision to take part or withdraw will not affect any treatment your child may be receiving from their doctor or the hospital.

What will happen to my child if he/she takes part?
We would like to analyse and store sputum/cough samples from your child that we obtain, when possible, at every clinic appointment, annual review and hospital admission your child has. Using sophisticated laboratory techniques we will analyse the stored samples for bacteria, fungi and viruses but also molecules involved in airway inflammation called biomarkers. The research will in no way delay the appropriate treatment given to your child.

We would like to take and store a small amount of extra blood (size of 2 teaspoon measures: 10ml) at a time when your child is already scheduled to have a blood test for clinical reasons. We would like to collect this extra blood sample, when possible, at every clinic appointment, annual review and hospital admission your child has. We will then measure the relevant biomarkers in the blood. We will also use the information obtained from the specialist breathing tests that your child is doing as part of clinical management. If your child takes part in this study, the study team will need to look at their hospital notes.

Information Sheet for Parents(s)/Guardian(s)
Children with Cystic Fibrosis (6-16 years old)
Version 2, dated 23 July 2012

220
What are the possible benefits of taking part?
There are no direct benefits for your child by taking part in this study, however we will learn more about infections and lung inflammation in cystic fibrosis. This may influence our antibiotic management in the future.

What if something goes wrong?
There is no risk of harm from any parts of this research. However, if your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone’s negligence then you may have grounds for a legal action but you may have to pay for it.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the National Health Service complaints mechanism will be available to you. If you have any complaints about staff, or if anything serious happens during the procedure you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen Road, Leicester, LE3 4QF, where your concerns will be dealt with within 2 weeks.

Will I receive payment for participating in this research study?
No.

Will my taking part in this study be kept confidential?
Yes. All the results are confidential. There is no way in which anybody outside the research study team from University Hospitals of Leicester NHS Trust and the University of Leicester will be able to identify any patient from the results of the tests. All the results, names and addresses are kept on different files and are secure. We will only inform any outside individual (such as your general practitioner) with your permission. Dr Gaillard will have overall control of the sputum/cough and blood samples, i.e. act as Custodian.

What will happen to the results of the research study?
The results of the study will be presented at national and international scientific meetings and published in the medical literature in due course. In practice, publication takes about 1-2 years from the end of a study. Your child will not be identified in any report or publication. We will also provide you with a written summary of the results from this study.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Ethical approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information:
Please contact the following number to speak to or leave a message for Dr Gaillard or another member of the research team.
• Phone: 0116 252 5881 • Email: gae15@le.ac.uk • Fax: 0116 252 3282

Patient Information and Liaison Service
• Free phone line: 08031 788337

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this information leaflet

Dr Erol Gaillard, Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary

Information Sheet for Parents(s) or Guardian(s)
Children with Cystic Fibrosis (5-15 years old)

Version 2, dated 23 July 2012
University Hospitals of Leicester NHS

Information Sheet
For Young Children with Cystic Fibrosis (6 years – 10 years)

Study title: Longitudinal study of microbial diversity, viruses, fungi and biomarkers in sputum and blood of patients with cystic fibrosis.

Principal Investigator: Dr Erol Gaillard

We are trying to find out what happens in your lungs when you have a cough. We would like you to help us. Your parent(s)/guardian(s) will have some information too, so you can talk to them about it. Please take your time to read about the study.

Why?
We want to find out more about what is happening inside your lungs when you have a cough or cold and how this is different when you are well. We know a little about what is going on inside your lungs.

How will I take part in this study?
We would like to use and store the cough samples that we regularly obtain from you, when possible, at every clinic appointment, annual review and hospital admission, including those where you have the salty nebuliser to help you cough. On those samples we will do some tests to understand what is happening inside your lungs when you have a cough and you are well.

We would also like to ask you if we can have a few extra drops of blood, (2 teaspoon measures; 10ml). We would like to take these samples, when possible, at all of your clinic appointments, annual review and hospital admissions. This will only happen when you normally have a blood test in hospital. There will be no extra blood tests.

Problems?
There are no extra needles, no pain or discomfort and no other risks. The tests will not affect you in any way. Your parent(s)/guardian(s) will be with you all the time.

Anything else?
If you take part in this study the study team will need to look at your hospital notes.

What next?
If you and your parent(s)/guardian(s) are happy to take part in this study, your parent(s)/guardian(s) will sign a “consent form”. The important thing to remember is you do not have to take part if you don’t want to. After you take part we will give you a summary of the results from this study.

Any questions?
If you would like to ask the research team any questions please ring the below number and email Dr Erol Gaillard.
• phone (0116) 252 5881
• email eaq1@le.ac.uk
• fax (0116) 252 3282

Patient Information and Liaison Service
• Free phone line: 08081 788337
You will be given a copy of the information sheet and a signed consent form to keep.

Dr Erol Gaillard, Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary.

Information Sheet for Young Children with Cystic Fibrosis (6 years – 10 years)

Version 2, dated 23 July 2012
Information sheet for children with cystic fibrosis (11 years – 16 years)

Study title: Longitudinal study of microbial diversity, viruses, fungi and biomarkers in sputum and blood of patients with cystic fibrosis.

Principal investigator: Dr Erol Gaillard

We are inviting you to take part in a research study. Before you decide, it is important for you and your family to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask the study team if there is anything that is not clear, or if you would like more information. Please take time to decide whether or not you wish to take part.

What is the purpose of the study?
We want to find out more about what is happening inside your lungs when you have a cough or a cold and how this is different when you are well. Finding out what happens inside your lungs when you cough could help other children who have cystic fibrosis with coughs and colds.

Why have I been chosen?
You have been chosen because you have cystic fibrosis.

Do I have to take part?
It is completely up to you whether or not you are happy to take part in this study. You can keep this information sheet. If you decide to take part in this study you are still free to withdraw at any time and without giving a reason. This will not affect how we treat you in hospital.

What will happen to me if I take part?
We would like to use and store the cough samples that we regularly obtain from you, when possible, at every clinic appointment, annual review and hospital admissions. These include when you have the salty nebulizer to help you cough. On those samples we will do some tests to understand what is happening inside your lungs when you are unwell.

We would also like to use and store a few extra drops of blood (the size of 2 teaspoon measure; 10ml). This will only happen when you have a routine blood test at your clinic appointment. We would like to take these samples, whenever possible, at every clinic appointment, annual review and hospital admissions. There will be NO EXTRA blood tests.

Are there any risks of taking part?
There are no extra needles, no pain or discomfort and no other risks. The tests will not affect you in any way. Your parent(s)/guardian(s) will be with you all the time.

Anything else?
If you take part in this study the study team will need to look at your hospital notes.
What next?
If you and your parent(s)/guardian(s) are happy to take part in this study, your parent(s)/guardian(s) will sign a ‘consent form’. The important thing to remember is you do not have to take part if you don’t want to.

What are the benefits of taking part?
There are no direct benefits right now for you from taking part in this study. However we will find out what is happening inside your lungs which will help us to develop better treatments in the future.

What will happen to the results of the research study?
The results of the study will be presented at scientific meetings and in medical newspapers. We will also give you a written summary of the results from this study.

Thank you very much for reading this information leaflet.

Any questions?
If you would like to know any more information or leave a message for a member of the study team, please use the below telephone numbers and/or email address:
• phone (0116) 252 5881 • email: esgf15@e.ac.uk • fax (0116) 252 9282

Patient Information and Liaison Service
• Free phone line: 08081 788337

Dr Erol Gaillard
Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary

Information sheet for children
with cystic fibrosis (11 years – 16 years)
CONSENT FORM
For Parent(s)/Guardian(s) of Child with Cystic Fibrosis

Study Number:
Name of Child:

Title of Project: Longitudinal study of microbial diversity, viruses, fungi and biomarkers in sputum and blood of patients with cystic fibrosis.

Principal Investigator: Dr Erol Gaillard

Please initial box

1. I confirm that I have read and understand the Information Sheet for Parent(s)/Guardian(s) of Children with Cystic Fibrosis (6-16 years old), version 2, dated 23rd July 2012. I have had the opportunity to discuss the study and ask questions which have been answered to my satisfaction.

2. I understand that my child’s participation is voluntary and that my child is free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected.

3. I understand that my child’s participation in this trial will result in their routine clinical sputum samples being analysed and stored for future analysis by the research study team.

4. I understand that the extra 10ml of blood taken from my child’s routine clinical blood samples will be analysed and stored for future analysis by the research study team.

5. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from the research team from University Hospitals of Leicester NHS Trust and University of Leicester, and from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

6. I agree to allow my child to take part in the above study.

Name of Child ___________________________ Date ____________ Signature ____________

Name of Parent/Guardian ___________________________ Date ____________ Signature ____________

Researcher ___________________________ Date ____________ Signature ____________

1 for participant; 1 for researcher; 1 for site file

CONSENT FORM
For Parent(s)/Guardian(s) of Child with Cystic Fibrosis

Version 2, dated 23rd July 2012
Appendix B: Ethical approval, PIS, consent form and questionnaires for asthma study in which healthy controls recruited
15 June 2011

Dr Erol A Gailard
Senior Lecturer in Child Health
University of Leicester
Dept of Infection, Immunity &
Inflammation, University of
Leicester, RG05B, LRI, Leicester,
LE2 7LX

Dear Dr Gailard,

Study title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

REC reference: 09/H0403/52

Amendment number: Modified amendment

Amendment date: 03 June 2011

Thank you for submitting the above amendment, which was received on 03 June 2011. It is noted that this is a modification of an amendment previously rejected by the Committee.

The modified amendment was reviewed at the meeting of the Sub-Committee held on 14 June 2011. A list of the members who took part in the review is attached.

Ethical opinion

I am pleased to confirm that the Committee has given a favourable ethical opinion of the modified amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved are:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Consent Form: For Control Child and Parent (5 - 16 years)</td>
<td>2</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Participant Consent Form: For Children with Difficult-to-treat Asthma (5 - 16 years)</td>
<td>2</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Participant information Sheet: Brief information Sheet for Young Children with difficult-to-treat Asthma recruited from clinic (5 years - 19 years)</td>
<td>2</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Participant information Sheet: Information Sheet for Children with Difficult-to-treat Asthma (11 years - 15 years)</td>
<td>2</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Participant information Sheet: Information Sheet for Parents</td>
<td>2</td>
<td>02 June 2011</td>
</tr>
</tbody>
</table>

This Research Ethics Committee is an advisory committee to the East Midlands Strategic Health Authority. The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England.
children with Difficult-to-treat Asthma

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Modified Amendment</td>
<td>Date</td>
</tr>
<tr>
<td>3</td>
<td>02 June 2011</td>
</tr>
<tr>
<td>Covering Letter</td>
<td>Date</td>
</tr>
<tr>
<td>Email correspondence</td>
<td>03 June 2011</td>
</tr>
</tbody>
</table>

| Participant Information Sheet, information sheet for young control children (5 years - 16 years) | Date       |
| 2        | 02 June 2011 |
| Participant Information Sheet, study summary - control children | Date       |
| 2        | 02 June 2011 |
| Participant Information Sheet, information sheet for parents - control children | Date       |
| 2        | 02 June 2011 |
| Participant Information Sheet, information sheet for control children (11 years - 16 years) | Date       |
| 2        | 02 June 2011 |

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

09/0403/92: Please quote this number on all correspondence

Yours sincerely

Mr Robert Johnson
Chair

E-mail: catherine.dixon@nottspol.nhs.uk

Copy to: Mrs Carolyn Maloney, University Hospitals of Leicester NHS Trust
29 April 2010

Dr E. A. Galliard
Senior Lecturer in Child Health
University of Leicester
Dept of Infection, Immunity & Inflammation
RKGSS
Leicester Royal Infirmary
Leicester LE2 7LA

Dear Dr Galliard,

Study Title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

REC reference number: 09/M0403/92
Protocol number: 3

Thank you for your emailed correspondence of 17 March 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/SHSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study:

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research etc.

This Research Ethics Committee is an advisory committee to East Midlands Strategic Health Authority. The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England.
Governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at [http://www.crforum.nhs.uk](http://www.crforum.nhs.uk) Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC application</td>
<td>19364/6934771/1364</td>
<td>19 September 2009</td>
</tr>
<tr>
<td>Investigator CV</td>
<td></td>
<td>09 October 2009</td>
</tr>
<tr>
<td>Referees or other scientific critique report</td>
<td>Designated Referee 1</td>
<td></td>
</tr>
<tr>
<td>Questionnaire: Breathing problems in children aged 14 and over</td>
<td>1</td>
<td>26 October 2009</td>
</tr>
<tr>
<td>Questionnaire: Breathing problems in children</td>
<td>1</td>
<td>26 October 2009</td>
</tr>
<tr>
<td>Covering Letter</td>
<td></td>
<td>16 October 2009</td>
</tr>
<tr>
<td>Referees or other scientific critique report</td>
<td>Designated Referee 2</td>
<td></td>
</tr>
<tr>
<td>Referees or other scientific critique report</td>
<td>Designated Referee 3</td>
<td></td>
</tr>
<tr>
<td>Referees or other scientific critique report</td>
<td>Email correspondence from Peter Glisson</td>
<td>18 January 2010</td>
</tr>
<tr>
<td>Article from American Journal of Respiratory and Critical Care Medicine Vol 161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Article from Clinical and Experimental Allergy, 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to Request for Further Information Protocol</td>
<td>3</td>
<td>01 February 2010</td>
</tr>
<tr>
<td>Participant Information Sheet: Children with Asthma (11 - 16 years)</td>
<td>2</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Participant Information Sheet: Healthy Children (11 - 16 years)</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Participant Information Sheet: Brief Information Sheet for young children with Asthma (5 - 10 years)</td>
<td>3</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Participant Information Sheet: Brief Information Sheet for young healthy children (5 - 10 years)</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Participant Consent Form: For child with Asthma and parent</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Participant Consent Form: For healthy child and parent (6-16 years)</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Response to Request for Further Information</td>
<td></td>
<td>17 March 2010</td>
</tr>
<tr>
<td>Letter of invitation to participant</td>
<td>For healthy children V</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Lay Summary - Children with Asthma</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Lay Summary - Healthy Children</td>
<td>1</td>
<td>12 March 2010</td>
</tr>
<tr>
<td>Email correspondence</td>
<td></td>
<td>04 March 2010</td>
</tr>
</tbody>
</table>
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review.

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H0403/82 Please quote this number on all correspondence

Yours sincerely

Dr Kate Pointon
Chair

Email: trish.wheel@nhs.nhs.uk

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
‘After ethical review – guidance for researchers’

Copy to: R&D office for NHS care organization at lead site - UHL
INFORMATION SHEET FOR PARENTS-Control Children

Study title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

Principal Investigator: Dr Erol Galard

We are inviting (name of child or young person) to take part in a research study. Before deciding, it is important for (name of child or young person) and the family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
Acute asthma attacks in children are common and often require admission to hospital. Inflamed airways are an important feature of an acute asthma attack. This can be confirmed by collecting secretions that are coughed up and studying them under a microscope. In children there is very little data on the type of inflammation present during an acute asthma attack. It is also not clear if the lung remains inflamed during periods when children with asthma are completely well. Such information will be very helpful to increase our knowledge of the mechanisms that trigger an asthma attack in children. The purpose of this study is to understand the link between lung inflammation and acute asthma attacks. This knowledge may help us to predict an acute attack earlier and to commence appropriate treatment before hospital management becomes necessary. This information may help to keep children out of hospital in the future.

Why have I been chosen?
We need to study children with asthma and children that have no breathing problems. Your child has been chosen because he/she has no breathing problems.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide not to take part you can withdraw at any time, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if (name of child or young person) takes part?
We will invite your child to attend the children’s lung function laboratory at the Leicester Royal Infirmary with one or both parent(s) at an agreed date and time that is convenient for you. This can be arranged during school breaks. The visit will take approximately 2 hours and involve a number of tests. These tests are not in any way painful or uncomfortable but they require quite sophisticated and large equipment and experienced people to perform the tests and therefore can only be done in the setting of a dedicated laboratory. The tests give us very detailed information on the workings of the breathing tubes in your child.

Information Sheet for Parents - Healthy Children Version 2, dated 2 June 2011

Ref: 09/H0403/02
First, when you arrive, we will ask you and your child to complete an asthma questionnaire. We will ask whether your child had previous breathing problems and if there are any breathing problems in the family.

Then we will ask your child to do a very simple breathing test to assess your child’s airways. This will involve breathing hard into a tube. This test is done in all the children routinely attending our respiratory clinics. We will then ask your child to breathe into another tube to measure a substance in the air that is breathed out called nitric oxide. This substance is increased when there are inflamed breathing tubes like in asthma. Both these tests are done in the routine asthma clinic and take no longer than 5–10 minutes.

For the next test we will ask your child to sit in a glass box, not dissimilar in size to an old fashioned telephone box and to breathe in and out through a tube. This test will give us information on how much air is going into the different lung compartments.

For the next test your child breathes in and out of a balloon. The balloon is filled with oxygen and for a brief period your child will breathe in pure oxygen. We measure how quickly the oxygen gets cleared from the lungs called a lung clearance index.

Again, whilst this sounds complicated, none of the tests are difficult, uncomfortable or painful. These tests are performed in children routinely as part of a detailed lung assessment in some children all these tests are done on a yearly basis. Most children attending for these tests find them stimulating and fun.

Finally, we would like to obtain a sample of your child’s phlegm. We will give your child a salty mist solution to breathe in and ask your child to cough into a pot at regular intervals. This procedure is very safe and again neither painful nor uncomfortable. It is routinely and frequently performed in children with severe asthma. This procedure takes approximately 15 minutes.

Laboratory work: The secretion samples will be analysed to measure the number and type of inflammatory cell present. We will also study the secretions for consistency of phlegm and measure several biomarkers to test their ability to predict an asthma attack.

**What are the possible benefits of taking part?**

We will learn more about the link between inflamed airways and acute asthma. This will not provide any advantage to your child but may influence the way we treat asthma in the future.

**What if something goes wrong?**

There is no risk of harm from any parts of this research. However, if your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. If you have any complaints about staff, or if anything serious happens during the procedure, you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen Road, Leicester, LE5 4QF, where your concerns will be dealt with within 2 weeks.

**Will I receive payment for participating in this research study?**

You will be paid for any travelling expenses and other out-of-pocket costs, incurred in taking part in the investigations.
Will my taking part in this study be kept confidential?
Yes. All the results are confidential. There is no way in which anybody outside the Division of Child Health and Leicester University will be able to identify any participant from the results of the tests. All the results and names and addresses are kept on different files, and are secure. We will only inform any outside individual (such as your general practitioner) with your permission.
Dr Gaillard will have overall control of the cough secretions, i.e. act as Custodian.

What will happen to the results of the research study?
The results of the study will be presented at national and international scientific meetings and published in the medical literature in due course. In practice, publication takes about 1-2 years from the end of a study. You will not be identified in any report or publication.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information:
Please contact the following number to speak to or leave a message for Dr Gaillard or one of the other research team.

Telephone: (0116) 252 3262 / (0116) 258 5691
E-mail: ega15@le.ac.uk
Fax: (0116) 252 3282

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this information leaflet

Dr Erol Gaillard
Consultant in Paediatric Respiratory Disease
Children’s Hospital, Leicester Royal Infirmary
Brief information sheet for young control children (5 years – 10 years)

Study title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

Principal Investigator: Dr Erol Gaillard

We are trying to find out what happens in the lungs of children with asthma. You have NOT got asthma but we would like you to help us. Your parent(s) will have some information too, so you can talk to them about it. Please take your time to read about the study.

Why?
We want to find out what is different about your (healthy) lungs compared to the lungs of children with asthma.

How?
There are some blowing and breathing tests at the Children’s Hospital. We would also like to collect a cough sample and some urine. First you will be sitting in something like an old fashioned telephone box and breathe into a tube. This is a bit like breathing into a large straw.

For the second test you will be breathing in and out of a balloon, almost like blowing up a party balloon. Many children in Leicester have done these tests and most find it interesting and fun. None of the tests will make you uncomfortable or cause any pain.

Problems?
There are no needles, no pain or discomfort and no other risks. The tests will not affect you in any way. A parent will be with you all the time.

What next?
If your parent(s) and you are happy to participate in this study your parent(s) will sign a “consent form”. The important thing is that you do not have to take part if you do not want to.

Any questions?
Please get in touch to speak to or leave a message for one of us, if you have any questions.

Dr Erol Gaillard
Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary

Information sheet - healthy children 5-10 yrs

Version 2, dated 2 June 2011

Ref: 09/H04/03/92
Information sheet for control children (11 years - 16 years)

**Study title**: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

**Principal Investigator**: Dr Erol Gallard

We are inviting you (name of the child) to take part in a research study. Before you decide it is important for you (name of the child) and your family to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

**What is the purpose of the study?**

Many children of your age have breathing problems called asthma. You have been asked to participate in this study because you DO NOT have asthma.

We want to find out more about what is different inside your (healthy) lungs compared to the lungs of children with asthma. Although common, we know surprisingly little about what exactly is going on inside the lungs of children with asthma. This research may help in finding better treatments for children with asthma in the future.

**Why have I been chosen?**

You have been chosen because you do not have asthma.

**Do I have to take part?**

It is up to you to decide whether or not you are happy to take part in this study. You may keep this information sheet. If you decide to take part you are still free to withdraw at any time and without giving a reason.

**What will happen to me if I take part?**

We will invite you to our lung function laboratory to do a few relatively simple tests. All the tests we are asking you to participate in are done routinely. In some cases yearly, by children with lung problems. None of the tests are uncomfortable or painful and usually children find them fun and interesting to do. The tests will tell us a lot about what is going on in your lungs.

First, we will first ask you to fill in a short questionnaire together with your parent to tell us about your lungs and any lung problems in the family.

Information sheet - healthy children 11-16 yrs

Version 2, dated 2 June 2011

Ref: 09.I-04.03/92
We will then invite you to do 2 simple blowing tests done on every child over 5 years in the asthma clinic. These tests will give us some basic information on your breathing tubes.

Then we will ask you to sit in something like an old fashioned telephone box and to simply breathe in and out of a tube. The box has some very sensitive equipment attached to it that gives us information we cannot get with the simple tests.

For the next test you will be breathing in and out of a balloon.

If possible we will also ask you for a sample of urine but this is not critical for the study.

Finally, we will ask you to inhale some slightly salty mist for a few minutes. During this inhalation we will ask you to have a big cough and to drop a little bit of fluid into a pot. This is not uncomfortable and is not in any way painful. The whole thing will not take longer than 15 minutes.

Many children in Leicester have done these tests and most find it interesting and fun.

**Are there any risks of taking part?**

There are no needles, no pain or discomfort and no other risks. The tests will not affect you in any way. A parent will be with you all the time.

**What are the possible benefits of taking part?**

There are no direct benefits right now for you from taking part in this study, but we will find out what is happening inside your healthy lungs.

**What will happen to the results of the research study?**

The results of the study will be presented at scientific meetings and published in medical newspapers.

Thank you very much for your time taken to read this information leaflet.

**Any questions?**

Please get in touch to speak to or leave a message for one of us, if you have any questions.

- phone (0116) 252 5681  
  - email: eau15@de.ac.uk  
  - fax (0116) 252 32382

Dr Erol Gaillard
Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary
CONSENT FORM
For control child and parent (5-16 years)

Study Number:

Name of Child:

Title of Project: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

Principal Investigator: Dr Erol Gaillard

Please initial box

1. I confirm that I have read and understand the Parent Information Sheet dated 2 June 2011 Version 2 for the above study and have had the opportunity to ask questions.

2. I understand that my child's participation is voluntary and that I/my child is free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my child's medical notes (if there are any) may be looked at by responsible individuals from Leicester Royal Infirmary where it is relevant to this research. I give permission for these individuals to have access to those records.

4. I agree to/allow my child to take part in the above study.

Name of Child __________ Date __________ Signature __________

Name of Parent (if needed) __________ Date __________ Signature __________

Name of Person taking consent (if different from researcher) __________ Date __________ Signature __________

Researcher __________ Date __________ Signature __________

1 for participant; 1 for researcher

Consent Form – healthy children Version 2, dated 2 June 2011
Questionnaire on breathing problems in children

First Name of Child: _____________________________ Number: ______

Person completing questionnaire: Mother □ Father □ Other □ If other who? ______

Date questionnaire completed: Day ______ month ______ year ______ (please fill in today's date)

Wheeze

Wheeze is breathing that makes a squeaky whistling sound from the chest, not the throat.

1. Has your child ever had wheezing or whistling in the chest at any time in the past? yes □ no □

2. Has your child had wheezing or whistling in the chest in the last 12 months? yes □ no □

If you answered "no" to both questions please skip to question (19)

3. In which of the last 12 months, was your child's wheeze particularly bad? (Please tick which apply)
   Jan □ Feb □ Mar □ Apr □ May □ Jun □ Jul □ Aug □ Sep □ Oct □ Nov □ Dec □

4. In the last 12 months, was the wheezing worse during a particular time of day? yes □ no □
   □ yes, during the day □ yes, in the evening before falling asleep □ yes, at night □

5. In the last 12 months, has your child had wheezing or whistling in the chest during or soon after a cold or flu? yes □ no □

6. In the last 12 months, has your child had wheezing or whistling in the chest even without having a cold or flu? yes □ no □

7. How many attacks of wheezing has your child had during the last 12 months?
   none □ 1 to 3 □ 4 to 12 □ more than 12 □

8. Do these attacks cause him/her to be short of breath? yes, always □ yes, occasionally □ no, never □

9. How many of these attacks lasted for more than one day, and needed extra inhaler treatment?
   none □ 1 □ 2 □ 3 □ 4 to 6 □ more than 6 □

10. In the last 12 months, has your child's chest sounded wheezy during or after exercise? yes □ no □
    If yes: when does it happen? Only, during a cold, flu or temperature □ even without a cold □

11. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?
    never □ occasionally □ less than one night per week □ one or more nights per week □

12. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? yes □ no □

13. How old was he/she when the first attack of wheezing occurred? _______ years

14. If the wheezing has now stopped, at what age did it stop? _______ years

15. In the last months, did the following factors cause any wheezing in your child?

   - exercise (running, sports) yes □ no □ don't know □
   - laughing, crying, excitement yes □ no □ don't know □
   - pollen (grass, hay, trees, flowers) yes □ no □ don't know □
   - house dust yes □ no □ don't know □
   - contact with pets or other animals yes □ no □ don't know □
   - Food or drink yes □ no □ don't know □ (yes, which food or drinks? _______)

Version 1 – 26 October 2009 – 09/130402:92
16. In the last 12 months, how much did wheezing interfere with your child’s daily activities?
   - not at all
   - a little
   - a moderate amount
   - a lot

17. Looking back at the last 12 months, how severe was your child’s wheezing or asthma?
   - very mild
   - mild
   - moderate
   - severe

18. During the last 12 months, did your child miss school because of asthma or wheezing or bronchiolitis?
   - yes
   - no
   - if yes, how much time did your child miss during the last 12 months (all missing days counted together)?
     - Less than 1 week
     - 1 to 2 weeks
     - 3 to 4 weeks
     - more than 4 weeks

19. Have you ever been told by a doctor or a nurse that your child has asthma? yes no

**Coughing**

20. Do you think that your child coughs more than other children? yes no

21. Does your child usually cough with colds? yes no

22. Does your child have a cough even without having a cold? yes no
   - if yes, is the cough worse at a particular time of the day?
     - yes, during the day
     - yes, in the evening before falling asleep
     - yes, at night

**Ears, nose, throat and skin**

23. In the last 12 months, how many times has your child had cold or flu?
   - never
   - 1 to 3 times
   - 4 to 5 times
   - 6 to 10 times
   - more than 10 times

24. In the last 12 months, has your child had a problem with sneezing, or a runny or blocked nose when he/she did NOT have a cold or the flu? yes no

   If you answered “no”, please skip to question 27

25. In the last 12 months, has this nose problem been accompanied by itchy/watery eyes? yes no

26. In which months of the last 12 months did this nose problem occur? (tick off which apply)
   - Jan
   - Feb
   - Mar
   - Apr
   - May
   - Jun
   - Jul
   - Aug
   - Sep
   - Oct
   - Nov
   - Dec

27. In the last 12 months, has your child had hayfever? yes no

28. Has your child ever had eczema?
   - yes
   - no

   If yes, has your child had eczema in the past 12 months?
   - yes
   - no

**Treatment**

29. How often did your child see the GP for coughing or wheezing during the last 12 months?
   - never
   - once
   - 2 to 3 times
   - 4 to 6 times
   - 7 or more times

30. In the last 12 months, because of wheezing or asthma, has your child?
   - been referred to a consultant in hospital
     - yes
     - no
   - been admitted to hospital
     - yes
     - no
   - attended a casualty (A and E) department
     - yes
     - no
   - attended (or called) the GP in an emergency
     - yes
     - no

31. Did your child take any of the following drugs during the last 12 months?
   - Salbutamol, Ventolin, Bricanyl or other blue inhaler
     - yes
     - no
   - Pulmicort, Fluticort, Becotide, Qvar or other brown or orange inhaler
     - yes
     - no
don’t know
   - Serevent or Oxis (a green or green-white inhaler)
     - yes
     - no
don’t know
32. If your child has used a brown, orange, violet or red inhaler, please answer also the following two questions:
   - Did he/she use it regularly (every day for at least 2 months in a row)? yes □ no □
   - In total, how many months in the last year did he/she use it (adding up all episodes)? ___________ months

**Family and household**

33. Does the child’s mother smoke cigarettes (in or out of the house)?   yes □ no □
   - If yes: how many per day?
     1 to 10 □ 11 to 20 □ more than 20 □

34. Does any other household member smoke at all (in or out of the house)? yes □ no □
   - If yes: how many per day?
     1 to 10 □ 11 to 20 □ more than 20 □
   (please add up all cigarettes which are smoked by everybody except the mother)

35. Does your child have any brothers and sisters (including half-siblings)? yes □ no □
   - If yes: (a) how many brothers?
     What years were they born ___________ ___________ ___________ ___________
   - (b) how many sisters?
     What years were they born ___________ ___________ ___________ ___________

You can also write any other comments you might have in this space below.

Thank you very much for helping us again to study and improve health in children and young people!
Questionnaire on breathing problems in children aged 14 and over

Your First Name: ___________________________ Number: ___________________________

Please fill in these questions yourself. We promise that your answers are confidential. They will not be shown to anyone that you know. The answers will only be seen by the research team.

Date questionnaire completed: day _______ month ________ year _______ (please fill in today’s date)

**Wheeze**
Wheeze: We mean breathing that makes a squeaky whistling sound from the chest, not the throat.

1. Have you had wheezing or whistling in the chest in the last 12 months? yes □ no □

2. Have you had chest tightness with cough in the last 12 months? yes □ no □

If you answered “no” to both questions please skip to question 9

3. When was the last occasion that you were wheezy?
   - This week □
   - Last week □
   - More than a week, but less than a month ago □
   - More than a month ago □

In the next questions we will ask you about things that make you wheeze, or have chest tightness with cough. We will ask questions about the times you have attacks of wheeze, or chest tightness with cough lasting for more than one day, then in the next section, about the shorter attacks which last for less than a day (only minutes or hours).

First, we will ask you about attacks of wheeze or chest tightness with cough which last more than one day.

4. Do you have attacks of wheeze or chest tightness with cough? yes □ no □

If you answered “no” please skip to question 11

5. How many attacks like this have you had in the last year?
   - None □
   - 1 to 3 □
   - 4 to 12 □
   - More than 12 □

6. How bad were these attacks at their worst in the last year?
   - Caused difficulty sleeping or kept you awake at night yes □ no □
   - Caused you to miss school yes □ no □
   - Limited the amount of exercise you do yes □ no □
   - Bad enough to stop you talking normally yes □ no □

7. Do you have these attacks only when you have a cold? yes □ only with colds □ no, also without colds □

8. Do these attacks cause you to be short of breath? yes □ no □

9. Tick all the things that you think cause these attacks lasting for more than one day

   - Colds or flu yes □ no □ don’t know □
   - Running or sports yes □ no □ don’t know □
   - Laughing, crying, excitement yes □ no □ don’t know □

Questionnaire children aged 14+

Version 1 – 26 October 2009 – 05/ID401/02

242
10. Tick all the symptoms which you have during these attacks lasting for more than one day

- cough
- chest tightness
- difficult breathing
- wheezing
- other
- if other, what

Next, we will ask you about shorter attacks of wheeze or chest tightness with cough lasting for less than a day (only minutes or hours).

11. Do you have short attacks of wheeze or chest tightness lasting for less than a day? yes □ no □

If you answered "no" please skip to question 16.

12. How often do you have attacks of wheeze or chest tightness lasting for less than a day?
- every day
- several times a week
- about once per week
- once per month or less
- never

13. Do these attacks cause you to be short of breath? yes □ no □

14. Tick all the things that you think cause these attacks lasting for less than a day

- colds or flu
- running or sports
- laughing, crying, excitement
- pollen (grass, hay, trees, flowers)
- pet (dogs, cats, or other)
- house dust
- cigarette smoke from others
- food or drinks
- other
- if other, what

15. Tick all the symptoms that you have during these attacks lasting for less than a day

- cough
- chest tightness
- difficult breathing
- wheezing
- other
- if other, what
### Coughing

15. Do you think that you cough more than other children?  
   - yes □  no □

17. Do you usually cough with colds?  
   - yes □  no □

### Smoking

18. Have you ever smoked cigarettes or roll-ups (or any other form of tobacco)?  
   - yes □  no □

19. Have you ever been a regular smoker (smoking at least daily for a period of more than 3 months)?  
   - yes □  no □

20. In what form do you or did you usually smoke? (Tick as many as apply)
   - Cigarettes with tobacco □
   - Roll-ups □
   - Other □
   - If other please specify ____________________________

21. How old were you when you started smoking? ____________ yrs ____________ months

22. Are you still smoking?  
   - yes □  no □

   If no, how old were you when you stopped smoking? ____________ yrs ____________ months

23. How many cigarettes (or equivalent do (did) you usually smoke per day?  
   - [ ] (please fill in a number)

Thank you very much for helping us again to study and improve health in children and young people!
03 October 2012

Dr Erol A Gaillard
Senior Lecturer in Child Health
University of Leicester
Dept of Infection, Immunity & Inflammation, University of
Leicester, RKCSB, LRI, Leicester
LE2 7LX

Dear Dr Gaillard

<table>
<thead>
<tr>
<th>Study title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC reference: 09/H0403/92</td>
</tr>
<tr>
<td>Amendment number: Substantial Amendment 5</td>
</tr>
<tr>
<td>Amendment date: 19 September 2012</td>
</tr>
</tbody>
</table>

The above amendment was reviewed at the meeting of the Sub-Committee held on 28 September 2012 in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notice of Substantial Amendment (non-CTIMPs)</td>
<td>Substantial Amendment 5</td>
<td>19 September 2012</td>
</tr>
<tr>
<td>Protocol</td>
<td>8</td>
<td>19 September 2012</td>
</tr>
<tr>
<td>Participant Information Sheet: PIS for Young people (11-17 years) in the Leicester Respiratory Cohorts</td>
<td>2</td>
<td>19 September 2012</td>
</tr>
<tr>
<td>Participant Information Sheet: Information Sheet for Parent(s)/Guardian(s) of Young People in the Leicester Respiratory Cohorts (11-17 years)</td>
<td>2</td>
<td>19 September 2012</td>
</tr>
<tr>
<td>Participant Consent Form: Consent Form for Parent(s)/Guardian(s) of Young People in Leicester Respiratory Cohorts (11-17 years)</td>
<td>2</td>
<td>19 September 2012</td>
</tr>
<tr>
<td>Reply Slips for Young People in Leicester Respiratory Cohorts (11-17 years)</td>
<td>1</td>
<td>19 September 2012</td>
</tr>
</tbody>
</table>
Invitation Letter for young people in Leicester Respiratory Cohorts (11-17 years)

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

09/0403/02: Please quote this number on all correspondence

Yours sincerely

\[ Signature \]

Mr Robert Johnson
Chair

E-mail: Wendy.Rees@nottsp.nhs.uk

Enclosures: List of names and professions of members who took part in the review

Copy to: Mrs Carolyn Matoney, University Hospitals of Leicester NHS Trust
University Hospitals of Leicester NHS Trust

246
NRES Committee East Midlands - Nottingham 1

Attendance at Sub-Committee of the REC meeting on 28 September 2012

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Robert Johnson</td>
<td>Research Coordinator - Professional registration maintained</td>
<td>Expert - Chair</td>
</tr>
<tr>
<td>Reverend Keith Lackenby</td>
<td>Lay member</td>
<td>Lay Plus</td>
</tr>
</tbody>
</table>
10/10/2012

Dr Erol A Gallard
University of Leicester
Senior Lecturer in Child Health
Dept of Infection, Immunity & Inflammation
RiKCB, Leicester
Leicester Royal Infirmary
LE2 7LX

Dear Dr Erol A Gallard

Ref: UHL 10836

Title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

Project Status: Project Approved
End Date: 31/10/2012

Thank you for submitting documentation for Substantial Amendment 5 dated 19.09.12, for the above study.

I confirm that the amendment has the approval of the University Hospitals of Leicester NHS Trust R&D Department and may be implemented with immediate effect.

The documents received are as follows:

<table>
<thead>
<tr>
<th>Documents</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notice of Substantial Amendment Form</td>
<td>Substantial Amendment 5</td>
<td>19.09.12</td>
</tr>
<tr>
<td>Protocol</td>
<td>9</td>
<td>19.09.12</td>
</tr>
<tr>
<td>PIS: PIS for Young people (11-17 years) in the Leicester Respiratory Cohorts</td>
<td>2</td>
<td>19.09.12</td>
</tr>
<tr>
<td>PIS: Information Sheet for Parent(s) / Guardian(s) of Young People in the Leicester</td>
<td>2</td>
<td>19.09.12</td>
</tr>
<tr>
<td>Document Description</td>
<td>Quantity</td>
<td>Date</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>POF: Information Sheet for Parent(s)/Guardian(s) of Young People in the Leicester Respiratory Cohorts (11-17 years)</td>
<td>2</td>
<td>18.08.12</td>
</tr>
<tr>
<td>Reply Slip for Young People in Leicester Respiratory Cohorts (11-17 years)</td>
<td>1</td>
<td>18.08.12</td>
</tr>
<tr>
<td>Invitation Letter for Young People in Leicester Respiratory Cohorts (11-17 years)</td>
<td>2</td>
<td>18.08.12</td>
</tr>
<tr>
<td>REC Favourable Opinion Letter</td>
<td></td>
<td>03.10.12</td>
</tr>
</tbody>
</table>

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments MUST be followed. Failure to do so may invalidate the approval of the study at this trust.

Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Yours sincerely,

Carolyn Maloney
R&D Manager

Dr. David Hetmanski
Director R&D
Information Sheet for Parent(s)/Guardian(s) of Children and young people in the Leicester Respiratory Cohorts (11-17 years)

Study title: How do inflammation cells in the induced sputum of children with Asthma change between acute exacerbation and recovery?
Principal Investigator: Dr Erol Gulland

We are inviting (name of young person) to take part in a research study. Before deciding, it is important for (name of young person) and the family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
Asthma in children is common and inflamed airways are an important feature of asthma. This inflammation can be studied by collecting secretions that are coughed up and studying them under a microscope.

The purpose of this study is to understand if children with wheezing in the preschool years have normal airways, free of inflammation in later childhood and during teenage years. We also want to study lung function to see how it relates to inflammation.

We do not know if children with wheezing during the preschool years continue to have slightly inflamed airways. We also know little about their lung function. Such information is important to increase our knowledge of the natural history of asthma in children.

Why have I been chosen?
Your son/daughter has been chosen because he/she is part of the Leicester Respiratory Cohorts that includes children with wheeze, cough or asthma as well as many healthy children.

Does my child have to take part?
It is up to you and your son or daughter to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide not to take part you can withdraw at anytime, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if (name of young person) takes part?
We will invite your son or daughter to attend the lung function laboratory at the Leicester Royal Infirmary at an agreed date and time that is convenient for you. This can be arranged during school breaks. We expect parents to accompany children below the age of 16. Older teenagers may attend by themselves if they wish, but parents are always very welcome. The visit will take approximately 2 hours and involve a number of tests. These tests are not in any way painful or uncomfortable but they require quite sophisticated and large equipment and experienced people to perform the tests and therefore can only be done in the setting of a dedicated laboratory. The tests give us very detailed information on the workings of the lungs and airways (breathing tubes).

First, when you arrive, we will ask your son or daughter to complete a questionnaire asking about previous breathing problems and if there are any breathing problems in the family.

Information Sheet for Parent(s)/Guardian(s) of Young People in the Leicester Respiratory Cohorts (11 years - 17 years)

Version 2, 19 September 2012

Ref: 09/H0102/92
The tests are described in detail below.

1. First, we will ask your son or daughter to do a very simple breathing test to assess the airway function. This test is done in all patients routinely attending our respiratory clinics.

2. The next test involves breathing into another tube to measure a substance we all produce, called nitric oxide. This substance is increased when there are inflamed breathing tubes, as in asthma. Both these tests are done in the routine asthma clinic and take no longer than 5-10 minutes.

3. The next test involves sitting in a Perspex cabin and breathing in and out through a tube. This test will give us information on how much air is going into the different lung compartments.

4. The last breathing test involves breathing pure oxygen for a couple of minutes to study how efficiently the lungs are working. Again, whilst this sounds complicated, none of the tests are difficult, uncomfortable or painful. These tests are often performed routinely as part of a detailed lung assessment, sometimes on a yearly basis. Most children and young people attending for these tests find them stimulating and fun.

5. Finally, we would like to obtain a sample of phlegm. We will give your son or daughter a salty mist solution to breathe in and ask him or her to cough into a pot at regular intervals. This procedure is very safe and again neither painful nor uncomfortable. It is routinely and frequently performed in patients and takes approximately 15 minutes.

Laboratory work: The secretion samples will be analysed to look at the level of inflammation and to see if there are any bacteria, viruses or fungi. We will also study the thickness of the secretions.

What are the possible benefits of taking part?

We will learn more about the link between asthma and inflamed airways. This will not provide any advantage to your son or daughter but may influence the way we treat asthma in the future.

What if something goes wrong?

There is no risk of harm from any parts of this research. However, if your son or daughter is harmed by taking part in this research project, there are no special compensation arrangements. If your son or daughter is harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it.

Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. If you have any complaints about staff, or if anything serious happens during the procedure, you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen Road, Leicester, LE2 4QF, where your concerns will be dealt with within 2 weeks.

Will I receive payment for participating in this research study?

You will be reimbursed for any travelling expenses and other out-of-pocket costs incurred in taking part in the investigations.

Will my taking part in this study be kept confidential?

Yes. All the results are confidential. There is no way in which anybody outside the Division of Child Health and Leicester University will be able to identify any participant from the results of the tests. All the results and names and addresses are kept on different files, and

Information Sheet for Parent(s)/Guardian(s)  Version 2, 19 September 2012
Of Young People in the Leicester Respiratory Cohorts (11 years - 17 years)
Ref: 09/H0403/92
are secure. We will only inform any outside individual (such as your general practitioner) with your permission.

Dr Gaillard will have overall control of the cough secretions, i.e. act as Custodian.

**What will happen to the results of the research study?**
The results of the study will be presented at national and international scientific meetings and published in the medical literature in due course. In practice, publication takes about 1-2 years from the end of a study. You and your son or daughter will not be identified in any report or publication.

**Who has reviewed the study?**
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

**Contact for further information:**
Please contact the following number to speak to or leave a message for Dr Gaillard or one of the other research team.

Telephone: (0116) 252 3262 / (0116) 256 5691
E-mail: asa156@le.ac.uk
Fax: (0116) 252 3282

You will be given a copy of the information sheet and a signed consent form to keep.

Thank you for taking the time to read this information leaflet

Dr Erol Gaillard
Consultant in Paediatric Respiratory Disease
Children’s Hospital, Leicester Royal Infirmary
Information sheet for young people in the Leicester Respiratory Cohorts
(11 years - 17 years)

Study title: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?
Principal Investigator: Dr Erol Gaillard

We are inviting you (name of the young person) to take part in a research study. Before you decide it is important for you (name of the young person) and your family to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
Many teenagers and young adults have breathing problems called asthma. You have been asked to participate in this study because we want to find out how much inflammation there is in the lungs of young people with and without asthma and to see if this is influenced by any symptoms they may have had as young children.

Although asthma is common, we know surprisingly little about what exactly is going on inside the lungs of children with asthma. This research may help in finding better treatments for children with asthma in the future.

Why have I been chosen?
You have been chosen because you were part of the Leicester Respiratory Cohorts that included children with wheeze, cough or asthma as well as many healthy children.

Do I have to take part?
It is up to you to decide whether or not you are happy to take part in this study. You may keep this information sheet. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?
We will invite you to our lung function laboratory at the Leicester Royal Infirmary to do some simple breathing tests. All the tests we are asking you to participate in are done routinely, in some cases yearly, by older children and teenagers with lung problems. None of the tests are uncomfortable or painful and usually people find them fun and interesting to do. The tests will tell us a lot about what is going on in your lungs.

First, when you arrive, we will ask you to complete a questionnaire asking about previous breathing problems and if there are any breathing problems in the family.

The tests are described in detail below:
1. First we will ask you to do a very simple breathing test to assess the airway function. This will involve breathing hard into a tube. This test is done in all patients routinely attending our respiratory clinics.
2. The next test involves breathing into another tube to measure a substance we all produce, called nitric oxide. This substance is increased when there are inflamed breathing tubes.

Information sheet for young people in the Leicester Respiratory Cohorts (11 years - 17 years)
Ref: 09/H0403/92

Version 2, 19 September 2012
as in asthma. Both these tests are done in the routine asthma clinic and take no longer than 5-10 minutes.

3. The next test involves sitting in a Perspex cabin, and breathing in and out through a tube. This test will give us information on how much air is going into the different lung compartments.

4. The last breathing test involves breathing pure oxygen for a couple of minutes to study how efficiently the lungs are working. Again, whilst this sounds complicated, none of the tests are difficult, uncomfortable or painful. These tests are often performed routinely as part of a detailed lung assessment, sometimes on a yearly basis. Most children and young people attending for these tests find them stimulating and fun.

5. Finally, we would like to obtain a sample of phlegm. We will give you a salty mist solution to breathe in and ask him or her to cough into a pot at regular intervals. This procedure is very safe and again neither painful nor uncomfortable. It is routinely and frequently performed in patients and takes approximately 15 minutes.

Are there any risks of taking part?
There are no needles, no pain or discomfort and no other risks.

What are the possible benefits of taking part?
There are no direct benefits right now for you from taking part in this study, but we will find out what is happening inside your lungs and this may influence the way we treat asthma in the future.

What will happen to the results of the research study?
The results of the study will be presented at scientific meetings and published in medical journals. You will not be identified in any report or publication.

Thank you very much for your time taken to read this information leaflet.

Any questions?
Please get in touch to speak to or leave a message for one of us, if you have any questions.
• phone (0116) 252 5891 • email: eaq15@le.ac.uk • fax (0116) 252 3292

Dr Erol Gaillard
Consultant in Paediatric Respiratory Medicine
Children’s Hospital, Leicester Royal Infirmary

Information sheet for young people in the Leicester Respiratory Cohorts (11 years - 17 years)
Ref: 09/H0405/82

Version 2, 19 September 2012
CONSENT FORM
For Parent(s)/Guardian(s) of Young Person in the Leicester Respiratory Cohorts (11 years - 17 years)

Study Number:

Name of Young Person:

Title of Project: How do inflammation cells in the induced sputum of children with asthma change between acute exacerbation and recovery?

Principal Investigator: Dr Erol Gaillard

1. I confirm that I have read and understand the Information Sheet dated 19 September 2012 Version 2 for the above study and have had the opportunity to ask questions.

2. I understand that my son or daughter’s participation is voluntary and that my child is free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected.

3. I understand that relevant sections of my son or daughter’s medical notes and data collected during the study may be looked at by responsible individuals from the research team, from regulatory authorities or from the NHS Trust and Sponsor, where it is relevant to my child taking part in this research. I give permission for these individuals to have access to my child’s records.

4. I agree to allow my son or daughter to take part in the above study.

Name of Young Person __________________________ Date _______________ Signature __________________________

Name of Parent __________________________ Date _______________ Signature __________________________

Name of Person taking consent
(if different from researcher) __________________________ Date _______________ Signature __________________________

Researcher __________________________ Date _______________ Signature __________________________

1 for participant; 1 for researcher; 1 for site file

Consent form for Young People in the Leicester Respiratory Cohorts (11 years - 17 years) Version 2, 19 September 2012
Ref. 09/H0400/52
Appendix C: Ethical approval, PIS, consent form and questionnaires for measuring lung development using $^3$He MR study from which data from healthy controls (children and young adults) were used
University Hospitals of Leicester
NHS Trust

DIRECTORATE OF RESEARCH AND DEVELOPMENT

Director: Professor D Rowbotham
Assistant Director: John Hampton
Co-ordinator: Jayna Mistry
Direct Dial: 0116 258 4614
Fax No: 0116 258 4226
EMail: jayna.l.mistry@uhl-tr.nhs.uk

14 August 2008

Professor M Silverman
Professor of Child Health
Division of Child Health/Consultant Paediatrician
Department of Infection and Immunity
Faculty of Medicine and Biological Sciences
Robert Kilpatrick Clinical Sciences Building, LRI
LE2 7LX

Dear Professor Silverman

ID: Measuring lung development using Helium-3 magnetic resonance

SSA Ref: 04-Q2501-114

Funder: University Hospitals of Leicester (UHL) NHS Trust

Proposed End Date: 31/10/2010

Please notify R&D Office if an extension is required

Substantial Amendment dated 22.07.08 (Modified 4)

Documents Received

Ethics Letter dated 04.08.08

Documents Approved

- Questionnaire: Personal questions 14 years and over (boys), v2, 21.07.08
- Questionnaire: Personal questions 14 years and over (girls), v2, 21.07.08
- Questionnaire: Breathing Problems child, v1, 08.06.08
- Questionnaire: Birth and Early childhood, v1, 08.06.08
- Questionnaire: Breathing Problems adolescent, v1, 08.06.08
- Protocol, v4, 08.06.08
- Participant Information Sheet: Patient, v2, 21.07.08
- Participant Information Sheet: Children, v2, 21.07.08
- Participant Consent Form: Young person and parent, v2, 21.07.08
- Modified Amendment (4), 22.07.08
- Covering Letter, 22.07.08
- Changes to answers given on application form
- Questionnaire: Personal questions - 13 years old and under (boys), v2, 21.07.08
- Questionnaire: Personal questions - 13 years old and under (girls), v2, 21.07.08

Based on the information received and in accordance with published guidance from the National Research Ethics Service (NRES), we are pleased to confirm that this amendment has been reviewed. Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Trust Headquarters, Gwendolen House, Gwendolen Road, Leicester, LE5 4QW
Tel: 0116 258 8665 Fax: 0116 258 4666 Website: www.uhl-tr.nhs.uk
Chairman Mr. Martin Hindle Chief Executive Dr. Peter Reading

257
We can confirm that the study has full approval from the University Hospitals of Leicester, which confirms that you are authorised to proceed with the project, using all resources declared in your original application, and any subsequent approved amendments. The study continues to be covered by Trust indemnity, except for those aspects already covered by external indemnity (e.g. ABPI). It is a condition of approval that you discuss with the R&D Office any proposed significant changes protocol, funding or costs of the project.

We will continue to request annual and final reports on the progress of this project, on behalf of the Trust but will accept a report generated for another external body e.g Pharmaceutical Company, provided that it includes information specific to UHL sites.

Please note approval is only valid until the end date as reflected in A3 of the National Research Ethics Service (NRES) form. You are not authorised to conduct the study on UHL premises beyond this date. In order to extend the study, please either complete an Annual Report form which can be found on the UHL R&D Website, or write to the R&D Office requesting an extension, providing information to justify the requirement for an amended end date.

Please ensure that any Honorary Contracts issued by UHL for the purposes of conducting this study remain valid, and appropriate action is taken to apply to extend the contract in a timely manner if required. Please remember that individuals are not authorised to continue to conduct research without a valid substantive / honorary contract on UHL sites.

It is a condition of approval that you discuss with the R&D Office any proposed significant changes protocol, funding or costs of the project.

I look forward to the opportunity of reading the published results of your study in due course.

Yours sincerely

[Signature]

Carolyn Burden
Acting Asst. Dir. R&D / R&D Manager
Information Sheet for Parents

Study Title: Measuring lung development using $^3$He magnetic resonance

Principal Investigator: Professor Mike Silverman

We are inviting (name of child or young person) to take part in a research study. Before deciding, it is important for (name of child or young person) and the family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We have only one pair of lungs. Most of their development occurs before birth and in the first few years of life. After that, they simply get larger during childhood, with little ability to catch-up if things go wrong earlier. Exactly how the lungs grow and how illness in childhood can affect lung development is not clear. It is important to find out, so that we can prevent lung damage. New treatments are under development which could even restore damaged lungs to normal. The purpose of this study is to learn how the lungs grow in health and disease, and which genes are important in lung growth.

Why have I been chosen?
We have chosen your child to take part because (name of child or young person) has either been a part of the Leicestershire study of Cough Colds and Wheezes in Childhood, and you have been kind enough to fill in questionnaires in the past, or because of premature birth recorded in the Trent Perinatal Survey. A total of 200 children and young people will participate, some of them healthy and some of them with various symptoms or born prematurely, in order to investigate the normal growth of the lungs and the effect of various symptoms and illnesses.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide not to take part you can withdraw at anytime, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if (name of child or young person) takes part?
There are two visits, one to Leicester Royal Infirmary and the other to the physics department at the Nottingham University, each of which will take about 1½ hours. We can combine these if you wish to make it easier, and we can organise transport to Nottingham (visit 2).

Information Sheet for Parents Version 2, dated 21 July 2008

Ref: -4/Q2501/114
Last saved: 21 July 2008
At the visit to Leicester Royal Infirmary you will be asked to fill in questionnaires related to your child’s health and early childhood. If you are happy with it, we will ask your child to fill in a confidential questionnaire regarding personal habits. After this, your child will have breathing tests in the Lung Function Laboratory at the Leicester Royal Infirmary. Your child will be asked to carry out blowing and breathing tests, and if he/she has asthma or any other results are not normal, we will give him/her a dose of an asthma puffer (Salbutamol) and repeat the measurements.

The second visit will take place at the Physics Department at Nottingham University where a lung scan will be carried out. This is a completely harmless test, using a tiny quantity of gas called Helium-3. A small amount of Helium gas (completely harmless substance used in routine breathing tests in babies, children and adults) would be breathed in from a small bag, while lying still in a scanning machine. Your child will then be asked to hold their breath for about 5 seconds and the test is over. This will be repeated 3 times.

There are no special restrictions before the tests and your child should carry on taking any treatment which has been prescribed. We will collect a few cells from inside the cheek by lightly scraping with a wooden spatula, to extract DNA for gene studies, if you agree.

There are no side effects from any of the agents which we use. Your child will only be given an asthma puffer if he/she has asthma or any evidence of blockage of the tubes in the chest. The scan does not involve any x-rays or any other form of radiation, and there are no known risks or disadvantages.

Your child will be asked to produce either a sample of urine during one of the visits, or a sample of saliva (spit). We will use this to measure exposure to cigarette smoke in an accurate way.

What are the possible benefits of taking part?
We will learn how the lungs grow during early life, and whether simple and common problems such as asthma or prematurity can affect the way in which the lungs grow. This will not provide any advantage to your child, but to future generations. If we do find any problem with your child’s lungs, we will discuss it with you and ask whether you would like your general practitioner to be informed.

What if something goes wrong?
There is no risk of harm from any parts of this research. However, if your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. If you have any complaints about staff, or if anything serious happens during the procedure, you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen Road, Leicester, LE5 4QF, where your concerns will be dealt with within 2 weeks.

Will participation be confidential?
Yes all the results are confidential. There is no way in which anybody outside the Division of Child Health and Leicester University will be able to identify any participant from the results of the tests. All the results and names and addresses are kept on different files, and are secure. We will only inform any outside individual (such as your general practitioner) with your permission.
What happens to the results of the research?
The results of the research will be published in journals which are widely read by doctors and medical physicists. It will not be possible to identify your child within any report or publication.

Who is funding the research?
The research is completely independently carried out by medical and physics staff at Leicester and Nottingham Universities. External funding will be used. You will be paid for any travelling expenses and other out-of-pocket costs, incurred in taking part in the investigations.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information
Please contact the following number to speak to or leave a message for Professor Silverman or one of the other research team.

Telephone: (0116) 525 3262
E-mail: ms70@le.ac.uk
Fax: (0116) 252 3282

THANK YOU FOR READING THIS. YOU WILL BE ABLE TO KEEP A COPY OF THIS INFORMATION SHEET AND A SIGNED CONSENT FORM.

Professor Mike Silverman
Children’s Hospital, Leicester Royal Infirmary.
Brief information sheet for children

Study Title: Measuring lung development using $^3$He helium magnetic resonance

Principal Investigator: Professor Mike Silverman

We are trying to find out how people's lungs grow. We would like you to help us. Your parent(s) will have some information too, so you can talk to them about it. Please take your time to read about the study.

Why?
We have only one pair of lungs. They start to form when we are babies in the first few years of life. After that, they simply get bigger as we grow up. Doctors do not know exactly how the lungs grow. We need to find out, so that we can prevent damage caused by illness. We would like to find out which genes are involved in the growth of the lungs.

How?
A new method to measure how lungs grow has been developed by Nottingham University. It is really simple. You just breathe in a tiny amount of special air, called helium, while lying still inside a giant magnet. Then you hold your breath for a few seconds. You do this three times – that's all. Helium is a harmless and tasteless form of air. Also, there are some blowing and breathing tests and a short questionnaire to fill in at the Children's Hospital, Leicester. We would also like to collect a sample of urine or some spit, during one of the visits. We can use this to measure how much smoke you have breathed in, over the last few days.

Problems?
There are no needles, no pain or discomfort and no other risks. The tests will not affect you in any way. A parent will be with you all the time.

What next?
If your parent(s) reply "yes" on the reply letter, we will make arrangements for you to visit us for the tests. We will ask you and your parent(s) to sign a "consent form" when you come for the tests. The important thing is that you do not have to take part if you do not want to.

Any questions?
Please get in touch to speak to or leave a message for one of us, if you have any questions.

- phone (0116) 252 5881
- email ms70@le.ac.uk
- fax (0116) 252 3282

Professor Mike Silverman
Children's Hospital, Leicester Royal Infirmary

Information Sheet for Children Version 2, dated 21 July 2008

Ref: 04/Q2501/114
Last saved: 21 July 2008
CONSENT FORM for young person and parent

Study Number:

Name of young person or child:
Title of Project: Measuring lung development using $^3$Helium magnetic resonance
Principal Investigator: Professor M Silverman

Please initial box

1. I confirm that I have read and understand the information sheet dated 21 July 2008 Version 2 for the above study and have had the opportunity to ask questions.

2. I understand that my child's participation is voluntary and that I/my child is free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my/my child's medical notes may be looked at by responsible individuals from Leicester Royal Infirmary where it is relevant to this research. I give permission for these individuals to have access to these records.

4. I understand that my child will be asked to complete a personal questionnaire as a part of the study. I understand that I have an opportunity to see the questionnaire before completion. I understand that the answers are confidential. I agree to my child completing this questionnaire.

5. I agree to allow my child to take part in the above study.

Name of Young Person  Date  Signature

Name of Parent (if needed)  Date  Signature

Name of Person taking consent (if different from researcher)  Date  Signature

Researcher  Date  Signature

1 for participant; 1 for researcher

Consent Form version 2  Version 2 21 July 2008

Ref: 04/Q2501/114
Last saved: 20 December 2006
**Helium³ magnetic resonance**

**Questionnaire – Personal questions – 13 years old and under (Boys)**

Your first Name: ___________________________  No: ___________________________  2008_He_U14_M

Please complete by either ticking the appropriate box: ✔ or filling in a number: 93

⇒ Please fill in these questions yourself. We would like to assure you that your answers will remain confidential in all but the most exceptional circumstances, where we believe you are at risk of significant harm. We would not show this questionnaire to your parents unless we had your full agreement.

⇒ Date questionnaire completed: day______ month:______ year______ (please fill in today’s date)

## Smoking

1. Have you ever smoked cigarettes or roll ups (or any other form of tobacco)?  yes [ ] no [ ]

⇒ If you answered ‘no’ to this question please skip to question 7.

2. Have you ever been a regular smoker (smoking at least daily for a period of more than 3 months)?  yes [ ] no [ ]

3. In what form do you, or did you, usually smoke? (tick as many as apply)
   - Cigarette with tobacco [ ]
   - Roll ups [ ]
   - Other [ ]
   - If other, please specify ___________________________

4. How old were you when you started smoking? ______ yrs ______ months

5. Are you still smoking?  yes [ ] no [ ]

   If no, how old were you when you stopped smoking? ______ yrs ______ months

6. How many cigarettes (or equivalent) do (did) you usually smoke per day? ______ (please fill in a number)

## Hobbies, activities

7. On average, how many hours per week do you spend outside normal work/study periods on:
   - sport or vigorous physical activity? 0-1 hr [ ] 2-3 hrs [ ] 4-5 hrs [ ] 6hrs or more [ ]
   - TV, video or computer activities? 0-4 hrs [ ] 5-10 hrs [ ] 10-20 hrs [ ] 20hrs or more [ ]

8. In the last 12 months, how often, on average, did you eat fresh fruit?
   - less than twice a week [ ] 2 to 4 times a week [ ] daily [ ] twice daily [ ]

9. How do you normally sleep?
   - On your front [ ] On your back [ ] On your left side [ ] On your right side [ ] On either side [ ] I do not have any preference [ ]

10. Do you currently play any wind musical instruments (eg. trumpet, clarinet, flute…)?  yes [ ] no [ ]
    
    If yes: please specify ___________________________

11. Have you ever played any wind musical instruments (eg. trumpet, clarinet, flute…)?  yes [ ] no [ ]
    
    If yes: please specify ___________________________
    
    how long did you play it? ______ yrs ______ months.

21 July 08 personal pullout_under14_male.doc  version 2 dated 21 July 08
## Body development

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. We are asking these questions since they may have something to do with how your lungs grow. If you do not understand a question or do not know the answer, just mark "I don’t know.”

12. Would you say that your rapid **growth in height** that occurs in teenagers?
   - Has not yet begun
   - Has only just started
   - Is definitely underway
   - Seems complete
   - I do not know

13. Would you say that your **body hair growth** ("body hair" means hair in any other place than your head, such as under your arms)?
   - Has not yet begun
   - Has only just started
   - Is definitely underway
   - Seems complete
   - I do not know

14. Have you noticed any **skin changes**, especially pimples?
   - Skin has not yet started changing
   - Skin has only just started changing
   - Skin changes are definitely underway
   - Skin changes seem complete
   - I do not know

15. Have you noticed a **deepening of your voice**?
   - Voice has not yet started changing
   - Voice has only just started changing
   - Voice changes are definitely underway
   - Voice changes seem complete
   - I do not know

16. Have you begun to **grow hair on your face**?
   - Facial hair has not yet started growing
   - Facial hair has only just started growing
   - Facial hair growth has definitely started
   - Facial hair growth seems complete
   - I do not know

---

Thank you for completing the questionnaire

---

21 July 08 personal pullout_under14_male.doc  version 2 dated 21 July 08
Helium³ magnetic resonance
Questionnaire – Personal questions – 14
years old and over (Boys)

Your first Name: 
No: 

Please complete by either ticking the appropriate box: ✔ or filling in a number 93

⇒ Please fill in these questions yourself. We would like to assure you that your answers will remain confidential in all but the most exceptional circumstances, where we believe you are at risk of significant harm. We would not show this questionnaire to your parents unless we had your full agreement.

⇒ Date questionnaire completed: day _______ month _______ year _______ (please fill in today’s date)

Smoking
1. Have you ever smoked cigarettes or roll ups (or any other form of tobacco)? yes ☐ no ☐
   ⇒ If you answered “no” to this question please skip to question 7

2. Have you ever been a regular smoker (smoking at least daily for a period of more than 3 months)?
   yes ☐ no ☐

3. In what form do you, or did you, usually smoke? (tick as many as apply)
   • Cigarette with tobacco ☐
   • Roll ups ☐
   • Other ☐
   If other, please specify

4. How old were you when you started smoking? _______ yrs _______ months

5. Are you still smoking? yes ☐ no ☐
   If no, how old were you when you stopped smoking? _______ yrs _______ months

6. How many cigarettes (or equivalent) do you usually smoke per day? ___________ (please fill in a number)

7. Have you ever smoked Cannabis (also known as Marijuana, weed, grass, pot, hash...)? yes ☐ no ☐

8. In the last 12 months, how frequently have you smoked Cannabis (also known as Marijuana, weed, grass, pot, hash...)? never ☐ less than once a month ☐ monthly ☐ weekly ☐ daily ☐

Hobbies, activities
9. On average, how many hours per week do you spend outside normal work/study periods on:
   (a) sport or vigorous physical activity? 0-1 hr ☐ 2-3 hrs ☐ 4-5 hrs ☐ 6hrs or more ☐
   (b) TV, video or computer activities? 0-4 hrs ☐ 5-10 hrs ☐ 10-20 hrs ☐ 20hrs or more ☐

10. In the last 12 months, how often, on average, did you eat fresh fruit?
    less than twice a week ☐ 2 to 4 times a week ☐ daily ☐ twice daily ☐

11. How do you normally sleep?
    On your front ☐ On your back ☐ On your left side ☐ On your right side ☐ On either side ☐
    I do not have any preference ☐

12. Do you currently play any wind musical instruments (eg. trumpet, clarinet, flute...)? yes ☐ no ☐
    If yes: please specify

21 July 08 personal pullout_over14_male.doc
version 2 dated 21 July 08
13. Have you ever played any wind musical instruments (e.g., trumpet, clarinet, flute)?: [ ] yes [ ] no
If yes: please specify _____________________________.
How long did you play it? __________ yrs __________ months.

**Body development**

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. We are asking these questions since they may have something to do with how your lungs grow. If you do not understand a question or do not know the answer, just mark "I don't know".

14. Would you say that your rapid growth in height that occurs in teenagers?
   - Has not yet begun [ ]
   - Has only just started [ ]
   - Is definitely underway [ ]
   - Seems complete [ ]
   - I do not know [ ]

15. Would you say that your body hair growth ("body hair" means hair in any other place than your head, such as under your arms)?
   - Has not yet begun [ ]
   - Has only just started [ ]
   - Is definitely underway [ ]
   - Seems complete [ ]
   - I do not know [ ]

16. Have you noticed any skin changes, especially pimples?
   - Skin has not yet started changing [ ]
   - Skin has only just started changing [ ]
   - Skin changes are definitely underway [ ]
   - Skin changes seem complete [ ]
   - I do not know [ ]

17. Have you noticed a deepening of your voice?
   - Voice has not yet started changing [ ]
   - Voice has only just started changing [ ]
   - Voice changes are definitely underway [ ]
   - Voice changes seem complete [ ]
   - I do not know [ ]

18. Have you begun to grow hair on your face?
   - Facial hair has not yet started growing [ ]
   - Facial hair has only just started growing [ ]
   - Facial hair growth has definitely started [ ]
   - Facial hair growth seems complete [ ]
   - I do not know [ ]

*Thank you for completing the questionnaire*
Helium³ magnetic resonance
Questionnaire – Personal questions – 13 years old and under (Girls)

Your first Name: [ ]

No: [ ]

Please complete by either ticking the appropriate box: [ ] or filing in a number: [ ]

⇒ Please fill in these questions yourself. We would like to assure you that your answers will remain confidential in all but the most exceptional circumstances, where we believe you are at risk of significant harm. We would not show this questionnaire to your parents unless we had your full agreement.

⇒ Date questionnaire completed: day ______ month ______ year ______ (please fill in today’s date)

Smoking

1. Have you ever smoked cigarettes or roll ups (or any other form of tobacco)?
   - yes [ ] no [ ]
   ⇒ If you answered “no” to this question please skip to question 2.

2. Have you ever been a regular smoker (smoking at least daily for a period of more than 3 months)?
   - yes [ ] no [ ]

3. In what form do you, or did you, usually smoke? (tick as many as apply)
   - Cigarette with tobacco
   - Roll ups
   - Other

   If other, please specify__________________________

4. How old were you when you started smoking?
   _______ yrs _______ months

5. Are you still smoking?
   - yes [ ] no [ ]

   If no, how old were you when you stopped smoking?
   _______ yrs _______ months

6. How many cigarettes (or equivalent) do (did) you usually smoke per day?
   [ ] (please fill in a number)

Hobbies, activities

7. On average, how many hours per week do you spend outside normal work/study periods on:
   (a) Sport or vigorous physical activity? 0-1 hr [ ] 1-2 hrs [ ] 2-3 hrs [ ] 3-4 hrs [ ] 4-5 hrs [ ] 5+ hrs [ ]
   (b) TV, video or computer activities? 0-2 hrs [ ] 2-4 hrs [ ] 4-6 hrs [ ] 6+ hrs [ ]

8. In the last 12 months, how often, on average, did you eat fresh fruit?
   - less than twice a week [ ] 2 to 4 times a week [ ] daily [ ] twice daily [ ]

9. How do you normally sleep?
   - On your front [ ] On your back [ ] On your left side [ ] On your right side [ ] On either side [ ]

   I do not have any preference [ ]

10. Do you currently play any wind musical instruments (eg. trumpet, clarinet, flute...)?
    - yes [ ] no [ ]

    If yes: please specify__________________________

11. Have you ever played any wind musical instruments (eg. trumpet, clarinet, flute...)?
    - yes [ ] no [ ]

    If yes: please specify__________________________

    How long did you play it? _______ yrs _______ months

21 July 08 personal pullout_under14_female.doc

version 2 dated 21 July 08
Body development

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. We are asking these questions since they may have something to do with how your lungs grow. If you do not understand a question or do not know the answer, just mark "I don't know."

12. Would you say that your rapid growth in height that occurs in teenagers
   • Has not yet begun
   • Has only just started
   • Is definitely underway
   • Seems complete
   • I do not know

13. Would you say that your body hair growth ("body hair" means hair in any other place than your head, such as under your arms)
   • Has not yet begun
   • Has only just started
   • Is definitely underway
   • Seems complete
   • I do not know

14. Have you noticed any skin changes, especially pimples?
   • Skin has not yet started changing
   • Skin has only just started changing
   • Skin changes are definitely underway
   • Skin changes seem complete
   • I do not know

15. Have you noticed that your breasts have begun to grow?
   • They have not yet started growing
   • They have only just started growing
   • Breast growth is definitely underway
   • Breast growth seems complete
   • I do not know

16. Have you started to have your periods? yes [ ] no [ ]
   If yes: how old were you when you started having your periods? ________ yrs _________ months

Thank you for completing the questionnaire
Helium³ magnetic resonance
Questionnaire – Personal questions –
14 years old and over (Girls)

Your first Name: ____________
No: ____________

Please complete by either ticking the appropriate box: ✅ or filling in a number: 93

⇒ Please fill in these questions yourself. We would like to assure you that your answers will remain confidential in all but the most exceptional circumstances, where we believe you are at risk of significant harm. We would not show this questionnaire to your parents unless we had your full agreement.
⇒ Date questionnaire completed: day______ month______ year______ (please fill in today’s date)

Smoking

1. Have you ever smoked cigarettes or roll ups (or any other form of tobacco)?
   yes ☐ no ☐

⇒ If you answered “no” to this question please skip to question 7.

2. Have you ever been a regular smoker (smoking at least daily for a period of more than 3 months)?
   yes ☐ no ☐

3. In what form do you, or did you, usually smoke? (tick as many as apply)
   • Cigarette with tobacco ☐
   • Roll ups ☐
   • Other ☐

   If other, please specify ______

4. How old were you when you started smoking?
   yrs ____________ months ____________

5. Are you still smoking?
   yes ☐ no ☐

   If no, how old were you when you stopped smoking?
   yrs ____________ months ____________

6. How many cigarettes (or equivalent) do (did) you usually smoke per day? ______ (please fill in a number)

7. Have you ever smoked Cannabis (also known as Marijuana, weed, grass, pot, hash…)?
   yes ☐ no ☐

8. In the last 12 months, how frequently have you smoked Cannabis (also known as Marijuana, weed, grass, pot, hash…)?
   • never ☐
   • less than once a month ☐
   • monthly ☐
   • weekly ☐
   • daily ☐

Hobbies, activities

9. On average, how many hours per week do you spend outside normal work/study periods on:
   (a) sport or vigorous physical activity? 0-1 hr ☐ 2-3 hrs ☐ 4-5 hrs ☐ 6hrs or more ☐
   (b) TV, video or computer activities? 0-4 hrs ☐ 5-10 hrs ☐ 10-20 hrs. ☐ 20hrs or more ☐

10. In the last 12 months, how often, on average, did you eat fresh fruit?
    • less than twice a week ☐
    • 2 to 4 times a week ☐
    • daily ☐
    • twice daily ☐

11. How do you normally sleep?
    • On your front ☐
    • On your back ☐
    • On your left side ☐
    • On your right side ☐
    • On either side ☐
    • I do not have any preference ☐

12. Do you currently play any wind musical instruments (eg. trumpet, clarinet, flute…)?
    yes ☐ no ☐

   If yes; please specify ______

21 July 08 personal pullout_over14_female.doc
version 2 dated 21 July 08
13. Have you ever played any wind musical instruments (e.g., trumpet, clarinet, flute...)?
   - Yes □
   - No □
   If yes, please specify.
   - How long did you play it? _________ yrs _______ months

### Body development

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. We are asking these questions since they may have something to do with how your lungs grow. If you do not understand a question or do not know the answer, just mark "I don't know."

14. Would you say that your rapid growth in height that occurs in teenagers:
   - Has not yet begun □
   - Has only just started □
   - Is definitely underway □
   - Seems complete □
   - I do not know □

15. Would you say that your body hair growth ("body hair" means hair in any other place than your head, such as under your arms):
   - Has not yet begun □
   - Has only just started □
   - Is definitely underway □
   - Seems complete □
   - I do not know □

16. Have you noticed any skin changes, especially pimples?
   - Skin has not yet started changing □
   - Skin has only just started changing □
   - Skin changes are definitely underway □
   - Skin changes seem complete □
   - I do not know □

17. Have you noticed that your breasts have begun to grow?
   - They have not yet started growing □
   - They have only just started growing □
   - Breast growth is definitely underway □
   - Breast growth seems complete □
   - I do not know □

18. Have you started to have your periods? Yes □ No □
   If yes, how old were you when you started having your periods? _________ yrs _______ months

*It is common for girls and young women to be given contraceptives pills (birth control pills) for painful periods or other reasons. We would like to know if you are on these pills.*

19. Do you currently take contraceptive pills or birth control pills for any reason? Yes □ No □
   If yes, what age did you start using them? _________ yrs _______ months

20. Have you ever taken contraceptive pills or birth control pills for any reason? Yes □ No □
   If yes, what age did you start using them? _________ yrs _______ months
   What age did you stop using them? _________ yrs _______ months

Thank you for completing the questionnaire.

21 July 08 personal pullout_over14_female.doc

Version 2 dated 21 July 08
### Helium³ magnetic resonance
Questionnaire - Birth and early childhood

**First Name:**

No. 2008_He_Birth

Please complete by either ticking the appropriate box or filling in a number.

- Persons completing questionnaire: Mother [ ] Father [ ] Other [ ]

- Date questionnaire completed: day [ ] month [ ] year [ ] (please fill in today's date)

#### When the child was born

1. Immediately after the child was born, did he/she need to be admitted to a special care unit (the neonatal unit)?
   - Yes [ ] No [ ]
   - If yes, go to question 2.

2. Was the child admitted because he/she was premature? Yes [ ] No [ ]
   - If yes:
     - (a) How many weeks early (before the due date) was the baby born? [ ] weeks
     - (b) Many maternity units give steroids to the mother before the birth in order to make baby's lung mature. Was the child's mother given steroids (such as dexamethasone, betamethasone)?
       - Yes [ ] No [ ] Don't know [ ]
   - If no, what was the reason for admission to the neonatal unit?
     - Lung problems [ ] No [ ] Don't know [ ]
     - Feeding problems [ ] No [ ] Don't know [ ]
     - Infection [ ] No [ ] Don't know [ ]
     - Jaundice [ ] No [ ] Don't know [ ]
     - Heart problem [ ] No [ ] Don't know [ ]
     - Fits or other brain problems [ ] No [ ] Don't know [ ]

3. How long did your child stay in the neonatal unit? [ ] days
   - If yes, go to question 4.

4. Did the child need any support with breathing in the neonatal unit? Yes [ ] No [ ] Don't know [ ]
   - If yes, was the breathing supported by:
     - Breathing machine (ventilator) [ ] No [ ] Don't know [ ]
     - CPAP by nasal prong or mask [ ] No [ ] Don't know [ ]
     - Extra oxygen [ ] No [ ] Don't know [ ]
   - If yes, for how long? [ ] days or [ ] weeks

5. In the neonatal unit, did the child have any tube inserted in the chest (for treating air leak into the chest)? Yes [ ] No [ ] Don't know [ ]
6. During the period after the child’s birth, did the child have any operation on chest or heart?  
   - [ ] yes  
   - [ ] no  
   - [ ] don’t know

   If yes: please describe (what operation, when and how many days after birth):

7. Were there any other problems with the stay in the neonatal unit that have NOT been covered above?  
   - [ ] yes  
   - [ ] no  
   - [ ] don’t know

   If yes: please specify:
   - [ ] Bleeding into brain (intraventricular haemorrhage)
   - [ ] PDA (Patent Ductus Arteriosus – a heart problem)
   - [ ] Retinopathy of prematurity (an eye problem)
   - [ ] NEC (Necrotising enterocolitis – a bowel problem)
   - [ ] Severe lung infections
   - [ ] Other
   - [ ] if other, please specify ____________________________

8. Was the child breastfed? 
   - [ ] yes  
   - [ ] no  
   - [ ] don’t know

   If yes:
   (a) How many months? _______ months (please fill in number)

   (b) How many months was he/she exclusively breastfed? (meaning that the child received only breast milk without solids, formula milk or other drinks except water)? _______ months (please fill in number)

9. During the first year of life, in what position was the child usually put to sleep?  
   - [ ] on the tummy  
   - [ ] on the back  
   - [ ] on the side  
   - [ ] no specific position  
   - [ ] cannot remember

10. Did the child’s mother have asthma or wheeze when she was pregnant with her child?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

11. Did the child’s mother receive treatment for asthma when she was pregnant with her child?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

    If yes: did the child’s mother use steroid inhaler (brown, purple or orange inhaler) or steroid tablets (e.g., prednisolone) when she was pregnant with her child?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

**Symptom history**

12. Has the child ever had wheezing or whistling in the chest at any time in the past?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

    (By “wheeze” we mean breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat)

    If yes, during which periods?
    - [ ] When he/she was 0-3 years old
    - [ ] When he/she was 3-10 years old

13. Has the child ever used any type of inhaler?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

    ➞ If you answered “no” to this question please skip to question 15

14. Did he/she use a steroid (brown/purple/orange) inhaler?  
    - [ ] yes  
    - [ ] no  
    - [ ] don’t know

    If yes, during which period did he/she use the steroid (brown/purple/orange) inhaler?
    - [ ] When he/she was 0-3 years old
    - [ ] When he/she was 3-10 years old
15. Yes the child ever suffered from any other chest problems (other than wheeze or asthma)?
   yes [ ] no [ ] don't know [ ]

16. During which period did he/she suffer from the chest problems (other than wheeze or asthma)?
   • When he/she was 0-3 years old [ ] yes [ ] no [ ] don't know [ ]
     If yes:
     (i) please describe the chest problem(s).
     (ii) Was he/she admitted to the hospital for these problems? [ ] yes [ ] no [ ] don't know [ ]
     If yes: (i) How many times during this period? [ ] (please fill in number)
     (ii) Was he/she admitted to intensive care for these problems? [ ] yes [ ] no [ ] don't know [ ]
     (iii) If admitted to intensive care, was the child connected to a breathing machine (ventilator)? [ ] yes [ ] no [ ] don't know [ ]

   • When he/she was 3-10 years old [ ] yes [ ] no [ ] don't know [ ]
     If yes:
     (i) please describe the chest problem(s).
     (ii) Was he/she admitted to the hospital for these problems? [ ] yes [ ] no [ ] don't know [ ]
     If yes: (i) How many times during this period? [ ] (please fill in number)
     (ii) Was he/she admitted to intensive care for these problems? [ ] yes [ ] no [ ] don't know [ ]
     (iii) If admitted to intensive care, was the child connected to a breathing machine (ventilator)? [ ] yes [ ] no [ ] don't know [ ]

17. Did the child ever have any other diseases for which he/she has been taking regular (for a period of at least 2-3 months) treatment? [ ] yes [ ] no [ ]

18. During which period did he/she suffer from other diseases for which he/she has been taking regular treatment?
   • When he/she was 0-3 years old [ ] yes [ ] no [ ] don't know [ ]
     If yes: please specify
     (i) the disease __________________________ (please discuss with the study team if you are unsure)
     (ii) and the treatment __________________________

   • When he/she was 3-10 years old [ ] yes [ ] no [ ] don't know [ ]
     If yes: please specify
     (i) the disease __________________________ (please discuss with the study team if you are unsure)
     (ii) and the treatment __________________________

19. Did the child ever take oral steroid tablets (prednisolone) for any disease (e.g. renal disease, rheumatoid arthritis, tuberculosis, asthma)? [ ] yes [ ] no [ ]
   If yes: at what age? __________ years
   for which problem? __________ years (please discuss with the study team if you are unsure)
   for how long? [ ] weeks or [ ] months (please fill in number)
<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did the child's mother ever smoke cigarettes?</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, during which periods?</td>
<td></td>
</tr>
<tr>
<td>During pregnancy with this child</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 0-3 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 3-10 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>Currently</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did the child's father ever smoke cigarettes?</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, during which periods?</td>
<td></td>
</tr>
<tr>
<td>During mother's pregnancy with this child</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 0-3 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 3-10 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>Currently</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did any other household members ever smoke cigarettes?</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, during which periods?</td>
<td></td>
</tr>
<tr>
<td>During mother's pregnancy with this child</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 0-3 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>When the child was 3-10 years old</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
<tr>
<td>Currently</td>
<td>Yes ☐ No ☐ Don't know ☐</td>
</tr>
<tr>
<td>If yes, how many cigarettes per day?</td>
<td>Less than 1 ☐ 1 to 10 ☐ 11 to 20 ☐ More than 20 ☐</td>
</tr>
</tbody>
</table>

If the child does not live with his/her parents or has moved away from Leicestershire, please enter a contact address, telephone number or e-mail address.

Thank you very much for helping us again to study and improve health in children and young people!
Helium$^3$ magnetic resonance
Questionnaire on breathing problems

First Name: ___________________________ No: ___________________________

Please complete the questionnaire
Please complete by either ticking the appropriate box: ✓ or filling in a number: 93

Persons completing questionnaire: Mother [ ] Father [ ] Other [ ]

Date questionnaire completed: day ______ month ______ year ______ (please fill in today’s date)

Wheeze

By “wheeze” we mean breathing that makes a high-pitched whistling or squeaking sound from the
chest, or not the throat.

1. Has your child ever had wheezing or whistling in the chest at any time in the past? yes [ ] no [ ]
2. Has your child had wheezing or whistling in the chest in the last 12 months? yes [ ] no [ ]

If you answered “no” to these questions please skip to question 11.

3. In the last 12 months, has your child had wheezing or whistling in the chest during or soon after a cold or flu? yes [ ] no [ ]
4. In the last 12 months, has your child had wheezing or whistling in the chest even without having a cold or flu? yes [ ] no [ ]
5. How many attacks of wheeze has your child had during the last 12 months?
   none [ ] 1 to 3 [ ] 4 to 12 [ ] more than 12 [ ]
6. Do these attacks cause your child to be short of breath? yes [ ] always [ ] yes, occasionally [ ] no, never [ ]
7. In the last 12 months, how often, on average, has his/her sleep been disturbed due to wheezing?
   never woken with wheeze [ ] less than one night per week [ ] one or more nights per week [ ]
8. In the last 12 months, has wheezing ever been severe enough to limit his/her speech to only
   one or two words at a time between breaths? yes [ ] no [ ]
9. In the last 12 months, has his/her chest sounded wheezy during or after exercise? yes [ ] no [ ]
10. During the last 12 months, did your child miss school, college or work because of asthma or wheezing?
    yes [ ] no [ ]
    If yes: how many days did your child miss during the last 12 months (all missing days counted together)?
    less than 1 week [ ] 1 to 2 weeks [ ] 3 to 4 weeks [ ] more than 4 weeks [ ]
11. Have you ever been told by a doctor or a nurse that your child has asthma? yes [ ] no [ ]
12. Has he/she had any other chest or breathing problems over the last years? yes [ ] no [ ]
    If yes: please describe

08 June 08 child version questionnaire.doc version 1 dated 08 June 08

276
Ears, nose and throat

13. In the last 12 months, how many times has your child had a cold or flu?
   - never
   - 1 - 3 times
   - 4 -6 times
   - 7 or more times

14. In the past 12 months, has your child had hayfever? yes no

15. In the past 12 months, has your child had allergic rhinitis (sneeze, red eyes) caused by things other than grass pollen (for example pets or dust)? yes no

16. In the past 12 months, has your child had eczema? yes no

Coughing

17. Do you think that your child coughs more than other people? yes no

18. Does your child usually have a cough with colds? yes no

19. Does your child usually have a cough even without having a cold? yes no

20. Does your child usually bring up phlegm (spit, sputum) from your chest? yes no
   (a) If yes, about how many days a week? 1 2 - 3 more than 4
   (b) Do you bring up phlegm almost days over at least 3 consecutive months of the year? yes no

21. In the last 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection? yes no

Treatment

22. Has your child taken any inhalers or other treatment for asthma in the last 12 months? yes no
   If yes: did your child take any of the following drugs during the last 12 months?
   - Salbutamol, Ventolin, Bricanyl or any blue inhaler yes no don't know
   - Pimicort, Fludolate, Becotide, Qvar or other brown or orange inhaler yes no don't know
   - Seretide or Oxis (a green or green-white inhaler) yes no don't know
   - Seretide or Symbicort (a violet or red-white inhaler) yes no don't know
   - Singular tablets (Montelukast) yes no don't know
   - Steroid tablets (prednisolone) for attacks yes no don't know

23. Has your child had any other disease for which he/she has been taking regular (at least for a period of 12-3 months) treatment in the last 12 months? yes no
   If yes: please specify
   (a) the disease ___________________________ (please discuss with the study team if you are unsure)
   (b) and the treatment ___________________________

24. Has your child taken oral steroid tablets (prednisolone) for any disease (e.g. renal disease, rheumatoid arthritis, tuberculosis, asthma) in the last 12 months? yes no
   If yes: please specify for which disease: ___________________________ (please discuss with the study team if you are unsure)
   and for how long ____ weeks or ____ months (please fill in number)
Household and environment

25. How many rooms are there in the house where you live now? (not counting kitchen, bathroom and toilets) [please fill in a number]

26. How many people usually live in your household, including yourself? (please fill in numbers)
   (a) aged 16 and under [ ]
   (b) aged 17 and over [ ]

27. Which fuel is mainly used for cooking in your home? (tick as many as apply)
   electric [ ]
   gas [ ]
   other fuel [ ]
   if other, what [ ]

28. How is your home heated? (tick as many as apply)
   central heating [ ]
   gas heaters in rooms [ ]
   coal or wood fire [ ]
   other [ ]
   if other, what [ ]

29. What is the highest qualification of the child’s parents? [ ]
   Mother [ ]
   Father [ ]
   No qualifications at all [ ]
   Other qualification: level unknown [ ]
   GCSE/O level grade A*-C, vocational level 2 or equivalent [ ]
   Trade apprenticeships (or equivalent) [ ]
   A levels, vocational level 3 or equivalent [ ]
   Other higher education below degree level [ ]
   Degree or degree equivalent, and above [ ]

30. Has your child had any brothers and sisters (including half-siblings)? Yes [ ]
    No [ ]
    If yes:
    (a) how many brothers? [ ]
    what years were they born? [ ]
    (b) how many sisters? [ ]
    what years were they born? [ ]

Parents and grandparents

31. Has the child’s mother ever suffered from any of the following conditions?
   wheezing [ ] yes [ ]
   asthma [ ] yes [ ]
   COPD [ ] yes [ ]
   hayfever [ ] yes [ ]
   chronic cough [ ] yes [ ]
   other relevant lung disease [ ] yes [ ]
   If other, what [ ]

32. Has the child’s father ever suffered from any of the following conditions?
   wheezing [ ] yes [ ]
   asthma [ ] yes [ ]
   COPD [ ] yes [ ]
   hayfever [ ] yes [ ]
   chronic cough [ ] yes [ ]
   other relevant lung disease [ ] yes [ ]
   If other, what [ ]
33. | Mother | Father |
---|---|---|
(a) What is the child's **mother's and father's** year of birth? | | |
(b) What is the child's **mother's and father's** country of birth? | | |
(c) If born abroad, what year did **mother and father** immigrate? | | |
(d) What is the child's **mother's and father's** main spoken language? | | |
(e) What is the child's **mother's and father's** ethnic origin? | | |
(f) What is the child's **mother's and father's** religion? | | |

(With ethnic origin we mean the ethnic group your family originally comes from, for example European, Afro-Caribbean, south Asian, Chinese...)

34. In which country were the child's **grandparents** born?
(a) his/her **mother's father**? | | |
(b) his/her **mother's mother**? | | |
(c) his/her **father's father**? | | |
(d) his/her **father's mother**? | | |

If you have moved away from Leicestershire, please enter a contact address, telephone number or e-mail address.

You can also write any other comments you might have in the space below.

Thank you very much for helping us again to study and improve health in children and young people!
Helium³ magnetic resonance
Questionnaire on breathing problems

First Name: No: 2008_He_14+

Please complete the questionnaire (together with a parent or carer if you wish)
Please complete by either ticking the appropriate box: [ ] or filling in a number: [93]

Persons completing questionnaire: [ ] Mother [ ] Father [ ] Other [ ]
(tick all who helped to fill in) if other who

Date questionnaire completed: day [ ] month [ ] year [ ] (please fill in today’s date)

**Wheezing**

By “wheezing” we mean breathing that makes a high-pitched whistling or squeaking sound from the chest, not the throat

1. Have you ever had wheezing or whistling in the chest at any time in the past? [ ] yes [ ] no

2. Have you had wheezing or whistling in the chest in the last 12 months? [ ] yes [ ] no

⇒ if you answered “no” to these questions please skip to question (11)

3. In the last 12 months, have you had wheezing or whistling in the chest during or soon after a cold or flu? [ ] yes [ ] no

4. In the last 12 months, have you had wheezing or whistling in the chest even without having a cold or flu? [ ] yes [ ] no

5. How many attacks of wheezing have you had during the last 12 months?
   [ ] none [ ] 1 to 3 [ ] 4 to 12 [ ] more than 12

6. Do these attacks cause you to be short of breath? [ ] yes, always [ ] yes, occasionally [ ] no, never

7. In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing? [ ] never [ ] woken with wheezing [ ] less than one night per week [ ] one or more nights per week

8. In the last 12 months, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths? [ ] yes [ ] no

9. In the last 12 months, has your chest sounded wheezy during or after exercise? [ ] yes [ ] no

10. During the last 12 months, did you miss school, college or work because of asthma or wheezing? [ ] yes [ ] no

   If yes: how many days did you miss during the last 12 months (all missing days counted to either)?
   [ ] less than 1 week [ ] 1 to 2 weeks [ ] 3 to 4 weeks [ ] more than 4 weeks

11. Have you ever been told by a doctor or a nurse that you have asthma? [ ] yes [ ] no

12. Have you had any other chest or breathing problems over the last year? [ ] yes [ ] no

   If yes: please describe...
## Ears, nose and throat

13. **In the last 12 months, how many times have you had a cold or flu?**
   - never □
   - 1 - 3 times □
   - 4 - 6 times □
   - 7 or more times □

14. **In the past 12 months, have you had hayfever?** □ yes □ no □

15. **In the past 12 months, have you had allergic rhinitis (sneeze, red eyes) caused by things other than grass pollen (for example, pets or dust)?** □ yes □ no □

16. **In the past 12 months, have you had eczema?** □ yes □ no □

## Coughing

17. **Do you think that you cough more than other people?** □ yes □ no □

18. **Do you usually have a cough with colds?** □ yes □ no □

19. **Do you usually have a cough even without having a cold?** □ yes □ no □

20. **Do you usually bring up phlegm (spit, sputum) from your chest?** □ yes □ no □
   
   (a) If yes, about how many days a week? □ 1 □ 2 - 3 □ more than 4 □
   
   (b) Do you bring up phlegm on most days over at least 3 consecutive months of the year? □ yes □ no □

21. **In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or a chest infection?** □ yes □ no □

## Treatment

22. **Did you take any inhalers or other treatment for asthma in the last 12 months?** □ yes □ no □

   If yes: did you take any of the following drugs during the last 12 months?
   - Salbutamol, Ventolin, Bricanyl or any **blue** inhaler □ yes □ no □ don’t know □
   - Pulmicort, Fluticortide, Becotide, Quvar or other **brown or orange** inhaler □ yes □ no □ don’t know □
   - Seretide or Oxis (a **green** or green-white inhaler) □ yes □ no □ don’t know □
   - Seretide or Symbicort (a **violet** or red-white inhaler) □ yes □ no □ don’t know □
   - **Singulair tablets** (Montelukast) □ yes □ no □ don’t know □
   - **Steroid tablets** (prednisolone) for attacks □ yes □ no □ don’t know □

23. **Do you have any other disease for which you have been taking regular (at least for a period of 2 - 3 months) treatment in the last 12 months?** □ yes □ no □

   If yes: please specify ____________________________ (please discuss with the study team if you are unsure)

   (b) and the treatment

24. **Have you taken **oral steroid tablets** (prednisolone) for any disease (e.g., renal disease, rheumatoid arthritis, tuberculosis, asthma) in the last 12 months?** □ yes □ no □

   If yes: please specify for which disease: ____________________________ (please discuss with the study team if you are unsure)

   and for how long __________ weeks or __________ months (please fill in number)
26. Do you still live with your parent(s) or guardian(s)?
   yes, most of the time ☐  some of the time (e.g. only holidays) ☐  not at all or just visits ☐

26. How many rooms are there in the house where you live now? (not counting kitchen, bathroom and toilets)?
   ☐ (please fill in a number)

27. How many people usually live in your household, including yourself? (please fill in numbers)
   (a) aged 16 and under ☐  (b) aged 17 and over ☐

28. Which fuel(s) is mainly used for cooking in your home? (tick as many as apply)
   electricity ☐  gas ☐  other fuel ☐  if other, what ☐

29. How is your home heated? (tick as many as apply)
   central heating ☐  gas heaters in rooms ☐  coal or wood fire ☐  other ☐  if other, what ☐

30. What is the highest qualification of your mother and father?
   Mother ☐
   Father ☐
   - No qualifications at all, ............................................................................................................
   - Other qualification, level unknown, ............................................................................................
   - GCSE/O level grade A*-C, vocational level 2 or equivalent, ....................................................
   - Trade apprenticeships (or equivalent), ......................................................................................
   - A levels, vocational level 3 or equivalent, ..................................................................................
   - Other higher education below degree level, .............................................................................
   - Degree or degree equivalent, and above, ..................................................................................

31. Do you have any brothers and sisters (including half-siblings)?
   yes ☐  no ☐
   If yes, (a) how many brothers?
   ☐
   what year were they born?
   ☐
   (b) how many sisters?
   ☐
   what year were they born?
   ☐

Parents and grandparents

32. Has your mother ever suffered from any of the following conditions?
   □ wheezing
   □ asthma
   □ COPD
   □ hayfever
   □ chronic cough
   □ other relevant lung disease
   □ if other, what

33. Has your father ever suffered from any of the following conditions?
   □ wheezing
   □ asthma
   □ COPD
   □ hayfever
   □ chronic cough
   □ other relevant lung disease
   □ if other, what
34. What is your mother's and father's year of birth?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(b) What is your mother's and father's country of birth?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(c) If born abroad, what year did mother and father immigrate?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(d) What is your mother's and father's main spoken language?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(e) What is your mother's and father's ethnic origin*?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(f) What is your mother's and father's religion?

*('with ethnic origin we mean the ethnic group your family originally comes from, for example European, Afro-Caribbean, south Asian, Chinese...')

35. In which country were your grandparents born?

(a) Your mother's father?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(b) Your mother's mother?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(c) Your father's father?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

(d) Your father's mother?

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
</table>

If you don't live with your parents or have moved away from Leicestershire, please enter a contact address, telephone number or e-mail address.


You can also write any other comments you might have in the space below.


Thank you very much helping us again to study and improve health in children and young people!
Appendix D: Ethical approval, PIS and consent form for Helium-3 MR study for children and young adults with CF
DIRECTORATE OF RESEARCH & DEVELOPMENT

Research & Development Office
Leicester General Hospital
Gwendolen Road
Leicester
LE5 4PW

Director: Professor D Rowbotham
Assistant Director: Dr David Hetmanski
R&D Manager: Carolyn Maloney

Direct Ddi: (0116) 258 8851
Fax No: (0116) 258 4226

27/01/2010

Professor M Silverman
Department of Infection and Immunity
Faculty of Medicine and Biological Sciences
Robert Kilpatrick Clinical Sciences Building,
LRI
LE5 4PW

Dear Professor M Silverman

Ref: [UHL 06560]
Title: Measuring lung development using Helium-3 magnetic resonance
Project Status: Project Approved
End Date: 31/10/2010

Thank you for submitting documentation for Substantial amendment Number 3.1 for the above study.

I confirm that the amendment has the approval of the University Hospitals of Leicester NHS Trust R&D Department and may be implemented with immediate effect.

The documents received are as follows:

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Version Number</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Consent Form: Young Person and Parent</td>
<td>V3</td>
<td>15/01/10</td>
</tr>
<tr>
<td>Participant Information Sheet: Parents</td>
<td>V4</td>
<td>15/01/10</td>
</tr>
<tr>
<td>Participant Information Sheet: Children</td>
<td>V2</td>
<td>15/01/10</td>
</tr>
</tbody>
</table>

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments should be followed. Failure to do so may invalidate the approval of the study at this trust.

Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Yours sincerely,

Carolyn Maloney
R&D Manager
Dear Professor Silverman

Study title: Measuring lung development using Helium-3 magnetic resonance
REC reference: 04/Q2501/114
Amendment number: 3.1
Amendment date: 05 November 2009

The above amendment was reviewed at the meeting of the Sub-Committee held on 08 January 2010 with revised documents reviewed by the Sub-Committee in correspondence.

Ethical opinion

The Sub-committee did not have any ethical issues with the inclusion of children with Cystic Fibrosis. However, the information sheet for young adults was too long and technical for the age group. The parent information sheet included statements worded as though they would be the participant. The Sub-Committee have reviewed the revised version of these documents submitted on the 15 January 2010.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation as revised.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notice of Substantial Amendment (non-CTIMP)</td>
<td>3.1</td>
<td>05 November 2009</td>
</tr>
<tr>
<td>Covering Letter</td>
<td></td>
<td>14 December 2009</td>
</tr>
<tr>
<td>Email - response to changes requested</td>
<td></td>
<td>15 January 2010</td>
</tr>
<tr>
<td>Participant Consent Form: Young Person and Parent</td>
<td></td>
<td>16 January 2010</td>
</tr>
<tr>
<td>Participant Information Sheet Parents</td>
<td>4</td>
<td>16 January 2010</td>
</tr>
<tr>
<td>Participant Information Sheet Children</td>
<td>2</td>
<td>16 January 2010</td>
</tr>
</tbody>
</table>

This Research Ethics Committee is an advisory committee to East Midlands Strategic Health Authority. The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England.
Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

04/Q250/114: Please quote this number on all correspondence

Yours sincerely

Miss Jeannie D McKie
Committee Co-ordinator

E-mail: jeannie.mckie@motspec.nhs.uk

Enclosures: List of names and professions of members who took part in the review

Copy to: Sponsor / R&D office for NHS care organisation at lead site - UHL
**NOTICE OF SUBSTANTIAL AMENDMENT**

For use in the case of all research other than clinical trials of investigational medicinal products (CTIMPs). For substantial amendments to CTIMPs, please use the EU-approved voice of amendment form (Annex 2 to ENTR/CT) at [http://www.clinicaltrials.gu.eu.int/document.htm#guidance](http://www.clinicaltrials.gu.eu.int/document.htm#guidance).

Further guidance is available at [http://www.mres.casa.nhs.uk/applicants/review/alter/amendments.htm](http://www.mres.casa.nhs.uk/applicants/review/alter/amendments.htm).

<table>
<thead>
<tr>
<th>Details of Chief Investigator:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name:</strong></td>
<td>Professor Michael Silverman</td>
</tr>
</tbody>
</table>
| **Address:** | Department of Infection, Immunity & Inflammation  
                  (Child Health)  
                  University of Leicester  
                  Robert Kilpatrick Clinical Sciences Building  
                  Leicester Royal Infirmary, Leicester  
                  LE2 7LX |
| **Telephone:** | 0116 252 3261 |
| **Email:** | Mz79@le.ac.uk |
| **Fax:** | 0116 252 3262 |

<table>
<thead>
<tr>
<th>Full title of study:</th>
<th>Measuring lung development using Helium-3 magnetic resonance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of main REC:</td>
<td>Leicestershire, Northamptonshire &amp; Rutland Research Ethics Committee 1 &amp; 2</td>
</tr>
<tr>
<td>REC reference number:</td>
<td>04/Q2531/114</td>
</tr>
<tr>
<td>Date study commenced:</td>
<td>July 2006</td>
</tr>
<tr>
<td>Protocol reference (if applicable), current version and date:</td>
<td>Version 5 dated 06/08/08</td>
</tr>
<tr>
<td>Amendment number and date:</td>
<td>Amendment 8: Dated 3 November 2009</td>
</tr>
</tbody>
</table>
Type of amendment (indicate all that apply in bold)

(a) Amendment to information previously given on the ARES Application Form

Yes  No

If yes, please refer to relevant sections of the REC application in the "summary of changes" below.

(b) Amendment to the protocol

Yes  No

If yes, please submit either the revised protocol with a new version number and date, highlighting changes in bold, or a document listing the changes and giving both the previous and revised text.

(c) Amendment to the information sheet(s) and consent form(s) for participants, or to any other supporting documentation for the study

Yes  No

If yes, please submit all revised documents with new version numbers and dates, highlighting new text in bold.

Is this a modified version of an amendment previously notified to the REC and given an unfavourable opinion?

Yes  No

Summary of changes

Briefly summarise the main changes proposed in this amendment using language comprehensible to a lay person. Explain the purpose of the changes and their significance for the study. In the case of a modified amendment, highlight the modifications that have been made.

If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained.

Summary of the change:

Inviting participants with cystic fibrosis to study the impact of cystic fibrosis lung disease on microstructure of the distal airways and alveolar size in children aged 7 to 11 years.

Details:

We would like to recruit 10 children with cystic fibrosis into the HeMRI study for a pilot study investigating alveolar size and the presence of emphysema in cystic fibrosis lung disease. The MRI may be a relatively non-invasive way without the need for ionising radiation to monitor progression of cystic fibrosis lung disease.
Purpose of the change:

Over the last two years, we have collected data in a cross-section of children and young people using theHeliMit. We have been able to present our exciting new data that challenges some of the currently accepted paradigms of lung atelectasis development at the European Respiratory Society Annual Congress in Vienna 2008. In particular, we have looked exclusively at children who suffered from developmental and respiratory disorders in the perinatal period or early childhood and compared them with healthy children.

We would like to test children with persistent disease - cystic fibrosis - to test the hypothesis that lung development (and hence AEC) adversely affected compared to normal controls. Control data are already available.

Any other relevant information

Applicants may indicate any specific ethical issues relating to the amendment, on which the opinion of the REC is sought.

List of enclosed documents

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information Sheet for Parents 7-11 years old</td>
<td>3</td>
<td>3 November 2009</td>
</tr>
<tr>
<td>Consent Form</td>
<td>3.1</td>
<td>3 November 2009 21 July 2008</td>
</tr>
<tr>
<td>Information Sheet for Young Adults</td>
<td>2</td>
<td>3 November 2009</td>
</tr>
<tr>
<td>Consent Form for Young Adults</td>
<td>1</td>
<td>3 November 2009 24 October 2008</td>
</tr>
</tbody>
</table>

Declaration

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendment to be implemented.

Signature of Chief investigator:

[Signature]

Print name:

[Print name]

Date of submission:

[Date]
University Hospitals of Leicester

Leicester Royal Infirmary
Leicester
LE1 5WW

Brief information sheet for children

Study Title: Measuring lung development using \(^3\)He magnetic resonance

Principal Investigator: Professor Mike Silverman

We are trying to find out how people's lungs grow. We would like you to help us. Your parent(s) will have some information too, so you can talk to them about it. Please take your time to read about the study.

Why?

We have only one pair of lungs. They start to form when we are babies in the first few years of life. After that, they simply get bigger as we grow up. Doctors do not know exactly how the lungs grow. We also need to find out whether the lungs grow differently in children with cystic fibrosis, so that we can prevent damage caused by illness.

How?

A new method to measure how lungs grow has been developed by Nottingham University. It is really simple. You just breathe in a tiny amount of special air, called helium, while lying still inside a giant magnet. Then you hold your breath for a few seconds. You do this three times – that's all. Helium is a harmless and tasteless form of air.

Also, there are some blowing and breathing tests at the Children's Hospital, Leicester. We would also like to collect a sample of urine or some spit, during one of the visits. We can use this to measure how much smoke you have breathed in, over the last few days.

Problems?

There are no needles, no pain or discomfort and no other risks. The tests will not affect you in any way. A parent will be with you all the time.

What next?

If your parent(s) reply "yes" on the reply letter, we will make arrangements for you to visit us for the tests. We will ask you and your parent(s) to sign a "consent form" when you come for the tests. The important thing is that you do not have to take part if you do not want to.

Any questions?

Please get in touch to speak to or leave a message for one of us, if you have any questions.

- phone (0116) 252 5961
- email ms70@lc.ac.uk
- fax (0116) 252 3282

Professor Mike Silverman
Children's Hospital, Leicester Royal Infirmary

Information Sheet for Children

Version 2, dated 15 January 2010

Nuffield Department of Health

Trust Headquarters, Gwendolen House, Gwendolen Road, Leicester LE5 4QF
Website: www.le.ac.uk
Chairman: Mr. Philip Hammondsley CBE, Chief Executive Dr. Peter Reading

291
CONSENT FORM for young person and parent

Study Number:

Name of young person or child:

Title of Project: Measuring lung development using 3Helium magnetic resonance

Principal Investigator: Professor M Silverman

1. I confirm that I have read and understand the information sheet dated January 2010 Version 4 for the above study and have had the opportunity to ask questions.

2. I understand that my child's participation is voluntary and that if my child is free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my/ my child's medical notes may be looked at by responsible individuals from Leicester Royal Infirmary where it is relevant to this research. I give permission for these individuals to have access to these records.

4. I agree to allow my child to take part in the above study.

Name of Young Person

Date

Signature

Name of Parent (if needed)

Date

Signature

Name of Person taking consent (if different from researcher)

Date

Signature

Researcher

Date

Signature

1 for participant; 1 for researcher

Consent Form

Version 3: 16 January 2010
Study Title: Measuring lung development using 'Helium magnetic resonance'

Principal Investigator: Professor Mike Silverman

We are inviting (name of child or young person) to take part in a research study. Before deciding, it is important for (name of child or young person) and the family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We have only one pair of lungs. Most of their development occurs before birth and in the first few years of life. After that, they simply get larger during childhood, with little ability to catch-up if things go wrong earlier. Exactly how the lungs grow and how illness in childhood can affect lung development is not clear. It is important to find out, so that we can prevent lung damage. New treatments are under development which could even restore damaged lungs to normal. The purpose of this study is to learn how the lungs grow in health and disease, and which genes are important in lung growth.

Why have I been chosen?
We have chosen your child to take part because (name of child or young person) has cystic fibrosis and we want to study the impact of cystic fibrosis lung disease on the small airspaces in the lung where the blood is oxygenated. A total of 200 children and young people will participate, some of them healthy and some of them with various lung problems including premature birth, cystic fibrosis and others in order to investigate the normal growth of the lungs and the effect of various symptoms and illnesses.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide not to take part you can withdraw at any time, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if (name of child or young person) takes part?
There are two visits, each of which will take about 1½ hours. We can combine these if you wish to make it easier, and we can organise transport to Nottingham (visit 2).

At the first visit you will be asked to come for breathing tests in a Lung Function Laboratory at the Leicester Royal Infirmary. Your child will be asked to carry out blowing and...
breathing tests, and if he/she has asthma or any other results are not normal, we will give
him/her a dose of an asthma puffer (Salmeterol) and repeat the measurements.

The second visit will take place at the Physics Department at Nottingham University where
a lung scan will be carried out. This is a completely harmless test, using a tiny quantity of
a gas called Helium-3. A small amount of Helium gas (completely harmless substance
used in routine breathing tests in babies, children and adults) would be breathed in from a
small bag, while lying still in a scanning machine. Your child will then be asked to hold
their breath for about 5 seconds and the test is over. This will be repeated 3 times.

There are no special restrictions before the tests and your child should carry on taking any
treatment which has been prescribed. We will collect a few cells from inside the cheek by
lighty scraping with a wooden spatula, to extract DNA for gene studies, if you agree.

There are no side effects from any of the agents which we use. Your child will only be
given an asthma puffer if he/she has asthma or any evidence of blockage of the tubes in
the chest. The scan does not involve any x-rays or any other form of radiation, and there
are no known risks or disadvantages.

Your child will be asked to produce either a sample of urine during one of the visits, or a
sample of saliva (spit). We will use this to measure exposure to cigarette smoke in an
accurate way.

What are the possible benefits of taking part?
We will learn how the lungs grow during early life, and whether simple and common
problems such as asthma or prematurity can affect the way in which the lungs grow. This
will not provide any advantage to your child, but to future generations. If we do find any
problem with your child’s lungs, we will discuss it with you and ask whether you would like
your general practitioner to be informed.

What if something goes wrong?
There is no risk of harm from any parts of this research. However, if your child is harmed
by taking part in this research project, there are no special compensation arrangements.
If your child is harmed due to someone’s negligence, then you may have grounds for a legal
action but you may have to pay for it. Regardless of this, if you wish to complain, or have
any concerns about any aspect of the way you have been approached or treated during
the course of this study, the normal National Health Service complaints mechanisms
would be available to you. If you have any complaints about staff, or if anything serious
happens during the procedure, you are invited to get in touch with any of the researchers,
or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen
House, Gwendolen Road, Leicester, LE5 4QF, where your concerns will be dealt with
within 2 weeks.

Will participation be confidential?
Yes all the results are confidential. There is no way in which anybody outside the Division
of Child Health and Leicester University will be able to identify any participant from the
results of the tests. All the results and names and addresses are kept on different files,
and are secure. We will only inform any outside individual (such as your general
practitioner) with your permission.

What happens to the results of the research?
The results of the research will be published in journals which are widely read by doctors
and medical physicists. It will not be possible to identify your child within any report or
publication.

Information Sheet for Parents

Version 4, dated 15 January 2010

Ref: 4/Q2501/114
Last saved: 15 January 2010
Who is funding the research?
The research is completely independently carried out by medical and physics staff at
Leicester and Nottingham Universities. External funding will be used. You will be paid for
any travelling expenses and other out-of-pocket costs, incurred in taking part in the
investigations.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or
uses NHS premises or facilities must be approved by an NHS Research Ethics Committee
before it goes ahead. Approval does not guarantee that you will not come to any harm if
you take part. However, approval means that the committee is satisfied that your rights
will be respected, that any risks have been reduced to a minimum and balanced against
possible benefits and that you have been given sufficient information on which to make an
informed decision.

Contact for further information
Please contact the following number to speak to or leave a message for Professor
Silverman or one of the other research team.

Telephone: (0116) 525 3262
E-mail: mvis@le.ac.uk
Fax: (0116) 252 3262

THANK YOU FOR READING THIS. YOU WILL BE ABLE TO KEEP A COPY OF THIS
INFORMATION SHEET AND A SIGNED CONSENT FORM.

Professor Mike Silverman
Children's Hospital, Leicester Royal Infirmary.
296

Dear Dr Erol A Gaillard

Ref: UHL 09580

Title: Measuring lung development using Helium-3 magnetic resonance

Project Status: Project Approved
End Date: 31/05/2013

Thank you for submitting documentation for Substantial Amendment 14 dated 03.07.12, for the above study.

I confirm that the amendment has the approval of the University Hospitals of Leicester NHS Trust R&D Department and may be implemented with immediate effect.

The documents received are as follows:

<table>
<thead>
<tr>
<th>Documents</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCF: For children aged 12-17 and parent</td>
<td>3.1</td>
<td>04.07.12</td>
</tr>
<tr>
<td>PIS / CF: Adults with cystic fibrosis</td>
<td>1</td>
<td>03.07.12</td>
</tr>
<tr>
<td>PIS: Parents</td>
<td>4.1</td>
<td>03.07.12</td>
</tr>
<tr>
<td>Notice of Substantial Amendment Form</td>
<td>1.4</td>
<td>03.07.12</td>
</tr>
<tr>
<td>PIS: For children aged 12-17</td>
<td>2.1</td>
<td>03.07.12</td>
</tr>
<tr>
<td>REG Favourable Opinion Letter</td>
<td></td>
<td>16.07.12</td>
</tr>
</tbody>
</table>
Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments MUST be followed. Failure to do so may invalidate the approval of the study at this trust.

Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Yours sincerely

Carolyn Maloney
R&D Manager
Information Sheet for Parents

Study Title: Measuring lung development using 3He magnetic resonance

Principal Investigator: Dr Erol Gaillard

We are inviting your child to take part in a research study. Before deciding, it is important for your child and the family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We have only one pair of lungs. Most of their development occurs before birth and in the first few years of life. After that, they simply get larger during childhood, with little ability to catch-up if things go wrong earlier. Exactly how the lungs grow and how illness in childhood can affect lung development is not clear. It is important to find out so that we can prevent lung damage. New treatments are under development which could even restore damaged lungs to normal. The purpose of this study is to learn how the lungs grow in health and disease, and which genes are important in lung growth.

Why have I been chosen?
We have chosen your child to take part because your child has cystic fibrosis and we want to study the impact of cystic fibrosis lung disease on the small airways in the lung where the blood is oxygenated. A total of 200 children and young people will participate, some of them healthy and some of them with various lung problems including prematurity birth, cystic fibrosis and others in order to investigate the normal growth of the lungs and the effect of various symptoms and illnesses.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide not to take part you can withdraw at anytime, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if my child takes part?
There are two visits, each of which will take about 1½ hours. We can combine these if you wish to make it easier, and we can organise transport to Nottingham (via 2).

At the first visit you will be asked to come for breathing tests in a Lung Function Laboratory at the Leicester Royal Infirmary. Your child will be asked to carry out blowing and breathing tests, and if he/she has asthma or any other results are not normal, we will give him/her a dose of an asthma puffer (Salbutamol) and repeat the measurements.

The second visit will take place at the Physics Department at Nottingham University where a lung scan will be carried out. This is a completely harmless test, using a tiny quantity of a gas called Helium-3. A small amount of Helium gas (completely harmless substance used in routine breathing tests in babies, children and adults) would be breathed in from a small bag, while lying still in a scanning machine. Your child will then be asked to hold their breath for about 5 seconds and the test is over. This will be repeated 3 times.

Information Sheet for Parents
Version 4.1 dated 3 July 2012

Ref: G522001114
Last saved: 01 July 2012
There are no special restrictions before the tests and your child should carry on taking any treatment which has been prescribed. We will collect a few cells from inside the cheek by lightly scraping with a wooden spatula, to extract DNA for gene studies, if you agree.

There are no side effects from any of the agents which we use. Your child will only be given an asthma puffer if he/she has asthma or any evidence of blockage of the tubes in the chest. The scan does not involve any X-rays or any other form of radiation, and there are no known risks or disadvantages.

Your child will be asked to produce either a sample of urine during one of the visits, or a sample of saliva (spit). We will use this to measure exposure to cigarette smoke in an accurate way.

**What are the possible benefits of taking part?**
We will learn how the lungs grow during early life, and whether simple and common problems such as asthma or prematurity can affect the way in which the lungs grow. This will not provide any advantage to your child, but to future generations. If we do find any problem with your child’s lungs, we will discuss it with you and ask whether you would like your general practitioner to be informed.

**What if something goes wrong?**
There is no risk of harm from any part of this research. However, if your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. If you have any complaints about staff, or if anything serious happens during the procedure, you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHLS, Leicester General Hospital, Gwendoline House, Gwendoline Road, Leicester, LE5 4QF, where your concerns will be dealt with within 2 weeks.

**Will participation be confidential?**
Yes, all the results are confidential. There is no way in which anybody outside the Division of Child Health and Leicester University will be able to identify any participant from the results of the tests. All the results and names and addresses are kept on different files, and are secure. We will only inform any outside individual (such as your general practitioner) with your permission.

**What happens to the results of the research?**
The results of the research will be published in journals which are widely read by doctors and medical physicists. It will not be possible to identify your child within any report or publication.

**Who is funding the research?**
The research is completely independently carried out by medical and physics staff at Leicester and Nottingham Universities. External funding will be used. You will be paid for any travelling expenses and other out-of-pocket costs, incurred in taking part in the investigations.
Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information
Please contact the following number to speak to or leave a message for Professor Silverman or one of the other research team.

Telephone: (0116) 252 3262
E-mail: eag15@le.ac.uk
Fax: (0116) 252 3262

THANK YOU FOR READING THIS. YOU WILL BE ABLE TO KEEP A COPY OF THIS INFORMATION SHEET AND A SIGNED CONSENT FORM.

Dr Erol Gaillard
Consultant Paediatrician
Brief Information sheet for children (aged 12 to 17 years old)
Study Title: Measuring lung development using 3He magnetic resonance

Principal Investigator: Dr Erol Gaillard

We are trying to find out how people’s lungs grow. We would like you to help us. Your parent(s) will have some information too, so you can talk to them about it. Please take your time to read about the study.

Why?
We have only one pair of lungs. They start to form when we are babies in the first few years of life. After that, they simply get bigger as we grow up. Doctors do not know exactly how the lungs grow.
We also need to find out whether the lungs grow differently in children with cystic fibrosis, so that we can prevent damage caused by illness.

How?
A new method to measure how lungs grow has been developed by Nottingham University. It is really simple. You just breathe in a tiny amount of special air, called helium, while lying still inside a giant magnet. Then you hold your breath for a few seconds. You do this three times – that’s all. Helium is a harmless and tasteless form of air.

Also, there are some blowing and breathing tests at the Children’s Hospital, Leicester. We would also like to collect a sample of urine or some spit during one of the visits. We can use this to measure how much smoke you have breathed in, over the last few days.

Risks?
There are no needles, no pain or discomfort and no other risks. The tests will not affect you in any way. A parent will be with you all the time.

What next?
If your parent(s) reply “yes” on the reply letter, we will make arrangements for you to visit us for the tests. We will ask you and your parent(s) to sign a “consent form” when you come for the tests. The important thing is that you do not have to take part if you do not want to.

Any questions?
Please get in touch to speak to or leave a message for one of us, if you have any questions.

- phone (0116) 252 5381
- email eag10@e.ac.uk
- fax (0116) 252 3252

Dr Erol Gaillard
Children’s Hospital, Leicester Royal Infirmary
CONSENT FORM
For Children

Study Number:

Title of Project: Measuring lung development using Helium-3 magnetic resonance

Principal Investigator: Dr Eloi Galland

Please initial box

1. I confirm that I have read and understand the information sheet dated 06.02.2012 version 3 for the above study and have had the opportunity to ask questions. □

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. □

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from University Hospitals of Leicester NHS Trust or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. □

4. I agree to take part in the above study. □

Name of Participant: __________________________ Date: __________ Signature: _______________________

Name of Person taking consent (if different from researcher): __________________________ Date: __________ Signature: _______________________

Researcher: __________________________ Date: __________ Signature: _______________________

1 for participant; 1 for researcher; 1 for site file

Consent Form Version 2 dated 06.02.2012

Ref: Consent Form for Children 06.02.12 v2.doc.
Last saved 06 March 2012
DIRECTORATE OF RESEARCH & DEVELOPMENT

Director: Professor D Rowbotham
Assistant Director: Dr David Hetmansk
R&D Manager: Carolyn Maloney

Direct Dial: (0116) 258 8351
Fax No: (0116) 258 4228
23/03/2013

Dr Erol A Gaillard
University of Leicester
Dept of Infection,
Leicester Royal Infirmary
LE2 7LX

Dear Dr Erol A Gaillard

Ref: UHL 09560
Title: Measuring lung development using Helium-3 magnetic resonance
Project Status: Project Approved
End Date: 31/09/2013

Thank you for submitting documentation for Substantial amendment Number 17 Dated: 25th February 2013 for the above study.

I confirm that the amendment has the approval of the University Hospitals of Leicester NHS Trust R&D Department and may be implemented with immediate effect.

The documents received are as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Version</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant Consent Form: for Adults with CF (17 years+)</td>
<td>V2 Dated: 25.02.2013</td>
</tr>
<tr>
<td>Notice of Substantial Amendment (non-CTIMPs)</td>
<td>Dated: 25.02.2013</td>
</tr>
</tbody>
</table>

Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments MUST be followed. Failure to do so may invalidate the approval of the study at this trust.

Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Yours sincerely

David Hetmansk
R&D Assistant Director
Information Sheet for Adults with Cystic Fibrosis (Age more than 17 years)

Study Title: Measuring lung development using 'Helium magnetic resonance

Principal Investigator: Dr Erol Gailard

We are inviting you to take part in a research study. Before deciding, it is important for you and your family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We have only one pair of lungs made up of large airway branches and then smaller airway branches that are attached to sacs called alveoli. The purpose of this study is to learn whether the small branches and sacs of the lungs (peripheral lungs) are damaged in cystic fibrosis.

Why have I been chosen?
We have chosen you to take part because you are an adult with cystic fibrosis. The aim of the study is to investigate the structure of the small airways in the lung in adults with cystic fibrosis.

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you change your mind about taking part you can withdraw at any time, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

What will happen if I take part?
If you decide to take part we will ask you to attend 2 visits: 1 visit at the Leicester Royal Infirmary and 1 visit at the physics department at Nottingham University. Each visit will take about 1.5 hours. We can combine these if you wish to make it easier, and we can organise transport to Nottingham. We may ask later on if you are willing to carry out further visits.

Visit A will be at the Leicester Royal Infirmary, you will have breathing tests in the Lung Function Laboratory. You will be asked to carry out blowing and breathing tests, and we will give you a dose of an asthma puff (Salfutad) and repeat the measurements. We will perform two breathing tests called lung clearance index and total body plethysmography as well as the usual pulmonary function test. All that is required is for you to breathe in and out into the mouthpiece of specialist lung function equipment.

Visit B will take place at the Physics Department at Nottingham University, where a lung scan will be carried out. This is a completely harmless test, using a tiny quantity of a gas called helium 3. A small amount of Helium gas (completely harmless substance used in routine breathing tests in babies, children and adults) would be breathed in from a small bag while lying still in a scanning machine. You will then be asked to hold your breath for about 5 to 10 seconds and the test is over. This will be repeated 3-4 times.

Information Sheet for Adults with Cystic Fibrosis

Version 2 dated 30Jan2013

Ref: 04/Q23/Q11/124
There are no special restrictions before the tests and you should carry on taking any
treatment which has been prescribed.
There are no side effects from any of the agents which we use. You will only be given an
asthma puffer if you have asthma or any evidence of blockage of the tubes in the chest. The
scan does not involve any x-rays or any other form of radiation, and there are no known risks
or disadvantages.

What are the possible benefits of taking part?
We will learn how the small airways in the lung are affected in cystic fibrosis. If we do find
any problem with your lungs, we will discuss it with you and ask whether you would like your
general practitioner to be informed.

What if something goes wrong?
There is no risk of harm from any parts of this research. However, if you are harmed by
taking part in this research project, there are no special compensation arrangements. If you
are harmed due to someone’s negligence, then you may have grounds for a legal action but
you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns
about any aspect of the way you have been approached or treated during the course of this
study, the normal National Health Service complaints mechanism would be available to
you. If you have any complaints about staff, or if anything serious happens during the
procedure, you are invited to get in touch with any of the researchers, or to write to the
Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen
Road, Leicester, LE5 4GF, where your concerns will be dealt with within 2 weeks.

Will participation be confidential?
Yes all the results are confidential. There is no way in which anybody outside the Division of
Respiratory Medicine at University Hospitals Leicester will be able to identify any participant
from the results of the tests. All the results and names and addresses are kept on different
files, and are secure. We will only inform any outside individual (such as your general
practitioner) with your permission.

What happens to the results of the research?
The results of the research will be published in journals which are widely read by doctors
and medical physicists. It will not be possible to identify you within any report or publication.
Anonymous results may be shared with our academic and industry research partners, this
may include EU funded projects. Again it will not be possible to identify you within any data
shared.

Who is funding the research?
The research is completely independently carried out by medical and physics staff at
Leicester and Nottingham Universities. External funding will be used. You will be paid for
any travelling expenses and other out-of-pocket costs, incurred in taking part in the
investigations.

Who has reviewed the study?
All research that involves NHS patients or staff, information from NHS medical records or
uses NHS premises or facilities must be approved by an NHS Research Ethics Committee
before it goes ahead. Approval does not guarantee that you will not come to any harm if you
take part. However, approval means that the committee is satisfied that your rights will be
respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for further information
Please contact the following number to speak to or leave a message for Dr Erol Gaillard or one of the other research team.

Dr Erol Gaillard
Telephone: 0116 252 3251
Email: eaq15@le.ac.uk

Dr Simon Range
Email: simon.range@uhl-tr.nhs.uk

Dr Chandra Ohri
Email: Chandra.ohri@uhl-tr.nhs.uk

THANK YOU FOR READING THIS. YOU WILL BE ABLE TO KEEP A COPY OF THIS INFORMATION SHEET AND A SIGNED CONSENT FORM.

Dr Erol Gaillard
Children’s Hospital, Leicester Royal Infirmary.
CONSENT FORM for Adults with Cystic Fibrosis
(Age more than 17 years)

Study Number: UHL 09580

Name of the patient: ____________________________

Title of Project: Measuring lung development using \(^{3}\)He magnetic resonance

Principal Investigator: Dr Erol Gallard

1. I confirm that I have read and understand the information sheet dated 30\(^{th}\) January 2013 Version 2 for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, NHS Trust or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records.

4. I agree to take part in the above study.

Name of Adult ____________________________ Date ____________ Signature ____________

Name of Person taking consent (if different from researcher) ____________________________ Date ____________ Signature ____________

Researcher ____________________________ Date ____________ Signature ____________

1 for participant; 1 for researcher; 1 for study site file

Consent Form for Adults with Cystic Fibrosis
Version 2 25.02.2013
Ref: 04/Q2501/114
Appendix E: Ethical approval, PIS and consent for healthy adults>22 yrs. recruited for Helium-3 MR study
DIRECTORATE OF RESEARCH & DEVELOPMENT

Director: Professor D Rowbotham
Assistant Director: Dr David Hetmanski
R&D Manager: Carolyn Maloney

Direct Dial: (0116) 258 6351
Fax No: (0116) 258 4226

17/12/2012

Dr Erol A Gaillard
University of Leicester
Dept of Infection, Immunity &
Inflammation, University of
Leicester, RKGSB,
Leicester Royal Infirmary
LE2 7LX

Dear Dr Erol A Gaillard

Ref: UHL 09560
Title: Measuring lung development using Helium-3 magnetic resonance
Project Status: Project Approved
End Date: 31/05/2013

Thank you for submitting documentation for Substantial amendment Number 15
Dated: 20th November 2012 for the above study.

I confirm that the amendment has the approval of the University Hospitals of
Leicester NHS Trust R&D Department and may be implemented with immediate
effect.

The documents received are as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Version</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notice of Substantial Amendment (non-CTIMP*)s</td>
<td>Dated: 20.11.2012</td>
</tr>
<tr>
<td>Protocol</td>
<td>V0 Dated: 07.11.2012</td>
</tr>
<tr>
<td>Participant Consent Form: Asthmatic adults</td>
<td>V3 Dated: 07.11.2012</td>
</tr>
<tr>
<td>Participant Consent Form: Healthy Adults</td>
<td>V3 Dated: 07.11.2012</td>
</tr>
<tr>
<td>Participant Information Sheet: Asthmatic Adults</td>
<td>V3 Dated: 07.11.2012</td>
</tr>
</tbody>
</table>
Please be aware that any changes to these documents after approval may constitute an amendment. The process of approval for amendments MUST be followed. Failure to do so may invalidate the approval of the study at this trust.

Please ensure that all documentation and correspondence relating to this amendment are filed appropriately in the relevant site file.

Yours sincerely,

[Signature]

Carolyn Maloney
R&D Manager
Information Sheet for Healthy Adults (Age more than 22 years)

Study Title: Measuring lung development using ¹H Helium magnetic resonance

Principal Investigator: Dr Erol Gallard

We are inviting you to take part in a research study. Before deciding, it is important for you and your family to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Our contact details are on the enclosed invitation letter. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We have only one pair of lungs made up of large airway branches and then smaller airway branches that are attached to sacs called alveoli. The purpose of this study is to learn whether the small branches and sacs of the lungs (peripheral lungs) are damaged in severe asthmatics. We also need to determine what is ‘normal’ and therefore we will also look at healthy people without asthma.

Why have I been chosen?
We have chosen you to take part because you are an adult with healthy lungs who has no history of breathing problems. Up to 90 adults, will participate some of them healthy
and some of them with severe asthma, in order to investigate the structure of the small airways in the lung in adults.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you change your mind about taking part you can withdraw at anytime, without giving a reason. A decision to take part or withdraw does not affect any treatment you may be receiving from your doctor or the hospital.

**What will happen if I take part?**

There are two sites taking part in this study: Leicester Glenfield Hospital (site A) and the physics department at Nottingham University (site B). Each will take about 1½ hours. We can combine these if you wish to make it easier, and we can organise transport to Nottingham (visit 2).

There will be a minimum of 2 visits to both sites, i.e. visit 1A and 1B, plus a follow up visit 2A and 2B. We may ask later on if you are willing to carry out further visits.

**Visit A** will be at the Leicester Glenfield Hospital, you will have breathing tests in the Lung Function Laboratory. You will be asked to carry out blowing and breathing tests. A breathing test which will probably be new to most people, will be done as well as the usual pulmonary function tests. The new test will be done on a PEX (Particle Exhaled) Machine. All this requires is for you to breath out into the PEX machine which will then capture a sample of your exhaled breath on a filter. This will then be analysed in the labs to look for evidence of inflammation.

We will also collect a sputum sample from you. To help you to produce sputum you may need to inhale a salty solution for three 5-minute periods. This can cause some chest tightness, wheezing and/or cough. These can all readily be reversed by inhaling a bronchodilator (ventolin). The sputum will be investigated for inflammatory cells and substances and with your consent will be stored for further analysis in collaboration with academic and industry partners.

We will also ask you to complete health questionnaires.
Visit B. will take place at the Physics Department at Nottingham University where a lung scan will be carried out. This is a completely harmless test, using a tiny quantity of a gas called Helium-3. A small amount of Helium gas (completely harmless substance used in routine breathing tests in babies, children and adults) would be breathed in from a small bag, while lying still in a scanning machine. You will then be asked to hold your breath for about 3 seconds and the test is over. This will be repeated 3-4 times.

There are no special restrictions before the tests and you should carry on taking any treatment which has been prescribed.

There are no side effects from any of the agents which we use. You will only be given an asthma puffer if you have asthma or any evidence of blockage of the tubes in the chest. The scan does not involve any x-rays or any other form of radiation, and there are no known risks or disadvantages.

What are the possible benefits of taking part?
We will learn how the small airways in the lung are affected in severe asthma and healthy adults without any lung disease. If we do find any problem with your lungs, we will discuss it with you and ask whether you would like your general practitioner to be informed.

What if something goes wrong?
There is no risk of harm from any parts of this research. However, if you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you. If you have any complaints about staff, or if anything serious happens during the procedure, you are invited to get in touch with any of the researchers, or to write to the Complaints Department, UHL, Leicester General Hospital, Gwendolen House, Gwendolen Road, Leicester, LE5 4QF, where your concerns will be dealt with within 2 weeks.

Will participation be confidential?
Yes all the results are confidential. There is no way in which anybody outside the Division of Respiratory Medicine at Glenfield Hospital will be able to identify any participant from the results of the tests. All the results and names and addresses are
kept on different files and are secure. We will only inform any outside individual (such as your general practitioner) with your permission.

**What happens to the results of the research?**

The results of the research will be published in journals which are widely read by doctors and medical physicists. It will not be possible to identify you within any report or publication. Anonymised results and data may be shared with our academic and industry research partners, this may include EU funded projects. Again it will not be possible to identify you within any data shared.

**Who is funding the research?**

The research is completely independently carried out by medical and physics staff at Leicester and Nottingham Universities. External funding will be used. You will be paid for any travelling expenses and other out-of-pocket costs, incurred in taking part in the investigations.

**Who has reviewed the study?**

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

**Contact for further information**

Please contact the following number to speak to or leave a message for Dr Salman Siddiqui or one of the other research team.

(NIHR Clinical Lecturer: Leicester Glenfield Hospital)

Telephone: 0300 303 1573 (Ext 3788)

Email ss338@le.ac.uk

Dr Ruth Hartley

Information Sheet for Healthy Adults

Version 3, dated 07 Nov 2012

Ref: 04/Q2501/114
Telephone: 0116 259 2589
Email: rah45@le.ac.uk

Dr Sherif Gonem
Telephone: 0116 2582842
Email: sg330@le.ac.uk

THANK YOU FOR READING THIS. YOU WILL BE ABLE TO KEEP A COPY OF THIS
INFORMATION SHEET AND A SIGNED CONSENT FORM.

Dr Erol Gaillard
Children's Hospital, Leicester Royal Infirmary.
CONSENT FORM for Healthy Adults (Age more than 22 years)

Study Number: UHL 09580

Name of the adult:

Title of Project: Measuring lung development using 'Helium magnetic resonance

Principal Investigator: Dr Erol Gaillard

Please initial box

1. I confirm that I have read and understand the information sheet dated 07-Nov-2012 Version 3 for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, NHS Trust or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to access my records.

Consent Form for Healthy Adults

Version 3, 07-Nov-2012

Ref: 04/Q2501/114

Last saved:
4. I understand that I will be asked to complete a personal questionnaire as a part of the study. I understand that the answers are confidential.

5. I understand that my results and data may be used in other ethically approved research and by academic partners or industrial collaborators. In all instances my data will be anonymised.

6. I agree to take part in the above study.

_________________________  ____________  ____________
Name of Adult                  Date                  Signature

_________________________  ____________  ____________
Name of Person taking consent Date                  Signature
(if different from researcher)

_________________________  ____________  ____________
Researcher                  Date                  Signature

1 for participant; 1 for researcher

Consent Form for Healthy Adults

Version 3, 07-Nov-2012

Ref: 04/Q2501/11d

Last saved:
Appendix F: Results of Chapter 4

Power Calculation for Study 1:

![Power Calculation Output]

- **Critical t**: 1.99006
- **Noncentrality parameter δ**: 2.8477859
- **Critical t**: 1.9900634
- **Df**: 80
- **Total sample size**: 82
- **Actual power**: 0.8033045
Power Calculation for Study 2:
Table 1: Results of multivariate regression of subject characteristics against $S_{acin}$ in children with CF

<table>
<thead>
<tr>
<th>$S_{acin}$</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.249</td>
<td>0.383</td>
<td>0.520</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.022</td>
<td>0.015</td>
<td>0.156</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.002</td>
<td>0.004</td>
<td>0.673</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.003</td>
<td>0.004</td>
<td>0.533</td>
</tr>
<tr>
<td>Sex</td>
<td>0.086</td>
<td>0.039</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. All accounting for all variables, only sex make a significant contribution to the model fit. For the model as a whole, the RSD is 0.11, and the $r^2$ is 0.385, indicating that 38.5% of the variability of $S_{acin}$ in children with CF is explained by this regression model.

Table 2: Results of multivariate regression of subject characteristics against $S_{cond}$ in children with CF

<table>
<thead>
<tr>
<th>$S_{cond}$</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-0.172</td>
<td>0.074</td>
<td>0.027</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.002</td>
<td>0.003</td>
<td>0.616</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.002</td>
<td>0.001</td>
<td>0.025</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.001</td>
<td>0.001</td>
<td>0.115</td>
</tr>
<tr>
<td>Sex</td>
<td>0.003</td>
<td>0.008</td>
<td>0.714</td>
</tr>
</tbody>
</table>

Legend: Sex is coded as zero for female, and one for male. After accounting for all variables, height makes a significant contribution to the model fit. For the model as a whole, the RSD is 0.021, and the $r^2$ is 0.499, indicating that 49.9% of the variability of $S_{cond}$ in children with CF is explained by this regression model.
Appendix G: Results of Chapter 5

Regression equation for $F_{RCN_2}$

$F_{RCN_2}$ z-scores were calculated from data obtained from 90 healthy school-age children. These scores were based upon regression of $F_{RCN_2}$ against height. Addition of subject sex, age or weight to the model had no significant effect upon model fit (Table 7.2).

Table 1: Regression equation for $F_{RCN_2}$

<table>
<thead>
<tr>
<th></th>
<th>Constant</th>
<th>Coefficient (B)</th>
<th>SE (B)</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{RCN_2}$ (L)</td>
<td>-4.035</td>
<td>Ht. 0.037</td>
<td>0.003</td>
<td>0.394</td>
</tr>
</tbody>
</table>

$SE (B) = \text{standard error of the coefficient.}$

$RSD= \text{residual standard deviation for the regression.}$

This table allows calculation of z-scores for $F_{RCN_2}$ as follows:

Predicted value for parameter can be obtained from the equation

$Predicted\ value = \text{Constant} + (\text{height (cm)}*B)$

Standard deviation score (z-scores) can be calculated from the equation

$Z\text{-score} = (\text{observed value} - \text{Predicted value})/\text{RSD}$
Regression analysis for FRCN2:

Regression

Variables Entered/Removed:

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>height</td>
<td></td>
<td>Enter</td>
</tr>
</tbody>
</table>

a. Dependent Variable: FRCN2
b. All requested variables entered.

Model Summary:

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.42</td>
<td>0.181</td>
<td>0.181</td>
<td>0.3820</td>
</tr>
</tbody>
</table>

a. Predictors (Constant), height
b. Dependent Variable: FRCN2

ANOVA:

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>1</td>
<td>33.598</td>
<td>233.002</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>88</td>
<td>.157</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>99</td>
<td>47.419</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Dependent Variable: FRCN2
b. Predictors (Constant), height

Coefficients:

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std Error</td>
<td>Beta</td>
</tr>
<tr>
<td>1</td>
<td>Constant</td>
<td>-4.635</td>
<td>.402</td>
</tr>
<tr>
<td></td>
<td>height</td>
<td>0.257</td>
<td>.034</td>
</tr>
</tbody>
</table>

a. Dependent Variable: FRCN2
Residuals Statistics

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted Value</td>
<td>.54215</td>
<td>3.06226</td>
<td>1.61150</td>
<td>.614419</td>
<td>90</td>
</tr>
<tr>
<td>Std. Predicted Value</td>
<td>-2.066</td>
<td>2.036</td>
<td>.000</td>
<td>1.000</td>
<td>90</td>
</tr>
<tr>
<td>Standard Error of</td>
<td>.042</td>
<td>.096</td>
<td>.057</td>
<td>.015</td>
<td>90</td>
</tr>
<tr>
<td>Predicted Value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted Predicted</td>
<td>.53651</td>
<td>3.06681</td>
<td>1.60981</td>
<td>.614981</td>
<td>90</td>
</tr>
<tr>
<td>Residual</td>
<td>.991293</td>
<td>.957291</td>
<td>.000</td>
<td>.394072</td>
<td>90</td>
</tr>
<tr>
<td>Std. Residual</td>
<td>-2.476</td>
<td>2.416</td>
<td>.000</td>
<td>.994</td>
<td>90</td>
</tr>
<tr>
<td>Stud. Residual</td>
<td>-2.496</td>
<td>2.450</td>
<td>.002</td>
<td>1.004</td>
<td>90</td>
</tr>
<tr>
<td>Deleted Residual</td>
<td>-997019</td>
<td>.984510</td>
<td>.00166</td>
<td>.401775</td>
<td>90</td>
</tr>
<tr>
<td>Stud. Deleted Residual</td>
<td>-2.574</td>
<td>2.524</td>
<td>.002</td>
<td>1.015</td>
<td>90</td>
</tr>
<tr>
<td>Mahal. Distance</td>
<td>.000</td>
<td>4.268</td>
<td>.989</td>
<td>1.142</td>
<td>90</td>
</tr>
<tr>
<td>Cook’s Distance</td>
<td>.000</td>
<td>.687</td>
<td>.010</td>
<td>.015</td>
<td>90</td>
</tr>
<tr>
<td>Centered Leverage Value</td>
<td>.000</td>
<td>.460</td>
<td>.011</td>
<td>.013</td>
<td>90</td>
</tr>
</tbody>
</table>

a. Dependent Variable: FRCN2

Charts

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: FRCN2
Comparison of healthy children results obtained in the current study with those previously obtained in our laboratory:

The reason for comparing between our healthy controls data and the previously collected data in our laboratory as part of different study (Narayanan, Owers-Bradley et al. 2012) was because the two sets of data have been collected using different apparatus dead space and this may impact on VI indices obtained. The pre-capillary dead space we used with our N₂MBW set-up was larger than that used by the previous study because we have added bacterial filer with volume of 50ml for hygienic proposes. Table 7.2 and Figure 7.1 present comparisons of results obtained in healthy children in the current study with those previously collected in our laboratory.

Table 2 Comparison of healthy children results obtained in the current study with those previously obtained in our laboratory

<table>
<thead>
<tr>
<th></th>
<th>Healthy control data obtained during the current study (n=25)</th>
<th>Healthy control obtained as part of previous study (Narayanan, Owers-Bradley et al. 2012) (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M:F</td>
<td>9:16</td>
<td>32:34</td>
</tr>
<tr>
<td>Age(years)</td>
<td>14.9 (10.1,16.9)</td>
<td>12.7 (7.8, 17.1)*</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>164.9 (134.2, 184.0)</td>
<td>153.3 (122.6, 190.0)*</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>61.7 (27.5, 93.0)</td>
<td>48.8 (22.5, 87.4)*</td>
</tr>
<tr>
<td>LCI</td>
<td>6.5 (5.1, 8.2)</td>
<td>6.4 (5.2, 8.3)</td>
</tr>
<tr>
<td>$S_{\text{acin}}$ (L⁻¹)</td>
<td>0.082 (0.02, 0.23)</td>
<td>0.073 (-0.01, 0.15)</td>
</tr>
<tr>
<td>$S_{\text{cond}}$ (L⁻¹)</td>
<td>0.023 (-0.05, 0.06)</td>
<td>0.03 (0.0, 0.09)</td>
</tr>
</tbody>
</table>

Data presented as mean (ranges). *p-value <0.05.

Control population tested as part of the current study were significantly older, taller and heavier than control population tested previously. There was no significant difference in normal values for LCI, $S_{\text{acin}}$ and $S_{\text{cond}}$ between both set of healthy control data.
Figure 1 Comparison of LCI and phase III slope indices results obtained in the current study with those previously obtained in our laboratory.

Legend: Healthy children measured as part of the current study presented as black circles, and healthy children measured as part of previous study presented as green triangles. Note different scales used on Y-axes.
Regression analysis for LCI:

Regression

Variables Entered/Removed

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Entered</th>
<th>Variables Removed</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>height, weight</td>
<td></td>
<td>Enter</td>
</tr>
</tbody>
</table>

- a. Dependent Variable: LCI
- b. All requested variables entered.

Model Summary

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.584</td>
<td>.347</td>
<td>.332</td>
<td>.603837</td>
</tr>
</tbody>
</table>

- a. Predictors: (Constant), height, weight
- b. Dependent Variable: LCI

ANOVA

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>2</td>
<td>8.269</td>
<td>23.997</td>
<td>.0009</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>87</td>
<td>.269</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49.409</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- a. Dependent Variable: LCI
- b. Predictors: (Constant), height, weight

Coefficients

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>.018</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td>weight</td>
<td>.046</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td>height</td>
<td>.046</td>
<td>.007</td>
</tr>
</tbody>
</table>
**Residuals Statistics**

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted Value</td>
<td>5.29890</td>
<td>7.16984</td>
<td>6.28933</td>
<td>.434251</td>
<td>90</td>
</tr>
<tr>
<td>Std. Predicted Value</td>
<td>-2.281</td>
<td>2.028</td>
<td>.000</td>
<td>1.000</td>
<td>90</td>
</tr>
<tr>
<td>Standard Error of Predicted Value</td>
<td>.065</td>
<td>.203</td>
<td>.106</td>
<td>.031</td>
<td>90</td>
</tr>
<tr>
<td>Adjusted Predicted Value</td>
<td>5.30108</td>
<td>7.21042</td>
<td>6.28901</td>
<td>.434515</td>
<td>90</td>
</tr>
<tr>
<td>Residual</td>
<td>-1.124673</td>
<td>1.762679</td>
<td>.000000</td>
<td>.596025</td>
<td>90</td>
</tr>
<tr>
<td>Std. Residual</td>
<td>-1.866</td>
<td>2.924</td>
<td>.000</td>
<td>.989</td>
<td>90</td>
</tr>
<tr>
<td>Stud. Residual</td>
<td>-1.908</td>
<td>2.951</td>
<td>.000</td>
<td>1.004</td>
<td>90</td>
</tr>
<tr>
<td>Deleted Residual</td>
<td>-1.176117</td>
<td>1.795725</td>
<td>.000321</td>
<td>.614835</td>
<td>90</td>
</tr>
<tr>
<td>Stud. Deleted Residual</td>
<td>-1.938</td>
<td>3.093</td>
<td>.006</td>
<td>1.022</td>
<td>90</td>
</tr>
<tr>
<td>Mahal. Distance</td>
<td>0.050</td>
<td>9.068</td>
<td>1.978</td>
<td>1.782</td>
<td>90</td>
</tr>
<tr>
<td>Cook's Distance</td>
<td>0.000</td>
<td>.087</td>
<td>.011</td>
<td>.018</td>
<td>90</td>
</tr>
<tr>
<td>Centered Leverage Value</td>
<td>0.001</td>
<td>.102</td>
<td>.022</td>
<td>.020</td>
<td>90</td>
</tr>
</tbody>
</table>

a. Dependent Variable: LCI

**Charts**

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: LCI
Appendix H: Personal documents: Honorary Contract, certificates for GCP training and consent for research training

Assessment Certificate
Issued to
Noor Al-Khathlan
For completion of the University Hospitals of Leicester NHS Trust
Good Clinical Practice in Research
Training and Assessment on 28th October 2010
Authorised on behalf of the University Hospitals of Leicester

................................
Joanne Thompson

Valid until 27th October 2012
Certificate of Achievement

This is to certify that

Noor AL-Khathlan (na206@le.ac.uk)

successfully completed

Good Clinical Practice Training for Research (eLearning) (398965)

Date: Friday 14th September, 2012

Valid for: 24 months
Training Certificate

Issued to

Noor Al Khathlan

For completion of the University Hospitals of Leicester NHS Trust

Consent for Research Training for 2 years
25th June 2013

Authorised on behalf of the University Hospitals of Leicester

Julie James
Aldona Kirkham
Anne Moore

Valid until 24th June 2015
CONTRACT OF EMPLOYMENT
between
UNIVERSITY HOSPITALS OF LEICESTER NHS TRUST
and

Surname: Al-Khathlan
Forenames (in full): Noor Ali Rashed

APPOINTMENT OF HONORARY RESEARCH SCIENTIST

1. I am instructed by University Hospitals of Leicester NHS Trust (the Trust) to confirm the offer of a placement as an Honorary Unpaid Doctor in the Childrens Division- Infection, Immunity and Inflammation (Childrens Health) at Leicester Royal Infirmary, from 18th January 2011 until 31st January 2014.

2. The placement is subject to the Terms and Conditions of Service of Hospital Medical and Dental Staff (England and Wales) as amended from time to time. Any reference in those Terms and Conditions to an employing Authority shall be construed as if it were to include a reference to an employing Trust.

3. You are required to be registered with General Medical Council, and hold a licence to practise

4. You are normally covered by the NHS Hospital and Community Health Services indemnity against claims of medical negligence. However, in certain circumstances (especially in services for which you receive a separate fee) you may not be covered by the indemnity. The Health Departments therefore advise that you maintain membership of your medical defence organisation. See also Note 1.

5. The placement does not require you to reside in hospital accommodation; but if you have chosen to do so, a deduction from salary for lodgings will be made, in accordance with the Terms and Conditions of Service. See also Note 2.

6. The Trust accepts no responsibility for damage to or loss of personal property, with the exception of small valuables handed to their officials for safe custody. You are therefore recommended to take out an insurance policy to cover your personal property. Notwithstanding this, The Trust undertakes, so far as is reasonably possible, to ensure that lodgings are maintained in a secure
condition. You should, through the exercise of normal diligence, also seek to maintain the security of your lodgings.

7. Should you have any grievance relating to your attachment you are entitled to discuss the matter in the first instance with the Consultant(s) to whom you are responsible, and where appropriate to consult, either personally or in writing, with Human Resources/Personnel – Medical Staffing Department. The Trust has agreed Grievance and Disputes Procedure, which provides for the consideration of individual and collective grievances through successively higher levels of management. Copies of the full procedure are available for inspection within the Medical Staffing Department.

8. (a) The Trust expects its staff to observe a high standard of personal and professional conduct and to adhere to Human Resources/Personnel policies and procedures. The Trust’s Disciplinary / Performance and Conduct Procedure forms part of your contractual terms of attachment. It is your responsibility to familiarise yourself with this procedure, a copy of which may be obtained from your Clinical Chairman/Director, Specialty Manager, Staff Representative or HR/Personnel Department. Your attention is particularly drawn to the Disciplinary Rules which give examples of serious offences which are likely to result in summary dismissal. Rights of appeal are as stated in the above procedure.

(b) Issues relating strictly to your personal conduct will be dealt with under the above procedure.

(c) Matters of professional conduct or competence will be dealt with under a separate procedure (Medical Staff Disciplinary and Review Procedure for Matters of Professional Conduct and Competence) in accordance with the principles of HC(80)9, HC(82)13 and HM(58)17.

9. If you agree to accept the appointment on the terms specified above, please sign the form of acceptance on the following page and return it to the Medical Staffing Department. A second signed copy of this is attached, which you should also sign, and retain for your future reference.

Nita Patel
HR Administrator

For K Harris
MEDICAL DIRECTOR
University Hospitals of Leicester NHS Trust
References


BALL, I., 2011. Functional pulmonary MRI using hyperpolarised \( ^3 \)He, University of Nottingham.


THE UK CYSTIC FIBROSIS TRUST MICROBIOLOGY LABORATORY STANDARDS WORKING GROUP, 2010. *Laboratory Standards for Processing Microbiological Samples from People with Cystic Fibrosis.*


348


