

Simpler methods of assessing respiratory function and their application in infancy

A thesis submitted by
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For my son, Hamish

Abstract

The need to develop and evaluate simple non-invasive tests to measure respiratory function in wheezy infants, in order to investigate the physiological basis of lung disease and move towards a more rational basis for the treatment of airway disease in infancy was well recognised at the inception of this work. The main aims of this thesis were:

- to evaluate simpler methods of assessment of lung function such as the tidal breathing parameter $t_{PTEF}:t_E$, passive respiratory mechanics using the Single Breath technique and the rapid thoraco-abdominal compression technique (RTC) by comparison with established “gold standard” techniques, and:
- to address applications of such assessments, when comparing respiratory function in survivors of each limb of the Collaborative ECMO trial.

$t_{PTEF}:t_E$ and specific airway conductance were measured in healthy infants and those with recurrent wheezing and it was found that both parameters were significantly lower in the wheezy compared to the healthy group. There was a significant although weak association between these variables in infants, irrespective of prior wheezing status, which was also confirmed in the ECMO Respiratory Follow-up population.

Although a variable relationship between respiratory and airway resistance was found, both were significantly higher in infants with prior wheeze. Measurements obtained using the Single Breath technique had a relatively high failure rate.

Inter observer variability was compared within and between two specialised infant lung function testing centres and a strategy developed for performing and analysing infant respiratory function tests to facilitate future similar trials. A collaborative approach to trials with infant respiratory function as an outcome measure appears feasible, providing close attention is paid to study design.

Airway function was compared in survivors of the Collaborative ECMO trial. Respiratory function outcomes in those managed conventionally suggested that the larger proportion of these infants receiving respiratory medication and reporting respiratory symptoms may be attributed to subtle impairment of small airway function, relative to those assigned to ECMO. These findings probably reflected differences in neonatal management, since initial disease severity and background characteristics were similar in both groups.

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Symbols, abbreviations and units

The symbols, abbreviations and units used in this thesis are based on the ATS-ERS Working Party on “Infant Pulmonary Function Testing” recommendations (Quanjer et al 1997). The main abbreviations used and conversion factors between S.I. (System International) and traditional units are summarised in the table below.

conversion factors

Pressure $1 \text{ cmH}_2\text{O} = 0.098 \text{ kPa}$ $1 \text{ mmHg} = 0.133 \text{ kPa}$

Compliance $1 \text{ mL} \cdot \text{cmH}_2\text{O}^{-1} = 10.2 \text{ mL} \cdot \text{kPa}^{-1}$

Resistance $1 \text{ cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s} = 0.098 \text{ kPa} \cdot \text{L}^{-1} \cdot \text{s}$

Table of abbreviations and conversion factors

abbreviation	description	abbreviation	description
A, Alv	alveolar	CDH	congenital
AB	abdomen		diaphragmatic hernia
AC	analysis centre	CI	confidence intervals
ANOVA	Analysis of variation	cm	centimetre
ao	airway opening	CM	conventional
app	apparatus		management
ATS	American Thoracic Society	cmH ₂ O	centimetre of water
aw	airway(s)	CO ₂	carbon dioxide
B	barometric	CPU	central processing unit
bar	unit of pressure	CV	coefficient of variation
Br	bronchial	cw	chest wall
BTSPS	body temperature, barometric pressure and saturated with water vapour	ds	dead space
C	compliance	dyn	dynamic
		E	elastance
		E, exp	expiratory
		ECMO	extracorporeal
			membrane
			oxygenation
		EE	end expiratory

abbreviation	description	abbreviation	description
EEL	end expiratory level	IL₂	Interleukin 2
el	elastic	IL₄	Interleukin 4
EPP	equal pressure point	inf	infant
ERS	European Respiratory Society	IQR	inter quartile range
F	female	IRDS	idiopathic respiratory distress syndrome
FEV_t	forced expiratory volume in <i>t</i> seconds	J	jacket
F_{I,O₂}	fractional inspired oxygen concentration	JV	jet ventilation
FRC	functional residual capacity	kg	kilogram
FVC	forced expiratory vital capacity	kPa	kilopascal
G	conductance (1/ resistance)	L	length
g	gram	L	litre
GA	gestational age	LEIC	Leicester
G_{aw}	airway conductance	LRI	lower respiratory illness
GOS	Great Ormond Street Hospital	M	male
H₂O	water	m	metre
HBIR	Hering-Breuer Inflation reflex	max	maximal
He	helium	mB	mega byte
HFO	high frequency oscillation	MEFV	maximal expiratory flow-volume curve
Hz	hertz	min⁻¹	per minute
I, insp	inspiratory	MIT	multiple interruption technique
IgE	Immunoglobulin E	mL	millilitre
IgG	Immunoglobulin G	MLR	multiple linear regression
II	initial inspiratory	mm	millimetre
		mmHg	millimetre of mercury

abbreviation	description	abbreviation	description
MOT	multiple occlusion technique	r²	coefficient of determination
ms	millisecond	RAR	rapidly adapting stretch receptor
N₂	nitrogen	RASP	Respiratory Analysis Program
NO	nitric oxide	RC	ribcage
NPEU	National Perinatal Epidemiology Unit	RIP	respiratory inductance plethysmograph
O₂	Oxygen	RR	respiratory rate
occ	occluded/occlusion	rs	respiratory system
P	pressure	RTC	rapid thoraco-abdominal compression technique
p	probability	RV	residual volume
Pa	pascal	RVRTC	raised volume rapid thoraco-abdominal compression technique
PC	personal computer	s	second
PEEP	positive end expiratory pressure	SAR	slowly adapting stretch receptor
PEF	peak expiratory flow	SBT	single breath technique
PEFV	partial expiratory flow-volume curve	SD	standard deviation
pl	pleural	SEM	standard error of the mean
pleth	plethysmographic	sG_{aw}	specific airway conductance (G_{aw}/FRC)
PNA	postnatal age	SpO₂	pulsatile oxygen saturation
PNT	pneumotachograph	T	tidal
P_{TCO₂}	transcutaneous CO ₂ tension	t	time
PTEF	peak tidal expiratory flow		
PTIF	peak tidal inspiratory flow		
PVD	pulmonary vasodilator		
QS	quiet sleep		
R	flow resistance		
R	respiratory		

abbreviation	description	abbreviation	description
TAA	thoraco-abdominal	τ	time constant
	asynchrony	°C	degree Celsius
TC	test centre	μg	microgram
t_E	expiratory time		
t_I	inspiratory time		
ti	tissue		
tm	transmural		
TOGV	total occluded gas volume		
tot	total		
t_{PTEF}	time to peak tidal expiratory flow		
URTI	upper respiratory tract infection		
V	gas volume		
V	volts		
V'	gas flow		
V'_{max}	maximal expiratory flow		
$V'_{\text{max,FRC}}$	maximal expiratory flow at FRC		
VA	veno-arterial		
V_T	tidal volume		
VV	veno-venous		
WRLI	wheezing lower respiratory illness		
%	percentage		
Δ	delta; change in variable		
°	degree		
δ	delta; change in variable		

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Declaration

I have undertaken all the measurements presented in Sections 4 and 5, with the exception of a few infants who were recruited as controls and measured before my arrival at the Institute of Child Health, London. All the data analysis, however, was performed by myself.

The study design of the ECMO Respiratory Follow-up presented in Section 6 meant that 36 of the 78 infants attended for respiratory function testing at Leicester Royal Infirmary. The data from all 78 infants was analysed both by myself and Dr Caroline Beardsmore and colleagues at Leicester Royal Infirmary and cross-reported (details in Section 6.3.2.1). In Section 3 I based the description of methods on the relevant Chapters of Infant Respiratory Function Testing (Stocks et al 1996) with the consent of the editors, to which standards of data collection and analysis within this thesis adhere. The data presented in this thesis has formed the basis for several related publications which are appended. This work has not been accepted in any previous application for a degree.

Publications

Publications related to the work presented in this thesis are shown here. Those that are based directly on work presented within this thesis are appended and indicated by an asterix*.

*Dezateux CA, Stocks J, **Dundas I**, Jackson EA, Fletcher ME. The relationship between tPTEF:tE and specific airways conductance in infancy. *Pediatr.Pulmonol.* 1994;18:299-307.

***Dundas I**, Dezateux CA, Fletcher ME, Jackson EA, Stocks J. Comparison of single-breath and plethysmographic measurements of resistance in infancy. *Am.J.Respir.Crit.Care Med.* 1995;151:1451-8.

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Dezateux C, Stocks J, **Dundas I**, Fletcher ME. Impaired Airway Function and Wheezing in Infancy. The influence of maternal smoking and a genetic predisposition to asthma. *Am.J.Respir.Crit.Care Med.* 1999;159(2):403-10.

Dezateux CA, Fletcher ME, **Dundas I**, Stocks J. Infant respiratory function after RSV-proven bronchiolitis. *Am.J.Respir.Crit.Care Med.* 1997;155:1349-55.

Jackson E, Stocks J, Pilgrim L, **Dundas I**, Dezateux CA. A critical assessment of uncalibrated respiratory inductance plethysmography (Respirtrace) for the measurement of tidal breathing parameters in newborns and infants. *Pediatr.Pulmonol.* 1995;20:119-24.

1. Introduction and aims

The hypothesis that respiratory illness during the first year is associated with increased respiratory morbidity later in childhood and adult life continues to gather support (Strachan 1990, Barker et al 1986, Samet et al 1983, Barker et al 1991, Shaheen et al 1994, Barker 1995, Sayer et al 1997). The search for improved methods of detecting, treating and preventing respiratory disease in infants has resulted in an increasing interest in the physiology and pathophysiology of the respiratory system during the first year of life. Infant lung function tests have been used to define normal growth and development, to assess gender differences and to identify infants who may be at subsequent risk of respiratory disease, as well as to evaluate the impact of the environment and genetics on the developing lung.

Abnormalities in lung volume and airway function have been identified shortly after birth, which may predict wheezing respiratory illness in the first year of life (Martinez et al 1988, Martinez et al 1991). Measurements of respiratory function have helped to assess progress of the individual infant with respiratory disease, and their response to therapy (Helms et al 1982, Gutkowski 1990, Werchowski et al 1990, Beardsmore et al 1994, Numa et al 1995). Lung function tests would ideally help in the diagnosis and clinical management of these patients, but to date there is no equivalent to the “gold standard” of spirometry in adults and older children: a rapid, reproducible and sensitive method of assessing airway function, where the physiological determinants are well characterised (American Thoracic Society 1995, Castile 1998). An additional problem is the variation in specificity and sensitivity between the various methods that are applicable during the first year of life.

However, conventional methods such as whole body plethysmography not only require sophisticated equipment and highly trained personnel, but are also unsuitable for routine clinical assessment. In recent years attention has focused on simpler methods of measuring airway function that may be applied in both clinical and epidemiological settings. These methods include the single breath technique for measuring respiratory resistance and compliance (LeSouëf et al 1984b, Marchal et al 1988, Prendiville et al 1986, Gerhardt et al 1989, Haouzi et al 1991, Henderson et al 1995, Gappa et al 1993), tidal breathing parameters (Lødrup et al 1992, Lødrup 1992, Kummer et al 1977, Aston et al 1994, Clarke et al 1994, Eid et al 1994, Stick et al 1996, Lødrup Carlsen 1997) and the rapid thoraco-abdominal compression

technique (Tepper et al 1986, Taussig et al 1982)

The aims of this thesis were to evaluate simpler methods of assessment of lung function by comparing them to those of the established “gold standard” techniques, with respect to validity, applications and error of measurement, and to address applications of such assessments, together with some of the methodological issues raised, when comparing respiratory function in survivors of each limb of the UK Collaborative ECMO trial (UK Collaborative ECMO Trial Group et al 1996).

The specific objectives were:

1. In 50 healthy infants, and 50 infants with recurrent wheezing:
 - To compare measurements of plethysmographic airway resistance (R_{aw}) and total respiratory resistance (R_{rs}), using the Single Breath technique.
 - To examine the association between the ratio of the time to reach tidal peak expiratory flow to total expiratory time ($t_{PTEF}:t_E$) and specific airway conductance. (sG_{aw}), and;
 - To evaluate the relative ability of each of the simpler methods as compared to the “gold standard” plethysmographic measurements to assess airway function in infants with recurrent wheezing.
2. In 78 survivors of neonatal respiratory failure measured at one year of age:
 - To compare inter observer variability within and between two specialised infant lung function testing centres with respect to these measures of airway function
 - To compare respiratory health and function at one year in infants who were assigned to receive ECMO with that of similar infants who were assigned to conventional management.
 - To investigate the association between measurements of forced expiration and specific conductance, and of $t_{PTEF}:t_E$ and specific conductance, in this group of survivors of neonatal respiratory failure

This section begins with a review of the growth and development of the respiratory system during early life (section 2.1), then the determinants of wheezing and methods of assessing wheezing are briefly surveyed (section 2.2). The background to the application of ECMO in infants is described in section 2.3. Finally, this review of the literature concludes with a short history of the development of infant respiratory function testing (section 2.4).

2. Review of the literature

2.1 Growth and development of the respiratory system

Recent work by Barker and colleagues suggests that impaired growth and development during fetal life due to undernutrition may be associated with morbidity in later life (Barker 1995). The expression of wheezing illness is usually the result of an underlying genetic predisposition influenced by environmental factors- such as infection, or exposure to tobacco smoke. To examine these associations, and in order to evaluate measurements of respiratory function in both healthy and wheezy infants, understanding of the normal pattern of growth and development is needed. Although alveolar development has been well investigated (Hislop et al 1972, Hogg et al 1970), little is known about the relative development of the various structures within the respiratory system and their relationship to wheezing in infancy (Jeffery 1995). The influence of the pattern of development of the pulmonary and bronchial circulation on wheezing disorders requires further work (Sly et al 1995). In this section some of the central features of growth, development and function of the human lung pre- and postnatally are summarised, and some of the mechanical adaptations employed by the human infant to overcome the structural problems of an immature, rapidly developing system described. Comprehensive reviews of lung growth and development form the basis of this section (Hislop et al 1972, Hislop 1995, Thurlbeck 1975, Thurlbeck 1981, Yip et al 1991, Hislop et al 1974, Thurlbeck 1992).

2.1.1 Prenatal growth

At birth, all the major structures of the lung have developed although the lung is unique in growing and developing *in utero* without fulfilling its postnatal role, which suggests that growth is mainly genetically rather than functionally determined, although influenced by environmental factors *in utero* such as amniotic fluid circulation, space available for lung growth or maternal smoking during pregnancy (Hislop 1995). Lung development has classically been described in four main stages, although there is considerable variation between individuals and development occurs as a gradual, rather than phasic, process (Table 2-1).

Table 2-1 Stages of lung development

Embryonic	0-7 weeks gestation	Lung buds form Blood vessels connect to heart
Pseudoglandular	6-17 weeks gestation	Preacinar airways and blood vessels develop.
Canalicular	16-27 weeks gestation	Respiratory (intra-acinar) region develops. Thinning of peripheral epithelium and mesenchyme. Type I and II pneumonocytes
Alveolar	27 weeks to term	Development of saccules and then alveoli
Postnatal	Up to 18 months Up to adulthood	Alveoli and small blood vessels multiply All structures increase in size- simple growth

2.1.1.1 Airway development

During the embryonic stage, the airways of the lung begin developing 26 to 28 days after conception (Jeffery et al 1994). Airway branching requires both epithelium and mesenchyme. If the mesenchyme is stripped away, the airway will continue to grow but will fail to divide (Hislop 1995). The branching process is dependent on interactions between cell substrate adhesion molecules and the extracellular matrix proteins such as proteoglycans and glycosaminoglycans (Masters 1976). The most rapid phase of airway branching occurs between 10 and 14 weeks of gestation, during the pseudoglandular stage. All airway branching to the level of the terminal bronchiolus, that is to the pre-acinar airways, is complete by the sixteenth week of gestation (Hislop et al 1974). Submucosal glands appear at 10 weeks (Hislop et al 1974), and smooth muscle cells are present in the trachea and lobar bronchi at between 8 and 10 weeks (McCray 1993). Following the completion of branching, during the canalicular stage, the airways continue to increase in both diameter and length. The amount of cartilage, gland and smooth muscle in the airway wall also increases (Hislop 1995). Muscle in the airway wall continues to extend to the periphery until around 26 weeks of gestation, when it has reached the level of the respiratory bronchi (Hislop et al 1989).

2.1.1.2 Blood vessel development

During the embryonic period, the lung is supplied by a plexus of blood vessels from the dorsal aorta. By the sixth week of gestation, there is a link from the heart via the pulmonary artery and the sixth aortic arch and to the heart via a pulmonary vein. Links remain to the dorsal aorta to and from the bronchial arteries. The pre-acinar arteries grow alongside the airways and the pre-acinar veins grow at the same rate as the airways so that all are present by 16 weeks of gestation, although blood flow through the vessels is low during this period (Hislop 1995). Intra-acinar vessels appear as the respiratory airways and alveoli develop. During fetal life, the pulmonary arteries are thick walled and the veins have very little muscle in their walls. There is a rapid drop in relative wall thickness of the arteries immediately after birth as pulmonary vascular resistance falls, shown in neonatal pigs to be due to reorganisation of the shape and orientation of muscle and endothelial cells (Haworth 1993).

2.1.1.3 Alveolar development

During the canalicular stage, thinning of the epithelium and mesenchyme takes place, and blood vessels come to underlie the airway epithelium. By 22 weeks gestation, sac shaped alveolar ducts (saccules) are present, lined by type I and II alveolar epithelial cells. By the end of the canalicular stage the blood-gas barrier is narrow enough to support gas exchange (about $0.6 \mu\text{m}$) but the gas exchanging units are large thin walled saccules, which will later develop into alveoli. However, infants delivered at this stage are viable although there are no true alveoli present. Between 28 and 32 weeks gestation, small crests around the luminal edge of each saccule formed by discrete bundles of elastin elongate to form primitive alveoli (Hislop et al 1974). By 29 weeks, there are around 30 million alveoli and this number increases to around 150 million by 40 weeks of gestation (Hislop et al 1986). Although there is considerable variation in number, between one third and half the adult complement of alveoli is present at term birth. Alveoli continue to form until around three years of age (Zeltner et al 1987). The rapid increase in lung volume seen during the first year of life is due mainly to the increase in number and size of the alveoli (Wilson et al 1995), the surface area of the lung increases and shows a linear relationship to both age and body weight.

2.1.1.4 Control of airway development

Factors affecting lung development include hormones and growth factors that affect proliferation of epithelium (insulin-like growth factor-1 and epidermal growth factor) and those that inhibit cell multiplication but promote protein synthesis (transforming growth factor β) (Hislop 1995). Effects in utero which constrain lung growth, such as malnutrition, congenital diaphragmatic hernia and oligohydramnios, have been associated with reduced alveolar and capillary surface area in animal models (Sly et al 1995), impaired airway function during late childhood (Holt et al 1995) and an increase in standardised mortality from chronic obstructive airways disease in adult life (Barker et al 1991). A key factor in regulating lung growth is the volume at which the lung is held, which is influenced by the rate of secretion of lung fluid, the outflow resistance and the space available for the lung to grow (LeSouëf 1995). Studies in fetal sheep have shown that the lungs are maintained in a hyperexpanded state by lung liquid, secreted by the epithelium of the fetal lung. Lung liquid may act as a template regulating the dimensions of the developing air spaces. This regulatory mechanism is particularly vulnerable to disruption during the canalicular phase of development (Landau 1995). Fetal breathing movements are also thought to be a determinant of normal lung development, and have been used as an index of fetal wellbeing in pregnancies complicated by oligohydramnios, with conflicting results (Blott et al 1990, Korsgaard 1983). The fetal larynx may participate in these breathing movements, as well as acting as a valve regulating the flow of lung fluid out into the amniotic sac (Bartlett 1989).

2.1.2 Lung growth and development during infancy

Immediately after birth, catecholamine driven clearance of lung fluid takes place and a resting lung volume is established, with a corresponding rise in lung compliance and reduction in resistance (Stocks 1995). Adrenaline also triggers the release of surfactant by the lamellar bodies of the type II pneumonocytes. Surfactant serves to stabilise the alveoli, to increase lung compliance and hence reduce the work of breathing, and to prevent transudation of fluid into the alveolar space (Yee et al 1991, Jobe 1993). During the period of rapid growth in infancy which follows, major developmental changes in respiratory physiology occur, which will affect the interpretation of respiratory mechanics and lung volume measured in this age group

(American Thoracic Society/European Respiratory Society 1993). These are summarised in section 2.1.2.1 below.

2.1.2.1 The relationship of chest wall to lung during infancy

The chest wall is highly compliant at birth and during early infancy. In neonates, lung compliance is very similar to respiratory compliance (Papastamelos et al 1995), at around $40 - 50 \text{ mL.kPa}^{-1}$, but during the first two years of life, the chest wall gradually stiffens, undergoing ossification and changes in configuration (Openshaw et al 1984, Bryan et al 1986). The chest wall is therefore less effective during early infancy, because of its shape, high compliance and deformability, and this may limit the contribution of the rib cage in relation to that of abdominal movement during tidal breathing. In the newborn, the ribs are relatively horizontal at rest which limits the potential increase in thoracic cross-sectional area (Openshaw et al 1984). The floppy chest wall limits expansion, which may predispose to inward movement (recession) of the rib cage during diaphragmatic contraction during inspiration (Gerhardt et al 1980) and this, together with the reduced mechanical coupling between the diaphragm and rib cage may lead to reduced mechanical efficiency of breathing (Heldt et al 1987), with work expended on distorting the rib cage.

A degree of thoracoabdominal asynchrony (TAA) is also seen in both premature and wheezy infants (Hershenson et al 1990, Allen et al 1990, Heldt et al 1987, Allen et al 1991), during episodes of acute upper airway obstruction (Sivan et al 1990, Davis et al 1993), and during active sleep in normal premature and newborn infants (Gaultier et al 1987, Kirkpatrick et al 1976). The significance of thoracoabdominal asynchrony depends on the contribution of the chest wall to respiratory efforts, the mechanically inefficient strategy is of less consequence if there is only a small chest wall contribution (Hershenson et al 1990).

The efficiency of the respiratory pump is further reduced by the relative paucity of Type I skeletal muscle fibres (slow twitch, high oxidative fibres) which increases the risk of developing respiratory fatigue (Muller et al 1979). The net effect of all these factors is to result in an increased risk to infants and young children of developing respiratory muscle fatigue in the face of respiratory muscle loading, whether due to decreased lung compliance or increased resistance to airflow (Polgar et al 1979).

The chest wall in infancy also influences the function of the underlying lungs, the highly compliant chest wall with low outward recoil (Gerhardt et al 1980, Colin et al 1989) resulting in relatively low transpulmonary pressures (Helms et al 1981). The low outward recoil allows the lungs to deflate to low volumes where the small peripheral airways may close during late expiration, thus impairing gas exchange and ventilation-perfusion balance (Davies et al 1992) but also, with the absolute small size of the airways, rendering the infant vulnerable to intra-thoracic airway obstruction (Martinez 1991).

One strategy observed in infants that serves to defend lung volume during this period is modulation of expiratory flow by a combination of laryngeal and postinspiratory diaphragmatic braking, which can prolong the expiratory time constant sufficiently to dynamically elevate functional residual capacity (FRC or resting lung volume) above that which would be passively determined by the balance between outward recoil of the chest wall and inward recoil of the lung (Kosch et al 1984, Stark et al 1987, Kosch et al 1988, Mortola et al 1985). Transition to a more relaxed pattern of expiratory flow occurs between 6 and 12 months of age (Colin et al 1989, Ratjen et al 1989).

2.1.2.2 Growth and development of the airways

Although airway branching is complete by the 16th week of gestation, the airways continue to increase in size as lung volume increases. From 22 weeks of gestation until birth there is a linear increase in diameter in all airways from the bronchus to the terminal bronchiole, continuing after delivery with airways increasing in diameter and length up to threefold between birth and adulthood (Hislop et al 1989, Jeffery 1995). In addition to the effects on the airways of low transpulmonary pressures, the growth pattern of the airways and parenchyma may themselves influence airway function in early infancy. Airway conductance (the reciprocal of airway resistance) has been found to be relatively higher at birth compared with that at around 2 to 3 months of age (Doershuk et al 1974, Tepper et al 1986). As described above, there are temporal differences between multiplication of the alveoli (mainly after term birth) and airway branching (complete by 16 weeks gestation) which may result in larger airways relative to lung volume soon after term birth. The term dysanapsis was coined to describe this type of disproportionate growth. Mead et al postulated that

airway and parenchymal lung growth must proceed at unequal rates because the intersubject variability of maximal expiratory flow in normal adults was not explained by the variability of lung volumes (Mead 1980). In a term infant lung volume doubles by 6 months and triples by one year (Stocks 1977) and lung compliance shows a similar increase.

Although infants are obligate nose breathers and nasal resistance may account for up to 50% of total resistance (Stocks et al 1978b), specific airway conductance (conductance divided by lung volume) is greater in neonates than adults (Stocks et al 1977). Pathologic studies suggest that small airways (beyond the 12th to the 15th generation) have relatively greater resistance in young children compared with adults (Hogg et al 1970). This observation is supported by physiological studies of airway conductance in infants with congenital pulmonary hypoplasia (Helms et al 1982), although not by observations in newborn infants, as assessed by forced expiratory flows (Taussig et al 1982). There have also been gender differences reported with airways being narrower and hence conductance lower relative to FRC in male infants (Tepper et al 1986).

The major determinants of respiratory resistance are airway calibre, lung volume and tissue and chest wall resistance. Airway calibre is of particular importance- resistance of a tube is critically dependent on the diameter of the tube rather than the length, and theoretically if the radius is halved resistance increases by a factor of 16 and therefore factors influencing airway calibre- such as airway smooth muscle tone, mucus or oedema will in turn affect measured values of airway resistance. Airway resistance is also affected by the tethering effect of the lung parenchyma and transmural pressures across the airway wall (Shen et al 1997, Adler et al 1998).

Tissue resistance has recently evoked more interest, and it has been suggested that bronchoconstrictors may affect the parenchymal tissues of the lungs of mature and immature animals differently (Tepper et al 1995). Animal studies have demonstrated that a large component of the increase in pulmonary resistance that occurs in response to challenge with bronchoconstrictors is due to increases in lung tissue resistance (Robatta et al 1993). The mechanism for this is not fully understood yet, but may reflect maturational changes in the coupling of small airways and alveolar smooth muscle, or the presence of contractile elements in the parenchyma.

2.1.2.3 Airway smooth muscle and reactivity

Airway smooth muscle cells first appear in the trachea and in the primary and lobar bronchi at around 6 to 8 weeks gestation (Jeffery 1995). At birth, there is relatively less airway smooth muscle than in the adult (Hislop et al 1989, Matsuba et al 1972). 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 (Hislop et al 1972). 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 (Hislop et al 1989).

Smooth muscle tone is modulated by both slowly adapting stretch receptors (SARs), which normally evoke smooth muscle relaxation, and rapidly adapting receptors (RARs) and pulmonary C fibres, which normally promote cough and bronchoconstriction (Stocks 1995). In newborn infants, the coupling between smooth muscle and SARs is affected by the mechanical properties of both the cartilage of the large airways as well as the elastic recoil of the lungs and chest wall, and also by the relatively small number of RARs. Any given increase in airway resistance may reflect the differing contributions of both airway smooth muscle shortening and the relative thickness of the airway wall. Therefore, airway inflammation will increase and potentiate the effect of smooth muscle shortening on airway resistance, although the contribution to increased resistance may vary with airway generation, being opposed in the small airways by elastic lung recoil. The sum effect of these structural and physiological differences between infant and adult airways may result in different patterns of airflow limitation.

The stimulus for rapid development of airway smooth muscle after birth is attributed to the onset of air breathing (Hislop et al 1989). Additionally, an increase in the quantity of airway smooth muscle is also seen in premature and ventilated infants (Hislop et al 1989) as well as in children with a history of bronchiolitis (Matsuba et al 1972). The functional consequence of this is a greater increase in resistance in the

small airways for a given amount of smooth muscle shortening.

The mechanism underlying the response of infants to bronchial challenge appears to be similar to that of adults, and is also present in healthy infants with no history of lower respiratory illness (Montgomery et al 1990, Geller et al 1988, Prendiville et al 1987a, Tepper 1987). The structure and the innervation of airway smooth muscle is similar in infants and adults (Hislop et al 1990). The infants response to bronchial challenge includes oxygen desaturation, tachypnoea without hypoventilation, increased respiratory resistance, decreased forced expiratory flows (often accompanied by development of a concave flow-volume curve) and increased end-expiratory lung volume (FRC) (Prendiville et al 1988, Maxwell et al 1988, Young et al 1993).

The response to bronchial challenge with growth and maturation of the respiratory system is confounded by other age-related factors such as airway size, dose delivered and the differing methodologies used to assess response (Tepper 1995). Some studies suggest that infants are more sensitive to equivalent doses of bronchial challenge agents than adults (Morrison et al 1979, Myers et al 1986) and that airways responsiveness declines during infancy (Myers et al 1986), but this remains a controversial area.

However, the ability to limit maximal airway narrowing during bronchial challenge is also impaired in infants and, in contrast to healthy adults, no plateau of respiratory response to increasing challenge doses is seen, which has important practical implications when conducting bronchial challenges in this group (Tepper 1995).

Infants with a history of recurrent wheezing did not demonstrate heightened airway responsiveness, when compared with age and size matched controls, (Stick et al 1991, Clarke et al 1992). The authors of both studies suggested that the associated lower respiratory tract symptoms were related to airway mechanics such as reduced calibre and/or increased compliance rather than to increased responsiveness. In contrast, asymptomatic post-bronchiolitic infants (Tepper et al 1992), or those with cystic fibrosis (Ackerman et al 1991) did show increased airway responsiveness, when compared with controls. The relationships between baseline respiratory function, bronchodilator responsiveness and airway responsiveness have not yet been

fully investigated but these results suggest that airways responsiveness does not play a key role in the pathogenesis of wheezing in early infancy. Rather, the contribution made by airway geometry, respiratory mechanics or the adverse effects of mucus hypersecretion and airway oedema may be of more importance in wheezing illnesses in this age group.

2.1.2.4 Effects of prematurity

Premature delivery normally occurs during the period of rapid alveolar multiplication. Although there is no evidence that prematurity affects alveolar multiplication in itself, ventilation at either high or low pressures reduces alveolar number and increases production of elastin and collagen (Hislop et al 1987). The vulnerability of premature infants to infection is related in part to the structure and size of the airways. Infants born prematurely demonstrate normal airway diameter for postconceptional age, but reduced dimensions for postnatal age. They show the normal postnatal increase in bronchial smooth muscle and the absolute amount of muscle and submucosal glands are more closely related to postnatal, than postconceptional age (Sly et al 1995). Taken together, these alterations in normal airway development in premature infants mean that the calibre of already small airways is further reduced. These changes are even more pronounced in infants requiring ventilatory support (Hislop et al 1987)

2.2 Recurrent wheezing during infancy and early childhood

2.2.1 Background

Wheezing illnesses during infancy and early childhood have an estimated prevalence of between 30 to 60% in the industrialised world (Silverman et al 1995). Within England and Wales, one-third of general practitioner visits and one-fifth of hospital admissions during childhood are accounted for by lower respiratory illnesses (Dezateux et al 1997(b)). The existing burden of childhood asthma and other wheezing illnesses is considerable, and there are studies conducted worldwide which suggest the prevalence may be increasing (Burney et al 1990, Burr et al 1989, Ninan et al 1992). The mounting evidence of a link between childhood respiratory illness and chronic respiratory disease in later life (Shaheen et al 1994, Burrows et al 1977, Barker et al 1991) implies that a current increase in the prevalence of childhood

respiratory disease may generate a great public health burden in years to come.

Although asthma may develop soon after birth, the natural history of the disease remains obscure. Childhood asthma is associated with immunological processes that induce an increased production of total IgE (Burrows et al 1995). The association is poor during infancy, though (Ruiz et al 1990, Martinez et al 1995) and the current consensus is that recurrent wheezing during infancy and early childhood is not one entity but several which are largely independent of later childhood asthma. Wheezing is a symptom rather than a diagnosis and, although estimated to account for only around 6% of all wheezing disorders, may also be present as a symptom of other conditions, (Silverman et al 1995). A summary of causes of wheezing, excluding asthma and bronchiolitis, which make up the estimated 6% throughout infancy and childhood, is shown in Table 2-2.

2.2.2 What is a wheeze?

It is useful to clarify what is meant by the term “wheezing”. Wheezing has been described as a non specific physical sign associated with restriction of airflow through narrowed airways, and is thought to be initiated by turbulent airflow causing oscillations of the bronchial wall (McKenzie 1995). Audible wheezing is considered to originate almost exclusively in the larger airways, as flow within the small airways generates insufficient energy to result in oscillation of airway walls within audible frequencies. Although narrowing of the small airways is silent, it is often associated with dynamic compression of the large airways and, therefore, with wheezing if there is consequent obstruction below a critical airway diameter. Breath sounds resulting from turbulent flow within the large airways are damped by lung hyperinflation (McKenzie 1995). Wheezes are further defined as continuous adventitious lung sounds, where continuous is defined as greater than 250 ms. The American Thoracic Society (ATS) has also proposed a definition of wheeze as a high pitched continuous sound with a dominant frequency of 400 Hz or more and rhonchi as low pitched continuous sounds with a dominant frequency of 200 Hz or less (Meslier et al 1995). Wheezing is a non specific sign, and as suggested by Table 2-2, may be considered as a final common pathway for a variety of conditions associated with airway obstruction of varying significance and prognosis.

Table 2-2 Causes of recurrent wheezing other than asthma and bronchiolitis

Developmental abnormalities

- Congenital heart disease
- Tracheoesophageal fistula
- Oesophageal atresia
- Tracheomalacia
- Bronchomalacia

Lesions causing compression and/or obstruction of airways

- Vascular ring
- Congenital lobar emphysema
- Mediastinal cysts and/or tumours

Genetic disorders

- Cystic fibrosis

Immune deficiencies

- Severe combined immune deficiency
- Combined IgA and IgG2 deficiency
- Primary ciliary dyskinesia

Recurrent aspiration

- Gastroesophageal reflux
- Neuromuscular disease
- Mechanical and/or swallowing disorders

Infection

- non viral
- m pneumoniae
- Tuberculosis

Perinatal disorders

- Bronchopulmonary dysplasia/chronic lung disease
- Congenital infection
- Sub glottic stenosis

Other

- Foreign body aspiration
- Laryngeal dysfunction
- Pulmonary oedema

2.2.3 What is asthma?

Childhood asthma has a pragmatic definition, “ recurrent wheeze and/or persistent cough in a setting where asthma is likely and other rarer conditions are excluded” (Warner 1992). Asthma is considered to be primarily a disease of airway inflammation, but the difficulty in obtaining information on airway pathology in early childhood prevented inclusion of the concept of eosinophil mediated airway inflammation within the working definition (Warner 1992). At a workshop on early

childhood asthma, it was agreed that further definition of the terms *asthma* and *bronchiolitis* was not of benefit at the time (Silverman et al 1995).

2.2.4 The determinants of wheezing

Studies published both before and during the production of this thesis have delineated several distinct wheezing phenotypes during infancy (Silverman et al 1997). They have also provided convincing evidence that both infants with wheezing related lower respiratory illnesses (WRLIs), and children and adults who had WRLIs as infants, have lower levels of lung function than those unaffected by such illnesses. There were, however, several confounding factors identified in these studies. Exposure to maternal smoking, which affects lung development in utero and less strongly during infancy has been associated with increased prevalence of wheezing during infancy (Tager et al 1995). Other relevant exposures include gender, fetal nutrition, family atopic history, ethnic group and socio-economic status (Dezateux et al 1997 (b)). Recently published results from the Tucson Children's respiratory study (Martinez et al 1995) suggest that the majority of infants grow out of their wheezing. These transient early wheezers had lower forced expiratory flows ($V'_{\max, \text{FRC}}$), but normal bronchial responsiveness shortly after birth, suggesting that they had pre-existing smaller airways which as they grew in absolute size no longer resulted in wheezing symptoms with lower respiratory infections, although respiratory function did remain diminished when compared with those who had never wheezed. Maternal smoking was significantly associated with transient early wheezing. Children who either continued to wheeze, or developed wheezing after infancy had impaired lung function by 6 years of age and raised IgE levels, together with increased bronchial responsiveness.

During the past 20 years, there have been several epidemiological studies documenting the increased prevalence of asthma in the developed world (Duhme et al 1998, The European Community Respiratory Health Survey Group 1997, Cunningham et al 1996, Strachan et al 1996). In the Tucson children's respiratory cohort study, between 1973 and 1993, the percentage of subjects with active asthma rose from 8.1 to 14.2%, while those "ever wheezing" rose from 31 to 43%. The results from this study also suggested that persistent wheezers did not have reduced forced expiratory flows ($V'_{\max, \text{FRC}}$) at birth, but did so by 6 and 11 years of age, so

that wheeze without low specific conductance (i.e. without congenitally narrowed airways) but with a family history of asthma was the greatest risk combination.

Asthma that begins in early life and persists is associated with the development of structural changes. Methacholine responsiveness is highest in persistent wheezers, whereas those who are late wheezers show no increase in bronchial reactivity. This raises the question whether the increased bronchial reactivity in persistent wheezers is due to the virus interacting with the immune system, or that the children are genetically programmed. Chronic inflammation may lead to significant changes in airway structure. If the link could be broken, by preventing inflammation, then the chronic changes of the pathway leading to asthma, via inception of asthma, initial phases and exacerbations with development of chronic asthma, may be diverted to one of remission and protection from further challenges.

There is also an increasing body of evidence from epidemiological studies establishing a relationship between childhood lower respiratory illness (LRI) and wheezing and the development of chronic respiratory disease during later life (Shaheen et al 1994, Barker et al 1991). Research interest has been directed towards elucidating the nature of this relationship, the genetic and environmental exposures, which may modulate the expression of chronic lung disease and the associated underlying biological mechanisms.

Evidence of inflammation has been suggested by varying IgE levels, low anti IgE-IgG complexes, elevated IL₄ and decreased IL₂ and interferon γ levels in subjects at risk for atopy at birth (Busse 1998). These early changes may be the result of gene expression or alternatively may be due to programming - described as the permanent alteration of the structure and function of organs and tissues by factors operating during sensitive periods in fetal or early postnatal life (Barker 1990). Mediators of programming include fetal nutrition and fetal exposure to maternal smoking during pregnancy. During infancy, environmental factors influencing the development of asthma include allergen exposure, infant feeding practices and viral infections (Henderson et al 1992, Wright et al 1989, Murray et al 1992).

A genetic predisposition to asthma or atopy may be present in up to 40% of the population. Genetic markers on chromosomes 5, 11 and 14 have been reported to be

associated with immunoglobulins present alongside atopy and asthma, such as IgE; IL₄ and a high affinity IgE receptor (Bleecker 1997). This genetic vulnerability, together with exposure to allergens both pre- and postnatally, may trigger the inflammatory process and heightened airway responsiveness characteristic of asthma during early childhood. The role of viral infections during infancy is also relevant, and it has been suggested that infants experiencing fewer respiratory infections show a higher prevalence of atopy (Martinez 1994). Animal data suggest that T lymphocytes may develop a TH₁ response to viral infections, which results in the expression of protective antibodies, or a TH₂ response, in which cytokines and inflammatory mediators characteristic of asthma are expressed. There is counter-inhibition of each response, such that lack of challenge by viral infection may result in a predominance of the TH₂ response to environmental allergens with resultant allergic sensitisation (Openshaw P.J. et al 1994).

2.3 ECMO Background

Extracorporeal membrane oxygenation (ECMO) is a technically demanding and expensive means of providing temporary support during respiratory failure in infancy, particularly during the neonatal period. In Section 6 some aspects of the Respiratory follow-up of a group of ECMO survivors at around one year of age using methods evaluated within this thesis are evaluated. The technique and applications of ECMO are, however, described in this section.

Following the introduction of intensive care for the sick neonate, the need to evaluate the response to therapy and assess the respiratory function of survivors has been recognised (Ahlstrom 1975, Benoist et al 1976, Bryan et al 1973, Beardsmore et al 1983). With continued advances in neonatal care, such as surfactant therapy and antenatal glucocorticoids, the survival rate for prematurely born infants with respiratory disease is increasing. There have been many studies designed to assess respiratory mechanics in this group. These have shown changes in early life (Stocks et al 1976, Stocks et al 1978a, Lindroth et al 1980, Yuksel et al 1991), which may resolve with time (de Kleine et al 1990). However, long term studies of respiratory morbidity in survivors of neonatal ventilation show persisting abnormalities (Silverman et al 1991, Doyle et al 1991, Chan et al 1989). In contrast, the later outcome of more mature infants with neonatal respiratory disorders has been relatively under investigated.

In recent years, new techniques have been introduced for the management of neonatal respiratory failure, with the aim of reducing barotrauma and volutrauma, and the subsequent chronic lung disease, which has been observed in survivors of conventional ventilation. One of these techniques is extracorporeal membrane oxygenation (ECMO), which is increasingly being used in the treatment of reversible cardiorespiratory failure refractory to conventional medical treatment. During the past 10 years, ECMO has been introduced for the management of infants with respiratory failure that has generally been complicated by pulmonary hypertension and significant extra-pulmonary right to left shunting of blood (Kanto, Jr. 1995). The conditions for which ECMO is most commonly used include meconium aspiration syndrome, congenital diaphragmatic hernia, respiratory distress syndrome (in more mature infants) and persistent pulmonary hypertension of the newborn.

Surgical access to the circulation is established through the large vessels of the neck-cannulation is either venoarterial (VA) or venovenous (VV), according to the size of the infant and the type of support required, cardiopulmonary and pulmonary alone respectively. Blood is withdrawn from the venous catheter sited in the right atrium and driven through the ECMO circuit by a pump. Oxygenation of the blood and removal of carbon dioxide takes place in the silicone rubber membrane “lung”, and the blood is returned to the patient via a heat exchanger. In VV ECMO, blood is both drained and returned to the right atrium through a double lumen cannula, return flow being directed across the foramen ovale. In VA ECMO, the oxygenated blood is returned to the patient through the arterial cannula and into the arch of the aorta. As respiratory function improves, there is a gradual increase in oxygenation across the infant’s lungs (minimal conventional ventilation continues during ECMO) and the blood flow through the ECMO circuit is accordingly reduced until native respiratory function alone is supporting adequate gas exchange. Recovery is accompanied by an improvement in lung compliance and aeration, an increase in pulmonary capillary blood flow and surfactant protein A levels and a decrease in plasma prostanoid levels (Kanto, Jr. 1995). The infant is then decannulated and the internal jugular vein and common carotid artery (VA) or internal jugular vein alone (VV) ligated or repaired. To prevent clotting within the ECMO circuit, heparin is added.

The treatment has proved to be a valuable “rescue therapy”, with survival rates in some North American centres exceeding 90% (Kanto, Jr. 1995). However, randomised trials conducted in North America (Bartlett et al 1980, O'Rourke et al 1989) have been criticised because of their study design, with the established success of the technique in moribund infants precluding unbiased randomisation in controlled trials. In addition, concerns were raised that ECMO might improve survival at the cost of long-term disability. For these reasons a national randomised trial of ECMO (UK Collaborative ECMO Trial Group et al 1996) for mature neonates with reversible respiratory disease was undertaken, in which the main outcome measures were death or severe disability at one year of age. This showed that ECMO conferred a survival advantage over conventional management, without a concomitant rise in severe disability (UK Collaborative ECMO Group. 1998). The Respiratory follow-up of surviving infants described in section 6 was an additional element of the Collaborative ECMO trial.

2.4 Historical review of infant respiratory function

2.4.1 Early Background

Attempts were made from as early as the seventeenth century to measure lung volume in adults (Hutchinson 1846), employing an improved spirometer to measure and name subdivisions of the lung volumes, which today still forms the basis of routine adult respiratory function testing. Indirect measurements of lung volume were first performed at the beginning of the nineteenth century, using hydrogen as a tracer gas by Davy.

Initial attempts to quantify respiratory function parameters in infants were made over 100 years ago (Eckerlein 1890, Dohrn 1895), using variations of spirometry to assess tidal volume and respiratory frequency. These early attempts used equipment that was either unwieldy (Dohrn's multiple pen displacement system) or had an unphysiologically large dead space and required prolonged periods of rebreathing during recording of measurements (Eckerlein), and it was not until the introduction of the head-out plethysmograph by Murphy and Thorpe in 1931 that reasonably accurate measurement of tidal volume and respiratory frequency was achieved. Improvements to the technique that were introduced by Cross in 1949, when the rubber collar neck seal was replaced by an inflatable cuff, enabled widespread application in determination of baseline respiratory function in infants (Bolton et al 1970), as well as their ventilatory response to stimuli (Cross et al 1951) and allowed measurements in premature infants (Cross et al 1952). Although Fleisch had developed the first pneumotachograph in 1927, which had been widely used in adult studies, it was not until minaturisation with a consequent reduction in dead space (Swyer et al 1960) that pneumotachographs largely replaced the head-out (or trunk) plethysmograph for measuring respiratory parameters in infants.

When the first attempts to measure respiratory function in infants were underway, the basis for modern respiratory mechanics was also concurrently being established following the work of Donders and Hutchinson (Hutchinson 1846). This was continued by Rohrer, who developed a “unifying and quantitative approach to the subject” (Rohrer 1915), by establishing the fundamental relationship between pleural pressure, lung elastic recoil pressure and alveolar pressure, which was developed

further by Neergaard and Wirz in man (Neergaard et al 1927).

2.4.2 Measurement of lung volume

Measurements of lung volume in infants using gas dilution were first described in the late 1950's (Berglund et al 1956, Cook et al 1958). The use of helium as a tracer gas had previously been introduced in adults (Willmon et al 1948). Measurements in sick infants were not possible until 1970 when a system incorporating a rebreathing bag was introduced by Krauss and Auld (Krauss et al 1970). Gas dilution estimations of lung volume using nitrogen as a tracer gas were reported in infants (Strang et al 1962) and a rebreathing method later developed (Ronchetti et al 1975) which, however was rather impractical for routine measurements and assumed conditions that were difficult to verify. Modifications using an open circuit with lower dead space, suitable for neonates, were later introduced (Gerhardt et al 1985). Methodological difficulties and lack of standardisation still pose problems with both helium dilution and nitrogen washout techniques (American Thoracic Society/European Respiratory Society 1993).

Early plethysmographic estimations of lung volume in adults (Gerhardt et al 1985, Gad 1881, Pfluger 1882, Hitchcock et al 1946, Willmon et al 1948, Dejours et al 1953) were initially encumbered by technical difficulties and required a high degree of co-operation from trained adult subjects. In 1956 a new technique for measuring thoracic gas volume and airway resistance was described (DuBois et al 1956a, DuBois et al 1956b), and plethysmography became established as an accurate, reliable method. This technique was described for use in infants for the measurement of FRC (Klaus et al 1960, Klaus et al 1962, Auld et al 1963) and later adapted to measure airway resistance as well as FRC (Polgar 1961). Polgar's results contained technical errors, but demonstrated that the measurement of airway resistance was feasible in infants, and it was later modified (Radford 1974, Stocks et al 1977a, Stocks 1977b) with the addition of a heated rebreathing system. Plethysmography remains a valuable research tool but requires substantial operator training and expensive equipment, that needs careful calibration and characterisation of measurement conditions before use, that has limited its application.

2.4.3 Measurement of respiratory mechanics

During the late 1950's and 1960's, rapid developments in measurements of respiratory mechanics led to increasing emphasis on the application of electrical and mechanical models to the respiratory system, to further understand its function in health and disease. During this era, when "measurements of respiratory mechanics and gas exchange held the excitement which molecular biology holds today" (Wohl 1995), paradigms were developed which are still used as the framework for current measurement techniques. The approximation of pleural pressure by measurement of oesophageal pressure using an air filled catheter mounted balloon (Fry et al 1952) allowed partitioning of respiratory mechanics and was followed by a new approach to analysis using an occlusion test to demonstrate that changes in oesophageal pressure accurately reflected those of pleural pressure, requiring the subject to make respiratory efforts against an occluded airway (Mead et al 1953). These innovative tests allowed a description of dynamic mechanics measurements in adults, and were then adopted for use in infants by McIlroy and Tomlinson in 1955. Advances in measurement techniques were facilitated by the development of electrical recording apparatus (Boutourline-Young et al 1950) Following further validation, oesophageal manometry became widely accepted as a useful investigative tool (Beardsmore et al 1980, Beardsmore et al 1980) before interest waned following reports of potential inaccuracies invalidating the technique amongst small, sick or preterm infants (LeSouëf et al 1983, Thomson et al 1983, Heaf et al 1986).

DuBois described a technique whereby oscillatory changes in flow were applied at the airway opening, and respiratory impedance calculated, which reflected both resistive and elastic properties (the forced oscillation technique) (DuBois et al 1956). More recently, it has been suggested that this may be a useful approach for measurements of dynamic mechanics in ventilated infants (Lanteri et al 1995).

Compliance and resistance are rarely measured in adults, respiratory function being assessed by standardised indirect tests that do, however, require co-operation and some training to perform (American Thoracic Society/European Respiratory Society 1993). In infants, alternative approaches were required which did not rely on voluntary manoeuvres. Techniques for the measurement of compliance and resistance, may be considered as either passive (or quasistatic) or dynamic. The

development of dynamic mechanics measurements has been described above. In contrast, passive mechanics measurements were developed primarily for use in infants.

Comroe et al in 1954 described measurements of respiratory mechanics using a passive expiration in anaesthetised cats and in man. Passive respiratory mechanics measurements were initially restricted to paralysed or highly trained subjects because of the need for muscle relaxation (McIlroy et al 1963, Bergman 1966, Bergman 1969). The observation of apnoea resulting from prolonged inflation in neonates (Cross et al 1960), was attributed to the vagally mediated Hering-Breuer reflex (Breuer 1868, Hering 1868). Although the potential for applying an induced relaxation to enable passive respiratory mechanics measurements had been postulated (McIlroy et al 1963), it was not until the association between the induced apnoea and muscle relaxation was described (Younes et al 1973) that the technique was applied in infants (Olinsky et al 1974, Thach et al 1978). The technique was extended (Olinsky et al 1976), using brief inspiratory occlusions at varying lung volumes to construct a pressure-volume plot, the slope representing static compliance, thereby obtaining passive mechanics in unsedated infants.

In 1982 the technique was modified (Mortola et al 1982) using expiratory rather than inspiratory occlusions, which improved both the likelihood of successful measurement and the reproducibility of measurements. The multiple occlusion technique has recently been adapted to obtain the static pressure-volume relationship by making multiple occlusions during a single expiration: the multiple interrupter technique (Fletcher et al 1992), and by performing occlusions on two successive breaths, referencing the volume of the occlusion to the volume of the preceding occlusion (Mortola et al 1993).

2.4.4 Simpler methods of assessing respiratory mechanics in infants

During the past 10-15 years there has been an upsurge of interest in assessment of respiratory function in infants and young children. Although partly driven by an increasing recognition of the impact that respiratory disease has on childhood morbidity and mortality, and of the association between early life events and adult health, the development of information technology and the increasing availability of automated systems for performing measurements has also played an important rôle (Stocks et al 1996). The increase in interest has resulted in the development and application of specialised tests for infants and young children which are less complex than plethysmography, and less invasive than oesophageal manometry.

Tidal flow patterns in children with respiratory disease were first described 50 years ago (Kaye et al 1949), but no further progress in this area was reported until Morris and Lane (Morris et al 1981) described the use of simple ratios derived from the analysis of tidal expiratory flow, and correlated these with indices of airway function (FEV₁) in adults with and without chronic airway obstruction. Interest in the application of these methods in infants was subsequently evoked when quantitative analyses of tidal flow demonstrated that infants with bronchopulmonary dysplasia reached peak expiratory flow more rapidly than controls (Morgan et al 1984). In 1988 Martinez and colleagues published the very promising observation that one simple parameter, the ratio of the time to reach tidal peak expiratory flow to total tidal expiratory time, T_{me}/T_e (now known as $t_{PEF}:t_E$), was a predictor for subsequent wheezing lower respiratory illness in a cohort of young infants, recruited at birth. A technique by which infant respiratory function could be assessed with minimal equipment, possibly without sedation, during quiet tidal breathing, would represent a significant advance and it was not surprising that much research attention was directed towards assessing this and related parameters (Clarke et al 1995).

The techniques developed primarily for use in self-ventilating infants generally require a face mask and pneumotachograph. As well as the methodological problems of avoiding leaks, and requiring the infant to be in quiet sleep, usually sedated, the additional dead space and resistance, together with direct facial stimulation by the mask have measurable physiological effects (Dolfin et al 1983, Fleming et al 1982). Devices were developed which measured changes in partial circumference (strain

gauges), (Andersson et al 1983) anterior-posterior diameter of the rib cage and abdomen (magnetometers) (Lopes et al 1981, Stark et al 1979) and changes in chest wall impedance (impedance pneumography) (Daily et al 1969), with the aim of measuring tidal ventilation non-invasively. These techniques were not without their limitations, chiefly related to change of position and calibration (Adams et al 1993). Respiratory inductance plethysmography (RIP) (Daily et al 1969), which was later validated in infants (Duffty et al 1981) has been reported as the most reliable of the non invasive methods (McCool et al 1986, Baird et al 1991). Semi-quantitative and quantitative calibration methods have improved the accuracy of RIP (Dolfin et al 1982, Sackner et al 1989).

Following the development of the multiple occlusion technique (Olinsky et al 1976, Mortola et al 1982), various adaptations were introduced, including the expiratory volume clamping technique (Grunstein et al 1987) and alternative methods of assessing passive mechanics, such as weighted spirometry (Tepper et al 1984). The most widely applied adaptation of the MOT was the passive flow-volume, or single breath, technique (SBT). This was first studied in cats (Zin et al 1982), following the original idea of McIlroy (McIlroy et al 1963), then reported in infants (Mortola et al 1982), and later further developed (LeSouëf et al 1984a). A description of the theoretical basis for the SBT is contained in section 3.3.3.1.

A novel approach to the measurement of forced expiration parameters in infants analogous to spirometry in older children and adults was first reported in 1978 (Adler et al 1978). The rapid thoraco-abdominal compression technique was further modified, by the addition of an inflatable jacket (Tepper et al 1986, Taussig et al 1982) and has subsequently become one of the most widely applied methods, partly due to its relative simplicity but also because airway function may be assessed in both healthy infants and those with respiratory disease, in contrast to other techniques which may be invalid in the disease state (Morgan et al 1988).

2.4.5 Assessment of wheezing during infancy

During the last decade, therefore, many novel methods for assessing lung function have been introduced, while classical techniques have continued to evolve. Classical methods such as plethysmographic measurements of lung volume and airway

resistance have been used to describe the normal growth and development of the infant lungs and airways. The newer methods such as passive mechanics and the rapid thoraco-abdominal compression technique have been applied to characterise the effects of various pre- and postnatal biological, mechanical and environmental insults that disrupt normal development and may result in the infant with recurrent wheezing. There remains a need for development of tests to detect changes in respiratory mechanics, to assess response to therapy, bronchial responsiveness, and the effects of disease, and for use in epidemiological surveys of samples of the population.

3. Subjects, equipment and methods

3.1 Subjects and measurement conditions

3.1.1 Healthy and wheezy infants

Healthy infants were recruited shortly after birth as part of an ongoing epidemiological study (Dezateux et al 1999). Infants who were Caucasian (defined as both parents being White and European), with a gestational age of at least 36 weeks, without major congenital, respiratory, neuromuscular or cardiac abnormalities, and who did not require ventilatory support during the neonatal period were eligible for inclusion. Initial measurements were made between 5 and 13 weeks, prior to any respiratory illness and, when possible, were repeated at around 1 year of age. Some infants participated in additional measurements between 3 and 18 months of age for other studies (Dezateux et al 1997 (a)).

Written informed consent to participate was obtained from parents during a home visit, before attending the laboratory. Following the last laboratory visit, the primary medical care record of each infant was then examined, and any episodes of physician-diagnosed wheezing noted. For the current study, reported in sections 4 and 5, infants were retrospectively excluded from the healthy group from the first date that any wheezing was diagnosed and recorded, when the study population was selected.

Measurements were also made on one occasion in infants with physician-diagnosed recurrent wheezing recruited from the wards and clinics of the Great Ormond Hospital for Children NHS Trust and the Queen Elizabeth Hospital Hospital for Sick Children, Hackney. Infants were eligible for inclusion if no major congenital abnormalities were present and the parents command of the English language was adequate to obtain written informed consent to participate in the study.

For all infants, measurements were made at least 3 weeks from the onset of any upper respiratory tract infection. Information regarding respiratory morbidity and treatment, family history of asthma and relevant social history was obtained from parents at the time of recruitment (healthy infants), or on the date of laboratory visit (infants with recurrent wheezing). Data from each group of infants were then entered and validated using a database package (Epi-info, version 5.01b, Atlanta) on an IBM compatible PC. Details entered included lung function parameters, sex, ethnic group,

gestational age, birthweight and age, weight, crown-heel length and symptom status at testing. Maternally reported smoking in pregnancy and the presence of asthma in first degree relatives, obtained by questionnaire, were also included. For infants with recurrent wheezing, current medication (if any), numbers of episodes of wheezing and number of visits to General Practitioners and Hospitals were also recorded.

3.1.2 Survivors of neonatal respiratory failure

Infants entered during the neonatal period into the UK Collaborative ECMO Trial- a randomised controlled trial of maximal conventional respiratory support with or without ECMO (UK Collaborative ECMO Trial Group et al 1996), were measured at one year of age as part of the Respiratory Follow-up. Details of families of infants who had agreed to participate and attend for respiratory function testing were supplied by the ECMO Trial co-ordinator and arrangements were made for travel to the laboratory on a mutually acceptable date. On arrival, parents were requested to replace the infant's clothing with a high necked smock (to disguise any neck scars, if infants had received ECMO) and to withhold details of the limb of the trial that their child had been randomised to. This maintained the "blinded" status of laboratory staff and avoided potential bias during data collection.

All studies described in this thesis were approved by the local ethics committee. Written informed consent was obtained from one or both parents, who were often present during measurements. Recruitment and measurement of infants took place between February 1992 and December 1996 for all studies reported in this thesis, except for 10 infants recruited as part of a related project (Dezateux et al 1997 (a)).

3.1.3 Preparation of infants

The measurement conditions adhered to during infant lung function measurements at the Institute of Child Health, London, are described here as they applied to the studies presented in sections 4 and 5. There were some differences in approach for the ECMO Respiratory Follow-up, and therefore methods specific to that study are described separately in section 6.3.1.

Within the infant laboratory, room temperature was maintained between 20-25 °C and the infant's clothing adjusted to maintain warmth without overwrapping. During measurements, the infant was dressed in a vest or Babygro that was unbuttoned at the crutch to avoid any potential restriction of the thorax.

No attempts were made to measure lung function within three weeks of the onset of an upper respiratory tract infection, because of the potential influence of secretions and upper or lower respiratory tract reactivity (Gaultier et al 1996). Prior to measurements, the nostrils were examined and any debris gently removed, using a moistened cotton bud.

After an accurate weight was obtained (reported in kg to one decimal place), a urine collection bag was applied and the nappy replaced. All infants underwent clinical examination prior to measurements with sedation. Infants were not sleep deprived before attending the laboratory (Canet et al 1989). To avoid the potential influence of circadian rhythm changes on lung function parameters, all measurements were attempted in the late morning or early afternoon, although appointments were made, when possible, to coincide with normal sleep time.

The infants were sedated using triclofos sodium 75 mg·kg⁻¹ (up to 8 weeks) or 100 mg·kg⁻¹ (older infants) given orally (Turner et al 1990, Jackson et al 1990, Gaultier et al 1995). One gram of triclofos sodium is equivalent to 660 mg chloral hydrate. As it would have been impractical, given the number of measurements already in the data collection protocol, neurophysiological assessment of sleep state was not attempted. Behavioural assessment of sleep state according to well established criteria (Prechtl 1974) was made at frequent intervals throughout the measurements. Comments were made on the saved data as necessary. All measurements were made during quiet sleep when posture was stable, respiration regular and no eye

movements observed. At the onset of sleep, the infant was placed supine within the open plethysmograph with the neck slightly extended and head in the midline, supported on either side by small sandbags (Reiterer et al 1994). A transparent Rendell-Baker face mask, size 1 or 2 as appropriate, was gently lowered in place after applying therapeutic putty around the rim to obtain an airtight seal. Latex cheek strapping was attached to the face mask mount to provide additional support.

3.1.4 Completion of measurements

3.1.4.1 Crown-heel length

Immediately following measurements of lung function, while the infant remained drowsy, crown-heel length was measured by two adults using an infant stadiometer (Harpenden Infantometer, London, UK). Placing the small flat pillow from the plethysmograph under the infant's head and a cot sheet on the stadiometer to prevent discomfort, one adult supported the infant's head and, with the infant lying in the midline without trunk rotation, the other adult gently depressed their knees and adjusted the footplate of the stadiometer until it was resting against the soles of the feet. Measurements were repeated until two recordings were obtained within 0.5cm of each other, length being reported in cm to one decimal place (Cox 1992, Gaultier et al 1995). Measurements were made, whenever possible, by two laboratory staff although occasionally assisted by the infant's parents.

3.1.4.2 Questionnaire

The project specific questionnaire was completed, allowing time for questions and discussion, by the operator and parent(s). Parents were assured that information given was confidential and would be stored securely. The questionnaire, together with a copy of the consent form, a hard copy of oximetry data: heart rate and pulsatile oxygen saturation (SpO_2) during measurements and a hard copy of the analysis was stored in a lockable filing cabinet.

3.1.4.3 Urine Cotinine samples

If this had not already taken place, the urine sampling bag was gently removed and the infant's perineum cleansed before replacing their nappy. Urine was aspirated from the bag, using a syringe, from the sampling port on the bag and aliquots of at least 2mL were stored in a freezer at -40 °C prior to analysis. The stored urine was

periodically sent in batches to the Nicotine Laboratory, New Cross Toxicology Unit, London for measurement of urine cotinine levels, standardised to urine creatinine as an estimate of renal clearance of the samples.

3.1.4.4 Discharge advice

Following measurements, transport home by taxicab was arranged once the infant had roused (which usually occurred during measurements of crown-heel length, if not before) and had taken a feed, or some fluids. Parents were given an advice letter regarding effects of sedation and the telephone number of the paediatrician or nursing sister who performed the measurements, should they have any concerns after returning home.

3.2 Equipment

3.2.1 Apparatus

3.2.2 Whole body plethysmograph

The 100 L variable pressure whole body plethysmograph depicted in Figure 3-1 was built by the Biomedical Engineering Department, Great Ormond Street Hospital for Children NHS Trust. The plethysmograph was constructed of an aluminium base with a transparent perspex domed lid, the base being trimmed with compressible rubber. These materials provided adequate thermal conductivity. The dome was secured with safety release handles and maintained visibility of the infant. Pressure changes within the plethysmograph were measured using a low range differential pressure transducer (Validyne MP45 \pm 0.2 kPa). A temperature probe (Ellab) gave a digital display of temperature within the plethysmograph chamber. A vent operated by a tap remained open while the lid was closed, except during plethysmographic measurements of functional residual capacity (FRC_{pleth}) and airway resistance (R_{aw}), and a port on the outside of the base allowed connection of calibration syringes via narrow stiff tubing and a three-way tap. Within the chamber of the plethysmograph, suspended by an adjustable steel arm were the rebreathing circuit and shutter block-pneumotachometer-mask mount assembly as described below. The shutter block was operated by remote control bellows. A pulse oximeter probe (Ohmeda Biox 3740) within the chamber allowed continuous recording of heart rate and SpO_2 during measurements.

Figure 3-2 shows a circuit diagram of the monitoring and recording equipment which completed the plethysmograph apparatus. Signals were displayed in real time on the computer screen and as X-Y plots of pressure at the airway opening (P_{ao}) vs plethysmographic volume (V_{pleth}) (FRC_{pleth}) or flow (V) vs V_{pleth} (R_{aw}) on a cathode ray oscilloscope (Feedback Lanscope LAN521, Feedback Instruments Ltd.). As an additional safety measure, and for quality control, flow and P_{ao} signals were continuously displayed on a Digital Storage Oscilloscope (Gould Advance OS4100) during measurements.

Figure 3-1 Whole body infant plethysmograph

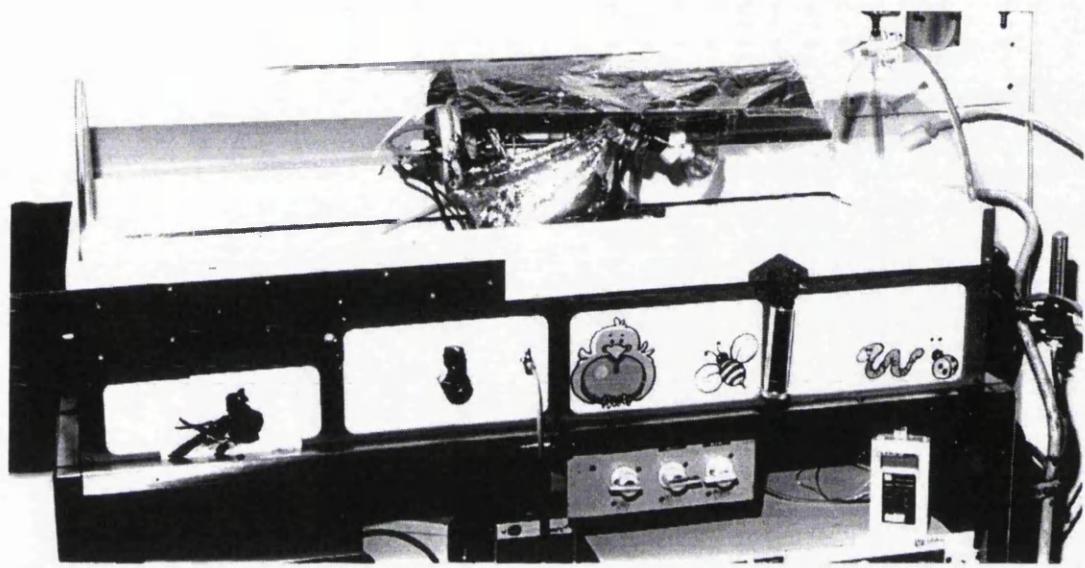
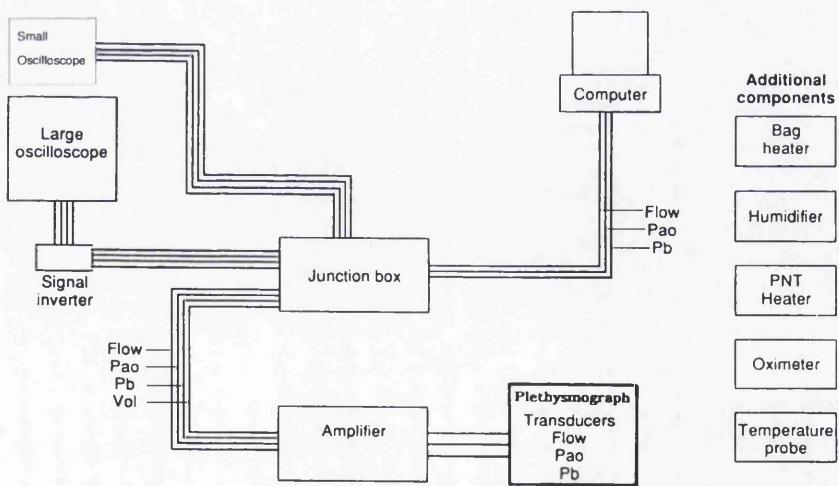


Figure 3-2 Circuit diagram of equipment for infant plethysmographic studies



Rebreathing circuit for collection of R_{aw} data

Two circuits were used in the course of this study:

1. A highly compliant transparent 2 L reservoir bag, open at both ends, supported by a perspex mounting plate with a humidified air inlet/outlet placed over the shutter block outlet. Attached to the mounting plate, ceramic heating resistors within a protective cage were heated by a variable power supply (Farnell Instruments). Air and oxygen were passed through a humidifier (Cape) to partially inflate the bag prior to measurements of R_{aw} via a three way tap, which remained closed during recordings. It was possible to remove most of the oxygen enriched air following each recording of R_{aw} using a 100 mL syringe at the three way tap.

A probe within the bag near the shutter block inlet displayed gas temperature which was between 40 and 50°C to achieve conditions at the mask at around BTPS* according to the respiratory rate and tidal volume of the infant. The resistors were heated by a 0-30 V Voltmeter and required frequent adjustment to maintain the desired temperature.

2. The design of the heater and temperature controller was improved by the provision of a servo controlled heater and fan with a digital display of temperature. The quality of data collected was equal to that of the previous system, but the time required to reach the desired temperature was reduced to around two minutes and once set, only small adjustments were needed. The time taken to collect technically satisfactory data was greatly reduced.

3.2.3 Shutter block, pneumotachograph and mask

The perspex shutter block (Biomedical Engineering, Great Ormond Street Hospital for Children NHS Trust) shown in Figure 3-3 had outlets into the rebreathing bag and plethysmograph which were opened or closed by lubricated pneumatically operated bobbins. The infant could therefore be directed to breathe air at room temperature or oxygen enriched, humidified air at around BTPS*. The heated Fleisch

* Body temperature, standard barometric pressure saturated with water vapour under these conditions.

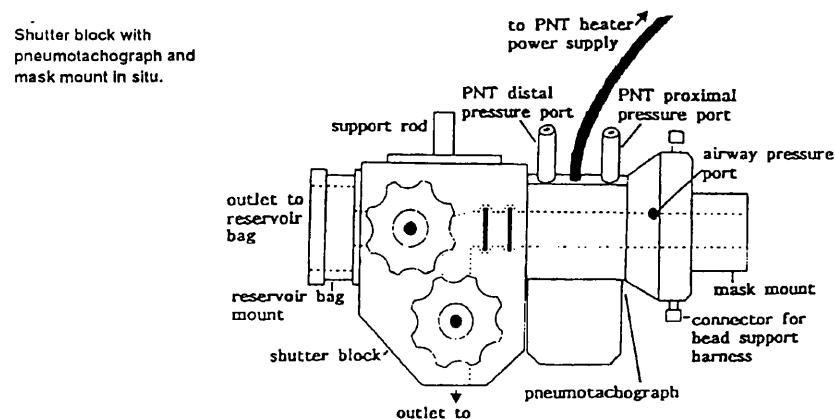
size 0 or 1 pneumotachograph was connected directly into the shutter block or via a tapered connector, respectively. The pneumotachograph was then attached to an appropriately sized mask mount with an airway pressure port. A transparent (Rendell - Baker, Soucek) face mask, size 1 or 2, according to the size of the infant completed the circuit. The pneumotachograph pressure ports proximal and distal to the resistance were connected by equal lengths of narrow gauge stiff polyethylene tubing (Portex Ltd., internal diameter 3mm, external diameter 5mm) to a Validyne MP45 differential pressure transducer range ± 0.2 kPa. This ensured that the coefficient of displacement was equal on both sides of the transducer diaphragm (Stocks et al 1991) (Sly et al 1996). Identical tubing connected the airway opening pressure port to a Validyne MP45 differential pressure transducer range ± 5.0 kPa referenced to atmospheric pressure. The dead space (excluding face mask) and resistance of the complete assembly are shown in Table 3-1

Table 3-1 Pneumotachograph characteristics

Fleisch PNT	Dead Space	Resistance at 100 $\text{mL} \cdot \text{s}^{-1}$
size 0	7.6 mL	$0.78 \text{ kPa} \cdot \text{L}^{-1} \cdot \text{s}$
size 1	26.0 mL	$0.48 \text{ kPa} \cdot \text{L}^{-1} \cdot \text{s}$

Details of apparatus assessment, including linearity, resistance and frequency response are given in Section 0

Figure 3-3 Shutter block



3.2.4 Parameters measured by whole body plethysmography

The PNT-shutter block-rebreathing circuit contained within the whole body plethysmograph as described enabled measurements of airflow and airway opening pressure, with volume being digitally integrated from flow. The plethysmographic pressure transducer measured pressure variations within the chamber during tidal or occluded breathing movements. By operating the remote controlled shutter: closed to rebreathing bag, open to atmosphere, data were collected for analysis of tidal parameters and passive respiratory mechanics. By closing both shutters, data were collected for FRC_{pleth} . By closing the shutter to atmosphere, open to the rebreathing bag and adjusting the temperature of the warmed, humidified oxygen enriched air, measurements of R_{aw} were obtained.

3.2.5 Rapid thoraco-abdominal compression Technique

Flow, airway opening pressure and volume were measured using a Fleisch 1 pneumotachograph. Partial expiratory flow volume curves were generated with the infant lying supine within the open plethysmograph with the PNT, mask mount and attached face mask being disconnected from the shutter block for these manoeuvres. The dead space of the apparatus was 12mL.

Inflatable jacket

The jackets were of a cummerbund design (Hannover Medical School Engineering Department) with an outer layer of cotton lined PVC which was non expansible, allowing volume increase during inflation to be directed onto the infant. A detachable vinyl pillow with a 30 mm diameter cuff was inserted within the jacket, which was wrapped around the infant's chest and fastened with velcroTM. Jackets were available in three sizes, and were adjustable in circumference so that a snug fit could be obtained in each infant, allowing one finger's passage when fitted, and extended from axillae to pubis.

Pressure Source

This consisted of a 200 L reservoir (metal beer barrel) with an adjustable air inflow (up to $20 \text{ L} \cdot \text{min}^{-1}$) connected to 27 mm internal diameter wide bore tubing, with an

adjustable pressure blow-off valve set at 10 kPa. A manually operated three way tap of equivalent diameter was incorporated in the circuit close to the jacket. A port between the three way tap and jacket recorded jacket pressure via a ± 10 kPa differential pressure transducer (Validyne MP45). The jacket inflation time was < 0.1s.

3.2.6 Signal processing

Analogue signals from the flow, pressure at the airway opening, plethysmograph and forced expiration jacket differential pressure transducers were amplified (Validyne MC 1-3 Amplifier). They were then channelled via a junction box and further amplified and digitised by an Analogue to Digital interface card (A/D) (Analog Devices RTI-815) installed within the central processing unit (CPU) used for data collection, analysis and storage. The output from the amplifier was matched to the input range of the A/D card (Bipolar -5V to +5V). Initially the CPU used was a Compaq 386 IBM compatible personal computer, later upgraded to an Elonex PC 433 when interim analysis of partial expiratory flow volume curves during data collection required faster processing speed. Data from all measurements were displayed in real time, processing occurring with minimal time delay, using specialised software for processing physiological signals (Respiratory Analysis Program [RASP], Physiologic Ltd. UK).

Data were displayed on a VGA colour monitor and paper copies of signals printed with an Epson fx-850 dot matrix printer.

Sampling Frequency

According to the respiratory rate of the infant and detail of processed signals required, sampling rates were 50-200 Hz. Details of sampling rates for each type of measurement are specified in the Analysis section.

3.2.7 Respiratory Analysis Program (RASP)

The Respiratory Analysis Program (RASP) was developed by PhysioLogic Ltd. in conjunction with the Institute of Child Health and The Royal Postgraduate Medical School at Hammersmith Hospital, London. Data streams from input transducers, as well as derived streams of integrals or filtered inputs (such as end-tidal CO_2) were displayed as time series or X-Y plots during data collection. On completion of measurements, data were stored on floppy and optical disks, and could also be printed or plotted. Analysed events could be stored or printed, and parameters used for analysis recalled.

RASP contained a library of physical channels, each of which referred to a physical entity such as respiratory rate. Each channel contained one entry for every variable monitored during data collection or computed by one of the analysis modules. The program was used for all data collection and analysis of respiratory function parameters in this thesis, with each measurement such as passive mechanics or plethysmography being analysed in a customised collection of channels called a “profile”. Physical channels were classified into several types:

- analogue inputs: Input from pressure transducers;
- derived inputs: Channels derived mathematically from the analogue inputs during data collection, such as tidal volume (integrated from tidal flow), or expiratory time (t_E);
- analysed results: Channels computed from data collected during measurements such as respiratory compliance or airway resistance.

The RASP algorithms for each of the data collection and analysis channels were fully validated within the Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit. An ASCII file output of data was printed for each separate analysis strategy (such as tidal breathing, $\text{FRC}_{\text{pleth}}$, C_{rs}). The ASCII output provided a complete listing of parameters for analyses such as time, flow, tidal volume, pressure at the airway opening or plethysmographic pressure collected at the specified sampling rate. It was then possible to manually calculate each parameter to ensure that the algorithm was correct, using a previous version of RASP as the “gold standard” and ASCII output of identical data to compare analysis results. This

process was repeated for each analysis channel within each profile when a new version of RASP was issued.

3.2.8 Pulse Oximetry

Arterial oxygen saturation and heart rate were displayed and recorded during all infant lung function measurements using an Ohmeda Biox 3740 oximeter. The oximetry probe was attached using crepe non adhesive tape (Coban, 3M) to the outside border of the foot, or great toe in older infants. The alarm was set to trigger audibly should SpO₂ fall below 90%. Stored data were reported and graphically displayed using the Biox program developed within the Respiratory Laboratory, Great Ormond Street Hospital for Children NHS Trust. A hard copy of the results was filed with the infant's paper records.

3.2.9 Resuscitation equipment

Suction and resuscitation equipment were checked before each measurement. The emergency trolley was stocked and maintained according to Hospital policy. An emergency bell was situated inside the infant laboratory to summon help. Prior to each study, medical cover was arranged.

3.2.10 Amplifier and pneumotachograph

All equipment was switched on and allowed to warm up for at least 15 minutes before calibration, except the amplifier which was left on permanently, to ensure thermal stability of output signals. The Fleisch pneumotachograph appropriate to the size of the infant was selected. Gain settings on the amplifier were also adjusted accordingly (Table 3-2). The amplifier offset was adjusted to ensure zero output with no signal input. This ensured that the full scale deflection was equal around zero, maximising the range over which data were collected.

Table 3-2 Amplifier settingsSmall infants: Peak flow $< 200 \text{ mL}\cdot\text{s}^{-1}$ use Fleisch size 0 PNT

Signal	Filter (Hz)	Amplifier gain
P_{pleth}	10	1
P_{ao}	10	5
Flow	10	5
Volume		(flow on 5)
P_j	10	10

Larger infants: Peak flow $>200 \text{ mL}\cdot\text{s}^{-1}$ use Fleisch size 1 PNT

Signal	Filter (Hz)	Amplifier gain
P_{pleth}	10	2.5
P_{ao}	10	10
Flow	10	2.5
Volume		(flow on 2.5)
P_j	10	10

The shutter block was then assembled: bobbins were greased lightly with silicone spray and placed into their respective cavities, the bellows ports with “O” ring seals were then hand tightened in place. The shutter block was attached to the supporting arm within the plethysmograph and the pneumotachograph connected. The Fleisch size 0 PNT connected directly between the shutter block and mask mount, but the size 1 PNT required adapters with internal coning to prevent turbulence, hence the proportionately larger dead space (7.6 mL versus 26 mL respectively) of the assembled apparatus. Tubing was connected to the port on the mask mount for measurement of P_{ao} . Flow transducer tubing was connected to each PNT port, ensuring correct polarity. The bobbins were primed with air from the bellows and checked to ensure that they could hold a pressure seal to 2 kPa without leaking. Reasons for leaks normally included either bellows slipping off the mounting plate, ageing diaphragms, loose tubing or moisture within the shutter block.

3.2.11 Calibration

Calibration of the assembled equipment was performed prior to each infant measurement. Although the output from the Validyne transducers and amplifier were stable, small inter-operator differences meant that fine tuning was occasionally required and checking known signals allowed prompt troubleshooting of faults

within the measurement system. Periodically, equipment was fully assessed and validated: details are given in section 9.

3.2.11.1 Plethysmograph calibration

Depending on the atmospheric conditions and the infant's weight, the plethysmograph pressure calibration required adjustment by recalibrating with the approximate weight (to the nearest kg) and estimated respiratory rate of the infant to be measured (Stocks 1977b).

3.2.11.1.1 Time constant of the plethysmograph

Bags of saline with a total weight approximately equal to that of the infant were placed in the plethysmograph. The lid of the plethysmograph was closed and time allowed for equilibration as shown by a stable plethysmographic pressure signal. An adjustable calibrated syringe (Hans Rudolph, USA) set at 20 or 40 mL according to amplifier gain (1 or 2.5 respectively) was used to inject air into the plethysmograph via the calibration port. The time taken for the signal to decay to 37% of initial amplitude was recorded (Figure 3-4) (Stocks et al 1996) to assess the combined mechanical and thermal time constant of the plethysmograph. The volume was then withdrawn and the measurement repeated. The value was normally between 10 and 14 seconds but was affected by atmospheric conditions. Calibration of the plethysmographic signals was performed in the infant measurement room with the door closed and air conditioning maintaining room temperature between 20 to 25 °C to avoid artefact caused by unstable environmental conditions.

Figure 3-4 Mechanical time constant of the plethysmograph

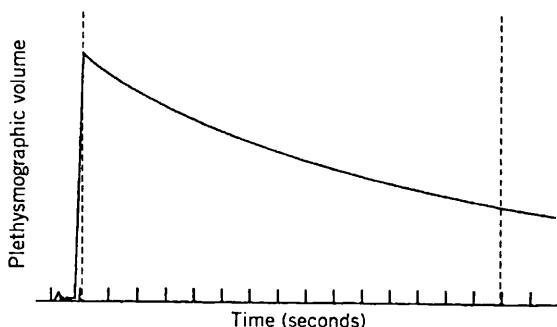


Figure 3.4. Calculation of the mechanical time constant (τ) of the plethysmograph. Following injection of 10 mL of air into the plethysmograph, it took 15 s for the V_{pleth} signal to decay to 37% of its original amplitude; that is $\tau = 15\text{s}$. Adapted from Stocks et al 1996 p206.

3.2.11.1.2 Plethysmograph volume

Prior to measuring each infant, the 100 L variable pressure plethysmograph was calibrated in terms of volume change. To approximate pressure changes occurring during measurements, 20 mL of air was syringed into and out of the chamber at a frequency equivalent to the infant's respiratory rate. Using RASP, the peak to trough signal size was measured and recalibrated if not within 1%, i.e. between 19.8 and 20.2 mL. The RASP time based calibration was used, where the desired signal amplitude was selected (20 mL), the average amplitude calculated, and a scale factor and offset computed to convert the actual signal to the desired signal, oscillating around a zero value.

3.2.11.1.3 Airflow

Within the RASP program, a zero flow signal was recorded for a few seconds then the rotameter was connected to the mask mount-pneumotachometer-shutter block assembly and signals of 50,100 and 150 $\text{mL} \cdot \text{s}^{-1}$ delivered in the expiratory direction (that is, into the apparatus). Signals were checked using the wall air supply (4 bar) through the rotameter and with a heated PNT as used during measurements. Following the recording of known flows, another zero flow was recorded and saved. Mean values during zero and known flows were then measured. The orientation of known signals was also noted, expiratory flow conventionally was displayed as negative (below zero on the display) in RASP. If the flow transducer tubing was connected incorrectly signals may have been inverted. If the mean measured signals were not within 2% of the delivered signals, the accuracy of the delivered signals was checked then, if still unsatisfactory, recalibrated. Using the calibration menu, a two point calibration was performed where a zero flow was recorded and a high setpoint signal of 150 $\text{mL} \cdot \text{s}^{-1}$ ($9 \text{ L} \cdot \text{min}^{-1}$) delivered in the expiratory direction. The calibration was accepted and known signals of 50, 100 and 150 $\text{mL} \cdot \text{s}^{-1}$ were again recorded. It was particularly important to select an accurate zero value when calibrating because a slightly negative or positive value (offset) would result in a steady drift in tidal volume when the flow signal was integrated within RASP, which could potentially disguise drift due to face mask leaks.

3.2.11.2 Pressure at airway opening (P_{ao})

A known signal of 20 cm H₂O (1.964 kPa) was recorded using a water manometer (read from the base of the meniscus) and a 10 mL syringe attached via a three-way tap to pressurise the P_{ao} transducer. Mean values for zero and 1.964 kPa were then checked. If recalibration was needed, then a two point method was used where atmospheric pressure was the zero reference (transducer tubing open to atmosphere). Transducer tubing was then connected to a water manometer using a three way tap and a 1.964 kPa signal delivered, checked for accuracy and the RASP profile saved with these calibration factors. The manometer tubing was changed periodically (6 monthly) and the level topped up with sterile water regularly to avoid any bacterial contamination which, if present, caused water to adhere to the sides of the glass making accurate recording difficult.

3.2.11.3 Volume

A 60 mL (for Fleisch “0” PNT) or 100 mL (for Fleisch “1” PNT) volume signal was recorded on RASP and checked for peak to trough accuracy (to be within 2%) and amount of drift over time. Slight drift was filtered out using RASP but, if significant, flow needed to be recalibrated after the zero voltage signal was checked to eliminate offset. The signal delivered using the Hans Rudolph calibrated syringe approximated the infant’s respiratory rate (30-40) and was as sinusoidal as possible, avoiding a square wave pattern, to simulate the physiological volume signal. The syringe was lubricated with silicone grease periodically to maintain a smooth piston action which helped the operator to deliver sinusoidal signals.

3.2.11.4 Jacket pressure

A similar method was used as for P_{ao} except that a larger known signal of 30 cm H₂O was used to check and recalibrate when necessary. The jacket transducer tubing was connected to a three-way tap and water manometer and the jacket pressure transducer pressurised using a 20 mL syringe.

3.2.12 Post measurement signals

Saline bags of a weight equivalent to the infant were wrapped in a pillowcase and placed within the plethysmograph, which was then closed and allowed to stabilise for

at least 10 minutes. A known signal of 20mL of air was injected into and out of the chamber at a frequency equivalent to the infant's respiratory rate. The time constant was checked by injecting 20 mL of air into or out of the chamber and recording the decay of the resulting plethysmographic pressure signal. The data were saved and the time taken for the signal to decay to 37% of the initial value noted. It was necessary to repeat these manoeuvres to correct for the infant's actual weight being significantly different from that predicted, and to ensure that amplifier settings and software profiles had been appropriately selected during the measurements.

Similarly, known expiratory flows of 50, 100 and 150 $\text{mL}\cdot\text{s}^{-1}$ and volume of 60 or 100mL, according to amplifier settings were recorded, together with a pressure signal of 1.964 kPa. Should the post measurement signals differ by more than 2%, after determining the cause, data were recalibrated within the RASP program as detailed in section 3.2.11. In addition, the weight and crown-heel length of the infant, apparatus dead space and barometric pressure were noted and saved to disk.

3.2.13 Cleaning of equipment

Following measurements, equipment was disassembled and the shutter block, mask mount, face mask and rebreathing bag were washed in dilute chlorhexidine solution ($>60^\circ\text{C}$). Pneumotachographs were irrigated with 90% ethanol while still warm then left to dry. Surfaces within the plethysmograph, cot and stadiometer were wiped with dilute chlorhexidine solution. Therapeutic putty was autoclaved by the Hospital Sterile Services department using a low temperature method.

3.2.14 Data storage

Raw data were copied onto one set of infant specific 3.5" disks, and two project specific 128 mB magneto-optical disks, using an external SCSI device (Ocean). Following data analysis, analysis files were copied onto the infant's disks and a project specific 3.5" master analysis disk. Once full, one of each pair of project specific magneto-optical disks was stored at a separate site. A hard copy of each analysis file was filed with the infant's questionnaire and oximetry data.

3.3 Methods

3.3.1 General methods

For all types of analyses, similar criteria for technically acceptable data were applied.

In this section, requirements for acceptable data for all analyses are described.

Criteria specific to each measurement are presented in the subsequent sections.

- Data were collected during behaviourally assessed quiet sleep (Prechtl 1974). During data collection, comments were saved with each 18 to 72 second epoch. Should active or transitional sleep state be suspected (by the presence of frequent startles or small body movements, eye movement or irregular respiratory pattern, a brief comment was added so that data could then be excluded from subsequent analyses.
- Flow through the PNT was zero during an occlusion.
- A stable end expiratory baseline (EEL) for tidal volume was recorded before, and after occlusions. EEL was often elevated for several breaths post occlusion, particularly following FRC_{pleth} manoeuvres. A step up in EEL following release of occlusions suggested a leak around the face mask and affected data were excluded. A minimum of 5 breaths were recorded and saved following release of airway occlusions to demonstrate return to baseline EEL, for quality control.
- Following sighs and coughs, a minimum of 10 breaths were recorded to reestablish EEL and tidal volume.

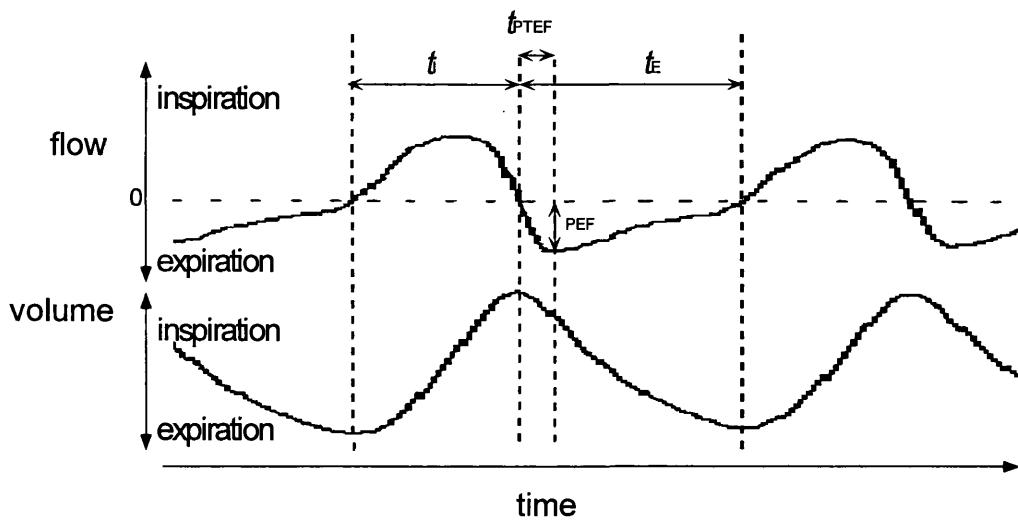
3.3.2 Tidal Parameters

3.3.2.1 Principles

Measurements of a number of indices, such as inspiratory time, t_I , have helped further understanding of the development of respiratory control during infancy. (Colin et al 1989, Fisher et al 1982, Kosch et al 1986). Around the time of inception of this thesis, several studies had suggested that in addition, analysis of other tidal breathing indices were potentially useful as measures related to respiratory mechanics and airway function in infants (Martinez et al 1988, Clarke et al 1995). The index “time to peak tidal expiratory flow (t_{PTEF}) as a ratio of total expiratory time (t_E)” had been shown to relate to other measures of airway calibre in adults (Morris et al 1981) and children (Cutrera et al 1991) and was diminished in male babies who went on to develop wheezing illnesses in later infancy (Martinez et al 1988).

Timing measurements derived from the respiratory cycle may refer either to mechanical events during the cycle, such as change in lung volume or flow, or to neuromuscular events that reflect central neural drive, such as phrenic nerve activity, diaphragmatic electromyogram or intercostal electromyogram (Stick 1996). The timing parameter, $t_{PTEF}:t_E$, is an indirect index reflecting both mechanical and control factors. Values of $t_{PTEF}:t_E$ are influenced by the decay in post-inspiratory diaphragmatic muscle activity, the presence of active expiration and the pattern of expiratory resistance. Expiratory time, t_E , is influenced by the time constant of the respiratory system, τ_{rs} , the integrated respiratory drive, tidal volume and the degree of dynamic elevation of lung volume present; particularly in younger or wheezy infants (Kosch et al 1984). Therefore, although this index appeared to relate to measures of airway function, as well as being simple to measure, it was necessary to evaluate its usefulness in comparison to a “gold standard” test of known variability and clinical relevance. Figure 3-5 shows a time based trace of tidal flow and volume showing how the tidal breathing parameters evaluated in this thesis were defined.

Figure 3-5 Tidal breathing parameters



Time based data showing flow and volume signals. t_i ; inspiratory time, t_E ; expiratory time, PEF; peak expiratory flow and, t_{PTEF} ; time to peak expiratory flow.

3.3.2.2 Measurement

Once the face mask was applied and data collection had commenced, at least 60 s of undisturbed breathing was recorded, then the mask seal and occlusion valve were checked for leaks by performing a brief end inspiratory occlusion after a stable end expiratory level (EEL) had been established (Stocks et al 1987). For subsequent test occlusions (when the face mask was reapplied) at least 10 breaths were recorded prior to occlusion, and a minimum of 5 afterwards. Mask and shutter block leaks were identified by a step up in EEL following occlusion, often accompanied by a decay in airway opening pressure during occlusion. Leaks around the face mask were readily corrected by repositioning the mask or applying more therapeutic putty. Within the occlusion valve, leaks were resolved by checking all connections, regreasing the bobbins and repriming the occlusion circuit with air (Dezateux et al 1991).

3.3.2.3 Analysis

A minimum of 20 breaths were selected over at least 2 epochs of tidal breathing (except when the infant breathed warmed, humidified air during collection of data for measurement of R_{aw}) (Stocks et al 1994). The RASP software allowed selection of a run of consecutive breaths for analysis, using cursors to select the range of data. Individual breaths could be excluded, for reasons of quality control, if a specific

reason was noted on the analysis file. Up to 70 parameters were identified for each breath, including respiratory rate (RR), inspiratory and expiratory time (t_I and t_E), peak tidal expiratory flow (PTEF), time to reach peak tidal expiratory flow as a ratio of total expiratory time ($t_{PTEF}:t_E$), and tidal volume (V_T). The start and end of expiration were defined as the last zero crossing of flow during inspiration and expiration respectively. Zero flow crossings were estimated using sample to sample linear interpolation. This strategy ensured that any expiratory pause was incorporated into expiratory time. An adjustable scan period (normally set to 0.3 seconds) prevented identification of false troughs and peaks. Peak expiratory flow was defined (using RASP) as the first sample at which maximal flow was recorded.

3.3.3 Passive Respiratory Mechanics

3.3.3.1 Principles

Measurements of passive respiratory mechanics: where the respiratory muscles are temporarily silenced by eliciting the vagally mediated Hering Breuer inflation reflex, have been increasingly used during the past decade to assess respiratory compliance (C_{rs}), resistance and the time constant of the respiratory system. Prior to the development of these non-invasive techniques (known as the occlusion techniques), assessment of respiratory mechanics were confined to paralysed and ventilated infants. The occlusion techniques have become increasingly popular and have been applied in a wide variety of settings (Landau 1990, Prendiville et al 1986, Koumbourlis et al 1992, Couser et al 1993)

3.3.3.1.1 Respiratory compliance

In contrast to dynamic measurements of lung mechanics, such as oesophageal manometry, passive (or quasistatic) respiratory mechanics do not enable partitioning of compliance and resistance into chest wall, lung and airway components. In early infancy, the chest wall is highly compliant with minimal outward recoil, and provides only a small component of respiratory compliance. As the rib cage stiffens and becomes more stable, chest wall compliance contributes increasingly to C_{rs} and by six months of age exceeds that contributed by lung tissue (Papastamelos et al 1995).

Compliance is an index of distensibility and may be defined as the change in volume per unit change in pressure expressed in $\text{mL}\cdot\text{kPa}^{-1}$;

$$C = \frac{\Delta V}{\Delta P}$$

Lung compliance (C_L) is the change in lung volume divided by the change in transpulmonary pressure measured in the absence of airflow by oesophageal manometry.

Total respiratory system compliance (C_{rs}) is a measure of the combined elastic recoil of the lung and chest wall and may be expressed as:

$$1/C_{rs} = 1/C_L + 1/C_W$$

where C_L is compliance of the lung and C_W that of the chest wall.

3.3.3.1.2 Respiratory resistance

Using the passive flow-volume (or single breath) technique, the resistance of the respiratory system, R_{rs} , may be determined. Respiratory resistance is the sum of the airway, lung tissue and chest wall resistance, expressed as:

$$R_{rs} = R_{aw} + R_{ti} + R_w$$

where R_{aw} is airway resistance, R_{ti} is visco-elastic tissue resistance and R_w resistance of the chest wall. R_{ti} is thought to make only a small contribution to total respiratory resistance (Stocks et al 1985, Helms 1982), but partitioning of R_{rs} is complex and, for example, it is not possible to calculate chest wall resistance simply as the difference between respiratory and airway resistance measured using the Single breath technique and plethysmography respectively. These issues will be examined in detail in Section 5.

3.3.3.1.3 Measurement of passive respiratory mechanics

Simultaneous values for airflow, volume and pressure are required regardless of the technique used. Airflow and volume are measured using a pneumotachograph. During the assessment of passive respiratory mechanics, recoil pressure is obtained by a brief airway occlusion above FRC when vagal stretch receptor stimulation induces the Hering Breuer Inflation reflex (HBIR), resulting in a brief respiratory pause and prolongation of expiration. During this period of no flow, pressure equilibrates throughout the respiratory system. Providing the respiratory muscles are relaxed, recoil pressure of the entire respiratory system is equivalent to pressure at the airway opening (P_{ao}) which may be readily recorded via a pressure transducer port at the mask.

3.3.3.1.4 Modelling the Respiratory system

Most techniques for assessing respiratory mechanics assume the respiratory system may be represented by a single balloon on a pipe (single compartment model). This model has only one degree of freedom: that is it can only move in one dimension, and can be described by a first order differential equation. The model has two parameters: elastance of the balloon and resistance of the pipe. If the balloon is inflated and allowed to empty, the relationship between pressure applied at the airway opening, at time t : $P(t)$, and the volume in the model $V(t)$ can be expressed as:

$$P(t) = EV(t) + RV'(t)$$

where E is the elastance of the balloon (i.ei the reciprocal of compliance), R is the resistance of the pipe and $V'(t)$ is the rate of change of volume at the airway opening (flow). The general equation of motion of the lung includes a term of inertance and acceleration, but for these purposes inertance is assumed to be negligible and the term excluded from this model.

3.3.3.2 Modelling a relaxed expiration

During a relaxed expiration, $P(t) = 0$ and the equation for the single compartment lung model can be expressed as:

$$E \cdot V(t) + R \cdot V'(t) = 0$$

This can be re-written as a first-order differential equation:

$$\delta V(t) \delta t + E/R \cdot V'(t) = 0$$

$$\delta V(t)/V(t) = -E/R \delta t$$

This can be solved by integration to yield:

$$V(t) = A e^{-t/\tau_{rs}}$$

where $V(t)$ is the volume in the lungs at time t , A is the initial volume at $t = 0$, and $\tau_{rs} = R/E$

This demonstrates that the volume-time profile of a single-compartment model of the respiratory system will describe a single exponential, with a time constant (i.e. the time required for volume to decrease by 63%), equal to R/E (or $R.C$) during a passive "expiration".

It can be inferred that, as the expiratory time constant is the product of resistance and compliance, during a passive expiration if resistance is increased as a result of airway obstruction, then expiratory τ_{rs} will be prolonged. In contrast, increased elastance (reduced compliance) will result in rapid emptying during expiration as observed in the short time constants of infants with stiff lungs.

3.3.3.2.1 The single breath technique (SBT)

This technique was first developed by Comroe and McIlroy for measuring respiratory resistance in adults (Comroe et al 1954, McIlroy et al 1963). Application of the SBT in infants followed when it became apparent that brief end inspiratory occlusion frequently evoked the HBIR resulting in relaxation of respiratory muscles not only during occlusion (as for the MOT) but also during the subsequent expiration

(LeSouëf et al 1984a, Mortola et al 1982, Thomson et al 1985).

Based on the model of a relaxed expiration, the infant airway is briefly occluded at end inspiration, evoking the HBIR and allowing passive expiration (Figure 3-6). Pressure is measured at the airway and τ_{rs} obtained from the flow-volume slope. By extrapolating the flow-volume curve to zero flow, C_{rs} may be calculated. It is assumed that the respiratory system behaves as a single compartment linear model, with negligible inertance, and is therefore described by the equation;

$$P = \left(\frac{1}{C_{rs}} \cdot V \right) + (R_{rs} \cdot V')$$

Where P is pressure, V is volume and V' is flow.

During a passive expiration, the applied pressure P is zero and therefore;

$$\frac{1}{C_{rs}} \cdot V = -R_{rs} \cdot V'$$

rearranging

$$\frac{V}{V'} = -R_{rs} \cdot C_{rs}$$

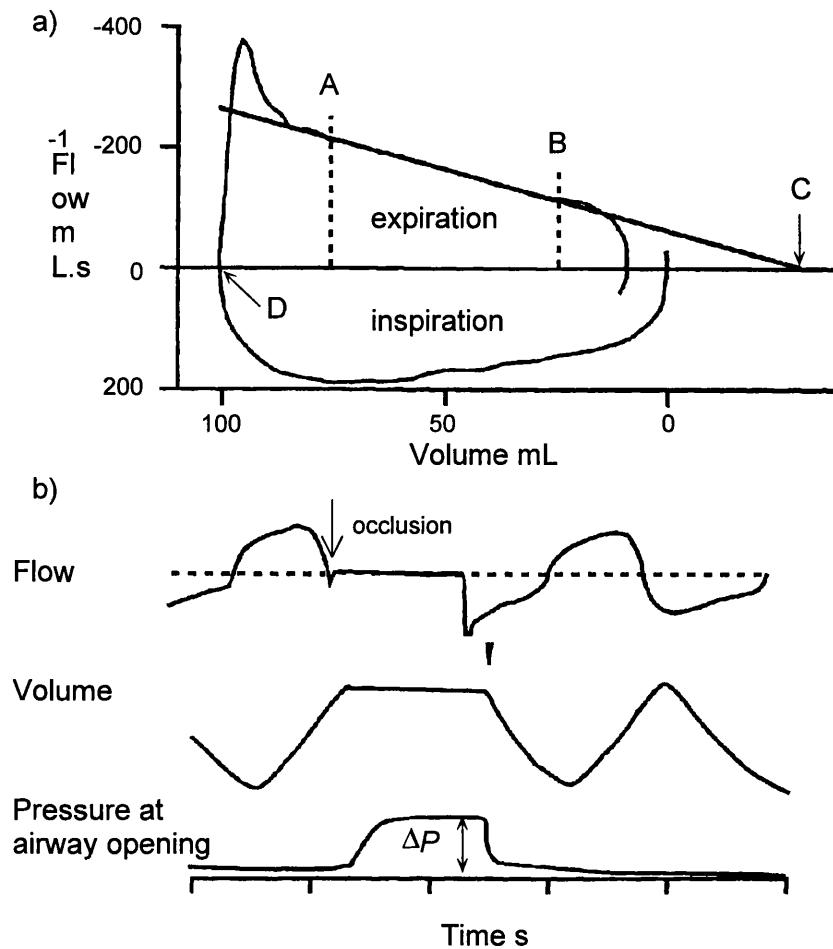
C_{rs} is calculated by measuring the recoil pressure at the moment of occlusion and extrapolating the flow volume curve to zero flow;

$$C_{rs} = \frac{\text{Volume above elastic equilibrium volume}}{\text{Plateau pressure at airway opening}}$$

As $\frac{V}{V'}$ i.e. τ_{rs} , is the slope of the expiratory passive flow volume curve and C_{rs} is

known, R_{rs} can be calculated.

Figure 3-6 The single breath technique



3.6 a): Flow-volume curve. **3.6 b):** Time based trace of flow, volume and pressure. The slope of the flow-volume curve is determined across the linear portion (AB) and the line extrapolated to zero flow (C). C_{rs} is calculated from the volume above elastic equilibrium at the time of airway occlusion (D-C) and the plateau pressure during airway occlusion (ΔP). R_{rs} is calculated from C_{rs} and the slope of the expiratory flow-volume curve. Adapted from Fletcher et al. 1996.

3.3.3.2.2 Underlying assumptions

For these techniques to be applied in clinical and research settings, rigorous quality control needs to be applied. The validity of these measures of respiratory compliance and resistance may be questioned if the underlying assumptions are not met due to poor data collection, respiratory disease or sleep state.

- Respiratory muscles are relaxed during occlusion

Infants with rapid respiratory rates or lung disease may not pause following a brief occlusion if a powerful respiratory drive exists. Inspection of the pressure profile will identify occlusions when muscles are not relaxed i.e pressure continuing to rise with no true plateau.

- Inertance is negligible

This assumption appears valid, at least during tidal breathing (Turner et al 1991)

- Elastic recoil pressure of the lungs equilibrates throughout the respiratory system during occlusion

This may not occur in airway disease or if the respiratory rate is rapid, resulting in failure to reach a plateau pressure. See analysis section for further details.

In addition, for the SBT:

- there is no respiratory muscle activity during expiration following release of the occlusion, and;
- the respiratory system is described by a single compartment model, that is described by a single time constant, τ_{rs} , where a linear flow-volume relationship exists over a large proportion (>50%) of the expired breath.

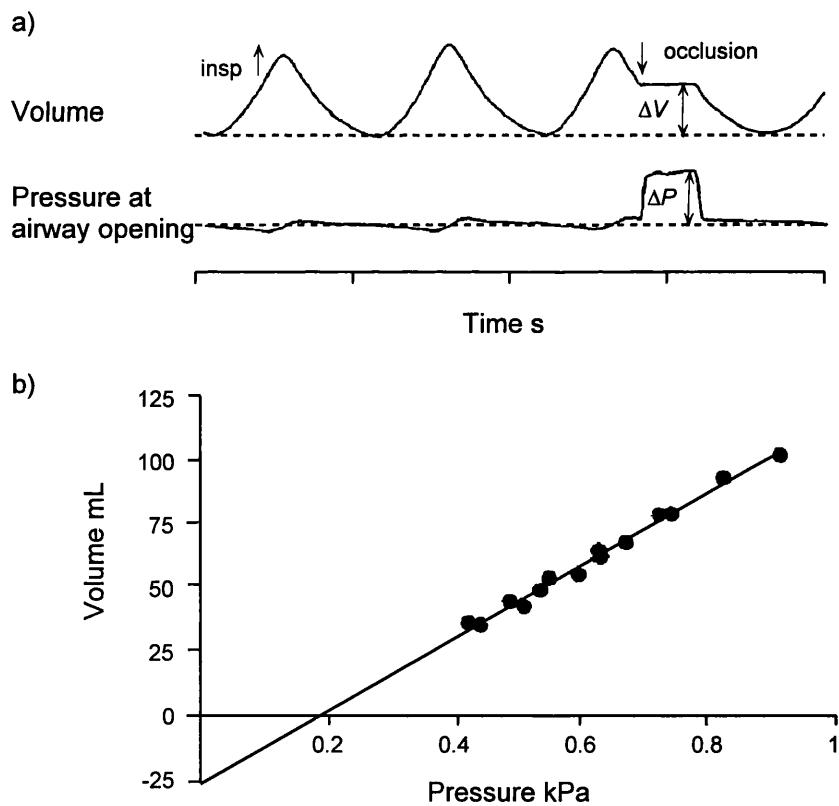
3.3.3.2.3 Multiple occlusion technique

This technique was first described by Olinsky et al in 1976. If the infant's airway is briefly occluded during expiration, the Hering Breuer Inflation reflex (HBIR) may be induced, resulting in a brief respiratory pause. In the absence of airflow, the pressure throughout the airways equilibrates and, providing no respiratory muscle activity occurs, the increase in pressure at the airway opening, ΔP_{ao} , reflects the elastic recoil of the respiratory system. C_{rs} can then be calculated by dividing the volume occluded above the elastic equilibrium volume by the airway opening pressure (P_{ao}):

$$C_{rs} = \frac{\Delta V}{\Delta P_{ao}}$$

Changes in airflow, volume and pressure at the airway opening are measured while a series of airway occlusions over the first two-thirds of expiration. Olinsky originally described the method using inspiratory occlusions but they are now more usually made during the first two thirds of expiration. Occlusions during inspiration and the final part of expiration lead to potentially unreliable estimates of compliance as the recorded pressures achieved frequently overestimate the elastic recoil pressure of the respiratory system due to muscle activity. Occlusion pressures are plotted against lung volume above end expiratory level (EEL) (Figure 3-7). A linear relationship is usually assumed (i.e. that a single compartment model applies) and a straight line of best fit calculated, its slope being C_{rs} . A negative volume intercept is normally present in infants, reflecting dynamic elevation of FRC (Fletcher et al 1996). Details of data collection and analysis are given in sections 3.3.3.2.3 and 3.3.3.4.2.

Figure 3-7 The multiple occlusion technique



3.7a): Time based trace of volume and pressure showing a mid-expiratory occlusion.
 3.7b): Pressure-volume plot constructed from multiple occlusions. $V = (C_{rs} \cdot P) + \text{volume intercept}$.
 $C_{rs} = 138.4 \text{ mL} \cdot \text{kPa}^{-1}$, $r^2 = 0.99$, 95% CI = 129.5 to 147.2 $\text{mL} \cdot \text{kPa}^{-1}$, volume intercept -25.5 mL.
 Adapted from Fletcher et al 1996.

3.3.3.3 Measurement

3.3.3.3.1 Single Breath Technique

Measurements of R_{rs} and respiratory system compliance (C_{rs}) by the SBT were attempted in all infants using the pneumatically operated valve system employed during plethysmographic measurements. End inspiratory occlusions (at end inspiration or within 10% of beginning of expiration) were held until a stable pressure plateau was seen. Occlusions that were too brief failed to elicit the HBIR and subsequently expiration was not passive, additionally, if equilibration was not attained then recoil pressure was not estimated accurately thus overestimating C_{rs} . Occlusions that were held for too long often resulted in rapid onset of inspiration, rather than passive expiration to elastic equilibrium volume. The ideal duration for equilibration of pressures has not been established, and varies significantly between infants (Steinbrugger et al 1993), but seems to be around 400 to 1000 ms (Mallol et al 1994). A minimum of 5 successful occlusions were obtained, allowing 5 to 10 breaths between occlusions to re-establish the end expiratory level and to detect air leaks around the face mask (Stocks et al 1987). When possible, occlusions were also performed while the infant was rebreathing warmed, humidified air to obtain SBT measurements of R_{rs} under the same conditions as R_{aw} . Sampling rate for the SBT was 100 Hz (200 Hz for respiratory rate greater than 50 min^{-1}).

3.3.3.3.2 Multiple Occlusion Technique

C_{rs} was also measured by the multiple occlusion technique (MOT) using previously published methods (Gappa et al 1993, Fletcher et al 1992) for comparison with SBT data (England 1988, American Thoracic Society/European Respiratory Society 1993). Brief airway occlusions during the first two thirds of expiration were performed, holding the occlusion until a plateau of at least 0.2 s was obtained (Figure 3-7). The range of recoil pressure recorded should be at least 0.3 kPa, therefore a spread of occluded volume from end inspiration to approaching EEL was recorded. In infants with a stable EEL, 5 breaths were allowed between occlusions, more if EEL was variable. Data were collected during continuous epochs whenever possible, to avoid the effects of a change in volume baseline. However, this was not always possible and if the infant stirred or other measurements took place, then the effect

over time of EEL change needed close examination. The sampling rate was 50-100 Hz according to the infant's respiratory rate.

3.3.3.4 Analysis

3.3.3.4.1 Single Breath technique

Criteria for technically satisfactory data:

- To enable accurate analysis of the slope of the flow-volume curve (i.e. τ_{rs}) and volume intercept, any constant tidal volume drift was removed before analysing data. Following release of the occlusion, passive expiration should proceed to at least 80% of the volume expired during pre-occlusion tidal breathing.
- Pressure at the airway opening should have risen rapidly to a plateau, which was then maintained for at least 0.1 s (0.2 s for the multiple occlusion technique, except in infants with very rapid respiratory rates or a strong respiratory drive when 0.1-0.15 s was accepted). A pressure plateau was defined as an SD of ≤ 0.01 kPa over 0.1 s. Figure 3-6 shows an occlusion for the SBT.

The flow-volume curve was visually inspected before analysis to reject any obviously unsatisfactory data. Curves were analysed when occluded tidal volume was at least 85% (normally $\geq 90\%$) relative to the EEL. Using linear regression analysis, a straight line was fitted to the expiratory portion. The initial part of the curve was discarded due to transients following release of the occlusion. The last portion at low lung volume may be distorted due to active expiration. However regression through 50% of expired volume was the minimum considered technically acceptable, where the default strategy was set to analyse between 60 and 10% expired volume (when 100% is the volume of air in the lungs at end inspiration and 0% the EEL). The defaults could be adjusted to within 80% to 5%, according to the individual infant data. Passive flow-volume data were accepted when a correlation coefficient of at least 0.99 (normally ≥ 0.995) was obtained.

Apparatus resistance, at the flows attained during expiration, was subtracted from R_{rs} .

Two strategies were used over the period of data collection for this thesis:

- After analysing each occlusion, the mean flows obtained over the portion of passive expiration used for analysis were noted. A scatterplot of R_{app} versus expiratory flow using the appropriate shutter and PNT was used to calculate R_{app} and this was subtracted from R_{rs} during automatic rework of the data after

entering the value onto the “options” menu.

- The RASP software was upgraded to automatically calculate R_{app} and subtract the value from each occlusion during analysis. The strategy identified mid expiratory flows and P_{ao} of the analysed expiratory portion to calculate R_{app} from $P_{ao} \div V'$.

To report an SBT analysis of R_{rs} and C_{rs} a minimum of 5 satisfactory occlusions were required, according to the quality control criteria described. When an analysis was rejected, a representative flow-volume loop was analysed and the reasons for failure were then documented and saved with the infant data. Failed analyses were grouped into 5 categories:

1. Failure to attain a relaxed P_{ao} plateau

Occlusion during early expiration, with a subsequent prolongation in expiratory time, usually evoked the Hering-Breuer inflation reflex (HBIR), resulting in a prolongation of expiratory time. A pressure plateau was obtained when pressures within the respiratory system equilibrated during conditions of no gas flow and respiratory muscles were relaxed. Under these conditions, P_{ao} reflected elastic recoil pressure. If a plateau was not attained, recoil pressure could not be estimated accurately and measurements of C_{rs} were invalid. This could be due to active expiration, failure to equilibrate or face mask leak.

2. A linear flow-volume relationship during passive expiration

A linearity of the descending limb of the passive flow-volume loop could occur due to the presence of multiple time constants within the respiratory system, as commonly seen in infants with respiratory disease. The single compartment model of the lung was thus invalid, thereby precluding accurate reporting of C_{rs} or R_{rs} as single values.

3. Early inspiration post occlusion

Data were considered technically unsatisfactory if inspiration occurred before the infant had passively expired towards EEL. This was a potential problem in infants with airway disease in whom it was necessary to hold the occlusions longer in an

attempt to achieve pressure equilibration at the airway opening.

4. Failure to sleep

When the infant woke before attempts to collect sufficient data to report at least 5 occlusions for SBT were made, and failed to return to sleep on that measurement occasion, no results were reported.

5. Technical failure/other

Possible reasons for causes of failure would include equipment failure: amplifier or shutter problems, or incorrect calibration prior to measurements, which were not possible to correct subsequently.

3.3.3.4.2 Multiple Occlusion technique

Criteria for technically acceptable data were similar to that of the SBT (section 3.3.3.4.1), with the additional requirement of a stable EEL over time to enable relation of each set of V/P data to the same baseline. Data were collected over a short period of time, attempting to avoid collection of data pre- and post rebreathing warmed, humidified air. C_{rs} from the MOT was calculated from the slope of the least squares linear regression through the volume-pressure data. Data were accepted when the P_{ao} plateau was of ≥ 0.15 s duration with a SD of ≤ 0.01 kPa and the slope of the volume-pressure regression line had an $r^2 \geq 0.95$. Although the correlation coefficient was used for quality control, high values could be obtained even in the presence of marked scatter, when occlusions were made over a wide pressure range. Therefore the 95% confidence intervals of the slope were also noted before accepting analyses.

At least 20 occlusions were made over the first 2/3 of expiration (Stocks et al 1987), and a minimum of 7 technically acceptable occlusions over a pressure range of at least 0.30 kPa were required before the analysis was reported. The volume intercept was noted and if ≥ 4 mL.kg⁻¹, occlusions were reinspected for evidence of active expiration which would invalidate results.

3.3.4 Whole body plethysmography

3.3.4.1 Principles

Infant whole body plethysmography remains the gold standard for both research and clinical measurements of lung volume and airway resistance, although limited in application by the requirements of complex, expensive equipment and training of the operator. The technique is not suitable for measurement of the acutely ill infant in an intensive care setting, though it may be readily adapted to infants with chronic oxygen dependency (Doershuk et al 1969). Current methods were established by Radford in 1974 and Stocks in 1977. In variable pressure plethysmography, the method used in this study, the infant lies supine within a rigid, closed container (the plethysmograph) and breathes through a pneumotachograph, recording flow, volume and airway opening pressure. During a brief occlusion at the airway opening, with a remotely controlled shutter, the infant makes respiratory efforts which compress and rarefy the thoracic gas volume. Thoracic gas volume at the moment of occlusion can be calculated by relating the resultant changes in alveolar pressure, which are equivalent to changes in airway opening pressure following equilibration in the absence of air flow, to changes in alveolar volume which are reflected by changes in the plethysmographic volume or pressure.

Airway resistance may be measured during unoccluded breathing by relating changes in alveolar pressure to simultaneous changes in flow at the airway opening (DuBois et al 1956a). If the temperature and humidity of the respired gas can be kept saturated at body temperature and pressure, (BTPS conditions), changes in plethysmographic pressure during spontaneous breathing will be inversely proportional to changes in alveolar pressure.

3.3.4.1.1 Lung volume

Resting lung volume is currently the only reliable and accurate estimation of lung volume that can be routinely measured in infancy. The established methods of measurement of resting end expiratory lung volume, or functional residual capacity, are whole body plethysmography and gas dilution techniques: nitrogen washout and

helium dilution. Gas dilution techniques measure the volume of gas in direct communication with the airway, in contrast to plethysmography where the total volume of gas within the lung at end expiration is measured, including that trapped behind closed airway.

Recently, within-subject comparisons of functional residual capacity (FRC) using plethysmographic and gas dilution techniques suggested that consistently smaller values of FRC were obtained by gas dilution (Gappa et al 1993). Although similar results using the two methods were seen in healthy adults and older children (Christensson et al 1981), these results support previous findings in healthy infants and young children (Borsboom et al 1993, Stocks et al 1995) where consistently higher values of FRC were reported using plethysmography. FRC measurement methods are indicated by a suffix i.e. FRC_{pleth} , FRC_{He} or FRC_{N_2} denoting plethysmographic, helium dilution and nitrogen washout measures of lung volume respectively.

In children and adults, functional residual capacity usually coincides with the end expiratory elastic equilibrium volume (EEV), which is determined by the passive respiratory mechanics of the lung and chest wall, being a balance between the outward recoil of the chest wall and inward recoil of the lungs. In adults and older children FRC is approximately 40% of total lung capacity and 25% of vital capacity. During early infancy the highly compliant chest wall with less outward recoil at end expiration results in FRC approaching residual volume, so that a degree of airway closure may occur during tidal breathing. However FRC may be dynamically elevated above the passively determined EEV in infants (Kosch et al 1984). The mechanisms for dynamic elevation of lung volume include post inspiratory diaphragmatic activity and laryngeal adduction, i.e. expiratory “braking”, in combination with a rapid respiratory rate, and consequently short expiratory time (t_E). Infants with airway disease may exhibit a more extreme form of dynamic elevation of FRC where elevated airway resistance prolongs the expiratory time constant (lung emptying being almost complete within three expiratory time constants) and consequently tidal volume cannot be fully expired in the time available for expiration, resulting in a rise in end expiratory level (EEL), until equilibrium is reached and an increased FRC attained (pulmonary hyperinflation).

3.3.4.1.2 Calculation of FRC_{pleth}

Measurement of FRC by plethysmography is based on Boyle's Law that states (in essence) that when gas in a closed container is compressed under isothermal conditions, its volume decreases as the pressure inside the container increases giving a constant product of volume and pressure, ie:

$$P \cdot V = \text{constant}$$

$$P_1 \cdot V_1 = P_2 \cdot V_2$$

Where the subscripts ₁ and ₂ indicate the initial and final conditions of the gas respectively.

At end-expiration, the volume in the lungs (V_1) is the FRC, and pressure within the alveoli (P_1) is equal to barometric pressure (P_b).

Following an inspiratory effort against the closed shutter, there will be a decrease in alveolar pressure ($P_2 = P_b - \Delta P$) and a corresponding increase in lung volume ($V_2 = FRC + \Delta V$), with opposite changes occurring during expiratory efforts.

Substituting terms, the equation now reads:

$$P_b \cdot FRC = (P_b - \Delta P) \cdot (FRC + \Delta V)$$

$$\text{Solving: } P_b \cdot FRC = P_b FRC + P_b \Delta V - \Delta P FRC - \Delta P \Delta V$$

$$0 = \Delta V (P_b - \Delta P) - \Delta P FRC$$

$$FRC \cdot \Delta P = -\Delta V (P_b - \Delta P)$$

$$\text{Thus: } FRC = -\Delta V (P_b - \Delta P) / \Delta P$$

Since ΔP is very small compared with P_b it may be omitted from the term $(P_b - \Delta P)$, giving:

$$FRC \approx (\Delta V / \Delta P) P_b$$

The gas in the lung is saturated with water vapour ($P_{H_2O} = 6.27 \text{ kPa or } 47 \text{ mmHg}$ at

37°C), and does not behave as a compressible gas. It must then be subtracted from the equation, giving:

$$\text{FRC} \approx (\Delta V/\Delta P) (P_b - P_{H_2O})$$

The calculated value for FRC includes apparatus dead space and volume occluded above end expiratory level (total occluded gas volume, or TOGV). These must be subtracted to obtain FRC

Therefore, if $\text{TOGV} = (\Delta V/\Delta P) (P_b - P_{H_2O})$

Then: $\text{FRC} = \text{TOGV} - V_{ds,app} - V_{occ}$

3.3.4.1.3 Underlying assumptions

Assessment of $\text{FRC}_{\text{pleth}}$ is based on a number of underlying assumptions (Stocks et al 1996):

1. ΔP_{ao} equals ΔP_{alv} during airway occlusion

During respiratory efforts against an occluded airway there is no air flow and therefore no flow-resistive loss in pressure. In adults with airway disease the upper airway may act as a shunt capacitor during airway occlusion, allowing gas to flow within the airway and to underestimate ΔP_{alv} by ΔP_{ao} , in turn overestimating FRC (Rodenstein et al 1982, Stanescu et al 1982). These conditions are frequency dependent and are of significance only at frequencies above 1 Hz (Brown et al 1984), and are therefore of minimal consequence in a normal infant during spontaneous breathing (Shore et al 1983). Similarly the presence of unequal time constants within the lung, as in airway disease, may create a pressure gradient and hence, during an occlusion, flow (Eber et al 1994) again overestimating FRC.

Inhomogeneous pressure swings during airway occlusion may be a consequence of airway disease when chest wall distortion is present, leading to unpredictable errors in the estimation of FRC (Kimball et al 1982, Stefano et al 1986).

2. Only thoracic gas undergoes rarefaction and compression

Changes in intra-abdominal pressure during airway occlusion are small relative to changes in alveolar pressure. For the purpose of FRC calculation, the assumption is made that abdominal gas volume is either uncompressed or that volume changes are insignificant (Hatch et al 1975, Hedenstierna et al 1985).

3. Changes in volume and pressure are isothermal

Boyle's law remains valid only when conditions are isothermal, that is during expansion or compression of gas in a container (lungs or plethysmograph) heat is transferred to or from the gas instantaneously maintaining constant temperature. Within the lungs, the large surface area available for gas exchange allows rapid heat transfer and meets those conditions. In contrast, conditions within the plethysmograph, where the thermal time constant is lengthened may be adiabatic or polytropic (i.e. partly adiabatic). Therefore accuracy of measurements may be affected by frequency dependence. To compensate for this, calibration of the plethysmograph in terms of volume is made at the approximate respiratory rate of the infant.

3.3.4.1.4 Calculation of airway resistance

In contrast to measurements of airway function during expiration, whether passive (single breath technique) or forced expiratory flows, airway resistance (R_{aw}) provides an assessment of resistance to airflow throughout the respiratory cycle. In combination with FRC_{pleth} measurement, information about both lung volume and respiratory mechanics may be made on the same occasion under similar conditions, assisting with interpretation of the effect of these related variables on infant lung function.

Airway resistance may be defined as the pressure difference that must be applied between the alveoli and atmosphere to produce a gas flow of $1 \text{ L}^{-1} \cdot \text{s}$ at the airway opening, expressed in units of $\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$. It is often expressed as its reciprocal, conductance which varies linearly with lung volume and growth in $\text{L} \cdot \text{s}^{-1} \text{kPa}^{-1}$.

Airway resistance may be expressed as:

$$R_{aw} = \Delta P_{alv} / \Delta \text{Flow}$$

Flow is measured through the pneumotachograph (PNT). ΔV_{pleth} is proportional to ΔP_{alv} and is calibrated in terms of pressure.

3.3.4.1.5 Calibration of the plethysmograph in terms of alveolar pressure change.

During airway occlusion, when there is no gas flow, assuming pressures equilibrate throughout the respiratory system:

$$\Delta P_{\text{ao}} = \Delta P_{\text{alv}}$$

If the total mass of gas in the system remains constant, changes in alveolar pressure will be inversely proportional to changes in plethysmographic pressure and directly proportional to changes in plethysmographic volume. The plethysmograph can therefore be calibrated in terms of alveolar pressure change by calculating the ratio of ΔP_{ao} (ie ΔP_{alv}) to ΔV_{pleth} during an airway occlusion. A calibration factor is derived:

$$CF = \Delta P_{\text{ao,occ}} / \Delta V_{\text{pleth,occ}}$$

The calibration factor (CF) assumes a constant ratio of lung to plethysmographic volume and only applies to the lung volume at which $\Delta P_{\text{ao,occ}} / \Delta V_{\text{pleth,occ}}$ was actually measured, which is the total occluded gas volume (TOGV). Correction to the actual lung volume at which resistance was measured is required.

TOGV comprises FRC, the volume of the apparatus dead space ($V_{\text{ds,app}}$) and any quantity of tidal volume above end-expiratory level included at the moment of occlusion (V_{occ}). In addition, during tidal breathing it is only the intrathoracic gas volume that is subjected to compression and expansion, not that within the extrathoracic airway or apparatus dead space. To calculate the correct ratio of $\Delta P_{\text{ao}} : V_{\text{pleth}}$ for calibration of P_{alv} during spontaneous breathing, the volume of these airways, which is calculated to be approximately half the anatomical dead space of the infant, or 1.15 per kg body weight should also be subtracted ($V_{\text{ds,inf}}$). Absolute lung volume during R_{aw} analysis equals FRC plus VR_{aw} , the latter being the tidal volume above end-expiration at the moment of analysis. VR_{aw} is negligible during early inspiration and late expiration, but significant at higher lung volumes during

late inspiration and early expiration and should therefore be added to FRC when R_{aw} is being measured at these points. During computerised analysis, the CF can be automatically corrected for VR_{aw} throughout the breathing cycle. A corrected CF to convert ΔV_{pleth} to ΔP_{alv} can then be calculated as:

$$CF = (\Delta P_{ao,occ} / \Delta V_{pleth,occ}) TOGV / (FRC + VR_{aw} - 1/2V_{ds,inf})$$

This CF can be derived from airway occlusions at any lung volume and may be applied to R_{aw} calculation throughout the respiratory cycle.

The resistance of the apparatus (R_{app}) must also be subtracted from the calculated value of total resistance. This can be calculated by the pressure drop across the apparatus at flows equal to those used for R_{aw} calculation.

$$R_{app} = \Delta P_{ao} / \Delta Flow$$

The final equation used for calculation of R_{aw} may be expressed as:

$$R_{aw} = (\Delta V_{pleth} / \Delta Flow) (\Delta P_{ao,occ} / \Delta V_{pleth,occ}) (TOGV / [FRC + VR_{aw} - 1/2V_{ds,inf}]) - R_{app}$$

3.3.4.2 Measurement

Figure 3-8 The infant plethysmograph

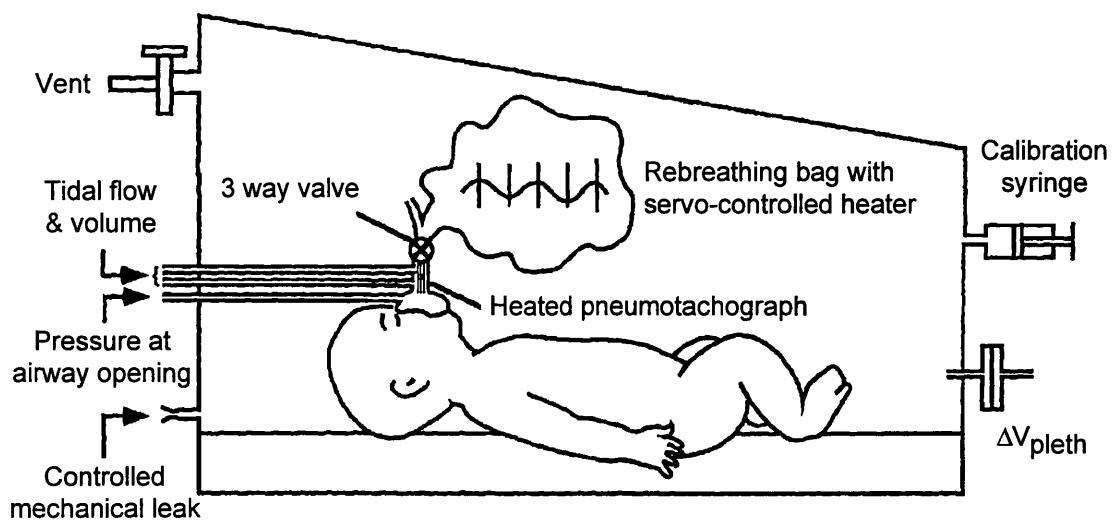


Diagram of the equipment used to measure FRC_{pleth} and airway resistance. Adapted from Stocks et al. 1996

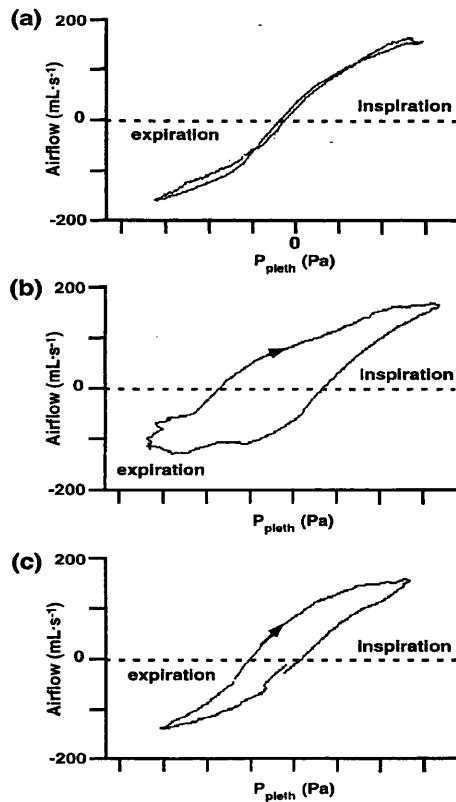
The plethysmograph was closed (Figure 3-8) and the infant allowed to breathe room air from the plethysmograph while thermal equilibrium within the chamber was attained, as indicated by a stable V_{pleth} signal. During this time, data for analysis of tidal breathing parameters were collected, once quiet sleep was established. The plethysmograph vent was then closed and measurements of thoracic gas volume at functional residual capacity were made (FRC_{pleth}) according to well established criteria as described previously in Section 3.3.4.1.1.

For FRC_{pleth} measurements, at least 5 early expiratory occlusions were made (within 10% of start of expiration), after a stable end expiratory level had been established, each being held for 2 to 3 respiratory efforts (Figure 3-10). Changes in V_{pleth} and P_{ao} were displayed on a cathode-ray oscilloscope. Data were considered acceptable if no leaks were evident and changes in V_{pleth} versus P_{ao} were in phase during occlusions. At least 5 breaths were recorded after each occlusion to provide evidence of a return to EEL. A sampling rate of 50 Hz was used for older infants (respiratory rate below around 30 min^{-1}) and 100 Hz for younger or more rapidly breathing infants.

The infant was then allowed to rebreathe from a highly compliant 2 litre bag containing warmed, humidified and oxygen enriched air (temperature at the mask $\approx 37^\circ\text{C}$). The phase relationship between V' and P_{pleth} was inspected on the oscilloscope and temperature within the rebreathing bag adjusted if necessary, until a

satisfactory pressure-flow loop was obtained (Stocks et al 1977a) (3.3.4.1.4) (Figure 3-9 (a)). Oscilloscope gains were adjusted to display the pressure-flow loop at 45°. A closed loop indicated that BTPS conditions were met. If the loop rotated anticlockwise and P_{pleth} led V' on the time based trace, as during air breathing, conditions were too cool (Figure 3-9 (b)). In contrast, if the loop rotated clockwise, and P_{pleth} lagged behind V' , the respiration gas was too warm (Figure 3-9 (c)). This situation was avoided whenever possible, as the warm gas tended to stimulate the infant into active sleep. Only a few breaths were required to assess phasing, and if conditions were obviously not at BTPS, data collection was paused while the temperature was adjusted. Three epochs of data during breathing of warmed, humidified air were collected, each of 36 s duration. The rebreathing bag was flushed with humidified oxygen enriched air between each epoch. A sampling rate of 100 Hz was used (200 Hz for respiratory rates greater than 50 min^{-1}).

Figure 3-9 Airway resistance flow-pressure loops during rebreathing showing (a) correct temperature and humidity, (b) too cool and (c) too warm



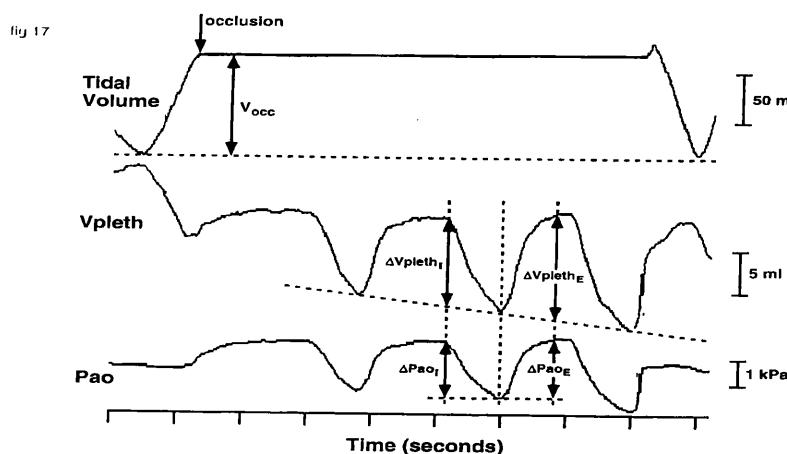
3.3.4.3 Analysis

Criteria for technically acceptable data:

- A stable EEL recorded prior to, and following, occlusions for FRC measurements, with no evidence of leaks.
- During an occlusion, changes in P_{ao} and V_{pleth} should be in phase. Constant drift during airway occlusion should be filtered to allow inspection of the phase relationship. The phase relationship should be inspected on an X-Y plot and occlusions were not analysed if there was any evidence of glottic closure or leaks through or around the apparatus (Stocks et al 1991, Sherrill et al 1996).

FRC_{pleth} was calculated as the mean of a minimum of 3 (maximum of 5) occlusions, where the infant was occluded at end inspiration or within 10% of the start of expiration. Each occlusion was the mean of 1 to 3 respiratory efforts, with each complete effort being the inspiratory and expiratory mean. Analysis defaults were set to measure each effort between 95 to 5% of the peaks and troughs thus limiting measurements to the rapidly changing period of the phase relationship. The subsequent correction to FRC was performed by subtracting the volume occluded above end expiratory volume and the apparatus dead space from the total occluded gas volume. The derivation of the algorithm used to calculate FRC_{pleth} is in section 3.3.4.1.2. Figure 3-10 shows an example of a satisfactory lung volume occlusion as a time based trace, identifying analysis parameters.

Figure 3-10 Calculation of FRC_{pleth}



3.3.5 Airway resistance

Criteria for technically satisfactory data:

- There should be only minimal drift of the plethysmographic pressure signal during measurements of airway resistance. Measurements of R_{aw} should be made after thermal equilibrium has been reached. Signals were affected adversely by changing weather conditions, which at times led to drift even when adequate time had been allowed for stable conditions to be met.
- Flow and plethysmographic pressure and volume signals should be in phase on a time series axis, and closed at points of zero flow when viewed on an X-Y plot (Figure 3-11 (a)). Failure to achieve a good phase relationship between V' and P_{pleth} may be due to several factors including failure to achieve BTPS conditions, face mask leaks, obstruction either from within the infant's airways, or from imposed obstruction by neck flexion or obstructed nostrils (such as following an upper respiratory tract infection).

Default strategies for reporting values of R_{aw} using RASP interactive operator analysis allowed selection and filtering (plethysmographic signals and tidal volume) on a breath-by-breath basis. Epochs of data were analysed when a time based trace suggested that V' and P_{pleth} were in phase. R_{aw} was reported as a minimum of 5 and maximum of 25 breaths, where the pressure-flow loops were accepted if closed at points of zero flow. R_{aw} was initially calculated from the beginning and end of both inspiration and expiration, i.e. at 4 points throughout the respiratory cycle, giving initial and end inspiratory and expiratory R_{aw} , at 50% maximum tidal flows (Figure 3-12). Data were then reworked using the facility within the RASP program at mean initial and end inspiratory and expiratory flows.

Figure 3-11 Airway resistance time based, flow-pressure loops and flow-volume loops

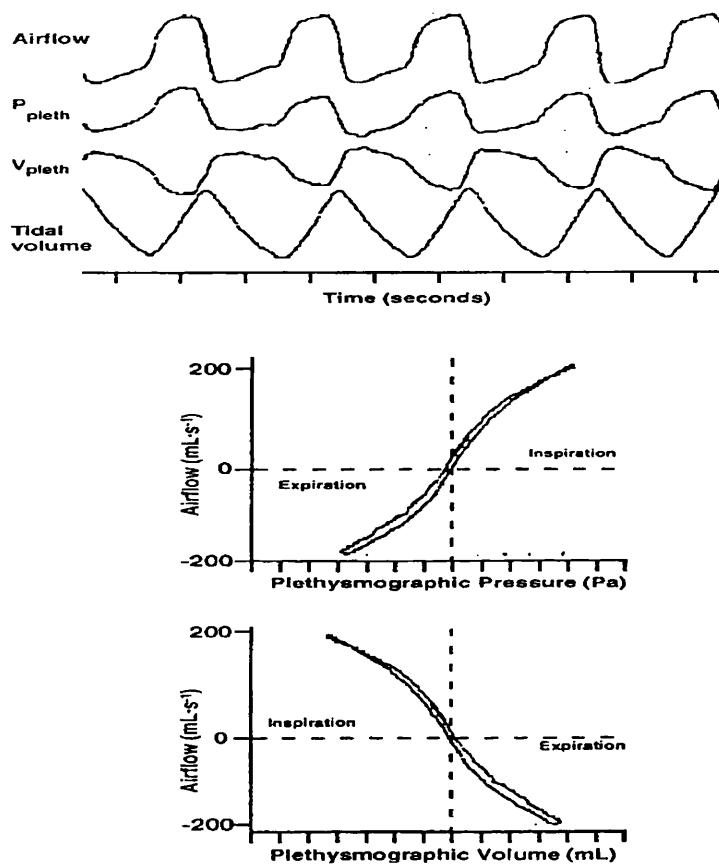
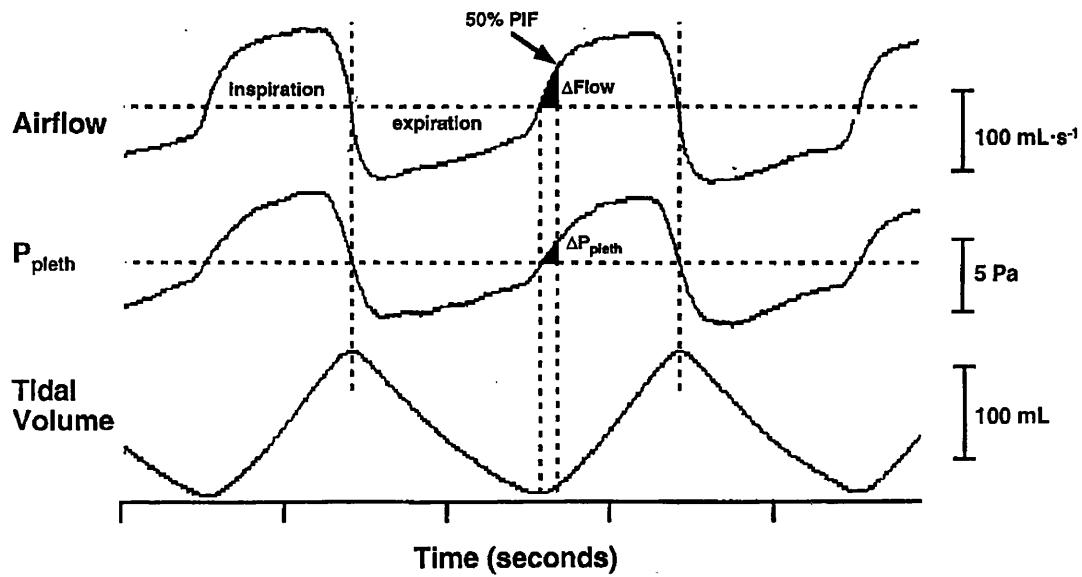


Figure 3-12 Calculation of airway resistance



3.3.6 Rapid Thoraco-abdominal Compression technique

3.3.6.1 Principles

In adults and older children, measurements of forced expiration are the gold standard approach to assessment of airway function, in both clinical and research settings. These include measurements of total volume expired: forced vital capacity (FVC), volume expired over time: forced expiratory volume at time t (FEV $_t$), peak expiratory flow: (PEF) and maximal expiratory flow at given lung volumes. The forced vital capacity manoeuvre, FVC, consists of a maximal inspiration followed by a rapid forced complete expiration and was first described last century (Hutchinson 1846). A further development of the method allowed improved assessment of respiratory function in obstructive airway disease by measuring expiratory flow during a specific period of time. The volume exhaled during the first second is called the forced expiratory volume, FEV $_1$ and is normally around 80% of FVC in adults.

Expiratory flow limitation during forced expiratory manoeuvres is considered to be the mechanism for the FEV $_t$ being the most useful measure of lung function in adults and older children. The concept was described by Fry et al in 1954 when, using iso-volume pressure flow curves, it was shown that flow is limited at fairly low transpulmonary pressures on the descending portion of the maximum expiratory flow volume curve (MEFV), with expiratory flow increasing as driving pressure increased to a maximum beyond which a further increase in pressure did not achieve increased flows. Thus, much of the MEFV curve is effort independent and it may be considered to represent airway conductance peripheral (upstream) to the flow-limiting segment of the tracheo-bronchial tree.

Three major mechanisms have been proposed to explain the concept of flow limitation, each of which is based on ideas developed from the previous theory:

- Equal pressure point theory (Mead et al 1967);
- Starling resistor theory (Pride et al 1967), and;
- Wave-speed theory (Dawson et al 1977).

Mead's equal pressure point theory proposed that flow limitation occurs at a given

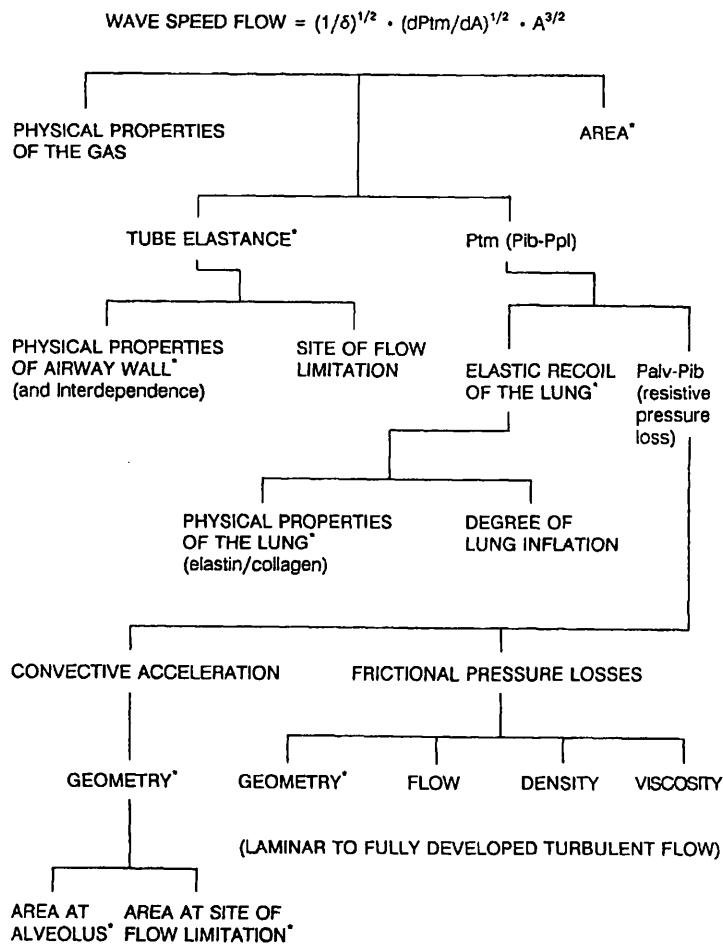
lung volume at a part of the airway when intrabronchial and intrapleural pressures are equal and opposite and that this site acts as a fixed resistance (equal pressure point, or EPP). At that part of the airway, flow remains constant despite increasing driving pressure, secondary to a proportional increase in airway resistance. Downstream of the equal pressure point (towards the mouth), pleural pressure (P_{pl}) exceeds intrabronchial pressure (P_{br}) and airway compression results. Upstream of the equal pressure point, towards the alveoli, driving pressure results from alveolar pressure (P_{alv}) less pleural pressure, which is the elastic recoil of the lung (P_{el}) driving expiration. As expiration continues, the EPP moves upstream towards the periphery as elastic recoil diminishes and airway resistance increases, as lung volume falls. Similarly, the equal pressure point is further upstream in infants with intrathoracic airway obstruction, in whom airway resistance is elevated during expiration. Determinants of maximal expiratory flow according to the equal pressure point theory are P_{el} , airway resistance upstream of the EPP and airway compliance at the site of compression.

The Starling resistor theory (Pride et al 1967) compares the large intrathoracic airways to the collapsible tube within the resistor used to control blood flow in the Starling heart-lung preparation. The site of flow limitation is determined by a critical transmural pressure where the large airways are dynamically compressed, narrowing to form a flow limiting orifice.

Wave-speed theory (Dawson et al 1977) was developed from studies of flow limitation within the urethra, and linked theories of wave-speed velocity in compliant tubes transporting incompressible fluids and maximal forced expiration. The determinants of flow limitation according to this theory are summarised in Figure 3-13 and the expression that predicts wave speed flow has terms of gas density, the slope of the transmural pressure-area characteristic (a measure of tube stiffness), and the cross sectional area at the site of flow limitation. Hence, higher flows within a tube will be seen with low density fluid, a stiffer tube wall and a larger cross sectional area. Flow limitation in this model is seen at the point within the bronchial tree where local wave speed flow is minimal for a given lung volume and this is termed the “choke point”. Multiple choke points may be present in parallel airways during a forced expiration, and expiratory airflow is the sum of these wave-speed

limited airflows.

Figure 3-13 Determinants of wave-speed airflow limitation



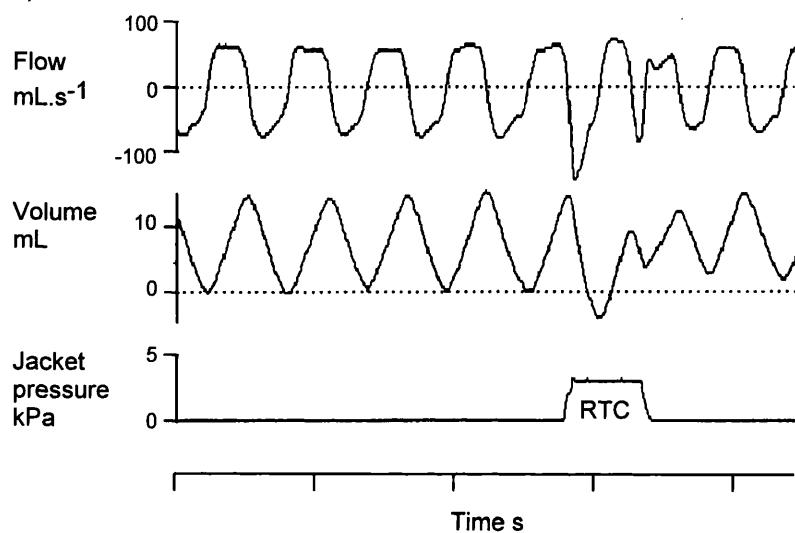
Indirect methods of achieving forced expiration during infancy include the application of thoraco-abdominal pressure to produce a passive forced expiration, both within the tidal range and at raised lung volumes (Adler et al 1978, Turner et al 1995). Alternatively, in tracheally intubated infants, negative pressure may be applied to produce a forced deflation (Motoyama 1977)

Partial expiratory flow-volume (PEFV) curves are generated when pressure is applied using a rapidly inflatable thoraco-abdominal jacket to the chest wall, producing a passive expiration. The technique was first described in 1978 (Adler et al) and later modified (Taussig et al 1982). Expiratory flow is measured at functional

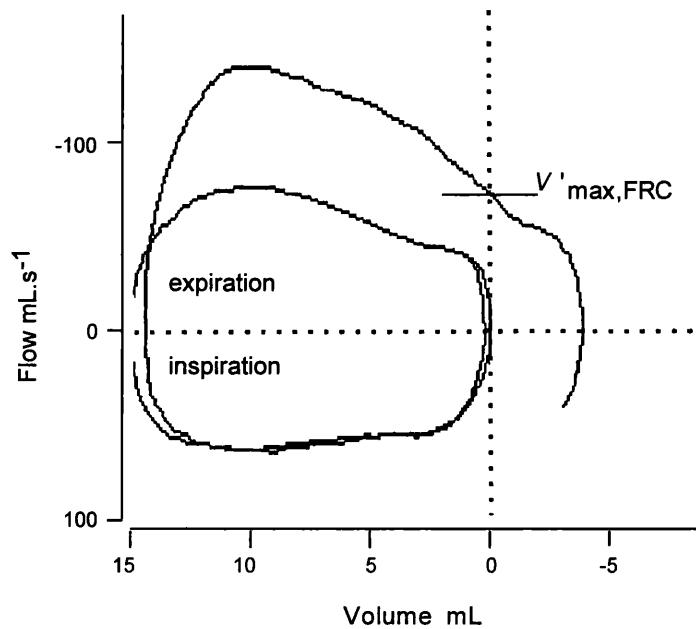
residual capacity, as a reference point, assuming that flow limitation is achieved at these low volumes. Maximal flow recorded at FRC is denoted $V'_{\max, \text{FRC}}$. Jacket pressure is incrementally increased until no further increase in flow is seen, avoiding excessive pressures which may cause negative effort dependence

Figure 3-14 Measurement of $V'_{\max, \text{FRC}}$

a) Real time data



b) Flow - volume curve



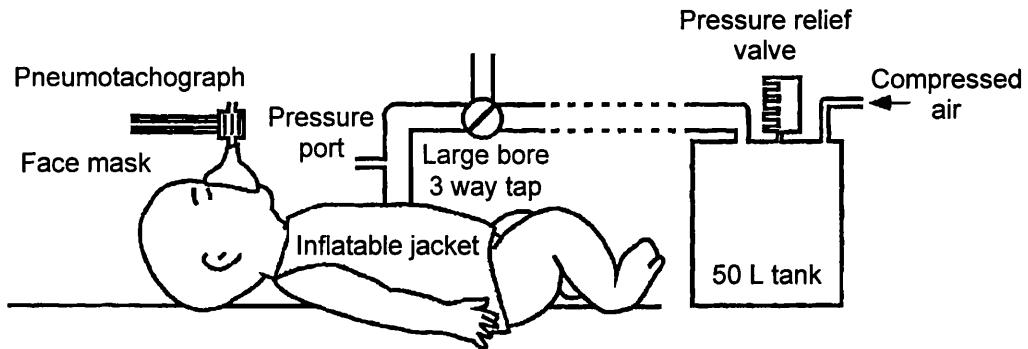
3.13a): Time based recording of flow, volume and jacket pressure during a rapid thoraco-abdominal compression. **3.13b):** Flow-volume curve to demonstrate calculation of $V'_{\max, \text{FRC}}$.

secondary to dynamic airway compression. However in normal infants maximal flows may exceed those obtained using modest compression pressures of around 10 kPa particularly as a substantial amount of jacket pressure is not transmitted to the chest wall (Stick et al 1994). A further concern with the technique is the use of FRC as a volume landmark, which is affected by sleep state and dynamic elevation of lung volume.

Together with examining the shape of the expiratory flow-volume curve, $V'_{\max, \text{FRC}}$ has been widely used in assessment of intrathoracic airway function, as a simple non-invasive gauge of small airway size (Taussig et al 1982).

3.3.6.2 Measurement

Figure 3-15 Set up for the Rapid Thoraco-abdominal compression technique



The face mask and pneumotachometer were disconnected from the shutter block and applied to the infant's face, using a rim of therapeutic putty. The inflatable jacket was applied around the infant's chest and abdomen. A epoch of tidal breathing was collected and a brief end inspiratory occlusion performed to detect face mask leaks. At least 10 breaths were recorded to establish a stable EEL, then the jacket was inflated rapidly at end inspiration, held for one second until all volume was expired (or inspiration commenced) and then pressure was vented to atmosphere and the jacket rapidly deflated. Using RASP the data could be viewed as a time based trace or as an X-Y plot of flow-volume, to assist with timing of the forced expiration (Figure 3-14) The jacket inflation pressure commenced at approximately 2.5 kPa, and was increased in increments of 0.5 kPa to 10 kPa, or until the highest flow at

FRC ($V'_{\max, \text{FRC}}$) was obtained. After each incremental increase in jacket pressure, the flow at FRC was rapidly analysed and when no further increases in flow at FRC were seen with increasing pressure, pressures were decreased until flows again decreased. Once optimal jacket pressure had been established, at least 5 further inflations were recorded at that pressure. The optimal jacket pressure was the minimum at which $V'_{\max, \text{FRC}}$ was obtained consistently. Caution was exercised in those infants who were flow limited at low inflation pressures or even within the tidal range where increasing applied jacket pressure could actually reduce expiratory flows below maximal for that infant. This negative effort dependence is probably related to dynamic airway compression. A further consequence of higher jacket pressures, seen in both healthy and wheezy infants, was glottic closure during expiration. This could be reduced by extending the neck and using a small neck roll to fully open the upper airway.

The transmission of jacket pressure to the pleural space was assessed, whenever possible, by occluding the infant at end inspiration, to evoke the HBIR and record a relaxed pressure plateau, from which the elastic recoil pressure could be measured. As soon as this had been achieved, the jacket was rapidly inflated which resulted in a second higher plateau. The difference between the two plateaux estimated the pressure actually transmitted to the pleura (Stick et al 1994).

3.3.6.3 Analysis

Criteria for technically acceptable data:

- Jacket inflation pressures were over a sufficient range to obtain maximal flows in the infant, up to a maximum of 100 kPa as applied from the pressure source.
- Time to peak jacket (P_J) inflation was < 0.1 s, from the onset of inflation to peak jacket pressure. Slow jacket inflation time may result in pressure being applied to the pleura during mid to late expiration. Loose jackets or a stiff jacket pressure tap were among the reasons for P_J inflation time exceeding 0.15 s.
- Jacket inflation lag time (the interval between end inspiration and onset of jacket inflation) should be within 0.1 s.

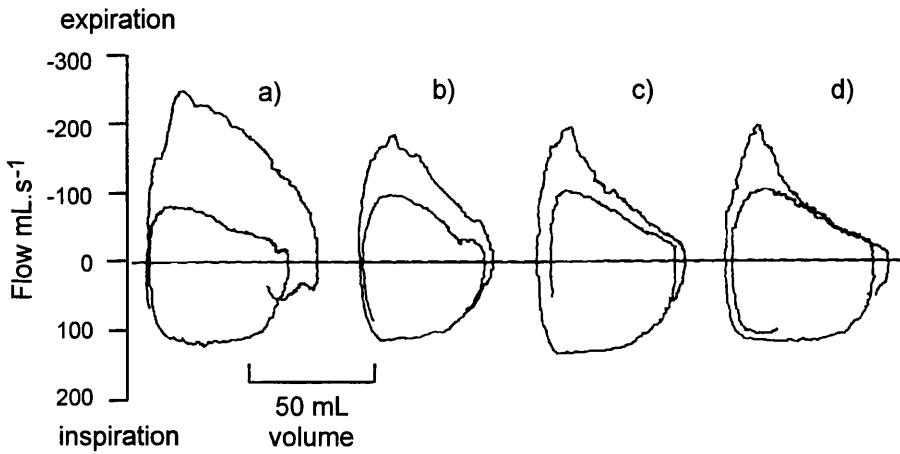
- A rapid rise to peak expiratory flow should be seen, with no evidence of glottic closure, although transient alterations in flow prior to mid expiration were accepted, if in agreement with similar data with a smooth expiratory flow pattern.

A stable EEL was particularly important for analysis of forced tidal expiratory flows. During the rapidly changing part of expiration, poor selection of EEL could markedly affect values of $V'_{\max, \text{FRC}}$. Peak expiratory flow volume (PEFV) curves were accepted for analysis if no evidence of braking of expiratory flows was seen (as indicated by a flattened expiratory curve, with peak expiratory flow attained late during expiration). When inspiration occurred before FRC was reached it was not possible to analyse affected data. If the PEFV curve was shifted to the left (step up in volume) the presence of a face mask leak was suggested.

PEFV curves were analysed using interactive operator quality control, by RASP software. A minimum of 4 technically acceptable curves were reported, together with the “best” (highest technically acceptable) $V'_{\max, \text{FRC}}$ value. The maximal and mean jacket pressures were noted, and the number of inflations recorded to enable reporting of analyses. The coefficient of variation [100·(SD/mean)] was calculated for each infant.

Figure 3-16 shows examples of different flow-volume curves, ranging from the convex shape seen in healthy infants to the concave, flow limited pattern often seen in infants with airway disease (LeSouëf et al 1986).

Figure 3-16 Rapid Thoraco-abdominal compression technique flow-volume curves



Examples of flow-volume curves of different shapes. [Le Souëf et al, 1986]

4. The relationship between $t_{\text{PTEF}}:t_E$ and specific airway conductance in infancy

4.1 Introduction

Tidal breathing measurements are being increasingly applied to population based studies of the determinants of early respiratory morbidity (Martinez et al 1988, Martinez et al 1991). The time taken to achieve peak tidal expiratory flow as a proportion of total expiratory time ($t_{\text{PTEF}}:t_E$), when measured in healthy infants in the first 3 months of life, has been shown to be predictive of subsequent wheezing in boys during the first 3 years (Martinez et al 1988, Martinez et al 1991). In addition, $t_{\text{PTEF}}:t_E$ has been reported to be significantly related to indices of airway size in adults and children (Morris et al 1981, Cutrera et al 1991). However, the extent to which this parameter of tidal breathing is associated with established measures of airway function in infants remains unclear.

The aim of this study was to examine the association between $t_{\text{PTEF}}:t_E$ and specific airway conductance (sG_{aw}), when measured on the same test occasion, in both healthy infants and those with recurrent wheezing.

4.2 Study Population

4.2.1 Recurrent wheezing group

Recruitment of the population for this study was described in section 3.1.1. Of the eligible infants whose details were supplied, the families of 58 were contacted either by telephone or by visiting the ward. A further 8 families who had no telephone number (and for whom a number could not be obtained) or whose referral forms were incomplete were not contacted. Of the families who were approached, 3 infants were found to be ineligible for inclusion (command of English not adequate to obtain informed consent) and 3 agreed to attend but their infants contracted respiratory tract infections repeatedly, so that appointments to visit for measurements were cancelled several times. The family of one infant withdrew after initially agreeing to take part. The remaining 51 infants with recurrent wheezing attended the laboratory for respiratory function measurements during the period of data collection for this thesis

(March 1992 to July 1995) and results from all infants are reported in this and the next section.

Background details of the group were obtained from parents at the time of their visit and, if necessary, from the Hospital notes. The mean age of onset of wheezing reported by parents was 12 weeks (range: 0 to 32 weeks), and the number of episodes ranged from 1 to 15. For 35 infants, parents reported continuous wheezing and for 5 infants parents were either unable to state the number of episodes (3 infants) or were unable to identify any episodes of wheezing (2 infants). Parents reported that a physician had diagnosed asthma in 33 infants (65%), wheezy bronchitis in 5 infants (10%), bronchiolitis in 35 infants (69%) and allergies in 13 infants (26%).

Episodes of wheezing were associated with "coughs and colds" in 46 infants (90%), with "allergy" in 4 (8%) and with "change of weather" in 25 (49%). There was "no obvious reason" in 19 subjects (37%) and "other" reasons for wheezing in 23 subjects (45%). "Other reasons" for wheezing included onset after feeding and in relation to physical activities such as crawling, walking and crying.

49 of 51 families had consulted their General Practitioner about their infant's wheezing on a median of 5 occasions (mean 9, range 0 - 40), 2 had not been discharged from hospital since birth. Mean (range) number of admissions to hospital in 43 infants was 3 (1 - 18). Thirteen (26%) had visited a Hospital Casualty department and 35 (67%) had attended a Respiratory Out - patient clinic

At the time of measurements, 18 infants had recently received beta-agonists (range 1 - 96 hours), 4 had received ipratropium bromide (range 6 - 18 hours), 12 had received inhaled steroids (range 1 -18 hours) and one had received theophylline (4 hours before measurements).

4.2.2 Control group

Available for comparison with this group were 106 infants who had been recruited shortly after birth to an ongoing epidemiological study (Dezateux et al 1999), most of whom had attended the laboratory for plethysmographic measurements of respiratory function on two occasions, or a small number who had been recruited at a later age for additional studies (Gappa et al 1993) (section 4.2.1). Using Excel version 5.0, data files were constructed for both groups listing a unique identifier for each infant with sex, age, and whether measurements of both the simpler methods of

passive mechanics and tidal parameters together with whole-body plethysmography had been attempted. For the healthy infants, the data file was coded to indicate those infants who had developed physician-diagnosed wheezing by their laboratory visit at around one year of age, and they were excluded from the selection of healthy infants. The remaining group of healthy infants were then sorted by sex, then ranked in terciles according to age in weeks. Healthy infants were then selected by matching with wheezy infants within each tercile, as far as possible selecting those of a similar age. Selection of the population was made without reference to the relative success rates of each method, but preferentially selecting, whenever possible, those infants where the techniques had been attempted on the same occasion. Data from infants in the healthy group (who normally attended on two occasions) were reported from one measurement occasion only. Baseline characteristics of the 51 control group infants were compared with the entire unselected population. There were no significant differences in birthweight, gestational age, prevalence of maternal smoking or family history of asthma between the two groups ($p > 0.10$ for all parameters).

4.3 Statistical analysis

Unpaired t-tests and a two sample test for proportions were used to compare baseline characteristics and lung function parameters between healthy and wheezy groups of infants. Comparisons of lung function parameters, obtained by different techniques or under different conditions, were made using the method of Bland and Altman (Bland et al 1986). Linear interactive modelling was used to examine the extent to which variation in $t_{\text{PTEF}}:t_E$ was explained by $\text{FRC}_{\text{pleth}}$ and specific airway conductance (sG_{aw}), measured at initial inspiration (II) and end expiration (EE), both before and after adjusting for age, sex, weight and length at testing.

4.4 Study Design

Measurements were made, following sedation with triclofos sodium, as described in section 3.1.3. Plethysmographic measurements of $\text{FRC}_{\text{pleth}}$ and R_{aw} and data for the analysis of tidal breathing parameters were collected and analysed as described in Section 3.3.

Data for tidal breathing and $\text{FRC}_{\text{pleth}}$ were successfully obtained on all occasions in each group of infants within the study population selected. Failure to measure R_{aw}

occurred on one occasion in the healthy group when the infant woke before this could be attempted.

Data for comparison of $t_{\text{PTEF}}:t_{\text{E}}$ and sG_{aw} were therefore available for 101 infants, a complete set of measurements having been obtained in 50 healthy infants and in 51 infants with recurrent wheezing.

4.5 Results

Details of the infants included in each group are given in Table 4-1. There were no significant differences between healthy and wheezy infants with respect to sex distribution and age, weight or length at testing. Significantly fewer wheezy infants were of Caucasian origin relative to healthy infants, reflecting the differing criteria for inclusion for wheezy infants. Mean birthweight and gestational age were significantly lower in wheezy infants compared with healthy (mean difference; 95% CI: -510 g: -205, -815; and -2.71 weeks: -1.53, -3.89 respectively). There were no significant differences in the prevalence of maternal smoking during pregnancy in wheezy compared to healthy infants (37 vs 30% respectively). However, a family history of asthma was reported in 49% of the wheezy infants, this being more common than in the healthy group (mean difference; 95% CI: 29%; 10, 48). Four healthy infants (5%) were symptomatic at testing, all of whom had minor signs of upper respiratory illness only. Of the 21 (41%) wheezy infants reported as symptomatic at testing, 5 had minor signs of an upper respiratory tract infection, while audible wheeze or expiratory rhonchi were present in 16. In the 4 (8%) healthy infants reported as symptomatic, all showed only minor signs of upper respiratory tract infection.

Table 4-1 Infant characteristics

	Healthy n=50	Wheezy n=51	95% CI (wheezy-healthy)
Male n (%)	34 (68)	35 (69)	-17, 19%
Caucasian n (%)	48 (96)	35 (78)	-42, -12%***
Age (weeks) [range]	47.1 (16.2) [9.6-70.9]	44.7 (19.0) [15.1-94.6]	-9.4, 4.5
Weight (kg) [range]	9.1 (1.9) [4.5-13.8]	9.3 (1.4) [7.7-11.0]	-0.74, 0.86
Length (cm) [range]	74.4 (6.7) [58.1-83.4]	72.6 (7.1) [51.6-90.5]	-4.6, 0.94
Birthweight (g) [range]	3544 (460) [2575-4600]	3034 (977) [864-6875]	-815, -205**
Gestation (weeks) [range]	40.3 (1.3) 38-43	37.5 (4.0) 25-42	-3.89, -1.53***
Antenatal smoking n (%)	15 (30)	19 (37)	-11, 26%
Family history of asthma n (%)	10 (20)	25 (49)	11, 48%***
Symptomatic at testing n (%)	4 (8)	21 (41)	16, 50%***

Key to Table: Values are shown as mean (SD) and [range] or n (%) as appropriate.

SD: Standard Deviation; CI: Confidence Interval.

P values for differences Wheezy -Healthy: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

Lung function parameters for each group are summarised in Table 4-2. Mean (range) FRC_{pleth} was 261.7 mL (133.7 - 375.6) and 292.5 mL (109.9 - 538.1) for healthy and wheezy infants respectively. The interquartile range (IQR) was 203.5 to 311.9 mL (healthy infants) and 244.9 to 349.4 mL (wheezy infants). The group mean difference (wheezy - healthy) was significant (30.7 mL: *P* = 0.049). When corrected for body weight, the group mean difference (wheezy - healthy) was 4.4 mL.kg⁻¹, mean (range) being 28.8 mL.kg⁻¹ (19.7 - 37.2) and 33.2 mL.kg⁻¹ (20.4 - 63.1) for healthy and wheezy infants respectively. Weight correction increased the significance of the difference between groups, to give *P* = 0.006, 95% CI of the difference (wheezy - healthy): 1.3, 7.5 mL.kg⁻¹. Mean (range) airway resistance at initial inspiration ($R_{\text{aw,II}}$) and at end expiration ($R_{\text{aw,EE}}$) were significantly higher in the wheezy group of infants, (3.09 kPa.L⁻¹.s (1.19 - 10.50), and 4.6 kPa.L⁻¹.s (1.06 - 17.28)) than in the healthy group (1.95 kPa.L⁻¹.s (0.89 - 4.40), and 2.35 kPa.L⁻¹.s (0.77 - 10.27)) respectively.

Table 4-2 Lung Function parameters

	Healthy n=50	Wheezy n=51	95% CI (wheezy-healthy)
FRC _{pleth} (mL)	261.8 (65.6)	292.5 (87.8)	0, 61.3*
FRC _{pleth} (mL.kg ⁻¹)	28.8 (3.9)	33.2 (10.3)	1.3, 7.5**
$R_{aw,II}$ (kPa.L ⁻¹ .s)	1.95 (0.86)	3.09 (1.70)	0.60, 1.67***
$R_{aw,EE}$ (kPa.L ⁻¹ .s)	2.35 (1.65)	4.61 (3.38)	1.21, 3.31***
$sG_{aw,II}$ (s ⁻¹ kPa ⁻¹)	2.31 (0.74)	1.40 (0.56)	-1.16, 0.65***
$sG_{aw,EE}$ (s ⁻¹ kPa ⁻¹)	2.13 (0.81)	1.12 (0.61)	-1.30, -0.73***
RR (min ⁻¹)	32.4 (8.0)	36.0 (9.7)	0.1, 7.1*
t_{PTEF} (s)	0.326 (0.110)	0.245 0.110	-0.125, 0.037***
t_E (s)	1.13 (0.25)	1.04 (0.27)	-0.185, 0.020
$t_{PTEF}:t_E$	0.292 (0.082)	0.235 (0.088)	-0.091, -0.024**
V_T (mL.kg ⁻¹)	10.3 (1.8)	9.4 (1.8)	-1.59, -0.21*
R_{rs} (kPa.L ⁻¹ .s)	3.16 (0.84) ^a	4.33 (1.52) ^a	0.59, 1.75***
C_{rsSBT} (mL.kPa ⁻¹ .kg ⁻¹)	14.9 (2.6) ^a	11.3 (2.5) ^a	-4.89, -2.46***
C_{rsMOT} (mL.kPa ⁻¹ .kg ⁻¹)	14.3 (2.6) ^b	12.7 (2.4) ^c	-2.62, -0.62**

Key to Table: SD: Standard Deviation; CI: Confidence Interval.

P values for differences Wheezy -Healthy: * P < 0.05; ** P < 0.01; *** P < 0.001.

FRC_{pleth} : plethysmographic functional residual capacity;

$R_{aw,II}$, $sG_{aw,II}$, initial inspiratory airway resistance and specific conductance respectively;

$R_{aw,EE}$, sG_{awEE} , end expiratory airway resistance and specific conductance respectively;

RR: respiratory rate;

t_{PTEF} : time to peak tidal expiratory flow

$t_{PTEF}:t_E$: time to peak tidal expiratory flow as a proportion of total expiratory time;

t_E : total expiratory time;

V_T : tidal volume;

R_{rs} total respiratory resistance;

C_{rsSBT} : total respiratory compliance as measured by the single breath technique;

C_{rsMOT} : total respiratory compliance as measured by the multiple occlusion technique.

n=36 for parameters indicated by a; n=48 for b and n=49 for c.

Mean $sG_{aw,II}$, $sG_{aw,EE}$ and $t_{PTEF}:t_E$ were significantly lower among wheezy than healthy infants (Table 4.2). Mean (range) $sG_{aw,II}$ for healthy and wheezy groups were $2.31 \text{ s}^{-1} \cdot \text{kPa}^{-1}$ (0.97 - 4.40) and $1.40 \text{ s}^{-1} \cdot \text{kPa}^{-1}$ (0.25 - 3.01) respectively, whereas mean (range) $sG_{aw,EE}$ was $2.13 \text{ s}^{-1} \cdot \text{kPa}^{-1}$ (0.39 - 4.34) and $1.12 \text{ s}^{-1} \cdot \text{kPa}^{-1}$ (0.21 - 3.07), showing marked between-subject variability.

Mean (range) $t_{PTEF}:t_E$ was 0.292 (0.138 - 0.481) and 0.235 (0.107 - 0.469) in the healthy and wheezy groups respectively. The group mean difference for $t_{PTEF}:t_E$ was not explained by a difference in t_E , (Group mean difference, wheezy-healthy: -0.09; 95% CI - 0.185, 0.020). Respiratory rate was greater in the wheezy group: Group mean difference (95% CI), 3.6 min^{-1} (0.1, 7.1); range $24.3 - 66.4 \text{ min}^{-1}$ (wheezy) and $21.7 - 65.4 \text{ min}^{-1}$ (healthy), $P = 0.043$. Group mean t_{PTEF} was also significantly lower in wheezy infants. There were also significant differences in tidal volume, when expressed per kg body weight: Group mean difference (wheezy - healthy) $-0.9 \text{ mL} \cdot \text{kg}^{-1}$, 95% CI $-1.59, -0.21$; $p = 0.011$.

Using linear regression, and with $t_{PTEF}:t_E$ as the outcome variable, a significant but weak association was found between $t_{PTEF}:t_E$ and $sG_{aw,II}$ and $sG_{aw,EE}$ (Table 4-3). The relationship between $t_{PTEF}:t_E$ and $sG_{aw,II}$ and $sG_{aw,EE}$ is shown in Figure 4-1 and Figure 4-2 identifying wheezy and healthy infants separately. After adjusting the regression equation for the effects of age, sex, weight and length, little effect was seen.

When wheezing status was added in, the relationship between $t_{PTEF}:t_E$ and $sG_{aw,EE}$ was weakened, then accounting for only 7% of the total variance in $t_{PTEF}:t_E$. The inclusion of wheezing status for $t_{PTEF}:t_E$ and $sG_{aw,II}$, reduced the relationship between those variables to below the accepted level of significance ($p=0.158$).

Table 4-3 Regression analyses for $t_{\text{PTEF}}:t_E$ in 101 infants

Unadjusted	Regression coefficient (95% CI)	r^2	p value
$sG_{\text{aw,II}}$	0.0373 (0.0160, 0.0586)	0.109	0.0008
$sG_{\text{aw,EE}}$	0.0444 (0.0260, 0.0628)	0.189	0.000
Adjusted for age, sex, weight and length			
$sG_{\text{aw,II}}$	0.0364 (0.0155, 0.0573)	0.108	0.0008
$sG_{\text{aw,EE}}$	0.043 (0.0250, 0.0612)	0.185	0.000
Adjusted for the above and wheezing status			
$sG_{\text{aw,II}}$	0.0147 (-0.006, 0.0352)	0.200	0.158
$sG_{\text{aw,EE}}$	0.0242 (0.006, 0.0420)	0.066	0.009

Key to table: abbreviations as for Table 4.2

Figure 4-1 Scattergram of $t_{\text{PTEF}}:t_E$ and initial inspiratory specific conductance

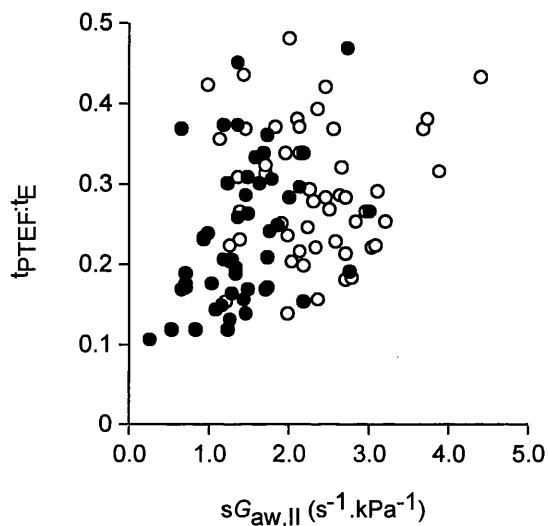
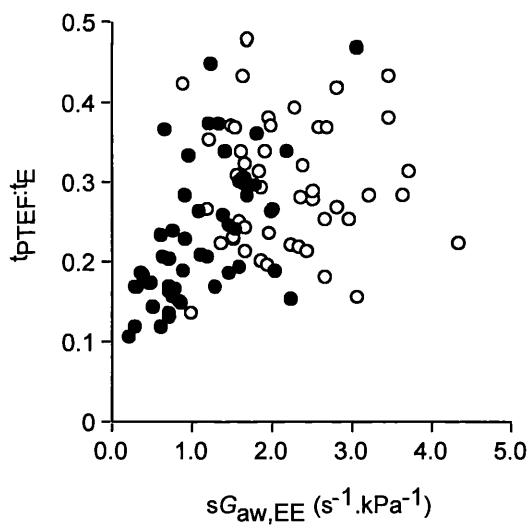


Figure 4-2 Scattergram of $t_{\text{PTEF}}:t_E$ and end expiratory specific conductance



Key to Figures 4.1 and 4.2: solid circles denote wheezy infants, open circles denote healthy infants.
Abbreviations as for Table 4.2

4.6 Discussion

Tidal expiratory flow patterns in infancy may reflect both age and underlying lung mechanics. During passive expiration, the elastic recoil pressure of the respiratory system is highest at onset of expiration, a time when the airways are also well distended and hence resistance relatively low. Theoretically, peak tidal expiratory flow should therefore occur at onset of expiration, giving rise to a low $t_{PTEF}:t_E$. In infants, this pattern may be evoked by inducing muscle relaxation with a brief end inspiratory occlusion, but is otherwise unusual, indicating that some braking of tidal expiration is normal. It has been suggested that the low values of $t_{PTEF}:t_E$ observed in adults with airway obstruction reflect alterations in control of breathing, with a reduction of expiratory airflow braking in response to underlying respiratory mechanics, rather than being simply a direct index of lower airway size (Morris et al 1981).

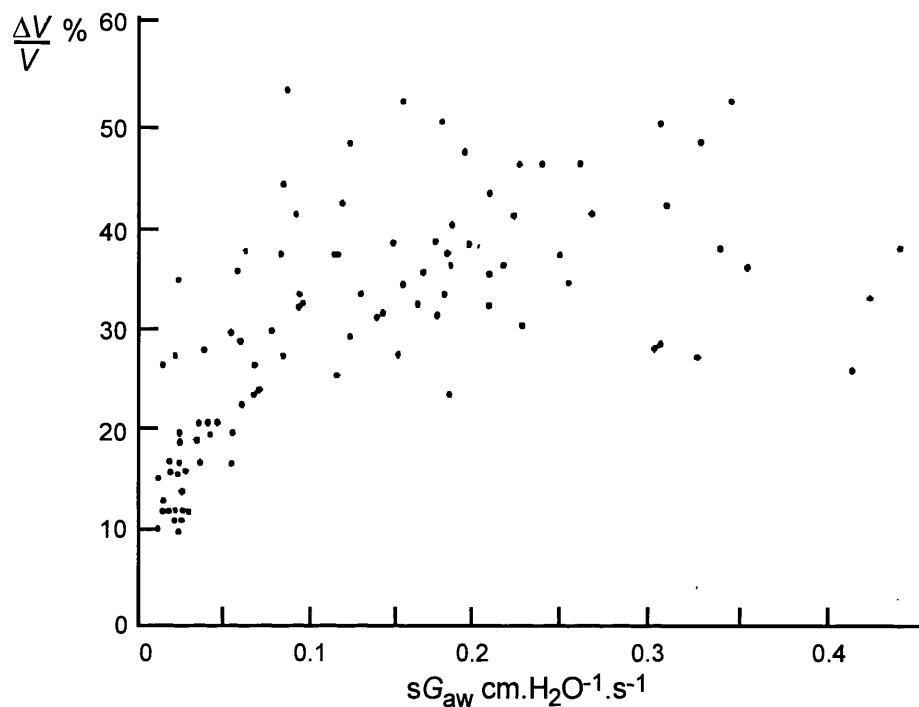
In this study, measurements of $t_{PTEF}:t_E$, FRC_{pleth} and airway resistance were made on the same test occasion in healthy infants and those with prior physician diagnosed wheezing. In the group with a prior history of physician diagnosed wheezing associated LRI, many of whom were asymptomatic at testing, $t_{PTEF}:t_E$ was significantly lower when compared with a group of healthy infants of similar age and body size. A lower $t_{PTEF}:t_E$ has also been reported in adults with airflow obstruction (Morris et al 1981) and in infants with bronchopulmonary dysplasia (Kosch et al 1988), but was not observed in asthmatic children who were asymptomatic at testing (Cutrera et al 1991). However, in our study, although group mean $t_{PTEF}:t_E$ was significantly lower in infants with recurrent wheezing, there was considerable between subject variation, suggesting that $t_{PTEF}:t_E$ discriminates well between groups but not individuals. Martinez et al have shown that infants with a $t_{PTEF}:t_E$ in the lower tercile of the distribution, when measured before 13 weeks of age, are at significantly greater risk of developing wheezing associated LRI by 3 years of age (Martinez et al 1991). However, the purpose of this study was to report cross-sectional associations of this parameter with an established measure of airway function: sG_{aw} . Recent analysis of the longitudinal population study, from which the healthy controls in this study were drawn, confirms Martinez' finding, with $t_{PTEF}:t_E$ being lower shortly after

birth in otherwise healthy infants who subsequently develop wheezing in their first year (Dezateux et al 1999).

In this study, group mean sG_{aw} , during initial inspiration and late expiration, was significantly lower in infants with recurrent wheezing when compared with healthy infants of a similar age. However, within each group there was considerable between infant variation. The range of values observed in healthy infants in our study is greater than previously reported (Stocks et al 1977). This may reflect differences in technique, as measurements made in the healthy infants studied by Stocks et al (Stocks et al 1977) were undertaken after more prolonged periods of rebreathing than is current practice in our laboratory.

$t_{PTEF}:t_E$ has been reported to be significantly associated with inspiratory sG_{aw} , in healthy adults and those with airflow obstruction (Morris et al 1981), with FEV_1 in healthy and asthmatic children (the latter asymptomatic at testing) (Cutrera et al 1991) and with $V'_{max,FRC}$ in healthy infants and those with bronchopulmonary dysplasia (Morgan et al 1984). We have shown previously that, in healthy infants aged 13 weeks or less, FRC_{pleth} , but not $sG_{aw,II}$ or $sG_{aw,EE}$, is significantly but weakly associated with $t_{PTEF}:t_E$. (Dezateux et al 1994) The lack of a significant relationship between $t_{PTEF}:t_E$ and both $sG_{aw,II}$ or $sG_{aw,EE}$ in younger healthy infants suggests that, in the absence of airflow obstruction, there is relative freedom to vary tidal expiratory flow patterns. Laryngeal and post inspiratory diaphragmatic braking, which are accompanied by late onset of peak expiratory flow and hence prolongation of $t_{PTEF}:t_E$, help to maintain a dynamically elevated FRC and maximise gas exchange in newborn infants (Kosch et al 1988). The weak relationship between FRC_{pleth} and $t_{PTEF}:t_E$ observed amongst the younger healthy infants in the previously reported study (Dezateux et al 1994) suggests that this phenomenon may persist to some extent throughout the first 3 months of life. The importance of establishing a stable FRC, together with interdependence between respiratory timing, modulation of expiratory flow and dynamic elevation of lung volume, may confound any relationship between $t_{PTEF}:t_E$ and indices of airway function, especially in healthy infants. With increasing postnatal age, the need to modulate expiratory flow and timing to maintain a stable FRC diminishes.

Figure 4-3 The relationship between tidal breathing pattern and specific airway conductance



Key to Figure: Scattergram of percentage of expiratory volume at peak tidal expiratory flow ($\Delta V/V$) and inspiratory specific airway conductance (sG_{aw}) in healthy adults and adults with lung disease. Adapted from Morris and Lane, 1981.

While Morris and Lane (Morris et al 1981) have found that the percentage of expiratory volume expired at peak tidal expiratory flow is significantly associated with inspiratory sG_{aw} , in healthy adults and those with airflow obstruction, the published data (Figure 4-3) suggest that this relationship may have been influenced by individuals with sG_{aw} , of less than $0.10 \text{ s}^{-1} \text{ cm H}_2\text{O}^{-1}$ (equivalent to $1.0 \text{ s}^{-1} \cdot \text{kPa}^{-1}$), those with values greater than this showing both higher mean values and greater variability in $t_{PTEF}:t_E$. While direct comparisons are impossible, since measurements in infants include a variable component due to the resistance of the nasal passages (Stocks et al 1978b), mean sG_{aw} , does appear to be relatively constant from the end of the first year of life to adulthood (Stocks et al 1977). In our study, all but 4 of the healthy infants had values of $sG_{aw,EE} \geq 1.0 \text{ s}^{-1} \cdot \text{kPa}^{-1}$, as did a significant proportion of those with recurrent wheezing, reflecting the fact that many of these infants were asymptomatic at testing. In the absence of current airflow obstruction, there should be relative freedom to vary tidal expiratory flow patterns, and hence no strong relationship between $t_{PTEF}:t_E$ and other measures of airway function would be

expected. Had measurements been confined to only those infants with more severe degrees of airflow obstruction, as evidenced by a low sG_{aw} , a stronger relationship might have been found, as indicated by inspection of the data in the current study from infants with $sG_{aw} < 1.0 \text{ s}^{-1} \cdot \text{kPa}^{-1}$ (Figure 4-2). However, we felt it important to examine the association for wheezy infants across the whole spectrum of disease severity encountered in clinical practice, and thus employed a clinical rather than laboratory based definition for prior airways disease.

The results in this chapter extend somewhat beyond the previously published findings (Dezateux et al 1994), although the group of healthy infants below 13 weeks of age has not been included in this chapter for reasons of simplicity, and this section includes a larger proportion of more severely symptomatic infants who were recruited from the wards and clinics of participating hospitals towards the end of the study. This is reflected in the somewhat lower values for sG_{aw} and $t_{PTEF}:t_E$ than previously reported. Despite the inclusion of these more severely wheezing infants, the association remains diluted by the number of relatively asymptomatic wheezers although almost half of the wheezy group had values of $t_{PTEF}:t_E$ below 0.250 and sG_{aw} below $1 \text{ s}^{-1} \cdot \text{kPa}^{-1}$, similar to that of Morris and Lane's adults with airflow obstruction. The relationship between variables and the conclusions drawn are, however, similar.

It has been suggested that the sensitivity of the ratio $t_{PTEF}:t_E$ is reduced by the independent effect of airway obstruction on respiratory rate during infancy (Clarke et al 1995). An increase in respiratory rate with increased airway obstruction, as occurs in infants but not adults, will mask a reduction in t_{PTEF} . In this study, respiratory rate was significantly greater in the wheezy group ($36.0 \text{ vs } 32.4 \text{ min}^{-1}$) and t_E consequently reduced. The group mean difference (95% CI of the difference wheezy - healthy) for t_{PTEF} was $0.081 \text{ (-0.125, -0.037) s}$ and for $t_{PTEF}:t_E$ $0.057 \text{ (-0.091, -0.024)}$ the significance of the difference between groups being greater for t_{PTEF} . Although considering t_{PTEF} and t_E separately may help to improve discrimination between healthy and wheezy infants, inspection of the relationship between $t_{PTEF}:t_E$ and $sG_{aw,II}$ or $sG_{aw,EE}$ in Figure 4-1 and Figure 4-2 reveals the wide variability of sG_{aw} in both healthy and wheezy infants, which limits the extent to which any tidal parameter can sensitively estimate airway obstruction.

The wide range of values for sG_{aw} in this study may be partly explained by biological variability, and by the algorithms used for analysis of R_{aw} , whereby values were calculated at 4 specific points throughout the respiratory cycle (section 3.3.4.1.4) rather than using all the available data by, for example, regressing through ensembled breaths, which would reduce the intrasubject variability.

In conclusion, we measured $t_{PTEF}:t_E$, and sG_{aw} during both initial inspiration and end expiration, in both healthy infants and those with physician diagnosed wheezing and found that both $t_{PTEF}:t_E$ and sG_{aw} were significantly lower in the wheezy compared to the healthy group. There was a significant though weak association between $t_{PTEF}:t_E$ and $sG_{aw,EE}$, in healthy infants. However, this association reported here was not found to be significant in healthy younger infants (Dezateux et al 1994), in whom the pattern of expiratory flow may reflect dynamic maintenance of FRC as much as a response to airway calibre. Further work is needed to elucidate the factors influencing tidal expiratory flow patterns in infancy.

5. Comparison of Single-breath and plethysmographic measurements of resistance in infancy

5.1 Introduction

The association between respiratory illness in the first year of life and subsequent respiratory morbidity is being increasingly recognised (Samet et al 1983, Strachan 1990). The consequences of environmental insults, both pre and post-natal, to the developing lung at a time of rapid growth are of particular importance. Objective measurements of infant lung function are required if the mechanisms underlying these associations are to be elucidated.

Plethysmographic measurements of lung volume (FRC_{pleth}) and airway resistance (R_{aw}) may be used to identify functional and developmental abnormalities in infants (Helms et al 1982, Kao et al 1985, Stocks et al 1976, Gutkowski 1990, Stocks et al 1978a, Kraemer 1993). However, the complexity of these tests limits their epidemiological and clinical applications. During the past decade, new approaches to infant lung function testing have simplified methods of measurement in this age group (Gerhardt et al 1989, Landau 1990). The Single Breath Technique (SBT) which measures the resistance, compliance and time constant of the respiratory system during passive expiration following brief airway occlusions (LeSouëf et al 1984a, Mortola et al 1982, Gappa et al 1993, Fletcher et al 1992) is simple and rapid to perform, and is being increasingly applied in clinical settings (Landau 1990, Prendiville et al 1986, Koumbourlis et al 1992, Couser et al 1993). Despite its increasing popularity, the relationship between SBT measurements of total respiratory resistance (R_{rs}), which includes chest wall and lung tissue components, and those of airway resistance had yet to be fully established in infants at the inception of this thesis. The aim of this study was to compare measurements of airway and total respiratory resistance obtained on the same occasion in both healthy infants and those with prior airway disease.

5.2 Study Population

Recruitment of the population of healthy and recurrently wheezy infants is described in section 3.1.1. Selection of the healthy group of infants, for comparison with the

group of 51 infants with recurrent wheezing is described in section 4.2. For this study, measurements of airway resistance (R_{aw}) and total respiratory resistance (R_{rs}) were attempted in 102 infants (51 in each group). Successful measurements of R_{aw} were obtained in all but 1 healthy infant, who woke before measurements could be completed. Measurements of R_{rs} were not attempted in 1 wheezy, and 3 healthy infants, where the infants again woke before completion of measurements. The resulting study population was therefore 47 healthy and 50 wheezy infants. The study protocol differed slightly between groups: for the infants with recurrent wheezing, equal emphasis was given to data collection for the SBT and plethysmographic measurements of lung volume and airway resistance. In the healthy group, priority was given to plethysmographic measures of FRC and R_{aw} , since these were the outcome variables for the epidemiological study to which they had been recruited, and the slightly higher numbers of infants where SBT or MOT data were not collected reflect this. Healthy infants were aged from 9.6 to 70.8 weeks and weighed from 4.5 to 13.8 kg at testing, and wheezy infants ranged in age from 15.1 to 94.6 weeks and weighed from 7.7 to 11.0 kg.

5.3 Results

Technically satisfactory results for both R_{aw} and R_{rs} were obtained in 72 of the 97 infants in whom they were attempted. As summarised in Table 5-1, a significantly higher percentage of R_{rs} measurements failed relative to plethysmographic measurements of R_{aw} (weighted mean from all occasions 26% and 0% respectively; 95% confidence intervals (CI) of the difference R_{rs} - R_{aw} : 16%, 35%).

However, there was no significant difference in the percentage of failed SBT measurements between infants with physician diagnosed wheeze and healthy infants; (95% CI for the difference: -20%, 15%). Alinearity of the flow volume curve (Figure 5.3) and failure to equilibrate or achieve a relaxed plateau during airway occlusion (Gappa et al 1993, Fletcher et al 1992) were the major reasons for failure to obtain satisfactory estimates of R_{rs} (27% and 35% of all failures respectively). Further details of reasons for failure of the SBT are contained in Section 3.3.3.4.1.

Table 5-1 Number of infants in whom measurements of airway and respiratory resistance were attempted but unsuccessful

	Healthy (n = 47)	Wheezy (n = 50)	Total (n = 97)
Failed R_{rs} n (%)	12 (25)	14 (28)	26 (27)
Failed R_{aw} (%)	0	0	0
Both successful n (%)	36 (77)	36 (72)	72 (74)

Key to Table: R_{aw} , airway resistance; R_{rs} total respiratory resistance.

Therefore, comparison of R_{rs} and R_{aw} was possible in 72 of 102 infants (74%), and details of these infants are summarised in Table 5-2. Of the 36 infants with prior wheeze, 10 were born at or below 36 weeks gestation, 2 required supplemental oxygen, and 15 were symptomatic at time of testing. There were no significant differences between those successfully studied and those in whom one or more measurements failed with respect to sex, maternal smoking during pregnancy, family history of asthma, body size or specific conductance (reciprocal of resistance divided by FRC_{pleth}) ($p>0.10$ for all parameters). For infants with successful measurements of R_{rs} and R_{aw} , weight and length at time of testing were similar between healthy and wheezy infants ($p>0.10$). There were no significant differences in the prevalence of maternal smoking during pregnancy in wheezy compared to healthy infants (44 vs 31% respectively). The prevalence of postnatal maternal smoking was within 3% of that reported during pregnancy for each of the groups (data not shown).

Table 5-2 Details of infants in whom satisfactory measurements of both airway and respiratory resistance were attempted[†]

	Healthy (n = 36)	Wheezy (n = 36)	95% CI of the difference (wheezy - healthy)
Age (weeks)	47.9 (16.3)	46.0 (19.1)	-10.4, 6.3
Weight (kg)	9.1 (2.0)	9.0 (2.2)	-1.07, 0.89
Length (cm)	74.6 (6.9)	72.6 (7.2)	-5.2, 1.4
Birthweight (g)	3550 (512)	3032 (1070)	-915, -121*
Gestation (weeks)	40.4 (1.2)	37.4 (4.2)	-4.4, -1.4***
Male n (%)	22 (61)	23 (64)	-20, 25
Maternal smoking during pregnancy n (%)	11 (31)	16 (44)	-8, 36
Family history of asthma n (%)	9 (25)	19 (53)	5, 50***
Symptomatic at testing n (%)	3 (8)	15 (42)	13, 53***

Key to Table: [†] Values are shown as mean (SD). CI: Confidence interval of the difference (wheezy - healthy). P values for differences Wheezy -Healthy: * p < 0.05; ** p < 0.01; *** p < 0.001

Results from the lung function measurements are summarised in Table 5-3. Both R_{rs} and R_{aw} were significantly higher amongst wheezy infants than healthy infants of a similar age. These differences were most significant during end expiration.

There were small but statistically significant differences between C_{rs} calculated by the MOT and SBT in both groups (Table 5-3). Mean difference (95% CI) was -1.0 (-1.7, -0.4) $\text{ml.kPa}^{-1}.\text{kg}^{-1}$ ($p < 0.002$) for healthy infants and 0.9 (0.3, 1.5) $\text{ml.kPa}^{-1}.\text{kg}^{-1}$ ($p < 0.01$) for the wheezy group. The mean intercept ($[\text{MOT} + \text{SBT}]/2$) was approximately $3\text{ml}.\text{kg}^{-1}$ for both groups of infants.

Table 5-3 Lung Function results[†]

	Healthy n=36	Wheezy n=36	95% CI (wheezy-healthy)
FRC _{pleth} (mL)	259.6 (63.1)	281.0 (82.6)	-13, 56
FRC _{pleth} (mL.kg ⁻¹)	28.6 (3.6)	31.7 (8.5)	0.1, 6.24*
R_{aw} (kPa.L ⁻¹ .s)			
<i>Initial inspiration</i>	2.26 (1.26)	3.18 (1.75)	0.20, 1.64*
<i>End inspiration</i>	1.60 (0.95)	2.37 (1.49)	0.18, 1.37*
<i>Initial expiration</i>	1.90 (0.75)	2.44 (1.02)	0.11, 0.13*
<i>End expiration</i>	2.54 (1.04)	4.34 (1.87)	1.09, 2.52***
sG_{aw} (s ⁻¹ .kPa ⁻¹)			
<i>Initial inspiration</i>	2.27 (0.63)	1.47 (0.52)	-1.08, -0.54***
<i>End expiration</i>	2.14 (0.85)	1.13 (0.53)	-1.34, -0.67***
RR (min ⁻¹)	31.7 (7.9)	35.3 (9.5)	-0.5, 7.7
t_{PTEF} (s)	0.327 (0.115)	0.238 (0.094)	-1.339, -0.674***
t_E (s)	1.162 (0.250)	1.072 (0.281)	-0.214, 0.035
$t_{PTEF}:t_E$	0.285 (0.081)	0.227 (0.076)	-0.094, -0.021*
V_T (mL.kg ⁻¹)	9.4 (1.9)	10.4 (1.7)	-1.9, -0.2*
R_{rs} (kPa.L ⁻¹ .s)	3.16 (0.84)	4.30 (1.5)	0.59, 1.75***
C_{rsSBT} (mL.kPa ⁻¹ .kg ⁻¹)	14.9 (2.6)	11.3 (2.5)	-4.89, -2.46***
C_{rsMOT} (mL.kPa ⁻¹ .kg ⁻¹)	14.2 (2.4)	12.6 (2.4)	-2.77, -0.47**

Key to Table 5.3: [†] Values are shown as mean (SD) **SD**: Standard Deviation; **CI**: Confidence Interval of the difference.

P values for differences Wheezy -Healthy: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

FRC_{pleth}: plethysmographic functional residual capacity;

R_{aw}, **SG_{aw}**, airway resistance and specific conductance respectively;

t_E : expiratory time; **t_{PTEF}**: time to peak tidal expiratory flow;

t_{PTEF}:t_E: time to peak tidal expiratory flow as a proportion of total expiratory time;

V_T: Tidal volume; **R_{rs}** total respiratory resistance;

C_{rsSBT}: total respiratory compliance as measured by the single breath technique;

C_{rsMOT}: total respiratory compliance as measured by the multiple occlusion technique.

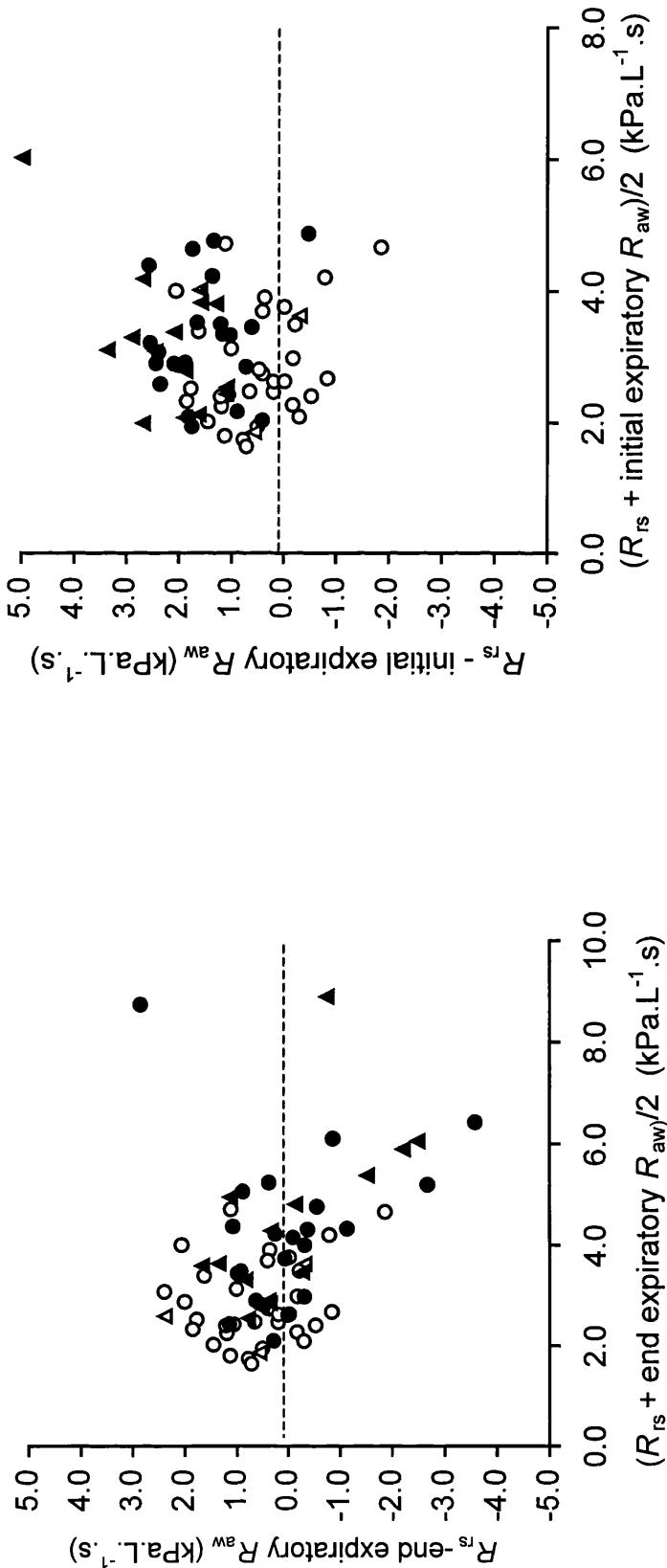


Figure 5-1 The relationship between respiratory resistance and initial and end expiratory resistance

Key to Figure 5-1: solid circles denote healthy infants, open circles denote wheezy infants who were symptomatic at testing.
Abbreviations as for Table 5.3

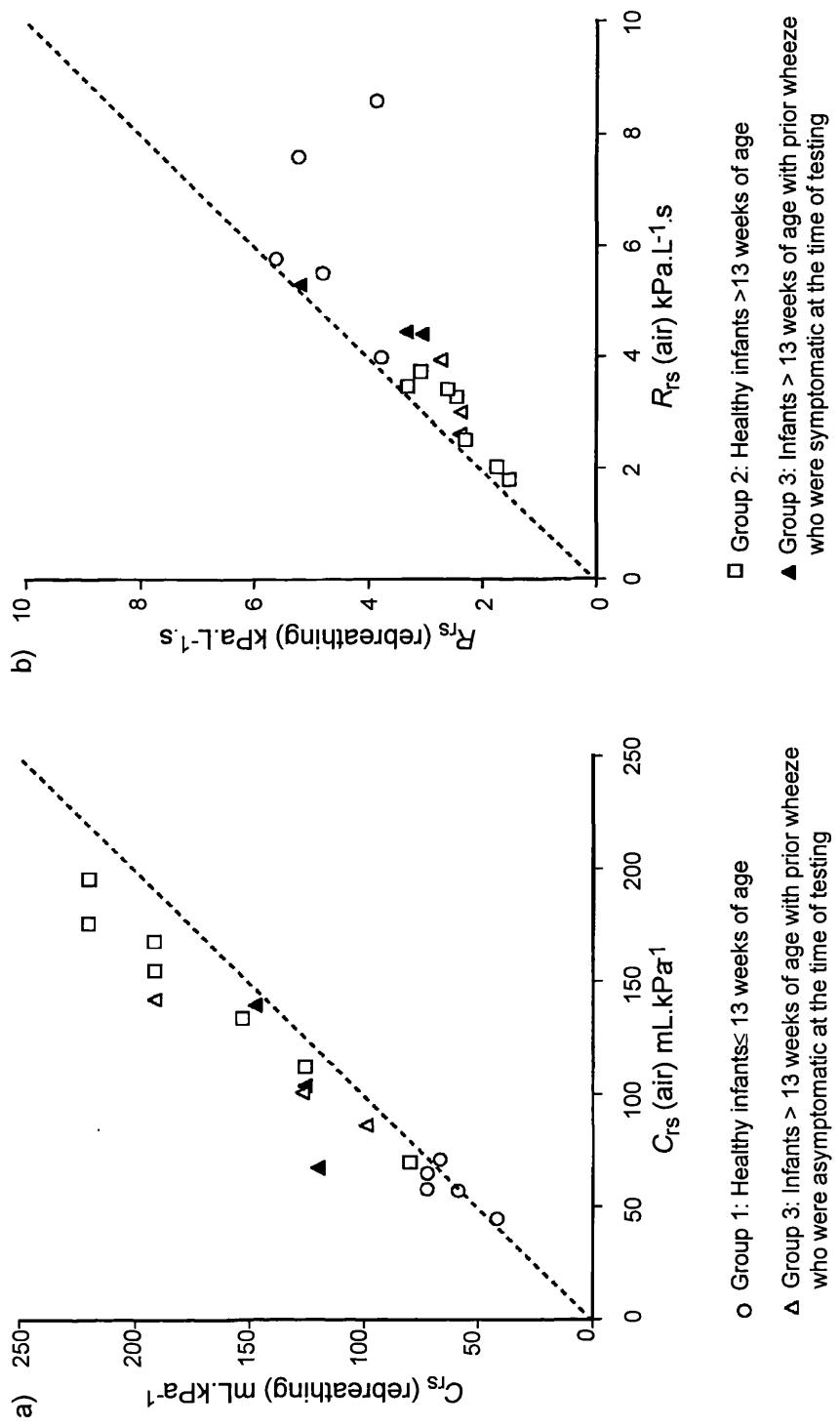


Figure 5-2 The effect of rebreathing on a) respiratory compliance and b) respiratory resistance assessed by the single breath technique

The relationship between R_{rs} and R_{aw} calculated at matched flows during initial and end expiration for each group (Bland et al 1986) is shown in Figure 5.1, with limits of agreement shown in Table 5-4. Despite the wide scatter of results, R_{rs} was significantly ($p<0.001$) higher than initial expiratory R_{aw} in each group. R_{rs} exceeded initial expiratory R_{aw} in all but 2 healthy infants. Mean R_{rs} was also significantly ($p<0.001$) higher than R_{aw} calculated at end expiration in healthy infants. However, there was no significant difference between the two techniques in wheezy infants ($p>0.10$). End expiratory R_{aw} exceeded R_{rs} in 17% of healthy infants, and in 44% of wheezy infants.

Table 5-4 Comparison of respiratory and expiratory airway resistance (kPa.L⁻¹.s)

	Mean difference (SD)	95% Limits of agreement	95% CI
$R_{rs}-R_{aw}$ (initial expiration)			
Healthy	1.25 (0.88)	-0.51, 3.01	0.96, 1.55***
Wheezy	1.89 (1.16)	-0.43, 4.21	1.50, 2.28***
$R_{rs}-R_{aw}$ (end expiration)			
Healthy	0.62 (0.97)	-1.32, 2.56	0.30, 0.94***
Wheezy	-0.01 (1.29)	-2.57, 2.59	-0.44, 0.42

Key to Table:

SD: Standard Deviation;

95% CI: 95% Confidence Interval of the difference $R_{rs} - R_{aw}$.

P values for differences: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

R_{aw} : airway resistance;

R_{rs} : total respiratory resistance;

During measurements of R_{aw} the infant breathed from a bag containing warmed, humidified, oxygen enriched air. This was accompanied by statistically significant increases in respiratory rate, tidal volume and peak tidal expiratory flow (PTEF) (Table 5-5). At any given lung volume, flows recorded following release of airway occlusions for the SBT were also significantly higher than those during tidal breathing (data not shown). When approximately 40% of tidal volume had been expired, the point at which analysis of the linear portion of the flow-volume curve

normally commenced, flows were approximately 22% higher than those recorded in that infant during tidal breathing (ranging from 30% less to 110% greater). The mean flow recorded for each infant during the calculation of R_{rs} , which was used to denote the point of analysis for R_{aw} , was equivalent to 71% (SD 16%) of PTEF during R_{aw} measurements, a similar mean and range of percentages being obtained from both groups of infants.

Table 5-5 Effect of rebreathing on tidal parameters

	Baseline	Rebreathing	95% CI [†]
<i>Healthy, n=36</i>			
RR (min ⁻¹)	31.7	34.3	1.8, 3.4*
V _T (mL)	95.6	125.6	21.2, 38.8*
PTEF (mL.s ⁻¹)	126.7	175.0	37.4, 59.0*
<i>Wheezy, n=36</i>			
RR (min ⁻¹)	35.3	37.6	-1.56, 4.0
V _T (mL)	85.3	111.9	11.7, 29.2*
PTEF (mL.s ⁻¹)	129.9	170.9	21.9, 50.5*

Key to Table:

[†]: 95% Confidence Interval of the difference in rebreathing values compared with baseline values.

P values for differences (rebreathing - baseline -) : * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001

Repeat measurements of C_{rs} and R_{rs} by the SBT while breathing warmed humidified air, as for the R_{aw} measurements, were achieved in 18 infants (mean (SD) weight 8.3 (2.5) kg, age range 8-61 weeks) (Table 5.6). There was a significant increase in C_{rs} during rebreathing, the mean increase (95% CI) being 19.9 (11.5, 28.3) mL.kPa⁻¹ Figure 5-2 (a). This was accompanied by a significant reduction in mean R_{rs} of -0.89 kPa.L⁻¹.s (95% CI of difference: -1.45, -0.33 kPa.L⁻¹.s Figure 5-2 (b). There was also a tendency for the volume intercept to increase significantly during rebreathing, both in absolute terms and when corrected for body weight (mean increase (95% CI): 1.2 (0.4, 2.0) mL.kg⁻¹).

Table 5-6 Effect of rebreathing on assessment of respiratory compliance and resistance by the single breath technique

	Subjects (n)	Baseline	Rebreathing	95% CI [†]
C_{rs} (mL.kPa ⁻¹)	18	108.2	128.1	11.5, 28.3*
R_{rs} (kPa.L ⁻¹ .s)	18	4.20	3.30	-1.45, -0.33***
Intercept (mL)	18	19.3	31.2	4.7, 19.0***

Key to Table:

[†]: 95% Confidence Interval of the difference in rebreathing values compared with baseline values;
 P values for differences (rebreathing - baseline) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

5.4 Discussion

The results from this study indicate that, despite the apparent simplicity of the SBT for measuring R_{rs} , relative failure rate is higher than that encountered during plethysmographic measurements of R_{aw} in the same infants. The failure rate for the SBT in the current study was considerably lower than that previously reported in preterm infants (Gappa et al 1993). This probably reflects fewer exclusions due to distortion of the flow-volume curve by excessive modulation of expiratory flow, which was noted in preterm neonates. Nevertheless failure to achieve an adequate plateau, or alinearity of the flow-volume curve resulted in technical failures on 26% of occasions. Although the SBT did fail in 2 of the infants with the highest R_{aw} values, there was no apparent relationship between failure rate and either respiratory symptoms or R_{aw} values. This may reflect the fact that less than half of the wheezy infants studied were symptomatic at testing.

Despite the fact that most of the infants were asymptomatic at time of testing, both R_{rs} and end expiratory R_{aw} were significantly higher amongst infants with prior wheeze than healthy infants of similar age. However, an extremely variable relationship between R_{rs} and R_{aw} was observed within infants. Partitioning of airway resistance from that contributed by the visco-elastic properties of the lung and chest wall is extremely complex. The degree of variability between techniques observed in this study can largely be explained by the numerous factors known to influence the assessment of resistance.

5.4.1 Flow and volume dependency of resistance:

Airway resistance is known to be both flow and volume dependent, values tending to increase due to turbulence at higher flows, and decrease due to increased elastic recoil and hence distension of the small airways at higher volumes (Barnas et al 1992, Tantucci et al 1991, Barnas et al 1991). In addition, at any given lung volume or flow, R_{aw} is generally lower during inspiration than expiration due to the distending influence of the negative inspiratory intrapleural pressure on intrathoracic structures. In adults and healthy infants, in whom the chest wall has begun to stiffen, such changes may be fairly minimal over the tidal range so that stable values of R_{aw} are achieved. However, the highly compliant chest wall of young infants results in a low transpulmonary pressure at end expiration (Helms et al 1981), such that dynamic airway closure with marked elevation of end expiratory resistance can occur within the tidal range. This tendency may be more pronounced in infants with airway disease due to an increased pressure gradient along the airways in the presence of airway obstruction and/or the rise in intrapleural pressure which will accompany any active expiratory efforts. In adults, the stiffness of the bronchial wall protects the conducting airways from deformation and collapse due to positive intrathoracic pressures that develop during forced expiratory efforts. However, airways from immature animals are far more compliant than those of adults and hence vulnerable to collapse at smaller transmural pressures (Panitch et al 1992). Consequently changes in lung volume which alter the interdependence between airways and parenchyma may act as a major determinant of airway calibre and resistance in infants. In this study, changes in R_{aw} were found to occur throughout the respiratory cycle in both groups of infants (Table 5-3).

In this study, we attempted to minimise potential sources of variability when making comparisons between techniques by analysing R_{rs} and R_{aw} at similar flows and phases of respiration. Since R_{rs} is measured during passive expiration following an end inspiratory occlusion, it may reflect the mechanics of the airways during initial expiration (together with a chest wall and tissue component) most closely (Springer et al 1993). However, since analysis is performed on the descending portion of the flow-volume curve after peak expiratory flow has been attained, it could also reflect airway characteristics towards end expiration. Consequently we compared R_{rs} with both initial and end expiratory R_{aw} . R_{aw} was calculated at the average flow recorded during R_{rs} analysis in each infant which proved to be approximately 71% of PTEF,

i.e. similar to R_{aw} at two-thirds PTEF, which has been conventionally calculated in the past (Stocks et al 1985, Stocks et al 1977). However, these attempts to match flows can at best only be an approximation, with slight discrepancies being potentially responsible for much of the observed variability.

5.4.2 Potential influence of the rebreathing bag:

Even when attempting to match flows and phase of respiration, we could not control for the potential changes in resistance induced by rebreathing warm humidified air. Adults are conventionally requested to pant during plethysmographic assessments of R_{aw} : both to minimise pressure changes due to alterations in temperature and humidity of respired gas and to keep the glottis wide open, thereby minimising upper airway resistance. Since infants cannot be asked to pant, they are allowed to rebreathe warm humidified air but this will inevitably result in some build up of CO_2 and thereby increase ventilatory drive (Table 5-5).

It is not clear from the current study whether the reduction in R_{rs} which occurred when measurements were repeated during rebreathing (Table 5-6) reflect a bronchodilator effect of CO_2 per se (Kaise et al 1991), increased tidal volume or increased dynamic elevation of lung volume subsequent to the increase in minute ventilation (ie volume dependence of R). The accompanying rise in C_{rs} and volume intercept during rebreathing (Figure 5-2, Table 5-6) suggests that a change in lung volume may be a contributory factor. If so, this has important implications with respect to timing of lung volume measurements during plethysmography.

5.4.3 Influence of changes in gas composition

During this study the pneumotachograph was calibrated with air which may have introduced a slight error into measurements during the rebreathing of oxygen enriched air ($FiO_2 \approx 0.40$). However, such errors are likely to be small and would not account for the differences reported in Table 5-6. Theoretically, C_{rs} could be overestimated and R_{rs} underestimated by 11% if an infant breathed 100% O_2 through a pneumotachograph calibrated in air, due to the relatively high viscosity of O_2 compared with air (Helms et al 1981, Yeh et al 1984). However, this error falls to 2% at an FiO_2 of ≈ 0.4 , and would be further compensated by the $FiCO_2$ of ≈ 0.06 commonly observed in the bag during rebreathing, since the viscosity of CO_2 is lower than that of air.

It was not feasible to measure R_{rs} under identical conditions to R_{aw} in all infants, nor to repeat the MOT assessment of C_{rs} during rebreathing, since this part of the study was only attempted if infants remained asleep at the end of the main measurement protocol. Furthermore, many infants failed to relax adequately under conditions of stimulated breathing. Nevertheless the observations in the sub-group of 18 infants emphasise the marked influence of measurement conditions on measured values of respiratory function and hence variability between techniques.

5.4.4 Partitioning of respiratory system resistance

Previous studies in which pulmonary and airway resistance have been measured simultaneously and analysed at identical flows suggest that lung tissue resistance makes a relatively small (<15%) contribution to total pulmonary resistance (Stocks et al 1985, Helms 1982). This may be related to the fact that the major component of lung tissue resistance is thought to be due to hysteresis of the lung which, since it is inversely proportional to breathing frequency, will make a smaller contribution in rapidly breathing infants than adults.

The contribution of the chest wall is more controversial, since differences between techniques often preclude direct comparison. Theoretically C_{rs} and R_{rs} can be partitioned into lung, airway and chest wall components by measuring the relevant driving pressure. However, under normal functional conditions the driving pressure across the chest wall is a combination of static elastic properties and the pressure generated by activity of the chest wall muscles. Accordingly, the passive mechanical properties of the chest wall can only be derived when muscle activity is inhibited. In a comparative study of pulmonary and total respiratory resistance, Gerhardt et al found that R_{rs} was approximately 24% higher than pulmonary resistance ($p < 0.001$) in infants at approximately 1 year of age (Gerhardt et al 1989). However, no significant difference was observed in preterm neonates, a finding which was attributed to the highly compliant chest wall in immature infants.

Within individuals in this study, R_{rs} was, on average, 80% greater than R_{aw} during initial expiration and 27% greater than R_{aw} during end expiration in healthy infants, these values being 100% and 15% respectively amongst the wheezy infants. However, there was huge individual variability and it is not meaningful to calculate a value for tissue resistance or visco-elasticity simply as the difference between the two techniques.

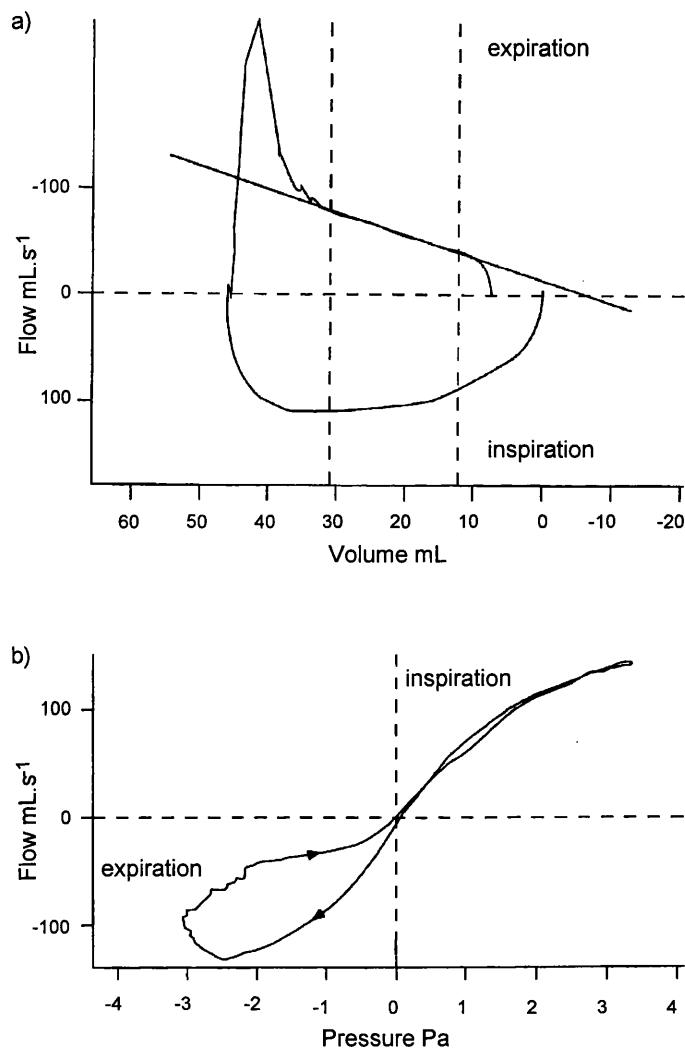
5.4.5 Passive versus dynamic resistance

In addition to differences in measurement conditions discussed above, R_{rs} assesses expiratory resistance under passive conditions, whereas R_{aw} reflects the dynamic changes that occur during tidal breathing. Despite obvious alinearities of the pressure-flow loop during R_{aw} measurements, a linear flow-volume relationship was frequently obtained from infants with airway disease (Figure 5-3). Indeed the failure rate of the SBT due to alinearity was no greater amongst the group with prior wheeze than in healthy infants of a similar age. Following end inspiratory occlusions, infants frequently inspire earlier than usual on the subsequent breath, such that any rise in end expiratory resistance due to dynamic airway closure may be missed. Furthermore, analysis of the “passive” time constant is limited to the “linear” portion of the flow volume curve, when any muscle activity has supposedly been inhibited - consequently measurements of R_{rs} may reflect the dimensions of the airways under passive conditions, but cannot reflect the dynamic changes which normally occur throughout the breath. This would explain why values of end expiratory R_{rs} exceeded those of R_{rs} in approximately 25% of infants studied.

Similar findings have been reported by Springer et al (Springer et al 1993). This group found virtually identical values of weight corrected FRC_{pleth} in 15 “normal” and 9 post-bronchiolitic infants as in the current population. Springer et al did not tabulate absolute values of R_{rs} and R_{aw} , making direct comparisons difficult, but their published illustrations reveal that initial expiratory R_{aw} was equal to or exceeded R_{rs} in approximately one third of the mixed population and that end expiratory R_{aw} was significantly greater than R_{rs} amongst the wheezy infants, many of whom were symptomatic at time of testing.

During the SBT, time is allowed during the end inspiratory occlusion for relaxation of the diaphragm and other inspiratory muscles. It could therefore be argued that the level of activity of the inspiratory and laryngeal expiratory muscles during analysis of the passive flow volume curve most closely resembles that occurring at mid-expiration during tidal breathing and that R_{rs} should therefore be compared to mid-expiratory R_{aw} . Although we did consider this approach, the higher flows occurring towards mid-expiration were associated with increased values for R_{aw} , presumably due to increased turbulence (Stocks et al 1985) and therefore tended to increase rather than diminish any discrepancies between R_{aw} and R_{rs} .

Figure 5-3 a) An apparently linear flow-volume curve obtained with the SBT. b) Example of a pressure-flow curve obtained in the same infant showing elevated end expiratory airway resistance



5.4.6 Validity of the SBT

Concern has been expressed regarding the use of the SBT in unintubated infants due to possible laryngeal modulation of expiratory air flow resulting in a falsely elevated volume intercept and hence overestimation of C_{rs} and underestimation of R_{rs} (Gappa et al 1993, Fletcher et al 1992, American Thoracic Society/European Respiratory Society 1993). In addition, a linear shape on the flow-volume curve does not necessarily indicate relaxation of respiratory muscles or the presence of a single time constant, since an even, descending slope could represent balanced respiratory muscle contraction and/or reciprocal changes in compliance and resistance as lung volume decreases. We measured C_{rs} using both the MOT and SBT in an attempt to validate measurements of R_{rs} with respect to these potential problems and found small differences between the techniques, which appeared to be primarily attributable to small variations in the size of the volume intercept. Nevertheless, it should be noted that although the differences between techniques were statistically significant, the magnitude of these differences is probably too small to be of physiological significance or to markedly influence results.

5.5 Conclusions

In conclusion, despite the difficulties in interpreting results from individual infants and the relatively high failure rate, the SBT was simple to use and is potentially far more applicable than plethysmography which is essentially limited to specialised laboratories and generally unsuitable for critically ill infants. Although it has been suggested that R_{rs} may reflect initial expiratory airway mechanics, this is still open to debate. In this study both R_{rs} and end expiratory R_{aw} were significantly higher in infants with prior wheeze, most of whom were asymptomatic at time of testing, than in healthy infants of similar age and weight. This suggests that measurements of R_{rs} may be of value in epidemiological studies although further work is needed to define the extent to which elevated values of R_{rs} correctly identify those infants with airflow obstruction as determined by clinical symptoms or other objective measures of airway function. However, the clinical value of measurements of R_{rs} within individual infants may be limited by its failure to detect the dynamic changes in resistance throughout the breath that are clearly evident during plethysmographic studies.

6. Assessment of airway function at around one year of age in survivors of the UK Collaborative ECMO trial

6.1 Introduction

Between 1993 and 1995 ECMO was only available for neonates in the UK within the context of a randomised controlled trial of ECMO support for relatively mature infants. The collaborative ECMO trial compared a policy of transfer for consideration of ECMO with continued conventional management (CM) without transfer. The principal outcome measure was death or severe disability at one year of age, chosen because of concern about the quality of survival of this group of neonates with severe respiratory failure. Infants who had potentially reversible respiratory failure were randomly allocated to one of two policies: ECMO or CM. Those allocated to the ECMO arm of the trial were considered for transfer to one of the five ECMO centres, those allocated to the conventional arm of the trial normally remained in the referral centre and continued with full ventilatory support.

Entry criteria for the trial were:

- greater than 35 completed weeks gestation;
- birth weight above 2000 g;
- less than 8 days high pressure ventilation;
- oxygenation index > 40 or $Pa,CO_2 > 12$ kPa for 3 or more hours.

Exclusion criteria were:

- congenital or acquired central nervous system abnormality;
- irreversible cardiopulmonary disease;
- period of asystole;
- proven necrotising enterocolitis;
- other reason to question continuation of intensive care.

In summary, 185 infants were enrolled in the trial (93 allocated ECMO and 92 allocated CM) (UK Collaborative ECMO Trial Group et al 1996). Recruitment to the trial was stopped early in November 1995 when interim analysis of data showed a clear advantage with ECMO. The overall mortality rate was significantly different between the ECMO and conventional management groups (32 vs 59%, $p=0.0005$), the relative risk being 0.55: equivalent to one extra survivor for every three to four

infants allocated to ECMO. Follow-up studies included neurodevelopmental assessment at one year of age, respiratory follow-up, information from parents' and health visitors about health and development and parents' experience of their involvement in the trial (UK Collaborative ECMO Group. 1998). A follow-up of children at 4 years of age is underway, and follow-up to 7 years is being planned. Given that there was a clear benefit of ECMO in terms of survival and that developmental disability was of similar proportions (UK Collaborative ECMO Group. 1998) (26% ECMO group vs 29% conventional group), respiratory status during infancy, as well as later in childhood, is of particular interest in this group of infants who were in severe respiratory failure at birth.

At the inception of the main ECMO trial it was envisaged that one possible outcome would be similar rates of survival and severe disability in the two groups, but better respiratory status in one group or the other. Whether or not ECMO conferred a survival advantage, it was considered essential to investigate the respiratory function in survivors of both limbs of the trial. ECMO could potentially result in survival of infants with severe respiratory dysfunction who would otherwise have died, which would result in poorer respiratory status in this group. Alternatively, those infants receiving ECMO might be spared aggressive ventilation and consequent barotrauma, which has been shown to be associated with subsequent alterations in respiratory mechanics (Ahlstrom 1975, Stocks et al 1976, Tammela et al 1991). In this case the infants in the conventionally managed control group would be at a disadvantage.

In existing reports of respiratory function in infants receiving ECMO, measurements have been made during or shortly after ECMO (Koumourlis et al 1992, Kugelman et al 1995, Greenspan et al 1997), or at age 6 months (Garg et al 1992). None of these have included appropriate control groups. Follow-up studies of respiratory function in subjects receiving intensive care in the neonatal period have shown abnormalities extending into childhood and beyond (Gerhardt et al 1986, de Kleine et al 1990, Chan et al 1989, Doyle et al 1991). The end-point of one year was chosen because at this age the acute effects of disease and treatment would have receded, and any effect on growth of lungs or airways should be apparent. In addition, this timing of respiratory function tests provided an opportunity to gain an overview of clinical status and respiratory morbidity during the first year of life.

6.1.1 Respiratory follow-up

All infants who were recruited into the main ECMO trial and survived to one year were eligible for the respiratory follow-up. One hundred and eighty-five infants were entered into the main ECMO trial of whom 101 survived to one year, and 99 were followed up.

In view of the wide geographical area involved, the number of infants to be measured, and the potential benefits of a collaborative study, it was decided that the respiratory measurements should be performed at 2 specialised centres in the UK, based at Great Ormond Street Hospital for Children NHS Trust and the Institute of Child Health, London, (hereafter referred to as GOS) and at the Department of Child Health, Leicester Royal Infirmary (hereafter referred to as LEIC). The follow-up provided the opportunity to compare measurements of forced expiration with plethysmographic measurements in infants with a history of respiratory failure during the neonatal period. In addition, participation in this multicentre trial offered the opportunity to address methodological problems of such trials.

6.2 Aims

The main aims of the study were:

- to compare inter observer variability within and between two specialised infant lung function testing centres with respect to these measures of airway function
- to compare respiratory health and function at one year in infants who were assigned to receive ECMO with that of similar infants who were assigned to conventional management.
- to investigate the association between measurements of forced expiration and specific conductance, and between $t_{PTEF}:t_E$ and specific conductance, in a group of survivors of neonatal respiratory failure

6.3 Equipment and Methods

6.3.1 Recruitment, collection of questionnaire data and clinical assessment

The administrative side of recruitment was undertaken by staff involved in the collaborative ECMO trial (National Perinatal Epidemiology Unit). The referring paediatrician was contacted prior to approaching parents of survivors in order to establish the current status of the infant.

As the surviving infants approached one year of age, arrangements were made for clinical assessment and respiratory function testing at GOS or LEIC. Parents who elected to take part in Respiratory follow-up were given their choice of venue, and their details were passed on to the respective department. Where attendance involved travelling for over 3 hours, accommodation was offered in a nearby hotel, immediately prior to testing. In addition, travel tickets were purchased for families and meal expenses supplied. An appointment letter with details of travel and, if appropriate, accommodation arrangements together with an information leaflet giving details of the Respiratory follow-up was sent to each family following recruitment by telephone by staff at each test centre. Laboratory staff were blinded to the infants' management status (ECMO or CM) and parents were requested to withhold any information regarding neonatal history during their visit. On arrival in each laboratory, infants were dressed in a specially designed smock of high neck design to obscure any neck scars received during ECMO treatment. A detailed history was taken with reference to respiratory disease and the infant was examined clinically by a staff member not directly involved in measurement and analysis. The Respiratory questionnaire was designed to provide information concerning possible confounding variables such as smoking in the home or a family history of asthma.

6.3.2 Respiratory function testing

On arrival in the respiratory function laboratory, the nature of the tests was again explained to parents.

This study was approved by the local ethics committee at each centre. Written informed consent was obtained prior to measurements from one or both parents, who were usually present during measurements.

Following clinical assessment as described previously, a baseline S_pO_2 was recorded and the infant was weighed, wearing only the smock, then sedated with triclofos sodium (100 - 150 mg.kg⁻¹) at GOS or chloral hydrate (80-100 mg.kg⁻¹) at LEIC before being fed, and having their nappy changed if needed.

Following completion of measurements, the infants' length was recorded, and infants were allowed home, once awake. At GOS, parents were given an information leaflet with a contact telephone number to use if they had any questions or concerns after leaving the laboratory.

6.3.2.1 Between centre methodology.

The methodology for performing respiratory measurements at each test centre was already well established when the Respiratory follow-up commenced (Beardsmore et al 1994, Dundas et al 1995). Full details of the standard GOS equipment, data collection and analysis are given in Section 3. However there were differences in equipment, software set-up and methodology between centres that could potentially influence the approach to collection and analysis of data. These differences were examined prior to starting the Respiratory follow-up and at regular intervals subsequently by arranging inter-laboratory visits when equipment, infant measurements and analytical techniques were observed and compared, including cross analysis of the same data. Following an interim analysis of results, 7 months after commencement of the study, minor amendments to the protocol, with respect to data collection, analysis and reporting results, were implemented as described below.

Details of equipment are summarised in Table 6-1, with details of equipment assessment at GOS presented in Section 0.

Table 6-1 Details of equipment at each centre

	GOS	LEIC
Infant plethysmograph	100 L custom built	85 L Jaeger
Pneumotachograph model	Fleisch '1' capillary type	Jaeger infant screen type
Effective Deadspace: mask+PNT+shutter (mL)	41*	19*
Linear range of PNT	$\pm 500 \text{ mL.s}^{-1}$	$\pm 1000 \text{ mL.s}^{-1}$
Resistance: PNT and shutter at 100 mL.s^{-1} (kPa.L $^{-1}$.s)	0.5	0.23
Time constant [†] (s)	10 - 14 s	10 - 14 s
RTC measurements:		
Deadspace face mask-PNT (mL)	27*	16*
Resistance: PNT at 100 mL.s^{-1} (kPa.L $^{-1}$.s)	0.20	0.18
Jacket model	Hannover arms out adjustable size	Hammersmith size 2 to 4 arms in

Key to Table : ICH = Institute of Child Health, London; LEIC = Department of Child Health, Leicester Royal Infirmary; PNT = pneumotachograph; RTC = rapid thoraco-abdominal compression technique.

* using Rendell-Baker size 2 mask

[†]Combined mechanical and thermal time constant of the plethysmograph.

Both plethysmographs were assessed using an identical test lung, made of copper tubing filled with copper wire, attached to a piston driven pump with a stroke volume of 7 mL. The volume of the test lung was approximately 200 mL (depending on the connectors used) and both centres obtained results to within 2% of the volume measured by water displacement. Following measurements using a face shaped out of putty or a nasal cast to simulate the space potentially occupied by an infant's nose

and cheeks, the effective mask deadspace was considered to be 15 mL at ICH (i.e. 50% of the water displacement volume) and 7 mL at LEIC. The remaining difference in apparatus deadspace of 14 mL arose mainly from the style of the pneumotachograph (PNT: capillary at ICH and screen at LEIC), and partly from the slight differences in shutter configuration.

For measurements of $V'_{\max,FRC}$ the deadspace and apparatus resistance presented to the infant was reduced, consisting of mask and PNT alone. The inflatable jacket (Medical Engineering, Royal Postgraduate Medical School, Hammersmith Hospital, London) used for RTC measurements at LEIC was wrapped around the infant with their arms within and the entire anterior section was inflated. GOS infants wore a cummerbund style fully adjustable jacket (Hannover Medical School, Germany) with their arms remaining outside.

RASP software for data collection and analysis (Section 3.2.7) used by each centre was configured in a similar manner so that original data could be inspected and analysed between centres, and calibration factors examined.

6.3.2.2 Data collection

Once asleep, the infant was wrapped in an adjustable inflatable jacket for measurements of $V'_{\max,FRC}$ and a face mask and PNT was applied over the nose and mouth and made leakproof with therapeutic putty. Measurements of maximum flow at functional residual capacity ($V'_{\max,FRC}$) were then performed (Section 3.3.6). The inflatable jacket was loosened before plethysmographic measurements of FRC_{pleth} and R_{aw} were made. Details of general equipment and measurement methods at GOS are given in section 3.2. Methods were applied in a similar manner by both GOS and LEIC during the Respiratory follow-up.

The raw data from each infant, including calibration checks, were exchanged between centres by disk, soon after measurements were complete. Results were analysed, by both the test centre and the analysis centre, using the protocol agreed following an interim analysis. Minor adjustments to usual practice were made by both centres following this analysis, including standardisation of sampling frequencies and the method by which end expiratory baselines were assessed, together with the recording of (rather than simply just performing) calibration checks and test occlusions with several pre and post manoeuvre breaths, the latter being to

provide evidence on disk regarding absence of any leaks (Stocks et al 1996, Beardsmore et al 1982). The aim of these adjustments was to minimise inter-observer variability, and facilitate analysis by an operator who had not been present during the measurements, and therefore required evidence of quality control during data collection.

6.3.2.3 Data Analysis

Interactive software (RASP), which recorded data in real time throughout the measurement period and allowed subsequent off-line analysis, was used by both centres. It was possible to customise both data collection and analysis to individual preference with respect, for example, to sampling frequency, number of breaths analysed, thresholds for event recognition, and methods of assessing end expiratory baselines. All data were stored on disk, including the original raw data channels (time, flow, volume and pressure signals), details of each analysed event, alongside the selected user options at time of analysis, and prevailing measurement conditions such as ambient pressure, apparatus dead space and calibrations at the time of data recording. Exchange of the raw data alone on disk allowed inspection of original signals and enabled reanalysis of results, blind to that of previous investigators. However, in the event of inter-centre discrepancies in calculated results, additional exchange of the analysis files facilitated rapid identification of the source of any bias.

Based on the inherent intra-subject variability of each of the respiratory parameters, the aim of inter-observer analysis of each infant respiratory function measurement was to report values analysed by each centre to within 10% of each other for FRC_{pleth} , $V'_{\text{max,FRC}}$, tidal volume (V_T), and respiratory rate (RR), and 20% for the more variable measures of R_{aw} (Dezateux et al 1994, Dundas et al 1995), using the test centre result as the numerator and the analysis centre result as the denominator. Where agreement was not within these agreed limits, analysis files were examined and amended where appropriate.

Following interim analysis, it also became apparent that, when a considerable amount of data had been collected, selection of the optimal data for analysing R_{aw} with respect to achievement of BTPS conditions and minimal drift was difficult without some reference to quality control at time of data collection. It was therefore

agreed that the test centre should indicate the best epochs of data from which to select data for analysis prior to sending the data to the test centre for re-analysis.

For tidal breathing parameters, RR and V_T , values were reported from data collected immediately prior to measurements of forced expiration, which were generally slightly lower than those obtained during plethysmography (data not shown). The former were considered to be closer to baseline values, by virtue of the smaller apparatus dead space and resistance. The mean of 25 breaths was reported. Both centres based the analysis on the first 5 savesets of data containing a forced expiration manoeuvre, with LEIC reporting a mean value based on 5 breaths from each saveset, extracted from the $V'_{max,FRC}$ analysis report, while GOS analysed the same savesets but used a specific tidal breathing analysis profile which allowed individual weighting of each breath, and hence the calculation and reporting of an SD for the 25 breaths. Tidal volume was reported to the nearest mL and RR to one decimal place.

$V'_{max,FRC}$ values were reported both as the highest, and the mean of the 4 highest, values of technically acceptable data. Data were technically acceptable when: jacket inflation pressures were over a sufficient range to obtain maximal flows in the infant, time to peak jacket (P_J) inflation was < 0.1 s, and the jacket inflation lead time (the interval between end inspiration and onset of jacket inflation) was within 0.1 s. For the flow-volume curves, data were accepted when a rapid rise to peak expiratory flow was seen, with no evidence of glottic closure. The maximal and mean jacket pressures (P_J) were noted, together with the pressure during the best manoeuvre (optimal P_J), % P_J transmission, and the total number of manoeuvres performed.

FRC_{pleth} was reported as the mean of all technically acceptable occlusions, where the infant was occluded at end inspiration or within 10% of the start of expiration. Each occlusion was the mean of 1 to 3 respiratory efforts, with each complete effort being the inspiratory and expiratory mean. Analysis defaults were set to measure each effort between 95 to 5% of the peaks and troughs thus limiting measurements to the rapidly changing period of the phase relationship. The subsequent correction to FRC was performed by subtracting the volume occluded above end expiratory volume and the apparatus dead space from the total occluded gas volume.

If a degree of glottic closure was present, it was permissible to cautiously “truncate” the measured effort, measuring over a shorter period e.g. between 80 to 20% peak to trough. A minimum of three occlusions was initially required to report results, but later relaxed following the interim analysis to allow reporting of otherwise good quality data, where fewer than 3 end inspiratory occlusions were available.

The revised quality control criteria were:

- minimum of 3 end inspiratory occlusions, no maximum if data were of good quality.
- if 3 high volume occlusions (within 10% of end inspiration) were not analysable, then mid volume data (above 50% tidal volume) were included provided that such values were within 10% of the high volume occlusion.
- if sufficient high/mid volume data were not available to report on 3 after applying these criteria, then n=2 was acceptable if both occlusions were of good technical quality and within 10% of each other.

R_{aw} was reported from data where there was only minimal drift of the plethysmographic pressure signal. Measurements of R_{aw} were made only after thermal equilibrium was reached. Signals were affected adversely by changing weather conditions, which at times led to drift even when adequate time had been allowed for stable conditions to be met.

Flow and plethysmographic pressure and volume signals were technically acceptable when in phase on a time series axis, and closed at points of zero flow when viewed on an X-Y plot (Section 3.3.4.2). Default strategies for reporting values of R_{aw} using RASP interactive operator analysis allowed selection and filtering (plethysmographic signals and tidal volume) on a breath-by-breath basis. Epochs of data were analysed when a time based trace suggested that V' and P_{pleth} were in phase. R_{aw} was reported as a mean of 7 breaths for Respiratory follow-up, where pressure-flow loops were accepted if closed at points of zero flow. R_{aw} was initially calculated from the beginning of inspiration and end of expiration, i.e. at 2 points throughout the respiratory cycle, giving inspiratory and expiratory R_{aw} , at 50% maximum tidal flows (Figure 3-12). Data were then reworked using the facility within the RASP program at mean inspiratory and expiratory flows. Analysis centre reporting was confined to data from savesets recommended by the test centre when data were exchanged, which met the criteria stated above.

Where technically satisfactory measurements were not obtained or data were not collected, the reasons for failure were coded as:

- Technical: when failure was related to the equipment used;
- Physiological: when measurements were invalidated by the infant; or
- Woke: when the infant did not remain in quiet sleep during measurements.

6.3.2.4 Analysis of within-infant, inter-centre differences

Infant details and results were double entered on an Excel spreadsheet (version 5.0, ©Microsoft Corporation) and checked for data transcription errors, by both the test centre and the analysis centre independently, before comparing results between centres. The aim was to minimise differences in measured parameters that were not due to biological variability alone: for example data transcription and entry errors, data selection bias or differing analysis strategies.

6.3.3 Reporting of Results

A short clinical report, based on the results obtained from the test centre, was sent to the referring paediatrician and infant's family doctor as soon as possible after testing.

The definitive results with respect to comparisons of respiratory function between the ECMO and CM groups, which are described in Section 6.5 (Makkonen et al 1997, Dundas et al 1997), were reported from the analysis centre, i.e. the centre that did not test the infant, and therefore had no contact with the family. This ensured complete blinding to management status. The management status at trial entry of the whole group of infants was not revealed until both data collection and analysis were complete, when background details of surviving infants, including a description of their status at trial entry and management (ECMO or CM), were obtained from the ECMO trial data co-ordinator.

6.3.4 Statistical methods and control data

Outcome variables obtained from respiratory function measurements were FRC_{pleth} , R_{aw} (also expressed as the lung volume corrected reciprocal: sG_{aw} reported during early inspiration and late expiration) and $V'_{\text{max,FRC}}$. V_T and RR are also presented as explanatory variables.

The description of the 78 infants who attended Respiratory follow-up at trial entry and at one year is by treatment allocation group: ECMO vs CM. Respiratory function outcome measures and background details are presented both by allocation group and by test centre: GOS vs LEIC. Where comparisons of airway function between ECMO and CM groups or the association between measurements are described, the definitive results were reported from the analysis centre (AC). The association between measurements of forced expiration and specific conductance and of $t_{PTEF}:t_E$ and specific conductance was evaluated for the whole group of 78 infants.

6.3.4.1 Comparison of infants studied in the two different centres

For comparisons between the two groups of infants studied at GOS and LEIC, unpaired t tests were used to examine background details and respiratory function results where data were normally distributed. The Mann Whitney U test was used to assess differences between groups of non normally distributed data. A p value of less than 0.05, and 95% Confidence intervals of the differences between groups or paired data not encompassing zero, were considered significant.

6.3.4.2 Comparison of within-subject, inter-centre differences

Inter-centre, within-subject differences, which would reflect variation in analytical techniques, were evaluated according to the method of Bland and Altman (Bland et al 1986), by calculating the mean and standard deviation (SD) of the difference between pairs of measurements (GOS - LEIC). Results were displayed graphically by plotting the differences between pairs against their mean, together with the group mean difference and limits of agreement (± 2 SD of the within pair differences).

To examine the association between $V'_{max,FRC}$ or $t_{PTEF}:t_E$ and sG_{aw} , simple linear regression was used (SPSS for windows version 7).

6.3.4.3 Power of study

To achieve 85% power of study at the 5% level to detect differences in the 3 respiratory outcome measures (FRC_{pleth} , R_{aw} (or sG_{aw}) and $V'_{max,FRC}$), equivalent to one standardised difference, it was calculated that at least 30 infants should be studied in each management group. However, early termination of the main trial, when interim analysis demonstrated the survival advantage of ECMO, together with

80% follow-up of survivors meant that this target was not quite reached, the numbers in each group being 51 (ECMO) and 27 (CM).

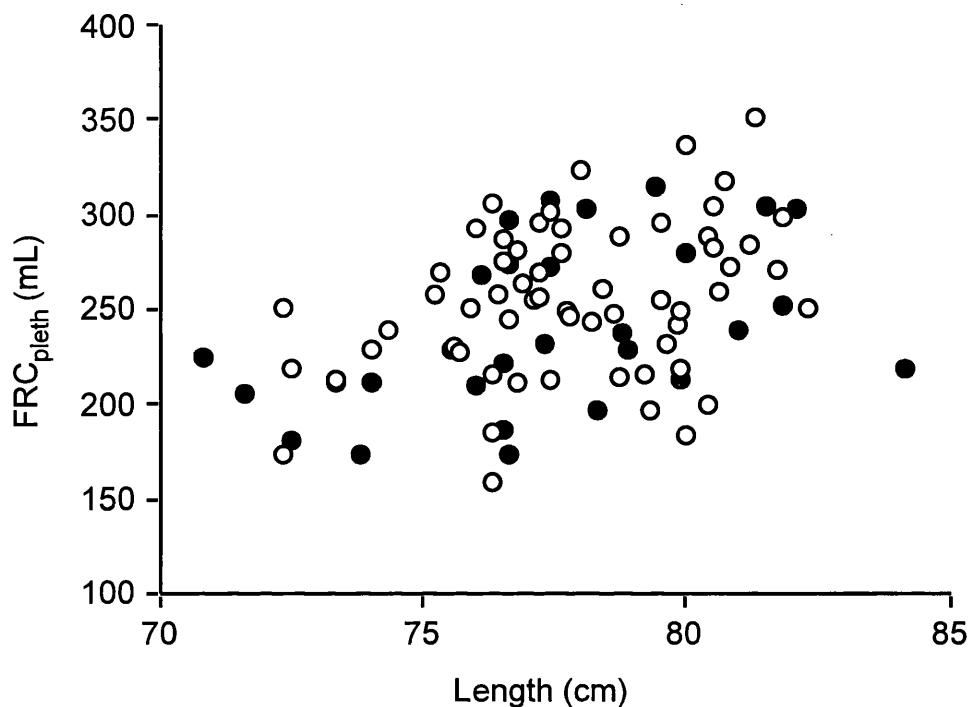
6.3.4.4 Control infants

Available for comparison with Respiratory follow-up data were selected reference groups of fullterm infants, matched for age. For plethysmographic measurements the comparison group comprised 93 infants recruited to an epidemiological study of respiratory function and acute respiratory illness (Dezateux et al 1999). Sixty-three infants were healthy and wheeze-free and 30 had experienced at least one episode of physician confirmed lower respiratory illness. The eligibility criteria are summarised in Section 3.1. Twenty-five of these infants of the comparison group were also included as healthy controls in Section 4 and Section 5. The comparison group was measured in the same laboratory and with the same equipment as the GOS group in the ECMO Respiratory follow-up. Data from healthy controls (controls) and infants with at least one episode of LRI (LRI controls) are described and shown separately. Values of FRC_{pleth} and length are shown in Figure 6-1, with healthy and LRI controls identified separately.

For the outcome measure of $V'_{\text{max,FRC}}$, two separate populations were available for comparison with Respiratory follow-up subjects. Forty-seven infants from a population of 112 North American healthy fullterms measured to obtain reference values for forced expiratory flows and lung volume by helium dilution recruited by Tepper and colleagues (Tepper et al 1993) selected by age (all infants between 11 and 19 months) and values of $V'_{\text{max,FRC}}$ were reported as the mean of up to 3 manoeuvres.

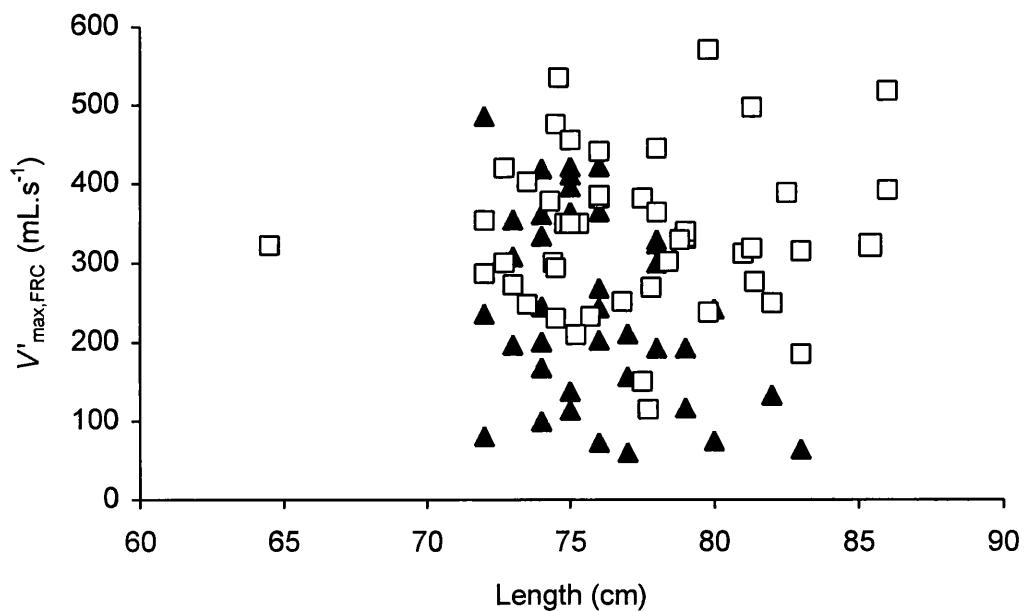
Also available for comparison were 41 infants from an initial cohort of 73 recruited by Clarke and colleagues (Clarke et al 1995) to investigate the association between bronchial responsiveness and LRI. All infants had at least one atopic parent. Values are reported from their third laboratory visit, at around one year of age. This group was measured using similar equipment and identical software to infants in the ECMO Respiratory follow-up and was also UK based. Figure 6-2 shows values of $V'_{\text{max,FRC}}$ and length for both UK and USA groups.

Figure 6-1 Plethysmography reference group: FRC_{pleth} vs length



Key to Figure 6.1: open circles LRI controls, solid circles healthy controls.

Figure 6-2 $V'_{\text{max},\text{FRC}}$ reference group: $V'_{\text{max},\text{FRC}}$ vs length



Key to Figure 6.2: Solid triangles; UK controls, Open squares; USA controls.

6.4 Inter observer variability within and between two specialised infant lung function testing centres

6.4.1 Introduction

Infant respiratory function tests are time consuming and relatively complex, but there is an increasing interest in their use as outcome measures of interventions, or indicators of disease severity. However, accrual of adequate numbers of infants to studies based in one test centre or geographical area may take many years, while reporting of smaller numbers will result in insufficient power to evaluate the statistical significance of outcome measures. In contrast, large multicentre clinical trials may accrue more infants than can reasonably be measured at one centre. Collaboration between centres who perform these specialised tests would be the most realistic alternative, but a common methodological approach and compatible equipment and software are a prerequisite to pooling of data.

When the opportunity arose to undertake the Respiratory follow-up of the Collaborative ECMO trial (Section 6.1.1), measurements of infant respiratory function were well established at both centres. However, the complexity of measurement procedure and methods of analysis led to considerable potential for differences in approach. Participation in this two centre trial, which lasted from August 1994 to December 1996, offered the opportunity to address methodological issues, and to develop a standardised approach which could be applied within future multicentre trials.

The specific aims of this aspect of the study were :

- a) to develop a strategy for performing and analysing infant respiratory function tests to facilitate future multi-centre trials, and;
- b) to compare inter observer variability within and between two specialised infant lung function testing centres with respect to plethysmographic measurements of functional residual capacity (FRC_{pleth}) and airway resistance (R_{aw}), and assessments of maximal flow at FRC ($V'_{\text{max,FRC}}$), using the tidal rapid thoraco-abdominal compression technique (RTC), these being the main outcome variables of the Respiratory Follow up.

6.4.2 Results

6.4.2.1 Population by test centre

Between August 1994 and December 1996, of the 78 infants who took part in the Respiratory follow-up with their parents, 42 attended GOS and 36 LEIC. Those who participated in the respiratory follow-up were similar to whole group of survivors with respect to gestational age, birthweight, sex distribution, primary diagnosis, age and severity of disease at trial entry (UK Collaborative ECMO Trial Group et al 1996, Dundas et al 1997, Makkonen et al 1997) (data not shown). Details of infants attending each centre are summarised in Table 6-2. Age, weight and length of infants attending each test centre were similar, as were birthweight and gestational age.

The reported prevalence of maternal tobacco smoking during pregnancy and postnatally was similar at each centre, although a greater proportion of infants attending GOS were reported to be exposed to tobacco smoke from any sources (parental and/or other regular exposures), 69% vs 47%, (95% CI of the difference GOS vs LEIC -0.1%, 44%; $p=0.051$). More infants who attended LEIC were symptomatic at testing (respiratory symptoms included rhinorrhea, coryzal illness, cough and wheeze): 25% vs 7%, $p < 0.05$. There were also more infants reported as having had recent upper respiratory tract infections; 53% (LEIC) vs 17% (GOS), $p < 0.001$. The majority of infants, however, were coded as normal following examination by a paediatrician blinded to management status.

Table 6-2 Details of infants attending each Test Centre

	Test centre		
	GOS n=42	LEIC n=36	95% CI of the group mean difference (GOS - LEIC)
Age (months)†	13.6 (1.8)	13.9 (2.0)	-1.1, 0.6
Weight (kg)‡	9.9 (1.5)	10.5 (1.6)	-1.2, 0.2
Length (cm)‡	78.1 (3.5)	78.4 (3.6)	-2.0, 1.2
Birthweight (g)‡	3378 (645)	3521 (468)	-400, 116
Gestation (wks)‡	39.3 (2.4)	39.6 (2.0)	-1.4, 0.7
Male†	24 (57%)	24 (67%)	-31, 12%
Caucasian†	32 (76%)	28 (78%)	-20, 17%
Afro Caribbean†	5 (12%)	0	1, 23%*
Indo-Pakistan†	4 (10%)	7 (19%)	-25, 6%
Other†	1 (2%)	1 (3%)	-7, 7%
Smoking during pregnancy†	7 (17%)	4 (11%)	-10, 21%
Current maternal smoking†	13 (31%)	12 (33%)	-23, 18%
Any smoke exposure†	29 (69%)	17 (47%)	-0.1, 44%
Current resp. medicine†	10 (24%)	7 (19%)	-14, 23%
Symptomatic at testing†	3 (7%)	9 (25%)	-34, -2%*
Recent URTI†	7 (17%)	19 (53%)	-57, -15%***
Normal medical exam†	29 (69%)	29 (81%)	-31, 8%

Key to table: †Values are given as mean and SD; ‡Values are given as n and %.

* p<0.05, ** p<0.01, *** p<0.001

6.4.3 Technically acceptable measurements

Table 6-3 shows the percentage of successful measurements for each outcome parameter, grouped by test centre. Tidal parameters were reported for all LEIC infants, and all but one GOS infant (who woke before measurements of $V'_{max,FRC}$ were attempted, tidal parameters being reported from data collected prior to forced expiratory manoeuvres). Most infants had resting lung volume (FRC_{pleth}) successfully measured, in similar proportions at each test centre. However, a significantly smaller proportion of infants measured in Leicester had successful R_{aw} results reported ($p = 0.014$).

For infants attending GOS, failure to report FRC_{pleth} or R_{aw} was in most cases due to waking before measurements were complete: 3 infants woke following $V'_{max,FRC}$ but before successful FRC_{pleth} measurements and 2 during plethysmography following recording of FRC_{pleth} but prior to attempting to measure R_{aw} . In a further 3 infants R_{aw} could not be reported for technical reasons, signals being adversely affected by weather or building work in the area.

In contrast, only one infant in LEIC woke during plethysmographic measurements of R_{aw} , but FRC_{pleth} was unsuccessful in 3 infants (one technical failure, two attempts invalidated by glottic closure during expiratory efforts) and R_{aw} was unsuccessful in 14 infants. The latter was due to 10 technical failures, subsequent to the introduction of a new amplifier part way through Respiratory follow-up (7 occasions) or insufficient humidification in the rebreathing bag (3 occasions), and 1 failure for physiological reasons (glottis closure). The remaining 3 measurements failed because FRC_{pleth} was unsuccessful, as noted previously, so that the occlusion data could not be used to calibrate R_{aw} data.

Successful measurements of $V'_{max,FRC}$ were achieved in 37 (88%) infants attending GOS and 34 (94%) from LEIC. At GOS, 1 infant woke, in 3 others there was persistent early inspiration during each manoeuvre, and in the remaining infant suboptimal jacket pressures were applied, such that flow limitation was not demonstrated. In the 2 LEIC infants in whom measurements were unsuccessful, the jackets were of poor fit resulting in late application of thoraco-abdominal pressures

in all manoeuvres, thereby failing to meet quality control criteria with respect to jacket inflation time.

Table 6-3 Number and percentage of successful measurements at each test centre

	<i>Test centre</i>		
	<i>GOS</i> <i>n=42</i>	<i>LEIC</i> <i>n=36</i>	<i>95% CI</i> (<i>GOS - LEIC</i>)
FRC_{pleth}	39 (93%)	33 (92%)	-10%, 13%
R_{aw}	35 (83%)	21 (58%)	5%, 45%*
V'_{maxFRC}	37 (88%)	34 (94%)	-13%, 9%
Tidal breathing parameters	41 (98%)	36 (100%)	-7%, 3%

Key to Table: Values are shown as n (%). FRC_{pleth} = functional residual capacity by plethysmography; R_{aw}: airway resistance; V'_{max,FRC}: maximum expiratory flow at functional residual capacity. *p=0.014

6.4.4 Comparison of respiratory function in infants studied in the two centres

There were no significant differences for any of the outcome measures i.e. FRC_{pleth}, R_{aw} or V'_{max,FRC} between the infants studied at the 2 centres (Table 6-4). The group mean (range) FRC_{pleth} was 284 (160 - 450) mL for infants attending GOS, and 281 (145 - 492) mL for those attending LEIC. The number of FRC_{pleth} manoeuvres reported by each centre following cross-centre analysis, expressed as median (range), was 4 (2 - 9) for GOS analysis of LEIC data and 7 (2 - 12) for LEIC analysis of GOS data. However, only one infant from each centre had lung volume reported on only 2 manoeuvres. When corrected for weight, the group mean (range) FRC_{pleth} of infants attending GOS was 29 (17 - 46) mL.kg⁻¹, and that at LEIC 27 (15 - 49) mL.kg⁻¹.

Although airway resistance tended to be higher and Specific airway conductance (the inverse of resistance, corrected for lung volume) lower amongst those infants attending GOS (Table 6-4), these differences did not reach significance, (95% CI [GOS - LEIC] for R_{aw,II} and R_{aw,EE} : 0.14, 0.77 and -0.15, 1.12 kPa.L⁻¹.s respectively).

Table 6-4 Respiratory function and measurement details of infants attending each test centre

	GOS n=42	LEIC n=36	95% CI [GOS-LEIC]
FRC _{pleth} (mL)	284 (59)	281 (73)	-28, 35
$R_{aw,II}$ (kPa·L ⁻¹ ·s)	2.30 (0.89)	1.98 (0.76)	-0.14, 0.77
$R_{aw,EE}$ (kPa·L ⁻¹ ·s)	2.94 (1.18)	2.45 (1.12)	-0.15, 1.12
$sG_{aw,II}$ (s ⁻¹ .kPa ⁻¹) [‡]	1.68 (1.17-2.30)	1.92 (1.12-2.72)	-0.84, 0.2
$sG_{aw,EE}$ (s ⁻¹ .kPa ⁻¹) [‡]	1.28 (0.87-1.73)	1.80 (0.90-2.26)	-0.87, 0.14
Mean $V'_{max,FRC}$ (mL·s ⁻¹)	171 (73)	145 (75)	-9, 61
Best $V'_{max,FRC}$ (mL·s ⁻¹)	176 (80)	158 (82)	-20, 56
Optimal P _j (kPa)	4.80 (1.70)	2.56 (0.85)	1.61, 2.87***
Mean P _j (kPa)	5.49 (1.59)	2.99 (0.94)	1.89, 3.31***
Highest P _j	6.49 (1.62)	4.30 (0.79)	1.61, 2.75***
% P _j transmitted	29%	58%	
RTC manoeuvres: median (range)	18 (8 - 40)	18 (11 - 24)	-2, 3
V_T (mL)	94 (17)	83 (16)	3, 18**
V_T (mLkg ⁻¹)	9.5 (1.5)	8.0 (1.2)	0.9, 2.1***
RR (min ⁻¹)	33 (8)	33 (10)	-4, 4

Key to table: [‡]Values are given as mean (SD), except for sG_{aw} , when group differences were assessed using the non parametric Mann-Whitney U Test, where values are shown as median (interquartile range) and 95%CI of the difference between group medians.

** p<0.01 *** p < 0.001

$V'_{max,FRC}$, whether expressed as the highest value obtained or as the mean of the three highest technically acceptable manoeuvres, was similar for infants attending both test centres. Mean (range) $V'_{max,FRC}$ was 171 (48 - 415) mL.s^{-1} at GOS and 145 (27 - 320) mL.s^{-1} at LEIC, when expressed as a mean, with similar values for the highest technically acceptable $V'_{max,FRC}$ (Table 6-4). However, despite the similarity of results between the two centres, the applied jacket pressures were significantly higher at GOS than at LEIC ($p<0.001$ for each variable), reflecting the lower percentage of jacket pressure transmitted to the infant at GOS (29% vs 58% at LEIC). The median number of manoeuvres was 18 (range 8-40) at each centre.

Tidal breathing parameters were not selected outcome measures, but were recorded and analysed to provide additional evidence for quality control and baseline information regarding respiratory function. Respiratory rate was not significantly different between centres mean (range) being 33 (19 - 53) min^{-1} at GOS and 33 (24 - 74) min^{-1} at LEIC. However, group mean V_T was larger for infants attending GOS than LEIC (mean [range] 94 [64 - 134]) and 83 [52 - 121] mL) respectively, $p=0.006$. These differences persisted when corrected for weight. When examined according to test centre, subdivided into treatment groups (ECMO and CM), no differences were seen between treatment groups for any parameter (data not shown).

6.4.5 Comparison of within-subject respiratory function results analysed by each centre

In addition to comparing respiratory function results between measurement centres, within-infant inter-observer variability was also assessed. As stated previously, the aim was to obtain values within 10% of each other for tidal breathing parameters, $V'_{max,FRC}$ and $\text{FRC}_{\text{pleth}}$, and within 20% for R_{aw} . Data obtained from infants in the Pilot study, have been described in section 0 but will also be summarised in this section for clarity. Results of the cross-analysis of each centre's data are shown in Table 6-5.

Table 6-5 Comparison of within- subject respiratory function results analysed by each centre

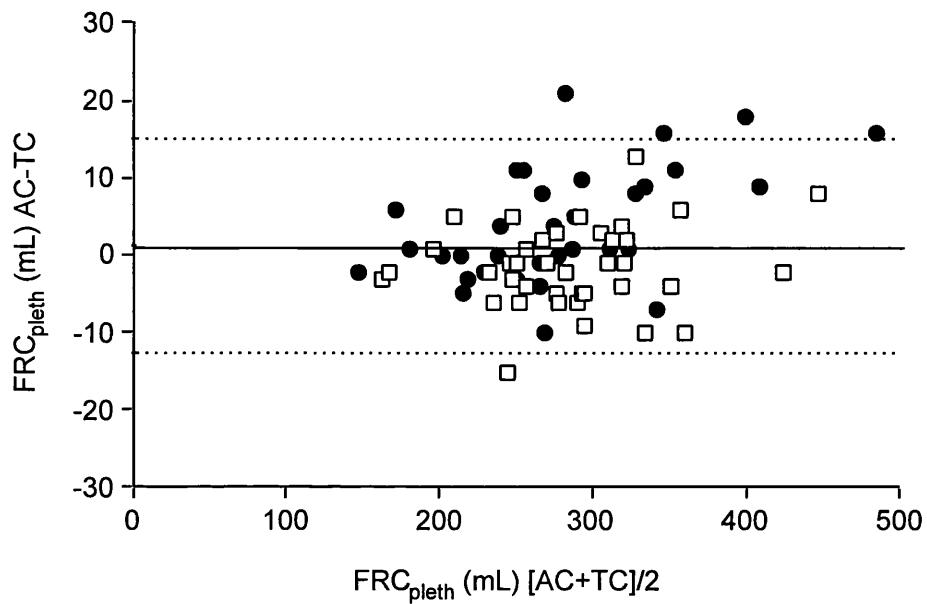
Test centre (TC)	GOS n=42		LEIC n=36		mean, (95% CI) [AC-TC] n=78
Analysis centre (AC)	GOS	LEIC	GOS	LEIC	
FRC _{pleth} (mL)	286 (58)	284 (59)	281 (73)	277 (67)	1.11 (-0.53, 2.75)
$R_{aw,II}$ (kPa·L ⁻¹ ·s)	2.19 (0.79)	2.30 (0.89)	1.98 (0.76)	1.86 (0.84)	0.04 (-0.02, 0.10)
$R_{aw,EE}$ (kPa·L ⁻¹ ·s)	2.91 (1.22)	2.93 (1.18)	2.45 (1.12)	2.32 (1.16)	-0.02 (-0.12, 0.08)
Mean $V'_{max,FRC}$ (mL·s ⁻¹)	168 (73)	171 (73)	145 (75)	146 (75)	0.91 (-0.94, 2.77)
Best $V'_{max,FRC}$ (mL·s ⁻¹)	178 (77)	176 (80)	158 (82)	156 (80)	1.43 (-1.42, 4.28)
V_T (mL)	94 (17)	94 (17)	83 (16)	83 (16)	0.04 (-0.57, 0.64)
RR (min ⁻¹)	33 (7)	33 (8)	33 (10)	33 (10)	-0.13 (-0.39, 0.14)

Key to Table : Values are given as mean (SD). For definition of abbreviations, see Table 6.3 and $R_{aw,II}$ = airway resistance during initial inspiration; $R_{aw,EE}$ = airway resistance during late expiration; V_T = tidal volume; RR = respiratory rate

FRC_{pleth}

FRC_{pleth} analyses were generally within 10% agreement. On initial comparison of the results from the 2 centres, discrepancies greater than 10% occurred in 12 infants. In 4 of these infants this was due to data collection in which it had not been possible to report values based on at least 3 occlusions, either due to the infant waking early (1), or insufficient collection of end inspiratory occlusion data (3). Following revision of guidelines and reanalysis by each centre, the definitive results reported were all within agreed limits, although it was necessary to report FRC_{pleth} as the mean of only 2 manoeuvres in 2 infants. In the remaining 8 infants, discrepancies were due to data transcription errors (2 infants), different epochs of data being selected for analysis (1 infant) and differing analysis strategies, which were mainly related to “truncating” glottic expiratory efforts by varying amounts (5 infants). All were readily resolved after critical inspection of data by each centre, so that final agreement for FRC_{pleth} was within 10% all infants. The between centre agreement is shown in Figure 6.3.

Figure 6-3 Between centre agreement for resting lung volume



Key to Figure: AC: Analysis centre; TC: Test centre; Solid line denotes mean between-centre difference; dotted lines denote 95% limits of agreement of the mean difference; □ infants tested at GOS; • infants tested at LEIC

Airway Resistance

For R_{aw} analyses, there were initially discrepancies $> 20\%$ in 10 infants. These were due to operator errors in selecting analysis options within the software that did not adhere to the agreed protocol (3 infants) or selection of different epochs of data for analysis and reporting (7 infants) i.e. biological variability. Of the infants in whom R_{aw} measurements were not reported, 1 of 3 technical failures at GOS was failed by LEIC alone, while the remaining 2 were failed by both centres. Similarly 4 of 14 technical failures at LEIC were failed by GOS alone and the remainder by both centres. After inspection and amendment of the analysed data by each centre, agreement was improved to within 20% in all but 3 of the 56 infants. The between centre agreement, for R_{aw} calculated during initial inspiration and late expiration, is displayed in Figure 6-4 and Figure 6-5 respectively.

Figure 6-4 Between centre agreement for initial inspiratory airway resistance

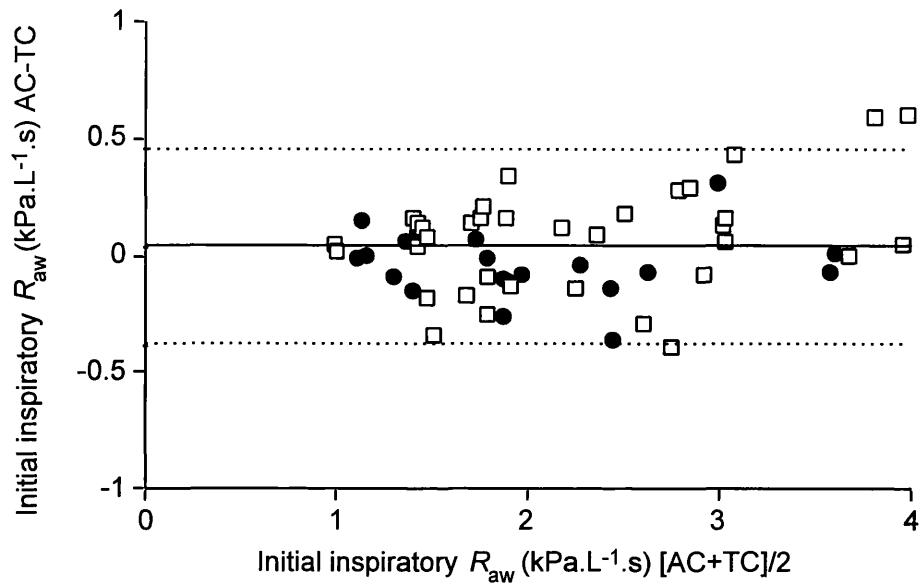
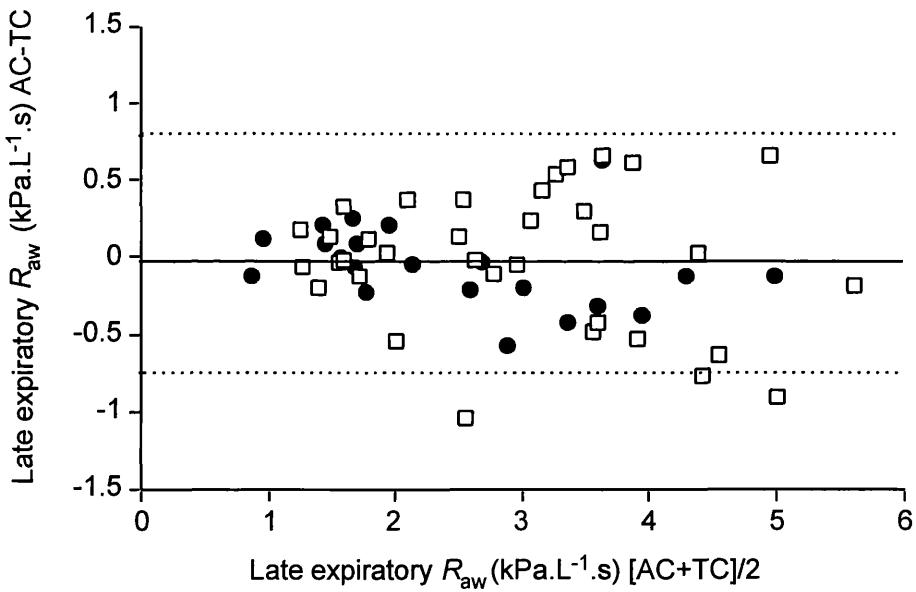


Figure 6-5 Between centre agreement for late expiratory airway resistance

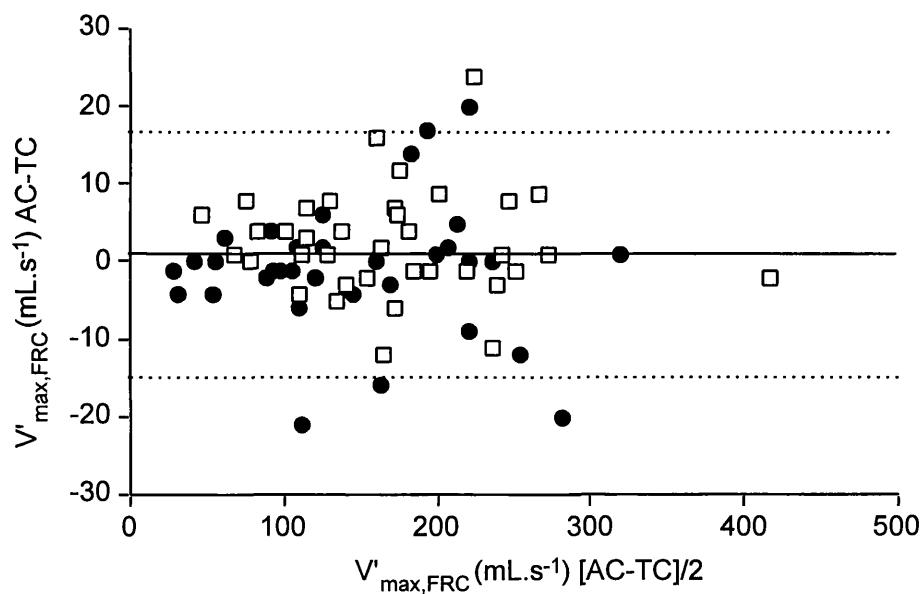


Key to Figure 6-4 and Figure 6-5: AC: Analysis centre; TC: Test centre; Solid line denotes mean between-centre difference; dotted lines denote 95% limits of agreement of the mean difference; □ infants tested at GOS; ● infants tested at LEIC

$V'_{\max,FRC}$

Following the interim analysis, when a common volume baseline filtering strategy was adopted by both centres, and it was agreed to establish the FRC level by regression through the end expiratory points preceding the RTC manoeuvre, agreement was generally good for $V'_{\max,FRC}$ analyses. Initially analyses of $V'_{\max,FRC}$ were outwith agreed limits of 10% for 6 infants. One was due to a data transcription error, 2 to the selection of different epochs of data from which the best 4 manoeuvres were reported, one was attributed to volume baseline filtering strategies, while data from 2 infants required recalibration to adjust a zero offset of the flow signal before good agreement could be achieved. Following inspection and amendment of the analysed data by each centre, 3 infants remained outwith agreed limits. Two of these had very low, flow-limited values of $V'_{\max,FRC}$, so that although the discrepancy between laboratories exceeded 10%, absolute values from the 2 centres were within 6 mL.s^{-1} of each other. The poor between-centre agreement in the remaining infant (20%) arose from the inclusion of data by 1 centre, but not the other, of one manoeuvre where instability of breathing patterns made establishment of the FRC level very difficult to determine. The between-centre agreement for within -subject values of $V'_{\max,FRC}$ is displayed in Figure 6-6.

Figure 6-6 Between centre agreement for $V'_{\max,FRC}$



Key to Figure: AC: Analysis centre; TC: Test centre; Solid line denotes mean between-centre difference; dotted lines denote 95% limits of agreement of the mean difference; \square infants tested at GOS; \bullet infants tested at LEIC

Tidal breathing parameters

For tidal breathing parameters, between centre differences were seen in 3 infants. In 2 of these infants, different epochs of data had been selected for analysis, and agreement was improved to within agreed limits by selecting data which appeared more representative of that displayed during the part of the measurement session from which the definitive $V'_{\max, \text{FRC}}$ data had been obtained. The remaining infant displayed a variable respiratory rate with insufficient control breathing from the first 5 epochs of tidal breathing prior to forced expiratory manoeuvres, with each centre then selecting slightly different epochs of data such that agreement was 12% rather than below 10%. The between centre agreement for tidal parameters RR and V_T is displayed in Figure 6-7 and Figure 6-8.

Figure 6-7 Between centre agreement for respiratory rate

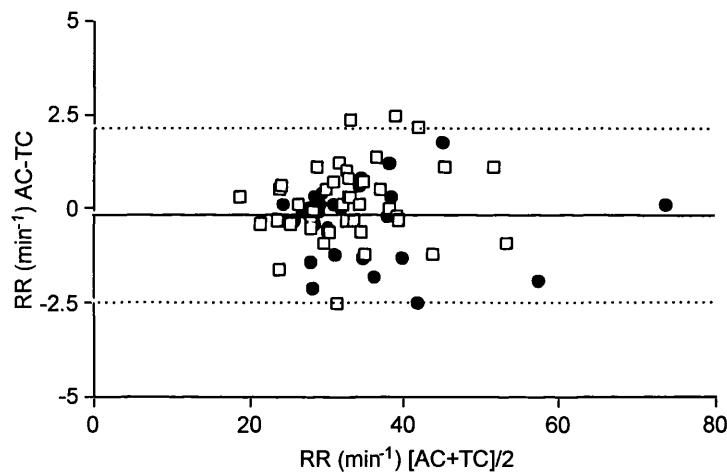
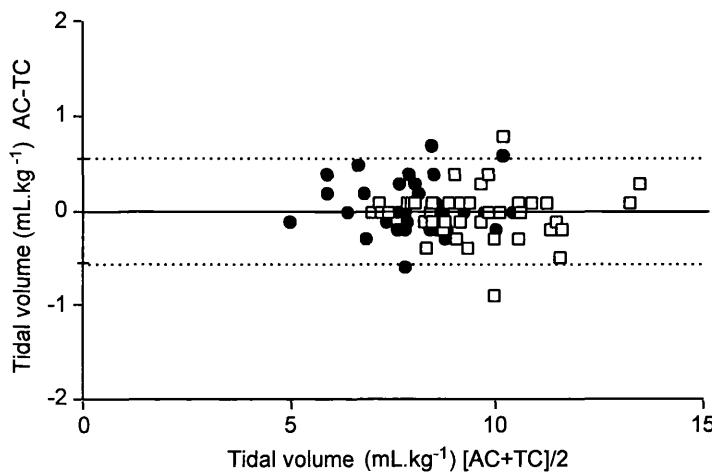


Figure 6-8 Between centre agreement for weight corrected tidal volume



Key to Figure 6-7 and Figure 6-8: AC: Analysis centre; TC: Test centre; Solid line denotes mean between-centre difference; dotted lines denote 95% limits of agreement of the mean difference; □ infants tested at GOS; • infants tested at LEIC

6.4.6 Discussion

One of the major advantages of the Respiratory follow-up study design was that the analysis of respiratory function in survivors recruited from the ECMO trial could be made fully blinded to their management status. However, before combining data from each centre, it was necessary to ensure that there was no bias either within or between laboratories that might confound interpretation of the results. It was therefore necessary to assess the agreement between centres of both the background and respiratory function data.

The aim of this component of the study was to evaluate inter-observer variability within and between two specialised infant lung function testing centres. We found that, after amendment of measurement and analysis protocols, conducting follow-up from a randomised controlled trial with infant respiratory parameters as outcome measures was possible. Additionally, after adjustment for local measurement conditions, respiratory function results from both centres were pooled, avoiding the potential bias of results by one centre.

This section contains a critical evaluation of the methodology employed within the study. The characteristics of the study population attending each centre are addressed briefly, a more detailed discussion with respect to their trial management status is contained within section 6.5. The results obtained between centres, and the inter observer variability of within infant results are then discussed. Finally, the feasibility of conducting this, and other similar trials with infant respiratory outcome parameters is considered.

6.4.6.1 Population

Infants and their families attended Respiratory follow-up from all over the United Kingdom. The majority of infants attending each test centre were of Caucasian ethnicity. Amongst the remaining ethnic groups there were 5 infants of Afro-Caribbean origin who all attended GOS; while more Asian infants attended LEIC than GOS. However, when examined according to management group (ECMO vs CM) there were similar proportions of infants from each ethnic background within each group (Makkonen et al 1997).

The prevalence of maternal smoking during pregnancy was similar at each centre, but lower than that reported elsewhere (Laube et al 1996, Trigg et al 1990). In particular, the proportion of infants reported to be exposed who attended GOS was 17% (7 of 42) compared with 43% (43 of 101) of infants in an East London based prospective study (section 6.3.4) who also attended GOS for similar measurements during an overlapping time period. This suggests that there may have been under reporting of maternal smoking during pregnancy by mothers of the Respiratory follow-up population at each test centre. The suggestion is reinforced by the increase in reported maternal smoking at the time of the laboratory visit at around one year, when 31% and 33% (GOS and LEIC respectively) of infants were exposed to maternal tobacco smoke: thereby approaching other reported prevalences (Laube et al 1996, Trigg et al 1990). The proportion of infants exposed to tobacco smoke from any source postnatally was significantly greater at GOS and was similar to that reported in the sub-population of East London infants who did not wheeze in the first year of life (Dezateux et al 1999).

Although efforts were made to avoid testing infants within three weeks of onset of URTI symptoms at GOS and within four weeks at LEIC, this was not always possible, particularly amongst infants who suffered recurrent respiratory infections, or when long distance travel arrangements had been made. The proportion of infants who had had recent symptoms of URTI was greater at LEIC, which may have reflected the longer symptom free time period stipulated at that centre. However, a greater proportion of infants were also symptomatic at testing in LEIC (25% vs 7%). Local laboratory practice with respect to booking and, if necessary, postponing appointments would also have influenced these findings. There was no evident correlation between recent URTI symptoms and reported values of R_{aw} , with the 18 infants who had symptoms of recent URTI and successful measurements of R_{aw} having values which ranged from 1.10 - 3.68 kPa.L⁻¹.s during initial inspiration, the phase of respiration most likely to be adversely affected by such infections. In future, greater standardisation with respect to the duration of a symptom free period should be adhered to. However, in clinical follow-up studies such as this, it may be preferable to emphasise absence of current symptoms, rather than a prolonged symptom free period, since the latter is difficult to achieve in a population prone to recurrent infections.

In conclusion, background characteristics of infants attending each centre were similar, and unlikely to confound interpretation of respiratory function parameters between management groups (ECMO and CM).

6.4.6.2 Evaluation of methodology

To our knowledge, this is the first report of a multicentre study using established methods of infant lung function testing where results have been included as outcome measures. The North American multicentre HIV study has included measures of forced expiration ($V'_{\max, \text{FRC}}$) and passive respiratory mechanics amongst the outcome measures but results had not been published at the time of producing this thesis.

Within the field of infant respiratory function assessment, considerable variability exists with respect to equipment and techniques. However, efforts have recently been directed towards standardising nomenclature (Quanjer et al 1997), measurement conditions (Gaultier et al 1995) and assessments of respiratory function in infants (American Thoracic Society/European Respiratory Society 1993, Stocks et al 1996). One of the unique features of the Respiratory follow-up was the opportunity to critically evaluate variability within infant for technically complex and operator controlled analysis which was only partly automated, such that selection of data and analysis strategy could markedly influence results.

Ideally, an assessment of analysis variability would have preceded this study but limited time and funding precluded this approach. Operator selection of the most suitable data for analysis, the relatively low signal to noise ratio of respiratory signals in infants, and the inherent biological variability of certain parameters, all increase within and between subject variability in this age group. Consequently, careful selection of a limited number of relevant outcome measures, together with recruitment of adequate numbers of infants are essential to achieve an adequate power of study (Dezateux et al 1996).

The aim of the study design was to reduce both systematic and random errors and prevent measurement and analysis bias affecting reported results. Statistical assessment of differences between the two groups of infants studied at GOS and LEIC aimed to detect any sampling differences, for example in background characteristics, management status, disease severity, and any measurement bias arising from differences in apparatus or technique. Statistical assessment of the whole group of infants, analysed by each centre, in contrast, aimed to highlight

biological variability together with inter- observer variability in analytical approach and data selection.

Careful assessment and evaluation of both equipment and measurement and analysis techniques, with subsequent amendments, led to improved between-centre agreement. With both laboratories also concurrently involved in other studies, limitations were however placed on the extent to which adaptations could be made to existing equipment and protocols.

In vitro assessment of both plethysmographs, using a copper tubing test lung, showed that lung volume was measured accurately at both centres. The effective dead space that the infant breathed through was greater at GOS, mainly due to the different style of PNT and shutter configuration, but also due to the different volume subtracted for effective deadspace of the face mask. The latter may have been due to the application of larger quantities of therapeutic putty at LEIC, or simply to dead space measurement techniques. Differences in tidal volume between centres may have been attributable to either dead space or style of PNT. In a small group of infants, tidal volume and end tidal CO_2 were measured using PNTs of both styles on the same measurement occasion (data not shown). CO_2 values were similar but V_T larger using a Fleisch "1" compared with a Hans Rudolph 100 PNT. Despite the effect on tidal volume, the PNT style did not appear to influence between centre interpretation of the selected outcome measures of respiratory function and lung volume. However, for future collaborative studies we would try to use equipment that was as similar as possible.

The linearity and apparatus resistance of measurement equipment used at each centre was assessed and found to be adequate over the range of flows and pressures used in these measurements. Had the study involved healthy infants with correspondingly higher expiratory flows during forced expiration measurements, a PNT with greater range may well have been required. Future studies will be able to use a new generation of screen PNTs, which have considerably lower deadspace but greater linear range.

Prior to each study, known signals, including the functional time constant of each plethysmograph (i.e. combined mechanical and thermal) were recorded and checked at each centre, and these data were exchanged between centres. Weight and length of

each infant were recorded, using regularly calibrated equipment, thereby avoiding any systematic errors in anthropometry at or between centres. The influence of jacket style during measures of forced expiration at each centre on the range of pressures used to obtain $V'_{\max,FRC}$ was addressed. The Hammersmith jacket used at LEIC was almost twice as efficient at transmitting pressure to the pleura when compared with the Hannover jacket used at GOS. Consequently, higher jacket inflation pressures were used at GOS. Despite these methodological differences, the mean and wide range of forced expiratory flows obtained at each centre suggested that the driving pressures (the product of jacket inflation pressure and jacket efficiency less elastic recoil of thoracic structures) used at each centre were similar (Stick et al 1994).

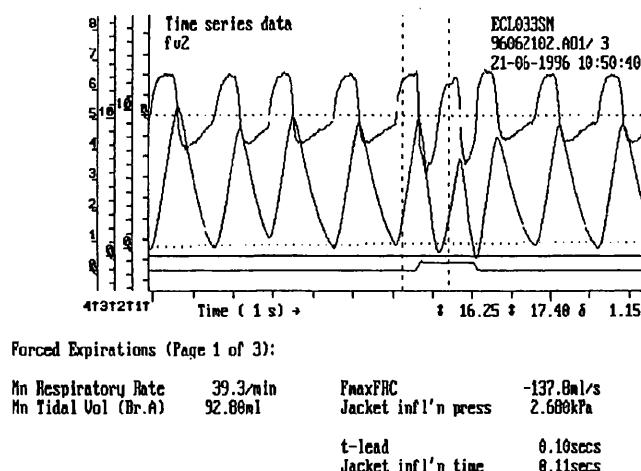
The success rate of measurements at each test centre was similar, with the exception of R_{aw} where the increased failure rate at LEIC was mainly attributable to untimely problems with a replacement amplifier which adversely affected quality of the plethysmographic pressure signals in a number of infants. Success rates of infant respiratory function parameters are rarely reported, but compared with a younger group of infants measured at GOS (Dundas et al 1995, Dezateux et al 1999, Dezateux et al 1997 (a)), in whom 85% of measurements of R_{aw} were successful, a similar rate of 83% was reported in the GOS Respiratory follow-up population. A significant number of plethysmographic measurements at GOS were lost when the infants woke before completion of the protocol, despite increasing the usual dose of sedation from 100 to 150 mg.kg⁻¹ triclofos sodium (equivalent to 100 mg.kg⁻¹ chloral hydrate) when necessary. This was still somewhat lower than maximum doses used at LEIC, which may explain the fewer failures due to early awakening at the latter. A number of the infants recruited from the ECMO trial were above one year of age, taking longer to settle into quiet sleep and waking earlier. The time available for measurements during sedated sleep is short and this limitation should be borne in mind when designing future protocols which include infant respiratory function. However, with similar numbers of infants attending each centre, and similar overall failure rates at both, predominance of one centre when reporting and interpreting results was avoided.

The process of exchanging raw data on disk between centres was a valuable opportunity to critically evaluate approaches to analysis using software which, although identical at each centre, could be readily customised to user preference with

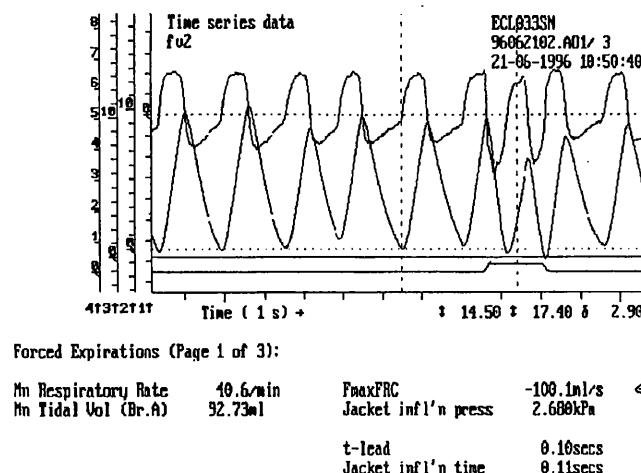
respect to collection, display, calibration, filtering and reporting of results. The effect, for example of different strategies of filtering volume baselines prior to analysis of $V'_{max,FRC}$ (regression over 5 breaths vs single determination of EEL) on within-infant between-centre analysis, was of concern although readily standardised. Even after adhering to regression over 5 breaths, the difficulties of accurate determination of EEL (reflecting FRC) that may be encountered are illustrated by inspecting the analysis of infant ECL033SN, in whom between centre agreement was 20%. The effect of slight adjustment of the volume baseline on calculated values of $V'_{max,FRC}$ is illustrated for this infant (Figure 6-9). The improved regression of baseline EEL has reduced $V'_{max,FRC}$ from 137 mL.s^{-1} to 100 mL.s^{-1} .

Figure 6-9 Effect of adjusting volume baseline on $V'_{max,FRC}$

a) $V'_{max,FRC}$ 137.8 mL.s^{-1} . Note slightly unstable EEL.



b) Effect of slight adjustment of baseline (dotted line under volume minima) is to reduce values to of $V'_{max,FRC}$ to 100.1 mL.s^{-1} .



Approximately 10% of analyses needed to be re-examined when cross-analysis between centres showed poor agreement. Although in several cases differences were due to selection of data, there were also 4 apparent random errors during analysis and reporting of the results. We can speculate that most of these errors would not have been detected had exchange of data and comparison of analyses not taken place, thus emphasising the need to develop software where such transmission errors are minimised.

The design of the Respiratory follow-up served to increase confidence in the Respiratory follow-up findings and was readily incorporated within the existing data collection and analysis protocol of laboratories at each test centre.

6.5 Comparison of respiratory function in ECMO and CM groups

6.5.1 Study population: from trial entry until Respiratory follow-up

Details of the primary diagnosis and management until initial discharge from hospital of the 103 surviving infants are shown in Table 6-6. There were no significant differences for any recorded parameters between infants who attended Respiratory follow-up, and the remaining 25 infants who were discharged home but did not take part in the follow-up, in terms of primary diagnosis and initial treatment. Four infants were effectively lost to the main trial follow-up: 2 died after discharge from hospital and 2 were withdrawn by their parents. Eight infants were excluded from Respiratory follow-up for primarily medical reasons: 5 with congenital heart disease, one due to gross developmental impairment and 2 who were still in hospital at around one year of age. Thirteen potentially eligible infants did not attend due to: parental refusal (8), inability to attend before the child was 18 months old (2), adverse social circumstances (1) or repeated (>3 occasions) cancellations due to URTI onset within 3 weeks of their appointment (2). **Thus, Respiratory follow-up was possible in 78 (77%) of survivors.**

The design and analysis of the Collaborative ECMO trial was based on “intention to treat”; a policy that was continued when comparing respiratory function in the two management groups. The condition of 3 of the infants allocated to ECMO improved between trial entry and arrival at an ECMO centre, and they no longer met trial criteria for cannulation and connection to the ECMO circuit. To remain consistent with other publications arising from the trial, data from these 3 infants have been included in the ECMO group.

In the study population of 78, the most common primary diagnosis was persistent pulmonary hypertension due to meconium aspiration, which occurred in 33 (42%) infants. There were no survivors with congenital diaphragmatic hernia (CDH) in the conventional group, and 3 in the ECMO group, a difference which could not be assessed statistically due to the small absolute numbers. Between ECMO and CM groups, there were no significant differences for any primary diagnoses ($p>0.10$ in all categories).

At trial entry, a similar proportion of each group had received between 1 to 6 days high pressure ventilation (defined as > 30 cm H₂O), ECMO: 56%, CM: 43%. Twenty-nine percent of infants in the ECMO group and 37% in the conventional management group had an oxygenation index of 60 or above indicating very severe respiratory disease. Most infants had received pulmonary vasodilators other than NO: (85% of Respiratory follow-up group) but, at trial entry, Nitric oxide (NO) had been used in relatively few infants (12%). Similarly high-frequency ventilation had been used sparingly in only 8% of the Respiratory follow-up group.

The clinical management of each group post randomisation is presented in Table 6-7. Following randomisation to ECMO or to conventional management, 86% had been treated with pulmonary vasodilators but NO had been used in only 12%, with no significant differences present between groups. Ten conventionally managed infants were treated with high frequency oscillation (or jet ventilation) compared with 4 ECMO infants ($p=0.0025$). All infants with CDH had surgical repair during the neonatal period and a further 9% and 8% had surgery for other reasons after trial entry in the ECMO and CM groups respectively (data not shown). Post randomisation, ECMO group infants required 90% oxygen for a significantly shorter period (the treatment schedule was to reduce to 21 - 30% O₂ following cannulation), and were also ventilated for fewer days, requiring supplementary oxygen following extubation and decannulation for a shorter time than their conventionally managed counterparts. The mean, range and 95% confidence intervals for the difference (ECMO - CM) are shown on Table 6-6. Median (interquartile range) for ECMO and CM groups respectively were 0.5 (0.5 - 1.0) and 3 (2 - 6) days for oxygen requirement $> 90\%$; 3 (2 - 5) and 9 (5 - 13) days ventilated and 9 (5 - 21) and 18 (12 - 30) days in supplementary oxygen.

Table 6-6 Description of surviving infants at trial entry

values are shown as n (%)	Infants who attended Respiratory Follow-up		
	All n=103	ECMO N=51	CM N=27
Primary diagnosis:			
Congenital diaphragmatic hernia	5 (5)	3 (6)	0
Isolated persistent fetal circulation	19 (18)	11 (22)	4 (15)
persistent pulmonary hypertension	77 (75)	36 (71)	22 (81)
PPH due to:			
meconium aspiration	48 (47)	19 (37)	14 (52)
sepsis	10 (10)	8 (16)	1 (4)
IRDS	14 (14)	6 (12)	5 (19)
other	5 (5)	3 (6)	2 (7)
Other	2 (2)	1 (2)	1 (4)
At trial entry:			
Days on high pressure ventilation			
0	47 (46%)	22 (43%)	14 (56%)
1-6	56 (54%)	29 (57%)	11 (44%)
Oxygenation index [†]			
≥60	33 (32%)	15 (29%)	10 (37%)
40-59	69 (67%)	36 (71%)	17 (63%)
Pulmonary vasodilators			
NO	14 (14%)	6 (12%)	3 (11%)
Other PVDs	86 (84%)	45 (88%)	21 (78%)
HFO/JV	11 (11%)	5 (10%)	1 (4%)

Key to table:

IRDS; idiopathic respiratory distress syndrome:

[†]one infant met randomisation criteria $\text{Pa CO}_2 < 40$ for more than 12 hours:

NO; nitric oxide:

Other PVDs; all other pulmonary vasodilators:

HFO; high frequency oscillation:

JV; jet ventilation.

Table 6-7 Management of infants who attended respiratory follow-up: overall status at discharge home from hospital and at around one year of age

values are shown, except where indicated, as n (%)	ECMO n=51	CM n=27	95% CI (ECMO - CM)
<i>Management between trial entry and discharge</i>			
pulmonary vasodilators:			
nitric oxide	5 (10%)	4 (15%)	-20%, 10%
all	41 (80%)	26 (96%)	-32%, 0.3%
HFO/JV	4 (8%)	10 (37%)	-47%, -11%**
Surfactant	17 (33%)	13 (48%)	-38%, 8%
Days Fi O ₂ ≥ 0.90: median (IQR)	0.5 (0.5-1.0)	3.0 (2.0-6.0)	-24%, -2%*
Days ventilated: median (IQR)	3 (2-5)	9 (5-13)	-8%, -4%***
Days supplementary O ₂ : median (IQR)	9 (5-21)	18 (12-30)	-19%, 11%
<i>Status at discharge home</i>			
Respiratory status -			
normal	38 (74%)	23 (85%)	-30%, 9%
questionable	9 (18%)	0	3%, 32%*
abnormal	4 (8%)	4 (15%)	-21%, 7%
home O ₂	3 (6%)	1 (4%)	-8%, 12%
bronchodilators	0	1 (4%)	-10%, 2%
inhaled steroids	0	3 (11%)	-16%, 0.2%
time to discharge home: median (IQR)	23 (18-37)	25 (17-42)	-10, 24
<i>Overall status at around one year</i>			
Respiratory:			
cough at night	13 (26%)	12 (44%)	-41%, 3%
wheeze	4 (8%)	5 (19%)	-25%, 4%
regular bronchodilators	4 (8%)	8 (30%)	-38%, -5%*
home O ₂	2 (4%)	1 (4%)	-9%, 9%
chest infection requiring antibiotics	32 (63%)	21 (78%)	-36%, 7%
Growth:			
weight below 3rd centile	7 (14%)	2 (7%)	-9%, 21%
head circumference below 3rd centile	2 (4%)	0	-4%, 11%
head circumference above 97th centile	4 (8%)	2 (7%)	-12%, 13%

Key to table: *** p<0.0001; ** p<0.005, * p<0.05: All; all other pulmonary vasodilators: HFO; high frequency oscillation: JV; jet ventilation: IQR; interquartile range.

At the time of discharge home, the respiratory status of the survivors was assessed by a physician who had been involved in the infant's management (and was therefore *not* blinded to their management status). Overall, there were no significant differences between groups with respect to respiratory status, although in the ECMO group more infants were categorised as abnormal/questionable. A small number of infants in the ECMO group were discharged requiring oxygen and other respiratory medication. The proportion of infants with a diagnosis of broncho-pulmonary dysplasia (defined as oxygen requirement at 28 days postnatal age together with characteristic chest radiography changes (Northway et al 1967)) was not formally recorded on the information supplied from the NPEU. The time to discharge for both groups was similar, data being skewed by one infant who remained within hospital for 419 days.

At around one year of age, there was a tendency for more conventionally managed infants who attended Respiratory follow-up to cough at night, wheeze and have had more chest infections requiring antibiotic treatment, but differences between treatment groups did not reach significance. However, a significantly greater number of CM infants were treated with bronchodilators at one year ($p = 0.014$). There was a small and similar number of infants in each group receiving other respiratory medication, mainly inhaled steroids (data not shown). There were no significant differences between groups for weight below the 3rd centile or for head size either large (above 97th centile) or small (below 3rd centile).

6.5.2 Population at Respiratory follow-up

Infant details are presented according to allocation group in Table 6-8, together with 95% CI of the difference between groups for each presented variable. When the 78 infants attending Respiratory Follow-up were grouped according to their initial management, following completion of follow-up visits and analysis of data, it was noted that the proportion of infants having received ECMO was significantly greater than those managed conventionally, reflecting overall survival (UK Collaborative ECMO Trial Group et al 1996). Fifty-one (65%) received ECMO and 27 (35%) were managed conventionally. Forty-eight of 78 infants were male, equally distributed between groups, and this distribution was also seen in the initial group of 103 survivors.

Ethnic background, age and body size was similar in each group (Table 6-8). Infants were eligible for trial entry only if above 35 weeks completed gestation and 2000 g

Table 6-8 Infant details according to allocation group

management group	ECMO n=51	CM n=27	95% CI of the group mean difference (ECMO-CM)
Male n (%)	31 (61)	17 (63)	-25, 21%
Caucasian n (%)	37 (73)	23 (85)	-32, 7%
Age (months) mean (SD)	13.6 (1.8)	13.9 (2.0)	-1.1, 0.7
Weight (kg) mean (SD)	10.2 (1.6)	10.1 (1.3)	-0.5, 0.9
Length (cm) mean (SD)	78.1 (3.9)	78.4 (2.8)	-1.9, 1.4
Birthweight (g) mean (SD)	3392 (531)	3542 (639)	-421, 120
Gestation (weeks) mean (SD)	39.3 (2.3)	39.7 (2.2)	-1.4, 0.7
Smoking during pregnancy n (%)	6 (12)	5 (19)	-23, 9%
Current maternal smoking n (%)	15 (29)	10 (37)	-29, 14%
Other smoke exposure n (%)	17 (33)	12 (44)	-34, 11%
Family history of atopy n (%)	25 (49)	17 (63)	-37, 9%
Current respiratory medication n (%)	7 (14)	10 (37)	-43, -4%*
Symptomatic at testing n (%)	6 (12)	6 (22)	-27, 6%
Recent URTI n (%)	18 (35)	8 (30)	-16, 28%
Normal medical examination n (%)	39 (77)	21 (78)	-21, 18%

Key to Table: Values are given as mean and SD or n and % as indicated.

* p= 0.014

weight, and the mean birthweight and gestational age of ECMO subjects (3392 g and 39.3 weeks) and CM subjects (3542 g and 39.7 weeks). The low variance (SD) of each parameter, reflected these criteria.

The two groups were similar with respect to exposure to smoking both during pregnancy and from maternal and other sources at around one year of age. There was no significant difference between groups for a family history of atopy. More infants in the CM group were receiving respiratory medication at around one year of age (95% CI of the difference [ECMO-CM]: -43, -4%, $p=0.014$), which comprised inhaled steroids and bronchodilators (data not shown). Proportionately more infants were symptomatic at testing in the CM group, although this trend did not reach significance. Both groups were very similar with respect to a history of recent URTI, with around one third of infants having had recent symptoms of greater than 3 days of nasal symptoms with cough and/or sneeze. Similarly, the majority of infants were categorised as normal on medical examination and respiratory assessment.

6.5.3 Respiratory function results by management group

The number of technically acceptable measurements for each variable according to management groups is shown in Table 6-9 and reasons for unsatisfactory measurements presented in Section 6.4.3. There were no significant differences in failure rates for any parameter according to either ECMO or CM management. The respiratory function results are summarised according to management group in Table 6-11, together with the 95% CI of the difference between groups for each variable. Although measurements were made in all 78 infants, not all outcome variables were successfully measured in all infants.

Table 6-9 Percentage of successful measurements in each management group: ECMO and CM

	management group		
	ECMO (n=51)	CM (n=27)	95% CI (ECMO-CM)
FRC _{pleth}	94	89	-7, 18
R_{aw}	69	78	-30, 12
$V'_{max,FRC}$	92	96	-16, 7
Tidal breathing parameters	100	96	-2, 9

There were no significant differences between management groups with respect to the number of successfully completed measurements ($p > 0.10$ for each parameter) and reasons for failure (waking, physiological or technical failures) also appeared similar.

The respiratory function outcome parameters of FRC_{pleth} , sG_{aw} (derived from FRC_{pleth} and R_{aw}) and $V'_{\text{max,FRC}}$ are compared both between the ECMO and CM groups, and also with respect to control groups (Section 6.3.4). Table 6-10 summarises infant details and respiratory parameters for ECMO, CM and control population groups. The groups were closely matched with respect to weight and length at test. With respect to $V'_{\text{max,FRC}}$ reference data, the control infants (Tepper et al 1993) were slightly older with a mean age of 15.2 months, while infants studied in Clarke's group were slightly shorter.

When length, weight and test age were compared between ECMO and CM infants, together with either the plethysmography group or the USA and UK $V'_{\text{max,FRC}}$ control groups, using one way ANOVA (Altman et al 1996), no significant differences were found (data not shown). The majority of infants in all groups were Caucasian, with 7 of 47 infants being of Hispanic origin in Tepper's group, whereas in Clarke's London based control group there were small numbers of Afro-Caribbean, Indo-Pakistan and Oriental infants. The eligibility criteria for the plethysmography group included Caucasian ethnicity, and all but one infant (who was of mixed ethnic origin) of that group met the requirement.

Table 6-10 Study and control Population comparison

n	ECMO	CM	Plethysmography reference group [‡]	$V'_{\text{max,FRC}}$ reference groups			
				Controls	LRI controls	USA Controls [†]	UK Controls [§]
51	27	62		30		47	39
Age (months)	13.6 (1.8)	13.9 (2.0)	13.7 (1.9)	13.4 (1.7)		15.2 (2.6)	12.5 (0.6)
Weight (kg)	10.2 (1.6)	10.1 (1.3)	9.9 (0.9)	9.9 (1.3)		10.3 (1.2)	10.2 (1.3)
Length (cm)	78.1 (3.9)	78.4 (2.8)	77.8 (2.5)	77.3 (3.2)		77.3 (4.2)	75.9 (2.8)
% male	61	63	40	60		48	51
% Caucasian	73	85	98	100		85	79
FRC _{pleth} (mL)	271 (65)	306 (60)	256 (40)	242 (44)		-	-
sG _{aw,II} (s ⁻¹ .kPa ⁻¹)	2.18 (1.15)	1.62 (0.73)	2.14 (0.78)	1.81 (0.65)		-	-
sG _{aw,EE} (s ⁻¹ .kPa ⁻¹)	1.70 (0.98)	1.55 (1.09)	2.04 (0.80)	1.67 (0.53)		-	-
mean $V'_{\text{max,FRC}}$ (mL.s ⁻¹)	170 (77)	137 (65)	-	-		337 (14)	244 (19)

[†]Dezeux et al 1999[†] (Tepper et al 1993)[§]Clarke et al 1995

Values are shown as mean and SD or % as indicated.

FRC_{pleth}

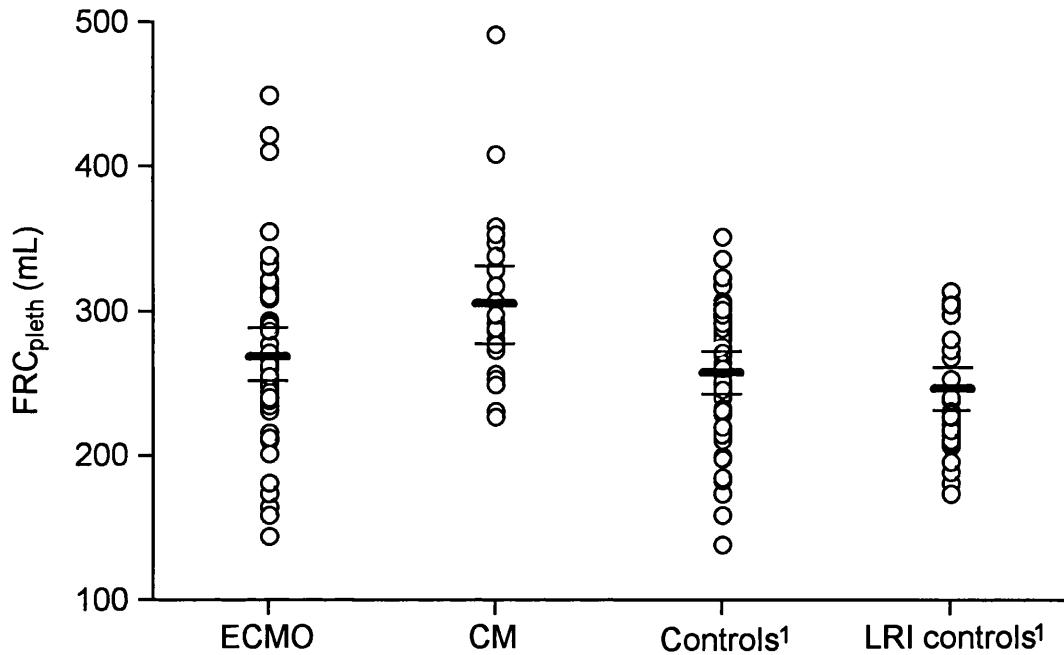
Resting lung volume, measured at FRC, ranged from 145 - 450 mL in the ECMO group and 228 - 492 mL in the CM group. FRC_{pleth} was significantly lower in the ECMO group, compared with those managed conventionally: 271 mL vs 306 mL, mean difference 35 mL (95%CI -67, -4 p = 0.025) (Table 6-11). Figure 6-10 shows individual values of FRC_{pleth} for ECMO, CM and control groups. For the control groups of infants recruited to an epidemiological study (Dezateux et al 1994, Dezateux et al 1999, Dezateux et al 1998) values of lung volume were similar for both healthy infants and those who had one or more episodes of wheezing associated physician diagnosed LRI. One way ANOVA¹ for this data indicated significant differences between the 4 groups, with F=0.001. The Bonferroni² method was used as a post hoc Multiple Comparison test to determine which group differed significantly from the mean, set at a significance level of 0.05. Only the CM group had a significantly different mean from each of the remaining 3 groups.

Weight corrected individual values of FRC_{pleth} are shown in Figure 6-11 where the difference between the CM and ECMO and control groups remains evident. The mean (95% CI of the weight corrected difference: ECMO-CM) was 4.3 (-7.8, -0.7) mL.kg⁻¹, p=0.020 (Table 6-11).

¹ Used for 2 or more independent groups to test the null hypothesis that data are a sample from a population in which the mean and variance of a test variable is equal in each group, where a single grouping variable is considered. Tables of the F distribution are used to test the ratio of sample variances between groups. Post hoc multiple comparisons may then be used to test the significance of any group mean differences.

² The Bonferroni method uses t tests to perform pairwise comparisons between group means, but controls the overall error rate by setting the error rate for each test to the experiment-wise error rate divided by the total number of tests. It is a conservative method of comparison suited for groups of 5 or less.

Figure 6-10 Dot Plot of FRC_{pleth} for management and control groups

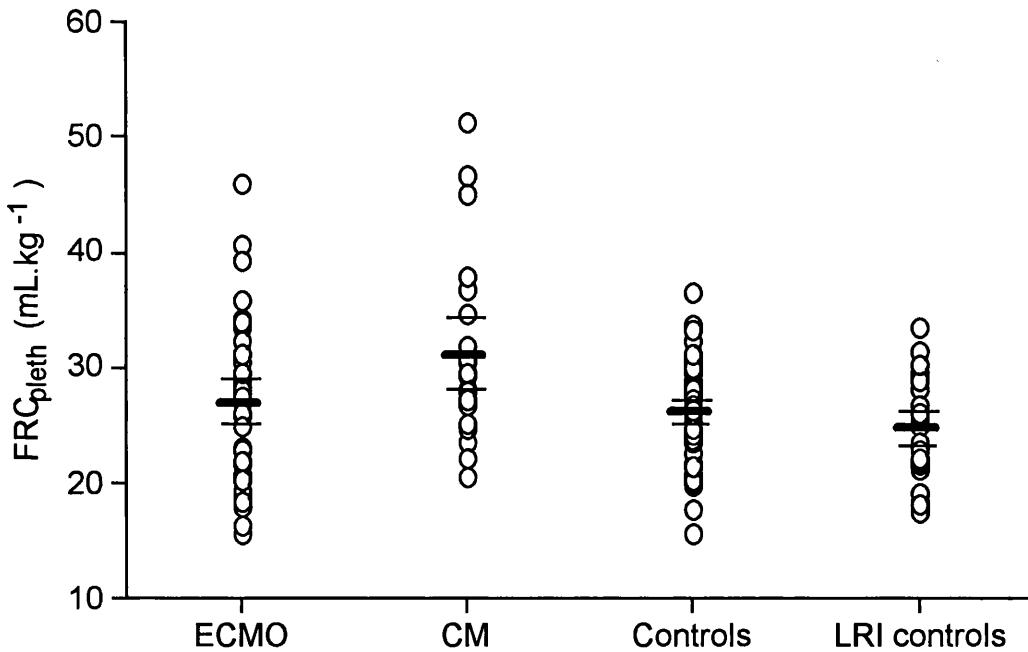


Key to Figures 6.10 and 6.11: Solid bars denote mean values for each group and lines indicate standard errors of the mean (SEM).

¹controls are from (Dezateux et al 1999)

¹LRI controls are from (Dezateux et al 1999)

Figure 6-11 Dot Plot of weight corrected FRC_{pleth} for management and control groups



R_{aw} and sG_{aw}

Airway resistance, measured during initial inspiration or late expiration, was not significantly different between groups during either initial inspiration: $R_{aw,II}$ was mean (range) 2.05 (1.01 - 4.11) $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$ in the ECMO group compared with 2.38 (1.16 - 4.28) $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$ in the CM group, or during late expiration: mean (range) R_{aw} was 2.70 (1.01 - 4.54) $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$ in the ECMO group and 2.85 (0.79 - 5.51) $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$ in the CM group (Table 6-11). There was large intra-subject variation within each group, with the mean (range) coefficient of variation (CV: 100 x SD/mean %) being 15.8 (6.0 - 28.1) % in the ECMO group and 13.4 (5.9 - 29.1) % in the CM group for initial inspiratory R_{aw} . Similar CV values were found for late expiratory R_{aw} ; 18.3 (3.4 - 34.9) % in the ECMO group (apart from one outlier of 49.4%, in whom poor inter centre agreement was also reported) and 19.2 (3.9 - 33.7) % in the CM group for expiratory R_{aw} .

When expressed as specific airway conductance, a measure of the conducting ability of the airways corrected for lung volume, $sG_{aw,II}$, initial inspiratory specific conductance, was significantly higher in the ECMO group: mean (range) 2.22 (0.86 - 5.70) $\text{s}^{-1}\cdot\text{kPa}^{-1}$ than in the CM group, 1.62 (0.78 - 3.77) $\text{s}^{-1}\cdot\text{kPa}^{-1}$ (Table 6-11). The significance of this difference increased, when 2 outliers, whose values were above 5.0 $\text{s}^{-1}\cdot\text{kPa}^{-1}$ were excluded, the 2-Tailed p value obtained from the Mann-Whitney U test falling from 0.041 to 0.022. $sG_{aw,EE}$, late expiratory specific conductance was also higher in the ECMO group, but not significantly so, mean (range) 1.74 (0.51 - 5.48) $\text{s}^{-1}\cdot\text{kPa}^{-1}$ and 1.55 (0.54 - 5.54) $\text{s}^{-1}\cdot\text{kPa}^{-1}$ for ECMO and CM infants respectively, $p=0.348$. Data were of skew distribution for both R_{aw} and sG_{aw} within ECMO and CM groups and are shown as median and interquartile range in Table 6-11, non parametric methods were consequently used for statistical analysis.

Figure 6-12 and Figure 6-13 show individual values of initial inspiratory and late expiratory sG_{aw} for each group of infants: ECMO, CM and controls. One way ANOVA for the variable sG_{aw} (measured during initial inspiration and late expiration) was used to compare ECMO, CM and healthy and wheezing controls, using the non-parametric Kruskal-Wallis test. P values of 0.021 (initial inspiratory sG_{aw}) and 0.011 (late expiratory sG_{aw}) were calculated, indicating that there were significant differences between group means. No outliers were excluded during data analysis.

For initial inspiratory sG_{aw} , in addition to the significantly lower group mean value reported in CM compared with ECMO infants, values of sG_{aw} in CM compared with healthy control infants were also lower ($p = 0.005$). Neither ECMO nor CM groups were significantly different from LRI controls. However, when both control groups were compared, initial inspiratory sG_{aw} was significantly lower in the LRI controls ($p = 0.044$, 95% CI of the difference: Control - LRI Control:).

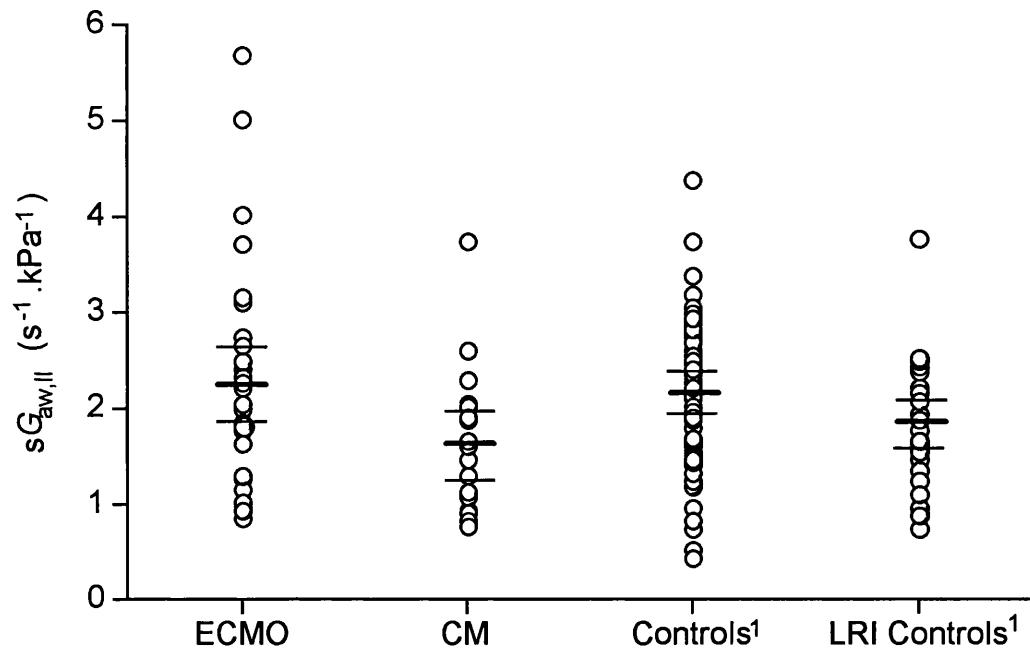
When values of late expiratory sG_{aw} were compared between groups, both CM and ECMO were significantly lower compared with healthy control infants ($P = 0.020$, 95% CI of the difference: Control - ECMO; $P = 0.006$, 95% CI of the difference: Control - CM:). Again, neither ECMO nor CM groups were significantly different from LRI controls. When both control groups were compared, late expiratory sG_{aw} was also significantly lower in the LRI controls ($p = 0.067$, 95% CI of the difference: Control - LRI Control:).

Table 6-11 Respiratory function results: ECMO and CM groups compared

	Allocation group		
	ECMO (n=51)	CM (n=27)	95% CI of group mean difference (ECMO-CM)
FRC _{pleth} (mL)	271 (65)	306 (60)	-67, -4*
R _{aw,II} (kPa.L ⁻¹ .s)	2.05 (0.78)	2.39 (0.92)	-0.83, 0.15
R _{aw,EE} (kPa.L ⁻¹ .s)	2.70 (1.06)	2.85 (1.36)	-0.85, 0.56
sG _{aw,II} (s ⁻¹ .kPa ⁻¹) (IQR) [‡]	median 2.03 (1.30-2.68)	1.63 (1.02-1.98)	0.03, 0.98*
sG _{aw,EE} (s ⁻¹ .kPa ⁻¹) (IQR) [‡]	median 1.51 (1.01-2.13)	1.33 (0.78-1.93)	-0.20, 0.63
Mean $V'_{max,FRC}$ (mL.s ⁻¹)	170 (77)	137 (65)	-2, 67
Highest $V'_{max,FRC}$ (mL.s ⁻¹)	179 (85)	147 (70)	-5, 69
V _T (mL)	90 (17)	88 (17)	-7, 10
RR (min ⁻¹)	32 (9)	34 (8)	-6, 3

Key to Table: [‡]Values are given as mean (SD), except for sG_{aw} , for which group differences were assessed using the non parametric Mann-Whitney U Test, for which values are shown as median (interquartile range).

Figure 6-12 Dot Plot of inspiratory sG_{aw} for management and control groups

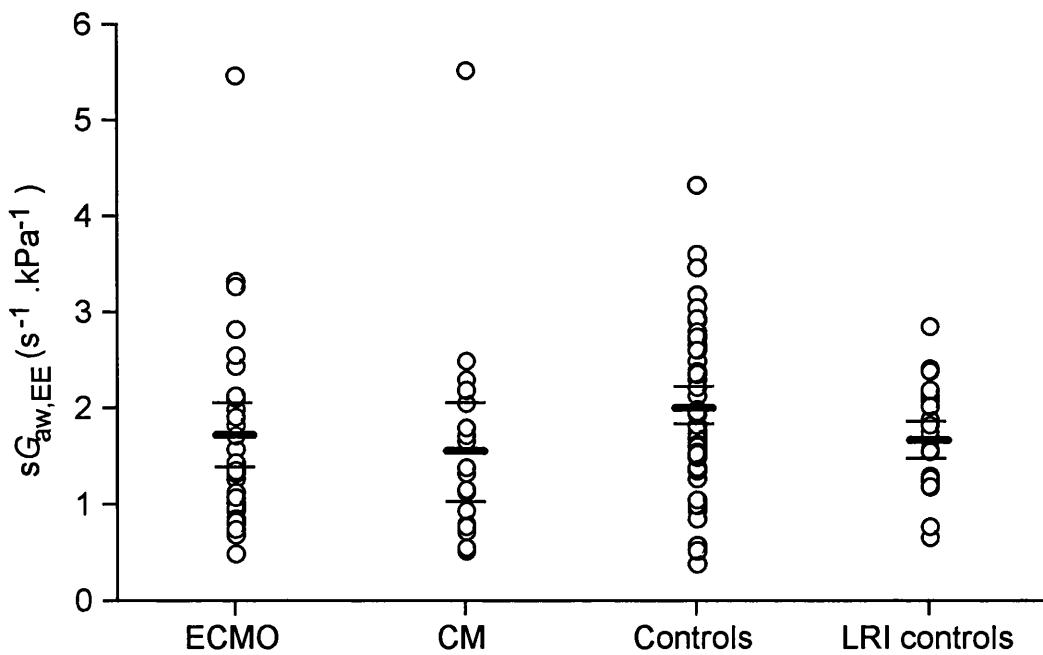


Key to Figures 6.13 and 6.14: Solid bars denote mean values for each group and lines indicate standard errors of the mean (SEM).

¹controls are from (Dezateux et al 1999)

¹LRI controls are from (Dezateux et al 1999)

Figure 6-13 Dot Plot of expiratory sG_{aw} according to management and control groups



Maximal expiratory flow at FRC ($V'_{\max,FRC}$)

$V'_{\max,FRC}$ reported separately as the mean of the highest three technically acceptable manoeuvres and as the single highest (technically acceptable) value obtained tended to be higher in the ECMO group, 170 mL.s^{-1} vs 137 mL.s^{-1} (CM), with wide variability in measured values ranging from 30 to 420 mL.s^{-1} within both groups. Similar differences were observed with respect to the group mean results for the highest values obtained: 179 mL.s^{-1} (ECMO) vs 147 mL.s^{-1} (CM). These differences just failed to reach statistical significance, ($p = 0.079$ for the difference between groups for mean $V'_{\max,FRC}$) (Table 6-11).

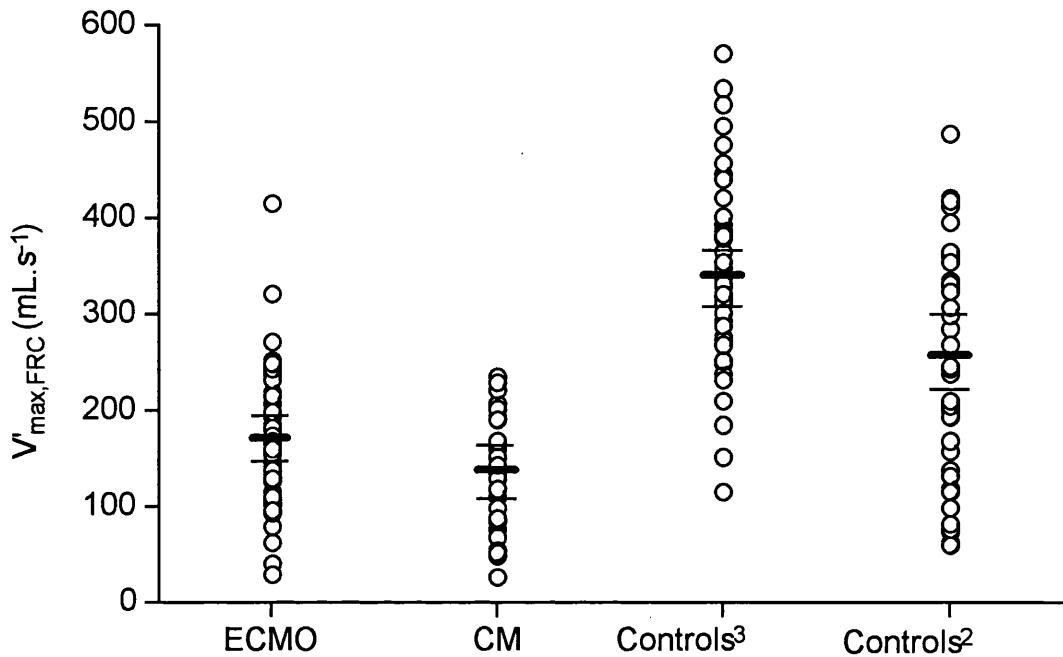
Figure 6-14 shows individual values of mean $V'_{\max,FRC}$ for ECMO, CM and control groups (Dezateux et al 1994), (Dezateux et al 1998, Marsh et al 1994, Clarke et al 1995) (Dezateux et al 1999), (Tepper et al 1993) displaying the wide variation within and between groups. One way ANOVA was used to compare group means, and showed highly significant differences between groups ($F = 0.000$). Using the Bonferroni method, with a significance level set at 0.05, significant differences were demonstrated between all combinations of groups, with the exception of ECMO and CM infants. These differences are summarised in Table 6-12.

Table 6-12 Summary of unpaired t-tests for ECMO, CM and control groups for mean $V'_{\max,FRC}$

Groups	Mean group difference (mL.s^{-1})	95% CI of difference between groups
ECMO - CM	33	-5, 69
USA Controls - ECMO	167	131, 204***
USA Controls - CM	200	157, 243***
UK Controls - ECMO	78	34, 120**
UK Controls - CM	110	58, 162***
USA Controls - UK Controls	90	43, 138***

Key to Table: ** $p < 0.01$, *** $p < 0.001$

Figure 6-14 Dot Plot of $V'_{\max,FRC}$ values for management and control groups*



Key to Figure: Solid bars denote mean values for each group and lines indicate standard errors of the mean (SEM).

²controls are from (Tepper et al 1993)

³LRI controls are from (Clarke et al 1992)

Tidal breathing parameters

Mean tidal volume was 90 mL and 88 mL in ECMO and CM infants respectively.

Weight corrected tidal volume was 9 mL·kg⁻¹ in both groups and respiratory rate was 32 min⁻¹ in ECMO and 34 min⁻¹ in CM infants. The 95% CI of the group mean difference for each parameter was not significant.

6.5.4 Discussion

6.5.4.1 Introduction

This was the first Respiratory follow-up study of survivors of a randomised controlled trial of ECMO. A unique opportunity was provided to assess the respiratory outcome of a group of infants following neonatal respiratory failure, in whom comprehensive data describing their status at trial entry and throughout infancy had been collected, and to evaluate the longer term effects of ECMO management.

Infants from both groups demonstrated a broad spectrum of respiratory function, from entirely normal to major functional disturbances. These results confirm those of others, who have demonstrated that ECMO does not prevent sequelae of severe respiratory disease in the newborn period (Garg et al 1992). In contrast to previous studies (Koumbourlis et al 1992, Kugelman et al 1995, Garg et al 1992) the present investigation includes a contemporary control group of infants not assigned to ECMO, which facilitated comparison of the effect of ECMO on respiratory outcome.

Interpretation of the effects of ECMO treatment may have been affected by a variety of confounding variables, which needed to be examined to evaluate their potential influence. For results of multicentre studies to be robust all centres should study groups which are representative of the whole population under test. The comparison of between-centre methodology and analysis in section 6.4 showed that similar numbers and almost identical proportions of ECMO and CM infants were studied at each centre. Furthermore, results from the two centres were very similar (6.4.5). The remaining potential sources of bias are considered in the following section.

6.5.4.2 Potential sources of bias

Within the main trial, potential bias was kept to a minimum by strict random allocation at trial entry, complete follow-up of the whole surviving cohort, and assessment of outcome without knowledge of treatment allocation. Random allocation by use of a computerised minimisation algorithm ensured that balanced numbers were entered within each trial group with respect to the key prognostic variables of primary diagnosis, disease severity, referral centre and the ECMO centre in which a cot was reserved. Avoidance of bias at trial allocation was reflected in the

similar background details of infants attending Respiratory follow-up at trial entry (Table 6-2). Following trial entry, not surprisingly more infants in the conventional group were treated with surfactant, high frequency ventilation or both, particularly during the latter part of the study. It is uncertain whether wider use of these treatments would have introduced bias with respect to improved outcome after conventional management, as there are few published studies of their effectiveness in mature newborn infants with severe respiratory failure (Mrozek et al 1998, Sandberg et al 1997, Todd et al 1995, Clark et al 1994, The Neonatal Inhaled Nitric Oxide Study Group (NINOS) 1997).

Bias may also have been introduced by selection of an unrepresentative population with selective inclusion by participating centres of those infants considered to be most gravely ill, and who may have potentially benefited the most from ECMO treatment. ECMO was not available during this time except within the UK collaborative randomised trial. However, restriction of entry criteria to mature neonates in severe respiratory failure, and inclusion of a large number of infants from 55 of 83 participating hospitals reduced the potential source for bias. After trial entry, follow-up of infants up to and including their one year neurodevelopmental assessment was virtually complete, except for two infants. From this population of 101, the 78 of 99 infants surviving at one year who attended Respiratory follow-up had received ECMO or CM in similar proportions when compared with those not attending. Completeness of follow-up and absence of any attendance bias between management groups (i.e. parents of ECMO subjects being more motivated to attend) served to further increase confidence in the reported results.

The assessment of outcome without knowledge of treatment allocation by blinding both paediatricians and laboratory staff at the home visit for neuro-developmental assessment at around one year and Respiratory follow-up, together with the cross exchange of data between centres without knowledge of management group allowed objective reporting of both clinical observations and respiratory function data, with definitive results (for publication) being reported by the centre that did not measure the infants, thus avoiding bias during data collection, analysis and reporting of results.

As discussed in section 6.4, there were no significant between-centre differences with respect to the background details of infants, their management group or their

respiratory function outcome measures that would confound interpretation of results according to their management at birth: whether CM or ECMO. This was a particularly important conclusion, as there were some differences in equipment for testing and methodology that could potentially have affected results obtained from each centre. Again, these factors are discussed in more detail in section 6.4.6 and will be addressed in a more general fashion in the overall discussion concluding this thesis (Section 7.4).

6.5.4.3 ECMO and CM groups compared

An endpoint of around one year was chosen for Respiratory follow-up of the surviving infants able to attend each centre. At one year, any initial remodelling of the respiratory system following neonatal respiratory failure, should be complete (Stocks 1995). Control data collected from one year old infants was available for close matching of groups (Dezateux et al 1997(a), Dezateux et al 1998, Dezateux et al 1999) and additional information including respiratory status and growth and development was extracted from the one year paediatric assessment (UK Collaborative ECMO Trial Group et al 1996).

At Respiratory follow-up, despite an increased proportion of survivors in the ECMO group (UK Collaborative ECMO Trial Group et al 1996), the respiratory status of each group of infants was similar and overall satisfactory, with only 2 infants remaining oxygen dependent. At the inception of the main ECMO trial it was envisaged that one possible outcome would be similar rates of survival and severe disability in the two groups, but better respiratory status in one group or the other. Whether or not ECMO conferred a survival advantage, it was considered essential to investigate the respiratory function in survivors of both limbs of the trial. The use of ECMO could potentially result in the survival of infants with severe respiratory function who would otherwise have died, which may have led to poorer respiratory status within this group. Conversely, the ECMO group may have been exposed to less endo-tracheal ventilation at high pressures with high inspired oxygen fractions, which have been associated previously with subsequent alterations in respiratory mechanics (Ahlstrom 1975, Stocks et al 1976, Tammela et al 1991).

At around one year of age, there were more reports of cough and wheeze in the CM group, who were also receiving more respiratory medication. At the time of their

laboratory visit, medication was in most cases inhaled steroids and/or salbutamol (data not shown). The incidence of URTI was similar between groups and therefore did not confound reporting of symptoms. Similarly, the majority of infants (78%) in both groups were considered to be normal from a respiratory viewpoint when examined at their laboratory visit.

Interpreting the increased values of FRC in the CM group compared with the ECMO group, was further complicated by the potential for either decreased or increased lung volume, depending on the underlying pathology. Congenital pulmonary hypoplasia may lead to reduced FRC, together with low R_{aw} due to a reduction in number of airway generations (Helms et al 1982). Gas trapping secondary to increased R_{aw} following volutrauma and subsequent peripheral airway disease during the neonatal period may result in increased FRC. The absence of infants with FRC_{pleth} below 200 mL in the CM group may indicate that conventional treatment alone was insufficient to support survival of those infants with both severe respiratory failure and underlying pulmonary hypoplasia. Necropsy results from those infants whose parents consented to post mortem examination have not yet been published, but will include results of lung histology. The proportion of infants in each group with evidence of pulmonary hypoplasia is likely to be small but will be of great interest.

Airway resistance, which is a measure of absolute airway calibre, in the absence of any upper airway disease or abnormality, will approximate the optimal airway calibre when measured during inspiration. By contrast, when measured during late expiration at low lung volumes, airway resistance will be elevated, in the presence of peripheral airway closure or narrowing. Specific conductance provides a measure of effective airway calibre corrected for lung size and hence somatic growth, (Stocks et al 1977) and will be reduced if airway calibre is diminished and/or lung volume increased in relation to body size. One infant, identified as an high outlier on dot plots of initial inspiratory and late expiratory sG_{aw} (Figures 12 and 13) had low values of R_{aw} and an elevated lung volume (infant 36 in Appendix), compatible with reduced airway branching and underalveolisation but normal chest wall configuration, leading to an increase in airway calibre, relative to lung volume (this finding is frequently accompanied by a reduction in respiratory compliance as the small lungs expand to fill the thoracic cavity). This infant did not have confirmed pulmonary hypoplasia, but the 3 others who did had unremarkable values.

The lower values of initial inspiratory sG_{aw} measured in the CM group, compared with ECMO infants suggests some mild impairment in airway function, relative to the ECMO group. Differences were not related to the incidence of recent URTI in each group, or symptoms at time of testing. In contrast, for late expiratory sG_{aw} , there was no significant difference between ECMO and CM group medians. Wide variability in this parameter made interpretation of findings more difficult and the high failure rate in reporting R_{aw} (from which sG_{aw} was derived) reduced the power to detect differences.

$V'_{max,FRC}$ group mean differences narrowly missed statistical significance at the 5% level, but a trend was evident for lower values in the CM, compared with the ECMO, management group ($p=0.07$). Both groups again showed wide variability in measured values, with a small number of infants being flow limited within the tidal range while others had flows within the normal range ($>200 \text{ mL.s}^{-1}$).

Were the differences between outcome variables reflecting peripheral airway function apparent because $V'_{max,FRC}$ is more sensitive at detecting small changes in airway calibre ? A potential disadvantage of all measurements of resistance during infancy is that infants are preferential nose breathers and hence the resistance of the nasal passages, which can comprise as much as 50% of total resistance, is included (Stocks et al 1978b). This may reduce the relative sensitivity of plethysmographic measurements of airway resistance in detecting changes in peripheral airway function. The relationship between these two methods of assessing small airway function is considered in the following section (6.6)

In summary, taken together, the findings of larger lung volumes, a difference of 33 mL.s^{-1} between management groups for values of $V'_{max,FRC}$, and lower sG_{aw} in the CM group suggested that the larger proportion of CM infants receiving respiratory medication and reporting respiratory symptoms could have been attributed to a greater degree of impairment of small airway function in CM infants than in those allocated to ECMO. These findings probably reflect differences in management during the neonatal period, since initial disease severity, as well as prevalence of atopy and exposure to tobacco smoke were similar in CM and ECMO infants.

Between trial entry and discharge, infants in the ECMO group were exposed to high fractions of inspired O_2 (>0.90) for a significantly shorter time. They were also

ventilated for significantly fewer days which, following the agreed ECMO protocol, included ventilation at lung rest settings (including peak inspiratory pressures of <25 cm H₂O) (UK Collaborative ECMO Trial Group et al 1996), thus limiting barotrauma after trial entry.

The physiological sequelae of oxygen toxicity and barotrauma (or, more accurately, volutrauma) have mainly been studied using animal models. Bronchopulmonary dysplasia appears to start as an acute lung injury which initiates a series of inflammatory responses, subsequently evolving into chronic lung disease (Davis et al 1991). Hyperoxia and hyperventilation led to characteristic biochemical, cellular, physiological and pathological abnormalities in a neonatal piglet model of lung injury (Davis et al 1991). These changes appeared to be more strongly associated with hyperoxia than with barotrauma.

Panitch and colleagues investigated maturational changes in airway smooth muscle structure-function relationships in sheep trachealis (Panitch et al 1992). Their results suggested that airway smooth muscle from immature tracheae was unable to generate as much force as adult tissue. In contrast to adults, immature airways become deformed when exposed to distending pressures associated with positive pressure ventilation, and this pressure induced injury may be partly explained by the inability of the immature airway to generate sufficient tension to withstand such barotrauma (Panitch et al 1992).

Although the preterm group are particularly at risk of such injuries, the maturation process is a continuum and term infants, as in this Respiratory follow-up, still have immature airway smooth muscle, relative to that of adults. Therefore, in this group of infants, lower values of $sG_{aw,II}$ in conventionally managed infants may reflect subtle changes secondary to structural injury to the central airways, in addition to alveolar overdistension and subsequent peripheral airway obstruction. This may in turn at least partly explain why significant differences were detected as the airways were distended during inspiration, reflecting optimal airway calibre, rather than during late expiration, which mainly reflects peripheral airway closure or narrowing.

However, insults such as volutrauma and hyperoxia are conventionally associated with peripheral airways obstruction, following overdistension of terminal lung units and fibrosis following the acute inflammatory response (Hershenson et al 1994).

These effects are evident following respiratory function measurements in infants with chronic lung disease as increased expiratory airway resistance, particularly during expiration at low lung volumes, often accompanied by a characteristic pressure-flow loop (Figure 5-3), decreased expiratory specific conductance and decreased maximal expiratory flows.

Although there were no significant differences in late expiratory R_{aw} and sG_{aw} between ECMO and CM groups, these findings may be explained if the presence of gas trapping secondary to small airways narrowing in the CM group resulted in a reduction in sG_{aw} by lung hyperinflation, not reflected in the measured values of resistance. Gas trapping is associated with an increase in airway resistance by prolongation of the time constant determining the rate of lung emptying but differences in values of late expiratory R_{aw} , although sufficient to account for the mild gas trapping observed in the CM infants, may have been masked by both nasal and central airways (Stocks et al 1978b), as well as by the wide variability in measured values. The relative ability of these techniques in detecting small airway dysfunction is considered in the following section.

Improved survival was seen following ECMO treatment in all categories, including CDH although the overall outcome for CDH was poor, with all such infants allocated CM dying. Further studies of outcome after CDH repair including less severely ill infants, and the role of treatments such as ECMO, high frequency ventilation, nitric oxide and surfactant would help to evaluate effects on survival and respiratory morbidity.

At trial entry, the most common underlying diagnosis was persistent pulmonary hypertension which was due to meconium aspiration in 47% of the whole group. Infants with isolated persistent fetal circulation formed the next category with 18% of the whole group affected. Infants with a primary diagnosis of meconium aspiration have been shown to exhibit, at least in the short term, an increased prevalence of airways responsiveness, when assessed at around 14 days of age (Koumbourlis et al 1995). The assessment of airways responsiveness by methacholine challenge may have provided a more detailed picture of respiratory function in this sub group but limitations of study design such as the relatively short time period of sedated sleep and the initial selection of three outcome measures of respiratory function (which

had already required an adjustment to reduce the power of study to avoid reporting false positive results [Type 1 errors] precluded this additional measurement.

6.5.4.4 Comparison of Respiratory follow-up and control airway function

Overall, plethysmographic measurements in the 78 Respiratory follow-up infants were comparable to that of the LRI Controls, who were otherwise healthy infants, having been recruited at birth and followed prospectively (Section 6.3.4.4) and who had experienced repeated episodes of wheezing associated lower respiratory illnesses during infancy (Dundas et al 1995). Only the CM infants had a significantly larger group mean FRC_{pleth} , which persisted when corrected for weight.

There were no significant differences in FRC_{pleth} in healthy controls when compared with LRI controls whether results were expressed as absolute values or corrected for weight. The absence of any significant differences may have been related to the degree of impairment of airway function between the groups, as hyperinflation secondary to airway obstruction generally signifies more advanced airway disease, such as that seen in infants with cystic fibrosis (Nowotny et al 1998, Tepper et al 1988).

However, when sG_{aw} values were assessed using ANOVA, for initial inspiratory sG_{aw} , CM infants had significantly lower values than ECMO or LRI controls. In turn, LRI control values were significantly lower than healthy controls. For late expiratory sG_{aw} (reflecting primarily small airway function) both ECMO and CM group mean values were similar to LRI controls, and all 3 groups had significantly lower values when compared to healthy controls. These results place the Respiratory follow-up infants as a group at the “lower end” of the respiratory function spectrum, and suggest that, as a group they are similar to infants within the epidemiological study from which the control groups were selected who had pre-morbid diminished airway function (Dezateux et al 1997(a), Dezateux et al 1999, Dezateux et al 1998).

Many infants with wheezing LRI in the first year of life do not go on to develop asthma, the symptoms of wheeze being associated with lower levels of $V'_{\text{max,FRC}}$ reflecting smaller airway size rather than inflammatory changes (Martinez et al 1995). It remains uncertain, however, whether the diminished early airway function

associated with transient early wheezing LRI in early childhood is a risk factor for chronic obstructive airway disease in adult life (Dezateux et al 1997 (a)).

It was also possible to compare results obtained in ECMO and CM infants with the more symptomatic group of recurrently wheezing infants, recruited from the wards and clinics of Great Ormond Street Hospital (Section 4) whose details and respiratory function results are shown in Table 4-1 and Table 4-2. The Respiratory follow-up group was slightly older and heavier at testing. Mean lung volume in the wheezing infants (272 mL) was similar to that of the ECMO and LRI control groups, but lower than that of the CM infants (306 mL). However, mean initial inspiratory and late expiratory R_{aw} was higher in the wheezing infants than in both ECMO and CM groups (Table 4.2), with lower initial inspiratory and late expiratory sG_{aw} (1.40 and $1.12 \text{ s}^{-1} \text{ kPa}^{-1}$ respectively) in the wheezing infants. The significance of this observation is uncertain and may reflect the high variability of R_{aw} , or reflect simply the lower values of R_{aw} in infants attending LEIC, when compared to GOS.

The interpretation of values of $V'_{max,FRC}$ obtained from ECMO, CM and USA and UK control groups was influenced by the extent to which study and control populations were comparable, and to the large differences in group mean values noted between control and Respiratory follow-up and control groups. The original aim of the study was to study at least 30 infants in each group, to achieve 85% power of study at the 5% level to detect differences in the 3 respiratory outcome measures, equivalent to one standardised difference. However, early termination of the main trial, when the survival advantages of ECMO became apparent, meant that this target was not reached ($n = 27$ in the CM group). Recent developments in methodology and approach to the analysis of infant flow-volume curves may improve the reproducibility of forced expiratory flow measurements, and hence reduce the number of subjects that need to be studied to achieve statistical significance (Henschen et al 1999).

The issues surrounding the selection of use of control data including that reported here, are discussed in section 7.2.1. Values of forced expiratory flow showed considerable inter-subject variability in both ECMO, CM and UK and USA control groups. Within the ECMO, CM and UK groups values ranged from normal to those found alongside severely compromised small airway function. Healthy one year olds

usually have forced expiratory flows in excess of 150 mL.s^{-1} , considerably higher than that observed in many of the infants in the Respiratory Follow-up.

However, comparison of plethysmographic assessment of airway function with Healthy and LRI controls do not suggest such marked impairment. This may be explained by the reduced power to detect differences in sG_{aw} due to the high variability and failure rate in this study, or that despite the wide inter-subject variability also reported, $V'_{\max, \text{FRC}}$ is a more sensitive indicator of small airway function (Dezateux et al 1997(b)).

Tidal breathing parameters were similar to those reported in a group of 50/51 healthy/wheezy infants measured using similar equipment and are shown in Table 4.2.

6.5.4.5 Comparison with other studies of outcome after neonatal disease

The UK Collaborative ECMO trial design enabled follow-up of a cohort of infants surviving neonatal respiratory failure, in whom comprehensive data describing status at trial entry and throughout infancy had been collected. This study was the first Respiratory follow-up of survivors of a randomised controlled trial of ECMO at around one year of age. Follow-up studies of respiratory function in subjects receiving intensive care in the neonatal period have shown abnormalities extending into childhood and beyond (Gerhardt et al 1987, de Kleine et al 1990, Bader et al 1987, Silverman and Chan 1991, Doyle et al 1991), which may be associated with airway hyper-responsiveness (Koumbourlis et al 1996). A group of 31 children, treated with ECMO following meconium aspiration at birth, showed evidence of mild restrictive disease not significantly different from 11 controls (Judge et al 1997)

Infants from both groups showed a broad spectrum of abnormalities of respiratory function, from within the normal range to major functional disturbances. These results, therefore, support those of others who have shown that ECMO does not prevent sequelae of severe respiratory disease in the newborn period (Garg et al 1992). In contrast to previous work, however (Koumbourlis 1992, Kugelman 1995, Green 1996), this study included a control group of infants not assigned to ECMO which, having avoided confounding factors by careful study design (UK Collaborative ECMO Trial Group et al 1996) and examined between centre results

for any evidence of bias, then allowed documentation and interpretation of the additional effects of ECMO treatment alone. Although North American centres were unable to undertake a randomised controlled trial of ECMO, and therefore could not include a prospectively ascertained control group, follow-up studies have included control data for comparison obtained from "hospital normals" (Garg et al 1992), historical controls (infants measured prior to the introduction of ECMO) (Osiovich et al 1992) infants with respiratory failure not meeting ECMO criteria (Walsh-Sukys et al 1994) or reference to other published values (Greenspan et al 1997).

Previous reports of respiratory function in infants treated with ECMO, have reported measurements made during or shortly after ECMO (Koumbourlis et al 1992, Kugelman 1995, Greenspan 1997), or at 6 months of age (Garg et al 1992). Kugelman and colleagues measured FRC_{N2} , C_{rs} and tidal breathing parameters during weaning from ECMO, and Greenspan and colleagues used $V'_{max,FRC}$ as an outcome measure, to evaluate the effects of early vs late commencement of ECMO.

Garg et al assessed respiratory sequelae of ECMO in 19 of 42 eligible infants using outcome measures of C_{dyn} , R_{aw} , FRC_{pleth} and PaO_2 . The group mean values were: R_{aw} 5.07 $kPaL^{-1}.s$, and FRC_{pleth} : 18.5 $mL.kg^{-1}$ and an sG_{aw} of 1.7 $kPaL^{-1}.s$. Although as a group, ECMO and CM infants had lower values of R_{aw} and larger values of FRC_{pleth} , corrected sG_{aw} was similar within the range of 1.33 - 2.03 $kPaL^{-1}.s$ according to management group and phase of respiration to that of our study (Table 6-11), although age related changes during normal growth preclude direct comparison of values.

Since ECMO became an established therapy in neonatal intensive care, the past decade has witnessed development of other less invasive therapies such as nitric oxide and alternative modalities of ventilation. This has made comparison of the present group of infants with those reported in the literature of limited value, and may also lead to a decrease in demand for neonatal ECMO (Walsh-Sukys et al 1994). The proportion of infants treated with surfactant, NO and high frequency ventilation would be greater if the ECMO trial was repeated now, but it is uncertain what effect that might have on the numbers of conventionally treated infants surviving if the "ultimate safety net" of ECMO was removed.

6.6 Association between measurements of forced expiratory flows and specific conductance

In Section 5 of this thesis, the simpler method of assessment of respiratory function, using an index of tidal breathing: $t_{PTEF:t_E}$, was compared with an established “gold standard” method of airway mechanics: plethysmographic measurements of sG_{aw} , (Stocks et al 1977a). The Respiratory follow-up of survivors of the UK Collaborative ECMO trial offered the opportunity to investigate, in a similar manner, the association between the newer measurements of forced expiration ($V'_{max,FRC}$) and specific conductance (sG_{aw}) in a group of survivors of neonatal respiratory failure.

In addition, the association between $t_{PTEF:t_E}$ with sG_{aw} , and $t_{PTEF:t_E}$ with $V'_{max,FRC}$ was also examined in this population, to assess whether the findings in healthy and recurrently wheezy infants could be confirmed in another population measured under similar conditions, and, examining the relationship between $t_{PTEF:t_E}$ and $V'_{max,FRC}$, to observe any correlation between these two simpler methods of assessing small airway function.

To facilitate comparison of the relationships between these variables in the Respiratory follow-up and healthy infants, appropriately aged infants recruited to an epidemiological study of respiratory function and acute respiratory illness (Dezateux et al 1999) (Section 6.3.4) were selected as a control group.

6.6.1 Study Population and methods

The study population comprised the 78 surviving infants from the UK Collaborative ECMO Trial who attended for Respiratory follow-up as described previously (section 6.5.2) and 62 healthy and wheeze-free infants recruited to an epidemiological study of respiratory function and acute respiratory illness (Dezateux et al 1999) (section 6.3.4).

For the 78 Respiratory follow-up infants, data collection, analysis and reporting of results was as described in section 6.3.2.2, with the exception of $t_{PTEF:t_E}$, which was reported from tidal parameters analysed at GOS for the whole group, rather than that reported by each analysis centre.

For the healthy controls, data collection, analysis and reporting of results was as previously described (Section 3).

6.6.2 Results

Of 78 Respiratory follow-up infants, $t_{\text{PTEF}}:t_E$ was reported in 76: one infant woke before measurements were attempted, tidal parameters being reported from data collected prior to forced expiratory manoeuvres, and one infant could not be analysed (data on disk corrupted). sG_{aw} was successfully reported in 56 infants, and $V'_{\text{max,FRC}}$ in 71. In one infant, neither sG_{aw} nor $V'_{\text{max,FRC}}$ was reported. Thus, it was possible to investigate the association between $V'_{\text{max,FRC}}$ and sG_{aw} in 50 infants, between $t_{\text{PTEF}}:t_E$ and sG_{aw} in 53 infants and between $V'_{\text{max,FRC}}$ and $t_{\text{PTEF}}:t_E$ in 70 infants.

In the group of healthy controls, sG_{aw} was reported in 58 infants, tidal parameters being successfully reported in the whole group. $V'_{\text{max,FRC}}$ measurements were not included within the protocol for that study. It was therefore possible to compare measurements in 58 healthy infants. Table 6-13 presents the background details and respiratory function parameters for the whole study population.

Table 6-13 Background details and Respiratory function parameters of Respiratory follow-up and healthy controls for comparison of $V'_{max,FRC}$ and $t_{PTEF:t_E}$ with sG_{aw}

Values are shown as mean (SD), except where indicated	Respiratory follow-up infants n=78	Healthy Control infants n=62	95% CI (Resp follow-up - Controls)
Males n(%)	48 (62%)	25 (40%)	5, 38%*
Weight (kg)	10.2 (1.5)	9.9 (0.9)	-0.1, 0.8
Length (cm)	78.2 (3.5)	77.8 (2.5)	-0.6, 1.4
Age (months)	13.7 (1.9)	12.9 (1.3)	0.3, 1.3**
FRC_{pleth} (mL)	283 (65)	256 (40)	8, 46**
$sG_{aw,II}$ ($s^{-1} kPa^{-1}$) median (IQR)	1.86 (1.16, 2.33)	2.23 (1.55, 2.63)	-0.51, 0.17
$sG_{aw,EE}$ ($s^{-1} kPa^{-1}$) median (IQR)	1.40 (0.89, 2.10)	1.95 (1.56, 2.68)	-0.73, -0.05**
$V'_{max,FRC}$ (mL.s ⁻¹)	158 (74)	not measured	
RR (min ⁻¹)	32.7 (8.6)	29.5 (4.0)	0.9, 5.6**
$t_{PTEF:t_E}$	0.263 (0.085)	0.305 (0.087)	-0.072, -0.013**

Key to table: * p < 0.05, ** p < 0.01

The proportion of males in the Respiratory follow-up group was significantly greater than in the healthy controls, reflecting the excess of males who were entered onto the trial (UK Collaborative ECMO Trial Group et al 1996). They were also slightly older at testing, but there were no significant differences in body weight or length between the two groups. Maternal smoking following pregnancy was similar in each group, at around 32% (data not shown). In the Respiratory follow-up group, 12 infants were symptomatic when attending for measurements.

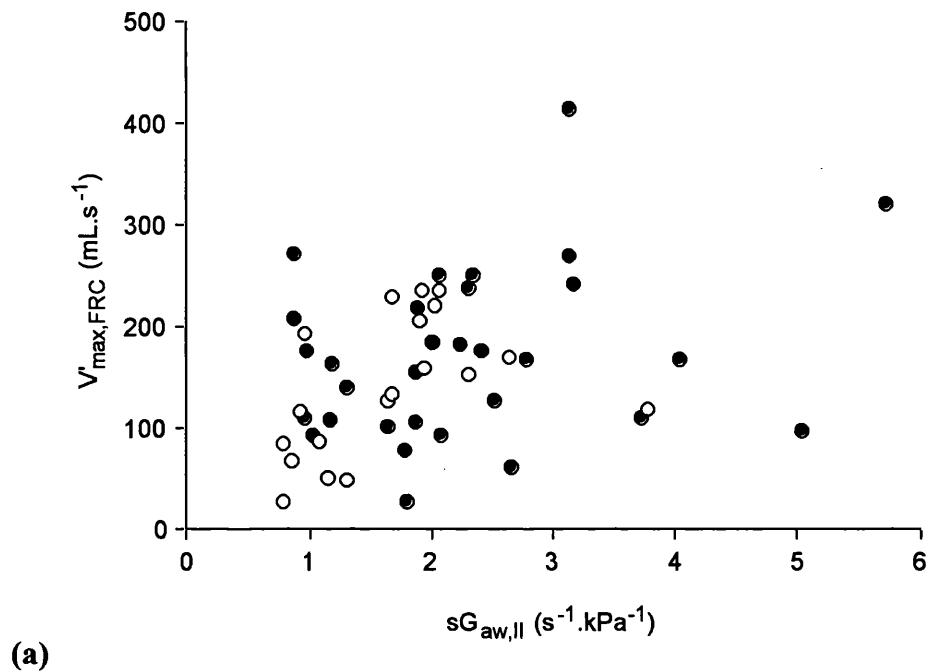
Mean (range) for FRC_{pleth} for Respiratory follow-up and healthy groups was 283 mL (145 - 492) and 256 mL (156 - 352), respectively, and this group mean difference of 17 mL was significant (Table 6-13). Mean $sG_{aw,EE}$ and $t_{PTEF:t_E}$ were significantly lower in the Respiratory follow-up group (Table 6-13). There was marked between-

subject variation for specific conductance measures, and $sG_{aw,II}$ was not significantly lower when data from the whole group of 78 infants were pooled for comparison with healthy controls. Respiratory rate was, however, significantly higher amongst the 78 infants, compared with the healthy control group. Mean (SD) $V'_{max,FRC}$ was 158 (74) for the Respiratory follow-up group (not measured in the healthy controls).

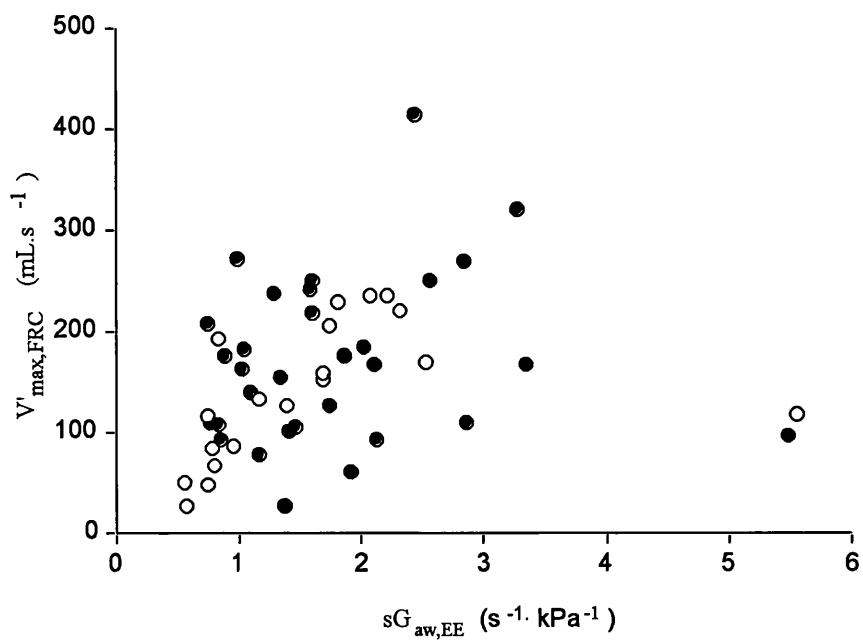
Using linear regression and with $V'_{max,FRC}$ as the outcome variable, $sG_{aw,EE}$ was not significantly associated with $V'_{max,FRC}$ in the 78 Respiratory follow-up infants (Table 6-14). A weak, but significant association was seen between $sG_{aw,II}$ and $V'_{max,FRC}$ which explained only 12% of the total variance in $V'_{max,FRC}$. A similar association was also observed between $t_{PTEF:t_E}$ and $V'_{max,FRC}$ accounting for 11% of the total variance in $V'_{max,FRC}$ (Figure 6-16). After removing the 2 outliers shown in Figure 6-15 (b), a scatterplot of $V'_{max,FRC}$ and $sG_{aw,EE}$, the significance of the association between these variables rose ($p = 0.0002$) and could then explain 26% of the total variance of $V'_{max,FRC}$.

Simple linear regression with $t_{PTEF:t_E}$ as the outcome variable amongst Respiratory follow-up groups and healthy matched controls, for the dependent variable sG_{aw} , found no significant relationship between with sG_{aw} in the Respiratory follow-up group. After removing the same outliers as previously, a significant but weak relationship between $t_{PTEF:t_E}$ and $sG_{aw,EE}$ was then observed. Similarly, for the healthy controls, a weak relationship between $t_{PTEF:t_E}$ and sG_{aw} was found. Only 5% of the total variance in $t_{PTEF:t_E}$ was explained by the dependent variables in the healthy controls and even less in the Respiratory follow-up infants, with the exception of $t_{PTEF:t_E}$ and $sG_{aw,EE}$ (9%).

Figure 6-15 Scatterplot of $V'_{max,FRC}$, and sG_{aw} during: (a) initial inspiration and (b) late expiration, in Respiratory follow-up infants



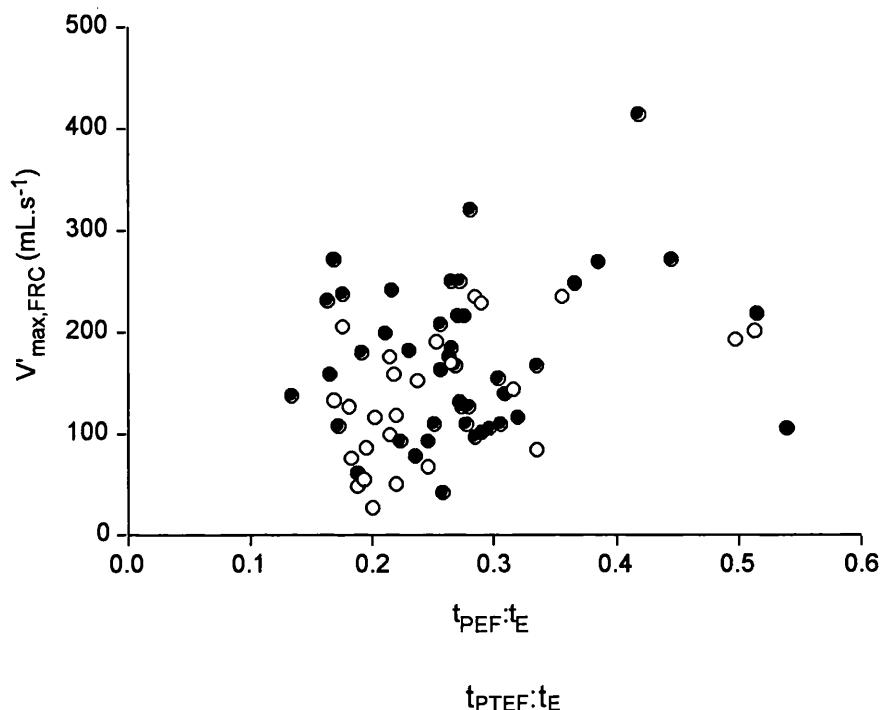
(a)



(b)

Key to Figure: Closed circles: ECMO Group; Open circles: CM group

Figure 6-16 Scatterplot of $V'_{max,FRC}$ and $t_{PTEF}:t_E$ in Respiratory follow-up infants



Key to Figure: Closed circles: ECMO Group; Open circles: CM group

Table 6-14 Regression analyses for $V'_{\max,FRC}$ in Respiratory follow-up infants

Unadjusted	Regression coefficient (95% CI)	r^2	p value
$sG_{aw,II}$	0.005 (0.0009, 0.002)	0.116	0.016
$sG_{aw,EE}$	0.003 (0.0006, 0.007)	0.058	0.092
$t_{PTEF:t_E}$	-0.0004 (-0.0001, -0.0007)	0.107	0.006
After removing 2 outliers			
$sG_{aw,EE}$	0.005 (0.002, 0.007)	0.264	0.0002

Table 6-15 Regression analyses for $t_{PTEF:t_E}$ in Respiratory follow-up and healthy control infants

Unadjusted	Regression coefficient (95% CI)	r^2	p value
Respiratory follow-up infants			
$sG_{aw,II}$	2.11 (-1.34, 5.56)	0.028	0.225
$sG_{aw,EE}$	2.29 (-1.11, 5.69)	0.034	0.183
After removing 2 outliers			
$sG_{aw,EE}$	2.55 (0.26, 4.84)	0.091	0.03
Healthy control infants			
$sG_{aw,II}$	2.36 (0.40, 4.32)	0.05	0.019
$sG_{aw,EE}$	2.53 (0.53, 4.52)	0.055	0.014

6.6.3 Discussion

These findings, comparing $t_{\text{PTEF}}:t_E$ and sG_{aw} in the respiratory follow up population confirm those of Chapter 5, where $t_{\text{PTEF}}:t_E$ and sG_{aw} were both significantly lower in wheezy, compared with healthy infants but were only weakly associated with each other. Both $t_{\text{PTEF}}:t_E$ and $sG_{\text{aw,II}}$ were lower in infants attending respiratory follow-up than healthy control infants, although there were no significant differences between the two groups for the variable group mean $sG_{\text{aw,EE}}$.

When the association between $V'_{\text{max,FRC}}$ and sG_{aw} and $V'_{\text{max,FRC}}$ with $t_{\text{PTEF}}:t_E$ was examined for the group of respiratory follow-up infants, again there was only a weak association between these variables. It was not considered to be appropriate to use more complex regression models to examine the relationship between variables, by adjusting for gender, body size or management group because of the initially poor association and relatively low numbers.

Using linear regression analysis, but correcting $V'_{\text{max,FRC}}$ for lung volume ($V'_{\text{max,FRC}}/\text{FRC}_{\text{pleth}}$), a significant correlation between $t_{\text{PTEF}}:t_E$ and $V'_{\text{max,FRC}}$ was found in a group of 21 infants with airway obstruction ($r=0.630$, $p=0.002$) (Bancovin et al 1995). In contrast, no association with R_{aw} was found in the same group of infants. No details were given by the authors regarding the phase of the respiratory cycle used to report R_{aw} and the results are expressed as % predicted using unspecified reference values, precluding further interpretation of these data. Issues surrounding the comparison of $V'_{\text{max,FRC}}$ and R_{aw} are again addressed in the overall discussion in Section 7.2.

7. General discussion

7.1 Introduction and summary of findings

At the inception of this thesis there was an pressing need to develop and evaluate simple non-invasive tests to measure respiratory function in infants, in order to investigate the physiological basis of lung disease and move towards a more rational basis for the treatment of airway disease in infancy.

The aims of this thesis were to evaluate simpler methods of assessment of lung function by comparison with established “gold standard” techniques, evaluating the validity, applications and error of measurements, and then to address applications of such assessments, together with some of the methodological issues raised, when comparing respiratory function in survivors of each limb of the Collaborative ECMO trial.

In Section 4, $t_{PTEF}:t_E$ and sG_{aw} were measured in healthy infants and those with recurrent wheezing and it was found that both parameters were significantly lower in the wheezy compared to the healthy group. There was a significant although weak association between $t_{PTEF}:t_E$ and $sG_{aw,EE}$ in healthy infants, irrespective of prior wheezing status. In Section 6.6, addressing the same question within the Collaborative ECMO Trial Respiratory follow-up population, these findings were confirmed. In Section 4 it was concluded that, although both R_{rs} and R_{aw} were significantly higher in infants with prior wheeze, the clinical value of measurements of R_{rs} within individual infants may be limited by its failure to detect the dynamic changes in resistance throughout the breath that are obtained by plethysmographic measurements of resistance.

Inter observer variability was compared within and between two specialised infant lung function testing centres and a strategy developed for performing and analysing infant respiratory function tests to facilitate future similar trials in Section 6.4. No significant between centre differences were found between any of the respiratory function outcome measures. A collaborative approach to trials with infant respiratory function as an outcome measure appears feasible, providing close attention is paid to

study design and participants in such trials maintain a standard approach to data collection and analysis.

In Section 6.5 airway function was compared at around one year in survivors of the UK Collaborative ECMO trial who had been allocated either ECMO or conventional ventilatory management (CM). The findings of elevated lung volumes, reduced specific conductance during inspiration, and a tendency for reduced forced expiratory flows in those managed conventionally, suggested that the larger proportion of CM infants receiving respiratory medication and reporting respiratory symptoms at around one year of age may be attributed to subtle impairment of small airway function in these infants, relative to those assigned to ECMO. These findings probably reflected differences in management during the neonatal period, since initial disease severity and all other background characteristics were similar in both groups.

When compared with selected control groups, matched for age, respiratory function in survivors of the ECMO trial was similar to that of infants in whom recurrent LRI's had been reported, and relatively poorer than healthy controls. Interpretation of measures of forced expiration in relation to UK and USA controls was rather more complex, but suggested some impairment in airway function in the Respiratory follow-up group as a whole.

One of the original aims of this thesis was to include a detailed comparison of respiratory inductance plethysmography with pneumotachography as an additional non-invasive simpler method suitable for infants. However, the invitation to participate in the Respiratory follow-up of the Collaborative ECMO trial arose, with the opportunity to address some of the methodological issues of using infant respiratory function parameters as outcome measures in a randomised controlled trial, as well as the chance to compare measurements of forced expiration with specific conductance and tidal parameters. The comparison of respiratory inductance plethysmography with pneumotachography was therefore delegated to Dr E. Jackson for inclusion in a PhD and related publication (Jackson et al 1995). The aim of that study was to assess the accuracy of uncalibrated respiratory inductance plethysmography for the computerised measurement of $t_{\text{PTEF}}:t_E$ in both newborns and infants. The technique was found to be of limited utility and it was concluded that computerised measurement of $t_{\text{PTEF}}:t_E$ using

uncalibrated respiratory inductance plethysmography should be used with caution particularly outside the neonatal period. The problems were thought to be greatest in infants who have had respiratory problems even if they were asymptomatic at the time of testing, who comprise the population likely to be of particular interest when such measurements are performed. Examination of the separate ribcage and abdominal signals of healthy infants suggested that some form of qualitative calibration would have been desirable if respiratory inductance plethysmography was to be used to determine $t_{\text{PTEF}}:t_{\text{E}}$. However, further limitations of this technique using a commercially available calibrated system (NIMS) were demonstrated during a subsequent study from this department (Brown et al 1998).

The purpose of the following section is to determine the extent to which the initial aims of the thesis were met; to consider some of the obstacles to the assessment of respiratory function when using simpler techniques in infancy and to address some of the key issues arising from the studies presented in this thesis. To conclude, some future challenges for respiratory function assessment in infants are presented and possible solutions suggested.

7.2 Critique of simpler methods of assessing airway function

7.2.1 Evaluation of simpler methods

In evaluating how one test variable predicts an outcome, the performance of the measurement can be described by its sensitivity, specificity and positive predictive value (Dezateux et al 1996). Sensitivity refers to the proportion of “positives”, that in the context of this thesis would be recurrently wheezing infants, who are correctly identified by the test. Specificity refers to the proportion of the other group i.e. healthy infants, who are correctly identified by the test. Specificity and Sensitivity are in a “trade-off” relationship, with an increase in one resulting in a decrease in the other. An important aspect of these measures of test performance is the threshold at which a test becomes “positive” i.e. the cut off level. The level is selected according to the relative importance of sensitivity and specificity. For example, in detecting infants who may be at risk of continuing to wheeze as a screening test, a cut off level with high sensitivity would be chosen. However, if screen positive infants were then to receive early intervention such as allergen avoidance or inhaled corticosteroids with the aim of preventing chronic lung disease then a cut off point with high

specificity would be needed to prevent the unnecessary treatment of infants who were not destined to develop disease. Positive predictive value is the probability that the subject with a positive test has the disorder, and is generally regarded as being more useful to both the subject and clinician. An additional term in this measure is the prevalence of the disorder, with increased prevalence resulting in a higher positive predictive value.

Ideally, the evaluation of simpler methods of assessing respiratory function in wheezy infants presented in this thesis would have included an estimate of the sensitivity and specificity of each method. One of the limitations of the thesis study design was the difficulty in formally evaluating specificity and sensitivity. It is helpful to consider the reasons why it was not considered feasible to undertake this evaluation, and to discuss other approaches that may have been used to evaluate the simpler methods.

In assessing the value of a “new” test applicable in wheezy infants, the primary interest is in airway calibre. The method selected within this thesis to examine the relationship between the simpler tests of airway function and the degree of airway obstruction was by comparison with the accepted “gold standard” benchmark of airway resistance. The simpler tests were also compared in both healthy controls and recurrently wheezing infants. As an alternative method, the measured response to induced changes in airway calibre following administration of bronchodilators or bronchoconstrictor agents may be measured.

Benoist et al (Benoist et al 1994) used methacholine challenge to compare the simpler tests of airway function (SBT measurements of C_{rs} and R_{rs} , $V'_{max,FRC}$ and tidal breathing parameters) with the “gold standard” P_{TCO_2} in response to mild bronchial obstruction and plotted individual responses in SD units, calculated from the standard deviation of the differences between the extreme values obtained in each infant for each parameter. A response to the methacholine challenge was defined as a change of 2SD or more from baseline, allowing comparison between the ability of different lung function tests to detect post methacholine airway obstruction.

Klug and Bisgaard, in an evaluation of 3 different techniques of respiratory function testing in young awake children, also used methacholine challenge as an intervention to determine the relative sensitivity of each technique (Klug et al 1998). Two “gold

standard" reference methods ($FEV_{1.0}$ and sR_{aw}) were also included in the protocol. To compare the performance of each technique, measurements were transformed to an SD index (calculated from [test value-pre test value]/within subject SD) and the number of methacholine doses inhaled by each subject ranked by the highest dose used. A dose response curve was constructed and the sensitivity of each technique assessed by taking the slope of the curve (representing ΔSD index/ Δ methacholine dose). This statistical approach had the advantage of obtaining an objective assessment of sensitivity that also included the intersubject variability of each measurement, and used the dose-related bronchoconstrictor effect of methacholine to induce airway calibre, then measured the effect of these changes by each method of assessing resistance.

It would not have been possible to incorporate methacholine challenge within the study protocol for this thesis. The epidemiological study to which the healthy controls were recruited included outcome measures of FRC_{pleth} and R_{aw} as a priority and insufficient time would have been available to include additional techniques, particularly those as time consuming as bronchial challenge, during periods of sedated sleep. The advantage of bronchial challenge is that relative changes in the parameters being measured may be assessed, avoiding the need for direct comparisons of methods that may not be directly comparable in the physiological sense i.e $t_{PTEF}:t_E$ and $sG_{aw,EE}$. In addition, the relative sensitivity of each technique may be estimated within a specific population. The disadvantage of such challenges is that, as Benoist et al discussed (Benoist et al 1994), no inferences may be made regarding the underlying physiological mechanisms of each technique or measure being compared. Other groups have reported rather inconclusive findings when incorporating methacholine challenge within methodological comparisons of forced expiratory flow techniques (Hayden et al 1997).

In future similar methodological studies, the incorporation of bronchial challenge in order to make direct comparisons of test sensitivity may be considered, in addition to more detailed evaluations of success rates, reasons for failure and physiological interpretation of results. However, there are major physiological and methodological problems in applying bronchial challenge testing in the wheezy infant population. There are safety considerations when bronchoconstricting wheezy infants with a potential fall in oxygen saturation levels during testing (Gaultier et al 1996, Mallol et

al 1988). The effect of bronchoconstriction of the airway parenchyma is not standard or uniform between infants. The tests themselves are not yet standardised with respect to dosage delivery, or testing protocols. Many recurrently wheezy infants would not be eligible, having forced expiratory flows below the threshold of 100 mL.s^{-1} commonly used as the minimum value considered to be acceptable for challenge testing.

The limitations of using R_{aw} as a “gold standard” for methodological comparisons within this thesis can be categorised as:

- The biological variability of airway resistance, particularly amongst healthy infants in whom there is relative freedom to vary respiratory pattern.
- The method of analysing R_{aw} using the Respiratory Analysis Program, where values were obtained from 4 points within the respiratory cycle rather than, for example, regressing through the breath, which led to increased variability of the measurement.
- Airway resistance gives an estimate of airway calibre, whereas R_{rs} includes chest wall and tissues. It is not altogether clear exactly what $t_{PTEF}:t_E$ is measuring (Section 4.6), therefore the methods are not directly comparable, and may incorporate differing physiological mechanisms within measured values.

Taken together, it can be seen that straightforward estimates of sensitivity and specificity would not have been possible, given the differing variability of each measurement and the difficulty in determining a physiologically meaningful threshold above which values were considered abnormal.

The potential disadvantages of selecting arbitrary post hoc cut offs for assessment of sensitivity and specificity were illustrated by a study designed to determine whether $t_{PTEF}:t_E$ measured in the first week of life predicted subsequent respiratory morbidity (Yuksel et al 1996). The authors concluded that $t_{PTEF}:t_E$ of less than 0.4 had 92% sensitivity, 64% specificity and 41% positive predictive value for respiratory symptoms in infancy. This cut off appears to have been arbitrarily chosen following data inspection, and furthermore values of $t_{PTEF}:t_E$ have also been shown to change with age (Stocks et al 1994). Selection of a cut off of 0.2 or 0.5 may have resulted in very different results, but in the absence of a physiologically meaningful threshold

would still be of limited use when extrapolating from the group to an individual infant. In addition, many previous studies have comprised highly selected groups and findings have rarely been applied to the general population.

7.2.2 Tidal breathing parameters

Studies presented in this thesis and published elsewhere have demonstrated a relationship between the ratio of peak tidal expiratory flow to expiratory time ($t_{PTEF}:t_E$) and indices of airway obstruction in infants, children and adults. This measurement of tidal breathing parameters is simple, does not require expensive and technically complex equipment and is potentially applicable even in unsedated infants. Furthermore, no assumptions are made when interpreting results, in contrast to, for example the SBT which assumes that the respiratory system behaves as a one compartment model which may be invalid in infants with airway disease.

Stocks and colleagues (Stocks et al 1994) have demonstrated changes in the ratio $t_{PTEF}:t_E$ during the first year of life and shown that, in common with most other tests of lung function, above a certain cut off value it has a very limited ability to detect changes in airway function, due to the wide within and between subject variability of the measurement in healthy infants. In addition, they demonstrated considerably greater within subject variability amongst newborn infants which may limit the clinical and epidemiological applications of $t_{PTEF}:t_E$ during the first few weeks of life. Furthermore, the marked expiratory braking that occurs during the first days of life may mask any association between $t_{PTEF}:t_E$ and airway function in neonates.

Clarke et al evaluated changes in $t_{PTEF}:t_E$ over the first year of life and also correlated $t_{PTEF}:t_E$ to $V'_{max,FRC}$ in healthy and asthmatic infants and infants with chronic lung disease (Clarke et al 1994). Their reported values for $t_{PTEF}:t_E$ at one year of age in the various groups are in close agreement with those reported in this thesis. In healthy infants, they reported $t_{PTEF}:t_E$ as 0.29 in 13 infants of similar size and gender distribution to the 62 healthy infants in Section 4, whose group mean $t_{PTEF}:t_E$ was 0.305. In 51 recurrently wheezing infants (Section 4) whose results are shown in Table 4-2, mean $t_{PTEF}:t_E$ was 0.235 compared to 0.25 in Clarke et al's 26 infants with recurrent LRI, of similar age and length, and 0.24 in 20 asthmatic infants who were slightly older and shorter (group mean values: 13.4 months and 74.7 cm). They did not demonstrate any significant differences between healthy, LRI and asthmatic

infants, in contrast to our reported results, which may have been due to the relatively small numbers in their study, together with the high variability of the measurement. Only in 16 infants with CLD, compared with healthy infants of a similar age was a significant difference demonstrated, and these CLD infants had severe airway obstruction with flow limitation present even during tidal breathing.

Adler et al (Adler et al 1995), in a longitudinal study investigating the relationship between alterations in $t_{PTEF}:t_E$ and the occurrence of wheezing LRI in the first year of life, reported values of $t_{PTEF}:t_E$ in infants up to 10 weeks ($n = 80$), in whom large within subject variability has been demonstrated (Stocks et al 1994) and in 18 infants between 10 weeks and 6 months of age, stratified by gender and presence or absence of LRI. Their results were in broad agreement with Clarke et al (Clarke et al 1994).

The absence of a significant relationship between $t_{PTEF}:t_E$ and $V'_{max,FRC}$ demonstrated in our population confirms the findings of Adler et al and Clarke et al.

Although only a weak relationship between $t_{PTEF}:t_E$ and other measures of airway calibre was demonstrated, differences between the ECMO Respiratory follow-up and healthy control groups were significant for both $t_{PTEF}:t_E$ and $sG_{aw,EE}$ in Section 6.7. Therefore although the underlying mechanisms may remain unclear, both techniques demonstrated differences in airway function between controls and survivors of neonatal respiratory failure.

The limitations of the use of tidal breathing as an assessment of airway function may arise from the variety of physiological determinants of $t_{PTEF}:t_E$. In the presence of severe airflow limitation, the tidal breathing curve is determined by intra-thoracic airway mechanics. In healthy infants, however, tidal parameters reflect rather the integrated response of the whole respiratory system. Expiratory flow is modulated by the summed output of a variety of inputs, such as those from laryngeal abductor and adductor, abdominal and intercostal muscles and post inspiratory activity of the diaphragm and intercostal muscles. Changes in these muscle activities in response to biochemical or neurological stimuli may alter the ratio of $t_{PTEF}:t_E$ independently of changes in the resistance or compliance of the airways and lung tissue (Mikkilineni et al 1994). Recent work on the neuromuscular control of breathing in cats demonstrated that t_{PTEF} was also highly influenced by inspiratory muscle activity during early expiration, associated with vagal receptor activity, (van der Ent et al

1998). Thus, there is a better relationship between sG_{aw} and $t_{PTEF:t_E}$ in the presence of airway narrowing than in healthy subjects (Dezateux et al 1994).

In summary, $t_{PTEF:t_E}$ is unlikely to meet the criteria previously stated for a test to be both useful and informative for clinical purposes. Although of limited use for the individual, it may have potential applications in epidemiological studies looking at large groups of infants. Low values (below 0.2) suggest airway narrowing but above that threshold give relatively little useful information and do not correlate with measures of airway calibre. The relationship between $t_{PTEF:t_E}$ and airway calibre is not a linear one, and in common with most other respiratory function tests has a lower limit of normality above which there is wide variation. Future approaches to tidal breathing analysis (Morris et al 1998, Williams et al 1998) incorporating the slope of the time constant for expiration, τ_{rs} , may avoid the reliance on detection of a single point of peak tidal expiratory flow which introduces some variability into $t_{PTEF:t_E}$ measurements (Stocks et al 1994). However extreme caution is required when attempting to extrapolate expiratory τ_{rs} from one slope in recurrently wheezing infants with multiple expiratory time constants where the single-compartment model of the respiratory system may not apply.

7.2.3 Single breath technique

In the assessment of the SBT, its validity, application and errors of measurement, the aims of the thesis were largely met. The conclusions of the study reported in Section 5 were that, despite the difficulties in interpreting results from individual infants, the SBT was simple to use and is potentially more widely applicable than plethysmography which remains essentially limited to specialised centres. Although suggestions have been made that R_{rs} may reflect initial expiratory airway mechanics, further work is needed to confirm the association. In this study, we found that both R_{rs} and end expiratory R_{aw} were significantly higher in infants with prior wheeze, most of whom were asymptomatic at time of testing, than in healthy infants of similar age and weight. This suggests that measurements of R_{rs} may be of value in epidemiological studies. However, the value of R_{rs} as an outcome measure may be limited by the high failure rate of the technique, particularly in younger infants, (Gappa et al 1993). In contrast to R_{aw} , the SBT is unable to detect the dynamic changes throughout the respiratory cycle and, as it includes both chest wall and lung

tissue components of resistance is rather a crude method of detecting subtle changes in airway calibre.

The limitations of the technique in the assessment of airway function in wheezy infants were discussed in Section 5. Although mean R_{rs} was significantly higher in healthy infants, this was not shown in the wheezy group (Table 5-4). End expiratory R_{aw} exceeded R_{rs} in 44% of wheezy infants and 17% of healthy infants. This finding was unpredicted, since R_{rs} includes chest wall and tissue resistance. In addition, when R_{rs} measurements were obtained in 18 infants during periods of rebreathing warmed, humidified air a significant reduction in measured values was noted (Table 5-6). This implies that the relative underestimation of R_{rs} compared to R_{aw} would have been even more marked if all measurements had been recorded under identical circumstances.

In addition, a linear shape on the flow-volume curve does not necessarily indicate relaxation of respiratory muscles or the presence of a single time constant, since an even, descending slope could represent balanced respiratory muscle contraction and/or reciprocal changes in compliance and resistance as lung volume decreases. A limitation of all measurements of resistance in infants is the inclusion of nasal resistance which reduces the discriminative ability to detect small changes in lower airway function (Stocks et al 1978b, Stocks 1980).

7.2.4 Rapid thoraco-abdominal compression techniques

$V'_{max,FRC}$ is thought to primarily reflect airway calibre upstream to the airway segment subjected to flow limitation and therefore gives a measure of airway function that is relatively uninfluenced by the resistance of the upper airways. In this respect it is the only measure of intrathoracic airway function in infants, in whom nasal resistance comprises around 50% of total resistance (Stocks et al 1978b). The calibre of the intrathoracic airways is determined not only by their anatomic dimensions but also by many other factors, including the distending pressures surrounding the airways (Figure 3-13). Therefore full interpretation of results often requires simultaneous measurements of lung volume and elastic recoil of the respiratory system. Whether flow limitation is usually reached in the healthy infant population remains an area of controversy (LeSouëf et al 1996). The technique also relies on FRC as a landmark, which is known to be unstable, especially in younger

infants (Henschen et al 1998). This technique remains a useful forcing function which has provided much valuable information (LeSouëf et al 1996).

In the ECMO Respiratory follow-up population, although not achieving significance at the 0.05 level, there was a trend for values of $V'_{\max, \text{FRC}}$ to be lower in the conventionally managed group, who also showed lower values of $sG_{aw, II}$. Had the trial not been halted, recruitment and measurement of a few more infants may well have resulted in significant differences between the 2 groups.

Adaptations to the RTC technique have been described since the inception of this thesis which aim to overcome some of its well recognised limitations. A recent approach: the raised-volume RTC (RVRTC) technique has been developed using an extended volume range analogous to the full vital capacity range used in adults and older children (Feher et al 1996). By delivering either one (Turner et al 1995) or a few (Feher et al 1996) large sigh-like breaths, lung volume may be elevated prior to performing RTC. The resulting full forced expiratory flow-volume curves may be used for derivation of either timing and flow indices (Feher et al 1996) or, by extrapolating to the elastic equilibrium volume calculated from the linear segment of the sigh-like breaths preceding the RTC, and overlaying successive curves relative to the EEV, flow may be determined at weight corrected volume points around EEV (Henschen et al 1998, Henschen et al 1999).

7.2.5 Influence of measurement conditions

Data from the 18 infants measured during periods of rebreathing (Sections 5.3 and 5.4.2) highlights the potential influence of measurement conditions, which includes factors such as the timing of measurements i.e. pre and post periods of rebreathing, the influence of equipment such as jackets for the measurement of forced expiration and the differing deadspace and resistance of equipment used for each measurement technique within the same measurement occasion. These factors may markedly increase the variability of techniques. Minimum acceptable standards for measurement conditions have already been developed (Gaultier et al 1996). For future multi-centre trials further standardisation is mandatory, and recently an ERS working party has been formed to address these (Frey et al 1999). Guidelines have been prepared by this working party and, if adhered to, should ensure that infant lung function measurements may be performed with an acceptable degree of safety,

precision, and reproducibility. The ultimate aim of these guidelines is to facilitate multi-centre collection of data and performance of clinical investigations.

7.2.6 So, which test is best?

An important distinction to be made between the various tests of airway function applicable in infancy is whether measurements are obtained during tidal breathing such as airway resistance and measures of impedance (Frey et al 1998a), during passive expiration, or requiring the respiratory muscles to be silenced (SBT and multiple linear regression) and those which are forcing functions such as forced expiratory measurements of $V'_{max,FRC}$.

Having evaluated some of the simpler approaches to the assessment of respiratory function in infancy, there remains a need to evaluate the relative benefits of each approach. The duration of a measurement occasion in infant respiratory function testing is limited and it is essential to make rational choices for both research protocols and clinical assessments. Although it has been suggested that $V'_{max,FRC}$ and t_{PTEF} may be the most sensitive measures of bronchial responsiveness or induced airway obstruction available (Benoist et al 1994) the “best test” to assess changes in baseline airway function has yet to be determined. The scope of this thesis was limited to spontaneously breathing infants and particularly those with wheezing illnesses, with both clinical and research applications in mind.

As previously stated, beyond the neonatal period, most of the more common respiratory diseases in infancy involve the airways rather than the parenchyma therefore measures of airway function are most likely to be relevant. Applications of infant respiratory function testing may be considered as primarily research or clinical, although this distinction is rather arbitrary. Research applications include epidemiological studies of growth and development of the respiratory system, and more recently have aimed to identify infants who may be subject to increased risk of respiratory morbidity. They have also attempted to interpret risk in relation to both environmental and genetic factors. The characteristics of the “ideal” test for epidemiological applications includes, in addition to being easy, safe, rapid to administer and not requiring costly, complex equipment or extensive expertise or training, the requirement that outcome measures should be consistent and reproducible and bear a direct relationship to the outcome of interest (Hanrahan et al 1996).

For clinical purposes, there are three main areas of application:

- evaluation of therapeutic intervention;
- monitoring of disease;
- detection of risk groups.

Each area has requirements in common, such as the need for a measure to be standardised within-infant in the same way as for epidemiological measurements. Measurement variables should ideally be of low variability and error between manoeuvres within a measurement occasion, between differing equipment set-ups and between laboratories. This may require substantial efforts to standardise protocols, for example in applying similar pressures to the pleura in forced expiration measurements regardless of jacket style or efficiency (Section 6.4).

Evaluation of therapeutic interventions, assessing relative changes in a parameter rather than absolute values requires a measure with good short term repeatability. Because of time constraints the test should be rapid and fairly simple to apply. Ideally, the measurement of airway function should relate closely to the clinical situation i.e. airway calibre after administration of bronchodilators. For most interventions of clinical interest in wheezy infants, measures of forced expiration in conjunction with lung volume would meet these criteria, however there are severe limitations of time when measuring sedated infants and it is not possible to repeat lung volume measurements each additional dose in bronchial challenge.

The most common interventions in this group are bronchial challenge, using agents such as inhaled methacholine or histamine. There are potential disadvantages of using $V'_{\max,FRC}$ to assess either baseline airway function or response to intervention. Modest increases in airway tone or wall thickness may reduce airway calibre (and therefore specific conductance) while protecting against airway closure, resulting in normal, or even slightly elevated $V'_{\max,FRC}$. Similar paradoxical responses to challenge have been reported in both bronchoconstrictor and bronchodilator studies (Prendiville et al 1987b). An alternative mechanism is that if airway calibre is already diminished, then the measured response to bronchial challenge may be apparently exaggerated (Section 2.1.2.3). In addition, interpretation of results may

also be confounded by concurrent changes in lung volume, which is why it is particularly important to include this measure.

Monitoring of wheezing illnesses and assessing their outcome using relevant measures, as in the ECMO Respiratory follow-up infants, requires a measurement which is robust and stable with good long term repeatability. Ideally, the parameter should be applicable at any age, allowing the follow-up of a group of infants into childhood and beyond. As with the evaluation of therapeutic intervention, it is important to always consider airway function in relation to lung volume, as the two are interdependent. The most appropriate current approach would be a combination of measures of forced expiration such as $V'_{\max, \text{FRC}}$ or raised volume RTC parameters together with $\text{FRC}_{\text{pleth}}$ to detect any lung hyperinflation and, in future, there is the prospect of measuring the serial distribution of airway resistance using impedance by the high frequency interrupter technique (Frey et al 1998a).

For the identification of high risk groups, the comments regarding epidemiological applications also apply. The ideal test would have good long term repeatability but a low intersubject variability, increasing its sensitivity. It should be simple to apply outside the setting of a lung function laboratory i.e in wards, clinics or at home and in order to apply to large cohorts of infants, not require sedation. However, the physiological meaning of such tests is less important than in other areas. The tidal breathing parameters of t_{PTEF} and $t_{\text{PTEF}}:t_E$, interrupter resistance (Frey et al 1998b) and high frequency impedance measurements (Frey et al 1998a) come closest to meeting these criteria, although none are ideal.

7.3 Reference data: use and misuse

Reference values in physiology are normally used to establish whether an individual falls within the range expected for healthy subjects of similar age, gender, body size and ethnicity. The significance of values that fall outside the expected range will partly depend on the range itself: the repeatability of the measurement within an individual, the number of subjects and the methods used to construct the range. Reference values with scatter (where there is relatively poor repeatability due to biological variability, or a small sample size, or poorly standardised methods) may result in disease or abnormality remaining undetected, if the subject remains within the wide limits of "normal". Reference data collected by one centre may not be applicable to others, and inappropriate application may also result in

misclassification of an infant as “abnormal”. This is more likely to happen when there are differences in methods, population distribution by age and/or weight, ethnicity or background characteristics such as smoking exposure or atopy. A recent ATS/ERS statement on reference values for lung volumes (Stocks et al 1995) advised that reference values for use in any laboratory should be obtained by

- selecting an appropriate published set of values, or;
- establishing specific values for that laboratory, or;
- combining data from several laboratories, in which a standardised approach to techniques, methods of analysis, and characteristics of the reference population has been adopted.

There are particular problems experienced when attempting to apply these recommendations to infants and children under five. In infant measurements, which are normally performed with the use of sedation outside the neonatal period, there may be ethical problems in recruitment of normal subjects to studies where there is no benefit to the individual child. A related problem is the relatively low accrual of normal infants to research studies, with rates of 18-30% being reported in major epidemiological studies (Dezateux et al 1997). Recruitment, measurement and analysis in this field remains time consuming. The selection of reference groups within the context of infant respiratory function testing is therefore beset with problems and without extreme care use of reference ranges can obscure, rather than clarify, interpretation of observed values.

In this thesis, some obstacles were encountered when attempting to interpret outcome measures in the Respiratory Follow-up infants in relation to data obtained from different centres. Interpretation of plethysmographic data alone (FRC_{pleth} and sG_{aw}), using local and prospectively collected reference data, would have sketched a picture of a group of infants with overall mild impairment of airway function, ranging from normal to, in some cases, severe dysfunction. In contrast, $V'_{max,FRC}$ values were below the normal range of the USA control group in almost all infants and, if interpreted only in relation to that data, the Respiratory Follow-up group would have moderate to severe impairment of airway function, which was not supported by their reported clinical status. This section addresses some of the issues surrounding the

selection and interpretation of control (or reference) data that arose within the Respiratory Follow-up of survivors of the UK ECMO Trial.

Control data, for comparison of respiratory function with ECMO and CM infants, were selected from three separate populations. Ideally, a group of normal infants of similar age, body size and ethnicity would have been recruited and measured concurrently with the Respiratory follow-up group. Logistically, that was not possible but the plethysmographic control groups (controls and LRI controls) came closest to meeting that ideal, being measured at GOS under identical conditions. For $V'_{\text{max},\text{FRC}}$ control data, there were few published series available for comparison, the “gold standard” being that of the USA controls (Tepper et al 1993) which have been widely cited by other centres when a comparison from with normal infants is required. Although the data of Clarke et al (Clarke et al 1995) was not collected with the intention of producing a reference population, there were several reasons why these data were included within the comparison. Firstly, the infants were measured using the same inflatable jackets as LEIC, with similar equipment and identical RASP[®] software. Secondly, the methodology used for data collection and analysis was similar and well documented (Aston et al 1994, LeSouëf et al 1996). Thirdly, the population was from a comparable urban environment to that of many of the Respiratory follow-up infants with similar exposures and, although eligibility criteria included parental atopy, a large proportion of the study group (53%) likewise had a reported family history of atopy.

For plethysmographic data, infants were selected by age and grouped according to history of lower respiratory illness. The criteria used for selection of a reference group in this field have been reviewed by Dezateux et al (Dezateux et al 1996). The GAP conference (Taussig et al 1980) suggested that suitable paediatric reference populations would exclude those with chronic respiratory disease, URTI within three weeks prior to tests and more than incidental smoking experience. If similar criteria were applied to an infant population, potentially those with recurrent wheezing and passive smoke exposure may be excluded from control groups. Comparisons with a group such as those infants in the Respiratory follow-up, who had a high incidence of parental atopy and passive smoke exposure may then be biased.

For this reason, it was considered inappropriate to limit comparison to Respiratory follow-up only with infants who had not experienced any wheezing- the so called “squeaky clean” population, but to include the LRI controls as a separate group.

Infants comprising the control group for interpretation of $V'_{\max,FRC}$ values reported in the Respiratory follow-up study population- the UK and USA controls as mentioned previously- were selected by age. When infant characteristics were examined, although there were no significant differences between groups for weight or length at test, and the proportion of infants who were male and Caucasian was similar, age at test was greater in the USA controls. The UK infants were measured at around one year of age with a mean (range) 12.5 (12.0 - 14.0) months. In contrast, USA infants, having been recruited specifically for construction of reference values, when selected over the same age range as the Respiratory follow-up group had a wider distribution of age than either the UK control or study groups with a mean (range) of 15.2 (10.5 - 19.8) months.

Additionally, interpretation was complicated by the fact that there were significant differences between the UK and USA physiological data. The group mean difference in $V'_{\max,FRC}$ between the USA controls and the UK control infants was 76 mL.s^{-1} . Such differences were potentially attributable to:

- methodological approaches
- equipment and measurement conditions
- infant characteristics: either differences in small airway function or confounding variables such as body size, age, race and family history of atopy.

There were no published differences in methodological approach which would help to explain these findings. Clarke et al (Clarke et al 1995) reported that the Hammersmith jacket used in their study had a static pressure transmission of 56 to 80%, they applied jacket pressures in the range 2.5 to 8 kPa and reported $V'_{\max,FRC}$ from 8 to 10 technically satisfactory measurements at the optimal jacket pressure. Tepper et al (Tepper et al 1993) reported the mean of 3 highest technically acceptable measurements of $V'_{\max,FRC}$ with a maximum jacket pressure of 8 kPa using an arms out jacket. The static pressure transmission of the jacket was not stated. Moreover, although jacket styles and applied pressures varied between centres, the results obtained between GOS and LEIC had similar group means and

ranges, suggesting that similar driving pressures were applied and that flow limitation was normally achieved in this particular group of infants despite different jacket styles.

The greater values reported in the USA controls, could be related to maturation, either via somatic growth alone or possibly secondary to attainment of a standing posture. After around one year of age, as the infant chest wall stiffens with maturation, chest wall compliance has been reported to fall to proportionately the same values as that found in adults (Papastamelos et al 1995). This could potentially lead to an increase in resting lung volume (FRC) and hence $V'_{\max, \text{FRC}}$ relative to length in toddlers compared with less mature, but similar sized infants. Far greater numbers of healthy infants and young children between 1 and 2 years of age would need to be measured to test this hypothesis but inspection of the scatterplot of $V'_{\max, \text{FRC}}$ and length for USA controls (Figure 6-2) suggests such a trend.

As described previously, the UK controls all had a history of parental atopy, and were recruited prospectively during the neonatal period because they were thought to be at increased risk of wheezing during childhood. Those with a history of maternal asthma had significantly lower values of $V'_{\max, \text{FRC}}$ (Clarke et al 1995). This may have reduced the group mean $V'_{\max, \text{FRC}}$, relative to the USA controls, and a scatterplot of $V'_{\max, \text{FRC}}$ and length (Figure 6-2) reveals 8 infants in the UK group with values below 100 mL.s^{-1} suggesting some impairment of small airway function.

The absence of flows above 400 mL.s^{-1} in the whole Respiratory follow-up group could also be related to close attention to quality control within and between laboratories, with the rejection of all late forced expiratory manoeuvres in which peak flow was not attained by 50% expiration. The inclusion of these data would have markedly increased mean and highest values of $V'_{\max, \text{FRC}}$. The number of variables affecting interpretation of control values demonstrates the problem of not collecting control data from within each laboratory, in contrast to plethysmographic data, where although there were differences observed, these were of an order of magnitude that could be reasonably attributed to characteristics of the study population under investigation.

If translated into Z scores using the USA controls as a “gold standard” reference group, then the Respiratory follow-up group would be comparable to a population of

infants with chronic lung disease, but this was not evident in most infants within the study, with the majority being considered “normal” on respiratory examination and only 17 of 78 infants requiring any form of respiratory medication (inhaled steroids and bronchodilators). Three of 78 infants participating in the follow-up required domiciliary oxygen.

7.4 Conducting a multicentre trial with infant respiratory function outcomes

Few multicentre trials have been conducted with infant respiratory function parameters as outcome variables. There are many challenges facing participants in multicentre trials involving infant respiratory function, in a field where reference values and definitive methodologies remain to be established. We have attempted one such study and have found that as well as increasing the power of study, by enabling more infants to be followed up, other benefits have accrued: such as detailed sharing of measurement strategies and protocols leading to critical evaluation and improvement of established methodologies. These studies demand considerable commitment both in financial terms and with respect to time and energy- of participants, but may strengthen confidence in the results obtained.

Ideally, an assessment of analysis variability would have preceded this study but limited time and funding precluded this approach. Operator selection of the most suitable data for analysis, the relatively low signal to noise ratio of respiratory signals in infants, and the inherent biological variability of certain parameters, all increase within and between subject variability in this age group. Consequently, careful selection of a limited number of relevant outcome measures, together with recruitment of adequate numbers of infants are essential to achieve an adequate power of study (Dezateux et al 1996).

The lack of adequate commercially available equipment for infant respiratory function tests, with many centres relying on custom built systems, contributes to the problems of ready access to essential spare parts. An additional component of study design that is a prerequisite for future similar trials, is thus the need for equipment to be standardised during the trial period, and to have ready access to replacement parts, so that no disruption to data collection occurs during critical time frames.

During the course of this study, we became aware of the difficulties in imposing appropriate criteria to maintain stringent quality control within clinical trials, particularly when attempts were being made to follow up an entire cohort, as in this study. In such circumstances, the individual data from each infant are unique and cannot simply be replaced by recruiting additional subjects. These difficulties led to our decision to relax our standard criteria, which normally specify a minimum of 3 technically acceptable measures before reporting FRC_{pleth} , to allow the mean of 2 such measures to be reported, providing they were within 10%. Controversy remains over the number of measurements that should be used to report the mean for the various respiratory parameters commonly measured in infants (Stocks et al 1996). Further objective evaluation is needed, so that the time required for data collection can be minimised in infants without compromising accuracy.

During the current study, prior commitment to other ongoing studies at each centre compromised full standardisation, but in future similar trials even greater consistency could be achieved. This will require the development of improved software and measurement equipment and adherence to mutually agreed international standards, which are currently evolving (ERS/ATS Task Force on Infant Respiratory Function). Some of the essential features required for successful design of future multi-centre trials in this field are summarised in Table 7-1.

Table 7-1 Methodological basis for multi-centre trials

- Comparison, assessment and standardisation of equipment
- pilot study to assess accrual rate and validate protocol
- Power of study calculation, based on the number of outcome measures, knowledge of within subject variability of each parameter and estimated sample size
- Frequent inter-laboratory visits during trial period
- Identical measurement and analysis protocol using similar software
- Exchange of raw data and cross-analysis of at least a sub sample of data
- Examination of population characteristics for between-centre bias

The study design presented here, despite the advantages presented, was time consuming and would not have been feasible with the available software had more

than a couple of laboratories participated. Nevertheless, the ability to record all raw data and compare a random selection may provide the means of improving quality control both within and between laboratories in future studies. This could be as relevant to studies of respiratory function in children and adults as those in infants. The selective use of appropriate, automated and validated software could potentially speed data analysis and reduce within subject and inter-observer variability (van der Ent et al 1996). Unfortunately, reproducibility does not equate to accuracy, and arbitrary exclusions to achieve low within subject variability may not provide a faithful representation of the infant's respiratory function. Some compromise is therefore needed whereby obvious artefacts are excluded while still retaining physiologically accurate data. While a fully automated system could ensure virtually identical results on reanalysis in a multi-centre setting which would be operator independent, infant signals, with their high variability and low signal to noise ratio, often require interactive operator input for correct interpretation. Consequently, it is unlikely that assessments of respiratory function in infants will ever become as routine as those in older subjects, and will continue to require operators with extensive training and experience if successful measurements are to be achieved.

In conclusion, a multicentre approach to trials with parameters of infant respiratory function as outcome measures appears feasible providing that close attention is paid to study design, and that participants in such trials maintain a standard approach to data collection and analysis. Collaboration of this nature, despite requiring considerable time and effort, is of great benefit with respect to quality control within, as well as between, specialised infant lung function testing centres. Furthermore, there is increasing recognition of the need for multicentre studies of infant respiratory function if we are to achieve sufficient statistical power to address clinically relevant questions regarding respiratory disease during early childhood over a realistic time period.

7.5 Conclusions and future directions

During the gestation period of this thesis, infant lung function testing has also been evolving. At the inception of this thesis there was widespread interest in applying simpler methods of measurement, possibly without the requirement for sedation, and workers in this field were well aware of the need for standardisation of both methods and equipment.

At the same time, the continuing development of specialised measurement devices and information technology was offering the potential opportunity for wider availability and (eventually) lower cost of equipment for use in this age group.

Although the simpler approaches to assessing respiratory function in infants have largely failed to live up to their early promise, much useful work has arisen from this, and similar studies. An improved understanding of the limitations of these techniques, particularly when applied to infants with airway disease, has been attained by critically evaluating their underlying assumptions. This has limited the continued inappropriate application of these techniques particularly when incorporated within automated measurement “carts”, used by clinicians with a poor understanding of the underlying physiology upon which these techniques rely. There is no doubt that measurements within the infant population will always be time consuming and complex, relying on the efforts of the individual investigator for an often fairly limited result! Despite these very real limitations, much can still be achieved of real benefit if meticulous attention to detail is given and a carefully thought out protocol is applied.

The ideal test of infant respiratory function would have a robust outcome measure that would be applicable not only during infancy but also continuing into early childhood and adult life. While conventional timing indices, such as FEV₁, are not applicable during early infancy since few young infants have a duration of forced expiration >0.5 s, nevertheless, this is an area where future research is needed.

The relative advantages of the tests evaluated in this thesis have been discussed (Section 7.2.6). Most protocols should include methods of measuring both airway function and lung volume, since there is a close relationship between elastic lung recoil, the volume at which the lungs are held and baseline airway calibre. Ideally, an

outcome measure should relate closely to the particular study question, and be physiologically meaningful i.e the physiological determinants are well characterised. The closer to “basic science” a measure of lung function is, the easier it is to interpret changes in that measure. Measurements of airway wall mechanics using the high frequency interrupter technique have shown great promise, but are still in the early stages of assessment and may show rather high variability for application as a clinical tool (Frey et al 1998b). The raised volume rapid thoraco-abdominal compression technique (RVRTC) has been briefly discussed, and is another method that shows promise, particularly as it offers the prospect of selecting an index of lung function that can be used throughout infancy into childhood and beyond. The optimal index has not yet been determined but, like the RTC technique, it offers the benefit of not relying on assumptions that may not be applicable in disease and may actually perform better in this population than in healthy infants where flow limitation may not be reached and variability is greater. Gas mixing indices offer another potential approach particularly as results are not related to age or body size and may be more robust measurements of respiratory mechanics (Gustafsson et al 1998b, Gustafsson et al 1998a). There is a need for a more multidisciplinary approach within the infant respiratory field combining some of these newer approaches to mechanics with gas mixing, ventilation-perfusion distribution and inflammatory markers to further characterise disease processes.

Future directions for research include the “dark ages” of the pre-school years where the difficulties in developing techniques that are suitable for children too old to sedate yet too young to co-operate have so far held back progress. Newer methods such as gas mixing indices and interrupter techniques may offer the potential to assess lung function more reliably in this important group.

The role of clinical lung function testing within the intensive care setting also requires further attention in order to justify its place in the management of ventilated neonates and infants. Again, progress in this field is reliant on the continuing co-operation of commercial manufacturers of ventilators and monitors to invest in research and development and to allow access to the underlying algorithms that are used to generate flow, volume and pressure data.

The critical importance of selecting a suitable reference group with which to interpret changes in disease was demonstrated (Section 7.3) and the issues surrounding the interpretation of reference values was discussed. Although the Respiratory follow-up of infants from the Collaborative ECMO trial would still have been valid had only outcome parameters between infants allocated to ECMO or conventional management been examined, the study design was improved, and the conclusions strengthened, by the comparison with reference groups. The benefits of a control group matched for age and sex and measured during a similar time frame with identical equipment were demonstrated by the “plethysmography group” comparison, in contrast to the “ $V'_{max,FRC}$ ” groups measured in different laboratories with whom direct comparison was difficult. Ideally, a group of control infants would have been measured prospectively although constraints of time and money did not allow this.

For large, multi-centre collaborative trials using infant respiratory function outcome measures it is probably mandatory to recruit such a group, until such time as adequate standardised reference values are available for the most useful tests. The difficulties of recruiting control infants have been referred to, and research proposals should include sufficient requests for funding, and allow adequate time to accrue sufficient numbers of healthy controls to realise the full potential benefits of a collaborative trial.

Collaborative trials require a common approach to measurement and analysis of results by participating centres and these issues were considered in Section 7.4. The potential of such trials is probably the most exciting prospect to arise from the studies presented in this thesis, with the limitations of single centre work being well recognised. Collaborative trials rely on an infrastructure of equipment and software (and training of investigators) common to all centres, even before a study protocol has been developed. There is now a realistic prospect of this occurring, with funding from the European Respiratory Society underpinning the development of hardware and software, in collaboration with an established manufacturer, to be commercially available in the short term (Frey et al 1999). The aim of the ERS task force is to summarise what is currently considered to be good laboratory practice, and to offer recommendations for both users and manufacturers of infant lung function equipment and software. The recommendations have been developed after extensive

communication on an international level and are directed towards future developments in this field, including the use of more automated equipment than has been used in many research centres in the past. Hopefully they will facilitate development of, and co-operation between, centres where infant respiratory function testing is undertaken.

Appendix A

Pilot study for the UK Collaborative ECMO trial Respiratory follow-up

Aims

The aims of the pilot study were to:

- assess the accrual rate of survivors of the UK collaborative ECMO trial to Respiratory follow-up and the numbers attending each test centre, which were the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust (GOS) and Leicester Royal Infirmary (LEIC).
- evaluate the initial measurement protocol
- determine the inter-observer variability of analyses

Methods

Accrual rate

The administrative side of recruitment was undertaken by staff involved in the collaborative ECMO trial based at the National Perinatal Epidemiology Unit in Oxford. The referring paediatrician was contacted prior to approaching parents of survivors in order to establish the current status of the infant. Infants with congenital heart disease, gross developmental problems or extremely adverse social circumstances were excluded from the Respiratory follow-up. Parents who elected to take part were given their choice of venue, and their details were then passed onto either GOS or LEIC, when arrangements were made for clinical assessment and respiratory function testing by each centre. The numbers of infants attending each centre during the pilot study, and those not referred by the NPEU were noted.

Measurement protocol

On arrival in each laboratory, infants wore a smock of high neck design to obscure any neck scars received during ECMO treatment. Clinical assessment was undertaken by a paediatrician who also remained blind to the status of each infant. Attempts were made to avoid measurements within 3 weeks of the onset of a respiratory tract

infection at GOS (4 weeks in LEIC). A baseline S_pO_2 was recorded and the infant was weighed, wearing only the smock, then sedated with triclofos sodium (GOS) at 100 mg.kg⁻¹ or chloral hydrate (LEIC) at 80-100 mg.kg⁻¹ before being fed, and made comfortable (Gaultier et al 1996). Once asleep, the infant was wrapped in an adjustable inflatable jacket and a face mask and PNT applied over the nose and mouth in order to measure maximum flow at functional residual capacity ($V'_{max,FRC}$) (Section 3.3.6). The inflatable jacket was then loosened and measurements of FRC_{pleth} and R_{aw} were then made according to criteria described in Sections 3.3.4 and 3.3.5 at GOS, and with similar criteria at LEIC (Beardsmore 1993, Beardsmore 1995, Beardsmore et al 1994, Hampton et al 1991).

Following completion of measurements, the infant's length was recorded (Section 3.1.4.1), and infants were allowed home, once awake. At GOS, parents were given an information leaflet with a contact telephone number to use should they have any questions or concerns after arriving home.

Analysis

Respiratory function measurements were analysed by the test centre (GOS or LEIC) then each centre exchanged data by disk, soon after measurements were complete. Results were analysed by both the test centre (TC) and the analysis centre (AC). Data were double entered on an Excel spreadsheet (version 5.0, [®]Microsoft Corporation), checked for entry errors, and compared between the two centres. Unpaired t-tests and a two sample test for proportions were used to compare baseline characteristics and lung function parameters between groups of infants attending each test centre.

Results and discussion

Accrual rate

Over the 7 month pilot study period, 38 of the survivors of the UK collaborative ECMO trial were of a suitable age for Respiratory follow-up. Included were several young children who had reached one year of age before the follow-up was underway, accounting for the relatively large numbers in the pilot study. Of 38 survivors, 2 were excluded due to CHD, one due to gross developmental impairment and 4 due to either parental refusal (2 infants) or inability to attend before the child was 18 months old (2 infants). The follow-up rate was therefore 81% of all surviving infants, reflecting the commitment of both NPEU staff and parents to the Respiratory follow-up and

suggesting that the study population would be an adequate sample of all surviving infants. Approximately equal numbers of parents elected to attend each centre, avoiding potential bias in measurement and reporting of results by one centre. Infants attending GOS travelled from all over Britain and Northern Ireland demonstrating that it was feasible to recruit this population, although travel expenses were high.

Measurement protocol

Of the 31 infants within the pilot study, 15 attended GOS and 16 LEIC. Background details of the infants are shown in Table A-1. Both groups were of similar age at test, weight and length ($p>0.10$ for all parameters). The majority of infants recruited during the pilot study were male :66% at GOS and 81% at LEIC. One infant in each group had documented respiratory symptoms when measured, although a greater proportion of LEIC infants were reported to have had a recent URTI (56 vs 7%). Although attempts were made to avoid measurements within 3-4 weeks of URTI onset, this was not always possible and data from infants having recent URTI symptoms, particularly inspiratory R_{aw} , were consequently interpreted with caution.

Of 15 infants attending GOS, the measurement protocol was completed in 12, the remaining 3 infants awoke during measurements and could not be settled back into quiet sleep. One further infant attended twice to complete the measurement protocol. There were no sedation failures within the LEIC group, reasons for failure being technical: insufficient humidification within the rebreathing bag for measurements of R_{aw} (3 occasions) or poorly fitting jackets for $V'_{max,FRC}$ measurements (1 occasion).) The age at testing of the infants attending GOS who woke early was from 12 to 19 months which may have partially explained these results. Consequently, the dosage of triclofos sodium was increased at GOS from $100 \text{ mg} \cdot \text{kg}^{-1}$ to $150 \text{ mg} \cdot \text{kg}^{-1}$ (equivalent to $100 \text{ mg} \cdot \text{kg}^{-1}$ chloral hydrate (Gaultier et al 1995)), although $125 \text{ mg} \cdot \text{kg}^{-1}$ was the usual revised starting dose, with a maximum dose of 1500 mg.

Exchanging data on disk between centres highlighted the need for meticulous attention to quality control during both calibration and data collection. Both centres agreed to record known signals of flow, airway opening pressure, time constant of the plethysmograph and V_{pleth} to demonstrate correct calibration of signals. Data requiring recalibration after measurements e.g. V_{pleth} was recalibrated at the Test centre and sent to the Analysis centre noting the correct files to use for analysis. Minor adjustments to customary practice were made by both centres following the pilot study which

included: recording test occlusions and several post manoeuvre breaths to demonstrate absence of face mask leaks on disk, performing only one forced expiratory manoeuvre during each epoch of data collection, performing only end inspiratory FRC_{pleth} manoeuvres (Eber et al 1992, Stocks et al 1996, Beardsmore et al 1982) and ensuring that RASP profiles were configured to give sufficient length of savesets at each chosen sampling rate (i.e. 36s at 100 Hz) to record pre and post manoeuvre tidal breathing. The aim of these adjustments was to facilitate analysis by an operator who was blinded to measurement conditions and required evidence of quality control during data collection. The overall success rate of pilot measurements, although lower than in similar studies reported elsewhere in this thesis (Dundas et al 1995, Dezateux et al 1997 (a), Dezateux et al 1994), demonstrated that it was feasible to apply the amended protocol in this particular group of survivors of neonatal respiratory failure, using the outcome measures of lung volume and airway function selected at the outset as the most relevant.

Table A-1 Pilot study infant details

	GOS n = 15	LEIC n = 16
Age (mo)		
mean	14.2	14.1
SD	2.8	2.5
range	10.7 - 18.6	11.7 - 19.9
test weight (kg)		
mean	10.6	10.5
SD	1.4	1.8
range	8.3 - 12.5	7.0 - 13.2
test length (cm)		
mean	79.2	78.1
SD	3.2	4.3
range	73.1 - 85.0	66.8 - 83.1
sex % male	66	81
gestational age (weeks)		
mean	39.1	40.2
SD	2.8	1.5
range	36 - 42	38 - 42
birthweight (g)		
mean	3.3	3.6
SD	0.8	0.6
range	2.3-4.6	2.4-4.5
recent URTI %	6.7	56.3
symptomatic %	6.7	6.7
normal exam %	87	75

Analysis

The pilot study results are summarised in Table A-2, giving group mean values of outcome measures and tidal breathing parameters analysed at each TC and AC, and displayed as scatterplots of TC and AC for each parameter for the whole group of 31 infants (Figure A-1). The results are shown as initially analysed, before any methodological problems or between centre discrepancies were addressed.

Infants tested at GOS had a larger V_T : 97.5 mL and 79.0 mL for GOS and LEIC respectively. FRC_{pleth} was also greater at GOS: 338.4 mL than LEIC: 283.6 mL. The differences were present when analysed by each centre, and were considered to be related to dead space and/or apparatus resistance together with the GOS practice of leaving the face mask applied for longer periods of time than was the custom at LEIC. In fact, subsequent assessment of the plethysmograph using a lung model revealed a

systematic error affecting GOS data due to failure to subtract deadspace of transducer tubing and mask. This was readily corrected and a short additional protocol was developed to investigate the V_T between centre differences. Values of R_{aw} during both initial inspiration and late expiration were higher at GOS, although these values did not reach significance, and were not explained by the lung volume correction which when subsequently applied, served to slightly increase, rather than decrease, R_{aw} . Group mean values of $V'_{max,FRC}$ were similar between centres: 145.4 (SD 57.8) mL and 136.5 (SD 53.0) mL for GOS and LEIC infants respectively.

Table A-2 Pilot study respiratory function results

Test centre	GOS data	Leic. analysis	Leic. data	GOS analysis
$V_T \cdot \text{kg} (\text{mL} \cdot \text{kg}^{-1})$				
mean	9.4	9.3	7.8	7.8
SD	1.6	1.5	1.1	1.1
range	7.1 - 11.8	7.2 - 11.3	5.8 - 10.4	4.9 - 10.3
$RR (\text{min}^{-1})$				
mean	31.8	31.8	34.8	34.5
SD	4.1	4.0	13.2	13.3
range	27.9 - 39.2	24 - 38.5	24.9 - 73.5	24.1 - 73.6
$V'_{max,FRC} (\text{mL} \cdot \text{s}^{-1})$				
mean	136.5	145.4	141.5	145.6
SD	53.0	57.8	84.4	86.1
range	66.8 - 252.4	71.0 - 255.0	28.0 - 323.0	27.2 - 319.8
$FRC_{\text{pleth}} \cdot \text{kg}^{-1} (\text{mL} \cdot \text{kg}^{-1})$				
mean	33.6	33.7	26.7	27.7
SD	6.4	6.6	8.9	9.7
range	23.2 - 48.3	23.5 - 49.5	15.7 - 49.6	15.7 - 51.3
$sG_{aw,II} (\text{s}^{-1} \cdot \text{kPa}^{-1})$				
mean	0.139	0.140	0.235	0.225
SD	0.049	0.053	0.161	0.137
range	0.081 - 0.296	0.085 - 0.234	0.062 - 0.517	0.069 - 0.536
$sG_{aw,EE} (\text{s}^{-1} \cdot \text{kPa}^{-1})$				
mean	0.133	0.126	0.171	0.194
SD	0.071	0.066	0.086	0.122
range	0.052 - 0.296	0.057 - 0.255	0.060 - 0.633	0.054 - 0.480

The initial goal of inter-observer analysis of each infant respiratory function measurement was to reach agreement on values calculated by each centre to within 5% for FRC_{pleth} , V_T and RR , 10% for $V'_{max,FRC}$ and 20% for R_{aw} , or sG_{aw} (Dezateux et al 1994, Dundas et al 1995) reflecting the relative intrasubject variability for the various parameters.

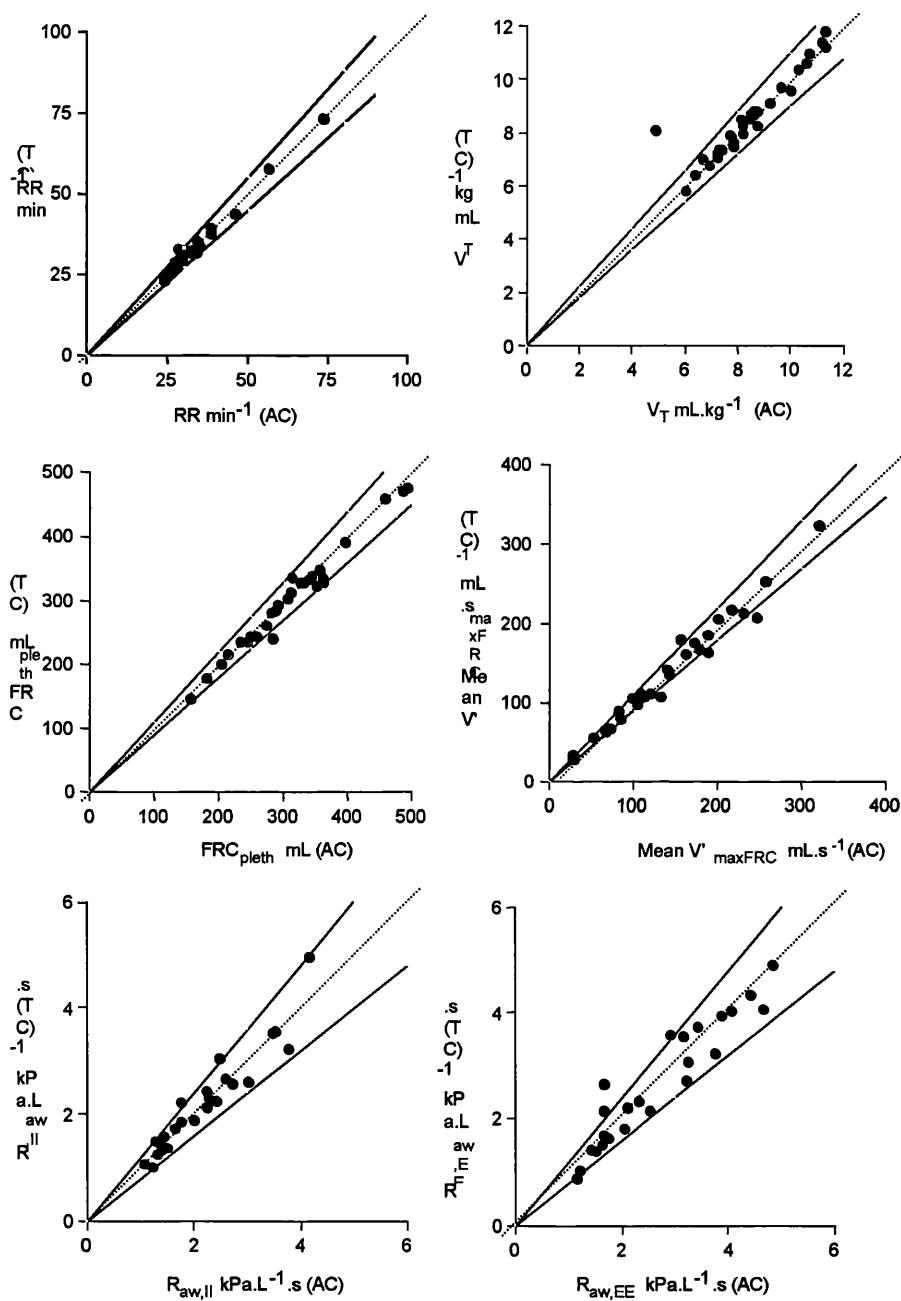
Inter-observer analyses of the pilot study data are shown in Figure A-1. The solid lines in each plot show the 5,10 or 20% agreement boundaries for each outcome measure and tidal parameter, where TC results were expressed as a percentage of AC results. Where agreement was not within agreed limits, analyses were examined and amended where appropriate.

For V_T and RR, reported values were generally based on the first 5 epochs of data collected during measurements of forced expiration. The between centre differences seen in two infants were due to selection of different data. In both these infants, data from the first 5 epochs were of a markedly different respiratory pattern from later data and the Analysis centre (GOS) selected later data with more a physiologically appropriate V_T which appeared more representative of that displayed during the subsequent measurement session. Differences were resolved by each centre reporting the later data.

The discrepancy in FRC_{pleth} was greater than 5% for 5 infants. All but one were, however within 10% of both centres analyses. Differences arose from data selection, where insufficient technically acceptable manoeuvres had been collected at end inspiration (2 infants), from either failing to “truncate” efforts with a degree of glottis closure (2 infants) or inappropriate truncation of respiratory efforts (1 infant). One infant woke immediately following the 3rd FRC_{pleth} manoeuvre and the analysis was failed by the AC. Differences were resolved by agreeing to report the result based on 2 reproducible manoeuvres (including an end expiratory occlusion) collected during the pilot study, and by excluding periods of mild glottic closure from analysed respiratory efforts, if necessary, to enable reporting of results. However, the protocol was subsequently amended to perform all occlusions at end inspiration.

In 4 infants, values of R_{aw} were outside agreed limits of 20% agreement, one affecting initial inspiratory values and 3 late expiratory values. A limitation of the algorithm on which RASP analysis of R_{aw} was based was the calculation of P_{pleth}/V' values at one sample point rather than a regressed or mean value. This increased the intrasubject variability of reported data both within and between centres. To minimise variability, it was agreed that the TC should indicate which savesets or epochs of data to use for analysis, based on pre-existing criteria (Dezateux et al 1991) and to report on a minimum of 5 and maximum of 7 individual breaths. By appropriate selection of data, prior differences in calculated results were resolved within the agreed limits.

Figure A-1 Pilot study comparison of test centre and analysis centre results



Key to Figure A-1: RR: respiratory rate; V_T : tidal volume; FRC_{pleth} : plethysmographic functional residual capacity; $V'_{\text{max,FRC}}$: maximum flow measured at FRC; $R_{\text{aw,II}}$: airway resistance at initial inspiration; $R_{\text{aw,EE}}$: airway resistance at end expiration. Dotted lines represent line of identity and solid lines represent % differences between groups

In 5 infants, reported values of $V'_{\max,FRC}$ differed by 10% to 20% between centres. On examination of analyses from each centre, the differences (which were as low as 7 mL.s^{-1} with respect to absolute flows) were explained by differing tidal volume filtering strategies. When data were reanalysed having determined the FRC baseline by regressing over 5 breaths prior to forced expiratory manoeuvres, rather than by selecting a fixed representative end expiratory level, the differences were reduced to within 10%.

7.5.1 Summary

- Accrual to the pilot study was satisfactory, with 80% follow-up of survivors, and families, electing to attend each centre in approximately equal numbers.
- The measurement and analysis protocol was amended following the pilot study, increasing sedation quantities at GOS and reducing the variability between centres during data collection and analysis.
- Respiratory function results showed a spectrum of abnormalities, ranging from those with little or no impairment to those with marked changes in lung volume and airway function

7.5.2 Power of study calculation

At the inception of the Respiratory follow-up, approximately 100 infants per year were entering the main trial, with a mortality rate of around 50%, over the three year trial period. Therefore 150 infants were available for follow-up of whom 80% could be expected to attend. It was not known, however, whether numbers in the two management groups (ECMO and CM) were equal. By January 1996, the data monitoring committee of the collaborative ECMO trial reported initial results showing that there was a survival advantage within the ECMO group (32% vs 59%, conventionally managed and ECMO respectively). Therefore, with an accrual rate to follow-up of 80%, of the 185 infants entered onto the main trial, approximately 24 conventionally managed infants and 44 infants who were allocated to ECMO would comprise the study population. To detect a difference in each parameter equivalent to 1 SD, with 95% power at the 5% level, a minimum of 20 infants would be required in each group. One SD for each parameter, based on the pilot study data, was approximately 8 mL.kg^{-1} for $\text{FRC}_{\text{pleth}}$, 72 mL.s^{-1} for $V'_{\max,FRC}$ and 0.09 ($\text{s}^{-1}.\text{kPa}^{-1}$) for sG_{aw} .

An additional adjustment was required to the power of study calculation to allow for the selection of 3 outcome measures and a slightly lower power of study was selected to trade off the far larger numbers of infants that would have been required in each group.

Therefore 30 infants were needed in each group to detect a significant difference in any of the 3 outcome measures, with 85% power of study at the 5% level.

Appendix B

Equipment assessment

Introduction

Greater demands are placed upon equipment used in these measurements than for adult work. For example, minaturisation of equipment is a compromise between minimising apparatus dead space and consequent increase in apparatus resistance.

Although an appreciation of the underlying theory is necessary for collection and interpretation of infant lung function data, it is also important to characterise and quantitate errors that are specific to the equipment used in infant lung function measurements. Equipment should be assessed as used during measurements, as the entire system may be degraded by a single component, such as a non standard connector or overly compliant tubing (Jackson et al 1995).

In physiological measurements, equipment (transducers and their attachments) must be able to faithfully produce an electrical analogy over the entire range of the physical changes being measured. This requires:

- Calibration, also known as static response, it describes a predictable relationship (normally linear) between the electrical output of the transducer and the magnitude of the physical parameter over the full range of the physical change. A description of routine equipment assessment and calibration of equipment used in this study may be found in section 2.4.
- Dynamic response: the dynamic response of a system is an assessment of its ability to measure rapidly changing parameters. The dynamic response of a transducer becomes important when the variable being measured changes significantly during the time that the measurement is being made (Jackson 1984), which is certainly true of the physiological parameters measured in infants. All transducers require some physical displacement in order to generate an electrical signal. In practice, the dynamic response of the electronic components is well above that required, and the major determinants of the response in gas filled systems are the displacement characteristics of the transducer coupled with the compressibility of the gas and the length and diameter of the connecting tubing. Most differential pressure

transducers may be modelled as second order systems, where the relationship between the change in diaphragm displacement and chamber pressure may be modelled as a compliance, the movement of the gas through the connecting tube as a resistance, and the mass of gas moving to and fro in the transducer tubing, which has some finite mass that must be accelerated, as inertance. The dynamic performance of a transducer is determined by these characteristics.

Assessment of frequency response

The ability of a measuring device to accurately estimate physical signals changing at various frequencies is known as its frequency response, and can either be assessed by response to a step function such as a balloon burst, or by assessing response to a periodic signal with appropriate frequency content. The frequency response of the flow and plethysmographic pressure transducers used in this thesis were assessed, using the pressure chamber method described by Vallinis (Vallinis et al 1993).

The apparatus consisted of an airtight 81 L rectangular box separated in the middle by a 30cm acoustic suspension loudspeaker, the membrane of which had been sealed with latex to reduce porosity. The test transducer was connected to one chamber and a reference transducer (Validyne MP45 ± 2 cmH₂O) was connected to the other with a wide bore connector. The frequency response of the reference transducer had been shown to be adequate in amplitude and phase to at least 40 Hz with this configuration (Vallinis et al 1993). The loudspeaker was driven by a sinusoidal wave form produced by a signal generator (Brookdeal Signal Source Type 471) connected to a low frequency power amplifier to generate sinusoidal pressure changes within the box. The volume displaced by the speaker movement was in the order of 200 mL per cycle. The volume of air entering or leaving the chamber through the attached flow transducer was negligible in comparison to the displacement volume, such that there was no distortion of the sinusoidal pressure wave form. The flow through the flow transducer was directly proportional to the pressure within the chamber. The amplifier was adjusted to keep the reference transducer pressure constant, which gave peak flows through the transducer of ≈ 130 mL·s⁻¹ which was within the manufacturer's stated working range. The direct current voltage outputs of the test and reference transducers were displayed on the X and Y axes respectively of a memory oscilloscope (Tektronix Model 2201). When the sinusoidal signal was

applied and the reference signal displayed on the Y axis, together with the test signal on the X axis, a Lissajou loop was generated (Figure B-1). With a frequency of 1 Hz from the signal generator, the gain of the reference transducer was adjusted so that the peak to trough voltage outputs of the test and reference devices were equal. The frequency was then increased by one Hz steps to 10 Hz. Any loss of amplitude (attenuation) and time delay (phase lag) was measured as shown on Figure B-1.

The following equipment used for studies reported in this thesis was assessed using the pressure chamber method of Vallinis et al:

- Validyne MP45 transducer $\pm 2 \text{ cmH}_2\text{O}$ used for measuring flow;
- Validyne MP45 transducer $\pm 2 \text{ cmH}_2\text{O}$ used for measuring plethysmographic pressure;
- Fleisch "0" pneumotachograph;
- Fleisch "1" pneumotachograph;
- Fleisch pneumotachographs sizes "0" and "1" with plethysmographic shutter block and connectors attached.

At frequencies up to 10 Hz, there was no phase lag or attenuation seen for the Validyne transducers and Fleisch pneumotachographs as listed above. When the shutter block and connectors were added, the frequency response was degraded slightly above 3 Hz with a phase lag of approximately 30 degrees.

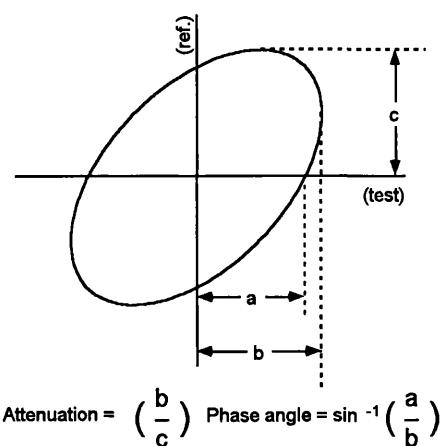


Figure B-1 Assessment of frequency response

Calculation of attenuation and phase angle from a Lissajous loop. The signal from the reference transducer (ref.) is plotted on the y axis and that from the transducer being tested (test) on the X axis. From Vallinis et al (Vallinis et al 1993).

Assessment of apparatus linearity

Respiratory equipment is specified to function over a certain range, and an instrument is regarded as linear when incremental changes in input and output are constant over the specified range. The working range of each piece of equipment should be known, and its linearity checked periodically.

Assessment of flow

The Fleisch pneumotachographs size “0” and “1” as used in studies reported in this thesis were assembled and calibrated as for normal respiratory function measurements. Known flows were then delivered via a calibrated rotameter (KDG 1100, Sussex, England) in step-wise increments in both directions through each PNT. The process was then repeated with the shutter block and connectors assembled for each PNT. An example of a plot of measured flow vs delivered flow for a Fleisch “1” PNT is shown in Figure B-2.

Assessment of airway opening pressure

Linearity of the transducers for both airway opening pressure and jacket pressure was assessed by delivering both positive and negative pressures in step-wise increments, using a water filled manometer. Measured pressure was plotted against delivered pressure, as shown in Figure B-3. The linearity of both transducers was satisfactory within the operating range of each transducer.

Figure B-2 Linearity of flow measured using a Fleisch “1” PNT

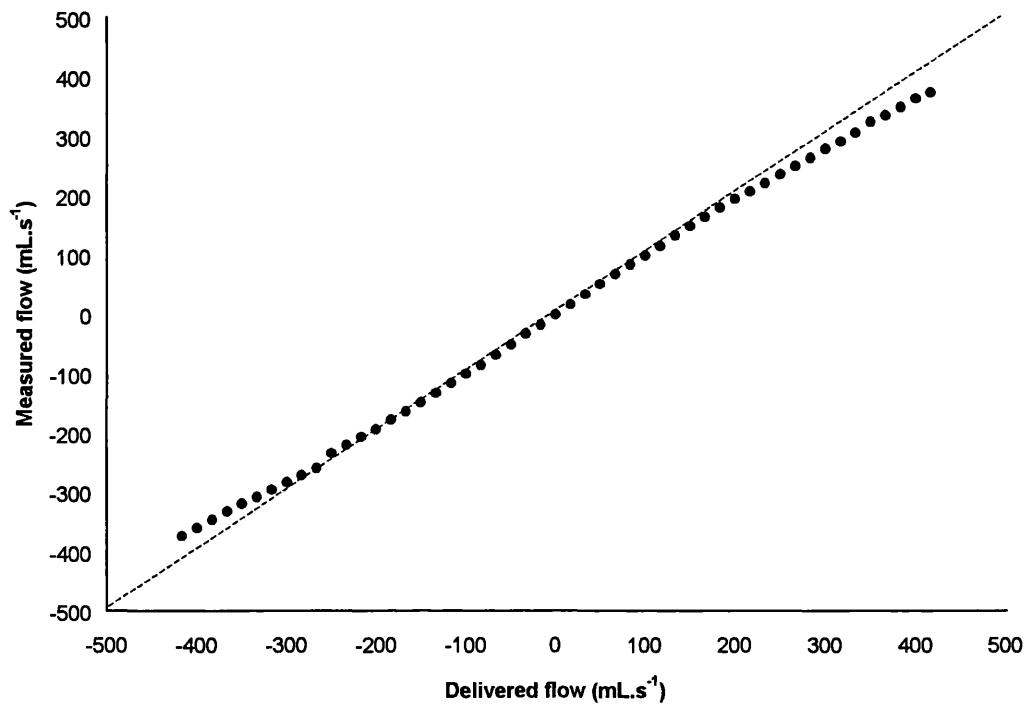
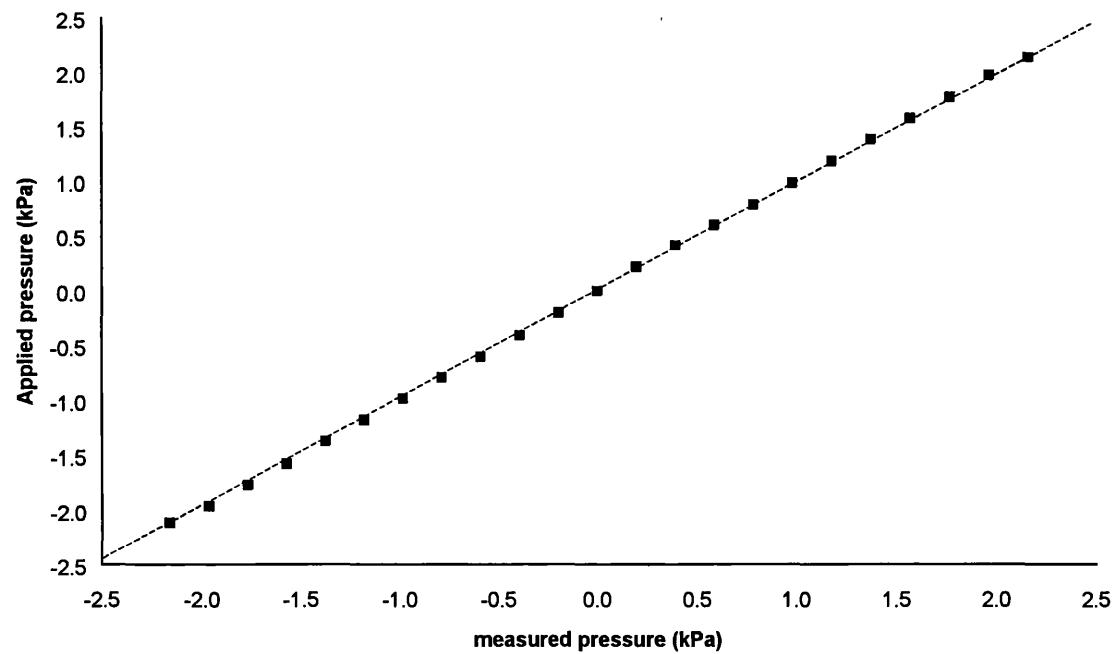


Figure B-3 Linearity of airway opening pressure



Assessment of apparatus resistance

The resistance of the apparatus (R_{app}) used for studies reported in this thesis was assessed for PNTs alone (Fleisch sizes “0” and “1”) and with the plethysmographic shutter block connected as for measurements, in both inspiratory and expiratory directions for each configuration (Figure 3-3).

A large gauge needle was inserted into the flow delivery tubing, close to the PNT, and connected to a Validyne differential pressure transducer ($\pm 2\text{cm H}_2\text{O}$). The pressure drop at this point was measured while flow through the rotameter (KDG1100) was set at between 300 and 400 mLs^{-1} . Flows were then reduced smoothly down to zero, recorded using RASP at 200 Hz. Data was exported to Excel version 5.0 and plots of apparatus resistance (pressure measured at the needle insertion point/ flow) versus flow were created.

There was little difference in measured values of R_{app} according to the direction of gas flow (“inspiratory” or “expiratory”) through the equipment for the PNT alone, or when including the plethysmograph shutter block (Table B-1). The addition of the plethysmographic shutter block markedly increased R_{app} , with a small increase in measurements through the “TGV” port, relative to the “RAW” port, probably due to the angular configuration of the shutter block (Figure 3-3, Table B-1). A selection of the scatterplots obtained from these measurements is shown.

Table B-1 Apparatus resistance

Equipment	Apparatus dead space presented to infant PNT [PNT-shutter app.]* (mL)	R_{app} measured at 100 mL.s^{-1} ($\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$)		
		PNT alone	PNT and shutter apparatus “TGV” port	PNT and shutter apparatus “RAW” port
Fleisch “0” PNT (insp)	2.5 [7.6]	0.36	0.69	0.64
Fleisch “0” PNT (exp)	2.5 [7.6]	0.37	0.95	0.67
Fleisch “1” PNT (insp)	10 [26]	0.15	0.48	0.35
Fleisch “1” PNT (exp)	10 [26]	0.17	0.58	0.38

*not including face mask

Figure B-4 Apparatus resistance plotted against flow for a Fleisch 0 PNT in the inspiratory direction

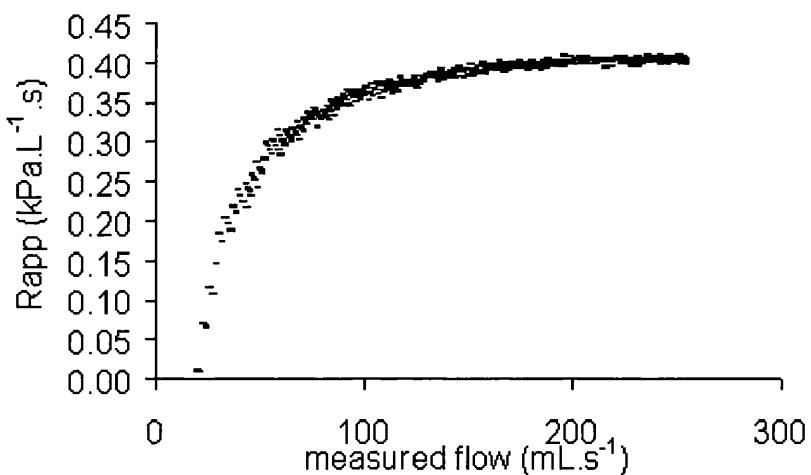


Figure B-5 Apparatus resistance plotted against flow for a Fleisch 0 PNT in the expiratory direction

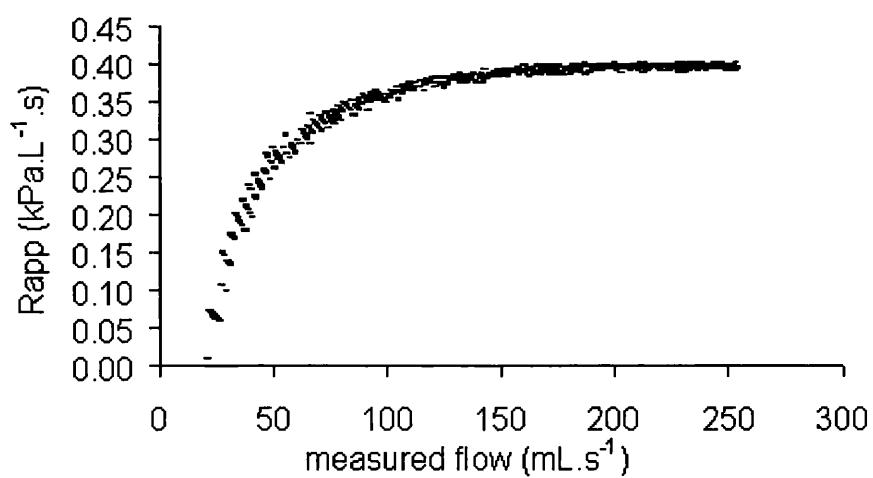


Figure B-6 Apparatus resistance plotted against flow for a Fleisch 1 PNT in the inspiratory direction

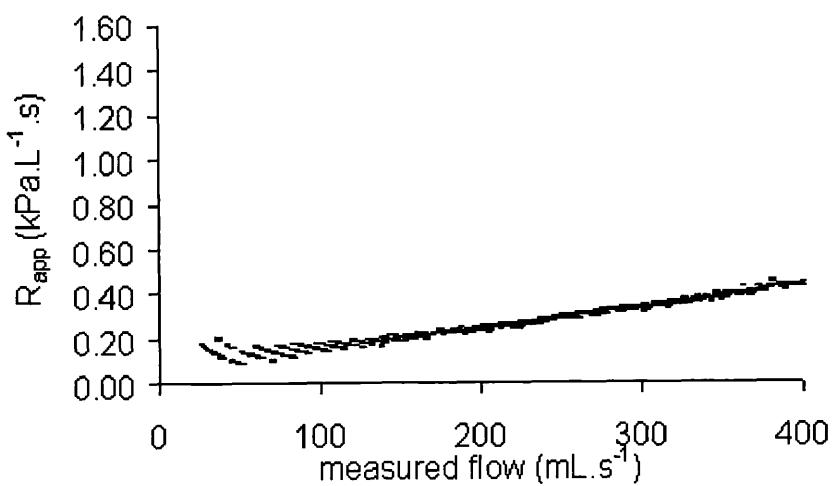


Figure B-7 Apparatus resistance plotted against flow for a Fleisch 1 PNT in the expiratory direction

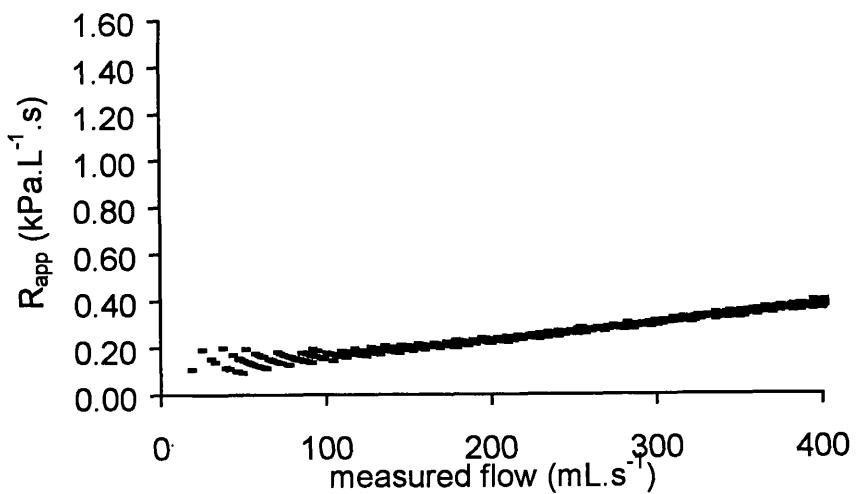


Figure B-8 Apparatus resistance plotted against flow for a Fleisch 1 PNT and plethysmograph shutter block in the inspiratory direction through the "TGV" port

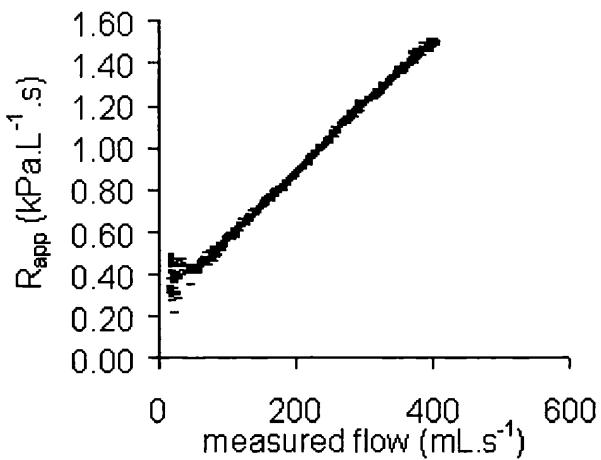


Figure B-9 Apparatus resistance plotted against flow for a Fleisch 1 PNT and plethysmograph shutter block in the expiratory direction through the "TGV" port

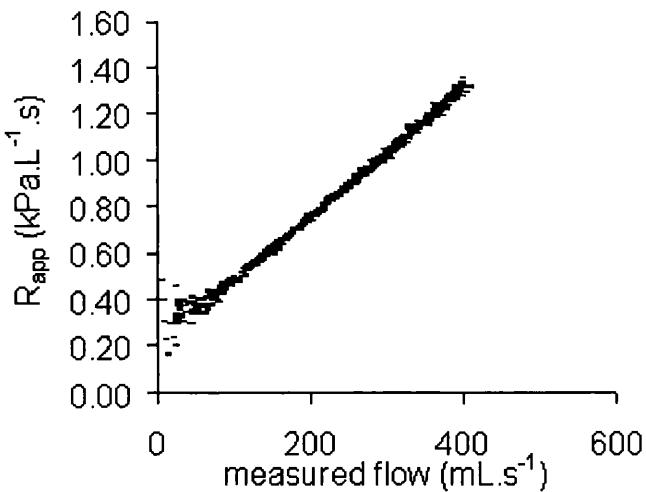


Figure B-10 Apparatus resistance plotted against flow for a Fleisch 1 PNT and plethysmograph shutter block in the inspiratory direction through the "RAW" port

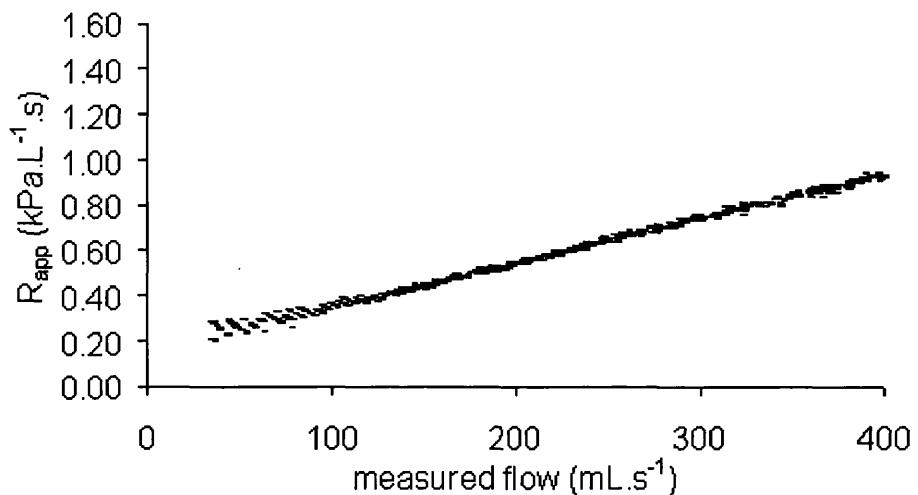
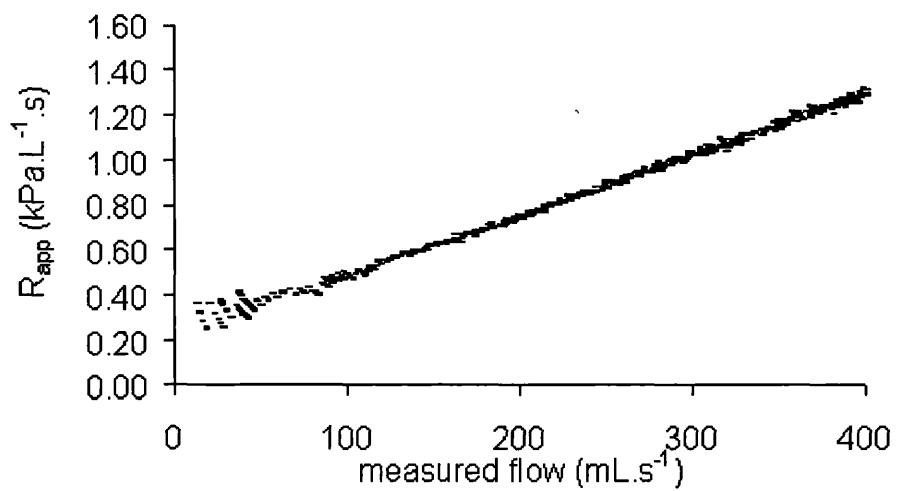


Figure B-11 Apparatus resistance plotted against flow for a Fleisch 1 PNT and plethysmograph shutter block in the expiratory direction through the "RAW" port



Plethysmograph assessment

The accuracy of lung volume measurements was assessed using a lung model developed by Leicester University, which comprised a sine pump attached to a loop of copper tubing, filled with copper wool to approach isothermal conditions during test lung measurements. The test lung measured the volume of the 'copper lung' alone to within 2% accuracy. The values obtained using the plethysmograph employed in the thesis were: water displacement volume of test lung = 203 mL, plethysmographically measured volume at 15, 30, 45 cycles per minute = 199 mL.

However, when measuring lung volume via the TGV shutter block apparatus, there was a consistent over-estimation of 20.5 mL when using either the Fleisch "0" PNT (estimated apparatus dead space 7.6 mL) or the Fleisch "1" PNT (estimated dead space 26 mL). This was concluded to be due to the dead space of the tubing leading to the flow and pressure transducers (3 tubes each of 7 mL dead space, half of which was within and half outside the plethysmograph i.e. a total of 20.5 mL) not having been included in the estimated apparatus dead space.

Although infants did not breathe through this tubing (that is, it was not part of the effective dead space during tidal breathing) it was proximal to the occlusion bobbins within the shutter block and therefore constituted additional volume which was subjected to pressure changes during airway occlusion.

Together with most other infant respiratory function testing centres, dead space for the face mask had also not been subtracted for any measurements performed within our laboratory. This was difficult to estimate because of the variable amount occupied by the infant's face. However, water displacement experiments showed that the total dead space of the Rendell-Baker masks used to be: 30ml for size 2 (used for infants approximately 6 months and above), 20ml for size 1 (used for infants between 1 and 6 months of age), and 12ml for size 0 (used for premature and new born infants). An infant face was fashioned out of putty and attached to the face masks, then the water displacement values measured on at least 3 occasions. On average, the effective dead space appeared to comprise 50% of the total face mask volume, ie 15ml, 10 and 6 ml for sizes 2, 1, and 0 Rendell-Baker masks respectively.

The additional dead space that was identified during the plethysmograph assessment was required to be subtracted from previously measured FRC_{pleth} data, so for infants below 6 months of age, an additional 30ml was subtracted from existing FRC_{pleth} values, whilst for older infants, an additional 35ml was subtracted.

Furthermore, since the ratio $\Delta P_{ao}/\Delta P_b$ during airway occlusion which was used to calibrate ΔP_b in terms of ΔP_{alv} , varied according to actual lung volume, a correction factor was needed to be applied to all values of R_{aw} . The magnitude of the correction factor varied according to the ratio between the amended and original values of FRC_{pleth} . Thus, the values of FRC_{pleth} were reduced and values of R_{aw} increased. The rationale behind this correction factors and the way in which it was calculated in any infant are described below:

Worked example:

If $TGV = 300 \text{ mL}$, $V_T 100 \text{ mL}$ Dead space = 26 mL :

then $TOGV = 426 \text{ mL}$

If ΔP_{ao} is assumed to be 2 kPa , and $P_{atm} = 100 \text{ kPa}$

$$TOGV = \frac{\Delta P_b \times P_{atm}}{\Delta P_{ao}} \quad \text{i.e. } 426 = \frac{\Delta P_b \times 100}{2}$$

$$\text{or } \frac{426 \times 2}{100} = \Delta P_b = 8.52 \text{ mL}$$

$$\text{Correction (CF) factor for } R_{aw} = \frac{\Delta P_{ao} \times TOGV}{\Delta P_b \times TGV}$$

In the original example:

$$\frac{2}{8.52} \times \frac{426}{300} = 0.3333$$

However, if the apparatus dead space was actually $26 \text{ mL} + 20 \text{ mL (tubing)} + 15 \text{ mL (mask)}$ i.e. 61 mL

$$\text{true correction factor} = \frac{2}{8.52} \times \frac{426}{265} = 0.3773$$

i.e. to correct previously calculated R_{aw} values, one would need to multiply by $0.3373/0.333$ i.e. 1.132

i.e. R_{aw} values will increase by 13% in this example

Similar calculations could be performed for any lung volumes. However, a simpler approach to obtaining the correction factor was to divide the original by the amended FRC_{pleth} .

$$\text{i.e. } 300/265 = 1.132$$

However, there was no change in calculated values of sG_{aw} since any errors in FRC_{pleth} were cancelled out once G_{aw} was divided by FRC_{pleth} .

Values of FRC_{pleth} were subsequently adjusted for all infant data reported by GOS in Section 6 (ECMO Respiratory Follow-up) including the reference group used for comparison of plethysmographic data (Dezateux et al 1999).

In Sections 4 and 5, the data on which the original paper was based had already been published and the values of FRC_{pleth} and R_{aw} were not adjusted. In Section 4, the main aim was to compare measurements of sG_{aw} with $t_{\text{PTEF}}:t_E$ and these were unaffected by the adjustment for dead space. In Section 5 comparisons of R_{rs} and R_{aw} were made, and the values of R_{aw} would have been affected by not subtracting the additional dead space, but the conclusions of that Section, showing a highly variable relationship between the two measures of resistance would not have been changed.

The amended raw values of data are contained within the Appendix, showing unadjusted and adjusted values for Sections 4 and 5, and the adjusted values of data reported in Section 6.

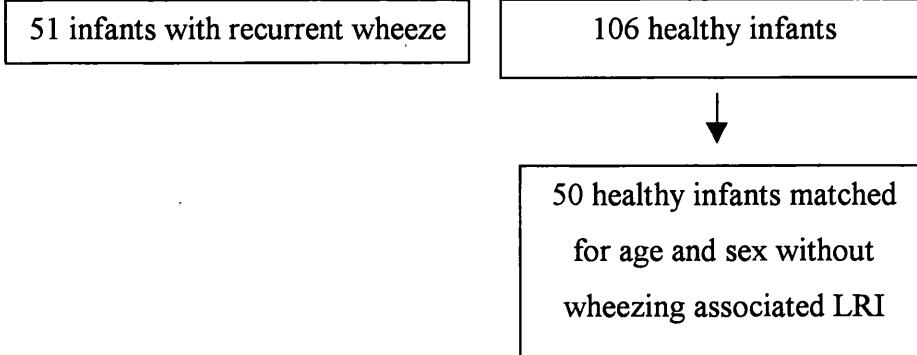
Appendix C Tables of infant details and respiratory function data

Values of FRC_{pleth} were adjusted for all infant data reported by GOS in Section 6 (ECMO Respiratory Follow-up) including the reference group used for comparison of plethysmographic data (Dezateux et al 1999) (Section 0).

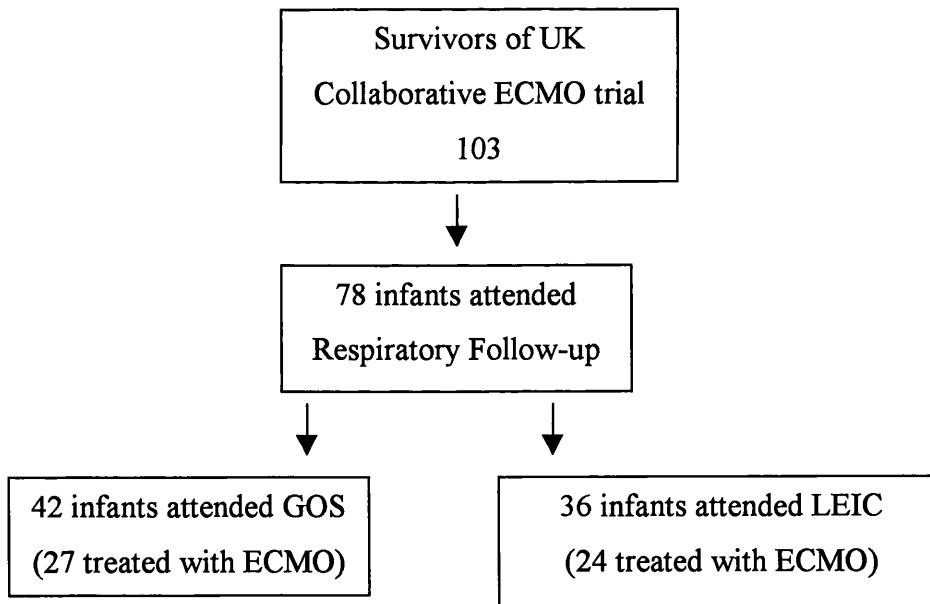
The amended raw values of data are contained within the Appendix, showing unadjusted and adjusted values for Sections 4 and 5, and the adjusted values of data reported in Section 6.

Appendix C Flow diagram of subjects included in thesis

Flow chart of infants recruited and measured in Sections 4 and 5:



Flow chart of infants recruited and measured in Section 6



Appendix C: Individual details and respiratory function results in control infants (Sections 4 and 5)

Infant no.	test weight (kg)	test length (cm)	sex	RR (min ⁻¹)	V _T (mL)	t _{PEF} .t _E (mL.kPa ⁻¹)	C _{rs,MOT} (mL.kPa ⁻¹)	C _{rs,SBT} (mL.kPa ⁻¹)	FRC _{pleth} (mL)	FRC _{pleth} corr. (mL)	R _{aw,II} corr. (kPa.L ⁻¹ .s)	R _{aw,EE} corr. (kPa.L ⁻¹ .s)	R _{aw,EE} corr. (kPa.L ⁻¹ .s)
1	4.5	59.3	f	65	39	0.316	74.1	59.9	2.58	134	104	1.93	2.49
2	4.9	58.2	f	38	44	0.382	56.3	68.6	3.39	137	107	1.95	2.50
3	5.1	58.1	f	60	40	0.369	57.3	54.5	3.98	136	106	2.00	2.57
4	5.7	59.0	m	48	47	0.356	63.3	62.9	3.82	202	172	4.40	5.17
5	5.7	60.2	m	40	51	0.216	67.1	60.3	3.76	146	116	3.24	4.08
6	6.1	65.3	f	33	61	0.138	71.7	70.1	3.74	159	124	3.17	4.07
7	6.7	67.2	m	36	63	0.203	110.7			188	153	2.63	3.23
8	6.7	65.1	m	40	56	0.340	73.9	77.1	5.28	155	120	3.30	4.27
9	7.4	69.9	m	39	84	0.322	86.4	106.9	5.04	205	170	1.84	2.22
10	8.0	72.0	f	33	72	0.215	115.1	109.0	2.82	203	168	1.83	2.21
11	8.0	76.3	f	34	61	0.222	147.0			194	159	1.71	2.09
12	8.3	67.1	m	34	64	0.294	90.1			204	169	2.19	2.64
13	8.5	76.4	m	23	111	0.157	168.3	158.9	2.85	273	238	1.56	1.79
14	8.7	74.3	f	31	95	0.267	121.6	105.3	4.10	225	190	3.21	3.80
15	8.7	77.1	m	28	110	0.279	168.0			291	256	1.50	1.70
												1.37	1.56

Appendix C: Individual details and respiratory function results in control infants (Sections 4 and 5) continued.

Infant no.	test weight (kg)	test length (cm)	sex	RR (min ⁻¹)	V _T (mL)	t _{PTEF:t_E}	C _{rs,MOT} (mL.kPa ⁻¹)	C _{rs,SBT} (mL.kPa ⁻¹)	FRC _{pleth} (mL)	FRC _{pleth corr.} (mL)	R _{aw,II} (kPa.L ⁻¹ .s)	R _{aw,II corr.} (kPa.L ⁻¹ .s)	R _{aw,EE} (kPa.L ⁻¹ .s)	R _{aw,EE corr.} (kPa.L ⁻¹ .s)
16	8.7	78.6	m	33	94	0.254	126.8	108.8	112.3	3.47	283	248	1.10	1.26
17	8.8	71.8	f	33	71	0.232	108.8			174	139	4.19	5.25	3.79
18	8.9	72.5	m	32	97	0.434	143.7			255	220	0.89	1.03	1.13
19	8.9	67.4	m	35	81	0.153	90.5			191	156	4.35	5.32	6.26
20	8.9	76.9	m	29	104	0.224	132.7	136.2	2.13	299	264	1.09	1.23	0.77
21	9.1	79.9	f	27	97	0.370	168.7	171.8	2.20	285	250	1.37	1.56	1.31
22	9.1	77.7	f	28	95	0.481	123.7	144.7	2.73	285	250	1.76	2.01	2.07
23	9.2	76.5	m	29	127	0.369	143.6	154.9	2.90	311	276	2.23	2.51	2.10
24	9.3	72.3	m	23	107	0.269	166.5	170.5	2.37	286	251	1.39	1.58	1.24
25	9.3	76.4	f	26	122	0.339	172.3	173.7	2.26	293	258	1.60	1.82	2.10
26	9.4	78.2	m	27	119	0.182	146.9	176.0	3.42	279	244	1.33	1.52	1.35
27	9.4	79.7	m	30	108	0.198	143.7	143.3	2.75	267	232	1.73	1.99	1.93
28	9.6	75.4	m	30	106	0.395	103.9	150.8	3.90	243	208	1.75	2.04	1.80

Appendix C: Individual details and respiratory function results in control infants (Sections 4 and 5) continued.

Infant no.	test weight (kg)	test length (cm)	sex	RR (min ⁻¹)	V _T (mL)	t _{PEF, t_E}	C _{rs,MOR} (mL.kPa ⁻¹)	C _{rs,SBT} (mL.kPa ⁻¹)	R _{rs,SBT} (kPa.L ⁻¹ .s)	FRC _{pl} (mL)	FRC _{pl} corr. (mL)	R _{aw,II} (kPa.L ⁻¹ .s)	R _{aw,II} corr. (kPa.L ⁻¹ .s)	R _{aw,EE} (kPa.L ⁻¹ .s)	R _{aw,EE} corr. (kPa.L ⁻¹ .s)
29	9.6	80.4	f	31	105	0.286	123.5	159.2	3.26	235	200	1.62	1.90	1.33	1.56
30	9.6	75.3	m	30	102	0.221	118.4	142.3	2.14	305	270	1.41	1.59	1.41	1.59
31	9.7	75.6	f	27	65	0.266	179.2	189.5	4.28	266	231	1.28	1.47	1.89	2.18
32	9.7	76.0	m	32	90	0.372	152.9			328	293	1.43	1.60	1.53	1.71
33	9.7	75.5	m	39	85	0.283	128.4	121.7	2.01	265	230	1.40	1.61	1.60	1.84
34	9.7	77.2	m	30	118	0.230	155.0	170.4	1.95	332	297	1.17	1.31	1.99	2.22
35	9.8	74.3	m	26	99	0.237	149.1	125.4	3.88	271	236	1.86	2.14	1.88	2.16
36	9.8	78.0	m	22	144	0.310	157.8			359	324	2.06	2.28	1.77	1.96
37	9.8	78.7	f	25	102	0.284	146.7	161.7	2.97	324	289	1.26	1.41	0.85	0.95
38	9.8	80.4	f	28	109	0.436	146.1	179.7	3.64	324	289	2.15	2.41	1.87	2.10
39	10.0	80.0	m	38	93	0.421				372	337	1.10	1.21	0.96	1.06
40	10.0	77.8	m	32	114	0.255	150.7	144.9	2.20	282	247	1.25	1.43	1.20	1.37
41	10.0	76.3	m	30	125	0.184	164.3	164.6	3.01	252	217	1.43	1.66	10.27	11.93
42	10.5	78.0	m	29	104	0.325	126.6			286	251	2.05	2.34	2.10	2.39

Appendix C: Individual details and respiratory function results in control infants (Sections 4 and 5) continued.

Infant no.	test weight (kg)	test length (cm)	sex	RR (min ⁻¹)	V _T (mL)	t _{PTEF:t_E} (mL.kPa ⁻¹)	C _{rs,MOT} (mL.kPa ⁻¹)	C _{rs,SBT} (mL.kPa ⁻¹)	R _{rs,SBT} (kPa.L ⁻¹ .s)	FRC _{pl} (mL)	FRC _{pl} corr. (mL)	R _{aw,II} (kPa.L ⁻¹ .s)	R _{aw,II} corr. (kPa.L ⁻¹ .s)	R _{aw,EE} (kPa.L ⁻¹ .s)	R _{aw,EE} CORR. (kPa.L ⁻¹ .s)
43	10.7	79.9	f	29	107	0.252	116.7	116.8	2.95	254	219	2.07	2.40	2.48	2.88
44	11.0	76.8	m	33	105	0.425				317	282	3.27	3.68	3.59	4.04
45	11.4	81.8	m	27	119	0.315	152.0	160.8	2.15	334	299	1.77	1.98	1.63	1.82
46	11.5	80.5	m	34	105	0.246	154.1	171.7	3.06	340	305	1.32	1.47	1.76	1.96
47	11.8	81.8	m	35	129	0.291	146.4			340	305	0.95	1.06	1.17	1.30
48	12.0	83.4	m	24	144	0.372	152.8	192.9	4.21	315	280	1.73	1.95	2.14	2.41
49	12.2	80.8	m	33	92	0.224	148.6	156.4	2.63	309	274	2.61	2.94	2.37	2.67
50	13.8	81.8	m	27	133	0.382	180.2	168.2	3.78	376	341	1.27	1.40	1.36	1.50

Appendix C: Individual details and respiratory function results in infants with recurrent wheezing (Sections 4 and 5)

Infant ID	test	sex	RR	V _T	t _{PTEF:IB}	C _{rs,MOT}	C _{rs,SBT}	R _{rs,SBT}	FRC _{pleth}	FRC _{pleth}	R _{raw,II}	R _{raw,II CORR.}	R _{raw,EE}	R _{raw,EE CORR.}	
	weight	length	(min ⁻¹)	(mL)	(mL.kPa ⁻¹)	(mL.kPa ⁻¹)	(mL.kPa ⁻¹)	(kPa.L ⁻¹ .s)	(mL)	corr. (mL)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	
	(kg)	(cm)													
1	3.6	51.6	f	66	24	0.308	32.9	32.0	10.2	110	80	6.19	8.51	5.52	7.59
2	4.7	57.4	m	56	28	0.165	46.8	44.6	8.5	140	110	5.58	7.10	9.93	12.64
3	5.4	64.9	m	48	53	0.176	57.0			341	311	4.24	4.65	6.51	7.14
4	6.3	68.8	m	43	46	0.107		38.7	4.6	384	349	10.50	11.55	12.69	13.96
5	6.3	65.7	f	30	86	0.209	110.0	111.0	4.1	213	178	2.73	3.27	4.25	5.09
6	6.4	63.1	m	48	69	0.34	81.1			196	161	2.36	2.87	2.33	2.84
7	6.4	65.0	f	54	46	0.297	65.7	57.7	4.4	142	107	3.30	4.38	3.97	5.27
8	6.7	61.7	m	34	61	0.17	90.9			206	171	7.42	8.94	17.28	20.82
9	6.7	68.4	f	38	60	0.285	98.9	86.4	4.0	248	213	2.01	2.34	2.37	2.76
10	7.2	63.7	m	35	69	0.267	96.7	86.4	4.9	147	112	2.26	2.97	3.39	4.45
11	7.3	71.2	f	25	81	0.119	83.7	84.4	4.8	235	200	3.47	4.08	7.00	8.22
12	7.3	67.1	m	45	58	0.149	81.2	73.7	4.7	266	231	3.29	3.79	4.35	5.01
13	7.4	67.9	f	32	75	0.145	100.3	69.2	4.8	246	211	3.80	4.43	7.76	9.05
14	7.4	70.4	m	47	72	0.307	91.9	91.4	3.0	304	269	1.85	2.09	1.98	2.24
15	7.4	66.6	f	30	61	0.368	108.0			251	216	6.03	7.00	6.02	6.99
16	7.6	67.7	f	40	92	0.171	87.4	58.9	3.9	376	341	3.86	4.26	8.26	9.11
17	7.7	62.1	m	43	45	0.12				205	170	5.89	7.10	16.32	19.68
18	8.1	68.0	m	31	67	0.375	100.1	102.2	5.5	245	210	3.47	4.05	3.40	3.97

Appendix C: Individual details and respiratory function results in infants with recurrent wheezing (Sections 4 and 5) continued.

Infant ID	test	test	sex	RR	V _T	t _{PEF:T_E}	C _{ra,MOT}	C _{ra,SBT}	R _{ra,SBT}	FRC _{pleth}	FRC _{pleth}	R _{aw,II}	R _{aw,II} CORR.	R _{aw,EE}	R _{aw,EE} CORR.
				(min ⁻¹)	(mL)		(mL.kPa ⁻¹)	(mL.kPa ⁻¹)	(kPa.L ⁻¹ .s)	(mL)	corr. (mL)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	
				(kg)	(cm)										
19	8.1	71.5	m	46	78	0.12	62.9			457	422	4.10	4.44	7.57	8.20
20	8.5	70.5	m	37	92	0.231	100.1	115.9	5.4	264	229	4.13	4.76	4.17	4.81
21	8.6	71.8	m	64	69	0.188	94.5			538	503	2.67	2.86	5.15	5.51
22	8.8	77.8	m	25	129	0.132	149.1	133.3	3.0	417	382	1.91	2.08	3.36	3.67
23	8.9	75.1	f	38	79	0.234	101.1	88.9	5.7	274	239	3.95	4.53	6.02	6.90
24	8.9	79.0	m	40	92	0.239	150.7			435	400	2.36	2.57	2.98	3.24
25	9.0	71.0	f	30	113	0.157	133.3	111.2	4.1	282	247	2.51	2.87	4.62	5.27
26	9.2	72.4	f	29	77	0.286	92.6	92.7	3.9	239	204	2.88	3.37	4.53	5.31
27	9.3	76.0	m	34	84	0.339	122.7			296	261	2.01	2.28	2.38	2.70
28	9.6	74.8	f	33	92	0.168	130.9			299	264	1.98	2.24	4.21	4.77
29	9.7	75.6	f	36	84	0.301	106.7	102.7	4.3	227	192	2.72	3.22	2.69	3.18
30	9.7	73.8	m	27	100	0.264	174.9	139.6	4.5	315	280	2.17	2.44	2.92	3.29
31	9.8	72.3	m	31	81	0.189	106.2	68.9	5.5	258	223	2.94	3.40	4.32	5.00
32	10.0	71.5	m	46	70	0.248	116.1	94.1	3.7	253	218	2.15	2.50	2.68	3.11
33	10.0	70.9	f	30	94	0.362	109.6	103.8	4.4	237	202	2.46	2.89	2.34	2.75
34	10.1	74.5	m	40	107	0.171	101.4	110.3	3.3	363	328	1.60	1.77	3.84	4.25
35	10.1	80.2	m	34	99	0.176	151.5	118.0	3.8	390	355	2.51	2.76	5.38	5.91

Appendix C: Individual details and respiratory function results in infants with recurrent wheezing (Sections 4 and 5) continued.

Infant ID	test	test	sex	RR	V _T	t _{PTEF:TB}	C _{rs,MOT}	C _{rs,SBT}	R _{rs,SBT}	FRC _{plth}	R _{raw,II}	R _{raw,II CORR.}	R _{raw,EE}	R _{raw,EE CORR.}	
	weight	length	(min ⁻¹)	(mL)		(mL.kPa ⁻¹)	(mL.kPa ⁻¹)	(mL.kPa ⁻¹)	(kPa.L ⁻¹ .s)	corr. (mL)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	(kPa.L ⁻¹ .s)	
	(kg)	(cm)													
36	10.3	74.4	f	25	106	0.451	110.7			286	251	2.57	2.93	2.85	3.25
37	10.6	78.8	m	37	105	0.334	120.1	131.8	3.9	384	349	1.66	1.83	2.67	2.94
38	10.7	77.7	m	24	109	0.154	148.7	142.2	3.0	277	242	1.67	1.91	1.61	1.84
39	10.7	81.1	m	26	114	0.259	174.7	185.4	3.2	349	314	2.11	2.34	2.07	2.30
40	10.9	80.6	m	34	109	0.138	163.2	117.1	2.8	403	368	1.71	1.87	3.54	3.88
41	10.9	78.7	m	33	83	0.205	117.8	94.9	4.5	256	221	3.10	3.59	5.48	6.35
42	10.9	79.7	f	29	112	0.188	169.8	149.9	3.1	311	276	2.41	2.72	2.20	2.48
43	11.0	76.7	m	31	105	0.191	145.4	131.9	2.9	266	231	1.36	1.57	1.84	2.12
44	11.2	73.5	m	26	85	0.207	113.8	111.6	4.6	265	230	3.22	3.71	5.88	6.77
45	11.2	80.8	m	27	113	0.374	163.6	142.1	3.8	277	242	2.67	3.06	2.68	3.07
46	11.2	80.5	m	32	133	0.469	171.7			307	272	1.19	1.34	1.06	1.20
47	11.5	81.3	m	29	117	0.242	153.4			301	266	1.91	2.16	2.16	2.44
48	11.7	78.5	m	31	104	0.169	127.4	100.8	2.6	265	230	2.55	2.94	2.92	3.36
49	11.8	79.5	m	29	102	0.207	177.2			353	318	2.24	2.49	2.38	2.64
50	12.6	78.8	m	31	111	0.196	138.7			330	295	2.31	2.58	1.90	2.13
51	14.3	90.5	m	31	102	0.302	137.3	155.5	2.2	487	452	1.69	1.82	1.30	1.40

Appendix C: Individual infant details and respiratory function results in infants allocated to conventional management (Section 6)

Infant ID	Group	Weight (kg)	Length(cm)	Sex	V_T (mL)	RR (min ⁻¹)	FRC_{pleth} (mL)	$R_{\text{aw,II}}$ (kPa.L ⁻¹ .s)	$R_{\text{aw,BE}}$ (kPa.L ⁻¹ .s)	Mean V' _{max,FRC} (mL.s ⁻¹)
1	control	7.7	74.5	m	65	39	359			143
2	control	8.2	72.2	m	68	46	250	3.06	5.51	48
3	control	8.3	73.1	f	94	29	254	4.28	5.28	117
4	control	8.7	78.0	f	W	W	329	2.07	2.29	
5	control	9.1	79.8	f	84	28	277	1.78	1.56	220
6	control	9.1	77.7	m	79	38	408	2.26	2.59	
7	control	9.1	79.1	f	103	32	289	3.00	2.93	
8	control	9.2	76.7	f	82	40	257	1.48	1.55	169
9	control	9.2	78.4	f	78	43	232	1.87	2.57	152
10	control	9.2	75.7	m	64	52	287	3.68	4.23	194
11	control	9.2	76.5	m	103	28	319	3.98	4.03	
12	control	9.5	79.4	m	129	19	349	1.51	1.65	206
13	control	9.6	77.9	m	66	46	492	1.76	3.76	51
14	control	9.8	77.5	m	113	24	274	2.18	3.18	134
15	control	9.8	78.2	m	102	24				190
16	control	10.1	77.5	f	97	32	274	2.24	2.62	
17	control	10.4	80.5	f	84	28				201

Appendix C: Individual infant details and respiratory function results in infants allocated to conventional management (Section 6) continued.

Infant ID	Group	Weight (kg)	Length(cm)	Sex	V _T (mL)	RR (min ⁻¹)	FRC _{pleth} (mL)	R _{aw,II} (kPa.L ⁻¹ .s)	R _{aw,BE} (kPa.L ⁻¹ .s)	Mean V' _{max,FRC} (mL.s ⁻¹)	100
18	control	10.6	79.6	f	91	35	249				
19	control	10.7	76.9	m	93	31	297	1.75	1.52	235	
20	control	11.2	80.2	m	68	34	228	1.16	0.79	119	
21	control	11.2	76.7	m	84	39				54	
22	control	11.5	83.5	m	100	39	307	1.59	1.58	236	
23	control	11.5	83.1	m	98	24	338	1.76	1.65	230	
24	control	11.6	83.0	m	56	29	354	3.61	4.92	27	
25	control	11.8	82.5	m	99	34	292	1.77	2.04	159	
26	control	12.2	79.5	m	88	35	359	3.29	3.53	68	
27	control	12.8	78.2	f	101	30	281			77	

Appendix C: Individual infant details and respiratory function results in infants allocated to ECMO management (Section 6).

Infant No.	Group	Weight (kg)	Length (cm)	Sex	V _T (mL)	RR (min ⁻¹)	FRC _{Pleth} (mL)	R _{aw,EE} (kPa.L ⁻¹ .s)	R _{aw,EE} (kPa.L ⁻¹ .s) Mean V' _{max,FRC} (mL.s ⁻¹)
1	ecmo	11.8	85.0	m	96	34	422	2.41	2.73
2	ecmo	10.5	77.4	f	76	34	212	2.88	3.38
3	ecmo	12.8	81.0	m	133	24	W	W	138
4	ecmo	11.2	78.7	m	96	33	245	W	W
5	ecmo	11.8	83.5	m	87	30	294	1.45	1.33
6	ecmo	12.5	81.5	f	105	28	W	W	251
7	ecmo	8.5	75.6	m	85	38	250	3.08	3.69
8	ecmo	10.3	78.0	m	109	33	269	1.85	1.84
9	ecmo	9.8	77.4	m	90	29	450	2.60	4.39
10	ecmo	10.7	77.5	m	105	30	334	2.55	3.65
11	ecmo	10.1	81.2	f	134	21	278	4.11	3.65
12	ecmo	8.7	74.3	f	75	32	160	1.97	2.72
13	ecmo	9.9	78.5	m	105	31.	267	1.67	3.96
14	ecmo	10.6	78.5	f	97	25	290	2.93	243
15	ecmo	10.9	80.9	f	103	28	195	T	3.37
16	ecmo	8.5	71.0	m	77	34	W	W	183
17	ecmo	8.2	76.3	m	80	37	249	3.11	116
18	ecmo	8.2	73.5	m	73	43	321	1.75	4.17
								2.72	78

Appendix C: Individual infant details and respiratory function results in infants allocated to ECMO management (Section 6) continued.

Infant No.	Group	Weight (kg)	Length (cm)	Sex	V_T (mL)	RR (min ⁻¹)	$FRC_{p\text{leth}}$ (mL)	$R_{aw,II}$ (kPa.L ⁻¹ .s)	$R_{aw,HE}$ (kPa.L ⁻¹ .s)	Mean $V'_{\text{max,FRC}}$ (mL.s ⁻¹)
19	ecmo	7.3	72.3	f	69	37	236	1.84	3.32	238
20	ecmo	8.2	74.5	m	89	39	231	1.38	1.53	270
21	ecmo	8.8	74.4	f	70	53	245	1.01	1.22	168
22	ecmo	9.1	76.6	m	104	23	309	2.45	4.54	
23	ecmo	11.1	81.5	f	112	33	313	1.33	1.73	177
24	ecmo	9.9	76.0	m	90	26	165	T	T	131
25	ecmo	7.8	75.5	m	77	33	317	1.01	1.29	415
26	ecmo	11.3	83.7	f	110	23	355	1.50	1.76	218
27	ecmo	10.4	84.7	f	92	33	323	1.51	1.95	250
28	ecmo	11.8	77.5	m	102	27	256	1.47	2.05	62
29	ecmo	10.0	80.2	m	78	26	338			159
30	ecmo	8.5	80.8	m	55	56	241			
31	ecmo	10.2	74.5	f	79	29	263			216
32	ecmo	13.1	80.4	m	78	30	213	2.60	3.43	28
33	ecmo	12.1	82.2	f	99	25	312	1.40	1.49	
34	ecmo	13.2	81.0	m	88	30	287	1.26	1.65	168

Appendix C: Individual infant details and respiratory function results in infants allocated to ECMO management (Section 6) continued.

Infant No.	Group	Weight (kg)	Length(cm)	Sex	V_T (mL)	RR (min ⁻¹)	$FRC_{p\text{leth}}$ (mL)	$R_{aw,II}$ (kPa.L ⁻¹ .s)	$R_{aw,EE}$ (kPa.L ⁻¹ .s)	Mean $V'_{\text{max},\text{FRC}}$ (mL.s ⁻¹)
35	ecmo	10.5	75.1	m	86	28	238	2.26	3.15	155
36	ecmo	11.5	80.4	f	84	35	181	1.10	1.01	97
37	ecmo	9.4	74.4	m	81	29	271			200
38	ecmo	7.0	66.8	m	52	74	145	1.21	2.11	320
39	ecmo	7.8	74.1	m	81	32	332	3.15	3.95	111
40	ecmo	10.4	78.7	m	80	34	202	1.33	1.74	109
41	ecmo	9.4	77.4	f	65	40	216	1.84	2.66	128
42	ecmo	11.0	78.2	f	82	30	213			248
43	ecmo	7.8	71.9	m	68	27	260			272
44	ecmo	13.2	84.8	m	106	25	412	2.37	2.91	92
45	ecmo	10.7	79.4	f	81	28	265	1.82	1.78	94
46	ecmo	12.1	81.3	m	99	28	277	1.93	2.49	106
47	ecmo	9.6	77.3	f	95	28	263			41
48	ecmo	12.7	81.8	m	109	30	291			126
49	ecmo	10.6	78.5	f	88	27	293			216
50	ecmo	9.2	75.4	f	72	38	174			105
51	ecmo	12.5	83.4	m	121	28	323	3.54	4.23	208

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The Relationship Between $t_{PTEF}:t_E$ and Specific Airway Conductance in Infancy

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Summary. This study examines the association between the time taken to achieve peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF}:t_E$) and specific airways conductance (SG_{aw}) in healthy infants and those with prior physician diagnosed, associated, lower respiratory illness with wheezing (prior LRI) during the first year of life. We compared $t_{PTEF}:t_E$ and SG_{aw} , the latter estimated during both initial inspiration (II) and end-expiration (EE), in 168 infants (94 males), measured on 220 occasions. Mean (range) $t_{PTEF}:t_E$ was 0.321 (0.150–0.522) in 73 healthy infants aged less than 3 months (mean, 7.8 weeks), in whom mean (range) EE SG_{aw} and plethysmographic thoracic gas volume at functional residual capacity (FRC_{pleth}) were $2.47 \text{ s}^{-1} \text{ kPa}^{-1}$ (0.6–5.8) and 141 mL (87–204), respectively. Both $t_{PTEF}:t_E$ and EE SG_{aw} were significantly lower in older infants with prior LRI ($n = 79$; mean age, 50.0 weeks) compared to a similarly aged group of healthy infants ($n = 68$; mean age, 48.5 weeks), the mean difference [95% confidence intervals (CI)] being -0.039 (-0.013 , -0.064) and $-0.48 \text{ s}^{-1} \text{ kPa}^{-1}$ (-0.24 , -0.72), respectively. A significant but weak association between $t_{PTEF}:t_E$ and EE SG_{aw} was found among infants above 3 months of age, irrespective of prior wheezing status. However, this relationship was not significant in healthy younger infants, in whom a significant but weak association with FRC_{pleth} was found. Further work is needed to elucidate the factors influencing tidal expiratory flow patterns in infancy. *Pediatr Pulmonol*. 1994; 18:299–307. © 1994 Wiley-Liss, Inc.

Key words: Lower respiratory illness, wheezing, healthy infants; lung volume, tidal expiratory flow pattern, specific airway conductance.

INTRODUCTION

Tidal breathing measurements are being increasingly applied to population-based studies of the determinants of early respiratory morbidity.^{1,2} The time taken to achieve peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF}:t_E$), when measured in healthy infants in the first 3 months of life, has been shown to be predictive of subsequent wheezing in boys during the first 3 years.^{1,2} In addition, $t_{PTEF}:t_E$ has been reported to be significantly related to indices of airway size in adults and children.^{3,4} However, the extent to which this parameter of tidal breathing is associated with established measures of airway function in infants remains unclear.

The aim of this study was to examine the association between $t_{PTEF}:t_E$ and specific airway conductance (SG_{aw}), when measured on the same test occasion, in both healthy infants and those with prior physician diagnosed lower respiratory illness with wheezing (prior LRI), i.e., wheezing, bronchiolitis, or asthma, during the first year of life.

MATERIALS AND METHODS

Study Population

Healthy Caucasian infants were recruited shortly after birth from the community as part of an ongoing epidemi-

ological study (unpublished data). Infants born before 36 weeks gestation, requiring ventilatory assistance at birth or suffering from major congenital abnormalities were ineligible. Infants with prior physician-diagnosed LRI with wheezing were recruited from the wards and outpatient clinics of the Hospitals for Sick Children, London. All infants with prior LRI, except those with major congenital abnormalities, were eligible for study.

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Study Design

Plethysmographic measurements of lung volume and airway resistance and measurements of tidal breathing parameters were undertaken on two occasions in infants recruited to the epidemiological study: between 5 and 13 weeks postnatal age prior to any lower respiratory illness, and again, at approximately 1 year of age. Additional measurements were made between 3 and 18 months of age in some infants who participated as healthy subjects in other ongoing studies.⁵ If necessary, measurements were postponed to allow an interval of at least 3 weeks from the onset of upper respiratory tract illness. Physicians' diagnosis of LRI with wheezing (wheezing, bronchiolitis, or asthma) in the interval between the first and subsequent measurements was ascertained by retrospective review of the infant's primary medical care record, which was undertaken as soon as possible after the last visit to the laboratory. Similar measurements were performed, on one occasion, in infants with prior LRI when between 3 and 18 months of age.

Written informed consent to participate in these studies was obtained from the infants' parents, who were commonly present when measurements were performed. All study protocols had received prior approval by the Ethics Committee of the Hospitals for Sick Children.

For the purposes of this study, infants were allocated into one of three groups according to their age and status at the time of lung function testing. Group 1 consisted of healthy infants aged ≤ 13 weeks at testing, Group 2 of healthy infants aged > 13 weeks at testing, and Group 3 of infants aged > 13 weeks at testing with prior LRI. Although some infants were studied on more than one occasion, the purpose of this study was to examine cross-sectional associations between $t_{PTEF:t_E}$ and SG_{aw} . Thus, while some infants included in Group 1 were also measured when above 3 months of age, and were included in Groups 2 or 3, depending on their status when older, longitudinal analysis was not undertaken for these infants. Groups 2 and 3 were mutually exclusive.

Lung function measurements were made on 241 occasions in 172 infants, during which data for tidal breathing and thoracic gas volume at functional residual capacity (FRC_{pleth}) were successfully obtained on all occasions and for airways resistance (R_{aw}) on 220 occasions. Failure to measure R_{aw} occurred because the infant awoke before this could be attempted (10 occasions in 10 infants), or before satisfactory data were collected (11 occasions in 11 infants). This mainly occurred in the youngest infants (17 occasions). However, a subsequent successful measurement was obtained in all but 4 of these infants, who failed to attend a further laboratory appointment.

Thus, complete data for comparison of $t_{PTEF:t_E}$ and

SG_{aw} were available for 168 infants (94 males) on 220 occasions. Measurements were obtained in 73 healthy infants aged ≤ 13 weeks (Group 1), in 68 healthy infants aged > 13 weeks (Group 2), and in 79 infants with prior physician diagnosed LRI with wheezing aged > 13 weeks (Group 3). Of the 73 infants included in Group 1, 39 (53%) and 13 (18%), who were measured again when aged more than 13 weeks, were included in Groups 2 and 3, respectively. Groups 2 and 3 were, however, independent.

Lung Function Measurements

Air flow (\dot{V}) was measured using a pneumotachograph [Fleisch (Lausanne, Switzerland): size 0 or 1, according to age and size of the infant] attached to a differential pressure transducer [Validyne MP 45; ± 0.2 kPa (Northridge, CA)]. Air flow was digitized, sampled at 100 Hz (Analog Devices RTI-815) and digitally integrated to yield volume. The start and end of expiration were defined as the last zero crossing of flow during inspiration and expiration, respectively. Zero flow crossings were estimated using sample-to-sample linear interpolation. This strategy ensures that any expiratory pause is incorporated into expiratory time. An adjustable scan period (usually set to 0.3 sec) prevented identification of false troughs and peaks. Peak expiratory flow was taken as the first sample at which maximum flow was recorded.¹³ Air flow measurements were calibrated prior to testing using known flows from calibrated rotameters (Series 1100, Fisher Controls Ltd). Plethysmographic pressure (P_{pleth}) and pressure at the airway opening (P_{ao}) were measured, the latter via a port in the face mask mount, with Validyne MP 45 pressure transducers (ranges: ± 0.2 and ± 5 kPa, respectively). Calibrations of P_{pleth} and P_{ao} measurements were also performed prior to testing. Data display, recording, and analysis were performed using RASP software (Respiratory Analysis Program, Physio Logic Ltd, Berks, England), which has been previously validated in our laboratory,¹³ on an IBM compatible PC. Deadspace of the apparatus, excluding face mask, was approximately 2 mL kg⁻¹ for all infants. Apparatus resistance, at a flow of 100 mL s⁻¹, was 0.78 kPa L⁻¹ s in the youngest and 0.48 kPa L⁻¹ s in older infants.

Measurements were made with the infant placed supine in the plethysmograph, following sedation with triclofos sodium (75–100 mg kg⁻¹: equivalent to 45–60 mg kg⁻¹ chloral hydrate) given by mouth. Data were collected, with room temperature controlled at 22–25°C by a servo controlled air conditioning unit, during behaviorally determined quiet sleep, i.e., when stable posture, regular respiration, and no eye movements were observed.⁶ With the neck slightly extended and the head supported in the midline with small sandbags, a transpar-

ent facemask (Rendell-Baker, Soucek: size 1 or 2 depending on size of the infant), to which a thin rim of therapeutic silicone putty (Carters, Wiltshire) had been applied, was positioned over the infant's nose and mouth to obtain an airtight seal. Additional support to the cheeks was provided by latex strapping attached to the face mask.

Measurements of thoracic gas volume at functional residual capacity (FRC_{pleth}) and airway resistance (R_{aw}) were made using a 100-L variable pressure infant plethysmograph by established techniques as previously described.⁷⁻¹⁰ Once an airtight seal around the face mask had been confirmed,¹¹ the plethysmograph was closed and the infant allowed to breathe room air while thermal equilibrium was attained. During this time, data for the analysis of tidal breathing parameters were collected in discrete 30–60 sec epochs.

Once thermal equilibrium was achieved, at least 5 measurements of FRC_{pleth} were obtained using end-inspiratory occlusions held for 2 to 3 respiratory efforts. Changes in P_{ao} and P_{pleth} during occlusion were displayed on a cathode ray oscilloscope to confirm that these were in phase during occlusions.

Measurements of R_{aw} were made while the infant re-breathed warmed, humidified, and oxygen-enriched air from a highly compliant 2-L bag. Temperature in the rebreathing bag was adjusted to obtain a satisfactory phase relationship between \dot{V} and P_{pleth} , as determined from the pressure-flow loop displayed on the oscilloscope. Once this was achieved, data for the analysis of R_{aw} were collected in discrete epochs of 18–36 sec duration, the rebreathing bag being flushed with humidified oxygen between each epoch.

Calculation of Lung Function Parameters

Lung function parameters were calculated using a computer assisted system with interactive operator control (RASP) according to previously established criteria.^{5,10,12}

For each infant, FRC_{pleth} was calculated as the mean of between 3 and 5 occlusions, where the infant's airway was occluded within the first 10% of expiration, the value from each occlusion being corrected by subtracting apparatus deadspace and the volume included above end-expiration (EE). Data were excluded from analysis if there was evidence of glottic closure or an air leak around the face mask.

R_{aw} was calculated as the mean of up to 25 breaths (minimum 5). Breaths were accepted for analysis if the pressure-flow loop was closed at points of zero flow. R_{aw} was reported from measurements made at 50% of peak \dot{V} at both initial inspiration (II) and EE. For each infant, the reciprocal of these values was computed to obtain G_{aw} and, after further division by FRC_{pleth} , SG_{aw} .

Tidal breathing parameters were calculated from a minimum of 20 breaths (range 20–50) collected in 2 discrete epochs, $t_{\text{PTEF}}:t_E$, respiratory rate and t_E being reported as the mean and standard deviation (SD).¹³ Periods of sighs or coughs were excluded from analysis.

Statistical Methods

For each infant, data were entered and validated with a double entry system using a database package (Epi-info, version 5.01b, Atlanta) on an IBM compatible PC. Details entered included lung function parameters, as described above, sex, ethnic group, gestational age, birth-weight and age, weight, crown-heel length, and symptom status at testing. Information on maternal smoking in pregnancy and the presence of asthma in first degree relatives was obtained questionnaire and also included. Unpaired *t* tests and a two sample test for proportions were used to compare baseline characteristics and lung function parameters between sexes within each group and between Groups 2 and 3. Analyses were performed separately for the infants included in Group 1, as measurements performed beyond 13 weeks of age in some of these infants were included in Groups 2 and 3. Data for Groups 2 and 3 were independent and initially analyzed together, the effect of status being examined subsequently. Linear interactive modelling was used to examine the extent to which variation in $t_{\text{PTEF}}:t_E$ was explained by FRC_{pleth} and II and EE SG_{aw} , both before and after adjusting for sex and age, weight, and length at testing (GLIM, Royal Statistical Society, London, 1985).

RESULTS

Details of the infants included in each group are given in Table 1. The prevalence of a family history of asthma and maternal smoking in pregnancy was 25 and 44%, respectively, for Group 1 infants. One infant in Group 1 was symptomatic at the time of testing, slightly crusted nostrils being the only abnormal finding on examination.

There were no significant differences between Groups 2 and 3 with respect to sex distribution and age, body weight, or length at testing. Significantly fewer infants in Group 3 were of Caucasian origin relative to Group 2, reflecting the differing criteria for inclusion for wheezy infants. Mean birthweight and gestational age were significantly lower in Group 3 infants compared to Group 2 (mean difference, 95% CI: -321 g; -99, -544; and -1.8 weeks; -0.9, -2.6, respectively). Mothers reported smoking in pregnancy in a significantly higher proportion for Group 3 than Group 2 infants (mean difference, 95% CI: 20%; 4, 36). Similarly, a family history of asthma was significantly more prevalent among infants in Group 3 (mean difference, 95% CI: 25%; 11, 40). Three

TABLE 1—Infant Characteristics¹

	Group 1 Healthy ≤13 weeks (n = 73)	Group 2 Healthy >13 weeks (n = 68)	Group 3 Prior LRI >13 weeks (n = 79)
Male:female	41:32	34:34	49:30
Caucasian (%)	97%	97%	86%*
Age (weeks)			
Mean (SD)	7.8 (1.3)	50.0 (10.9)	48.5 (15.0)
Range	5.0–12.6	22.9–72.9	15.1–78.1
Weight (kg)			
Mean (SD)	5.0 (0.8)	9.5 (1.4)	9.5 (1.7)
Range	3.6–8.2	6.1–13.8	3.6–13.4
Length (cm)			
Mean (SD)	57.8 (2.6)	75.7 (4.3)	74.5 (5.8)
Range	52.1–65.1	65.3–83.9	51.6–84.1
Birthweight (g)			
Mean (SD)	3440 (502)	3437 (495)	3115 (806)**
Range	2480–4680	2325–4600	864–4210
Gestation (weeks)			
Mean (SD)	40.1 (1.4)	40.1 (1.4)	38.5 (3.3)***
Range	37–43	36–43	25–42
Antenatal smoking (%)	44	32	53**
FH asthma (%)	25	16	42***
Symptomatic at testing (n)	1	3	12*

¹LRI, lower respiratory illness with wheezing; FH, family history. *P* values for differences Group 3 – 2:

P* < 0.05; *P* < 0.01; ****P* < 0.001.

TABLE 2—Lung Function Parameters¹

	Group 1 Healthy ≤13 weeks [mean (SD)] (n = 73)	Group 2 Healthy >13 weeks [mean (SD)] (n = 68)	Group 3 Prior LRI >13 weeks [mean (SD)] (n = 79)	Group 3 – 2 [mean diff; 95% CI]
FRC _{pleth} (mL)	141.1 (24.8)	262.4 (50.9)	261.9 (61.0)	-0.6 -19.1, 17.9
II SG _{aw} (s ⁻¹ kPa ⁻¹)	2.69 (1.05)	2.22 (0.69)	1.85 (0.88)	-0.37* -0.11, -0.63
EE SG _{aw} (s ⁻¹ kPa ⁻¹)	2.47 (1.05)	2.04 (0.70)	1.56 (0.77)	-0.48** -0.24, -0.72
t _{PTEF:t_E}	0.321 (0.075)	0.295 (0.077)	0.257 (0.079)	-0.039* -0.013, -0.064
Respiratory rate (breaths min ⁻¹)	46.5 (9.9)	30.5 (3.9)	32.8 (8.4)	2.3 -0.3, 5.0
t _E (sec)	0.76 (0.18)	1.17 (0.18)	1.13 (0.25)	-0.04 -0.12, 0.05

¹LRI, lower respiratory illness with wheezing; FRC_{pleth}, thoracic gas volume at functional residual capacity; II SG_{aw}, specific airway conductance at initial inspiration; EE SG_{aw}, specific airway conductance at end-expiration; t_{PTEF:t_E}, time to peak tidal expiratory flow:total expiratory time; t_E, total expiratory time; CI, confidence intervals. *P* values for differences Group 3 – 2: **P* < 0.001; ***P* < 0.0001.

infants (5%) in Group 2 were symptomatic at testing, all of whom had minor signs of upper respiratory illness only. Of the 12 infants (15%) in Group 3 reported as symptomatic at testing, 5 had minor signs of an upper respiratory tract infection, while audible wheezing or expiratory rhonchi were present in 7 infants.

Lung function parameters for each group are summarized in Table 2. Mean (range) FRC_{pleth} among healthy infants aged ≤13 weeks (Group 1) was 141.4 mL (87.2–203.9). This represented a mean (SD; range) of 28.6 mL kg⁻¹ (4.7; 16.5–38.4). Both II and EE SG_{aw} showed marked between-subject variability (mean (range): 2.7

$s^{-1} kPa^{-1}$ (1.1–6.2) and $2.5 s^{-1} kPa^{-1}$ (0.6–5.8), respectively. Mean (range) $t_{PTEF:t_E}$, respiratory rate and t_E for Group 1 infants was 0.321 (0.150–0.522), 46.5 breaths min^{-1} (30.8–81.6) and 0.76 sec (0.37–1.13), respectively.

Mean (range) FRC_{pleth} for Groups 2 and 3 was 262.4 mL (158.8–375.6) and 261.9 (109.9–435.4), respectively, and this group mean difference (Group 3 – 2) of -0.6 mL was not significant (Table 2). This represented a mean (SD; range) of 27.8 mL kg^{-1} (4.1; 19.7–40.7) in Group 2 and 28.0 mL kg^{-1} (7.1; 18.5–63.1) in Group 3. Mean II SG_{aw} , EE SG_{aw} , and $t_{PTEF:t_E}$ were significantly lower among Group 3 infants compared to Group 2 (Table 2). Mean (range) II SG_{aw} for Groups 2 and 3 was $2.2 s^{-1} kPa^{-1}$ (0.8–4.4) and $1.85 s^{-1} kPa^{-1}$ (0.7–5.0), mean (range) EE SG_{aw} was $2.0 s^{-1} kPa^{-1}$ (0.5–4.3) and $1.6 s^{-1} kPa^{-1}$ (0.3–4.2), and mean (range) $t_{PTEF:t_E}$ was 0.295 (0.138–0.500) and 0.257 (0.119–0.469) respectively. There were no significant differences in respiratory rate and t_E between Groups 2 and 3 (Table 2).

The data were then examined within each group to determine whether there were any significant sex differences for gestational age, birthweight, age, weight, and length at test as well as for lung function parameters (FRC_{pleth} , $FRC_{\text{pleth}} \text{ kg}^{-1}$, $t_{PTEF:t_E}$ and II, and EE SG_{aw}). Boys and girls in Group 1 were of comparable gestational age and age at testing. Girls in Group 1 tended to be significantly lighter at birth [group mean difference (95% CI): 270 g (42,500); $P = 0.02$], lighter at testing [group mean difference (95% CI): 0.6 kg (0.3, 0.9); $P < 0.001$] and shorter at testing [group mean difference (95% CI): 1.8 cm (0.6, 3.0); $p < 0.01$]. However, there were no significant sex differences in this group with respect to FRC_{pleth} , $FRC_{\text{pleth}} \text{ kg}^{-1}$, $t_{PTEF:t_E}$ and II, and EE SG_{aw} . There were no significant sex differences for infants in Group 2. Although of similar age and length at testing to boys, there was a tendency for girls in Group 3 to be lighter than boys [mean difference for boys–girls; (95% CI): 0.54 kg; -0.2 , 1.3; $P = 0.18$]. Thus, although absolute lung volume was lower in girls, $FRC_{\text{pleth}} \text{ kg}^{-1}$ was not significantly different [mean difference for boys–girls; (95% CI): 35.7 mL; 8.5, 62.8; $P = 0.01$ and 2.2 mL kg^{-1} ; $(-1.1$, 5.5); $P = 0.18$, respectively]. Data for boys and girls were, therefore, pooled within groups for the subsequent analyses.

Using linear regression and with $t_{PTEF:t_E}$ as the outcome variable, neither II nor EE SG_{aw} were significantly associated with $t_{PTEF:t_E}$ in Group 1 infants (Table 3). However, a weak but significant association was observed between FRC_{pleth} and $t_{PTEF:t_E}$, which could only explain a low proportion (11%) of the total variance in $t_{PTEF:t_E}$ (Fig. 1). This relationship remained significant after removing the outlier shown in Figure 1. A significant relationship was also found after prior adjustment for

TABLE 3—Regression Analyses for $t_{PTEF:t_E}$ in Healthy Infants ≤ 13 Weeks of Age (Group 1)

	Regression coefficient (95% CI)	r^2	χ^2 value (1 degree of freedom)
Unadjusted			
II SG_{aw}	0.015 (-0.002, 0.03)	0.04	3.0
EE SG_{aw}	0.016 (-0.0005, 0.03)	0.05	3.8
FRC_{pleth}	0.001 (0.0003, 0.002)	0.11	8.8*
Adjusted for age, length, weight at testing and sex			
II SG_{aw}	0.015 (-0.003, 0.03)	0.04	2.8
EE SG_{aw}	0.016 (-0.001, 0.03)	0.05	3.4
FRC_{pleth}	0.001 (0.0003, 0.002)	0.11	7.4*

¹For abbreviations see Table 2. * $P < 0.01$.

age, weight, and length at testing and sex of the infant (Table 3).

Among the older infants (Groups 2 and 3), a significant but weak association was found between $t_{PTEF:t_E}$ and both EE SG_{aw} and FRC_{pleth} , but not II SG_{aw} (Table 4). The relationship between $t_{PTEF:t_E}$ and EE SG_{aw} for infants in Groups 2 and 3 is shown in Figure 2, with separate identification of data points for those Group 3 infants who were wheezy at testing. After allowing for the effects of age, length, and weight at testing and sex, EE SG_{aw} alone remained significant but explained only a low proportion (9%) of the total variance in $t_{PTEF:t_E}$. There was, however, no significant effect of status on this relationship, the mean (95% CI) for the regression coefficient (intercept) for infants with prior LRI being -0.021 (-0.048 , 0.006); $P = 0.82$.

DISCUSSION

Tidal expiratory flow patterns in infancy may reflect both age and underlying lung mechanics. During passive expiration, the elastic recoil pressure of the respiratory system is highest at the onset of expiration, a time when the airways are also well distended and hence resistance relatively low. Theoretically, peak tidal expiratory flow should therefore occur at onset of expiration, giving rise to a low $t_{PTEF:t_E}$. In infants, this pattern may be evoked by inducing muscle relaxation with a brief end-inspiratory occlusion, but is otherwise unusual, indicating that some braking of tidal expiration is normal. It has been suggested that the low values of $t_{PTEF:t_E}$ observed in adults with airway obstruction reflect alterations in control of breathing, with a reduction of expiratory airflow

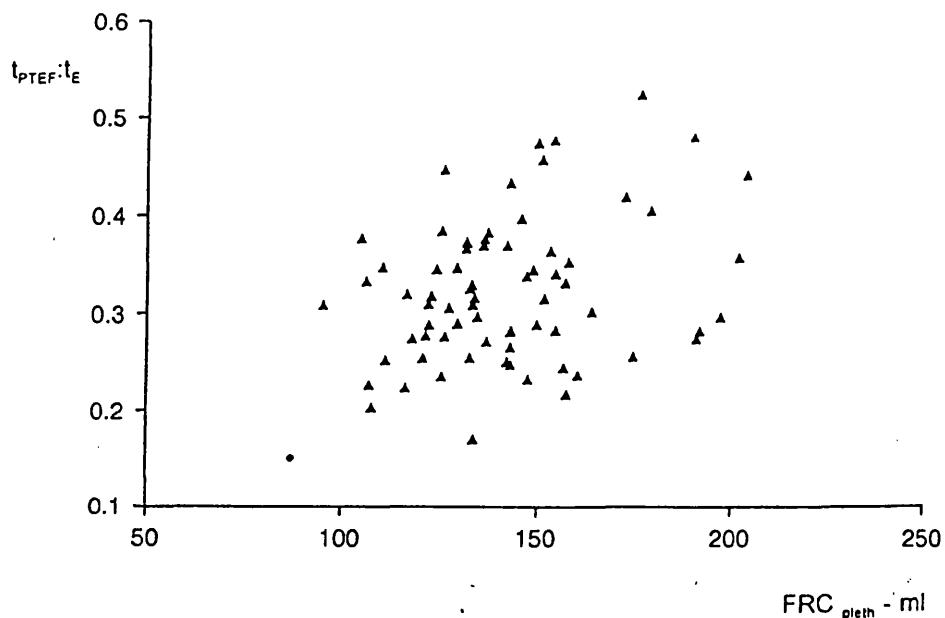


Fig. 1. Scattergram of the time to peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF}:t_E$) and thoracic gas volume at functional residual capacity (FRC_{pleth}) in healthy infants aged 13 weeks and less (Group 1). The closed circle on lower left identifies an outlier.

braking in response to underlying respiratory mechanics, rather than being simply a direct index of lower airway size³.

In this study, measurement of $t_{PTEF}:t_E$, FRC_{pleth} , and R_{aw} were made on the same test occasion in healthy infants and those with prior physician diagnosed LRI with wheezing. Group mean $t_{PTEF}:t_E$ in the younger healthy infants was comparable to that reported by Martinez et al. for a group of infants of similar age.¹ Among the older infants, mean $t_{PTEF}:t_E$ was significantly lower in the group of infants with a prior history of physician-diagnosed LRI with wheezing, most of whom were asymptomatic at testing, when compared to a group of healthy infants of a similar age. A lower $t_{PTEF}:t_E$ has also been reported in adults with airflow obstruction³ and in infants with bronchopulmonary dysplasia,¹⁴ but was not observed in asthmatic children who were asymptomatic at testing.⁴ However, in our study, although group mean $t_{PTEF}:t_E$ was significantly lower in infants with prior LRI, there was considerable between-subject variation, suggesting that $t_{PTEF}:t_E$ discriminates well between groups but not between individuals. Martinez et al. have shown that infants with a $t_{PTEF}:t_E$ in the lower tercile of the distribution, when measured before 13 weeks of age, are at significantly greater risk of developing LRI with wheezing by 3 years of age.² However, the purpose of this study was to report cross-sectional associations of this parameter with an established measure of airway

TABLE 4—Regression Analyses for $t_{PTEF}:t_E$ in Older Healthy Infants (Group 2) and Those With Prior Lower Respiratory Illness (Group 3)¹

	Regression coefficient (95% CI)	r^2	χ^2 value (1 degree of freedom)
Unadjusted			
II SG _{aw}	0.015 (-0.001, 0.03)	0.02	3.4
EE SG _{aw}	0.035 (0.018, 0.051)	0.11	18.4**
FRC_{pleth}	0.0003 (0.00006, 0.0005)	0.04	6.4*
Adjusted for age, length, weight at testing and sex			
II SG _{aw}	0.012 (-0.004, 0.03)	0.02	2.3
EE SG _{aw}	0.031 (0.014, 0.047)	0.09	14.1**
FRC_{pleth}	0.0002 (0.0001, 0.0005)	0.01	1.7
Adjusted for age, length, weight at testing and sex and status ²			
II SG _{aw}	0.008 (-0.008, 0.02)	0.01	1.1
EE SG _{aw}	0.027 (0.01, 0.04)	0.07	10.1**
FRC_{pleth}	0.0002 (0.00009, 0.0005)	0.01	2.1

¹For abbreviations see Table 2. * $P < 0.01$; ** $P < 0.001$.

²Status: infants with prior LRI (Group 3) compared to healthy infants (Group 2).

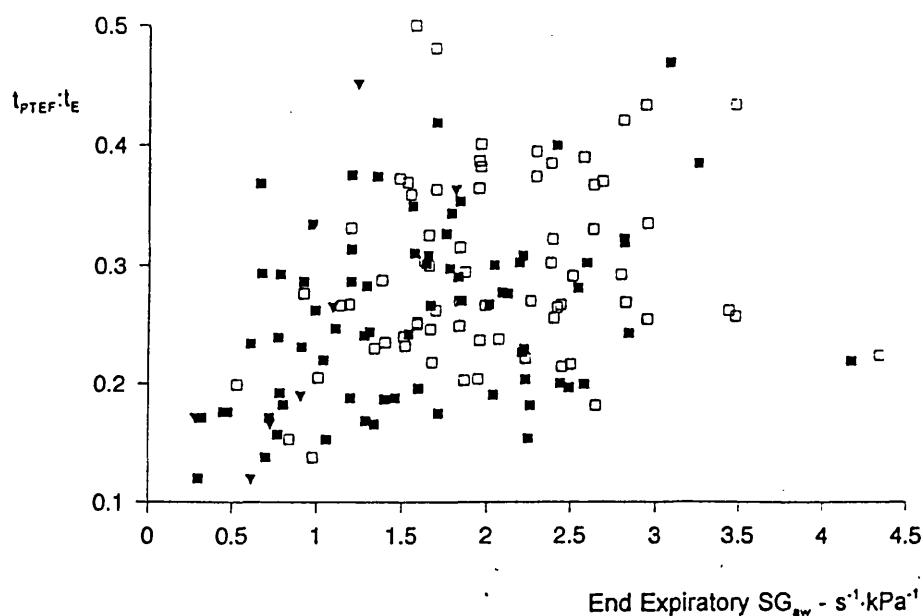


Fig. 2. Scattergram of the time to peak tidal expiratory flow as a proportion of total expiratory time ($t_{PTEF:t_E}$) and end-expiratory specific airway conductance (SG_{aw}) in infants aged more than 13 weeks. The open squares denote healthy infants (Group 2)

and the closed squares, infants with prior physician diagnosed lower respiratory tract illness with wheezing (Group 3) who were not wheezing when tested. Closed triangles indicate Group 3 infants who were wheezing when tested.

function— SG_{aw} , analysis of the longitudinal data not being feasible until all follow-up studies have been completed.

In this study, group mean II and EE SG_{aw} measurements were significantly lower in infants with prior wheezing LRI when compared to healthy infants of a similar age. However, within each group there was considerable between-infant variation. The range of values observed in healthy infants in our study is greater than previously reported.⁷ This may reflect differences in technique, as measurements made in the healthy infants studied by Stocks et al.⁷ were undertaken after more prolonged periods of rebreathing than is current practice in our laboratory.

A significant association of $t_{PTEF:t_E}$ with inspiratory SG_{aw} has been reported in healthy adults and those with airflow obstruction,³ with FEV₁ in healthy and asthmatic children (the latter asymptomatic at testing),⁴ and with \dot{V}_{maxFRC} in healthy infants and those with bronchopulmonary dysplasia.¹⁴ We have shown that, in healthy infants aged 13 weeks or less, FRC_{pleth} , but not II or EE SG_{aw} , is significantly but weakly associated with $t_{PTEF:t_E}$. The lack of a significant relationship between $t_{PTEF:t_E}$ and both II and EE SG_{aw} in younger healthy infants suggests that, in the absence of airflow obstruction, there is relative freedom to vary tidal expiratory flow patterns. Laryngeal and postinspiratory diaphragmatic braking, which are accompanied by late onset of peak expiratory

flow and hence prolongation of $t_{PTEF:t_E}$, help to maintain a dynamically elevated FRC and maximize gas exchange in newborn infants.¹⁵ The weak relationship between FRC_{pleth} and $t_{PTEF:t_E}$ observed among the younger healthy infants in this study suggests that this phenomenon may persist to some extent throughout the first 3 months of life. The importance of establishing a stable FRC, together with interdependence between respiratory timing, modulation of expiratory flow, and dynamic elevation of lung volume, may confound any relationship between $t_{PTEF:t_E}$ and indices of airway function, especially in healthy infants. With increasing postnatal age, the need to modulate expiratory flow and timing to maintain a stable FRC diminishes.

In older infants, whether healthy or with prior LRI with wheezing, EE SG_{aw} was most strongly associated with $t_{PTEF:t_E}$, the association with FRC_{pleth} being no longer significant after allowing for age, length, and weight at testing and sex. However, EE SG_{aw} explained only a low proportion of the total variance in $t_{PTEF:t_E}$. This relationship was not significantly different for infants with prior LRI compared to healthy infants. While Morris and Lane³ have found that the percentage of expiratory volume expired at peak tidal expiratory flow is significantly associated with inspiratory SG_{aw} in healthy adults and those with airflow obstruction, the published data (Fig. 3) suggest that this relationship may have been influenced by individuals with SG_{aw} of less than $0.10 \text{ cm H}_2\text{O}^{-1} \text{ s}^{-1}$

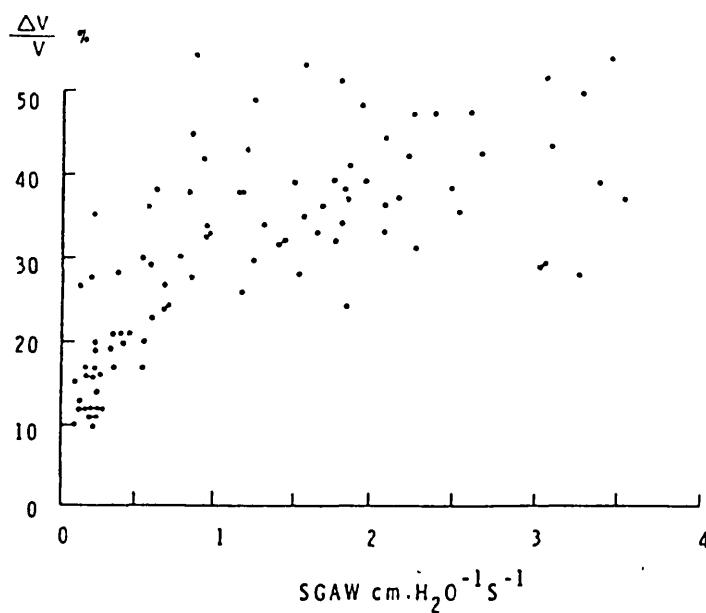


Fig. 3. Scattergram of percentage of expiratory volume expired at peak tidal expiratory flow ($\Delta V/V\%$) and inspiratory specific airway conductance (SG_{aw}) in healthy adults and those with airflow obstruction and restrictive lung disease. Reproduced with permission from Reference 3.

(equivalent to $1.0\text{ s}^{-1}\text{ kPa}^{-1}$). Those with values greater than this show both higher mean values and greater variability in $t_{PTEF}:t_E$. While direct comparisons are impossible, since measurements in infants include a variable component due to the resistance of the nasal passages,¹⁶ mean SG_{aw} does appear to be relatively constant from the end of the first year of life to adulthood.⁷ In our study, all but 3 of the healthy infants had values of EE $SG_{aw} \geq 1.0\text{ s}^{-1}\text{ kPa}^{-1}$, as did a large proportion of those with prior LRI, reflecting the fact that most of these infants were asymptomatic at testing. In the absence of current airflow obstruction, there should be relative freedom to vary tidal expiratory flow patterns and hence no strong relationship between $t_{PTEF}:t_E$ and other measures of airway function would be expected. Had measurements been confined to only those infants with more severe degrees of airflow obstruction, as evidenced by a low SG_{aw} , a stronger relationship might have been found, as indicated by inspection of the data in the current study from infants with $SG_{aw} < 1.0\text{ s}^{-1}\text{ kPa}^{-1}$ (Fig. 2). However, we felt it important to examine the association for wheezy infants across the whole spectrum of disease severity encountered in clinical practice, therefore we employed a clinical rather than laboratory-based definition for prior airways disease.

In conclusion, we have measured $t_{PTEF}:t_E$, II, and EE SG_{aw} in both healthy infants and those with prior LRI with wheezing above 3 months of age and found that both $t_{PTEF}:t_E$ and SG_{aw} are significantly lower in the wheezy than in the healthy group. There is a significant though

weak association between $t_{PTEF}:t_E$ and EE SG_{aw} in infants over 3 months of age, irrespective of their prior wheezing status. However, this association is not significant in healthy younger infants, in whom the pattern of expiratory flow may reflect dynamic maintenance of FRC as much as a response to airway caliber. Further work is needed to elucidate the factors influencing tidal expiratory flow patterns in infancy.

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Comparison of Single-Breath and Plethysmographic Measurements of Resistance in Infancy

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Single-breath technique (SBT) measurements of total respiratory resistance (Rrs) were compared with plethysmographic measurements of airway resistance (Raw) in healthy infants ≤ 13 wk of age (Group 1; n = 49) and > 13 wk of age (Group 2; n = 37) and in infants > 13 wk of age with prior wheeze (Group 3; n = 49). A significantly higher percentage of Rrs (19%) than of Raw (2%) measurements were technically unsatisfactory, a linearity of the flow-volume curve accounting for 54% of Rrs failures. Although both Rrs and Raw were significantly higher in Group 3 infants, between-subject variability was wide in all groups. Rrs was significantly higher than initial expiratory (IE) Raw in all groups. Mean difference Rrs - IE Raw (95% CI) values were 1.98 (1.51, 2.48), 1.29 (0.96, 1.62), and 1.97 (1.56, 2.38) kPa·L $^{-1}$ ·s for Groups 1, 2, and 3, respectively. Significant but smaller differences were seen for end-expiratory (EE) Raw in Groups 1 and 2 but not in Group 3. Mean difference Rrs - EE Raw (95% CI) values were 0.68 (0.11, 1.26), 0.55 (0.19, 0.92), and 0.31 (-0.06, 0.69) kPa·L $^{-1}$ ·s for Groups 1, 2, and 3, respectively. Despite wide between-subject variability in Rrs and a relatively high failure rate, the SBT is simple to use, and it may be applicable to epidemiologic studies. However, clinical applications in individual infants may be limited by failure to detect the dynamic changes in resistance throughout the breath evident from plethysmographic studies. **Dundas I, Dezateux CA, Fletcher ME, Jackson EA, Stocks J. Comparison of single-breath and plethysmographic measurements of resistance in infancy. Am J Respir Crit Care Med 1995;151:1451-8.**

The association between respiratory illness in the first year of life and subsequent respiratory morbidity is being increasingly recognized (1, 2). The consequences of environmental insults, both prenatal and postnatal, to the developing lung at a time of rapid growth are of particular importance. Objective measurements of infant lung function are required if the mechanisms underlying these associations are to be elucidated.

Plethysmographic measurements of lung volume (FRCpleth) and airway resistance (Raw) may be used to identify functional and developmental abnormalities in infants (3-8). However, the complexity of these tests limits their epidemiologic and clinical applications. Recently, new approaches to infant lung function testing have simplified methods of measurement in this age group (9, 10). The single-breath technique (SBT), which measures the resistance, compliance, and time constant of the respiratory system during passive expiration after brief airway occlusions (11-14), is simple and rapid to perform, and it is being increasingly applied in clinical settings (10, 15-17). Despite its increasing popularity, the relationship between SBT measurements of total respiratory resistance (Rrs), which includes chest wall and lung tissue components, and those of airway resistance has yet to be fully

established in infants (18, 19). The aim of this study was to compare measurements of airway and total respiratory resistances obtained on the same occasion in both healthy infants and in those with prior airway disease.

METHODS

Subjects

Measurements of Raw and total respiratory resistance (Rrs) were attempted on 135 occasions in 107 infants. Infants were recruited to the study from two sources. Healthy infants were recruited shortly after birth as part of an ongoing epidemiologic study (unpublished data). Infants with a gestational age of at least 36 wk, without major congenital, respiratory, neuromuscular, or cardiac abnormalities, and who did not require ventilatory support during the neonatal period were eligible for inclusion. Initial measurements were made between 5 and 13 wk of age, prior to any respiratory illness, and, when possible, they were repeated at around 1 yr of age. Some infants participated in additional measurements between 3 and 18 mo of age for other studies (20). After the last laboratory visit, the primary medical care record of each infant was examined, and any episodes of physician-diagnosed wheeze were noted.

Measurements were also made on one occasion in infants with physician-diagnosed recurrent wheezing recruited from the wards and clinics of the Hospitals for Sick Children, London. Infants were eligible for inclusion if no major congenital abnormalities were present. For all infants, measurements were made at least 3 wk from the onset of any upper respiratory tract infection. Information regarding maternal smoking and family history of asthma (first-degree relatives) was obtained from parents at time of recruitment.

To facilitate comparison between wheezy and healthy infants, infants were grouped according to their age at measurement and respiratory status. Group 1 comprised 49 healthy infants 13 wk of age or younger, Group

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2 comprised 37 healthy infants older than 13 wk of age, and Group 3 comprised 49 infants with physician-diagnosed wheeze older than 13 wk of age. The aim of this study was to compare measurements of Rrs and Raw obtained on the same occasion. Thus, although some infants were studied on more than one occasion, longitudinal analysis was not undertaken for these infants, who were included in Group 1 when younger than 13 wk of age and subsequently in Group 2 or in Group 3, according to their respiratory status. Groups 2 and 3 were, therefore, mutually exclusive.

Group 1 infants were 5.0 to 12.6 wk of age and weighed 3.8 to 6.7 kg at testing, Group 2 infants were 17.7 to 61.7 wk of age and weighed 6.1 to 12.7 kg at testing, and Group 3 infants were 15.1 to 74.3 wk of age and weighed 3.6 to 13.8 kg at testing.

This study was approved by the local ethics committee. Written informed consent was obtained from one or both parents, who were often present during measurements.

Procedures

Prior to measuring each infant, the 100-L variable pressure plethysmograph was calibrated in terms of volume change. Bags of saline with a total weight approximately equal to that of the infant were placed in the plethysmograph. To approximate pressure changes occurring during measurements, 20 ml of air (or 40 ml for infants older than 4 mo of age) were syringed into and out of the chamber at a frequency equivalent to the infant's respiratory rate. Pressure at the airway opening (Pao) and flow (\dot{V}) were also calibrated on each occasion (21). Airflow was measured using a Fleisch pneumotachograph (PNT) appropriate to the infant's size and peak flow (size "0" linear to ± 12 L/min, size "1" linear to ± 30 L/min) connected to a low range pressure transducer (Validyne MP45 ± 0.2 kPa; Validyne Corp., Northridge, CA); Pao, via a port in the mask mount, and plethysmographic pressure (PpIeth) were measured using Validyne MP45 transducers with ranges of ± 5 and ± 0.2 kPa, respectively. The apparatus resistance at a flow of 100 ml/s was 0.78 kPa·L⁻¹·s using the Fleisch "0" PNT and 0.48 kPa·L⁻¹·s with the Fleisch "1" PNT, with an apparatus dead space of 7.6 and 26 ml, respectively.

All measurements were made during quiet sleep when posture was stable, respiration regular, and no eye movements observed (22). The infants were sedated using triclofos sodium 75 mg/kg (up to 8 wk of age) or 100 mg/kg (older infants). One gram of triclofos sodium is equivalent to 660 mg chloral hydrate. Once asleep, the infant was placed supine within the open plethysmograph with the neck slightly extended and head in the midline, supported on either side by small sandbags. A transparent Rendell-Baker face mask, size 1 or size 2 as appropriate, was gently lowered in place after applying therapeutic putty around the rim to obtain an airtight seal. Latex cheek strapping was attached to the face mask mount to provide additional support.

After checking the mask seal by performing a brief end-inspiratory occlusion (23), the plethysmograph was closed, and the infant was allowed to breath room air while thermal equilibrium within the chamber was attained. Measurements of thoracic gas volume at functional residual capacity were made using the plethysmograph (FRCpIeth) according to well-established criteria as described previously (21, 24, 25).

For FRCpIeth measurements, at least five early expiratory occlusions were made after a stable end-expiratory level had been established, each being held for two to three respiratory efforts. Changes in PpIeth and Pao were displayed on a cathode-ray oscilloscope. Data were considered acceptable if no leaks were evident and changes in PpIeth versus Pao were in phase during occlusions. The infant was then allowed to rebreathe from a highly compliant 2-L bag containing warmed, humidified and oxygen-enriched air (temperature at the mask $\approx 37^\circ$ C). The phase relationship between \dot{V} and PpIeth was inspected on an oscilloscope, and temperature within the rebreathing bag was adjusted if necessary until a satisfactory pressure-flow loop was obtained (24). Three epochs of data during breathing of warmed, humidified air were collected, each of 18 to 36 s duration. The rebreathing bag was flushed with humidified oxygen between each epoch.

Measurements of Rrs and respiratory system compliance (Crs) by the SBT were attempted in all infants using the pneumatically operated valve system employed during plethysmographic measurements. End-inspiratory occlusions were held until a stable pressure plateau was seen. A minimum of five successful occlusions were obtained, allowing five to 10 breaths

between occlusions to reestablish the end-expiratory level and to detect air leaks around the face mask (23). When possible, occlusions were also performed while the infant was rebreathing warmed, humidified air to obtain SBT measurements of Rrs under the same conditions as Raw. Crs was also measured by the multiple occlusion technique (MOT) using previously published methods (13, 14) for comparison with SBT data (26, 27).

Data display, recording, and analysis were performed using RASP software (Respiratory Analysis Program, Physio Logic Ltd, Berks, UK) on an IBM compatible computer. \dot{V} , Pao, and PpIeth were digitized and sampled (Analog Devices RTI-815) at 50 to 100 Hz (FRCpIeth, MOT, and Raw) or 100 to 200 Hz (SBT) according to the infant's respiratory rate. Volume was digitally integrated from \dot{V} . Data were collected in discrete 18 to 72 s epochs.

Analysis of Lung Function Parameters

All data were analyzed using a computer-assisted system (RASP) with interactive operator quality control, according to previously established criteria (13, 14, 21).

Lung Volume

FRCpIeth was calculated as the mean of three to five occlusions when the infant was occluded at or within 10% of the start of expiration. Subsequent correction to FRC was performed by subtracting the volume occluded above end-expiratory volume and the apparatus dead space from the total occluded gas volume. The phase relationship between PpIeth and Pao was inspected on an X-Y plot, and occlusions were not analyzed if there was any evidence of glottic closure or leaks through or around the apparatus (21).

Airway Resistance

Values of Raw were reported as the mean of between five and 25 breaths (median, 11 breaths), where the pressure-flow loops were accepted if closed at points of zero flow. Raw was calculated from the beginning and end of both inspiration and expiration, i.e., at four points throughout the respiratory cycle, giving initial and end-inspiratory and initial and end-expiratory Raw (25), at a flow equivalent (± 20 ml/s) to the mean flow recorded during the calculation of Rrs by the SBT in each infant.

Respiratory Resistance and Compliance

Passive flow-volume data obtained from the SBT were accepted if the expiratory flow-volume plot was linear ($r^2 \geq 0.99$) over at least 40% of total expired volume and the standard deviation (SD) of the pressure plateau samples was ≤ 0.01 kPa over at least 0.1 s. Apparatus resistance at the flows attained during expiration was subtracted from Rrs. Crs from the MOT was calculated from the slope of the least-squares linear regression through the volume-pressure data. Data were accepted when the Pao plateau was of ≥ 0.15 s duration with a SD of ≤ 0.01 kPa and the slope of the volume-pressure regression line had an $r^2 \geq 0.95$ over a pressure range of at least 0.35 kPa.

Statistical Methods

Unpaired t tests and a two-sample test for proportions were used to compare baseline characteristics and lung function parameters between groups. Comparisons of lung function parameters, obtained by different techniques or under different conditions were made using the method of Bland and Altman (28).

RESULTS

Technically satisfactory results for both Raw and Rrs were obtained on 107 of 135 occasions. As summarized in Table 1, a significantly higher percentage of Rrs measurements failed relative to plethysmographic measurements of Raw (weighted mean from all occasions, 19 and 2%, respectively; 95% confidence intervals [CI] of the difference Rrs - Raw: 7%, 27%). This difference was particularly marked in infants older than 3 mo of age (i.e., Groups 2 and 3). However, there was no significant difference in the percentage of failed SBT measurements between infants with physi-

TABLE 1

PERCENTAGE OF INFANTS IN WHOM MEASUREMENTS OF AIRWAY AND RESPIRATORY RESISTANCES WERE UNSUCCESSFUL

	Group 1 (n = 49)	Group 2 (n = 37)	Group 3 (n = 49)	Total* (n = 135)
Failed Rrs, %	12	22	25	19
Failed Raw, %	4	0	2	2
Both successful, %	86	78	74	79

Definition of abbreviations: Group 1 = healthy infants 13 wk of age or younger; Group 2 = healthy infants older than 13 wk of age; Group 3 = infants with physician-diagnosed wheeze older than 13 wk of age; Rrs = total respiratory resistance; Raw = airway resistance.

* Weighted means from all occasions.

cian-diagnosed wheeze (Group 3) and healthy infants of a comparable age (Group 2) (95% CI for the difference Group 3 to Group 2: -15%, 20%). Alinearity of the flow volume curve (Figure 1) and failure to equilibrate or achieve a relaxed plateau during airway occlusion (13, 14) were the major reasons for failure to obtain satisfactory estimates of Rrs (54 and 23% of all failures, respectively). Successful measurements of Raw were obtained in all but three infants, two of whom woke before measurements could be completed, and in one of whom severe weather conditions precluded reliable plethysmographic data collection.

Thus, comparison of Rrs and Raw was possible on 107 of 135 occasions (79%), and details of these infants are summarized in Table 2. Of the 42 infants in Group 1, 21 and seven were included in Groups 2 and 3, respectively, when subsequently measured at more than 13 wk of age. Groups 2 and 3 were, however, independent. Of the 36 infants with prior wheeze, 11 were born at or below 36 wk of gestation, and two required supplemental oxygen. However, only 10 were symptomatic at time of testing.

There were no significant differences between those successfully studied and those in whom one or more measurements failed with respect to sex, maternal smoking during pregnancy, family history of asthma, body size, or specific conductance (reciprocal of resistance divided by FRCpleth) ($p > 0.10$ for all parameters). For older infants with successful measurements of Rrs and Raw, weight and length at time of testing were similar between healthy and wheezy infants ($p > 0.10$). The prevalence of maternal smoking during pregnancy and family history of asthma was significantly higher in wheezy (Group 3) than in healthy (Group 2) infants (95% CI of the differences Group 3 to Group 2: 10%, 59%; 3%, 50%, respectively). Of the seven infants from Group 1 sub-

TABLE 2

DETAILS OF INFANTS IN WHOM SATISFACTORY MEASUREMENTS OF BOTH AIRWAY AND RESPIRATORY RESISTANCE WERE OBTAINED*

	Group 1 (n = 42)	Group 2 (n = 29)	Group 3 (n = 36)
Age, wk	8.2 (1.2)	49.8 (10.5)	48.2 (15.0)
Weight, kg	5.1 (0.7)	9.5 (1.7)	9.4 (2.0)
Length, cm	58.3 (2.4)	75.8 (5.7)	73.4 (6.8)
Male, %	55	52	61
Maternal smoking during pregnancy, %	48	24	58†
Family history of asthma, %	29	21	47†

For definition of groups, see Table 1.

* Values are means with SD shown in parentheses.

† $p < 0.05$ for Group 3 compared with Group 2.

‡ $p < 0.001$ for Group 3 compared with Group 2.

sequently included in Group 3, six (86%) had mothers who smoked throughout pregnancy compared with only eight (38%) of the 21 infants who were subsequently included in Group 2. The prevalence of postnatal maternal smoking was within 3% of that reported during pregnancy for each of the groups (data not shown).

Results from the lung function measurements are summarized in Table 3. In Group 1, mean (SD) FRCpleth was 27.9 (5.0) ml/kg, and mean (SD) Rrs was 5.3 (1.7) kPa·L⁻¹·s, whereas Raw ranged from 2.4 to 4.5 kPa·L⁻¹·s throughout the respiratory cycle. Among the older infants there was a tendency for mean (SD) FRCpleth per kilogram to be higher in wheezy than in healthy infants: 29.9 (6.6) and 28.2 (4.1) ml/kg respectively, but this was not statistically significant (95% CI of the difference Group 3 to Group 2: -1.1, 4.3 ml/kg; $p > 0.10$). Both Rrs and Raw, calculated at either initial inspiration or end-expiration, were significantly higher among wheezy infants than among healthy infants of a similar age. However, these differences were not significant when Raw was calculated at higher lung volumes, i.e., during end-inspiration and initial expiration.

There were small but statistically significant differences between Crs calculated by the MOT and SBT in all three groups (Table 3). Mean difference (95% CI) was 0.7 (0.2, 1.2) ml·kPa⁻¹·kg⁻¹ ($p < 0.01$) for Group 1, -1.0 (-1.7, -0.4) ml·kPa⁻¹·kg⁻¹ ($p < 0.002$) for Group 2, and 0.9 (0.3, 1.5) ml·kPa⁻¹·kg⁻¹ ($p < 0.01$) for Group 3. The mean intercept [(MOT + SBT)/2] was approximately 3 ml/kg for all three groups of infants. However, while it was slightly higher during MOT for Groups 1 and 3 (0.6 and 0.9 ml/kg, respectively), for Group 2 infants it was, on average, 0.8 ml/kg higher during the SBT.

The relationship between Rrs and Raw calculated at matched flows during initial and end-expiration for Groups 2 and 3 (according to the method of Bland and Altman) is shown in Figures 2 and 3, with limits of agreement shown in Table 4. Despite the wide scatter of results, Rrs was significantly ($p < 0.001$) higher than initial expiratory Raw in all three groups of infants. Rrs exceeded initial expiratory Raw in all but two healthy older infants and three younger healthy infants. Although the differences were smaller, mean Rrs was also significantly ($p < 0.05$) higher than Raw calculated at end-expiration in Groups 1 and 2. However, there was no significant difference between the two techniques in Group 3 infants ($p = 0.10$). End-expiratory Raw exceeded Rrs in 28% of infants in both Groups 1 and 2, and in 22% of infants in Group 3.

During measurements of Raw the infant breathed from a bag containing warmed, humidified, oxygen-enriched air. This was accompanied by statistically significant increases in respiratory rate

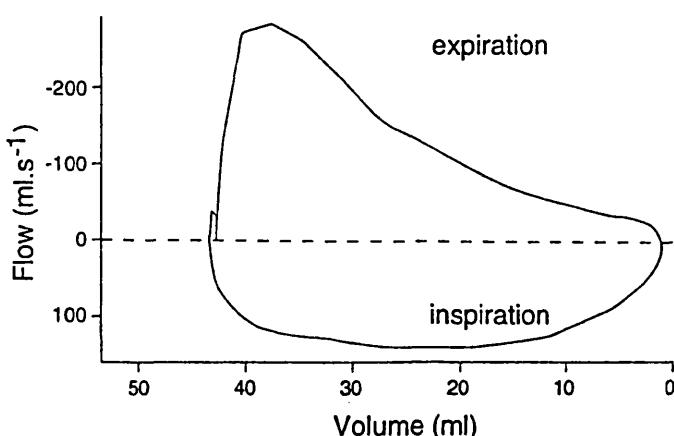


Figure 1. Example of an alinear flow-volume curve obtained using the single-breath technique.

TABLE 3
LUNG FUNCTION RESULTS*

	Group 1	Group 2	Group 3	95% CI†
FRCpleth, ml	142.1 (27.7)	265.3 (51.4)	278.4 (76.1)	-19.0, 44.9
Raw, kPa·l ⁻¹ ·s				
Initial inspiration	3.4 (1.1)	2.1 (0.9)	2.8 (1.5)	0.1, 1.4‡
End-inspiration	2.4 (1.3)	1.5 (0.6)	2.0 (1.3)	0.0, 0.9
Initial expiration	3.2 (1.4)	2.0 (0.8)	2.3 (0.9)	-0.1, 0.7
End-expiration	4.5 (1.7)	2.7 (1.0)	3.9 (1.7)	0.5, 1.9‡
Rrs, kPa·l ⁻¹ ·s	5.3 (1.7)	3.3 (1.0)	4.2 (1.7)	0.3, 1.6‡
Crs SBT, ml·kPa ⁻¹ ·kg ⁻¹	12.7 (2.3)	15.4 (2.7)	12.1 (2.5)	-4.6, -2.1§
Crs MOT, ml·kPa ⁻¹ ·kg ⁻¹	13.4 (2.0)	14.4 (2.5)	13.0 (2.4)	-2.6, -0.3‡

Definition of abbreviations: CI = confidence interval of mean difference; FRCpleth = functional residual capacity by plethysmography; Raw = airway resistance; Rrs = total respiratory resistance (single-breath technique); Crs = total respiratory compliance; SBT = single-breath technique; MOT = multiple occlusion technique. For definition of groups, see Table 1.

* Values are means with SD shown in parentheses.

† Group 3 compared with Group 2.

‡ p < 0.05.

§ p < 0.001.

(Groups 2 and 3 only), tidal volume, and peak tidal expiratory flow (PTEF) (Table 5). At any given lung volume, flows recorded after release of airway occlusions for the SBT were also significantly higher than those during tidal breathing (data not shown). When approximately 40% of tidal volume had been expired, the point at which analysis of the linear portion of the flow-volume curve normally commenced, flows were approximately 30% higher than the PTEF recorded in that infant during tidal breathing (ranging

from 30% less to 115% greater). The mean flow recorded for each infant during the calculation of Rrs, which was used to denote the point of analysis for Raw, was equivalent to 72% (SD 13%) of PTEF during Raw measurements, a similar mean and range of percentages being obtained from all three groups of infants.

Repeat measurements of Crs and Rrs by the SBT while breathing warmed humidified air, as for the Raw measurements, were achieved in 18 infants: mean (SD) weight, 8.3 (2.5) kg; age range, 8 to 61 wk (Table 6). There was a significant increase in Crs during rebreathing, the mean increase (95% CI) being 19.9 (11.5, 28.3) ml/kPa (Figure 4). This was accompanied by a significant reduc-

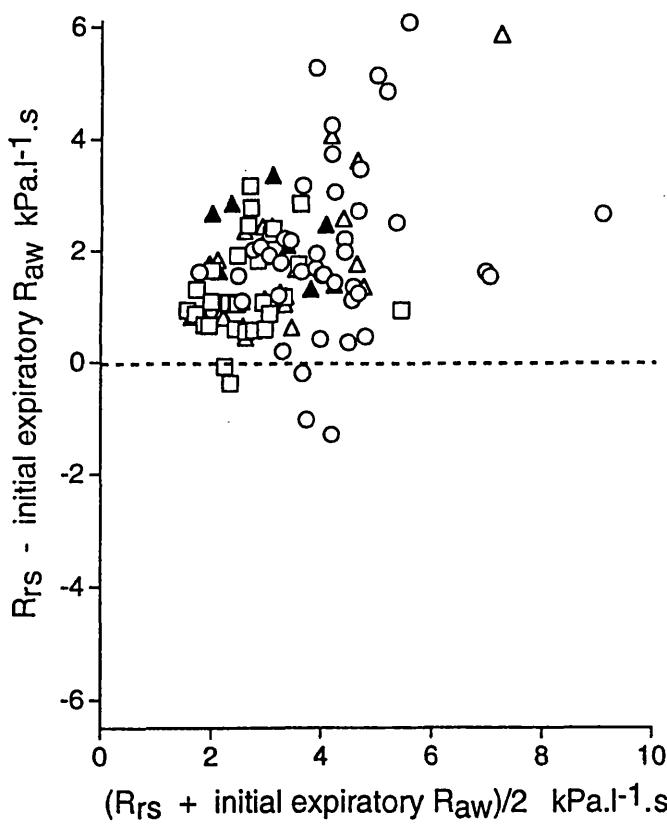


Figure 2. Scatter plot, according to the method of Bland and Altman (28), of the differences between total respiratory resistance and initial expiratory airway resistance and their mean. Open circles indicate Group 1 (healthy infants < 13 wk of age), open squares indicate Group 2 (healthy infants > 13 wk of age), open triangles indicate Group 3 (infants > 13 wk of age with prior wheeze who were asymptomatic when tested), closed triangles indicate Group 3 (infants who were symptomatic at testing).

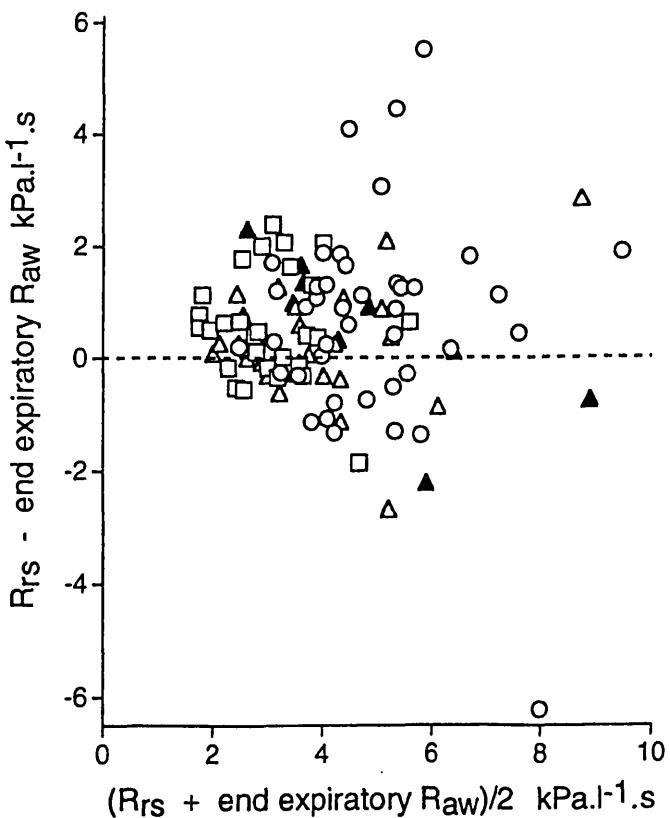


Figure 3. Scatter plot, according to the method of Bland and Altman (28), of the differences between total respiratory resistance and end-expiratory airway resistance and their mean. Symbols as for Figure 2.

TABLE 4

COMPARISON OF RESPIRATORY AND EXPIRATORY AIRWAY RESISTANCE (kPa·L⁻¹·s)

	Mean Difference (SD)	95% Limits of Agreement	95% CI
Rrs - Raw (initial expiration)			
Group 1	1.98 (15.6)	-1.12, 5.12	1.51, 2.48†
Group 2	1.29 (0.87)	0.45, 3.03	0.96, 1.62†
Group 3	1.97 (1.22)	-0.47, 4.41	1.56, 2.38†
Rrs - Raw (end-expiration)			
Group 1	0.68 (1.86)	-3.03, 4.40	0.11, 1.26*
Group 2	0.55 (0.96)	-1.37, 2.47	0.19, 0.92*
Group 3	0.31 (1.11)	-1.91, 2.54	-0.06, 0.69

For definition of abbreviations, see Table 1.

* p < 0.05.

† p < 0.001.

tion in mean Rrs of -0.89 kPa·L⁻¹·s (95% CI of difference: -1.45, -0.33 kPa·L⁻¹·s) (Figure 5). There was also a tendency for the volume intercept to increase significantly during rebreathing, both in absolute terms and when corrected for body weight: mean increase (95% CI), 1.2 (0.4, 2.0) ml/kg.

DISCUSSION

The results from this study indicate that, despite the apparent simplicity of the SBT for measuring Rrs, relative failure rate is higher than that encountered during plethysmographic measurements of Raw in the same infants. The failure rate for the SBT in the current study was considerably lower than that previously reported in preterm infants (13). This probably reflects fewer exclusions because of distortion of the flow-volume curve by excessive modulation of expiratory flow, which was noted in preterm neonates. Nevertheless, failure to achieve an adequate plateau or linearity of the flow-volume curve resulted in technical failures on 19% of occasions. Although the SBT did fail in two of the infants with the highest Raw values, there was no apparent relationship between failure rate and either symptoms or Raw values. This may reflect the fact that less than one third of the infants studied were symptomatic at testing.

Despite the fact that most of the infants were asymptomatic at time of testing, both Rrs and end-expiratory Raw were significantly higher among infants with prior wheeze than among healthy infants of similar age. However, an extremely variable relationship between Rrs and Raw was observed within infants. Partitioning of airway resistance from that contributed by the flow-resistive and viscoelastic properties of the lung and chest wall is extremely complex. The degree of variability between techniques observed in this study can largely be explained by the numerous factors known to influence the assessment of resistance.

Flow and Volume Dependency of Resistance

Airways resistance is known to be both flow- and volume-dependent, values tending to increase because of turbulence at higher flows and decrease because of increased elastic recoil and hence distension of the small airways at higher volumes (29-31). In addition, at any given lung volume or flow, Raw is generally lower during inspiration than during expiration because of the distending influence of the negative inspiratory intrapleural pressure on intrathoracic structures. In adults and in healthy infants in whom the chest wall has begun to stiffen, such changes may be fairly minimal over the tidal range so that stable values of Raw are achieved. However, the highly compliant chest wall of young in-

TABLE 5

EFFECT OF REBREATHING ON TIDAL PARAMETERS

	Baseline	Rebreathing	95% CI*
Group 1, n = 42			
RR, min	45.6	46.6	-0.9, 2.8
VT, ml	42.7	49.6	4.6, 9.2†
PTEF, ml/s	88.3	98.9	4.1, 16.9†
Group 2, n = 29			
RR, min	29.7	32.9	1.6, 4.7†
VT, ml	106.6	138.5	23.6, 40.4‡
PTEF, ml/s	137.4	182.6	31.5, 58.9‡
Group 3, n = 36			
RR, min	32.7	36.5	2.6, 4.8‡
VT, ml	93.7	114.5	12.5, 28.9‡
PTEF, ml/s	138.0	173.3	24.6, 46.1‡

Definition of abbreviations: RR = respiratory rate; VT = tidal volume; PTEF = peak tidal expiratory flow; Baseline = parameters measured with infants breathing room air; Rebreathing = parameters measured with infants rebreathing warmed, humidified, oxygen-enriched air. For definition of groups, see Table 1.

* Baseline values compared with Rebreathing values.

† p < 0.05.

‡ p < 0.001.

fants results in a low transpulmonary pressure at end-expiration (32), such that dynamic airway closure with marked elevation of end-expiratory resistance can occur within the tidal range. This tendency may be more pronounced in infants with airway disease caused by an increased pressure gradient along the airways in the presence of airway obstruction and/or the rise in intrapleural pressure that will accompany any active expiratory efforts. In adults, the stiffness of the bronchial wall protects the conducting airways from deformation and collapse because of positive intrathoracic pressures that develop during forced expiratory efforts. However, airways in immature animals are far more compliant than those in adults and hence vulnerable to collapse at smaller transmural pressures (33). Consequently, changes in lung volume that alter the interdependence between airways and parenchyma may act as a major determinant of airway caliber and resistance in infants. In this study, changes in Raw were found to occur throughout the respiratory cycle in all three groups of infants (Table 3).

In this study, we attempted to minimize potential sources of variability when making comparisons between techniques by analyzing Rrs and Raw at similar flows and phases of respiration. Because Rrs is measured during passive expiration after an end-expiratory occlusion, it may reflect the mechanics of the airways during initial expiration (together with a chest wall and tissue component) most closely (18). However, because analysis is performed on the descending portion of the flow-volume curve after peak expiratory flow has been attained, it could also reflect airway char-

TABLE 6

EFFECT OF REBREATHING ON ASSESSMENT OF RESPIRATORY COMPLIANCE AND RESISTANCE BY THE SINGLE-BREATH TECHNIQUE

Subjects (n)	Baseline	Rebreathing	95% CI*
Crs, ml·kPa ⁻¹	18	108.2	11.5, 28.3†
Rrs, kPa·L ⁻¹ ·s	18	4.20	-1.45, -0.33†
Intercept, ml	18	19.3	4.7, 19.0†

For definition of abbreviations, see Table 3.

* Baseline values compared with Rebreathing values.

† p < 0.05.

‡ p < 0.001.

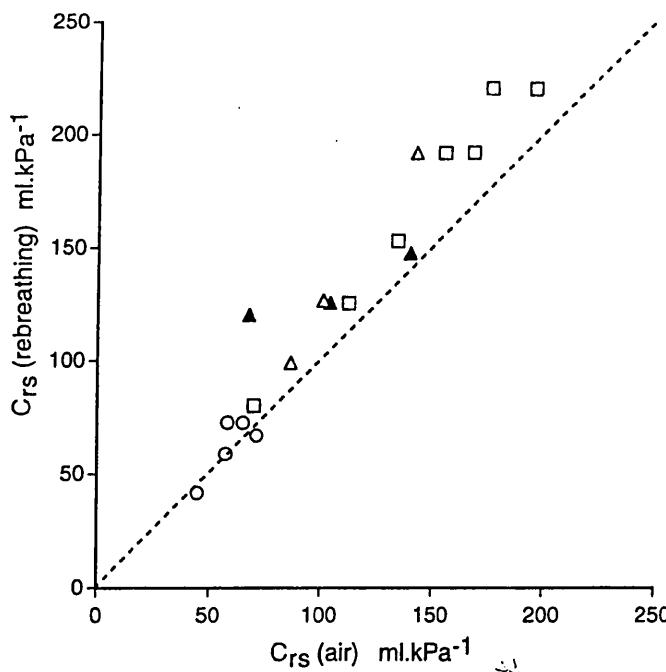


Figure 4. Scatter plot of respiratory compliance obtained by the single-breath technique during rebreathing against that measured in air. Symbols as for Figure 2. Dashed line is line of identity.

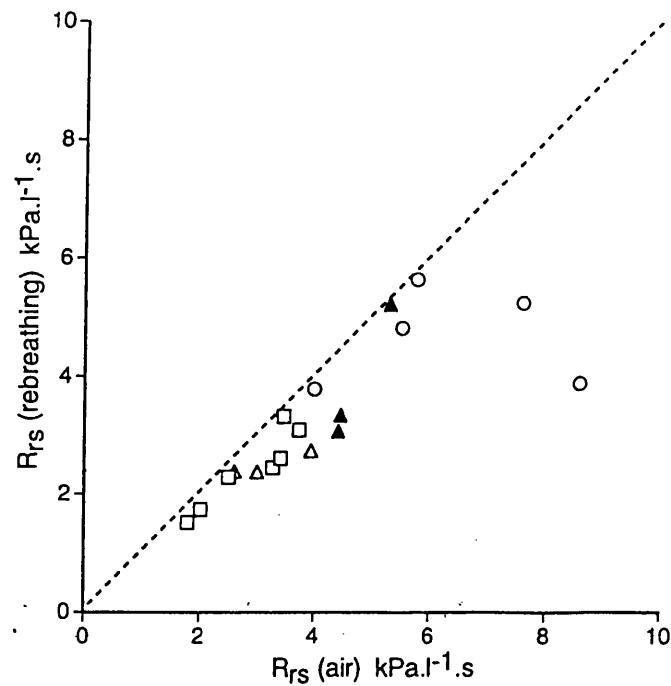


Figure 5. Scatter plot of total respiratory resistance obtained by the single-breath technique during rebreathing against that measured in air. Symbols as for Figure 2. Dashed line is line of identity.

acteristics toward end-expiration. Consequently, we compared Rrs with both initial and end-expiratory Raw. Raw was calculated at the average flow recorded during Rrs analysis in each infant, which proved to be approximately 72% of PTEF, i.e., similar to Raw at two-thirds PTEF, which has been conventionally calculated in the past (25, 34). However, these attempts to match flows can at best only be an approximation, with slight discrepancies being potentially responsible for much of the observed variability.

Potential Influence of the Rebreathing Bag

Even when attempting to match flows and phase of respiration, we could not control for the potential changes in resistance induced by rebreathing warm humidified air. Adults are conventionally requested to pant during plethysmographic assessments of Raw, both to minimize pressure changes caused by alterations in temperature and humidity of respired gas and to keep the glottis wide open, thereby minimizing upper airway resistance. Because infants cannot be asked to pant, they are allowed to rebreathe warm humidified air, but this will inevitably result in some build up of CO₂ and thereby increase ventilatory drive (Table 5). This effect was less marked among Group 1 infants, reflecting their smaller minute ventilation in relation to the volume within the rebreathing bag.

It is not clear from the current study whether the reduction in Rrs that occurred when measurements were repeated during rebreathing (Figure 5 and Table 6) reflect a bronchodilator effect of CO₂ per se (35), increased tidal volume or increased dynamic elevation of lung volume subsequent to the increase in minute ventilation (i.e., volume dependence of R). The accompanying rise in Crs and volume intercept during rebreathing (Figure 4) suggests that a change in lung volume may be a contributory factor. If so, this has important implications with respect to timing of lung volume measurements during plethysmography.

Influence of Changes in Gas Composition

During this study the pneumotachograph was calibrated with air, which may have introduced a slight error into measurements during the rebreathing of oxygen-enriched air (F_{O₂} ≈ 0.40). However, such errors are likely to be small, and they would not account for the differences reported in Table 6. Theoretically, Crs could be overestimated and Rrs underestimated by 11% if an infant breathed 100% O₂ through a pneumotachograph calibrated in air because of the relatively high viscosity of O₂ compared with that in air (36, 37). However, this error falls to ≈ 2% at an F_{O₂} of 0.4, and it would be further compensated by the F_{O₂} of ≈ 0.06 commonly observed in the bag during rebreathing since the viscosity of CO₂ is lower than that of air.

It was not feasible to measure Rrs under identical conditions to Raw in all infants nor to repeat the MOT assessment of Crs during rebreathing since this part of the study was attempted only if infants remained asleep at the end of the main measurement protocol. Furthermore, many infants failed to relax adequately under conditions of stimulated breathing. Nevertheless, the observations in the subgroup of 18 infants emphasize the marked influence of measurement conditions on measured values of respiratory function and hence variability between techniques.

Partitioning of Respiratory System Resistance

Previous studies in which pulmonary and airway resistance have been measured simultaneously and analyzed at identical flows suggest that lung tissue resistance makes a relatively small (< 15%) contribution to total pulmonary resistance (25, 38). This may be related to the fact that the major component of lung tissue resistance is thought to be due to hysteresis of the lung, which, since it is inversely proportional to breathing frequency, will make a smaller contribution in rapidly breathing infants than in adults.

The contribution of the chest wall is more controversial since differences between techniques often preclude direct compar-

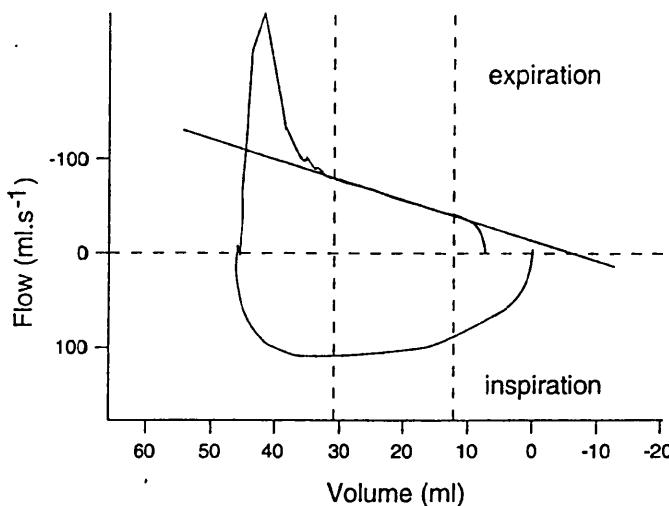


Figure 6. Example of an apparently linear flow-volume curve obtained using the single-breath technique in an infant with raised expiratory airway resistance.

son. Theoretically, C_{rs} and R_{rs} can be partitioned into lung, airway, and chest wall components by measuring the relevant driving pressure. However, under normal functional conditions the driving pressure across the chest wall is a combination of static elastic properties and the pressure generated by activity of the chest wall muscles. Accordingly, the passive mechanical properties of the chest wall can be derived only when muscle activity is inhibited. In a comparative study of pulmonary and total respiratory resistance, Gerhardt and colleagues (9) found that R_{rs} was approximately 24% higher than pulmonary resistance ($p < 0.001$) in infants at approximately 1 yr of age. However, no significant difference was observed in preterm neonates, a finding that was attributed to the highly compliant chest wall in immature infants.

Within subjects in this study, R_{rs} was, on average, 80% greater than R_{aw} during initial expiration and 27% greater than R_{aw} during end-expiration in both groups of healthy infants, these values being 100 and 15%, respectively, among the wheezy infants. However, there was huge individual variability, and it is not meaningful to calculate a value for tissue resistance or visco-elasticity simply as the difference between the two techniques.

Passive versus Dynamic Resistance

In addition to differences in measurement conditions discussed above, R_{rs} assesses expiratory resistance under passive conditions, whereas R_{aw} reflects the dynamic changes that occur during tidal breathing. Despite obvious nonlinearities of the pressure-flow loop during R_{aw} measurements, a linear flow-volume relationship was frequently obtained from infants with airway disease (Figures 6 and 7). Indeed, the failure rate of the SBT because of nonlinearity was no greater in the group with prior wheeze than in the group of healthy infants of a similar age. After end-inspiratory occlusions, infants frequently inspire earlier than usual on the subsequent breath, such that any rise in end-expiratory resistance caused by dynamic airway closure may be missed. Furthermore, analysis of the "passive" time constant is limited to the "linear" portion of the flow volume curve, when any muscle activity has supposedly been inhibited; consequently, measurements of R_{rs} may reflect the dimensions of the airways under passive conditions, but they cannot reflect the dynamic changes that normally occur throughout the breath. This would explain why values of

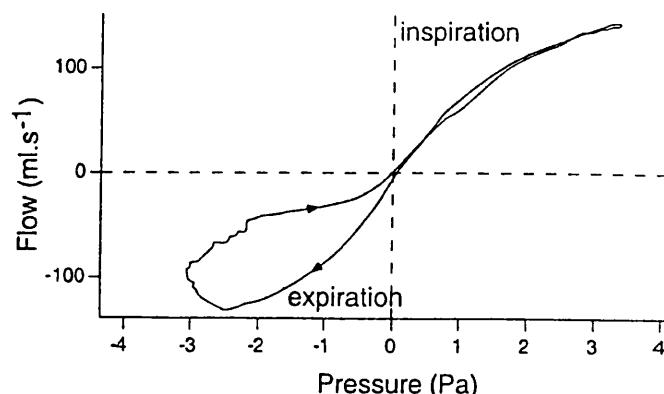


Figure 7. Example of a pressure-flow curve obtained in the same infant as in Figure 6 showing elevated end-expiratory airway resistance.

end-expiratory R_{aw} exceeded those of R_{rs} in approximately 25% of infants studied.

Similar findings were recently reported by Springer and colleagues (18). This group found virtually identical values of weight-corrected FRCpleth in 15 "normal" and in nine postbronchiolitic infants as in the current Groups 2 and 3 (healthy > 13 wk and prior wheeze, respectively). Springer and colleagues did not tabulate absolute values of R_{rs} and R_{aw} , making direct comparisons difficult, but their published illustrations reveal that initial expiratory R_{aw} was equal to or exceeded R_{rs} in approximately one third of the mixed population and that end-expiratory R_{aw} was significantly greater than R_{rs} among the wheezy infants, many of whom were symptomatic at the time of testing.

When using the SBT, time is allowed during the end-inspiratory occlusion for relaxation of the diaphragm and other inspiratory muscles. It could therefore be argued that the level of activity of the inspiratory and laryngeal expiratory muscles during analysis of the passive flow-volume curve most closely resembles that occurring at midexpiration during tidal breathing and that R_{rs} should therefore be compared with midexpiratory R_{aw} . Although we did consider this approach, the higher flows occurring towards midexpiration were associated with increased values for R_{aw} , presumably because of increased turbulence (25), and therefore tended to increase rather than diminish any discrepancies between R_{aw} and R_{rs} .

Validity of the SBT

Concern has been expressed regarding the use of the SBT in unintubated infants because of possible laryngeal modulation of expiratory air flow resulting in a falsely elevated volume intercept and hence C_{rs} and R_{rs} (13, 14, 27). In addition, a linear shape on the flow-volume curve does not necessarily indicate relaxation of respiratory muscles or the presence of a single time constant since an even, descending slope could represent balanced respiratory muscle contraction and/or reciprocal changes in compliance and resistance as lung volume decreases. We measured C_{rs} using both the MOT and SBT in an attempt to validate measurements of R_{rs} with respect to these potential problems, and we found small differences between the techniques, which appeared to be primarily attributable to small variations in the size of the volume intercept. Nevertheless, it should be noted that although the differences between techniques were statistically significant, the magnitude of these differences was probably too small to be of physiologic significance or to markedly influence results.

Conclusions

Despite the difficulties in interpreting results from individual infants and the relatively high failure rate, the SBT was simple to use and is potentially far more applicable than plethysmography, which is essentially limited to specialized laboratories and generally unsuitable for critically ill infants. Although it has been suggested that Rrs may reflect initial expiratory airway mechanics, this is still open to debate. In this study both Rrs and end-expiratory Raw were significantly higher in infants with prior wheeze, most of whom were asymptomatic at time of testing, than in healthy infants of similar age and weight. This suggests that measurements of Rrs may be of value in epidemiologic studies, although further work is needed to define the extent to which elevated values of Rrs correctly identify those infants with airflow obstruction as determined by clinical symptoms or other objective measures of airway function. However, the clinical value of measurements of Rrs within individual infants may be limited by its failure to detect the dynamic changes in resistance throughout the breath that are clearly evident during plethysmographic studies.

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A collaborative study of infant respiratory function testing

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ABSTRACT: The aims of this study were to compare inter-observer variability within and between two specialized infant lung function testing centres and to develop a strategy for performing and analysing infant respiratory function tests to facilitate future collaborative trials.

A protocol for data collection and analysis was developed using similar equipment and identical software. All raw data were exchanged on disk and analysed, blind to infant status. All data were cross-analysed by both centres to assess inter-observer variability. Outcome measures were functional residual capacity (FRC_{pleth}), airway resistance (R_{aw}) and maximal expiratory flow at FRC ($V'_{max,FRC}$). Subjects were recruited from the multicentre UK extracorporeal membrane oxygenation (ECMO) Trial and measured at around 1 yr of age. Forty-two infants attended the Institute of Child Health, London and 36 attended the Leicester Royal Infirmary. The proportion of infants treated with ECMO or conventional management at each centre was similar.

There were no significant differences between any of the outcome measures for infants tested at either centre. During a cross-analysis, the agreement between the two centres, within infant, was closer for $V'_{max,FRC}$ and FRC_{pleth} (within 10%) than for the more variable measurements of R_{aw} (within 20%).

A collaborative approach to trials with infant respiratory function as an outcome measure appears feasible, providing that close attention is paid to study design, and participants in such trials maintain a standard approach to data collection and analysis. *Eur Respir J 1998; 12: 944-953.*

Infant respiratory function tests are time-consuming and relatively complex, but there is increasing interest in their use as outcome measures of interventions, or indicators of disease severity. However, accrual of adequate numbers of infants to studies based in one test centre or geographical area may take many years, and reporting of smaller numbers will result in insufficient power to evaluate the statistical significance of outcome measures. In contrast, large multicentre clinical trials may accrue more infants than can be reasonably measured at one centre. Collaboration between centres that perform these specialized tests would be the most realistic alternative, but a common methodological approach and compatible equipment and software are a prerequisite to pooling data. In 1994, the opportunity arose to undertake a respiratory follow up of all survivors of a national randomized trial of extracorporeal membrane oxygenation (ECMO), hereafter referred to as the main ECMO trial [1].

Mature neonates with reversible respiratory disease were eligible for the main ECMO trial, and the aim of the respiratory follow up was to compare the respiratory health and function at 1 yr in infants who were assigned to receive ECMO with that of similar infants who were assigned to conventional management (CM). In view of the wide geographical area involved, the number of infants to be measured, and the potential benefits of a collaborative study, it was decided that the respiratory measurements should be

performed at two specialized centres in the UK, based at the Institute of Child Health, London (hereafter referred to as ICH) and at the Department of Child Health, Leicester Royal Infirmary (hereafter referred to as LEIC). Although the measurements of infant respiratory function were well established at both centres, the complexity of measurement procedure and methods of analysis led to considerable potential for differences in approach. Participation in this two-centre trial, which lasted from August 1994 to December 1996, offered the opportunity to address methodological issues, and to develop a standardized approach that could be applied within future multicentre trials.

The aims of this study were: 1) to develop a strategy for performing and analysing infant respiratory function tests to facilitate future multi-centre trials; and 2) to compare the inter-observer variability within and between two specialized infant lung function testing centres with respect to plethysmographic measurements of functional residual capacity (FRC_{pleth}) and airway resistance (R_{aw}), and assessments of maximal flow at FRC ($V'_{max,FRC}$), using the tidal rapid thoraco-abdominal compression technique (RTC), these being the main outcome variables of the respiratory follow-up [2].

Methods and subjects

All infants who were recruited into the main ECMO trial and survived to 1 yr were eligible for the respiratory

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follow-up. Entry criteria for the main ECMO trial are published elsewhere [1]. One hundred and eighty-five infants were entered into the main ECMO trial, and 101 survived to become eligible for the respiratory follow-up at 1 yr.

Arrangements were made for clinical assessment and respiratory function testing at ICH or LEIC. Parents who elected to take part in the respiratory follow-up were given their choice of venue. Laboratory staff were blinded to the infants' management status (ECMO or CM), and parents were requested to withhold any information regarding neonatal history during their visit. On arrival in each laboratory, infants were dressed in a specially designed smock of high neck design to obscure any neck scars received during ECMO treatment. A detailed history was taken with reference to respiratory disease, and the infant was examined clinically by a staff member not directly involved in measurement and analysis. The respiratory questionnaire was designed to provide information concerning possible confounding variables such as smoking in the home or a family history of asthma. Baseline oxygen saturation was recorded using pulse oximetry, and the infant was weighed, wearing only the smock, before being sedated with triclofos sodium (100–150 mg·kg⁻¹) at ICH or a similar dose of chloral hydrate (80–140 mg·kg⁻¹) at LEIC. The infant's length was recorded to the nearest 0.1 cm as described previously [3].

The methodology for performing respiratory measurements at each test centre was already well established when the respiratory follow-up commenced [4, 5]. However, there were differences in equipment, software set-up and methodology between centres that could potentially influence the approach to collection and analysis of data. These differences were examined prior to starting the respiratory follow-up and at regular intervals subsequently by arranging interlaboratory visits when equipment, infant measurements and analytical techniques were observed and compared, including cross-analysis of the same data. Following an interim analysis of results, 7 months after commencement of the study, minor amendments to the protocol, with respect to data collection, analysis and reporting results, were implemented as described below.

This study was approved by the local ethics committee at each centre. Written informed consent was obtained from one or both parents prior to measurements. Parents were usually present during the measurements.

Details of equipment and in vitro assessment

Details of the equipment are summarized in table 1. Both plethysmographs were assessed using an identical

test lung, made of copper tubing filled with copper wire, attached to a piston driven pump with a stroke volume of 7 mL. The volume of the test lung was approximately 200 mL (depending on the connectors used), and both centres obtained results to within 2% of the volume measured by water displacement. Following measurements using a face shaped out of putty or a nasal cast to simulate the space potentially occupied by an infant's nose and cheeks, the effective mask deadspace was considered to be 15 mL at ICH (*i.e.* 50% of the water displacement volume) and 7 mL at LEIC. The remaining difference in apparatus deadspace of 14 mL arose mainly from the design of the pneumotachograph (PNT: capillary at ICH and screen at LEIC), and partly from the slight differences in shutter configuration.

For measurements of $V'_{\max, FRC}$ the deadspace and apparatus resistance presented to the infant was reduced, consisting of mask and PNT only (table 1). The inflatable jacket (Medical Engineering, Royal Postgraduate Medical School, Hammersmith Hospital, London) used for RTC measurements at LEIC was wrapped around infants with their arms within, and the entire anterior section was inflated. ICH infants wore a cummerbund-style adjustable jacket (Hannover Medical School, Germany) with their arms remaining outside.

Data collection. All data were displayed and recorded on an IBM-compatible computer, using interactive, operator controlled software (Respiratory Analysis Program (RASP), Physio Logic Ltd, Newbury, UK). Measurements were made only during quiet sleep when posture was stable, respiration regular and no eye movements observed [6]. Once asleep, the infant was wrapped in an adjustable inflatable jacket, and a face mask and PNT were applied over the nose and mouth and made leakproof with therapeutic putty. Measurements of $V'_{\max, FRC}$ were then performed as described previously [4, 7]. Jacket inflation pressures were applied in increments of 1 kPa until flow limitation was reached (no increase in flows with increasing applied pressure, for technically acceptable curves) up to a maximum of 10 kPa. Curves were acceptable if the time to peak jacket pressure was ≤ 0.1 s, the interval between end inspiration and onset of jacket inflation was ≤ 0.1 s and there was a rapid rise to peak expiratory flow with no evidence of glottic closure.

Reasons for rejection of data included: inspiration before FRC was reached, a change in end-expiratory volume following the manoeuvre, suggestive of a face mask leak,

Table 1. – Details of equipment at each centre

	ICH	LEIC
Infant plethysmograph	100 L custom built	85 L Jaeger
Pneumotachograph model	Fleisch "1" capillary type	Jaeger infant screen type
Effective deadspace: mask+PNT+shutter mL	41	19
Linear range of PNT	± 500	± 1000
Resistance: PNT and shutter at 100 mL·s ⁻¹ kPa·L ⁻¹ s	0.5	0.23
Time constant* s	10–14	10–14
RTC measurements		
Deadspace face mask-PNT mL	27 (using Rendell-Baker size 2 mask)	16 (using Rendell-Baker size 2 mask)
Resistance: PNT at 100 mL·s ⁻¹ kPa·L ⁻¹ s	0.20	0.18
Jacket model	Hannover arms out adjustable size	Hammersmith size 2–4 arms in

ICH: Institute of Child Health, London; LEIC: Department of Child Health, Leicester Royal Infirmary; PNT: pneumotachograph; RTC: rapid thoraco-abdominal compression technique. *: combined mechanical and thermal time constant of the plethysmograph.

or evidence of expiratory braking during the forced expiratory manoeuvre. Whenever possible, the percentage of jacket pressure transmitted to the intrathoracic structures was assessed by performing an end inspiratory occlusion immediately prior to the jacket inflation and measuring the change in pressure at the airway opening during the subsequent jacket inflation relative to the applied jacket pressure [8].

The inflatable jacket was loosened before plethysmographic measurements were made. When measuring FRC_{pleth} at least five end inspiratory occlusions were performed, after a stable end expiratory level had been established, each being held for two to three respiratory efforts. Changes in plethysmographic and airway opening pressure were inspected and considered acceptable if no leaks were evident, and changes were in phase during airway occlusions. The infant was then allowed to rebreathe from a highly compliant 2 L bag containing warmed, humidified air (temperature at the mask ~37°C). The phase relationship between flow and plethysmographic signals was inspected and the temperature within the rebreathing bag adjusted if necessary, until a satisfactory pressure-flow loop was obtained [7, 9]. Three epochs of data, each of 18–36 s duration were collected, the rebreathing bag being emptied and refilled between each epoch.

The raw data from each infant, including calibration checks, were exchanged between centres by computer disk, soon after measurements were complete. Results were analysed, by both the test centre and the analysis centre, using the protocol agreed following an interim analysis. Minor adjustments to usual practice were made by both centres following this analysis, including standardization of sampling frequencies and the method by which end expiratory baselines were assessed, together with the recording of (rather than simply just performing) calibration checks and test occlusions with several pre- and post-manoeuvre breaths, the latter being to provide evidence in the data on disk regarding absence of any leaks [7, 10]. The aim of these adjustments was to minimize any inter-observer variability, and facilitate analysis by an operator who had not been present during the measurements, and therefore required evidence of quality control during data collection.

Data analysis

Interactive software (RASP), which recorded data in real time throughout the measurement period and allowed subsequent off-line analysis, was used by both centres. It was possible to customize both data collection and analysis to individual preference with respect, for example, to sampling frequency, number of breaths analysed, thresholds for event recognition, and methods of assessing end expiratory baselines. All data were stored on computer disk, including the original raw data channels (time, flow, volume and pressure signals), details of each analysed event, alongside the selected user options at time of analysis, and prevailing measurement conditions such as ambient pressure, apparatus dead space and calibrations at the time of data recording. Exchange of the raw data alone on disk allowed inspection of original signals and enabled reanalysis of results, blind to that of previous investigators. However, in the event of intercentre discrepancies in

the calculated results, additional exchange of the analysis files facilitated rapid identification of the source of any bias.

Based on the inherent intra-subject variability of each of the respiratory parameters, the aim of the inter-observer analysis of each infant respiratory function measurement was to report values analysed by each centre to within 10% of each other for FRC_{pleth}, $V'_{max,FRC}$, tidal volume (VT), and respiratory rate (RR), and 20% for the more variable measures of Raw [5, 11], using the test centre result as the numerator and the analysis centre result as the denominator. Where agreement was not within these agreed limits, the analysis files were examined and amended where appropriate. Following an interim analysis, it also became apparent that, when a considerable amount of data had been collected, selection of the optimal data for analysing Raw with respect to achievement of body temperature barometric pressure and saturated with water conditions (BTPS) and minimal drift was difficult without some reference to quality control at the time of data collection. It was agreed, therefore, that the test centre should indicate the best epochs of data from which to select data for analysis prior to sending the data to the test centre for reanalysis.

RR and VT were reported as the mean of 25 breaths, collected in five separate epochs, each consisting of five breaths immediately preceding the first five partial forced expiratory manoeuvres. FRC_{pleth} was reported as the mean of all technically acceptable occlusions, where the infant was occluded at end inspiration or within 10% of the start of expiration. The calculated value from each occlusion represented the mean of one to three complete respiratory efforts, using both inspiratory and expiratory excursions. Data samples within 5% of peak airway opening pressure (Pao) (*i.e.* the end expiratory plateau) were excluded, thus limiting analysis of the relationship between plethysmographic volume and Pao to periods of rapid change. The subsequent correction to FRC was performed by subtracting the volume occluded above end expiratory volume and the apparatus dead space from the total occluded gas volume. If a degree of glottic closure was present, it was permissible to cautiously "truncate" the measured effort, measuring over a shorter period, *e.g.* between 95 to 20% peak to trough thereby excluding portions where changes in Pao did not reflect changes in mean alveolar pressure. A minimum of three occlusions were initially required to report results, but this requirement was relaxed following the interim analysis to allow reporting of otherwise good quality data, providing that at least two technically satisfactory measurements within 10% each other were obtained.

Data for analysis of Raw were considered to be technically acceptable when flow and plethysmographic pressure (P_{pleth}) were in phase on a time-series axis, and closed at points of zero flow when viewed on an X–Y plot. Default strategies for reporting values of Raw using the RASP software allowed selection of data on a breath-by-breath basis. Raw was reported as a mean of 5–7 breaths at 50% maximum tidal flows during the beginning of inspiration and end of expiration for each selected breath. Although measurements of Raw were made only after thermal equilibrium was reached, signals could be adversely affected by changing atmospheric conditions, which at times led to

drift even when adequate time had been allowed for stable conditions to be met.

$V'_{\text{max}}\text{FRC}$ values were reported both as the highest, and as the mean of the four highest, values of technically acceptable data. The maximal and mean jacket pressures (P_J) were noted, together with the pressure during the best manoeuvre (optimal P_J), % P_J transmission [8], and the total number of manoeuvres performed.

Where technically satisfactory measurements were not obtained or data were not collected, the reasons for failure were noted.

Analysis of within-infant, intercentre differences. Infant details and results were double-entered on an Excel spreadsheet (version 5.0, Microsoft Corporation, Redmond, WA, USA) and checked for data transcription errors, by both the test centre and the analysis centre independently, before comparing results between centres. The aim was to minimize differences in measured parameters that were not due to biological variability alone, for example data transcription and entry errors, data selection bias or differing analysis strategies.

Reporting of results

A short clinical report, based on the results obtained from the test centre, was sent to the referring paediatrician and infant's family doctor as soon as possible after testing.

The definitive results with respect to comparisons of respiratory function between the ECMO and CM groups, which are described elsewhere [2, 12], were reported from the analysis centre, *i.e.* the centre that did not test the infant, and therefore had no contact with the family. This ensured complete blinding to management status. The management status at trial entry of the whole group of infants was not revealed until both data collection and analysis was complete, when background details of surviving infants, including a description of their status at trial entry and management (ECMO or CM), were obtained from the ECMO trial data coordinator.

Statistical methods

Comparison of infants studied in the two different centres. For comparisons between the two groups of infants studied at ICH and LEIC, unpaired t-tests were used to examine background details and respiratory function results where data were normally distributed. The Mann-Whitney U-test was used to assess differences between groups of non-normally distributed data. A p-value <0.05 , and 95% confidence intervals (CI) of the differences between groups or paired data not encompassing zero, were considered significant.

Comparison of within-subject, intercentre differences. Intercentre, within-subject differences, which would reflect variation in analytical techniques, were evaluated according to the method of BLAND and ALTMAN [13], by calculating the mean and standard deviation (sd) of the difference between pairs of measurements (ICH - LEIC). Results were

displayed graphically by plotting the differences between pairs against their mean, together with the group mean difference and limits of agreement ($\pm 2\text{sd}$ of the within pair differences).

Results

Demographic results

Of the 101 infants who survived to 1 yr, two were withdrawn from the main trial by their parents, eight infants were excluded from respiratory follow-up for primarily medical reasons: five due to congenital heart disease, one due to gross developmental impairment and two who were still in hospital at approximately 1 yr of age. Eight infants did not attend due to parental refusal, two because of the parents' inability to attend before the child was 18 months old, two due to repeated (more than three occasions) cancellations following onset of respiratory tract infections <3 weeks before their appointment and one due to adverse social circumstances. Thus, respiratory follow-up was possible in 78 (77%) of all survivors.

Between August 1994 and December 1996, of the 78 infants who took part in the respiratory follow-up with their parents, 42 attended ICH and 36 attended LEIC. Those who participated in the respiratory follow-up were similar to the whole group of survivors with respect to gestational age, birth weight, sex distribution, primary diagnosis, age and severity of disease at trial entry [1, 2, 12] (data not shown). Details of the infants attending each centre are summarized in table 2. The age, weight and length of infants attending each test centre were similar, as were birth weight and gestational age. The reported prevalence of maternal tobacco-smoking during pregnancy and

Table 2. – Infant characteristics according to test centre

	ICH n=42	LEIC n=36	95% CI of the group mean difference (ICH - LEIC)
Age months	13.6 (1.8)	13.9 (2.0)	-1.1, 0.6
Weight kg	9.9 (1.5)	10.5 (1.6)	-1.2, 0.2
Length cm	78.1 (3.5)	78.4 (3.6)	-2.0, 1.2
Birthweight g	3378 (645)	3521 (468)	-400, 116
Gestation weeks	39.3 (2.4)	39.6 (2.0)	-1.4, 0.7
Male n (%)	24 (57)	24 (67)	-31, 12
Caucasian n (%)	32 (76)	28 (78)	-20, 17
Smoking during pregnancy n (%)	7 (17)	4 (11)	-10, 21
Current maternal smoking n (%)	13 (31)	12 (33)	-23, 18
Any smoke exposure n (%)	29 (69)	17 (47)	-0.1, 44
Current respiratory medicine n (%)	10 (24)	7 (19)	-14, 23
Symptomatic at testing n (%)	3 (7)	9 (25)	-34, -2*
Recent URTI n (%)	7 (17)	19 (53)	-57, -15***
Normal medical exam n (%)	29 (69)	29 (81)	-31, 8

For definition of groups, see table 1. Values are given as mean and sd. CI: confidence interval; URTI: upper respiratory tract infection. *: $p \leq 0.05$; ***: $p \leq 0.001$

Table 3. – Number and percentage of successful measurements at each centre

Test centre			
	ICH n=42	LEIC n=36	95% CI (ICH - LEIC)
FRCpleth	39 (93)	33 (92)	-10, 13
Raw	35 (83)	21 (58)	5.45*
V'max,FRC	37 (88)	34 (94)	-13, 9
Tidal breathing parameters	41 (98)	36 (100)	-7, 3

For definition of groups, see table 1. Values are shown as n (%). FRCpleth: functional residual capacity by plethysmography; Raw: airway resistance; V'max,FRC: maximum expiratory flow at functional residual capacity; CI: confidence interval. *: p<0.05.

postnatally was similar at each centre, although a greater proportion of infants attending ICH were reported to be exposed to tobacco smoke from any source (parental and/or other regular exposures) at the time of follow-up. More infants who attended LEIC were symptomatic at testing (respiratory symptoms included rhinorrhea, coryzal illness, cough and wheeze) and had suffered recent upper respiratory tract infections. The majority of infants were, however, coded as normal following examination by a paediatrician blind to management status.

Causes of failure

The percentage of successful measurements for each outcome parameter, grouped by test centre is summarized in table 3. Tidal parameters were reported for all LEIC infants, and all but one ICH infant (who woke before measurements of V'max,FRC were attempted). Most infants (93%) had their resting lung volume (FRCpleth) successfully measured, in similar proportions at each test centre. However, a significantly smaller proportion of infants measured in Leicester had successful Raw results reported (p=0.014). For infants attending ICH, the failure to report FRCpleth or Raw was usually due to waking before measurements were complete (n=5). In a further three infants, Raw could not be reported for technical reasons, signals being adversely affected by weather or building work in the area. In contrast, only one infant in LEIC woke during plethysmographic measurements of Raw, but FRCpleth was unsuccessful in three infants (one technical failure, two attempts invalidated by glottic closure during expiratory efforts), and Raw failed in 14 infants. The latter was

due to 10 technical failures, subsequent to the introduction of a new amplifier partway through the respiratory follow-up (seven occasions) or insufficient humidification in the rebreathing bag (three occasions), and one failure for physiological reasons (glottis closure). The remaining three measurements failed because FRCpleth was unsuccessful, as noted previously, so that the occlusion data could not be used to calibrate Raw data. Successful measurements of V'max,FRC were achieved in 37 (88%) infants attending ICH and 34 (94%) from LEIC. At ICH, one infant woke, in three others there was persistent early inspiration during each manoeuvre, and in the remaining infant, suboptimal jacket pressures were applied, such that flow limitation was not demonstrated. In the two LEIC infants in whom measurements were unsuccessful, the jackets were of a poor fit, resulting in late application of thoraco-abdominal pressures in all manoeuvres, thereby failing to meet quality control criteria with respect to jacket inflation time.

Comparison of respiratory function in infants studied in the two centres

There were no significant differences for any of the outcome measures, *i.e.* FRCpleth, Raw or V'max,FRC between the infants studied at the two centres (table 4). The group mean (range) FRCpleth was 284 (160–450) mL for infants attending ICH, and 281 (145–492) mL for those attending LEIC. The number of FRCpleth manoeuvres reported by each centre following the cross-centre analysis, expressed as median (range) was 4 (2–9) for ICH analysis of LEIC data and 7 (2–12) for LEIC analysis of ICH data. However, only one infant from each centre had their lung volume reported on only two manoeuvres. When corrected for weight, the group mean (range) FRCpleth of infants attending ICH was 29 (17–46) mL·kg⁻¹ and that at LEIC 27 (15–49) mL·kg⁻¹. Although airway resistance tended to be higher amongst those infants attending ICH, these differences did not reach significance (95% CI (ICH - LEIC) for initial inspiratory Raw (Raw,II) and late expiratory Raw (Raw,EE) 0.14, 0.77 and -0.15, 1.12 kPa·L⁻¹·s respectively).

V'max,FRC, whether expressed as the highest value obtained or as the mean of the four highest technically acceptable manoeuvres, was similar for infants attending both test centres. The mean (range) V'max,FRC was 171 (48–415) mL·s⁻¹ at ICH and 145 (27–320) mL·s⁻¹ at LEIC, with similar values for the highest technically acceptable V'max,FRC when expressed as mean (table 4). However,

Table 4. – Comparison of within-subject respiratory function results analysed by each centre

Test centre	ICH n=42		LEIC n=36		Mean (95% CI) (Analysis centre - test centre) n=78
	ICH	LEIC	ICH	LEIC	
Analysis centre	ICH	LEIC	ICH	LEIC	
FRCpleth	286 (58)	284 (59)	281 (73)	277 (67)	1.11 (-0.53, 2.75)
Raw,II kPa·L ⁻¹ ·s	2.19 (0.79)	2.30 (0.89)	1.98 (0.76)	1.86 (0.84)	0.04 (-0.02, 0.10)
Raw,EE kPa·L ⁻¹ ·s	2.91 (1.22)	2.93 (1.18)	2.45 (1.12)	2.32 (1.16)	-0.02 (-0.12, 0.08)
Mean V'max,FRC mL·s ⁻¹	168 (73)	171 (73)	145 (75)	146 (75)	0.91 (-0.94, 2.77)
Best V'max,FRC mL·s ⁻¹	178 (77)	176 (80)	158 (82)	156 (80)	1.43 (-1.42, 4.28)
VT mL	94 (17)	94 (17)	83 (16)	83 (16)	0.04 (-0.57, 0.64)
RR min ⁻¹	33 (7)	33 (8)	33 (10)	33 (10)	-0.13 (-0.39, 0.14)

For definition of groups, see table 1. Values are given as mean (sd). For definition of abbreviations, see table 3; Raw,II: airway resistance during initial inspiration; Raw,EE: airway resistance during late expiration; VT: tidal volume; RR: respiratory rate.

despite the similarity of results between the two centres, the applied jacket pressures were significantly higher at ICH than at LEIC; mean (SD) 4.8 (1.7) kPa and 2.6 (0.9) kPa, respectively ($p < 0.001$), reflecting the lower percentage of jacket pressure transmitted to the infant at ICH (29% versus 58% at LEIC). The median number of manoeuvres was 18 (range 8–40) at each centre.

Tidal breathing parameters were not selected outcome measures, but were recorded and analysed to provide additional evidence for quality control and baseline information regarding respiratory function. The respiratory rate was not significantly different between centres, the mean (range) was 33 (19–53) min^{-1} at ICH and 33 (24–74) min^{-1} at LEIC. However, the group mean VT was larger for infants attending ICH than LEIC; mean (range) 94 (64–134) and 83 (52–121) mL respectively, 95% CI; 3–18 mL ($p < 0.05$). These differences persisted when corrected for weight.

Comparison of within-subject respiratory function results analysed by each centre

In addition to comparing results from infants studied at the two measurement centres, the within-infant inter-observer variability between centres was also assessed. As stated previously, the aim was to obtain values within 10% of each other for tidal breathing parameters, $V'_{\text{max},\text{FRC}}$ and $\text{FRC}_{\text{pleth}}$, and within 20% for Raw . The results of the cross-analysis of each centre's data are shown in table 4.

$\text{FRC}_{\text{pleth}}$. $\text{FRC}_{\text{pleth}}$ analyses were generally within 10% agreement. On initial comparison of the results from the two centres, discrepancies >10% occurred in 12 infants. In four of these infants, this was due to data collection in which it had not been possible to report values based on at least three occlusions, either due to the infant waking early (one infant), or insufficient collection of end inspiratory occlusion data (three infants). Following the revision of guidelines and reanalysis by each centre, the definitive results reported were all within the agreed limits, although it was necessary to report $\text{FRC}_{\text{pleth}}$ as the mean of only two manoeuvres in two infants. In the remaining eight infants, discrepancies were due to data transcription errors (two infants), different epochs of data being selected for analysis (one infant) and differing analysis strategies, which were mainly related to "truncating" glottic expiratory efforts by varying amounts (five infants). All were readily resolved after critical inspection of data by each centre, so that the final agreement for $\text{FRC}_{\text{pleth}}$ was within 10% all infants. The between-centre agreement for $\text{FRC}_{\text{pleth}}$ is shown in figure 1.

Airway resistance. For Raw analyses, there were initially discrepancies of >20% in 10 infants. These were due to operator errors in selecting analysis options within the software that did not adhere to the agreed protocol (three infants) or selection of different epochs of data for analysis and reporting (seven infants), *i.e.* biological variability. Of the infants in whom Raw measurements were not reported, one of three technical failures at ICH (not meeting quality control criteria) was failed by LEIC alone, whereas the remaining two were failed by both centres. Similarly, four of 14 technical failures at LEIC were failed by

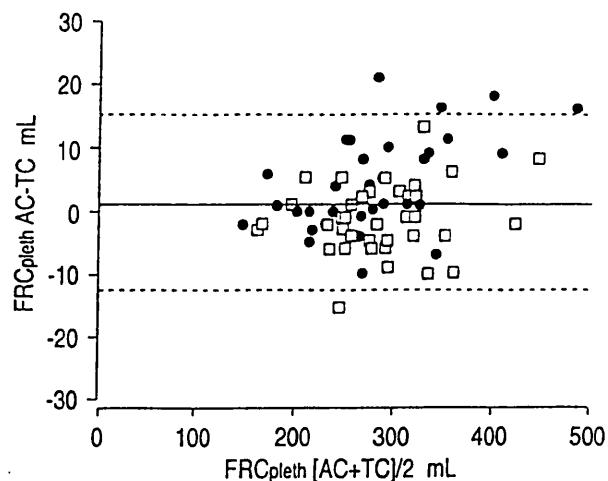


Fig. 1. – Scatter plot, according to the method of BLAND and ALTMAN [13], of the differences between functional residual capacity (FRC), analysed at each centre, and their mean. AC: analysis centre; TC: test centre; pleth: plethysmography. —: mean between-centre difference; - - -: 95% limits of agreement of the mean difference. Infants tested at Institute of Child Health (□); and Leicester Royal Infirmary (■).

ICH alone and the remainder by both centres. After inspection and amendment of the analysed data by each centre, agreement was improved to within 20% in all but three of the 56 infants. The between-centre agreement for Raw calculated during initial inspiration is displayed in figure 2.

$V'_{\text{max},\text{FRC}}$. Following the interim analysis, when a common volume baseline filtering strategy was adopted by both centres, and it was agreed to establish the FRC level by regression through the end expiratory points preceding the RTC manoeuvre, the agreement was generally good for $V'_{\text{max},\text{FRC}}$ analyses. Initially, analyses of $V'_{\text{max},\text{FRC}}$ were outside the agreed limits of 10% for six infants. One was due to a data transcription error, two to the selection of different epochs of data from which the best four manoeuvres were reported, one was attributed to volume baseline filtering strategies, whereas data from two infants

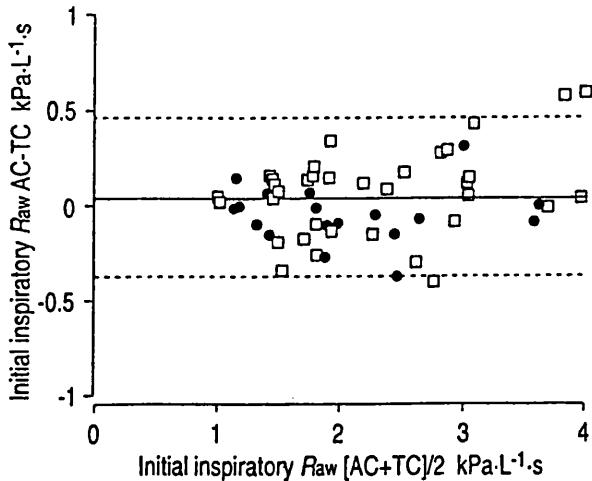


Fig. 2. – Scatter plot, according to the method of BLAND and ALTMAN [13], of the differences between initial inspiratory airway resistance (Raw), analysed at each centre, and their mean. AC: analysis centre; TC: test centre. —: mean. - - -: 95% limits of agreement of the mean difference. Infants tested at Institute of Child Health (□); and Leicester Royal Infirmary (■).

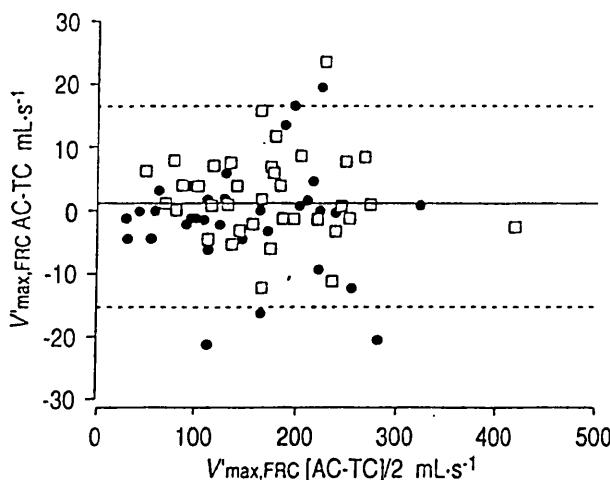


Fig. 3. — Scatter plot, according to the method of BLAND and ALTMAN [13], of the differences between maximum expiratory flow at functional residual capacity, analysed at each centre, and their mean. AC: analysis centre; TC: test centre. —: mean. - - -: 95% limits of agreement of the mean difference. Infants tested at Institute of Child Health (□); and Leicester Royal Infirmary (■).

required recalibration to adjust a zero offset of the flow signal before a good agreement could be achieved. Following inspection and amendment of the analysed data by each centre, three infants remained outside the agreed limits. Two of these had very low, flow-limited values of $V'^{\max,FRC}$, so that although the discrepancy between laboratories exceeded 10%, absolute values from the two centres were within $6 \text{ mL}\cdot\text{s}^{-1}$ of each other. The poor between-centre agreement in the remaining infant (20%) arose from the inclusion of data by one centre, but not the other, of one manoeuvre where instability of breathing patterns made establishment of the FRC level very difficult to determine. The between-centre agreement for within-subject values of $V'^{\max,FRC}$ is displayed in figure 3.

For tidal breathing parameters, between-centre differences $>10\%$ were seen in three infants, all of which were due to selection of different epochs of data (biological variability). A closer agreement was obtained by selecting alternative epochs of quiet breathing for analysis.

Discussion

One of the major advantages of the respiratory follow-up study design was that the analysis of respiratory function in survivors recruited from the ECMO trial could be made by operators who were fully blinded to their management status. However, before combining data from each centre, it was necessary to ensure that there was no bias either within or between laboratories that might confound interpretation of the results. It was necessary, therefore, to assess the agreement between centres with respect to both the background and respiratory function data.

Population

Infants and their families attended the respiratory follow-up from all over the UK. The majority of infants attending each test centre were of Caucasian ethnicity. Amongst the

remaining ethnic groups, there were five infants of Afro-Caribbean origin, all of whom attended ICH, whereas more Asian infants attended LEIC than ICH. However, when examined according to management group (ECMO *versus* CM) there were similar proportions of infants from each ethnic background within each group [12].

The prevalence of maternal smoking during pregnancy was similar at each centre, but lower than that reported elsewhere [14, 15]. In particular, of those attending ICH, the proportion of infants reported to be exposed to intrauterine tobacco was 17% (seven of 42) compared with 43% of infants in a recent East London-based prospective study [16] who attended ICH for similar measurements during an overlapping time period. This suggests that there may have been under-reporting of maternal smoking during pregnancy by mothers of the respiratory follow-up population at each test centre. The suggestion is reinforced by the increase in maternal smoking at the time of the laboratory visit at around 1 yr, when 31 and 33% (ICH and LEIC, respectively) of infants were reported to be exposed: thereby approaching other reported prevalences [14, 15].

Although efforts were made to avoid testing infants within 3 weeks of onset of upper respiratory tract infection (URTI) symptoms at ICH and within 4 weeks at LEIC, this was not always possible, particularly amongst infants who suffered recurrent respiratory infections, or when long-distance travel arrangements had been made. The proportion of infants who had had recent symptoms of URTI was greater at LEIC, which may have reflected the long symptom-free time period stipulated at that centre. However, a greater proportion of infants were also symptomatic at testing in LEIC (25% *versus* 7%). Local laboratory practice with respect to booking and, if necessary, postponing appointments would also have influenced these findings. There was no evident correlation between recent URTI symptoms and reported values of Raw , with the 18 infants who had symptoms of recent URTI and successful measurements of Raw having values that ranged $1.10\text{--}3.68 \text{ kPa}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$ during initial inspiration, the phase of respiration most likely to be adversely affected by such infections. In future, greater standardization with respect to the duration of a symptom-free period should be adhered to. However, in clinical follow-up studies such as this, it may be preferable to emphasize the absence of current symptoms, rather than a prolonged symptom-free period, since the latter is difficult to achieve in a population prone to recurrent infections.

Evaluation of methodology

To our knowledge, this is the first report of a multicentre study using established methods of infant lung function testing where results have been included as outcome measures. Within the field of infant respiratory function assessment, considerable variability exists with respect to equipment and techniques. However, efforts have recently been directed towards standardizing the nomenclature [17, 18], measurement conditions [19] and assessments of respiratory function in infants [7, 20].

One of the unique features of the respiratory follow-up was the opportunity to critically evaluate variability within infants for technically complex and operator controlled

analysis, which was only partly automated, such that selection of data and analysis strategy could influence results markedly. Ideally, an assessment of analysis variability would have preceded this study, but limited time and funding precluded this approach. Operator selection of the most suitable data for analysis, the relatively low signal-to-noise ratio of respiratory signals in infants, and the inherent biological variability of certain parameters all increase within and between-subject variability in this age group. Consequently, a careful selection of a limited number of relevant outcome measures, together with recruitment of adequate numbers of infants, is essential to achieve an adequate power of study [7].

The aim of the study design was to reduce both systematic and random errors and prevent measurement and analysis bias affecting reported results. Statistical assessment of differences between the two groups of infants studied at ICH and LEIC aimed to detect any sampling differences, for example in background characteristics, management status, disease severity, and any measurement bias arising from differences in apparatus or technique. The statistical assessment of the whole group of infants, analysed by each centre, in contrast, aimed to highlight the biological variability together with the inter-observer variability in the analytical approach and data selection.

Careful assessment and evaluation of both equipment and measurement and analysis techniques, with subsequent amendments, led to improved between-centre agreement. With both laboratories also concurrently involved in other studies, limitations were, however, placed on the extent to which adaptations could be made to existing equipment and protocols.

In vitro assessment of both plethysmographs, using the same copper test lung, showed that lung volume was measured accurately at both centres. The effective dead space that the infant breathed through was greater at ICH, mainly due to the different design of PNT and shutter configuration, but also due to the different volume subtracted for effective dead space of the face mask. The latter may have been due to the application of larger quantities of therapeutic putty at LEIC. Differences in tidal volume between centres may have been attributable to either dead space or design of PNT. In a small group of infants, tidal volume and end tidal CO_2 were measured using PNTs of both designs on the same measurement occasion (data not shown). CO_2 values were similar, but VT was larger using a Fleisch "1" compared with a Hans Rudolph 100 PNT. This presumably reflects the fact that any small increase in effective dead space will be compensated for by an increased VT to bring end tidal CO_2 back to normal values. Despite the effect on tidal volume, the PNT design did not appear to influence between centre interpretation of the selected outcome measures of respiratory function and lung volume. However, for future collaborative studies, we would try to use equipment that was of as low a dead space and as similar as possible.

The linearity and resistance of measurement equipment used at each centre was assessed and found to be adequate over the range of flows and pressures used in these measurements. Had the study involved healthy infants with correspondingly higher expiratory flows during forced expiration measurements, a PNT with greater range may well have been required. Future studies will be able to use

a new generation of screen PNTs, which have a considerably lower dead space but a greater linear range.

Prior to each study, known signals, including the functional time constant (*i.e.* combined mechanical and thermal) of each plethysmograph were recorded and checked at each centre, and these data were exchanged between centres. The weight and length of each infant were recorded, using regularly calibrated equipment, thereby avoiding any systematic errors in anthropometry at or between centres. The influence of jacket style during measures of forced expiration at each centre on the range of pressures used to obtain $V'_{\text{max},\text{FRC}}$ was addressed. The Hammersmith jacket used at LEIC was almost twice as efficient at transmitting pressure to the pleura when compared with the Hannover jacket used at ICH. Consequently, higher jacket inflation pressures were used at ICH. Despite these methodological differences, the mean and wide range of forced expiratory flows obtained at each centre suggested that the driving pressures (the product of jacket inflation pressure and jacket efficiency plus elastic recoil of thoracic structures) used at each centre were similar [8].

The success rate of measurements at each test centre was similar, with the exception of Raw , where the increased failure rate at LEIC was mainly attributable to untimely problems with a replacement amplifier which adversely affected the quality of the plethysmographic pressure signals in a number of infants. The lack of adequate commercially available equipment for infant respiratory function tests, with many centres relying on custom built systems, contributes to the problems of access to essential spare parts. An additional component of study design that is a prerequisite for future similar trials is thus the need for equipment to be standardized during the trial period, and to have ready access to replacement parts, so that no disruption to data collection occurs during critical time frames.

Success rates of infant respiratory function parameters are rarely reported, but compared with a younger group of infants measured at ICH [5, 21], in whom 85% of measurements of Raw were successful, a similar rate of 83% was reported in the ICH respiratory follow-up population. A significant number of plethysmographic measurements at ICH were lost when the infants woke before completion of the protocol, despite increasing the usual dose of sedation from 100 to 150 $\text{mg}\cdot\text{kg}^{-1}$ triclofos sodium (equivalent to 100 $\text{mg}\cdot\text{kg}^{-1}$ chloral hydrate) when necessary. This was still somewhat lower than maximum doses used at LEIC, which may explain the fewer failures due to early awakening at the latter. A number of infants recruited from the ECMO trial were >1 yr of age, taking longer to settle into quiet sleep and waking earlier. The time available for measurements during sedated sleep is short, and this limitation should be borne in mind when designing future protocols that include infant respiratory function. However, with similar numbers of infants attending each centre, and similar overall failure rates at both, predominance of one centre when reporting and interpreting results was avoided.

During the course of this study, the difficulties in imposing appropriate criteria to maintain stringent quality control within clinical trials became apparent, particularly when attempts were being made to follow up an entire cohort, as in this study. In such circumstances, the individual data from each infant are unique and cannot be simply replaced by recruiting additional subjects. These difficulties led to our decision to relax standard criteria, which

normally specify a minimum of three technically acceptable measures before reporting FRC_{pleth}, to allow the mean of two such measures to be reported, providing that they were within 10%. Controversy remains over the number of measurements that should be used to report the mean for the various respiratory parameters commonly measured in infants [7]. Further objective evaluation is needed, so that the time required for data collection can be minimized in infants without compromising accuracy.

The process of exchanging raw data on computer disks between centres was a valuable opportunity to evaluate approaches to analysis critically using software that although identical at each centre, could be readily customized to user preference with respect to collection, display, calibration, filtering and reporting of results. Exchanging data on disk between centres highlighted the need for meticulous attention to quality control during both calibration and data collection. The effect, for example, of different strategies of drift correction and determination of volume baselines prior to analysis of $V'_{max,FRC}$ (regression over five breaths *versus* selection of the most representative end expiratory level from a single breath over this period) on within-infant between-centre analysis, was of initial concern although readily standardized.

Approximately 10% of analyses needed to be re-examined when the cross-analysis between centres showed a poor agreement. Although, in several cases, differences were due to the selection of data, there were also four apparent random errors during analysis and reporting of the results. We speculate that most of these errors would not have been detected had exchange of data and comparison of analyses not taken place, thus emphasizing the need to develop software where such transmission errors are minimized.

The design of the respiratory follow-up served to increase confidence in the respiratory follow-up findings and was readily incorporated within the existing data collection and analysis protocol at each test centre. During the current study, prior commitment to other ongoing studies at each centre compromised full standardization, but in future similar trials, even greater consistency could be achieved. This will require the development of improved software and measurement equipment and adherence to mutually agreed international standards, which are currently evolving (European Respiratory Society/American Thoracic Society Task Force on Infant Respiratory Function). Some of the essential features required for successful design of future multicentre trials in this field are summarized in table 5.

The selective use of specialized, automated and validated software potentially could speed data analysis and

reduce within-subject and inter-observer variability [22]. Unfortunately, reproducibility does not equate to accuracy, and arbitrary exclusions to achieve a low within-subject variability may not provide a faithful representation of respiratory function, especially when a marked biological variability is present, as may occur in certain disease states. Some compromise is, therefore, needed, whereby obvious artefacts are excluded while still retaining physiologically meaningful data. While a fully automated system could ensure virtually identical results on reanalysis in a multicentre setting that would be operator-independent, infant signals, with their high variability and low signal-to-noise ratio, often require an interactive operator input for correct interpretation. Consequently, it is unlikely that assessments of respiratory function in infants will ever become as routine as those in older subjects, and that operators with extensive training and experience will always be required if successful measurements are to be achieved.

Conclusions

In conclusion, a multicentre approach to trials with parameters of infant respiratory function as outcome measures appears feasible, provided that close attention is paid to study design, and participants in such trials maintain a standard approach to data collection and analysis. Collaboration of this nature, despite requiring considerable time and effort, is of great benefit with respect to quality control within, as well as between, specialized infant lung function testing centres. Furthermore, there is increasing recognition of the need for multicentre studies of infant respiratory function if we are to achieve sufficient statistical power to address clinically relevant questions regarding respiratory disease during early childhood over a realistic time period.

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Table 5. – Methodological basis for multicentre trials

Comparison, assessment and standardization of equipment
Pilot study to assess accrual rate and validate protocol
Power of study calculation, based on the number of outcome measures, knowledge of within-subject variability of each parameter and estimated sample size
Frequent inter-laboratory visits during trial period
Identical measurement and analysis protocol using similar software
Exchange of raw data and cross-analysis of a subset of the data
Examination of population characteristics for between-centre bias.

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