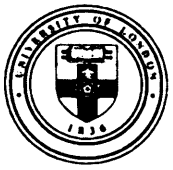

REFERENCE ONLY

UNIVERSITY OF LONDON THESIS

Degree PhD Year 2005 Name of Author HOOI A-F.

Application and interpretation of forced expiratory manoeuvres in infancy

A thesis submitted by

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For the degree of Doctor of Philosophy

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Abstract

This thesis comprised a series of population-based studies, based on two separate cohorts of healthy preterm and term infants, undertaken to examine factors determining respiratory function during infancy, together with the development of sex-specific reference data for maximal expiratory flow at functional residual capacity (V'_{maxFRC}).

108 preterm infants without respiratory disease were recruited to investigate whether, after adjustment for body size, respiratory function in the neonatal period is reduced in those exposed to maternal smoking prenatally. At ~36 weeks post-menstrual age, and prior to any postnatal smoke exposure, breathing patterns were altered and V'_{maxFRC} reduced in those whose mothers smoked antenatally, in white compared with black infants and in boys compared with girls. Repeat measures of V'_{maxFRC} in a subset of this preterm cohort (n=24) showed that, despite similar size-corrected values shortly after birth, at 1 year corrected postnatal age V'_{maxFRC} was significantly lower (-2 Z score) than in term infants, with marked 'tracking' of lung function between test occasions, suggesting that preterm delivery *per se* may adversely affect subsequent airway development.

Using the raised volume technique, airway function was assessed in 234 term infants (98 with low birth weight for gestational age [SGA]). At ~6 weeks postnatal age, after adjusting for relevant covariates including body size, airway function was significantly lower among SGA infants, in boys compared with girls and in those exposed to maternal smoking antenatally. Follow-up measurements at ~8-9 months in 80 infants (32 SGA) of non-smoking mothers demonstrated considerable 'tracking' of airway function, with forced expired volume in 0.4 s ($FEV_{0.4}$) being ~9% lower in SGA infants throughout the first year (95% CI: 2% to 16%). These results suggest that adverse prenatal events may constrain lung development with potential long-term effects of respiratory health. In summary, maternal smoking during pregnancy, sex, preterm delivery and being born SGA may exert independent effects on airway function during infancy.

Contents

Table of Contents

List of Tables

List of Figures

List of Appendices

Note: With the exception of Appendix C, of which a hard copy is provided, all appendices are stored in electronic format in the CD at the back of this thesis.

List of Abbreviations, Symbols and Conversion factors

Abbreviations/ Symbols	Description	Unit
AGA	appropriate birth weight for gestational age	
ASCII	American Standard Code for Information Interchange	
ATPS	ambient temperature, pressure, and saturated with water vapour	
ATS	American Thoracic Society	
bpm	breaths per minute	
AR	airway responsiveness	
BPD	bronchopulmonary dysplasis	
BR	bronchial responsiveness	
BTPS	body temperature, pressure and saturated with water vapour	
°C	degree in Celsius	
cf	Compared with	
CI	confidence interval	
CLDI	chronic lung disease of infancy	
cm	centimetre	
cmH_2O	centimetre of water	
COPD	chronic obstructive pulmonary disease	
CPAP	constant positive airway pressure	
C_{rs}	compliance of the respiratory system	$mL \cdot kPa^{-1}$
CV	coefficient of variance ([SD/mean] x 100)	%
EEL	end-expiratory level	
EEV	elastic equilibrium volume	mL
ERS	European Respiratory Society	
ERV	expiratory reserve volume	mL
FD	forced deflation	

Abbreviations/ Symbols	Description	Unit
FE	forced expiratory; forced expiration	
FEF	forced expiratory flow	$mL \cdot s^{-1}$
FEFV	forced expiratory flow-volume	
FEV	forced expiratory volume	mL
FEV_t	forced expiratory volume at time t	mL
FOT	forced oscillation technique	
FVC	forced vital capacity	mL
FRC	functional residual capacity	mL
FRC_{pleth}	FRC assessed by plethysmographic technique	mL
FRC_{He}	FRC assessed by helium dilution technique	mL
GA	gestational age	weeks
HBIR	Hering-Breuer inflation reflex	%
HIS	histamine	
HMD	Hyaline membrane disease	
Hz	Hertz	
IUGR	Intrauterine growth restriction	
kPa	kilopascal	
Kg	kilogram	
L	litre	
LBW	low birth weight	
LF	lung function	
LRI	lower respiratory illness	
M	month	
min	minute	
MCh	methacholine	
MEF	maximal expiratory flow	$mL \cdot s^{-1}$

Abbreviations/ Symbols	Description	Unit
MEF_{x}	maximal expiratory flow when x% of forced vital capacity remains in the lungs	$mL \cdot s^{-1}$
MEFV	maximal expiratory flow-volume (curve)	
mL	milli-litre	
MLR	multiple linear regression (multivariate) analysis	
MLM	multi-level modelling	
MOT	multiple occlusion technique	
N_2	nitrogen	
occ	occlusion	
P	pressure	kPa; cmH_2O
P_{ao}	pressure at the airway opening (i.e., mask, mouth and/or nose)	kPa; cmH_2O
ΔP_{ao}	changes in pressure at the airway opening	kPa; cmH_2O
PC_n	provocation concentration of an inhalational challenge agent causing n% fall in forced flow	
PMA	post-menstrual age (= sum of GA and PNA)	weeks
P_{def}	deflation pressure	kPa; cmH_2O
PEFV	partial forced expiratory flow-volume (curve)	
PEF	peak expiratory flow	$mL \cdot s^{-1}$
P_{el}	elastic recoil pressure	kPa; cmH_2O
P_{inf}	airway inflation pressure	KPa; cmH_2O
P_j	jacket pressure	kPa
pleth	plethysmographic	
P_{pl}	pleural pressure	kPa; cmH_2O
PNA	postnatal age	weeks; months
PNT	pneumotachometer	
P_t	(jacket) transmission pressure	kPa; cmH_2O
P_{tp}	transpulmonary pressure	kPa; cmH_2O

Abbreviations/ Symbols	Description	Unit
P-V	Pressure-volume (curve)	
r^2	coefficient of determination	
RASP	Respiratory Analysis Program	
R_{aw}	resistance of the airways	$kPa \cdot L^{-1} \cdot s$
RDS	respiratory distress syndrome	
ref	reference	
REM	rapid eye movement	
RFT	respiratory function test	
RIP	respiratory inductive plethysmograph	
RR	respiratory rate	min^{-1}
R_{rs}	resistance of the respiratory system	$kPa \cdot L^{-1} \cdot s$
RSD	residual standard deviation	
RSV	respiratory syncytial virus	
RTC	rapid thoraco-abdominal compression	
RV	residual volume	mL
RVRTC	raised volume RTC (technique)	
s	second	
SBT	single (breath) occlusion technique	
SD	standard deviation	
SDS	standard deviation (or Z) score	
SGA	small for gestational age	
SpO_2	pulsatile oxygen saturation	%
sG_{aw}	specific airway conductance	$s^{-1} \cdot kPa$
sR_{aw}	specific airway resistance	$kPa \cdot s^{-1}$
τ	tau; time constant	s
t	time	s

Abbreviations/ Symbols	Description	Unit
TC	threshold concentration of an inhalational challenge agent causing a x% fall in forced flow	
t_E	expiratory time	s
t_{FE}	duration of forced expiration	s
TGV	thoracic gas volume	mL
t_I	inspiratory time	s
TLC	total lung capacity	mL
t_{PTEF}	time to peak tidal expiratory flow	s
URTI	upper respiratory tract illness	
V	Volume	mL
V_{inf}	Inflation volume	mL
V'	flow	$mL \cdot s^{-1}$
V'_{maxFRC}	maximal expiratory flow at functional residual capacity	$mL \cdot s^{-1}$
V_T	tidal volume	mL
w	week	
y	year	
Z score	SD score	

Conversion factors	
Pressure	1 cmH_2O = 0.098 kPa
Pressure	1 mmHg = 0.133 kPa
Resistance	1 $cmH_2O \cdot L^{-1} \cdot s$ = 0.098 $kPa \cdot L^{-1} \cdot s$

Glossary of terms used in this thesis

Term	Definition
Airway conductance	Reciprocal of airway resistance ($L \cdot s^{-1} \cdot kPa^{-1}$)
Appropriate for gestational age (AGA) infant	Infant whose birth weight lies between the 20th and 95th centile for gestational age
Coefficient of variation (CV)	(Standard deviation/mean) x 100
Compliance (C)	A measure of distensibility, i.e., change in volume per unit change in pressure: C = volume/pressure ($mL \cdot kPa^{-1}$)
Elastance (E)	A measure of lung resistance to distension, i.e. a reciprocal of compliance: E = 1/ C ($kPa \cdot mL^{-1}$)
Heteroscedasticity	This is caused by non-normality of one of the variables, an indirect relationship between variables, or to the effect of a data transformation. Heteroscedasticity can be dealt with either by undertaking separate analyses for subgroups of subjects with similar typical errors (e.g., males and females), or by transforming the variable.
Homoscedasticity	The assumption that the variability in scores for one variable is roughly the same at all values of the other variable, which is related to normality. Homoscedasticity is detected with scatterplots and is rectified through transformation.
Jacket pressure transmission	Percent change in pressure at the airway opening (P_{ao})/jacket pressure (P_j) during a brief airway occlusion
Odds	The chance or balance of probability in favour of, or against, some outcome i.e., (the number of individuals with the outcome)/(number of individuals without the outcome)
Odds ratio (OR)	The ratio of the odds of having the target outcome (e.g., asthma [a]) in the index group (e.g., smokers [b]) relative to the odds in favour of, or against, having the target outcome [c] in the control group (e.g., non-smokers [d]) OR = (a/[b-a]) ÷(c/[d-c])
p-value	A measure of the strength of evidence against the null hypothesis
phenotype	the physical and biochemical characteristics of an organism (or specific individual) as determined by the interaction of its genetic constitution and the environment

Term	Definition
Relative risk (RR)	RR is a ratio of risk probabilities, i.e., = no. of individual with outcome (e.g., asthma) [a]/total no. of individuals in this group (e.g., smokers)[a+b]/no. of individuals with the outcome (c)/no. of cases in a 2nd group (e.g., non-smokers) [c+d]; i.e., RR between groups = (a/[a+b])/(c/[c+d])
Resistance (R)	A measure of pressure required to move gas/gases at a flow of one litre per second: R = pressure/flow ($kPa \cdot L^{-1} \cdot s$)
Specific airway conductance	Airway conductance/Functional residual capacity ($s^{-1} \cdot kPa^{-1}$)
Type I random error	Falsely concluding that the null hypothesis is false when in fact it is true. The p-value gives a measure of how likely this is to have occurred
Type II random error	Falsely concluding that the null hypothesis is true when in fact it is false. This type of error commonly occurs when the study is under-powered due to insufficient sample size

Peer reviewed publications associated with the studies presented in this thesis

a) Original papers

Stocks J, Henschen M, **Hoo AF**, Costeloe K, Dezateux C. [1997]

Influence of ethnicity and gender on airway function in preterm infants. Am J Respir Crit Care Med;156:1855-1862.

Hoo AF, Henschen M, Dezateux C, Costeloe K, Stocks J. [1998]

Respiratory function among preterm infants whose mothers smoked during pregnancy. Am J Respir Crit Care Med;158:700-705.

Lum S, **Hoo AF**, Dezateux C, Goetz I, Wade A, DeRooy L, Costeloe K, Stocks J. [2001]

The association between birthweight, sex, and airway function in infants of nonsmoking mothers. Am J Respir Crit Care Med;164:2078-2084

Hoo AF, Dezateux C, Hanrahan JP, Cole TJ, Tepper RS, Stocks J. [2002]

Sex-specific prediction equations for V'_{maxFRC} in infancy: a multicenter collaborative study. Am J Respir Crit Care Med;165:1084-1092.

Hoo AF, Dezateux C, Henschen M, Costeloe K, Stocks J. [2002]

Development of airway function in infancy after preterm delivery. J Pediatr;141:652-658.

Dezateux C, Lum S, **Hoo AF**, Hawdon J, Costeloe K, Stocks J. [2004

Low birth weight for gestation and airway function in infancy: exploring the fetal origins hypothesis. Thorax;59:60-66.

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Development of lung function in early life: influence of birth weight in infants of nonsmokers. Am J Respir Crit Care Med;170:527-533.

b) Methodological papers

Henschen M, Stocks J, **Hoo AF**, Dixon P. [1998]

Analysis of forced expiratory manoeuvers from raised lung volumes in preterm infants. J Appl Physiol;85:1989-1997.

Hoo AF, Lum SY, Goetz I, Dezateux C, Stocks J. [2001]

Influence of jacket placement on respiratory compliance during raised lung volume measurements in infants. Pediatr Pulmonol;31:51-58.

Ranganathan SC, **Hoo AF**, Lum SY, Goetz I, Castle RA, Stocks J. [2002]

Exploring the relationship between forced maximal flow at functional residual capacity and parameters of forced expiration from raised lung volume in healthy infants. Pediatr Pulmonol;33:419-428.

Lum S, **Hoo AF**, Stocks J. [2002]

Effect of airway inflation pressure on forced expiratory maneuvers from raised lung volume in infants. Pediatr Pulmonol;33:130-134.

Lum S, **Hoo AF**, Stocks J. [2002]

Influence of jacket tightness and pressure on raised lung volume forced expiratory maneuvers in infants. Pediatr Pulmonol;34:361-368.

Lum S, Hulskamp G, **Hoo AF**, Ljungberg H, Stocks J. [2004]

Effect of the raised lung volume technique on subsequent measures of V'_{maxFRC} in infants. Pediatr Pulmonol;38:146-154.

Note: All publications are available as PDFs in the CD at the back of this thesis.

Acknowledgements

At the Institute of Child Health, a joint programme of work, led by Professors Janet Stocks and Carol Dezateux, has been established over the last decade between the Portex Respiratory Unit and the Centre for Paediatric Epidemiology and Biostatistics, respectively, to examine the physiological and epidemiological aspects of respiratory function and disease in early life. I was privileged to have been involved in much of this research, which form the basis of this thesis. I thank Professors Stocks and Dezateux for their excellent supervision and guidance, their wisdom and scrutiny throughout the duration of the research.

Quite simply, it would be impossible to complete the studies without the countless unrecorded acts of support and assistance generously given to me by various colleagues over the years. I have enjoyed working with, and getting to know, many of them. Their cooperation, tolerance and good-humoured willingness to assist and labour on different aspects of the research studies have contributed considerably to the successful completion of various projects. I wish to record my grateful thanks, in particular, to Matthias Henschen, Isobel Dundas, Liane Pilgrim, Sooky Lum, Iris Goetz, Sarath Ranganathan, Sarah Davies, Anne Cantarella and Georg Hülskamp.

Half way through the writing of this thesis, a colleague and friend, Rosie Castle, passed away. Rosie single-handedly developed and maintained a complex relational Access database – without which it would have been next to impossible to store and manage the enormous amount of research data electronically. I am grateful for her assistance and friendship.

Immense thanks are also due to Dr Angie Wade and Professor Tim Cole, at the Centre for Paediatric Epidemiology and Biostatistics, for their invaluable statistical advice and collaboration on data analyses and interpretation. I should like to thank Dr Hui-qi Pan for developing the online Excel Add-in macros for the calculation of prediction values for maximal flow at functional residual capacity (V'_{maxFRC}) during infancy (Chapter 3).

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Declaration

The tasks of data collection and analysis for the research studies reported in this thesis had been undertaken in accordance with standardised study protocols; data management and interpretation were undertaken together with Professors Janet Stocks and Carol Dezateux. Professor Tim Cole and Dr Angie Wade (senior statisticians) were involved at all stages of these projects, including study design, data analysis and interpretation, through to the reporting of results. Dr Wade provided power calculations for the studies described in sections 3.3, 4.2 and 4.3.

- For the collaborative study described in section 3.2 – Professor Robert Tepper (Indianapolis, USA) and Dr John Hanrahan (Boston, USA) contributed their respective published dataset on maximal flows at functional residual capacity (V'_{maxFRC}). Professor Tim Cole (TC) provided statistical expertise and analysed the collated dataset, including data from our department, and developed sex-specific V'_{maxFRC} prediction equations.
- For the studies reported in sections 3.3 and 3.4 – I undertook all the recruitment and data analyses, with assistance provided by Matthias Henschen and Liane Pilgrim during data collection. Analysis of the follow-up data (section 3.4) was undertaken using V'_{maxFRC} prediction equations modified by Professor Cole, which excluded preterm infants from the collated reference population.
- For the studies presented in sections 4.2 and 4.3 – recruitment, data collection and analyses were undertaken jointly with Sooky Lum. Additional assistance for recruitment and data collection were provided by Sarah Davies, Iris Goetz, Anne Cantarella and Georg Hülskamp.

  Dr Angie Wade (AW) provided statistical advice on the analysis of cross-sectional data (section 4.2) and performed analyses of the follow-up data using multi-level modelling (section 4.3).

1. Introduction and literature review

1.1. OVERVIEW

Respiratory diseases are a major cause of morbidity and mortality during childhood, particularly during the first year of life. Wheezing in young children is extremely common in the industrialised world, particularly during viral infections. Assessment of respiratory function is an integral part of the diagnostic and clinical management of older children and adults with respiratory disease, but such tests are more difficult to apply during infancy and early childhood, as will be discussed below.

Assessment of respiratory function in infants and young children has significantly contributed to our understanding of respiratory physiology and normal development, not just during childhood but throughout life. This is particularly relevant since evidence from several epidemiological studies using respiratory function measurements have linked sub-optimal respiratory function shortly after birth with subsequent development of respiratory disorders [1-6]. Thus, knowledge of developmental physiology and growth of the respiratory system at birth and early infancy is an important component of the clinical interpretation, diagnosis and management of disease in infants and young children. To improve future treatment for airway diseases such as asthma, cystic fibrosis (CF) and chronic lung disease of infancy (CLDI), and to minimise the associated rate of morbidity and mortality, it is crucial that the body of knowledge regarding the physiological and pathological processes involved in these diseases is strengthened. Furthermore, an understanding of developmental anatomy and physiology is essential for clinical measurement and interpretation of respiratory function tests (RFTs) in infants and young children.

In the following sections, the burden of respiratory disease, the growth and development of the respiratory system and factors influencing respiratory function during infancy are outlined. The role and history of respiratory function measurements are briefly reviewed, leading to the development of tests for use in infancy. The differences in respiratory physiology between infants and older subjects are described. The application of RFTs in infancy and the theory of the most commonly used techniques to assess airway function (namely, those based on forced expiratory manoeuvres) are discussed in this chapter, together with a summary of published literature on studies using such techniques. This chapter concludes by summarising the aims and objectives of this thesis.

1.2. THE BURDEN OF RESPIRATORY DISEASE

In Europe, as well as the rest of the world, lung diseases are among the leading causes of mortality and morbidity. Recent publications reported the disturbing fact that currently in the UK, respiratory diseases have superseded cardiovascular disorders in becoming the leading killer [7;8]. The UK death rates due to respiratory diseases are among the highest in Europe, being nearly twice the European Union (EU) average. In 2000, respiratory disease cost the National Health Service (NHS) £2,576 million, more than any other disease area [7].

In Western, Central and Eastern Europe, pneumonia, chronic obstructive pulmonary disease (COPD) and lung cancer are the main respiratory causes of death in adults [8]. It is estimated that in 2020, of the 68.3 million deaths worldwide, 11.9 million will be caused by lung disease, with COPD becoming the major respiratory cause of death (Table 1.1). Globally, paediatric respiratory disease is a major cause of morbidity in children accounting for a large proportion of childhood hospitalisations, healthcare consultations and absenteeism from day care centres or schools [9-11]. In developed countries, although the number of deaths from respiratory illness is relatively low (~8% of all childhood deaths in England and Wales), nevertheless the burden is high particularly in children under five years of age, due to bronchiolitis, wheezing illnesses and CLDI [7;12]. The detrimental effects of smoking on lung health are well recognised [8;13]. However, lung problems are not just smoking-related; there is a wide variety of other causes ranging from genetic influences to nutritional, environmental and poverty-related factors. Since the associations between respiratory health status and these factors are potentially complex, different respiratory diseases require different approaches and treatments. During the last decade, there has been increasing recognition that altered lung growth and development *in utero* due to sub-optimal intrauterine conditions and/or lung injury associated with inflammation and infections during the first years of life may have important implications for long-term lung health [14-16]. Since lung growth and development occur most rapidly during the first few years of life, improvement of the knowledge base regarding early growth and development of the respiratory system is essential to the understanding of the effects of lung disease and how to prevent and minimise subsequent lung damage.

Table 1.1. Leading causes of death worldwide in 1990 and the prediction for 2020

Causes of death	1990 †	2020 †
Lung disease (all causes)	9.4 (18.7)	11.9 (17.4)
Pneumonia	4.3 (8.5)	2.5 (3.6)
COPD	2.2 (4.3)	4.7 (6.8)
Tuberculosis	2.0 (3.9)	2.4 (3.5)
Lung cancer	0.95 (1.8)	2.3 (3.3)
Ischaemic heart disease	6.3 (12.4)	11.1 (16.2)
Cerebrovascular disease	4.4 (8.7)	7.7 (11.3)
All causes of death	50.5 (100)	68.3 (100)

Data in millions and % in parentheses [8].

COPD: chronic obstructive pulmonary disease.

1.2.1. The burden of respiratory diseases in children

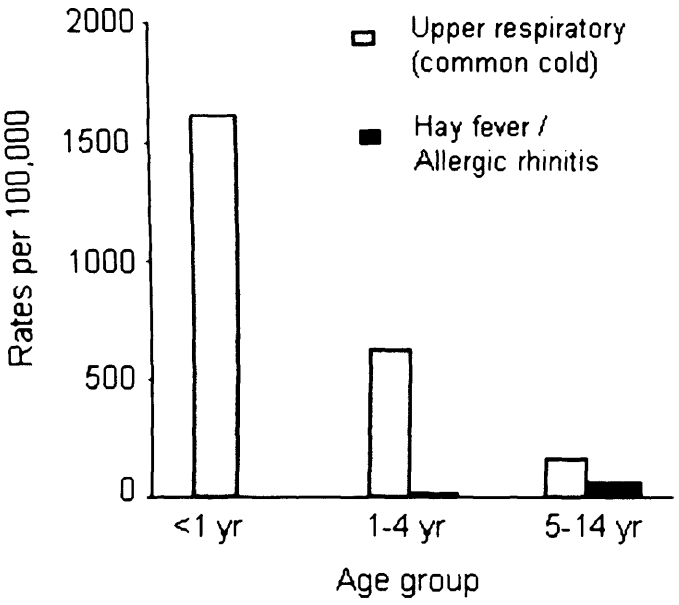
1.2.1.1. Incidence and prevalence of respiratory diseases

Despite advances in the understanding of the epidemiology of respiratory disorders in infants and young children, acute respiratory infections remain the commonest illnesses of childhood. The most frequently reported long-term illnesses in British children are conditions of the respiratory system, affecting 138 per 1000 in the 2-10 year old group [12]. In Britain, respiratory illnesses (both acute and chronic) account for 20% of weekly consultations between general practitioners (GPs) and children aged 0-14 years, with another 2% being for symptoms and signs involving respiratory disease [12;17]. Across this age range, the highest number of consultations is for upper respiratory tract infections (URTI) (Figure 1.1a). The peak incidence for URTI is at 6-12 months of age, with a second peak at around age 3-5 years, presumably reflecting attendance at day-care centre, nursery or school. Bronchiolitis is the commonest lower respiratory tract illness (LRI) in the under 1 year olds, and acute cough or bronchitis in the 1-4 year olds (Figure 1.1b) [12;17].

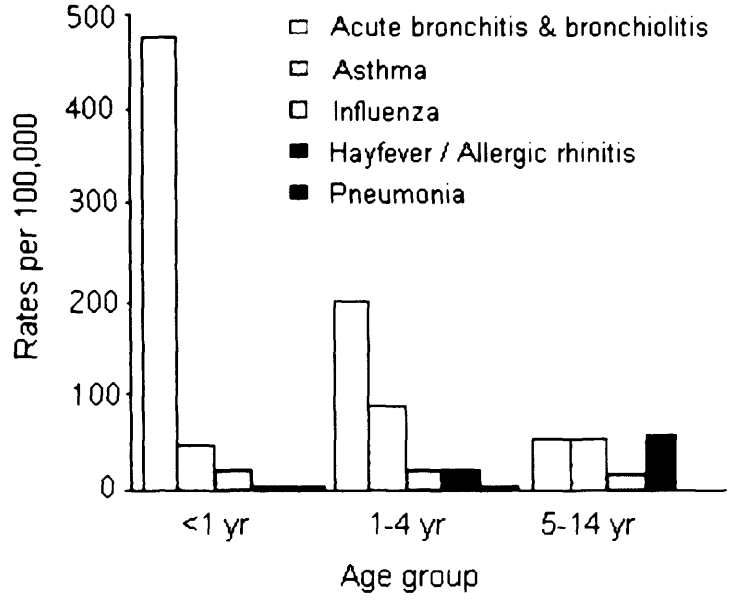
Asthma is the most common chronic disease in children and its prevalence has increased continuously during recent decades, particularly in Western Europe [8]. It has been suggested that this reflects changes in family size and life style [8;18]. The prevalence, causes, natural history and clinical presentation of asthma differ with age.

Figure 1.1. Mean weekly incidence of GP visits for respiratory diseases in children, 1999-2001.

a) Upper respiratory conditions



b) Lower respiratory conditions



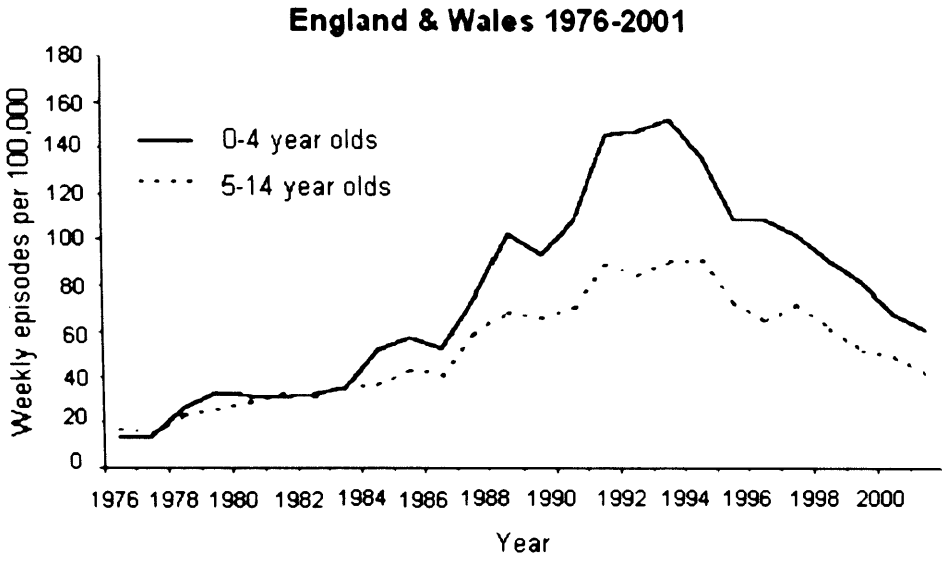
Figures adapted from LAIA [12].

In addition, differences in sex, genetic background and environmental exposure also influence both inter- and intra-individual variability in signs and symptoms of this heterogeneous condition, with wheezing as its major clinical expression [19]. A "wheeze" has been described as a non-specific sign and symptom associated with restriction of airflow through narrowed airways, which is thought to be initiated by turbulent flow causing oscillation of the bronchial walls, resulting in the characteristic high pitch, polyphonic 'whistling' sound [20].

The most recent figures for asthma across Europe, as reported from Phase I of the International Study of Asthma and Allergy in Childhood (ISAAC) and European Community Respiratory Health Survey (ECRHS) studies, suggest that the UK has the highest prevalence of asthma, being 20.7% in 13-14 year olds compared with 1.6% in Albania, and 22.9% among 6-7 year olds versus 1.4% in Estonia [8;21;22]. Currently, 24/30 study centres have completed fieldwork for Phase II of the ISAAC study, which aims to identify determinants of the observed differences in prevalence rates, including the role of atopic sensitisation and bronchial responsiveness, and the influence of indoor exposures and lifestyle factors [23]. In England and Wales, weekly GP consultation rates for acute asthma among both 0-4 year olds and 5-14 year olds have fallen rapidly in recent years (Figure 1.2), possibly reflecting increased use of prophylactic anti-inflammatory therapy and improved treatment for acute asthma [8;24]. In the recently published Aberdeen survey, 3,537 parents (84%) completed questionnaires on behalf of their children (around 9-12 years old) who attended primary schools that had previously participated in studies in 1964, 1989 and 1994. Findings with respect to respiratory symptoms suggested that the prevalence of childhood asthma appeared static between 1994 and 1999 [25]. However, reports from two further large surveys, conducted as part of ISAAC, in the UK (in 12-14 year old children during 1995-2002) and Melbourne (in 6-7 year olds between 1993-2002), respectively, provided evidence for an overall reduction in the prevalence of symptoms of asthma and/or atopy [26;27].

In most European countries, asthma mortality is low in children and decreases in later years [8]. The high prevalence of this condition nevertheless has a considerable impact on quality of life issues for children and their families, and places an economic burden on the health care services. Longitudinal population–based studies that followed up children from infancy or early childhood into adolescence and adulthood have improved our understanding of asthma [3;5;6;28-30]. Although most wheezing LRI commences during the first three years of life, it may be extremely difficult to distinguish viral-associated wheezing from childhood asthma in the pre-school child [31-33].

Figure 1.2. Mean weekly incidence of asthma GP episodes in children.

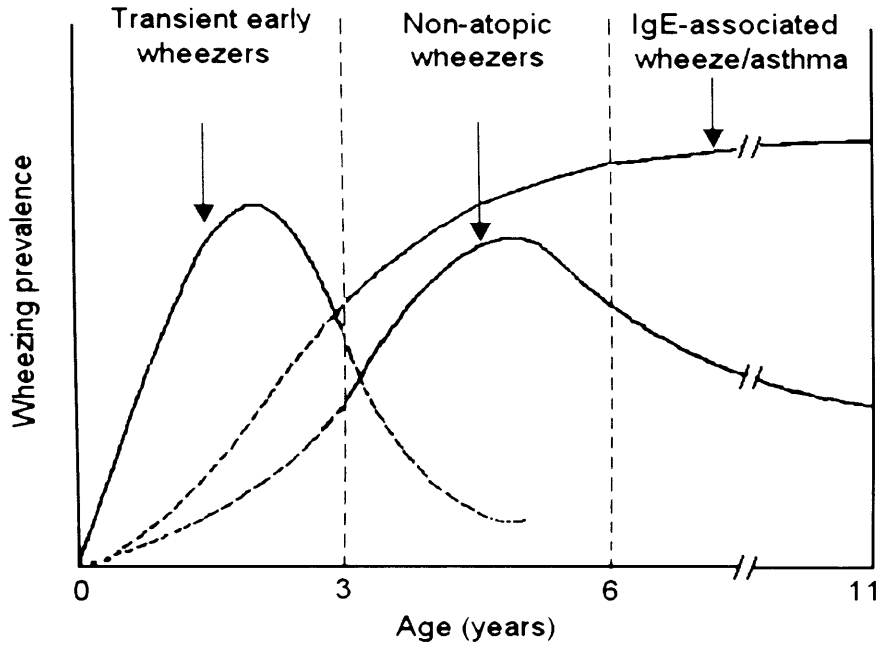


Figures and graph adapted from LAIA [12].

1.2.2. Phenotypes of childhood asthma

By recruiting healthy newborn infants, the prospective on-going longitudinal population-based Tucson Children's Respiratory Study (TCRS) has enabled identification of three phenotypes in children with asthma, namely: transient wheezing (up to 3–5 years of age but not thereafter); non-atopic wheezing of toddlers and children of pre-school age, and immunoglobulin E (IgE)-mediated wheezing [5]. Asthma phenotypes reflect a heterogeneous group of conditions, as determined by both genetic makeup and environmental influences, which is characterised by recurrent airway obstruction [34] and the three phenotypes in children are depicted in Figure 1.3. Although flow-limitation can occur without audible wheeze, expiratory wheezing is always a sign of expiratory flow-limitation [35].

Figure 1.3. Hypothetical yearly peak prevalence of wheezing according to phenotypes in childhood.



Adapted from Stein *et al* [32].

1.2.2.1. Transient early wheezing

In early life, wheezing is generally episodic and occurs during viral infections of the respiratory tract [5]. Children with transient wheezing are more likely to have diminished lung function early in infancy [3]. Prematurity [36], prenatal [1;37;38] and postnatal [39;40] exposure to tobacco smoke, and exposure to siblings or children at daycare centres [41] are all risks factors for transient wheezing. These observations are consistent with reports from Perth [4;42] and London [1;37].

1.2.2.2. Non-atopic wheezing

Recent reports suggested that children who present with intermittent wheezing during the first years of life and without a significant family history of asthma and/or allergies are most likely to remit after 10 years of age [5;32;43;44]. Respiratory syncytial viral (RSV) infections and pneumonia that occurred before three years of age are associated with an increased risk of developing asthma-like symptoms during school years, independently of family history of asthma or atopy [5;45]. However, the risk decreased with age and was no longer significant by 13 years [5], suggesting that RSV associated LRIs were not associated with an increased risk for allergic sensitisation or higher total serum IgE levels [34].

1.2.2.3. Atopic wheezing/asthma

Analysis from the Tucson study suggested that among children who wheezed with LRIs in the first three years of life, approximately 60% continue to wheeze and were atopic at 6 years [3]. Children who were still symptomatic at 6 years of age had experienced more wheezing episodes in early life than those whose symptoms had ceased by three years. They had more symptoms even without apparent viral infection, and were more likely to have atopy and to have mothers with asthma than children with transient wheezing or no wheezing [3]. Among these children, there was a significant association between an early onset of wheezing symptoms and severity of disease and airway responsiveness [5;34]. It has been suggested that early allergic sensitisation increases the prevalence of respiratory symptoms, chronic airway inflammation and the risk of changes in airway physiology and declining respiratory function early in life [3;46-48].

1.2.3. Hospital admissions

Table 1.2 lists childhood hospital admission rates for LRI in England. In 2000/2001, 15% of all children's hospital admissions in England were related to respiratory disease. Almost a third of all lower respiratory admissions in children and 40% in the 1-4 year olds were due to asthma, with croup and pneumonia also being important causes in the under 5 year olds. In contrast to the rising trend for asthma admissions in the 1970s and 1980s, there has been a rapid downward trend in admissions for asthma during the last decade, which parallels the decline in episodes of new asthma in GP surgeries (Figure 1.2) [12]. This may indicate improved treatment since there has been an accompanying increased use of anti-inflammatory drugs [49]. The second commonest cause for admission in all children is acute bronchiolitis, which peaks in winter and affects almost exclusively the very young. The high admission rate for the under 1-year olds for bronchiolitis is shown in Table 1.2.

Neonatal admissions are primarily related to the consequences of prematurity. In England & Wales, many preterm infants have associated respiratory disease. In 2000, for example, respiratory disease affected 4,200 per 100,000 live born infants, of whom approximately one third were due to respiratory distress syndrome of the newborn [12]. Those born very prematurely are at particular risk of developing CLDI or bronchopulmonary dysplasia (BPD) [50-55], which remains a common cause of severe morbidity and mortality. In England and Wales in 2000, there were 2,335 neonatal deaths, with diseases of the respiratory system accounted for one third of these [12]. Beyond the neonatal period, most respiratory illnesses involve the airways rather than the parenchyma.

Table 1.2. Hospital admission rates for lower respiratory diseases in England, April 2000-March 2001

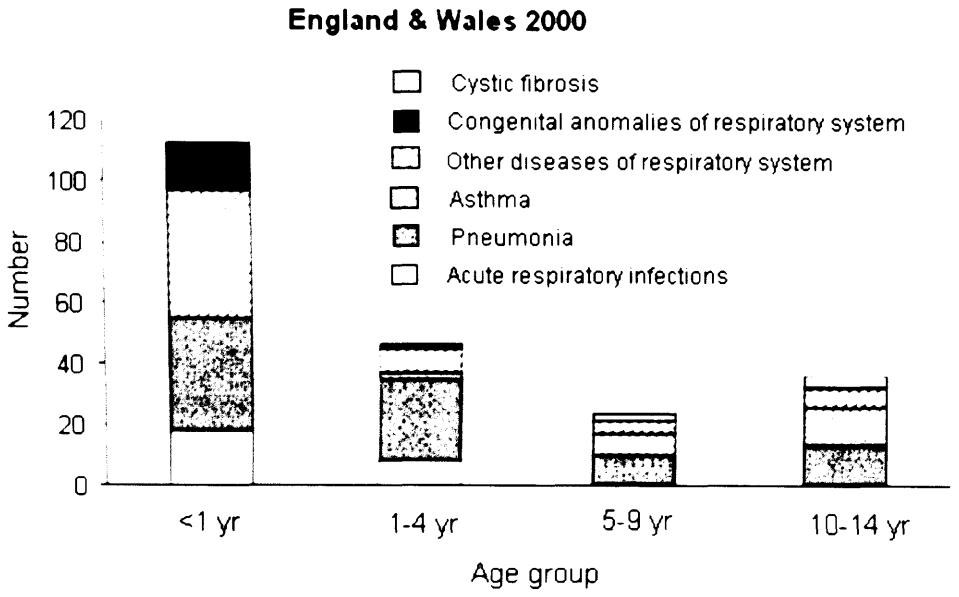
Per 100,000	Age in years				
	<1	1-4	5-9	10-14	All children
Asthma	179	559	211	135	272
Acute bronchiolitis	3,611	73	1	0	240
Pneumonia	373	246	55	24	113
Unspecified acute LRI	402	230	43	23	106
Croup	299	253	33	5	96
Cystic fibrosis	38	29	34	54	40
Acute bronchitis	48	12	2	1	7
Empyema	10	5	3	2	4
Bronchiectasis	3	2	2	2	2
Respiratory tuberculosis	4	2	0	2	2
Interstitial lung disease	3	2	0	0	1
Total	4,967	1414	385	249	882

Figures quoted for age categories are per 100,000 (Adapted from LAIA [12])
Definition of abbreviation: LRI: lower respiratory infections.

1.2.4. Mortality due to respiratory diseases

Deaths due to respiratory conditions in children (excluding neonates) in England & Wales in 2000 are shown in Figure 1.4. Over the age range between 0-14 years, pneumonia was certified as the cause of death four times more frequently than asthma (Figure 1.4). Three quarters of deaths from pneumonia occurred in the under 4 year olds, whereas 13 (60%) of the total of 22 asthma deaths in England and Wales occurred in children aged 10-14 years.

Figure 1.4. The number of deaths in children (excluding neonates) due to respiratory causes.



Data and graph adapted from LAIA [12].

1.2.5. Paediatric origins of adult respiratory disease

Numerous publications are available regarding the developmental origins of adult respiratory disease. An association identified by Barker and colleagues between birth weight and cardiovascular disease has led to the concept of 'programming' - the permanent alteration of the structure and function of organs and tissues by factors operating during sensitive periods in fetal and early postnatal life [56]. This concept has been used to explain associations between impaired fetal growth and risk factors for adult lung function, respiratory morbidity and death from COPD [57] and, more specifically, the association between early lung growth and chronic airflow obstruction [58]. From studies in North Hertfordshire, it has been postulated that early under-nutrition results in impaired development of molecular and cellular repair mechanisms which may affect all tissues but which are most critical in later life [59].

Similar investigations were conducted in two British national cohorts. Data from the 1946 birth cohort study suggested that LRI in the first two years of life was a significant risk factor for adult chronic obstructive pulmonary disease. Birth weight, overcrowded home circumstances at two years of age and a parental history of bronchitis were each independently associated with reduced peak expiratory flows at age 36 years, even after adjusting for smoking, level of education and social circumstance in adult life [60;61].

Incidence and prognosis of asthma and wheezing illness and their relation to perinatal factors were investigated in the 1958 cohort from birth to age 33 years. The incidence of wheezing during childhood was strongly and independently associated with pneumonia, hay fever and eczema, while that in young adults (17 to 33 years) was also strongly associated with a history of hay fever. Those with a history of wheezing illness in childhood were also shown to retain a risk of wheezing in adult life, above that of their healthy peers [62].

Factors implicated in programming are discussed later (section 1.5) and include fetal growth, nutrition, exposure to maternal smoking and family history of asthma. It is likely that outcome is determined by a combination of genetic, environmental and familial factors and that these factors will influence the interpretation and longer-term implications of respiratory function measurements during infancy and early childhood.

1.3. GROWTH AND DEVELOPMENT OF THE RESPIRATORY SYSTEM

Lung development occurs primarily during fetal life, a time when the lung is not required to fulfil its extrauterine function in gas exchange but has to function efficiently immediately after birth. It has been suggested that the pattern of lung growth is genetically determined [63]. However, several intrauterine environmental factors have been recognised as important modulating influences, including fetal breathing movements (with respect to changes in thoracic dimensions) [64;65], quantity of fetal lung liquid, its rate of secretion and absorption [66;67], maternal alcohol [68] and nicotine [69-71] intake, prenatal glucocorticoid administration [72] and infection [73;74]. While drainage of amniotic fluid as a result of premature rupture of membranes may lead to lung hypoplasia [75], restricted intrathoracic space, as in congenital diaphragmatic hernia and oligohydramnios, leads to a reduction in airway number [76;77]. Insulin-like growth factor promotes multiplication of epithelial cells, whilst transforming growth factor β inhibits multiplication but enhances protein synthesis [78]. In addition, data from animal models suggest that maternal malnutrition may inhibit lung growth [79]. Further details of factors influencing early lung growth and development will be presented in section 1.5.

There is increasing recognition that the level of airway function may be established during fetal development and the first year of life, with little subsequent improvement through the normal repair process [3;80;81]. Adverse events during fetal development and infancy may therefore have long-term and irreversible effects throughout life [82-84]. Since airway structure and function may be dependent on normal growth and development of the lung during early life, awareness of the sequence and timing of the major developmental changes is an important pre-requisite to understanding respiratory disease and its

treatment. Crucial to this understanding is the concept that the lung is not a mature organ at birth. The growth and development of the respiratory system is summarised in the following sections.

1.3.1. Early lung and airway development

The stages of human lung growth are listed in Table 1.3. Lung development begins in the 4th week after conception with a simple diverticulum of the foregut and lobar bronchi at the 5th week of gestation [63;85]. Based on the appearance of the lung tissue, lung growth has been classified into 4 phases, namely the embryonic, pseudoglandular, canalicular and alveolar periods. Each of these phases has a specific duration and each gradually evolves into the next phase (Table 1.3) [63;85]. The alveolar phase may be further divided into the early saccular or terminal sac stage and a later alveolar stage, which continues after birth in infants. There is considerable variation in the time span for lung development between different individuals and species [63]. An important event to note is that the full number of generations of conducting airways to the level of the terminal bronchioli is established by the 16th or 17th week of gestation [86]. Hence, the occurrence of any adverse events during the first half of the pregnancy may result in reduced numbers of airway generations. By further division, nearly all the respiratory airways are present at birth. By about 24 weeks gestation, type II cells in the respiratory region are capable of producing surfactant [63] and by 26 weeks, airway wall structure is mature [87;88]. Although gas exchange is feasible earlier (as witnessed by the survival of some extremely preterm infants from about 23 weeks gestation onwards), mature alveoli that are cup-shaped with a single capillary layer do not appear until approximately 28-30 weeks of gestation. By term (40 weeks post-menstrual age [PMA], where PMA is the sum of gestational and postnatal age), potential gas-exchanging surface has increased markedly, the interstitium has thinned and the capillary network expanded, resulting in increased capillary volume and diffusing surface. This increase continues up to 18 months postnatally [89]. Since fetal lung development appears to be highly sensitive to the intrauterine milieu, this may explain the wide range of alveolar number present at birth. The alveolar stage is followed by an additional and final stage in lung development, that of microvascular maturation [90;91]. In the human lung, rapid alveolar multiplication occurs during the last few weeks of intrauterine life and particularly the first few years of postnatal life.

Table 1.3. Stages of human lung growth

Phase	Gestation	Major events
Embryonic period	4 – 7 weeks	lung buds and segmental bronchi develop; epithelial tube appears, divides and grows into surrounding mesenchyme; vascular connections appear
Fetal period		
Pseudoglandular	6-17 weeks	Conductive airway tree develops in parallel with formation of vascular tree; smooth muscle cells present in trachea and lobar bronchi; cartilage plate appears in the trachea; epithelial lining cells differentiated into ciliated and goblet cells
Canalicular	16-27 weeks	intra- acinar (respiratory) airways develop; thinning of peripheral epithelial and mesenchyme; peripheral airways continue to divide forming prospective respiratory bronchioli and alveolar ducts; differentiation of type I and II pneumocytes cells & start of surfactant production; formation of distal pulmonary circulation
Saccular/alveolar	26 weeks to term	Formation of additional airway generations and alveoli; development of single-layered capillaries network lining saccules and alveoli; prospective gas exchanging airspaces dilate; maturation of surfactant system
Postnatal period		
Alveolar	Birth to 18 months	Formation of alveoli by outgrowth of secondary septa; symmetrical airway growth; small blood vessels multiply
Arteries & veins	Birth – 3 years	Reduction of double capillary network to single-layered network; reduction of interstitial tissue mass, fusions of capillaries, preferential growth of single-layered capillary network areas
	Up to adulthood	All structures increase in size

Adapted from Burri [85] and Hisplop & Pandya [90].

1.3.2. Postnatal lung and airway growth

Considerable remodelling of the lung occurs after birth [92]. Each of the structural components – airways, alveoli and blood vessels – has a different pattern of growth with respect to increase in number as well as size [93]. At birth, the airways are fully innervated and approximately 150 million alveoli, equivalent to $^1/_3$ to $^1/_2$ of the adult number, are present [94;95], with the blood gas barrier being of the same thickness as in the adult. In humans, alveolarisation is mainly a postnatal event, since approximately 50-80% of mature alveoli are formed after birth [85;96]. Lung growth occurs by alveolar multiplication and increase in tissue mass. By 6 months of life, a large proportion of the human lung appears mature in that many parenchymal septa no longer exhibit the double capillary network needed for the formation of new inter-alveolar walls. The question of the duration of the stage of alveolar formation has been much debated. In the human, a relatively rapid process (or 'bulk alveolar formation') starts around one month after birth but by 6 months, the intensity of alveolar formation has decreased [85;95]. At this age, numerous inter-alveolar walls and, by 1½ years, most septae, are at least partially of the mature type [85]. It has been suggested that human alveolar development may be virtually complete by 2-3 years of age [63;97]. After this, alveolar growth is by enlargement only [98]. In the first two years after birth, alveoli multiply with their accompanying arteries and veins. By 5 years of age, the lung has been found to correspond morphologically to a miniaturised adult lung [85].

The resting volume of the lungs increases from around 90 mL at birth in a term infant to 3,000 mL in adulthood [92;99], with lung weight increasing from 60 to 750 g over this period [100]. Lung size continues to increase at least until growth of the chest wall is complete [93]. The number of conducting airways is complete at birth, with airways only increasing in size postnatally [93]. These airways increase in diameter and length by two to three times between birth and adulthood [87] with growth occurring in a symmetrical manner, in a constant relation to the rest of the lung [87;101]. At birth, there is relatively less airway smooth muscle than in the adult [87;102]. During the first year of life, there is a rapid increase in both sub-mucosal glands and airway smooth muscle within the airway wall, relative to the more gradual, symmetrical, increase in airway length and diameter [101]. The increase in airway smooth muscle is particularly marked during the first weeks after birth and by 8 months of age, the relative proportion of muscle within the bronchioli is similar to that of adults. Within the larger airways, the proportion of airway smooth muscle continues to increase beyond infancy and therefore, during infancy, there is relatively more muscle within the small non-cartilaginous airways than in the bronchi [87]. The stimulus for rapid development of airway smooth muscle shortly after birth has been

attributed to the onset of air breathing [87]. An increase in the quantity of airway smooth muscle is seen in preterm and ventilated infants [87] as well as in children with a history of bronchiolitis [102]. The functional consequence of this is a greater increase in resistance in the small airways for a given amount of smooth muscle shortening. This is because airway calibre is one of the major determinants of airway resistance (R_{aw}), the latter being inversely proportional to the 4th power of the radius (Poiseuille's law)[103]. Thus, if the radius is halved, resistance increases by a factor of 16 whereas doubling the length of a tube only results in a 2-fold increase. Pulmonary inflammation and infection in infancy, though not affecting airway number, may have effects on airway growth resulting in diminished airway calibre. Stimulation provided by stretch may continue to influence lung growth after birth, since diaphragmatic and regional muscle activities have been reported to determine parenchymal growth by proliferation [104].

1.3.3. Dysanapsis

In the human, marked differences may exist in the relative size of the lungs and airways between individuals of similar age, height and sex. The term dysanaptic, or disproportionate (but physiologically normal), growth of the lung and airways has been used to explain the inter-subject variability in expiratory flow which can only be partly accounted by differences in lung elastic recoil or lung volume [105;106]. The disassociated growth patterns of the airways and parenchyma during fetal and early life result in airways that are relatively large in relation to lung volume at birth [83]. This is supported by the finding of relatively lower airway resistance at birth than at two to three months of age [107;108]. In another study in which FRC and maximal forced expiratory flows during the tidal breathing range were assessed in healthy infants, Tepper *et al* concluded that neonates have larger airways relative to their resting lung volume than older children [109]. A recent study using an infant lung model supported this finding [110]. Other studies have also provided evidence that dysanaptic growth occurs at least in the first few years of life [111;112] and again during adolescence [113]. The rate constant derived from forced expiratory manoeuvres, a measure of lung emptying, has been shown to be approximately four times greater in young infants than in adults [114].

By contrast, on the basis of the extent to which measurements of forced expiratory flow 'track' throughout childhood, and the fact that volume-adjusted flows correlate well within individuals with increasing age, Hibbert *et al* suggested that the relative rate of growth of different parts of the lung remains constant relative to one another at least between mean ages of 9 to 18 years [115]. However, from longitudinal data analysis, Merkus *et al* concluded that airways and airway spaces continue to grow isotropically (or

proportionally in all directions) in boys during adolescence, whereas in girls, airway growth lags behind that of the parenchyma during this period [116].

1.3.4. Increased vulnerability to respiratory illnesses

Hospital admission rates for lower respiratory diseases in infants and children demonstrate the marked increased vulnerability to LRIs during the first year of life (Table 1.2). This may be largely attributed to a combination of having airways that are small in absolute size and a relatively immature immune system. In addition, in infants, the tendency for dynamic closure of peripheral airways towards end-expiration during tidal breathing [83;117] increases their susceptibility to airway obstruction and wheeze in the presence of respiratory disease [118]. Willet and Sly [119] interpreted the differing patterns of wheezing illnesses, or phenotypes (section 1.2.2), expressed at different ages as an indication of the importance of dysanaptic growth. In the first 2 years of life, viral LRI can cause widespread airway obstruction, hyperinflation and wheezing, whereas in later childhood, the airway obstruction associated with viral LRI tends to be less severe and hyperinflation less common [119]. This difference may partly be explained by the increasing mechanical interdependence of the airways and air spaces with growth, which leads to increasing traction or "pull" on the peripheral airways, thereby 'enlarging' their calibre and diminishing the risk of wheezing [120].

The major aspects of developmental respiratory physiology which differentiate infants from adults, together with factors that may influence the growth and function of the respiratory system in early years, are presented in the next section.

1.4. DEVELOPMENTAL RESPIRATORY PHYSIOLOGY

In health, many parameters of respiratory function remain remarkably constant when related to either body size or surface area, reflecting the fact that respiration must remain closely attuned to the metabolic requirement of the body. However, the underlying factors determining these parameters may vary considerably according to age [83;84;121]. Interpretation of respiratory function measurements during infancy may be confounded by many factors, such as age, size, ethnic background, sex and environmental exposures. The rapid somatic growth that occurs during the first year of life is accompanied by major developmental changes in respiratory physiology, particularly with respect to the influence of control of breathing, the upper airways, dynamic elevation of FRC and chest wall compliance. An understanding of developmental respiratory physiology is essential to the success of measurements and interpretation of respiratory function data in this age group.

1.4.1. Transition to extrauterine breathing

Despite many anatomical similarities, the respiratory system in early life is by no means simply a miniaturised version of that of an adult. Within the first few minutes of birth, in order to establish efficient gas exchange, newborn infants must rapidly switch from intermittent fetal breathing movements, which play no role in gas exchange, to a continuous rhythmic respiratory pattern. Simultaneously, the respiratory system undergoes dramatic functional changes, such as clearance of lung liquid, rapid aeration of the lung, redistribution of blood flow and establishment of a resting lung volume. These changes are accompanied by a rapid rise in compliance and decrease in resistance, with relatively stable values being achieved within the first 12–24 hours after birth [122]. Blood gas and acid–base balance rapidly adjust to a steady state within the first few hours after birth [123].

To achieve effective ventilation at birth, the lungs must be developed to a stage where the alveoli can be inflated, and the forces opposing this must be overcome. Once regular respiration has been established, alveolar ventilation, minute ventilation and gas mixing efficiency are similar to that of an adult, once allowance has been made for differences in body size and metabolic rate [83]. However, the relatively large surface area to body mass ratio and rapid growth in infants are associated with relatively high oxygen consumption and metabolic rate when compared with those in adults [124]. The increased minute ventilation in relation to body size that is required to meet this increased demand for oxygen in early life is achieved primarily through an increased respiratory rate during the first months of life, with weight corrected tidal volume remaining relatively constant [99].

1.4.2. Control of breathing

Respiratory control and lung function undergo a series of inter-related dynamic changes during growth and development. These impact on measurements as well as interpretation of results, as outlined below.

1.4.2.1. Chemo-reflexes

Important differences in respiratory reflexes in young infants are related to maturational differences in chemo-sensitivity. At birth, chemoreceptor activity to hypercapnia is strong, whereas the hypoxic response is relatively weak, varying from a biphasic response with an overall decrease in ventilation during the early neonatal period to modest increases with advancing age [125;126]. Prematurity, intrauterine growth restriction, fetal exposure to maternal smoking during pregnancy and perinatal hypoxia have all been associated with

a diminished hypoxic and arousal response in young infants, thereby increasing their respiratory vulnerability [118;127-132].

1.4.2.2. Vagal mechano-reflexes

In 1960, Cross *et al* demonstrated the occurrence of apnoea following lung inflation, in newborn infants [133]. This apnoeic pause, or prolongation of expiratory time, was reported as the manifestation of inhibition of inspiratory effort as a result of stimulation of the pulmonary vagal stretch receptors, namely, the Hering-Breuer inflation reflex (HBIR) [134;135]). The original report was the first recognition of a self-steering regulatory mechanism or negative feedback for respiratory control [136]. In 1974, Younes *et al* confirmed that the apnoeic pause subsequent to lung distension was usually associated with relaxation of the respiratory muscles [137].

Three types of pulmonary receptors are regarded to be involved in vagally mediated, volume-related reflex control of breathing: slowly-adapting (stretch) receptors (SARs), rapidly-adapting (irritant) receptors (RARs) and pulmonary C-fibres [138]. SARs are responsible for the HBIR [139-142]. These receptors are located within airway smooth muscle and epithelial layers from the trachea to bronchioles, the concentration of which is greatest in the larger airways, decreasing progressively towards the terminal bronchioles [138;143;144]. In response to airway stretch (i.e., during lung inflation), they adapt slowly to the stimulus and provide an inspiratory off-switch once a certain volume threshold is reached, thus determining respiratory rate and depth [145].

It has been generally recognised that the HBIR is stronger and much more physiologically active in the newborn period and during infancy than in adult life, since a prominent reflex response has been observed in healthy infants during natural sleep within and above the tidal volume range [133;146-149]. It has also been recognised that the vagally mediated HBIR plays an important role in regulating the rate and depth of respiration in newborn mammals during the first weeks of life [150-152]. After birth, in both term and preterm infants, mechano-receptor activity is important in the maintenance of lung volume [146;153]. The presence of an active HBIR during tidal breathing in infants has facilitated measurements of passive respiratory mechanics in this age group, whereby airway occlusions at end-inspiration evoke a brief expiratory pause with muscle relaxation, during which, in the absence of flow, pressure equilibrates within the respiratory system allowing elastic recoil pressure to be measured at the airway opening. More recently, the ability to invoke HBIR has been used to evoke respiratory muscle relaxation and facilitate complete expiration during the raised lung volume technique [154] by delivering several augmented breaths prior to forcing expiration (section 1.10.2).

Another important aspect of the vagal mechano-reflex is with respect to the control of breathing. This is demonstrated when, in the presence of a reduced lung volume, expiratory time is reduced and respiratory rate increased in order to dynamically elevate resting lung volume. Further details of this mechanism are presented in section 1.4.5.

1.4.3. Influence of the chest wall

The highly compliant chest wall of the infant results in relatively low transpulmonary distending pressure when compared with older subjects [155]. The reduced outward recoil allows the lungs to deflate to relatively low lung volumes at the end of a passive expiration resulting in less tethering traction on the airways, and a tendency for peripheral airways to close at end-expiration. This not only impairs gas exchange and ventilation-perfusion balance, particularly in the dependent parts of the lungs [156] but, together with the absolute small size of the airways, renders infants and young children highly susceptible to airway obstruction and wheeze. In addition, the inability of the floppy chest wall (particularly in those born prematurely) to withstand normal negative intrapleural swings can lead to asynchronous movements of the rib cage and abdomen during inspiration, resulting in a less efficient system whereby the respiratory muscles expend energy not only in exchanging tidal volume but also in distorting the rib cage, resulting in fatigue [157]. Furthermore, the relative paucity of Type I skeletal muscle (slow twitch, high oxidative fibres) contributes to the further reduction of efficiency of the respiratory pump and increases the risk of developing respiratory fatigue [158;159]. The chest wall undergoes major structural and functional changes in the first five years of life [157]. During this period, ossification of the ribs, vertebrae and sternum lead to significant increase in chest wall rigidity with growth [157;160].

1.4.4. Upper airways

Nasal resistance comprises approximately 50% of total airway resistance at all ages [161]. Since infants are preferential nose breathers, the upper airways may have a major influence on the conduction of air to and from the lungs, particularly in the presence of upper respiratory infections [162]. For this reason, infant lung function tests should be delayed for at least 3 weeks following the onset of any respiratory infection. The role of the upper airway in modulating expiratory flow to preserve lung volume is described in the following section.

1.4.5. Dynamic elevation of end-expiratory level

Optimal pulmonary gas exchange requires an adequate resting lung volume or functional residual capacity (FRC). In order to overcome the potential difficulties imposed by their

highly compliant chest wall, infants tend to defend their lung volume by dynamically elevating the end-expiratory level, such that they breathe in before the passively determined FRC is reached. The emptying time of the lung, under passive condition, can be described by the expiratory time constant (τ) of the respiratory system, which is defined as the time taken for the lung to deflate passively in an exponential fashion to 37% of its original volume [163]. Three expiratory time constants are required to achieve 95% of lung emptying. τ is determined by the product of compliance and resistance. Stiff lungs (low compliance) will have a short τ and will empty and fill rapidly, whereas in the presence of high airway resistance, τ will be elevated and the lung will empty and fill slowly. Dynamic elevation of FRC occurs whenever the expiratory duration is less than three expiratory time constants. This may occur as the result of a rapid respiratory rate (i.e., short expiratory time) and/or an elevated airway resistance (increased τ) [164]. While pathological signs of hyperinflation may occur in infants with airway obstruction (asthma, bronchiolitis and cystic fibrosis), the phenomenon of dynamic elevation also occurs to a lesser extent among healthy young infants as a means of defending their FRC in the presence of a highly compliant chest wall [164;165].

During the first months of life, infants modulate expiratory flow using upper airway adduction and post-inspiratory diaphragmatic muscle activity to "brake" or slow expiratory flow, thereby lengthening the expiratory time constant of the respiratory system [164;166-169]. In addition, control of respiratory timing is mediated through tonic and phasic vagal stretch receptors which are exquisitely sensitive to changes in resting lung volume [170]. During the application of continuous negative or positive airway pressure, the instantaneous change in respiratory rate and pattern can be seen in response to small changes in resting lung volume [171]. Infants frequently exhale passively to a lower lung level (i.e., their "true" resting lung volume) during an apnoeic pause when the expiratory time is prolonged. Although the ability to modulate expiratory flow and timing is physiologically beneficial to the infant, this can complicate attempts to assess respiratory function. The variability of the end-expiratory level may hinder the assessment and interpretation not only of lung volumes, but also of respiratory mechanics and forced expiratory flows, since these indices are highly volume dependent [172].

It has been suggested that a shift from active to passive maintenance of FRC does not occur until the end of the first year of life, after which passive characteristics of the lung and chest wall are likely to determine resting end-expiratory lung volume as in older subjects [173].

1.5. FACTORS INFLUENCING LUNG GROWTH AND FUNCTION DURING INFANCY

Besides developmental differences in respiratory physiology, several factors are known to influence respiratory function in early life. These include -

1.5.1. Sex

Several investigators have reported differences in airway function and prevalence of respiratory illness during infancy and childhood according to the sex of the individual [84;113;174-180]. Although there appear to be no sex differences in the relationship of lung to somatic growth *in utero*, it has been reported that enhanced lung maturation has been observed in female compared to male fetuses both with respect to mouth movements [181] (a marker of fetal breathing that is thought to be a critical determinant of fetal lung development) [64;182] and phospholipid profiles (a marker of surfactant production) [183;184]. The higher degree of lung maturity in female than in male fetuses during the last two months of normal pregnancy may contribute to the higher reported incidence of respiratory distress syndrome (RDS) among male preterm neonates [183;185].

Functional studies have suggested that during both infancy [42;178;186-189] and up until puberty [116;180;190;191], girls have higher forced flows in relation to lung or body size than boys suggesting that airway calibre is greater in girls than in boys. This may be a contributory factor for greater severity [5] and higher prevalence of wheezy LRI and asthma observed in boys during both infancy [5;37] and childhood [5;192]. Another study has reported that sex differences in airway structure and responsiveness are present soon after birth, representing differences in fetal lung development and are associated with differences in the risk of subsequent LRI with wheezing [178]. Evidence also suggests that during childhood, boys have greater resting airway tone than girls and that this tone is less responsive to deep inspiration and isoproterenol (a β_2-agonist) [193].

Enhanced airway growth has been reported in adolescent males [111;179] which may partly explain the greater clinical improvement in males than in females with respect to respiratory disease as they become adults [116;194;195]. Recent evidence, however, suggests that relative to females with asthma, males with asthma are twice as likely to develop airway remodelling by 18 years [196] and have a lower FEV_1/FVC ratio at 26 years [30].

The implication of the biological differences between the sexes should be taken into consideration when interpreting respiratory function, particularly during rapid phases of growth and development [42;180].

1.5.2. Ethnic origins

Several studies have reported marked ethnic differences in infant mortality and respiratory morbidity. Although black infants have a higher risk of neonatal and postnatal deaths than white infants, among low birth weight infants, neonatal mortality is lower in black infants [197;198] who are also less likely to develop respiratory distress syndrome (RDS) than white infants of similar gestational age [199]. These relationships appear to persist even after allowing for socio-economic status [198] which suggests that the respiratory system is more mature or that airway function is enhanced in black preterm infants [200].

Ethnic differences in breathing patterns and respiratory mechanics have been observed during early life. Relative to white infants, black infants have lower airway resistance [161], lower respiratory rate, longer expiratory time and higher weight-corrected compliance of the respiratory system [189;201]. Maximal forced expiratory flows at FRC (V'_{maxFRC}) and the duration of time to reach peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF}:t_E$; an index of tidal expiratory flow pattern reflecting the control of lung emptying during expiration) are significantly lower in white compared with black infants [189;201]. This may reflect a transient difference in measurement of flows at a slightly higher end-expiratory lung volume among black infants due to their different pattern of breathing, with more marked braking or slowing of expiration (longer $t_{PTEF}:t_E$) during the neonatal period.

By contrast, based on values predicted from standing height, lung volume and forced expiratory flows are lower in black adults and older children, whereas no such discrepancies are observed when respiratory function is related to sitting height [190;202]. These differences may primarily reflect ethnic variation in the trunk to height ratio [190;203].

In the USA, relative to white children, a higher prevalence of asthma (1.1–1.7 times) [195;204;205], hospital admission (2–3.5 times) [204;206;207] and mortality rate (2–5 times) [204;208] associated with asthma have been reported in Hispanic and African American children, emphasising the need to take both ethnic group and socio-economic factors into account when interpreting lung function tests and their relation to respiratory disease in childhood.

1.5.3. Early life factors and socio-economic status

Socio-economic factors have been widely investigated as a 'fundamental' cause of disease and as such, may contribute to the trajectory of later disease over the life course [209;210]. A detailed review of this topic is beyond the scope of this thesis. It is generally

recognised that modifiable factors such as nutrition and smoking are associated with poor socio-economic conditions. Other indicators of poor socio-economic conditions include variables such as level of income, overcrowded households, family size and parental separation and/or divorce. Several studies have suggested that deprivation in childhood influences risk of mortality from cardiovascular disease and respiratory function in later life [210-217]. However, far less is known about the influence of socio-economic status (SES) on lung health since fewer studies have considered SES as a confounding factor when examining respiratory function, particularly during infancy and early childhood [218].

Over the past decades, epidemiological studies have been enormously successful in identifying risk factors for major diseases. As early as 1934, a paper by Kermack *et al* published in the Lancet hypothesised and discussed birth cohort influences regarding early-life exposures on adult diseases. Based on their analysis, they observed that "the expectation of life was determined by the conditions which existed during the child's early years", and concluded that "the health of the child is determined by the environmental conditions existing during the years 0–15, and ..... the health of the man is determined preponderantly by the physical constitution which the child has built up" [219]. Interestingly, Kermack *et al* also suggested that infant mortality was dependent upon the health of the mother (i.e., intergenerational effect), and thus that improvement in infant mortality followed the generational improvement in the vitality of women of childbearing age. However, based on the pattern of cohort-related mortality during the period covered by these authors, namely 1860–1932, they explicitly rejected the argument that very early-life development (before or within the first year of life) could play any role in determining the mortality risk of birth cohorts as they aged. Other recent research, taking a lead from the original work of Forsdahl [220], has investigated the possibility that exposures acting right across the growth and developmental phase, from prenatal growth through to the end of adolescence, may importantly influence later health [61;210;214;215;218;221]. Some epidemiologists maintain that the importance of social circumstances early in life may be in the way they influence employment, social position and exposure in adulthood, and thus it is the adult, rather than childhood, circumstances which are more important as predictors of disease outcome [217]. However, others have suggested that an individual's status for behavioural risk factors (exercise and smoking) is associated primarily with current socio-economic circumstances, while status for physiological risk factors (serum cholesterol, blood pressure, body mass index and FEV_1) is associated to varying extents with both past and present socio-economic circumstances [211]. Although retrospective longitudinal analyses from cohort studies support the notion that SES may contribute to the trajectory of respiratory function over the life course, they are limited by an inherent problem: namely, that socio-economic circumstances in childhood and adulthood are linked, and

the specific effects of socio-economic conditions in early life, as opposed to the continuing effects of deprivation throughout life, are difficult to isolate, and that the observed decline in lung function may be the result of several mechanisms [212;222].

It has been recognised that fetal and early life occurrences may prevent the lung from attaining its full potential, leading to increased risks of respiratory illnesses in later life [57;223]. A prospective study, reported in Chapter 4 of this thesis, was conducted to investigate the association between birth weight and airway function during infancy. Respiratory function data from this research was, in part, interpreted with SES as a distal cause, which was mediated by complex causal pathways including maternal smoking, nutrition, biological and environmental factors.

1.5.4. Fetal growth

Fetal or intrauterine growth retardation (IUGR) is associated with prematurity, intrauterine death and diminished fetal reserve during labour [224-227]. In a meta-analysis, the effect of birth weight has been attributed more to intrauterine growth retardation than to preterm delivery [228]. Cigarette smoking is the single most important factor affecting birth weight in developed countries [228], resulting in an average reduction of 150 to >300 g in infant birth weight [229-234]. Both maternal and paternal smoking are associated with low birth weight, with maternal smoking having the greater effect [235;236]. An improvement in infant birth weight has been demonstrated by reduction of smoking during pregnancy [237].

An increased risk of perinatal mortality and morbidity is associated with birth weight at or below the 10th percentile for gestational age [238-241]. During the first year of life, infants born with low birth weight for gestational age (commonly referred to as small for gestational age [SGA]) are more likely to be admitted to hospital due, mainly, to respiratory tract infections [242]. Hospital admissions that are directly related to respiratory infections have been found to be significantly associated with maternal smoking at the time of conception, rather than postnatal exposure [243]. Since maternal smoking is also strongly associated with SGA births, the association between SGA and respiratory infections may be confounded by maternal smoking. Low birth weight has also been associated with wheezing in children up to 5 years old [244] and reduced respiratory function in later childhood [245]. However, there are no similar studies examining the effect of SGA on airway function in infancy [80]. It has previously been reported that SGA infants have accelerated pulmonary maturation and a reduced risk of respiratory distress syndrome (RDS), relative to infants of appropriate birth weight for gestational age (AGA) [246;247]. However, findings from more recent studies challenged this concept of

accelerated pulmonary maturity and showed that, when compared with AGA infants of the same gestational age, sex and ethnic background, SGA infants are at increased risk of death and respiratory illness [242;248]. The relative risk for RDS and respiratory failure associated with being SGA (odds ratio [OR]: 1.9; 95% confidence interval [CI]: 1.2 to 3.2) was found to be of similar magnitude to that associated with male sex (OR: 2.0; 95% CI: 1.2 to 3.3) or white race (OR: 1.9; 95% CI: 1.1 to 3.2) [248]. This is corroborated by findings from a research study forming part of this thesis, which is presented in Chapter 4.

Several studies have confirmed a strong association between intrauterine growth restriction (IUGR) and sudden infant death syndrome (SIDS), although the extent to which this might be confounded by factors such as maternal smoking is not always clear [249-253]. It has been suggested that adverse intrauterine conditions are more important because it is birth weight, rather than growth velocity in early postnatal life, that is reduced in infants who die suddenly and unexpectedly [254]. It has been suggested that many cases of SIDS may be due to a final episode of progressive peripheral bronchial occlusion, which would be more likely to occur in infants with pre-existing diminished airway function [118]. Thus, minor alterations in lung structural development during fetal life could have marked postnatal consequences, as fetal insults may also exert effect on postnatal growth. In the presence of subsequent respiratory infections, airway calibre may be critically reduced, resulting in severe, and potentially fatal, respiratory compromise.

Over the last decade, the idea that adult disease is determined in early life is most influentially reflected in the 'fetal origins' work of Professor David Barker and his colleagues. In this context, the critical period of early-life development, which is influenced by environmental exposures and has long-term effects on later health, is the intrauterine period [255]. Extensive investigations by this group led to the suggestion that adult diseases are mediated by altered fetal nutritional status and defective perinatal growth [57;221;223;256;257]. The fetal origins hypothesis suggests that the structure and function of various organs in later life is "programmed" by impaired fetal nutrition or growth at critical periods of organ development [14]. This concept has been supported by evidence from animal studies [258-260]. Failure to reach growth potential during early life may be irreversible [56].

A population-based study, which formed part of the research reported in this thesis, was designed specifically to investigate the association between birth weight and airway function in early infancy and to explore the fetal origins hypothesis. Findings from this study are presented in Chapter 4.

1.5.5. Maternal smoking during pregnancy

The hazardous effects of cigarette smoke exposure on the fetus, infant and child with respect to growth and development and respiratory function have been widely reported [13;40;232;261-265]. Cigarette smoke is composed of some 3,000 different compounds and the main components are carbon monoxide (CO), nicotine, polycyclic aromatic hydrocarbons (PAHs) and others such as cyanide and cadmium [266]. Prenatal maternal smoking affects the fetus in several ways that may result in chronic hypoxia and low birth weight for gestation.

The affinity of CO for haemoglobin is 250 times greater than that for oxygen. Furthermore, it dissolves readily in the plasma leading to impaired oxygen exchange in the tissues [13;267]. During pregnancy, the dissolved CO in maternal plasma crosses the placental barrier by passive diffusion, subjecting the fetus to great risk since fetal haemoglobin has a higher affinity for CO than adult haemoglobin. Similarly, nicotine crosses rapidly from the maternal to fetal circulation via the placenta, with fetal concentrations being around 15% higher than maternal levels. Cotinine, the primary metabolite of nicotine, has a relatively long half-life of 14 to 24 hours and is frequently used to provide an index of nicotine exposure [268-271]. Cotinine has been detected in amniotic fluid as early as the 7th week of gestation in both active and passive smokers [272]. Throughout gestation, the fetus is exposed to increasing concentrations of nicotine through the maternal circulation and via gastrointestinal and skin absorption of nicotine from the amniotic fluid [273]. This is supported by the finding of significantly higher level of cotinine in the first urine from newborn infants born to smoking mothers compared to those not exposed to the chemical by-products of tobacco smoke *in utero* [274]. Placental vascular resistance is often increased when women smoke during pregnancy [275;276] and may result in placental dysfunction, impairing the transfer of nutrients to the fetus. The concomitant adverse effect of carboxyhaemoglobin on oxygenation, chronic exposure to CO and deprivation of nutrients *in utero* may result in significant growth restriction [13;277;278]. The primary mechanism mediating the effect of nicotine and CO on fetal growth is thought to be a combination of both the effects on oxygenation by carboxyhaemoglobin and the vasoconstrictive effects of nicotine on the uterine, and potentially the umbilical, artery [230;279;280].

The potential effects of smoking during pregnancy on lung and airway development may include structural alterations [69] and interference with the control of respiration [129;281], as well as the immune system [282]. One of the difficulties in assessing the adverse effect of *in utero* exposure to cigarette smoke on the developing respiratory system is that, in many studies, the extent to which exposure to environmental tobacco smoke (ETS) after birth

may have contributed is unclear, since measurements of infant respiratory function are often obtained some weeks following delivery. Several studies have reported the association between maternal smoking and reduced respiratory function in the neonatal period [40]; other studies have observed that the respiratory risk associated with parental smoking appears to be greatest in younger infants [14;283;284], providing further support that fetal development may represent a critical time of pulmonary vulnerability.

Over the last 20 years, the prevalence of maternal smoking in pregnancy has declined, particularly in the non-manual social classes, and perinatal mortality rates have also fallen [13]. However, after evaluation of the relationship between different patterns of smoking habits and changes in causes of perinatal death, smoking during pregnancy continues to be strongly associated with fetal and infant morbidity and mortality [13;38;40;232;285].

1.5.6. Nutrition

Several investigators have suggested that lipids, especially essential fatty acids, are crucial for early lung development in the rat [286-289] and that lipid deficiency could delay differentiation of epithelial cells and impair surfactant production [290-292]. Increased lung distensibility in rats has been associated with a defective synthesis or conformation of connective tissue [293] and vitamin E has an antioxidant role by preventing lipid peroxidation [294-296]. Selenium deficiency leads to lung growth retardation with alveolar septal attenuation [297]. Recently, vitamin A has been highlighted as important in the control of elastin expression in the lung, and hence the process of air-space septation by which new alveoli are formed in the growing lung [298]. Other animal models have demonstrated that absolute lung volumes in rat pups from protein restricted dams are reduced, though specific lung volumes are elevated [299] and that neonatal protein deprivation results in decreased recoil pressure and lower elastin concentration [300]. Different adverse effects on the lung may be associated with variations in timing and severity of these insults [79]. These findings suggest that, in humans, malnutrition in late fetal life and infancy is likely to have an important effect on lung size [301] and hence respiratory function. In rats, severe food restriction can induce 'nutritional emphysema' which, unlike true emphysema where there is destruction in the structural tissue of the lung, manifests by the presence of alveolar enlargement and septal attenuation [302]. This results in reduction in alveolar surface area, followed by subsequent emphysematous appearance [303]. Starvation has also been shown to aggravate elastase-induced injury. While refeeding appears to result in complete recovery of the mechanical changes, there is only partial recovery of the morphometric changes induced by starvation [304].

Following delivery, the lung parenchyma continues to undergo structural and functional changes such as the formation and multiplication of alveoli, maturation of the capillary network and the accumulation of connective tissues. Thus, neonatal starvation may have adverse consequences on the rate of cell division and lung growth [305]. Lack of nutritional substrates during fetal and early neonatal life may impede cellular growth and differentiation, surfactant synthesis, inactivation of free radicals, connective tissue synthesis and post-injury repair [306]. Evidence from post-mortem studies of newborn infants who were underweight suggested that although alveolar development appeared appropriate for gestational age, their lungs were lighter than expected for body weight [307].

Inadequate maternal intake of vitamin E during pregnancy may influence the fetal immune system in such a way as to modulate the risk of childhood atopy [308]. Similarly, low level of vitamin E intake has been associated with atopic disease in children [309] and adults [310]. Other studies have suggested that inadequate postnatal nutrition may be associated with the development of bronchopulmonary dysplasia (BPD) [311;312]. In children with CF, nutritional status and growth are associated with changes in percent predicted forced expiratory volume in one second (FEV_1), suggesting that nutritional intervention may enhance lung growth and optimise pulmonary function in children with CF [313].

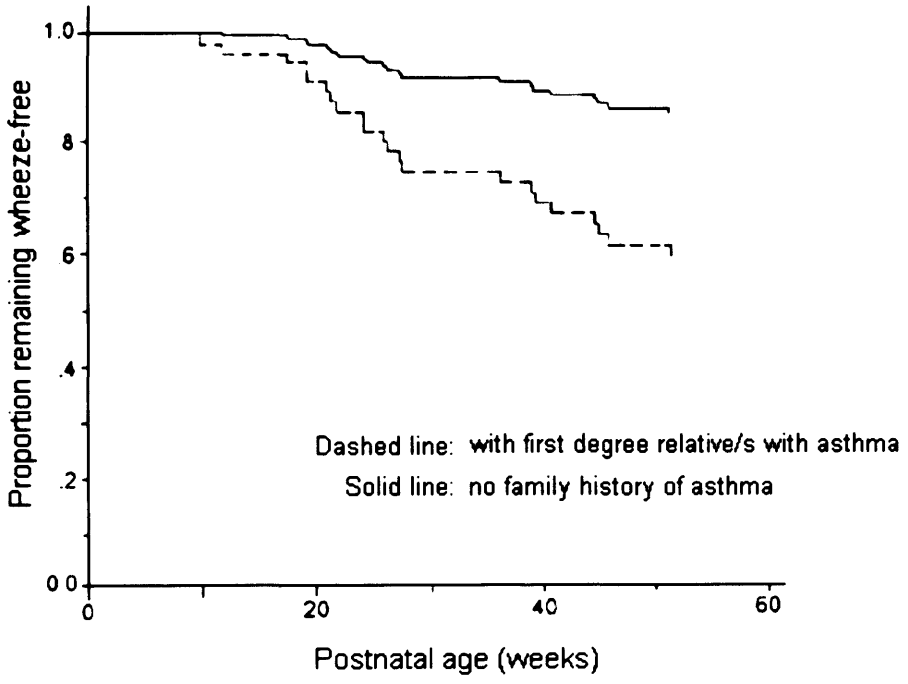
1.5.7. Family history of asthma

It has been suggested that genetic factors may influence airway development and function in early life [80]. There is good evidence for familial trends but until recently, the mode of inheritance was unclear [314]. It is estimated that genes and the environment contribute approximately equally to the development of asthma [315]. Atopy is characterised by a genetic predisposition for generating IgE against common environmental allergens, expressed clinically as asthma, eczema and rhino-conjunctivitis [315-317]. Several genetic studies indicate that multiple genes are involved in the pathogenesis of atopy and that different genes regulate the presence of increased levels of serum total IgE and specific IgE [317], which have been implicated in cross-sectional and longitudinal studies as one of the greatest risk factors for the development of asthma [47;315;318]. Cord blood IgE in newborn infants of atopic mothers is higher relative to those of non-atopic mothers, which is consistent with observations from genetic studies, reporting a higher risk of transmission of atopic disease from an affected mother than from an affected father [319-321]. Data from the Tucson longitudinal study suggested that children with maternal asthma were significantly more likely to have asthma if they had been exclusively breastfed (OR 8.7; 95% CI: 3.4 to 22.2) [322]. Most, though not all [323;324], studies have suggested that preferential inheritance for asthma occurs through

the maternal rather than paternal line [325;326]. Currently, intensive research using genetic and genomic methodology is underway to study polymorphisms in allergic disease, in order to identify the control or coding regions of genes that influence protein structure or expression and hence modify the presence and severity of disease [327-330].

Prospective data from our laboratory showed that premorbid specific airway conductance (sG_{aw}) and the time to peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF}:t_E$), a measure of the degree to which expiratory flow and timing are modulated to slow lung emptying, were significantly diminished at 8 weeks and one year of age among healthy term infants with a family history of asthma [37]. These infants were significantly more likely to develop wheezing at any given age during the first year of life than those without family history of asthma (proportional hazard ratio, 3.0, 95% CI: 1.3 to 7.3; p=0.013) (Figure 1.5) [37].

Figure 1.5. Proportion of infants remaining wheeze-free, according to family history of asthma, at any given age during the first year of life.



Adated from Dezateux *et al* [37].

In another study of wheezy infants, Sheik *et al* reported that those with a family history of asthma (FHA) showed a significant increase in the ratio of forced flows at low lung volumes to peak flows following administration of a bronchodilator, whereas no such

change was observed in those whose mothers smoked. However, healthy infants were not included as controls in this study [331].

An interim report from the Perth cohort study, based on measurements in 63 healthy fullterm infants measured between 2-10 weeks of life, suggested that bronchial responsiveness was increased in those with family history of asthma and those whose parents smoked [332]. However, the association between parental smoking and bronchial responsiveness was not confirmed when results from the entire cohort were analysed [333]. Findings from a London cohort study of 73 term infants with at least 1 atopic parent provided evidence that reduction in premorbid values of V'_{maxFRC} was associated with a history of maternal asthma, and at one year, analyses suggested that bronchial responsiveness assessed at ~1 month of age was significantly higher in infant girls who subsequently developed wheezy LRI compared with asymptomatic girls, whereas there was no apparent difference in BR between infant boys who did or did not develop wheezy LRI during the first year of life [178].

At follow-up at six years of age (n=95), increased bronchial responsiveness at 1 month in the Perth cohort was significantly associated with a reduction in FEV_1 and MEF_{25-75}, and with an increased risk of lower respiratory symptoms and the emergence of physician-diagnosed asthma by 6 year of age [333]. By 11 years of age, these children had significantly greater bronchial responsiveness and reduced MEF_{25-75}, when compared with other cohort members [6]. A link between neonatal bronchial responsiveness and lung function at 11 years was attributed to the arginine^{16} (Arg^{16}) allele of the β_2 adrenoceptor [334]. Results from follow-up assessment of the London cohort at age 10 years showed that early transient wheeze was associated with neonatal bronchial responsiveness but was not related to atopy or bronchial responsiveness at 10 years [335]. However, neonatal bronchial responsiveness was significantly associated with reduced FEV_1 % at 10 years, and reduced neonatal V'_{maxFRC} was found in those with the Arg^{16} or glutamine^{27} (Gln^{27}) alleles [335]. These findings suggest an increase in neonatal bronchial responsiveness and genotype-linked reduction in neonatal and later lung function among children born to at least one parent with a history of atopy or asthma.

1.5.8. Invasive prenatal procedures

Amniocentesis, or percutaneous transabdominal puncture of the uterus during pregnancy, to obtain amniotic fluid has been an established obstetric technique since 1973. It is commonly performed during pregnancy to obtain samples of amniotic fluid for fetal karyotype determination in order to diagnose abnormal fetal conditions or, at late gestation, to assay the concentration of sphyngomyelin and lecithin, the ratio of which

provides an index for fetal lung maturity, prior to decisions regarding induction of labour or surgical delivery. This technique has been considered by many to be a safe procedure. However, evidence from animal studies suggested that amniocentesis adversely impacts on fetal lung development. Following amniocentesis, a decrease in breathing movements for up to 48 hours has been observed, possibly due to the resultant increase in uterine activity [336]. The inhibition of fetal breathing movements may adversely affect the control of fetal lung growth [337]. In addition, the withdrawal of amniotic fluid has been associated with suboptimal lung growth [338;339]. Morphometric studies of lungs of (term gestation) newborn monkeys, whose mothers had undergone amniocentesis, with and without fluid withdrawal at mid and late gestations, showed that although the alveoli in experimental animals were of normal maturity, they were larger in size but fewer in number. Alveolar and alveolar duct diameters and respiratory bronchiolar length were reduced, and there was a reduction in gas exchange surface area [339]. These changes occurred regardless of the timing of amniocentesis, the amount of fluid removed and whether or not any fluid was removed after puncturing the amniotic membranes [339]. Similar observations were made by Moessinger *et al*, who reported that a single needle puncture of fetal rat membranes at late gestation resulted in persistent oligohydramnios, significant reduction in fetal weight and lung hypoplasia and hypotrophy [340].

In 1989, a method for diagnosis of fetal diseases by sampling the cells of the placental chorionic villi (CVS) for DNA analysis, presence of bacteria and concentration of metabolites was introduced. The effects of both the aforementioned invasive antenatal procedures on the incidence of neonatal respiratory problems and lung volume measurements have been investigated in newborn healthy, term, infants born to mothers who, during the first trimester of gestation, had undergone amniocentesis (n=74) or chorionic villi sampling (CVS; n=86) [341]. Six infants in the CVS group, but none in the amniocentesis group, required admission to the Special Care Baby Unit due to respiratory distress. Although there was no significant difference in the weight adjusted lung volume, 9% of the overall values were below the 2.5th centile of the normal range [341]. In a further study by the same group of investigators, wherein 25 infants were included as controls, higher airway resistance (R_{aw}) and lower specific airway conductance (sG_{aw}) were observed in infants whose mothers had early amniocentesis (n=47) or CVS (n=19) [342]. The authors concluded that both amniocentesis and CVS in early gestation may adversely impair prenatal lung and airway growth and development [341;342].

1.5.9. Prenatal glucocorticoids

Adrenal glucocorticosteroids are commonly administered in low doses to women in premature labour to accelerate late-gestation fetal lung maturation, to prevent neonatal

respiratory distress syndrome (RDS) and to improve perinatal survival of preterm infants [343-345]. However, research has suggested that high or repeated doses of glucocorticoids can inhibit somatic and lung growth [346;347]. Sex hormones appear to moderate the effects of glucocorticoids so that fetal lungs mature more rapidly in females than in males [185]. In animal studies, while maturation of the surfactant producing type II pneumocytes is accelerated by the use of glucocorticoids [348;349], rapid alveolarisation (i.e., accelerated thinning of the alveolar wall thickness and an increase in aerated parenchyma) was observed rather than increased production of alveolar surfactant [350;351]. The use of betamethasone has been associated with increased collagen and decreased elastin concentrations [352], which may prevent normal alveolar development [91;346;353] and reduce the multiplication of bronchial smooth muscle cells [354]. Adverse effects of the use of multiple doses of prenatal corticosteroids have been reported, which include significantly reduced birth weight and head circumference [355] and persistent suppression of cortisol and epinephrine postnatally [356].

1.6. ASSESSMENT OF RESPIRATORY FUNCTION

There is a wide range of techniques now available for measuring lung volumes, respiratory mechanics, ventilation inhomogeneity and the control of breathing in infants [357]. In the following sections, an overview of these techniques, with particular emphasis on how they differ from adult lung function tests, will be presented together with a more detailed description of the use of forced expiratory manoeuvres during infancy. Parameters derived from the latter were used as the primary outcome variables for the various studies described in this thesis.

1.6.1. Application in adults and older children

Respiratory function tests (RFTs) are integral to the clinical assessment and management of adults and older children with respiratory diseases. Their primary role is to identify the type and pattern of dysfunction presented by an individual and assess disease severity, which in turn helps to make or confirm a diagnosis, establish prognosis, or suggest and evaluate treatment. Respiratory function results are rarely diagnostic in their own right even among adults and therefore should always be interpreted in light of all other available information; for instance, clinical symptoms, chest x-ray or imaging and microbiology reports. Although isolated measurements of respiratory function may be useful, they are of limited value (if viewed as brief snapshots) compared to serial measurements when changes in function may be evaluated, particularly with respect to growth or therapeutic interventions.

Primarily, RFTs should be safe, simple and not unpleasant for the subject. A difficult procedure will have a high failure rate and may yield unreliable results, whereas one that is unpleasant will discourage participation both in the first instance and in any follow-up measurements. Acceptable tests should also be non-invasive and of short duration.

The result of a test should ideally be objective; in other words independent of the personality of the operator and motivation of the subject. It is important for the operator or observer to be aware of the reproducibility or repeatability of different tests when interpreting clinical or physiological data. The repeatability of a test is usually assessed as the variation between measurements either on a single test occasion or between test sessions. The within-test repeatability is a function of the intrinsic variability of the procedure, after allowance has been made for the extent to which the results may improve with practice. The between-test repeatability represents the additional variability due to factors which operate on the subject, operator or equipment over a longer period. Some of this variability can be eliminated by careful calibration of the equipment, standardisation of measurement conditions by controlling for ambient room temperature, posture, the time of day and interval since any meals or medication. Nevertheless, considerable within-subject and between-occasion variability may persist, particularly amongst those with respiratory disease, which may reduce sensitivity to detect significant changes within individuals in response to any intervention [358].

1.6.1.1. 'Ideal test': characteristics

In addition to being acceptable to the subject (and where applicable, to their parents), an ideal respiratory function test would be one that is -

- applicable to individuals of any age and arousal state;
- independent of subject co-operation;
- safe and easy for both the subject and operator to perform;
- reproducible both within and between subjects and between test occasions;
- sensitive enough to detect small changes with growth and distinguish clearly between health and disease;
- provide accurate and specific information about the structure and function of the lungs to clarify mechanisms underlying clinical status or disease, and to aid treatment management.

At present, no such test exists and even if it did, no single test is ever likely to provide all the necessary information for either a clinical or research study. Nevertheless, there is widespread use of RFTs amongst adults and older children as summarised in Table 1.4. By contrast, despite the high morbidity of respiratory disease in the first few years of life (section 1.2.1), the role of respiratory function testing in clinical management remains controversial in this age group [359;360] as discussed below.

Table 1.4. Some applications of respiratory function tests in adults

Field of application	Subdivision	Reason for testing
Clinical science	Diagnosis	Causes of wheeze, breathlessness, cyanosis and aspects of respiratory failure
	Clinical assessment	Disease of the lung, chest wall, heart and circulation, and central nervous system Serial assessment of disease state and/or function
	Medical treatment	Oxygen therapy, bronchodilator therapy, assisted ventilation
	Surgical treatment	Suitability for operation
	Anaesthetics	Suitability for and management during and after anaesthesia
	Research application	Evaluation of treatment (including surgical procedures) and new therapy Assessment of progress Relationship of deranged function to abnormal structure
Human biology & physiology	Physiology	Normal function; changes with posture, exercise, pregnancy, measurement conditions, growth and development
	Physiological determinants	Relation to age, sex, size, ethnic groups, activity variation due to time of day, season, climate, geographical location
Public health science	Epidemiology	Effects of smoking and air pollution Prevalence of respiratory impairment; prognosis (e.g., FEV_1 as predictor of all causes of mortality) Identification of high-risk cases
	Rehabilitation	Capability for physical work
Occupational medicine	Health in industry	Pre-employment, periodic and exit examinations, effects of respiratory hazards, suitability for strenuous work Establishment for safe conditions
	Diagnosis & assessment of occupational lung diseases	Asbestosis, farmer's lung, beryllium disease, pneumoconiosis of coal-workers and other occupational groups Lung disease in grain handlers, hard-metal workers, silo fillers, exposure to proteolytic enzymes, etc
Medicine and law	Assessment of disability	Function of the lungs and the capacity for exercise

Adapted from Cotes [358].

1.6.2. Measurement conditions: adults versus infants

As discussed in section 1.4, marked developmental changes in respiratory physiology occur during the first years of life and impact on both the measurements and interpretation of results. The important differences in undertaking RFTs in infants and young children ≤ two years of age relate to the need for miniaturised equipment, ethical issues, posture, sleep state, sedation and the fact that these subjects cannot be asked to undertake any specific breathing manoeuvres and tend to be preferential nose breathers [121;357]. Furthermore, techniques used to assess respiratory function in this age group have tended to be highly complex, requiring the operators to undergo intensive training, and have been expensive, hence limited to specialist laboratories.

In contrast to the upright, seated position adopted by adults and older children, the standard position for infant RFTs is usually supine, with the head in midline position, the neck and shoulders supported in slight extension to ensure patency of the airways, with measurements recorded in consecutive periods of relaxed, quiet sleep, when breathing patterns are regular [361]. The main differences in measurement conditions between adults and infants are summarised in Table 1.5.

Table 1.5. Comparison of differences in measurement conditions in adults and infants

Conditions	Adults	Infants
Position	Upright	Supine
Sleep state	Awake	Asleep
Sedation	Not required	Frequently used
Test duration	20–40 minutes	≥ 2 hours
Apparatus	Mouth piece & nose clip	Face mask

Unless clinically indicated, sedation is generally contra-indicated for RFTs in newborn infants. Although successful measurements in a full range of tests can be achieved in post-prandial natural sleep in infants in the first month of postnatal age (corrected for prematurity), it becomes increasingly difficult to perform any of the more complex measurements, such as plethysmography and forced expiratory manoeuvres, without some form of sedation. The dosage and use of sedation are dependent upon the age and condition of the infants, the reason for the test and the type of test being performed.

The most commonly used sedative agents are chloral hydrate and its derivative, triclofos sodium. Further details are presented in Chapter 2 (section 2.4.8). As infants are preferential nasal breathers (section 1.4.4), the presence of any upper airway respiratory symptoms will warrant cancellation and deferment of the test for at least 3 weeks, since nasal resistance and related parameters can change significantly due to mucosal swelling and excessive secretions.

In order to interpret tests of infant respiratory function, it is necessary to assess the validity of measurements, be knowledgeable regarding what constitutes a physiological or clinically significant change within or between infants and compare the results either with a specially selected control group or with a reference population [47;362]. However, the need for sedation in infant testing, together with the time-consuming nature of the tests, seriously limits the possibility of assessing short-term variability or repeatability of measurements (since it is not advisable to repeat dosage of sedation within relatively short intervals [363]), as well as attributing to a paucity of suitable reference values.

If reproducible measures of lung volume or respiratory mechanics are to be obtained, it is essential that a representative and stable end-expiratory level is established before commencing data collection. To achieve this, it is necessary to wait for the infant to be in behavioural quiet, non-rapid eye movement (non-REM) sleep and to ensure that data are collected during consecutive periods of this sleep phase. Consequently, each test session is relatively prolonged and lasts for approximately two hours or more. The need for sedation for respiratory function testing is a major contributory factor cited by parents (particularly of healthy infants) when declining to participate in such studies. Nevertheless, most population based studies achieve participation rates of about 25% [37;186;187;332].

1.7. RESPIRATORY FUNCTION TESTS IN INFANTS AND YOUNG CHILDREN: AN OVERVIEW

1.7.1. Introduction

Attempts to measure adult respiratory function date from the 1700s when Humphrey Davy made the first lung volume measurements. The water spirometer was introduced for use in adults in 1846 [364]. However, it was not until the 1890s that the first attempts to measure tidal ventilation in infants were made by Eckerlein [365] and Dohrn [366] using a small spirometer attached to a facemask. Their equipment had excessive dead space and reasonable accuracy was not obtained until the introduction of the head-out plethysmograph by Murphy and Thorpe in 1931 [367]. This allowed tidal ventilation to be determined without any apparatus dead space. In 1949, Cross refined this apparatus by

introducing a neck seal and reported tidal breathing parameters for term infants [368]. An early plethysmograph incorporating a water spirometer was introduced in 1950 [369]. A brief description of the main techniques used to assess lung function are presented below, with a more detailed account of forced expiratory manoeuvres in sections 1.9 and 1.10.

1.7.2. Measurements in infants and young children

The focus on the assessment of airway function during early life, using techniques to measure airway or respiratory resistance and forced expiratory parameters, reflects the fact that wheezing illnesses of either a transient or persistent nature account for majority of the respiratory disorders encountered in infants and young children beyond the neonatal period. More recently, it has been emphasised that measurements obtained from forced expiratory manoeuvres are determined not only by the calibre of the airways but also by airway wall compliance and elastic recoil of the surrounding parenchyma [370].

The lack of standardised equipment and techniques for testing infants in the past, together with the need for sedation, has resulted in a deficiency of normative data, which limits the objective assessment of many respiratory disorders in infancy. In particular, the lack of standardisation hampers collation of data from various centres, since custom-made equipment and different methods have been used to collect data. This has resulted in publication of relatively small individual data sets from which reference equations have been developed; consequently such equations are frequently not generalisable to populations outside the centres that developed them. In recent years, rapid technical advances and collaborative efforts by the European Respiratory Society and American Thoracic Society (ERS/ATS) international task force have begun to address this problem [371-378], and future multi-centre trials and collation of data should be greatly facilitated by the standardisation of equipment and protocols.

1.7.2.1. Oesophageal manometry

As a result of ingenious modifications of infant naso-gastric feeding tubes, oesophageal manometry was used to assess dynamic pulmonary mechanics (i.e., compliance and resistance) in many of the earlier studies in spontaneously breathing infants. When using this technique, changes in airflow and volume at the airway opening are related to simultaneous changes in oesophageal pressure, which reflect changes in pleural pressure (hence the transpulmonary pressure) due to the close apposition of the flaccid oesophagus to the pleural space [379].

The concept of mechanical analysis of the respiratory system is based on data from a series of studies published by Rohrer in 1915 in which he established the fundamental

relationship between pleural pressure, lung elastic recoil pressure and alveolar pressure [380]. However, it was not until the 1950s, following the introduction of electrical recording apparatus [369] and the acceptance of oesophageal pressure as an indirect measure of pleural pressure, that oesophageal manometry became an established technique and a significant number of measurements was recorded. The first oesophageal balloon was described in 1952 [381]. During the 1950s and early 1960s, a series of articles described improved techniques including the development of the "occlusion test" and the appropriate characteristics for oesophageal balloons when used in adults [382-385]. The occlusion test is used to demonstrate that changes in oesophageal pressure accurately reflect those of pleural pressure. Improvements in the use of oesophageal balloons for measurements in infants were described by Beardsmore *et al* in 1980 [386].

In addition to oesophageal balloon catheters, both liquid filled catheters [387;388] and flexible catheters mounted with miniaturised transducers at the tip [389-391] have also been used to measure oesophageal pressure in adults and infants. However, their use in infants beyond the neonatal period, which many parents view as relatively invasive, has been largely superseded by the introduction of the occlusion techniques to assess passive respiratory mechanics (section 1.7.2.5).

1.7.2.2. Static lung volumes

The first measurements of lung volumes in infants were published in the late 1950s [392;393] and used helium dilution which had first been described for use in adults by Willmon and Behnke in 1948 [394]. The early helium dilution technique was only suitable for spontaneously breathing infants. By 1970, measurements in sick infants became feasible when Krauss and Auld introduced a system incorporating a re-breathing bag [395].

Although the nitrogen (N_2) washout technique was described for use in adults by Darling *et al* in 1940 [396], its use in infants was not reported until 1962 [397], with a further report by Ronchetti *et al* in 1975 [398]. In 1985, this technique was simplified by the development of methods using open, rather than closed, circuit [399], and further adapted for use in ventilated infants by Sivan *et al* in 1990 [400].

At the same time as the helium dilution method was being developed for use in infants, whole body plethysmography was being developed to measure FRC and R_{aw} in adults by DuBois *et al* [401;402]. By 1960, this method had been adapted for measuring lung volume in infants [403] and for the assessment of R_{aw} the following year by Polgar [404]. With further modifications, including the addition of a heated re-breathing circuit in the 1970s [405;406], the whole body plethysmograph became established as a standard method for measuring FRC and R_{aw} in infants [407]. Airway function may also be expressed as

conductance (the reciprocal of resistance) or specific conductance by relating conductance to lung volume.

A potential advantage of the plethysmographic technique is that both FRC and R_{aw} can be computed from the plethysmographic data recorded during quiet tidal breathing, provided that BTPS (body temperature, pressure and saturated) conditions are achieved. However, the complexity of maintaining respired gas at BTPS condition using a re-breathing bag, and the related concern regarding infection control, remain a major obstacle. Besides being bulky and expensive, extensive training is essential in order to obtain technically satisfactory and accurate plethysmographic recordings, thus restricting the availability of this test to specialised centres. It should be noted that plethysmographic lung volume (FRC_{pleth}) includes the volume of gas trapped behind closed airways which do not contribute to tidal ventilation [407;408]. In health, similar FRC measurements should be obtained using the plethysmographic and gas dilution techniques within the same subject on one test occasion. However, in the presence of airway disease, estimates of FRC assessed by these different techniques are not interchangeable, since gas dilution techniques only measure the volume of thoracic gas that communicates between the alveoli and airway opening. Thus, in the absence of any technical problems, the presence of gas trapping may be inferred by the difference between the two FRC measurements.

The multiple-breath inert gas washout (MBW) technique was first introduced in the 1950s for measuring FRC (FRC_{MBW}) and for assessing overall ventilation inhomogeneity. The original test was the N_2 MBW test using 100% oxygen (O_2) for the washout [409]. More recently, this technique has been reappraised and modified for use specifically in spontaneously breathing infants and young children. Measurements require a wash-in phase during which the individual breathes a dry gas mixture containing one or more inert tracer gases of different molar mass (for example, the use of both 4% sulphur hexafluoride [SF_6] and 4% helium [He] in 21% O_2 and balance N_2) until equilibration of the concentration of the principal tracer gas is reached throughout the lungs. For the washout phase, the special gas mixture is disconnected during expiration and the individual breathes room air until the end-tidal tracer gas concentration is below $^1/_{40}{}^{th}$ of the starting concentration [410;411]. The gas concentration is measured at the mouth using an appropriate gas analyser while airflow is measured simultaneously by a pneumotachometer or similar flow sensor. In addition to the FRC measurement, several indices of ventilation inhomogeneity may be calculated, the main parameters being the lung clearance index (LCI), mixing ratio (MR) and moment ratio [411-414]. In general, elevated values of these indices are indications that ventilation distribution, as a measure of small airway function, is impaired [411;414].

1.7.2.3. Tidal breathing parameters

Abnormalities of tidal expiratory flow pattern were observed in the 1940's in adults with respiratory diseases [415-417] and were first reported in children with asthma and cystic fibrosis (CF) in 1949 by Kaye *et al* [418] who suggested that tidal flow recordings could be clinically useful. However, it was not until 1981 that Morris and Lane proposed the detailed analysis of tidal expiratory flow pattern as a quantitative method for assessing lower airway function based on measurements in adults [419]. Subsequently, many studies in infants and young children reported detailed analysis of various timed indices during tidal breathing, in particular the ratio of time to peak tidal expiratory flow (t_{PTEF}) to total expiratory time (t_E) which indirectly reflects airway calibre and/or control of breathing due to underlying respiratory mechanics. Infants with bronchopulmonary dysplasia [420] and those exposed to maternal smoking during pregnancy [421;422] have been reported to reach tidal peak expiratory flow more rapidly than age-matched controls. The tidal expiratory ratio (t_{PTEF}: t_E) has also been observed to be lower in male infants who subsequently developed wheezing illnesses [186;423].

Since it is a relatively simple procedure to record tidal breathing patterns in neonates and infants, this method of assessing airway function can potentially be applied to large epidemiological studies, without the need for sedation. This led to a surge of interest in the analysis of tidal breathing indices. However, due to rapidly changing breathing pattern and considerable intra- and inter-subject variability in tidal indices, particularly during early life, data must be collected during quiet sleep with analyses calculated on a large number of breaths [424]. Despite many reports, the relationship between the timing indices and respiratory mechanics remains unclear.

1.7.2.4. Body surface measurements

All the methods described above require accurate measurement of flow and volume changes at the airway opening by using a PNT or similar flow sensor. However, the use of a flow measuring device usually requires a tight-fitting face mask; the application of such equipment may itself affect ventilation due to the increased load and trigeminal stimulation [425;426] and necessitate considerable technical skill. Consequently, numerous efforts have been employed to develop less invasive methods of measuring tidal volume and respiratory timing. Several methods have been assessed for use in both spontaneously breathing and mechanically ventilated infants, including impedance pneumography, magnetometers, strain gauges and respiratory inductance plethysmography (RIP) [427-432], of which RIP has been used to assess airway obstruction and overall efficiency of the respiratory pump (chest wall and respiratory muscles) by analysis of qualitative data of thoraco-abdominal motions [430;433]. Different methods of calibration for RIP have been

described [434;435] including automated qualitative self calibration during tidal breathing (QDC) [436;437].

Although surface measurement has great potential for being a simple and non-invasive test, it has proved somewhat disappointing and failed to achieve widespread use in infants. This is mainly due to the difficulty in achieving optimal calibration in infants, particularly those born preterm, because of their highly compliant chest wall which frequently moves asynchronously with the abdomen [157;438]. Nevertheless, non-invasive RIP has recently been used in an animal model to demonstrate the feasibility of estimating lung volume recruitment in response to a range of positive pressures delivered at the airway opening during high frequency oscillatory ventilation [439]. Tidal ventilation profiles, obtained using RIP in a group of preterm infants receiving nasal continuous positive airway pressure support, have been characterised by a power law functional flow spectrum [440]. Ventilatory responses to hypoxia and hypercapnia, in 3-month old term infants with periodic breathing or obstructive apnoea, have also been investigated using RIP [441].

1.7.2.5. Passive respiratory mechanics

The relative complexity and invasiveness of whole body plethysmography and oesophageal manometry led to efforts in the 1980s to develop simpler tests specifically for infants, namely the airway occlusion techniques [442] and the rapid thoraco-abdominal compression (RTC) technique [443]. These methods permitted the assessment of the mechanical properties of the respiratory system, by measurements of total respiratory compliance and resistance, and assessment of peripheral airway function, respectively. An outline of methods for assessing passive respiratory mechanics is presented below, while the RTC technique is discussed in detail in section 1.9.

In 1954, Comroe *et al* first described measurements of respiratory mechanics during passive expiration in adults and in anaesthetised cats [444]. Such measurements were limited to paralysed or highly trained subjects because of the need for muscle relaxation [445;446]. In 1976, Olinsky *et al* reported the first measurements of passive mechanics in unsedated infants, by making use of the apnoeic pause, with respiratory muscle relaxation, induced in infants by stimulating the vagally mediated Hering-Breuer inflation reflex [447] (section 1.4.2.2). They developed the multiple occlusion technique (MOT) in which a series of brief occlusions at the airway opening were made during inspiration, thereby allowing a pressure-volume plot for the respiratory system to be created, with the slope representing static compliance.

In 1982, Mortola *et al* described the measurement of the respiratory time constant (τ_{rs}) from passive expirations following release of a brief airway occlusion at end-inspiration,

and calculated respiratory resistance (R_{rs}) using this time constant and compliance (C_{rs}), as assessed from the MOT (since $\tau_{rs} = C_{rs} \times R_{rs}$) [448]. In the same year the single breath technique (SBT), in which C_{rs} and R_{rs} were measured from the same breath in anaesthetised cats, was described [449]. Subsequently, Le Souëf *et al* [450] and Thomson *et al* [451] reported the use of the SBT in spontaneously breathing and mechanically ventilated neonates, respectively.

The advantage of the airway occlusion techniques is that data collection is relatively easy, requiring simple and comparatively inexpensive apparatus, which can be set up on a portable trolley allowing cot-side measurements. The occlusion techniques are also well tolerated by young infants in natural quiet sleep. However, it relies on the assumptions that:

- pressure equilibrates rapidly throughout the airways during airway occlusion, so that the recorded pressure plateau at the airway opening reflects the elastic recoil pressure of the respiratory system at any given volume above the end-expiratory level [442;452]
- on release of the occlusion, there is complete relaxation of the respiratory muscles such that expiration is passive
- the respiratory system can be described by a single time constant throughout expiration [450].

These assumptions may not always be valid, particularly in infants with respiratory disease who have elevated resistance and respiratory rates, wherein pressure equilibration is delayed, and inhomogeneous ventilation results in delayed lung emptying from some areas of the lung.

1.7.2.6. Forced oscillation technique (FOT)

The forced (or impulse) oscillation technique is another method of assessing dynamic respiratory mechanics. By applying a sinusoidal pressure waveform (termed a 'forcing' function) to the respiratory system via the tracheo-bronchial tree or thorax, and measuring the resulting flow at the airway opening, the respiratory impedance (Z_{rs}), which reflects both the resistive and elastic properties of the respiratory system, can be determined. Although the technique was first introduced by DuBois *et al* in 1956 [453], it has been used relatively little. Since a re-appraisal in the late 1980s, this technique has been applied to both ventilated and spontaneously breathing subjects of different ages.

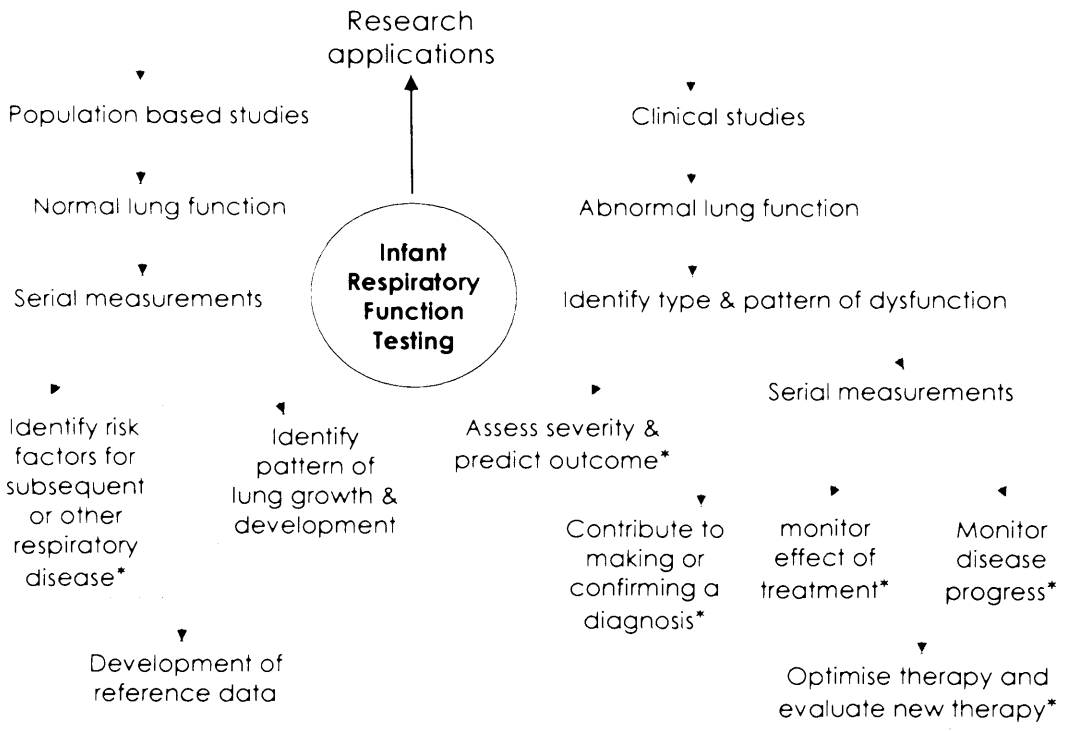
Potentially, this technique is ideal for use in infants and young children, since it imposes pressure oscillations (non-invasively) on the subject's spontaneous breathing, does not disturb the airways and requires no active co-operation [454-460]. However, this method assumes that both the measuring system and the mechanical properties of the respiratory system are linear during the time of measurement and remain so for the amplitude of pressures applied in order for respiratory resistance and reactance to be calculated for each frequency contained in the forcing function. The pressure oscillations may be applied at a single or multiple differing range of frequencies. The mechanical properties of the respiratory system vary depending on the range of frequencies used. In adults, frequencies between 2-32 Hz are generally used [461]. The lower frequencies (1-2 Hz) primarily reflect the behaviour of the parenchyma while the higher frequencies (>5-10 Hz) reflect the mechanical properties of the conducting airways [370;462]. At very high frequencies (>100 Hz), respiratory impedance will contain information on airway wall mechanics [463;464]. Currently, the optimal range of frequencies for use in infants is unclear. Complex mathematical models are also required for data analysis.

1.7.3. Application of RFTs in infants

Despite the difficulties associated with infant respiratory function testing (section 1.6.2), recent years have witnessed considerable advances in this field and widespread application of these tests, as summarised in Figure 1.6. Although infant RFTs are potentially useful in aiding diagnosis and contributing to the evaluation of treatment strategies, their main application has been in research, in both clinical and population based studies (Figure 1.6). The more controversial area in which RFTs are being used has been in the clinical management of individual sick infants [359;360].

Recent intensive international collaborative efforts, involving physiologists, scientists, clinicians and manufacturers, have facilitated consensus on standardisation of equipment, software for data collection and quality control issues regarding data analyses, and have culminated in a series of publications [371-374;376;378;409;465]. These endeavours should improve the feasibility of applying RFTs in clinical settings and permit multi-centre collaborative studies to develop more robust normative data [359;377].

Figure 1.6. Applications of respiratory function testing in infants.



Items marked with asterisks potentially are applicable to clinical studies.

1.8. FORCED EXPIRATORY MANOEUVRES

1.8.1. Introduction

Despite the wide range of RFTs now available for use in infants, the most commonly used approach remains assessment of airway function using either the partial or 'full' forced expiratory (FE) manoeuvres, which are the focus of this thesis. Detailed description of equipment and methodology used in conjunction with these techniques is presented in Chapter 2, with a discussion of relevant background theory and a literature review of their applications during infancy being presented in sections 1.9 and 1.10, respectively.

Measurements of vital capacity were originally made by Hutchinson in 1846 [364]. However, these measurements did not fully evaluate ventilatory function. In 1933, Hermannsen introduced the maximum breathing capacity (MBC) test [466] which became widely used after Baldwin *et al* published normal standards in 1948 [467]. MBC measurements are used to assess dynamic changes in lung volume and are considered

useful in evaluating overall ventilatory function. During the late 1940s and early 1950s, it became feasible to quantify respiratory disability in conditions such as asthma and emphysema by recording rate of exhaled air during a forced expiration [468;469]. This method, known as the forced expiratory manoeuvre, requires the subject to inspire maximally to total lung capacity (TLC) and then immediately exhale as rapidly, completely and as forcibly as possible to residual volume (RV).

Knowledge of the natural history and prognosis of diseases associated with airway obstruction in older subjects has mostly been derived using variables obtained from forced expiration. The FE manoeuvre is widely used, in part because it is relatively easy to perform and the flow-volume curves thus obtained tend to be highly reproducible and relatively easy to analyse. A robust and widely used index from spirometric measurements is the forced expiratory volume in one second (FEV_1), which is considered a reflection of central airway function. When expressed as a percentage of the forced vital capacity (FVC), FEV_1/FVC is a measure of overall airway obstruction. Maximal mid- or end-expiratory flows from the effort-independent portion of the flow-volume curve are considered more sensitive to obstruction towards the lung periphery [470;471]. These measurements are useful because, provided reasonable effort is made, expiratory flow is independent of the force driving flow over most of the expired vital capacity. By comparing forced flows and volumes, it is feasible to demonstrate that lung emptying is slower in individuals with airway obstruction relative to healthy subjects. However, changes in peripheral airway obstruction or resistance cannot be detected by spirometric measurements until the disease has progressed significantly. The theoretical background to these measurements is described in the following section.

1.8.2. Theoretical background

In adults and older children, expiratory flow-limitation is the underlying principle that allows sensitive and useful spirometric assessment of airway function to be made. FE manoeuvres are either performed from TLC, known as maximal forced expiratory manoeuvres, or from some lower volume, termed partial forced expiratory manoeuvres.

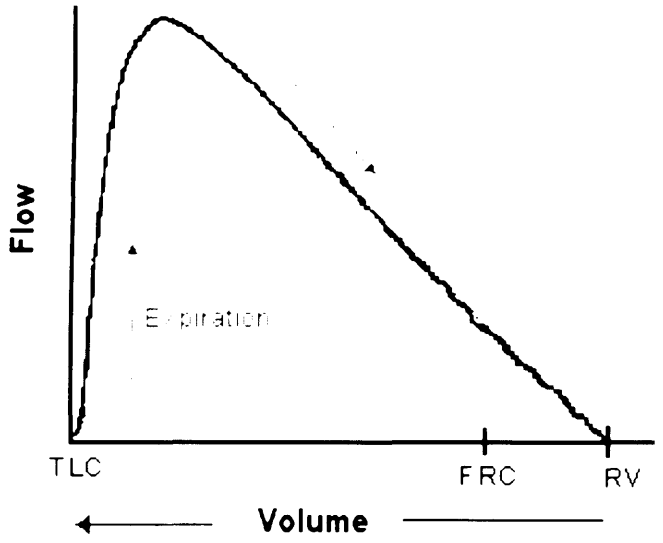
Figure 1.7 illustrates a classical maximal forced expiratory flow-volume (MEFV) curve derived from an adult exhaling as hard as possible to residual volume (RV), following an inspiration from FRC to TLC. The commonly reported indices from a MEFV curve include:

- measurement of the total volume expired (FVC),
- volume expired at a set time interval (FEV_t), most commonly taken at one second after onset of forced expiration (FEV_1),

- peak expiratory flow (PEF), and
- maximal expiratory flow measured at various proportions of FVC ($MEF_{\%}$): for example, maximal expiratory flow at 50% or 25% of FVC remaining in the lungs, which is abbreviated to MEF_{50} or MEF_{25}, respectively. When using the American notation, the latter are referred to FEF_{50} and FEF_{75}, reflecting forced expiratory flows when 50% and 75% of FVC has been exhaled.

These parameters are usually highly reproducible in older subjects.

Figure 1.7. A typical MEFV curve obtained from an adult.



Such data are obtained during a forced expiratory manoeuvre to RV, following a prior maximal inspiration from FRC to TLC.

Although maximum expiratory flow has been shown to be highly dependent on changes in intrathoracic airway size, forced expiration is also determined by the elastic resistance or recoil of the lung and thoracic cage and by airway compression or closure [472-475]. Thus, maximal flow will be reduced if there is loss of elastic recoil of the lung or an increase in airway compliance. Since assessments of forced expiration are likely to reflect the net effect of altered mechanical properties of the lung (for example, reduced airway size and/or lung elastic recoil), they could provide a better measure of the overall dysfunction in individuals with airway obstruction, compared with assessment of R_{aw} on its own [476].

During forced expiration, expiratory flow is independent of the driving pressure over most of the expired vital capacity, once a threshold value of driving pressure is exceeded. This phenomenon is known as expiratory flow-limitation. While FVC provides an indication of the lung volume and FEV_1 reflects central airway function, once flow-limitation is achieved, measurements of $MEF_{\%}$ would no longer be defined by respiratory effort but are exclusively dependent on the resistance of the intrathoracic airways, and according to earlier publications, should therefore represent the calibre of the airways through which air is expelled [473-475;477]. In the past, it has been widely accepted that minor degrees of airway obstruction may be reflected by diminished flows at lower lung volumes, hence flow measurements such as MEF_{25} have been taken to indicate peripheral airway function. This concept has, however, been challenged more recently by suggestions that in addition to airway resistance, flow-limitation is also dependent on airway wall compliance during a forced expiratory manoeuvre, such that the MEFV curve represents the 'integrated output' of the entire respiratory system. As such, it may not be easily partitioned into components to reflect different anatomical regions [476].

An understanding of the underlying physiology of forced expiration is necessary to enable correct application of the technique and valid interpretation of results. During the test procedure, in addition to inspiring to TLC, the subject must employ adequate expiratory effort to achieve flow-limitation, and such effort must be maintained long enough to empty the lungs to residual volume [478]. These manoeuvres are generally easy for adults and older children but more difficult for children < 5-6 years, and impossible without assistance for infants.

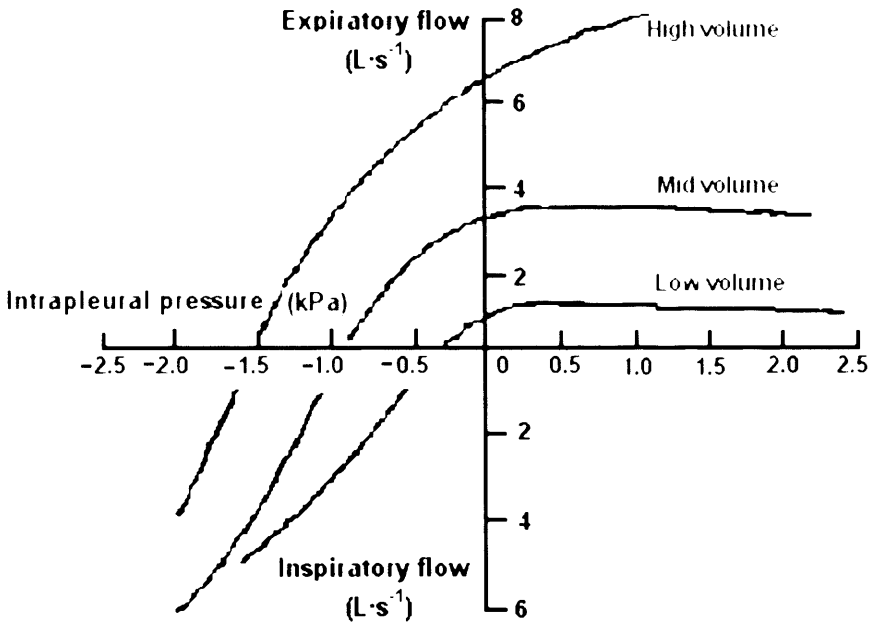
1.8.2.1. Flow-limitation

The mechanism for expiratory flow-limitation is complex. The principle governing the basic mechanism of this phenomenon has been recognised as a combination between airway compression and the pressure drop that occurs downstream along the airways, from the alveoli to the airway opening [472;475;479].

In 1958, Hyatt *et al* created a series of iso-volume pressure-flow curves by plotting intrathoracic pressures (measured using an oesophageal catheter) as a function of expiratory flows measured at the mouth at specific lung volumes for each maximal expiration and inspiration [477]. These iso-volume pressure-flow curves demonstrated that expiratory flow became limited, or effort-independent, at relatively modest positive intrathoracic pressures, as illustrated in Figure 1.8. It can be seen from this Figure that at high lung volumes, the expiratory flow continues to increase correspondingly with increasing effort (accompanied by rising intrapleural pressure). However, at mid or low

lung volumes, flows reach a plateau after a certain minimal or critical effort, and no increment in expiratory flow is achieved with further increase in intrapleural pressure. Under these conditions, flow is said to be limited or effort-independent.

Figure 1.8. Isovolume pressure-flow curves.



These curves were plotted for three lung volumes, each of which was derived from a series of forced expirations and inspirations. (Figure adapted from West [103])

The concept of flow-limitation is widely accepted and three theories have been postulated to explain the physiological mechanisms of this phenomenon:

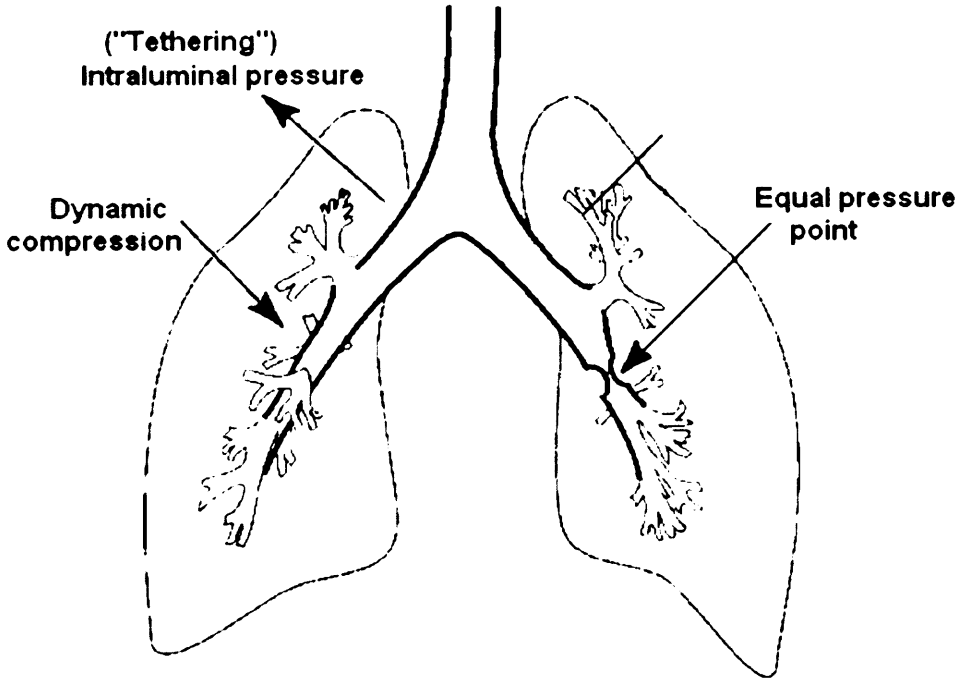
- the equal pressure point theory [472]
- the Starling resistor theory [480], and
- the wave speed theory [475].

These theories are briefly discussed below.

The equal pressure point theory

During inspiration, the intrapleural and alveolar pressures become increasingly negative as lung volume increases. This results in a corresponding increase in transmural pressure (i.e., the pressure difference between the inside and outside of the airways), which contributes to the distention of airway calibre. At end-inspiration, due to the mechanical inter-dependence of the airways and the lung parenchyma, high lung volume also maintains distention of airway calibre. During passive expiration, alveolar pressure (P_{alv}), which represents the sum of lung elastic recoil pressure (P_L) and pleural pressure (P_{pl}), provides the driving pressure for airflow along the airways downstream towards the mouth, where the pressure approximates atmospheric pressure. During forced expiration, the transmural pressure falls with decreasing lung volume and the airways become narrower. With increasing flow during a forced expiration, the driving pressure dissipates within the airways due to the need to overcome resistive forces. As expiration progresses, the intraluminal and extraluminal (or pleural) pressures become equal at certain sites along the intrathoracic airways, and such sites are known as the equal pressure points [472] (Figure 1.9). Distal (mouthward) to the equal pressure point, the intraluminal pressure is less than that surrounding the airway walls externally. Consequently, airway compression occurs and flow becomes limited Figure 1.9.

Figure 1.9. A schematic diagram illustrating the concept of equal pressure point.



At the site of the equal pressure point, intraluminal pressure = extraluminal pressure.

The Starling resistor theory

This theory, derived from the principle of circulatory blood flow, proposes that the driving pressure for maximal expiratory flow is the difference between alveolar pressure and a critical transmural pressure. As air flows downstream (towards the mouth), the driving pressure within the airway dissipates such that at certain point, where the critical transmural pressure exceeds the driving pressure, the airway collapses sufficiently to limit flow [480]. Thus, the Starling resistor and the equal pressure point models share similar concepts in that during forced expiration, intrathoracic airways distal to certain points become narrowed subject to the transmural pressure, and that the principal driving force at maximal flow is the lung's intrinsic elastic recoil [473;474].

The wave-speed theory

This theory postulates that the tracheobronchial tree, similar to other systems carrying flowing fluid, is unable to accommodate an airflow faster than the speed at which pressures can propagate along the airways. The velocity at which pressures will propagate in a given system is referred to as the wave-speed [475]. The tendency for extraluminal pressure to compress the airway is opposed by the stiffness of the airway wall. As lung volume decreases during expiration, the airway calibre decreases and airway compliance increases, both of which reduce the maximal flow that can be carried by the airways. When extraluminal pressure exceeds intraluminal pressure downstream, a more compliant airway will tend to narrow resulting in a segment of the airway acting as a "choke point" that limits flow.

During flow-limitation, the speed of gas molecules flowing in a tube or airway at the site of flow-limitation (choke point) equals the wave-speed and therefore bulk flow is the product of the wave-speed and the cross-sectional area at the choke point. The equation that predicts flow at wave-speed in the airway is:

$$\text{Wave-speed flow} = (1/d)^{1/2} \cdot (dPtm/dA)^{1/2} \cdot A^{3/2}$$

where d is the gas density, Ptm is the transmural pressure, A is the airway cross-sectional area at the site of flow-limitation and dPtm/dA represent a measure of the airway wall elastance at the same site [481]. Thus, in airways with flexible or compliant walls, forced expiratory flow limited by this mechanism is inversely proportional to the gas density and directly proportional to airway wall elastance and cross-sectional area at the choke point. As such, the larger and stiffer the airway and the lower the gas density, the greater the flow that would be achieved through the airway.

As forced expiration proceeds in healthy subjects, choke points multiply and cascade from central to peripheral airways. Flows measured at the mouth, however, reflect the integrated output of all the airways and therefore demonstrate only the stepwise movement of global sites of flow-limitation as these sites move from one level in the bronchial tree where flow has become limited in all contributing pathways to the next. A direct relationship cannot be made between flow measured at the mouth and the function of airways of any particular airway generation.

For the studies reported in this thesis, airway function was assessed using both partial and raised volume forced expiratory manoeuvres, by applying external thoraco-abdominal pressure at the end of a normal tidal inspiration, and after inflating the lungs to a pre-determined positive pressure of approximately 3 kPa, respectively.

1.9. THE TIDAL RAPID THORACO-ABDOMINAL COMPRESSION (RTC) TECHNIQUE

1.9.1. Background

Although measurements using spirometry rapidly became established as the gold standard for assessing airway function in older children and adults in both clinical and research studies, such measurements were not possible in infants until the late 1970s. Despite the fact that infants cannot be asked to take a deep breath and breathe out forcibly, such manoeuvres can nevertheless be achieved by applying an external compressive force around the thorax and abdomen, while measuring flows at airway opening.

In 1977, Taussig *et al* reported measurements of partial forced expiratory flows in 4-6 year old children [482]. In the same year, Motoyama *et al* obtained full MEFV curves in intubated infants by applying a negative pressure at the airway opening after several passive lung inflations [483]. The following year, Adler and Wohl first described the application of an external rapid thoraco-abdominal pressure compression (RTC) during spontaneous tidal breathing in healthy, sedated infants placed within a negative pressure chamber, and succeeded in recording partial forced expiratory flow-volume (PEFV) curves using a pneumotachometer (PNT) connected to a face mask placed over the nose and mouth [484]. The externally applied compression pressure substituted voluntary effort to force expiration. Although effective, this technique was relatively cumbersome and was modified in the 1980s by substituting the pressure chamber with an expandable jacket, which was wrapped around the chest and abdomen and inflated at the end of a tidal inspiration to force expiration [485]. Commonly known as the 'squeeze' technique,

the tidal RTC technique was the first practical, non-invasive method that was feasible for use in both healthy infants and those with lung disease.

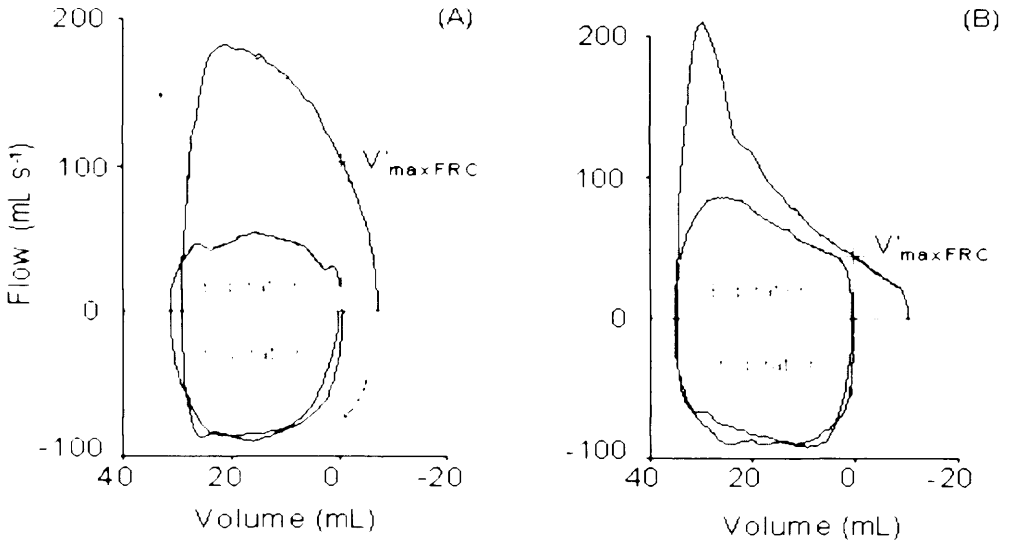
Similar to spirometric measurements in older subjects, the tidal RTC technique aims to assess airway function by achieving expiratory flow-limitation. Since this assessment is performed over the tidal volume range, it is considered to be a measure of partial forced expiratory flow only. The maximal expiratory flow at FRC (V'_{maxFRC}), the principal parameter determined from PEFV curves, is thought to reflect the function of peripheral airways [109;172;443;485]. Subject to flow-limitation, V'_{maxFRC} is independent of effort and upper airway resistance [172], whereas peak forced expiratory flow is not considered to be useful, as it is mainly determined by compression pressure [486]. Due to its relative simplicity, the tidal RTC technique has since become one of the most extensively used techniques in physiological, pharmaceutical and clinical studies involving infants (Table 1.7 to to Table 1.10). The body of information derived from these studies has contributed substantially to the knowledge of airway function in infancy.

1.9.2. Shape of PEFV curves

Previous studies using the tidal RTC technique reported marked within and between subject variabilities of V'_{maxFRC} [109;487]. It has, therefore, been suggested that the sensitivity of PEFV curves can be increased by assessing the shape of the curves rather than reporting V'_{maxFRC} values alone [488].

Having assessed the shape of the forced expiratory flow-volume portion as a reflection of airway status, Le Souëf *et al* [488] reported that convex (to the volume axis) curves are more common in normal infants, whereas concave curves are more frequently observed in infants with airway disease (Figure 1.10). In addition, infants with flow-limitation during tidal expiration, where application of external compression pressure does not increase expiratory flow, are likely to have more severe airway disease than those without flow-limitation on tidal expiration. Furthermore, these authors found that shape of the expiratory curve was determined by the external compression pressure and suggested that comparisons should be made at a standardised jacket transmission pressure whether or not flow-limitation had been reached [486;488]. As discussed later (section 1.9.3), this suggestion has, however, subsequently been challenged [489].

Figure 1.10. PEFV curves obtained from a healthy infant and one with airway disease.



(A): a convex flow-volume curve from a healthy infant and (B): flow-volume curve from an infant of similar age and weight with evidence of airway obstruction, who not only had a much lower V'maxFRC recorded but the trace has a characteristically concave shape.

1.9.3. Jacket compression pressure

During forced expiratory manoeuvres, the driving pressure (indicated by the percentage of pressure transmitted across the chest wall from the expandable jacket to the pleural space and hence the intrathoracic structures) varies between infants. It is influenced by the type of jacket used and how tightly it has been applied, the amount of reservoir pressure delivered to the jacket bladder, and the infant's respiratory compliance [443;486].

To assist data interpretation and quality control, it is essential to assess the transmission of jacket compression pressure. This can be measured using oesophageal manometry [490] or the airway occlusion technique to determine pressure throughout the respiratory system during an end-inspiratory airway occlusion prior to, and whilst jacket has been inflated [443;486]. In the studies presented in this thesis (Chapters 3 and 4), the latter procedure was used to assess the magnitude of jacket compression pressure (P_j) transmitted to the intrathoracic structures, i.e., the transpulmonary or driving pressure.

When an airway occlusion is performed during tidal breathing, under conditions of no air flow, pressure equilibrates within the lungs so that pressure at the airway opening (P_{ao}) is equal to alveolar pressure (P_{alv}) (shown as P1 in Figure 1.11). Provided the occlusion is