BRONCHIAL RESPONSIVENESS DURING THE
FIRST YEAR OF LIFE

A thesis submitted for the degree of Doctor of Medicine,
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by

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ABSTRACT

In older children and adults, asthma is associated with increased bronchial responsiveness (BR). In order to test the hypothesis that BR in the neonatal period is a risk factor for subsequent lower respiratory illness (LRI), a cohort of term healthy infants of atopic parents was studied during their first year of life, with questionnaires and lung function measurements. Using the "squeeze" technique to generate partial forced expiratory flow volume curves, \( \dot{V}_{\text{maxFRC}} \), the maximal flow at functional residual capacity, was calculated. BR to histamine aerosol was determined and expressed as PC_{30}, the provoking concentration of histamine which induced a 30% fall in \( \dot{V}_{\text{maxFRC}} \). Functional residual capacity (FRC), was measured using a small infant spirometer and the helium dilution technique. In a parallel study, a group of "asthmatic" infants and a group with severe chronic lung disease of prematurity (CLD) were studied during tidal breathing, to evaluate technically simpler methods of lung function assessment. During histamine challenge tidal breathing parameters and non-invasive measurements of oxygenation were recorded and any changes applied to BR assessment.

Both neonatal lung function and BR were risk factors for LRI in the first year of life. In boys, LRI was associated with decreased \( \dot{V}_{\text{maxFRC}} \), whereas in girls it was associated with increased levels of BR in the neonatal period. By the age of six months, both boys and girls with LRI had decreased lung function, but differences in BR were no longer apparent. Tidal expiratory flow measurements were less sensitive than forced expiratory flow measurements in detecting differences between health and disease, only falling outside the normal range in infants with CLD. During bronchial challenge, change in oxygenation, measured by a reduction in transcutaneous oxygen
tension, was a less sensitive index of bronchial responsiveness in healthy infants than in infants with a history of recurrent LRI.
DECLARATION

This work was performed while I was a Research Fellow in the Department of Paediatrics and Neonatal Medicine, Royal Postgraduate Medical School, Hammersmith Hospital. The work was performed by me, with help from research assistants Mrs Varsha Shah, Miss Mandi Reese and Miss Hazel Aston. The computer programming for the on-line data acquisition and analysis of lung function (Respiratory Analysis Software Programme, Physiologic Ltd, Newbury, Berkshire) was written by Mr Robert Cumberland. The pilot cohort study was carried out by Dr Bernadette Salmon and Dr Michelle Clay. The study described in section 6.2 was carried out on the main cohort, the data was collected and analysed jointly by myself and Hazel Aston, and a paper was written by Hazel Aston, under my supervision.

This work was composed by me and has not previously been submitted for a degree.
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CHAPTER 1 INTRODUCTION AND BACKGROUND

1.1 WHEEZING IN INFANCY

Lower respiratory tract illness and recurrent wheezing in early childhood are very common, affecting up to a third of infants in the first year of life (Wright 1989, Denny 1986, Glezen 1973). Hospital admission rates for "asthma" in the 0 to 4 age group have increased 13 fold over recent years (Anderson 1989). This increase appears to reflect a real increase in disease prevalence, and cannot be explained by differences in diagnostic labelling or lowering of thresholds for hospital admission (Anderson 1989).

1.1.1 The nature of wheeze and lower respiratory illness in infants

Recurrent cough and/or and wheezing, henceforth referred to as lower respiratory tract illness (LRI), in infancy, are usually associated with viral infections (Horn 1979, Pattemore 1992), but the reason why some infants wheeze with viral infections and others only develop coryzal symptoms is not known.

(a) Outcome

Children who develop LRI early in childhood are more at risk than healthy children for developing symptoms later in childhood (Mok 1982). These results, however, must be interpreted with caution, as the study was hospital based. A population-based study has shown that children who develop LRI early in childhood have impaired lung function in adolescence (Voter 1988). However, many children with lower respiratory symptoms in infancy "outgrow" their symptoms, and only a minority go on to have
childhood asthma (Sporik 1991). The risk for subsequently developing atopic asthma was similar for early LRI and symptom-free infants. In a study of boys, a history of wheezing in early childhood was not associated with increased bronchial reactivity measured during adolescence (Voter 1988). Passive smoking and allergy have been reported as risk factors for the development of wheezing later in life in those with LRI early in childhood. The remission in symptoms which occurs in most children may be due to lung growth and development.

1.1.2 Risk factors for LRI in infants

(a) Genetic and familial factors

In older subjects there is evidence for a strong genetic component to atopic asthma, demonstrated in studies of mono- and dizygotic twins reared together and apart (Hanson 1991). One group has proposed that atopy is inherited as an autosomal dominant trait (Cookson 1988), but there continues to be debate about the precise genetics of atopy. Little is known about whether or not infant LRI is familial. Whilst one study showed that infants with atopic parents had an increased incidence of wheezing (Stempel 1980), another large epidemiological study showed no relationship between familial atopy and infant wheezing (Mok 1982). Supporting the lack of association between atopy and infant LRI, a study looking at immunoglobulin E levels in the newborn period, measured from cord blood, found that levels of IgE were inversely related to the incidence of infant LRI (Halonen 1992). However, a study measuring lung function and bronchial responsiveness in the newborn period, showed that a positive family history of asthma was associated with increased levels of bronchial responsiveness (Young 1991). Therefore the interrelationship between
genetic and familial factors and infant LRI remains unclear.

(b) **Fetal and developmental factors**

Exposure to tobacco smoke during fetal life is associated with impaired neonatal lung function (Hanrahan 1992, Tager 1993). The only physiological predictor of wheeze in infancy identified to date, is altered neonatal or early infant, premorbid, lung function (Martinez 1988 & 1991, Hanrahan 1992, Tager 1993). Prematurity has been found to be a risk factor for asthma in *school children* (von Mutius 1993), and low birth weight has been found to be a factor associated with impaired lung function in *adulthood*, and later, with death from chronic obstructive airways disease (Barker 1991). The connection between these factors may be lung growth: an insult occurring during fetal life, such as premature birth or intrauterine growth retardation, could interrupt or disturb lung growth and development at a critical time, resulting in an adverse respiratory outcome later in life (Rona 1993, Shaheen 1994). Low maternal age has been found to predispose to wheezing during the first year of life (Martinez 1992a), but the reasons for this are unclear.

(c) **Postnatal environment and allergens**

Several studies have shown that postnatal passive smoking is a risk factor for lower respiratory tract illness in early childhood (Martinez 1992b, Holberg 1993). Early exposure to allergens, such as house-dust mite antigen, has been shown to be associated with subsequent development of childhood asthma. In a cohort study of children with a family history of allergy, a strong correlation was found between house dust-mite exposure in the first year of life, and sensitization at the age of 11
years (Sporik 1990). In this particular study this correlation was not apparent before the age of 5 years. There was, however a significant inverse relationship between the level of exposure (Der p 1 concentration) at the age of one and the age of onset of wheezing, which was stronger for the atopic children than for the group as a whole. A study looking at the benefits of early allergen avoidance demonstrated a significant reduction in "asthma" during the first year of life (defined as three or more episodes of cough and wheezing) in infants who avoided housedust mite antigen and who had, together with their lactating mothers, avoided food allergens, compared with controls (Arshad 1992). By the age of two years, although infants who had avoided allergens had less allergy and eczema than control infants, the reduced prevalence of asthma was no longer significant (Hide 1994). This study, however, as in Sporik's study, only involved infants with a family history of atopy, and the nature of their LRI could have been different from LRI in infants with no atopic family history. Ingested, as opposed to inhaled antigens, may be less important sensitisers in relation to atopic asthma. There was no reduction in the incidence of wheezing up to the age of seven years in children with a family history of allergic disease, who avoided cows' milk in infancy (Burr 1993). However, in the first year of life infants who had never been breast fed had twice the incidence of wheezing compared with infants who had some breast feeding, for however short a time. This effect, which was related to the duration of breast feeding, persisted to the age of seven years, but beyond the age of two years was restricted to non-atopics. These findings were independent of other risk factors such as maternal smoking, socio-economic class and overcrowding. In this study the annual incidence of wheezing tended to decline in those infants who were non-atopic, and increase in those who were atopic, suggesting that the pathogenesis of infant LRI
differs from that of atopic asthma in older children. One explanation for the findings of this study is that infant wheezing is related to infection rather than allergy, and that breast feeding may confer some protection against viral respiratory infection.

\textit{(d) Bronchial responsiveness}

Studies in older children and adults, have revealed an association between asthma and increased bronchial responsiveness (Cockcroft 1977, Juniper 1981, Woolcock 1987, Peat 1989). In New Zealand, a population of second-generation Polynesian migrants aged five to fifteen years has been studied (Crane 1989), to look at the relationship between atopy and bronchial responsiveness. The frequency of bronchial hyperresponsiveness in atopic children was constant at approximately 40\% for all age groups from seven to fifteen years, whereas bronchial hyperresponsiveness declined with increasing age in the non-atopic children, to a frequency of less than 5\% by the age of fifteen years. Comparisons of bronchial responsiveness between different age groups in childhood has potential problems (LeSouef 1992; see section 4.5.3), but despite this the study is important. A relationship between bronchial responsiveness and wheezing in infancy has not been established.

1.1.3 Conclusions

Infant LRI appears to be a distinct disorder, and is not synonymous with atopic asthma in older children (Silverman 1993). The two disorders have different outcomes, and it would therefore be logical to suppose that their aetiology and pathogenesis are different. There are several unanswered questions about infant LRI. Why do some infants have lower respiratory symptoms with viral infections, whereas others only
exhibit upper respiratory symptoms? Is bronchial responsiveness (BR) increased in infants with LRI, compared with asymptomatic infants? Is the impairment in neonatal lung function, reported to be present in infants who have LRI (Martinez 1988 & 1991, Hanrahan 1992, Tager 1993), due to disturbances of lung growth in fetal life? What factors are responsible for disturbing fetal lung growth and development? These questions can only be fully answered by detailed prospective epidemiological studies.

1.2 TECHNIQUES FOR INVESTIGATION

1.2.1 Clinical features

The selection of subjects varies between the different studies of infant LRI. Some investigators have selected only infants who have been hospitalised for LRI (Mok 1982, Wilson 1992). These studies have an inherent selection bias, as they exclude the great majority of infants who have LRI, who do not require hospitalization. Other large prospective epidemiological studies have been designed to be population based, recruiting mothers during pregnancy when they attended the local Health Centre for routine antenatal care, following them up throughout their pregnancy and subsequently following up their babies from birth through infancy (Hanrahan 1992, Tager 1993). The Tucson study population was also population based. Their target population was all the healthy children born to families registered with one of the largest health maintenance organisations in the locality (Martinez 1988 & 1991).

Studies of infant LRI have used a variety of methods for assessing clinical features. The two large prospective epidemiological studies described above (Martinez 1988, 1991, Hanrahan 1992, Tager 1993) have been designed such that infants are examined
by their paediatrician every time they have lower respiratory symptoms, so that the child's symptoms can be assessed, the child can be examined, and more accurate details of the LRI can be obtained. These studies have also made use of paediatric clinic records, details of well baby checks, and telephone calls to parents to find out details of the infants' respiratory symptoms and illnesses.

1.2.2 Questionnaire

Most epidemiological surveys of asthma employ questionnaires, which offer the advantages of being widely acceptable, cheap and convenient, requiring no special equipment and being easy to standardize (Burr 1992). The definition and description of LRI in infants varies, with some symptomatic infants having the label of asthma, and others wheezy bronchitis or virus-induced wheeze. It is therefore important to ask parents about symptoms, rather than just about the diagnosis. However, some of the symptoms are not readily understood by the layman. In particular, wheeze must be defined, as it is a term which parents may use to describe a variety of noises, not necessarily associated with lower respiratory tract illness. When obtaining information on respiratory symptoms, questionnaire data should be collected at frequent intervals in order to minimise any recall bias (Samet 1993).

Whilst questionnaires may be accurate in obtaining details of symptoms, parents may be less willing to admit and divulge their smoking habits to a medical researcher. Some studies have solved this problem by measuring urinary cotinine levels, rather than relying solely on verbal reports (Wright 1991, Hanrahan 1992). In both these studies there was a good correlation between reported maternal smoking and cotinine
measurements, suggesting that a careful history may be adequate for epidemiological purposes.

In order to avoid errors in recalling symptoms, some researchers use diary cards on which parents record their child's symptoms regularly. Whilst this method has the potential for being more accurate, it is more onerous for parents, and there may be problems with compliance, particularly in long term studies. Parental reports of some respiratory symptoms may be inaccurate. In a study comparing subjective with objective measures of night cough, diary card scores of night cough did not correlate with cough counts obtained from tape recordings (Archer 1985). No method of gathering data on respiratory symptoms in infants has yet been adequately validated, because of the lack of any "gold standard" with which to compare.

1.2.3 Physiological measurement

(a) Lung function in infancy

The measurement of lung function in infants places great demands on both the operator and the infant, and many of the techniques used in older children and adults cannot be used in infants, because of their inability to cooperate! Infant lung function testing is not practicable as a routine clinical aid to diagnosis and management, but is a useful research tool for studying lung mechanics in both health and disease, for assessing airway and lung growth and development and for measuring objectively the response to pharmacological agents.
(i) Forced expiratory flow

The measurement of forced expiratory flow provides a sensitive test of intrathoracic airway function. Two techniques have been described for infants. These are the rapid thoracoabdominal compression ("squeeze") technique and the forced deflation technique (England 1988, ATS-ERS statement 1993). These techniques will be described and discussed in more detail in section 4.2.

(ii) Elastic properties of the lungs

Compliance is defined as change in volume divided by change in pressure, and is a measure inversely related to the "stiffness" of the lung, hence it describes the elastic properties of the respiratory system. Dynamic compliance refers to compliance measured whilst air is flowing in and out of the respiratory system, and static compliance is measured when there is no air flow. Compliance is also separated into active and passive, according to whether the respiratory muscles are active or relaxed. Depending on the technique used, the compliance of the lung or the compliance of the whole respiratory system may be measured. Passive compliance, measured during relaxation of the respiratory muscles, reflects the compliance of the respiratory system, i.e. a combination of the elastic recoil of the lung and the chest wall.

Dynamic pulmonary compliance can be measured using an oesophageal balloon to determine oesophageal pressure, which represents average pleural pressure provided that certain criteria are met (Stocks 1991). Flow and volume are measured using a pneumotachograph connected to a face mask. The problem with this technique, which measures lung compliance, is that in the presence of chest wall distortion, oesophageal
pressure may not accurately reflect pleural pressure, as the pleural pressure is not uniform at any given tidal volume if the thorax is distorted (LeSouef 1983). The other disadvantage of this technique is that it is invasive, requiring placement of an oesophageal balloon or catheter. Despite these limitations, this technique has been used extensively, for example in studies investigating surfactant therapy in neonatal idiopathic respiratory distress syndrome (Davis 1988).

Dynamic compliance of the whole respiratory system can be measured non-invasively using weighted spirometry (Tepper 1984). The infant breathes through a well-fitting face mask, connected to a water-filled spirometer. During tidal breathing, a weight is applied to the spirometer bell, generating a positive pressure within the circuit, which can be measured with a pressure transducer. The volume change induced by this pressure is then determined from the change in baseline of the tidal volume tracing. In the presence of airway obstruction, a positive pressure applied to the lungs may actually open up obstructed airways, resulting in an underestimate of compliance, due to an underestimate of the volume change. This problem can be overcome by using several weights, and calculating compliance from the slope of a graph of change in volume against change in pressure. The technique assumes that the infant does not have any active reflex response to the increased positive pressure within the circuit, produced as weights are applied to the spirometer bell.

Static compliance of the respiratory system can be measured using occlusion techniques, which take advantage of the Hering Breuer reflex, in which, as a consequence of briefly occluding the airway at end inspiration, relaxation of the
respiratory muscles is induced (Dezateux 1991). This results in a brief respiratory pause and a longer passive expiration. The volume exhaled is related to the pressure at end-inspiration (measured at the airway opening). Two techniques have been described, both of which are non-invasive. In the single breath technique, the volume of air expired after release of the occlusion is measured using a pneumotachograph, and the pressure plateau within the face mask during the occlusion is measured with a pressure transducer. Static compliance of the respiratory system is calculated by dividing volume by pressure. A potential problem with this technique is that after a brief occlusion the infant tends to commence the next inspiratory effort prematurely, i.e. before functional residual capacity is reached (Bryan 1984, Stocks 1986). This results in an underestimate of volume, and hence of compliance. To overcome this problem the second technique, the multiple occlusion technique, can be used. In this technique several occlusions are made throughout the respiratory cycle, and volume and pressure measured. The slope of the regression of volume against pressure gives a measure of the compliance of the respiratory system. The multiple occlusion technique assumes that lung compliance is constant over the range of occluded volumes measured. Several other assumptions are made for both of the techniques described above. These are that the respiratory muscles are relaxed, that there is no laryngeal influence, that expiration is passive following the occlusion, and that during the occlusion the airway pressure at the mouth is equal to alveolar pressure. The last assumption is unlikely to be correct in the presence of small airway obstruction, when the airway pressure at the mouth tends to underestimate the true alveolar pressure. Both the single breath and multiple occlusion techniques measure total respiratory system compliance, and do not allow its components, namely chest wall and lung
compliance, to be measured separately. Examples of the application of these techniques include a study in which serial measurements of respiratory system compliance were used to evaluate steroid and chloroquine therapy in chronic interstitial pneumonitis in a group of infants (Kerem 1990) and a study of respiratory mechanics in infants with bronchiolitis (Seidenberg 1989).

Measurements of lung stiffness are important in assessing newborn babies with idiopathic respiratory distress syndrome, where the major problem is decreased compliance due to surfactant deficiency, or in interstitial lung disease, but compliance is less relevant in the investigation of infant LRI, where the major problem would appear to be one of airway obstruction.

(iii) Resistance

Resistance is expressed as pressure divided by flow, and as with compliance it can be classified as active and passive, and can be considered as the total resistance of the respiratory system, or separated into components, namely airway resistance and tissue resistance of the lung. Total pulmonary resistance may be measured using an oesophageal balloon to measure oesophageal pressure, as described above in the section on compliance. This technique ignores dynamic changes occurring during breathing. Particularly in the presence of disease and hyperinflation, compliance and resistance are unlikely to be constant throughout the respiratory cycle.

A less invasive method of measuring resistance in infants uses total body plethysmography (Dezateux 1991). The principle of total body plethysmography, is
that when an infant lies within a closed chamber, a "constant volume", any pressure changes within the chamber or box, occurring through the breathing cycle, reflect lung volume changes (Boyle's law). The thoracic gas volume (see below in section on lung volumes) and airway resistance can be measured by total body plethysmography. One of the difficulties in this technique is that the absolute changes in box pressure occurring as the infant breathes are miniscule. An advantage of this technique is that pressure-flow curves are obtained for the whole respiratory cycle, so it is possible to gain information about changes in resistance during the respiratory cycle. This technique has been used to obtain data about normal infants (Stocks 1977), changes in disease (Stokes 1981), as well as to look at airway responsiveness in infants (Gutkowski 1990), and to evaluate therapy for LRI (Orlowski 1991). A problem with the measurement of airways resistance in infants is that as they are generally nose breathers, the resistance of the upper airway, particularly the nose, constitutes a major proportion of the total airway resistance (Stocks 1978). Therefore the values obtained for airway resistance may not reflect the resistance of the lower airway. Likewise, the technique may not be sufficiently sensitive to detect changes in the resistance of the lower airways induced during bronchial challenge. Airway resistance is often converted to its reciprocal, airway conductance, as this has a linear relationship with lung volume. Conductance may then be corrected for lung volume by dividing it by the thoracic gas volume (see below in section on lung volumes), to give the specific conductance. This is a potentially more useful parameter, as in health, apart from increasing during early infancy, it remains fairly constant throughout childhood.

Another method of measuring resistance is by the forced oscillation technique
(Solymar 1989). This technique essentially consists of imposing an oscillation at the airway, producing oscillatory changes in pressure and flow at the airway. The resistance can be measured from the relationship between pressure and flow, and provides an estimate of the total respiratory system resistance. One potential problem is that oscillations applied at the mouth are affected by the compliance of the upper airway. Nevertheless, the technique has been applied in infants, comparing healthy infants with infants with bronchiolitis (Wohl 1969).

Total respiratory resistance can also be measured using the single breath technique, described above in the section on compliance. The slope of the flow-volume curve obtained during the passive exhalation relates inversely to the product of resistance and compliance, and can therefore be used to calculate the resistance of the respiratory system (Dezateux 1991).

(iv) Lung volume
A knowledge of lung volume plays an important role in the interpretation of other tests of lung function in infants, for example measurements of forced expiratory flow, compliance and resistance. The only lung volume that can accurately be measured in infants is functional residual capacity (FRC), or thoracic gas volume (TGV). Two techniques are available for measuring infant FRC or TGV. These are gas dilution and total body plethysmography. Gas dilution techniques are discussed further in section 4.3. When the infant is asleep and breathing quietly within the plethysmograph, an occlusion is applied to airway opening at the mouth. As the infant makes respiratory efforts against the occlusion, the gas within the thorax is
rarefied and compressed, and the swinging pressure at the mouth, reflecting the alveolar pressure may be measured. The resulting changes in the infant's thoracic cage volume can be measured indirectly from the pressure changes within the box. From Boyle's law the volume of gas within the thorax at end tidal expiration, the TGV, can be calculated (Dezateux 1991). The main advantage of plethysmography is that it measures all the gas within the thorax, including any distal to obstructed airways, unlike gas dilution techniques, which only measure the volume of gas within the lungs that is in direct communication with the airways. However, the technique is quite complicated and cumbersome, and is based on four assumptions. Firstly, during the airway occlusion the alveolar pressure should be equal to the airway opening pressure, measured at the mouth. Secondly, the lung parenchyma should be sufficiently elastic so as to allow pressure swings to be transmitted to all gas-containing spaces, with no pressure gradient across the tissues. Thirdly, it is assumed that the changes in pressure and volume are isothermal. To avoid potential adiabatic changes, the plethysmograph volume must be calibrated by injecting known volumes of air at a frequency which approximates to the frequency of the respiratory efforts during occlusion. The final assumption upon which plethysmography is based, is that only gas within the thorax undergoes rarefaction and compression. It is therefore assumed that any gas within the gut is insignificant. TGV has been measured in infants with lung disease, including bronchiolitis, (Stokes 1981, Henry 1983, Seidenberg 1989), and used to study the efficacy of therapy for various respiratory disorders in infancy (Kao 1984, Kraemer 1988). However, in the presence of airway obstruction, the first two assumptions upon which plethysmography is based, described above, may not hold. During the brief occlusion, airway opening pressure may not equilibrate with alveolar
pressure, hence alveolar pressure changes may be underestimated, resulting in an overestimate of TGV. Secondly, in infants with small airway obstruction, any areas of lung with very high resistance and low compliance may act like rigid spheres, so that the gas they contain is not rarefied and compressed during respiratory efforts, resulting in an underestimate of TGV. These issues were addressed in a study in which TGV, FRC, airways conductance and $V_{\text{max}FRC}$ were measured and compared in a number of infants, in health and disease (Godfrey 1986). In some of the infants with recurrent wheezing, despite clinical evidence of hyperinflation, and decreased $V_{\text{max}FRC}$, paradoxically values of TGV were lower. The validity of plethysmographic measurements of airway resistance and TGV in infants with airways obstruction is therefore questioned.

(v) General aspects of lung function measurement in infants

One of the main difficulties of testing infant lung function, is that sedation is required, as most infants will not tolerate a face mask. Due to the relatively brief duration of sedated sleep, there are limited opportunities to repeat technically unsatisfactory measurements. The equipment used for measuring infant lung function needs to be scaled down in size, but increased in sensitivity, as many of the measurements, for example static volumes and flows, are much smaller than in adults, so great attention to detail is required. In miniaturising equipment a balance has to be obtained between minimising the dead space and increasing the resistance (for flow-measuring devices, for instance). Infant lung function techniques have not been standardized, and there is still great debate about many aspects (ATS-ERS statement 1993).
(b) Bronchial responsiveness in infancy

Studies in normal healthy infants have demonstrated the presence of bronchial responsiveness (BR) to methacholine (Tepper 1987), cold dry air (Geller 1988) and histamine (LeSouef 1989). Other studies have looked at BR in wheezy infants (Prendiville 1987b, Gutkowski 1990). A small Australian study of recurrently wheezy infants showed that BR was independent of wheezing (Stick 1991). A larger study from the same group showed a family history of asthma or parental smoking to be associated with increased levels of BR to histamine in the newborn period, prior to the onset of any respiratory symptoms (Young 1991). No studies to date have looked at the relationship between BR in the neonatal period and subsequent lower respiratory symptoms.

The relationship between BR in the neonatal period and subsequent lower respiratory symptoms is an important issue, because the observation that increased BR antedates symptoms would have major implications for our understanding of the nature of infant LRI and its possible genetic or developmental basis. For example, fetal growth factors and genetic factors affecting neonatal airway physiology before exposure to the extrauterine environment would then be important determinants of LRI. In addition, assuming that BR at or soon after birth, must be independent of aeroallergen sensitisation, an association between BR and subsequent LRI would imply, contrary to the evidence from older children (Sears 1991, Crane 1989) that both BR and wheezing are independent of atopic sensitisation in the youngest age group. The search for a genetic basis would then be justifiable. The final implication of finding that BR was a risk factor for recurrent LRI in infancy is that this association could
provide the link between the respiratory disorders of early infancy and those of adult life (Strachan 1990).

In summary, the mechanisms of infant LRI remain unclear, and it is possible that antenatal factors are important. Certain lung function techniques, the choice of which has been presented above, may be useful in identifying differences in lung function and BR between infants who subsequently develop LRI and those who remain asymptomatic.

1.3 AIMS

1.3.1 Principal aim

The main aim of this thesis was to test the hypothesis that BR in the neonatal period is a risk factor for subsequent lower respiratory illness in infancy. A cohort of term, healthy infants was recruited in the neonatal period and studied during their first year of life in order to test this hypothesis.

1.3.2 Subsidiary aims

In order to investigate the principal hypothesis, methodological aspects of infant lung function had to be addressed. The secondary aims were:

1. To explore a range of technically simple methods for measuring lung mechanics based on tidal breathing parameters (Martinez 1988).

2. To evaluate non-invasive methods to detect the response to bronchial challenge

In addition to the cohort of healthy newborn infants, it was necessary to study other groups of children with obstructive lung disease in order to determine the sensitivity of the methods, by comparing with conventional measurements of infant lung function and BR.
CHAPTER 2 SUBJECTS

2.1 INTRODUCTION
The subjects included in these studies comprise three groups, a cohort of healthy infants of atopic parents recruited neonatally, a group of asthmatic infants who were recruited from a paediatric asthma clinic, and a group of infants with chronic lung disease of prematurity, who were recruited from a regional neonatal unit. Although testing the main hypothesis involved studying only the cohort, in order to evaluate some of the physiological measurements, it was necessary to include the other two groups of infants, to represent a wide spectrum of obstructive lung disease in infancy.

2.2 COHORT
Fifty four infants (28 boys and 26 girls) born between January 1990 and February 1991, were recruited in the neonatal period. In addition, another nineteen infants (8 boys and 11 girls) were recruited in a pilot study two years previously, making the total number of infants in the study seventy three, of whom ten were non-caucasian. Posters were displayed and invitation leaflets (Appendix 1) were available in the antenatal clinics and in the post natal wards of the hospitals involved. Criteria for entry into the study were that infants had to have at least one atopic parent (by history), be born at term (≥37 to ≤42 weeks gestation) and be otherwise normal, healthy infants. By recruiting infants with at least one atopic parent, it was hoped that a group at high risk of wheezing would be involved (Cogswell 1987). Previous studies have reported the incidence of wheezing in children up to the age of 11 years, with a family history of atopy to be greater than 60% (Sporik 1991, Burr 1993).
Ethical constraints prevented recruitment of a truly random population, because it was considered that infant lung function testing and bronchial challenge testing were too invasive for infants at low risk of developing subsequent lower respiratory problems. In addition to parents spontaneously volunteering, a research assistant visited the postnatal wards weekly and reviewed the maternal medical notes of recent deliveries, for details of atopy, which were routinely obtained at the antenatal booking clinic. Mothers were visited on the postnatal wards, if they and/or their partner had a history of atopy, i.e. previous or current asthma, eczema or hayfever, and the study was discussed. Mothers were given copies of the invitation leaflet, and encouraged to think about the study, discuss it with their partners, and decide whether they wished to participate in it. Parents were not tested for atopy. About a week after delivery, parents were contacted at home by telephone to obtain their decision regarding taking part in the study. For those who wished to participate in the study, the first visit to the infant lung function laboratory was arranged for when the infant was about 1 month old. Further visits to record symptoms and for pulmonary function testing were arranged at 6 and 12 months of age. The first visit took place prior to any upper or lower respiratory symptoms.

Parents were free to withdraw from the study at any stage. By the age of 6 months, 9 infants had withdrawn from the study, as they had either moved away from the area (n=1) or their parents no longer wished to participate in lung function testing (n=8). By the age of 12 months, a further 5 infants had withdrawn as they had either moved away from the area (n=2) or their parents no longer wished to participate in lung function testing (n=3). Questionnaires (see chapter 3) at 6 and 12 months were
completed by telephone or by post for all those infants who had withdrawn. The characteristics of the withdrawals compared with those who continued in the study, together with the possible bias induced as a consequence, are discussed later (chapters 3 & 5). The nineteen infants in the preliminary study only attended the hospital once at the age of 1 month, but were contacted during the first year of life to document symptoms on the questionnaire.

2.3 INFANTS WITH "ASTHMA"

A total of twenty five infants with recurrent episodes of wheezing, whose LRIs had been severe enough to warrant referral to the paediatric asthma clinic at Hammersmith Hospital, were included in these studies. All had a history of recurrent or persistent wheezing of at least one month's duration ("infantile asthma"), but prior to any physiological measurements, all infants had been free of upper and lower respiratory symptoms for at least four weeks. None had evidence of any congenital or systemic disorder, cystic fibrosis, gastroesophageal reflux or chronic lung disease of prematurity.

Twenty infants (11 boys and 9 girls) aged 8 to 23 months with infantile asthma (involved in the study described in chapter 6.1) were taking part in double blind, placebo controlled crossover studies, looking at the effect of salbutamol, given via metered dose inhaler and valved spacer, on bronchial responsiveness in wheezy infants (Clarke 1993). They underwent lung function testing on two occasions. Data from the placebo day are included in this thesis. Nineteen of the twenty infants were on anti-asthma medication. Ten infants were receiving intermittent inhaled or oral $\beta_2$
stimulants (salbutamol or terbutaline), fourteen were receiving intermittent inhaled ipratropium bromide, two infants were on regular inhaled sodium cromoglycate, and five infants were on regular inhaled corticosteroids.

The other five asthmatic infants (4 boys and 1 girl, aged 6 to 16 months) were taking part in a randomised, controlled study looking at lung function and bronchial responsiveness in wheezy infants before and after one month's treatment with sodium cromoglycate (Turner 1991). Data from the infants' initial visits are included in the study described in chapter 6.3. Four of these infants were taking salbutamol intermittently, but none had been treated with topical steroids or sodium cromoglycate.

2.4 INFANTS WITH CHRONIC LUNG DISEASE OF PREMATURITY

The twenty infants (12 boys and 8 girls) with severe chronic lung disease of prematurity (CLD) comprised all the infants from NW London discharged home on oxygen, from the neonatal units of Hammersmith Hospital and Queen Charlotte's and Chelsea Hospital between October 1989 and July 1992. This group of infants had particularly severe CLD, as they all required home oxygen therapy. Lung function tests formed part of their assessment either prior to discharge from hospital, or during out-patient follow up at the CLD clinic at Hammersmith Hospital. They were born at gestations of 23 to 32 weeks. At the time of lung function testing, these infants were aged 2 to 18.5 months.

2.5 SEDATION

On the day of the lung function study, parents brought their infants to the children's
day ward at Hammersmith Hospital, where they were examined, to identify any features which would preclude sedation. Length and weight were measured with the infant naked. Length was measured with the infant laid supine on the stadiometer (Holtain Infant Stadiometer), then whilst one observer gently positioned the infant's head to touch the top of the stadiometer in the mid-line, the other observer gently depressed the infant's knees, so that the legs were extended. The moving footplate of the stadiometer was then moved up to touch the soles of the infant's feet, also in the mid-line, and the lever on the foot plate was then fixed, so that the length could be read off the meter. The infants were then fed, then sedated with chloral hydrate (50 mg/kg for infants less than 3 months and 100mg/kg for those older than 3 months), or an equivalent dose of triclofos sodium (Gilman 1985).

2.6 ETHICAL STATEMENT

All the studies were approved by the local Ethics Committee. Written consent was obtained from the parents of all subjects (Appendix 2 for the cohort). The general practitioners of all the infants who took part in the studies were notified by letter, of the involvement of their patients in the research projects.
CHAPTER 3 QUESTIONNAIRE

3.1 INTRODUCTION

In these studies, family and infant data, and details of respiratory symptoms were obtained by questionnaire. The various methods available to record and collect historical data have been discussed earlier (see sections 1.2.1 and 1.2.2).

3.2 COHORT INFANTS

3.2.1 History by questionnaire

At the cohort infants' first visit at 1 month of age, a detailed birth history, family history, social and smoking history was obtained by direct interview, using a questionnaire (appendix 3). At the initial visit, parents were asked to record details of their infant's subsequent upper respiratory infections, and upper and lower respiratory and atopic symptoms. These details were collected by questionnaire at 6 and 12 months (appendix 4), on each occasion covering the previous 6 months. Parents were asked:

1. if their child had symptoms of cough and/or wheeze (described below);
2. dates of upper respiratory infections (defined below);
3. the number of cigarettes consumed by the parents, and separate details of antenatal and postnatal maternal smoking habits.

The infants who withdrew from the study after the 1 month visit, and therefore did not
attend the hospital for lung function tests subsequently, were contacted by telephone or visited at home, and the same questionnaire was administered, to obtain details of respiratory symptoms. Parents were encouraged to contact us directly if they were concerned about any respiratory symptoms. An out-patient appointment in the paediatric asthma clinic was offered to any infant who appeared to be having significant lower respiratory symptoms, as it was felt that parents should have some sort of extra support from the hospital, to acknowledge their invaluable help in taking part in the study. Four of the seventy three cohort infants attended the out-patient clinic or emergency department during their first year of life. After each infant's initial visit, their general practitioner was informed by letter about the study, and the involvement of their patient.

3.2.2 Definition of respiratory symptoms

Parents were asked whether their children had developed any symptoms of wheeze and cough, if any precipitants were apparent, and in particular whether they occurred only in association with "colds" or exercise and excitement, or whether symptoms were unprovoked. Wheeze was described to parents as a whistling noise, coming from the chest rather than the throat, and distinct from the predominantly inspiratory noise of croup. Cough was specified as a "chesty cough", meaning a wet cough from the chest, rather than a dry cough from the throat or upper airways. Parents were asked whether symptoms occurred in the daytime or at night or both. An upper respiratory tract infection was defined as episode of rhinitis, with an acute onset, lasting several days, and consistent with a viral aetiology.
3.3 CLINICAL SUBGROUPS

The cohort infants were classified according to the presence or absence of cough and/or wheezing, referred to as lower respiratory illness (LRI). The definition varied slightly between the studies for practical reasons, according to whether data were analysed for the first six months or the first year, so the definition is stated clearly each time, in the relevant sections of chapter 5, where the cohort subjects' data were subjected to various different analyses.

3.4 RESULTS OF COHORT QUESTIONNAIRE

The prevalence of maternal and paternal atopy and paternal smoking, in the group of infants who withdrew from lung function measurements, was similar to that of the infants who remained in the study (table 3.1). Although there was a trend towards more maternal smokers and families in possession of hairy or feathered pets amongst the withdrawals, neither reached statistical significance. Infants who withdrew from lung function measurements had significantly fewer upper respiratory tract infections than those who remained in the study. The results concerning the presence or absence of respiratory symptoms are given together with the results of the relevant studies (chapter 5). Most of the infants studied had repeat questionnaires at their 6 and 12 month visits, so it was possible to test the repeatability of the data collection and the reliability of the information given by parents at the first visit. There was absolute agreement about parental atopy and smoking habits, and the few differences in answers to questions concerning pets were consistent with new pets having been bought or old ones dying!
3.5 DISCUSSION

The main advantage of the questionnaire technique rather than the interview technique is that it is less susceptible to observer bias by the interviewer. Infants were not classified to symptom groups until all the six month studies had been completed. Classification was done independently of lung function data analysis. The history data obtained from the questionnaire was recorded on the computer independently from analysing the lung function data.

There has been debate about the reliability of parental reporting of symptoms. Some studies have shown a lack of agreement between parental reports of respiratory illness and general practitioners records (Watkins 1982) and between diary records of nocturnal cough and bedside tape recordings (Archer 1985). A recently reported cohort study of respiratory illnesses in the first 18 months of life compared symptom diaries with regular telephone surveillance and visits by nurses practitioners, and with outpatient clinic records (Samet 1993). Compared with both the nurse practitioner diagnoses and the outpatient records, parental report of "wet cough" and wheeze was very sensitive for detecting LRI, but not very specific. Most of the children where parents reported LRI symptoms which were not confirmed by either the nurse practitioners or the outpatient records ("false positives"), were reported by parents to have "wet cough" symptoms. This illustrates the difficulty in distinguishing between cough productive of secretions from the lower respiratory tract and cough due to post-nasal drip, secretions originating from nasal discharge that has drained into the oropharynx.
Reporting of symptoms in young children is very different from symptom reporting in older age groups, as it is totally second hand. This means that differences in perception and recall by individual parents may introduce bias. The difference in the occurrence of upper respiratory tract infections between those who withdrew from lung function testing after the initial one month visit, and those who remained in the study, may have been due to recall bias, although there was no significant difference between the incidence of lower respiratory symptoms during either the first six months or the first year of life between the two groups (see chapter 5). An alternative method which could have been used to collect symptom data, would have been to contact parents regularly by telephone to keep a constant record of symptoms, as done in the East Boston Neighbourhood Health Center Study (Hanrahan 1990, Tager 1993). Another method would have been to post out diary cards regularly to parents, but it was felt that this would add an unnecessary burden upon the families, and might lead to more withdrawals. The Tucson Children's Respiratory Study only included doctor-diagnosed symptoms for classification purposes (Martinez 1988 & 1991), but this may introduce bias, by only detecting the more severe symptoms.

In recording the parents' atopic state, the definitions were subjective and somewhat imprecise. No tests for atopy were carried out on parents, so their reported atopy was not verified. Atopy was not considered to be of major importance, as it was not one of the factors included in the hypothesis tested in this thesis. However, a difference in the incidence of parental atopy, and hence infant atopy, between the different infant symptom groups, or between boys and girls might have influenced the findings, particularly as in school children over 7 years old, bronchial responsiveness is
influenced by atopic status (Sears 1991, Crane 1989). Smoking history may have been inaccurate, and could have led to an underestimate of the true numbers of smokers. A method to avoid this would have been to measure the infants' urinary cotinine levels, but there may still be inaccuracies with this method, as it only records the infants' exposure to smoke immediately prior to the urine collection. Another London group, studying infant lung function, found a good correlation between reported smoking by mothers and infants urinary cotinine levels (J Stocks, personal communication), as have two groups from USA (Wright 1991, Hanrahan 1992). The numbers of smokers among the cohort parents was very low, so that it was not possible to look at parental or maternal smoking as a separate variable. Some of the other environmental data which were recorded, such as damp or cold housing, was rather vague and subjective. These data were not further analysed in this study, as the cohort size was small. Standardisation would be necessary if it had been included for analysis.
TABLE 3.1 Characteristics of infants who withdrew from lung function testing after their initial visit and those who remained in the study.

<table>
<thead>
<tr>
<th></th>
<th>Non-withdrawal (n=42)</th>
<th>Withdrawal (n=12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal atopy</td>
<td>27 (64)</td>
<td>10 (83)</td>
<td>0.21</td>
</tr>
<tr>
<td>Paternal atopy</td>
<td>32 (76)</td>
<td>10 (83)</td>
<td>0.60</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>7 (17)</td>
<td>5 (42)</td>
<td>0.07</td>
</tr>
<tr>
<td>Paternal smoking</td>
<td>8 (19)</td>
<td>2 (17)</td>
<td>0.85</td>
</tr>
<tr>
<td>Pets</td>
<td>10 (24)</td>
<td>1 (8)</td>
<td>0.24</td>
</tr>
<tr>
<td>No. of URTI's in first year (median)</td>
<td>3</td>
<td>1.5</td>
<td>*0.002</td>
</tr>
</tbody>
</table>

*The 19 infants in the pilot study are not included in this analysis

URTI = upper respiratory tract infection

Comparisons by Chi squared test (*Mann-Whitney U test)
CHAPTER 4 PHYSIOLOGICAL METHODS AND REFERENCE VALUES

4.1 INTRODUCTION

Lung function tests were used for two purposes in order to test the hypothesis that BR in the newborn period is a risk factor for subsequent lower respiratory illness in infancy. Firstly they were necessary to characterize neonatal lung function, and secondly they were used to measure the response to bronchial challenge. The different methods of measuring lung function were discussed earlier in section 1.2.3. Only two of those techniques, total body plethysmography (used to measure airways resistance) and the "squeeze" technique (used to measure $V_{maxFRC}$) have been used in the assessment of BR in infants. Plethysmography has disadvantages in that the upper airway, including the nose, is a major component of airways resistance in infants. In the presence of airway obstruction there are other disadvantages with plethysmography (see section 1.2.3). In contrast $V_{maxFRC}$ appears to accurately reflect the state of the small airways, without being very much affected by the upper airways, in particular any nasal obstruction which might occur when a nasally-breathing infant undergoes bronchial challenge (Tepper 1993b, Kano 1994). In addition to measuring forced expiratory flow, it was necessary to measure lung volumes, in order to assess lung function more fully. The technique chosen for this was helium dilution, rather than plethysmography, because of the potential problems of using plethysmography in infants with airways obstruction (Godfrey 1986 & 1991).
4.2 FORCED EXPIRATORY MANOEUVRES - PARTIAL EXPIRATORY FLOW-VOLUME CURVES

The maximal forced expiratory flow-volume (MEFV) curve was first described in 1958 in adults (Hyatt 1958). This procedure consists of having the subject inhale maximally and then immediately exhale as rapidly, completely and forcefully as possible. The test is reproducible and sensitive to disease. The configuration of the MEFV curve reflects small airway function, and has the great advantage that maximal expiratory flow, on the descending part of the MEFV curve, is independent of effort (Hyatt 1983 & 1986). Maximal flow (\(\dot{V}_{\text{max}}\)) is reached with relatively modest expiratory effort (ie. relatively low transpulmonary pressures). This is because \(\dot{V}_{\text{max}}\) is determined by the physical properties of the intrathoracic respiratory system.

Expiratory flow through the airway cannot exceed the product of the tube-wave speed (the velocity of propagation of pressure wave along a tube) and the tube cross-sectional area in the region where flow is limited. Flow at wave speed (\(\dot{V}_{\text{ws}}\)) is determined as follows:-

\[
\dot{V}_{\text{ws}} = \left[1/d\right]^{1/2} \left[dP_{\text{tm}}/dA\right]^{1/2} A^{3/2}
\]

where 
\[
\begin{align*}
d & = \text{gas density} \\
dP_{\text{tm}} & = \text{transmural pressure} \\
A & = \text{area at site of flow limitation}
\end{align*}
\]

The gas density is constant, so \(\dot{V}_{\text{max}}\) is dependent upon the transmural pressure and the airway area. Transmural pressure (\(dP_{\text{tm}}\)) can be separated into two components, the
elastic recoil of the lung and the pressure drop between the alveolus and the site of flow limitation within the bronchus. This pressure drop, the resistive pressure loss, depends upon the magnitude of the flow and the geometry of the airways between the alveolus and the site of flow limitation. The elastic recoil of the lung is dependent upon the physical properties of the lung, by which its pressure-volume relationship is determined, and the degree of inflation of the lung. Airway area \( (A) \) is equivalent to the size of the airway. To summarize, \( \dot{V}_{\text{max}} \) depends upon the gas density and viscosity, the size and specific compliance of the airways, peripheral airway geometry, lung elastic properties and the magnitude of the flow itself (Wohl 1991).

Forced expiratory techniques have been standardized, and MEFV loops are used widely to assess lung function, and to aid the diagnosis and treatment of various lung diseases in adults. Most children over the age of seven are able to cooperate and perform a forced expiratory manoeuvre, and reference ranges for children have been established. More recently two techniques have been described for measuring the forced expiratory flow-volume relationship in infants. The first method is the forced deflation technique (Motoyama 1977), which has the advantage of producing a full flow-volume curve over the entire range of the vital capacity, but has the disadvantage of requiring the infant to be intubated, anaesthetised and paralysed, so is not a very practicable technique for general use. The second technique, the rapid thoracoabdominal compression or "squeeze" technique (Adler 1978), has the advantage of being relatively non-invasive, but has the disadvantage of producing a partial rather than a full expiratory flow-volume curve. Partial expiratory flow-volume (PEFV) curves, however, still provide qualitative and quantitative information about
intrathoracic airways. A "squeeze" is applied to the sleeping infant's chest wall by means of a rapidly inflatable thoracoabdominal jacket at end inspiration, to produce a passive forced expiration. The PEFV curve is quantitated by measuring flow with reference to the functional residual capacity (FRC), the steady state end-expiratory lung volume determined during tidal breathing. Maximal expiratory flow at FRC ($\dot{V}_{\text{max FRC}}$) is the usual index reported. The lung volume at FRC corresponds approximately with 40% total lung capacity (TLC), or 25% vital capacity in older subjects (Bryan 1984).

The quantitation of $\dot{V}_{\text{max FRC}}$ assumes that towards end expiration flow limitation has been achieved. Provided that sufficient pressure is applied, and that the infant does not make an inspiratory effort, $\dot{V}_{\text{max FRC}}$ is probably achieved (see section 4.2.8). Under these circumstances, an increase in pressure in the jacket does not produce any further increase in expiratory flow.

4.2.1 Equipment for $\dot{V}_{\text{max FRC}}$ measurement

The equipment used to measure PEFV curves in infants is shown in figure 4.1. The squeeze jacket (Medical Engineering Department, Royal Postgraduate Medical School, Hammersmith Hospital, London), was an inflatable polythene jacket, available in a variety of sizes, extending from the shoulders to the upper thighs, with velcro straps for fastening and cut away at the neck to prevent tracheal compression. The pressure source consisted of a 200 litre plastic barrel, with an adjustable air inflow (up to 20 l/min), a pressure relief valve, tubing incorporating a 3 way tap to the jacket, and a port to measure jacket pressure close to the jacket, via a pressure transducer (Validyne
Flow was recorded with a heated low resistance screen pneumotachograph (Medical Engineering Department, Royal Postgraduate Medical School, Hammersmith Hospital, London; linear up to 40 l/min) and face mask (Rendell-Baker Soucek, size 1, Ambu International, Bath, Avon), connected to a differential pressure transducer and amplifier (Validyne MP45, Northridge, California, USA; range ± 2 cmH₂O). The resistance of the pneumotachograph was 0.1 kPa/l/s up to 450 ml/sec (figure 4.2). Its deadspace in conjunction with the facemask was 17.5 ml, measured by filling with water. Applied to the infant's face the deadspace was likely to be less because of facial structures. For flow calibration of the pneumotachograph a calibrated flowmeter (Rotameter, range up to 40 l/min) was used. Tidal volume was calibrated with a 100 ml syringe. To calibrate the jacket pressure transducer a water manometer (range up to 100 cmH₂O) was used.

4.2.2 Respiratory analysis program computer software (RASP)
The RASP program (RASP Software, Physiologic Ltd, Newbury, Berkshire) was written for MS-DOS personal computers in conjunction with the Department of Paediatrics and Neonatal Medicine, Royal Postgraduate Medical School, London, for measuring infant lung function. The direct data streams from the pressure transducers and the derived integrals were displayed as time series or X-Y plots on the computer screen, as they are collected. Additional plots were also displayed on an oscilloscope. All signals were digitised (100 Hz) and stored on computer (Compaq deskpro 386/20e, Houston, Texas, USA). RASP was used to calibrate the input signals from known reference values, to collect the raw data signals and to analyze the data, using
specified parameters, and to print and plot the data.

4.2.3 Calibration

(a) Pneumotachograph

(i) Flow at airway opening

The air supply was connected to the pneumotachograph, via the rotameter, noting the direction of the flow. Once a steady flow of 40 l/min had been achieved, the signal was recorded on computer, then the air flow was switched off in order to record zero flow. A two point calibration was thus achieved (Dezateux 1991). This two point calibration check was done before every infant study. The system was checked every 3 months for linearity, by applying increasing flows between 0 and ± 40 l/min, in increments of 10 l/min via the rotameter to the pneumotachograph, and recording the voltage output from the Validyne MP45 (figure 4.3). Linearity was checked by plotting flow against voltage (figure 4.4).

(ii) Volume

The calibrated 100 ml syringe was connected via non-compressible tubing to the pneumotachograph, and 100 ml of air was pushed in and out ten times at a rate of approximately 40/min, to mimic infant respiration. The flow signal was digitally integrated with respect to time to give the volume. The flow and volume signals were recorded on the computer, and the calibration was accepted if the volume signal was between 99 and 101 ml. This calibration check was carried out at three monthly intervals.
(b) Jacket pressure

The tubing from the jacket pressure transducer was connected to a water-filled manometer via a 3-way tap, and air was injected via a syringe into the 3-way tap, so that the column of water in the manometer was raised to 100 cmH₂O. This signal was recorded on the computer, then the syringe was removed and atmospheric pressure was recorded. A two point calibration was carried out before every study. Every 3 months 10 cmH₂O increments of pressure between 0 and 100 cmH₂O were applied to the transducer, and the voltage output from the Validyne MP-45 recorded. The linearity of the system was checked by plotting pressure against voltage (figure 4.5).

4.2.4 Monitoring

Throughout all lung function measurements, as a safety measure, oxygen saturation by pulse oximeter (SaO₂) and transcutaneous oxygen and carbon dioxide tensions (PecO₂ and PtcCO₂) were monitored continuously. The pulse oximeter probe (Ohmeda Biox 3740 pulse oximeter, Louisville, USA) was applied to the big toe and the combined O₂ and CO₂ skin electrode (Radiometer TCM3, Radiometer, Copenhagen, Denmark), heated to 44°C, was applied to the thigh. The electrode was calibrated with 20.9% oxygen and 5% carbon dioxide prior to each study.

4.2.5 Technique

(a) Preparation of the infant

The sleeping infant, wearing a loose vest, was laid supine on an appropriate sized jacket, extending from the neck to the pubis. The neck was extended slightly using a roll. The oximeter and transcutaneous monitors were applied. With the arms by the
infant's side, the jacket was wrapped snugly around the infant, fastening the velcro at the sides and across the shoulders. The neck cutout avoided compression or contact with the neck during a squeeze. The pressure reservoir was connected via the wide tubing to the jacket and the jacket pressure transducer tubing was connected to a port near the jacket. A rim of silicone putty (Carters, Bridgend, Mid-Glamorgan) was applied around the mouth and nose, and to the face mask. The pneumotachograph was inserted onto the mask, and the mask applied to enclose the infant's mouth and nose, with the silicone putty providing an air-tight seal (figure 4.1b).

(b) Measurement of PEFV curves

Flow-volume tracings were displayed on an oscilloscope, and time-based tracings of flow, volume and jacket pressure were displayed on the computer monitor. At least six breaths were recorded prior to the squeeze, ensuring a stable end expiratory level. Starting with a pressure of approximately 25 cm H$_2$O, the jacket was inflated at end inspiration, by rapidly turning the tap to the pressurized tank (figures 4.1a & 4.6). The inflation was held for approximately 1 second before releasing the pressure in the jacket. The jacket pressure (Pj) was increased from 25 cm H$_2$O by increments of 5 cm H$_2$O until a value of Pj had been obtained, at which $V_{maxFRC}$ was maximal (optimal Pj), or until the maximum jacket pressure of 80 cm H$_2$O had been reached. The maximum $V_{maxFRC}$ could be observed on the oscilloscope flow-volume curve as the highest flow at FRC (figure 4.7), and was confirmed by quickly analysing a PEFV curve. Negative pressure dependence of flow could be observed at pressures higher than the optimum in some infants with airway obstruction (LeSouef 1986, Silverman 1986). Under these circumstances, the flow volume curve tended to change from a...
convex to a concave configuration, as $P_j$ increased. Infants with airway obstruction needed a lower $P_j$ than healthy infants to achieve maximum flow (LeSouef 1988). A mean baseline value of $\dot{V}_{\text{maxFRC}}$ was derived from 8-10 technically satisfactory measurements made at the optimal jacket pressure.

(c) Technical quality of studies

PEFV curves where there was glottic closure, a premature inspiratory effort during the squeeze or an irregular respiratory pattern prior to the squeeze were technically unacceptable (figure 4.8, Silverman 1986, Beardsmore 1989). The importance of choosing the optimal $P_j$ has already been discussed. Excessive pressures not only produced negative pressure dependence, but tended to induce glottic closure. The time to peak jacket inflation (the interval from the onset of inflation to peak jacket pressure) needed to be sufficiently short for the $P_j$ to have reached a plateau before the lungs emptied to FRC, and was usually less than 120 msec. This was minimised by choosing the smallest suitable jacket, wrapping the jacket snugly around the infant, using wide bore tubing from the pressure source to the jacket and turning the tap rapidly. The jacket inflation lead time was the interval between end inspiration and the onset of jacket inflation. Ideally this should have been zero, but acceptable times varied according to the infant's respiratory frequency and airway characteristics. For healthy newborn infants the jacket inflation lead time should be within $\pm 100$ msec, so that the plateau of the jacket inflation pressure is reached well before the infant has expired to FRC. Beyond 100 msec, values of $\dot{V}_{\text{maxFRC}}$ may be spuriously low (personal observations).
Stability of the end expiratory level was assessed by inspecting, on the computer monitor, the time-based signals of at least six tidal breaths prior to the squeeze. The calculated end expiratory level of these breaths was displayed as a straight line on the computer monitor during RASP analysis, and if the end expiratory level was stable, it coincided with a horizontal line plotted on the computer screen, such that flow was equal to zero. Those PEFV curves which were not preceded by regular breathing around a stable end expiratory level were excluded from the analysis. The expiratory reserve volume (ERV; the volume below FRC to which the infant expired during the PEFV manoeuvre) was at least 10% of the tidal volume as some degree of reassurance that the infant had not begun to inhale before FRC was reached during the forced expiration. ERV was also likely to be low if there was irregular breathing, severe airway obstruction such that tidal expiration was close to being flow limited or if $P_j$ was sub-optimal.

4.2.6 Reference values of $V'_{\text{maxFRC}}$

It was important to establish reference values from these studies of healthy individuals for two reasons: firstly, to compare with reference values from other groups, and secondly to standardize lung function data for length in later studies (chapter 5). Fifty four infants had $V'_{\text{maxFRC}}$ measured at 1 month, (the nineteen infants who had preliminary studies with questionnaire follow-up, but no subsequent lung function tests are not included in this analysis, in order not to weight the regression lines towards the younger age group). Twenty two infants (thirteen boys and nine girls) had either no episodes of cough ± wheeze, or a single episode during the first year, and are the subjects included in this analysis as healthy infants. Of these infants sixteen had lung
function measurements at 6 months, and thirteen had lung function measurements at 1 year. Figure 4.9 shows the relationship between length and $V_{\text{ax}}$ for the healthy infants, (defined as having one or less episodes of LRI during the first year of life). The data are separated according to gender in figure 4.10. The regression equations for normal infants, boys and girls, combining 1, 6 and 12 month data, are as follows:

- all infants $V_{\text{ax}}$ (ml/s) = 7.22 (length in cm) - 238
- boys $V_{\text{ax}}$ (ml/s) = 5.41 (length in cm) - 152
- girls $V_{\text{ax}}$ (ml/s) = 8.09 (length in cm) - 271

The relationship between length and $V_{\text{ax}}$ was plotted again, excluding measurements made at 1 year of age (figures 4.11 and 4.12). The relationship between length and $V_{\text{ax}}$ over the latter part of the first year appeared to be curvilinear rather than purely linear, i.e. the rate of increase in $V_{\text{ax}}$ compared to length was more rapid than in early infancy. Therefore, for some of the studies described in chapter 5 in which subjects were less than 8 months old, $V_{\text{ax}}$ values were adjusted for length from the following regression equations for 1 and 6 month data:

- all infants $V_{\text{ax}}$ (ml/s) = 7.64 (length in cm) - 262
- boys $V_{\text{ax}}$ (ml/s) = 6.97 (length in cm) - 244
4.2.7 Repeatability

All the values of $\dot{V}_{\text{maxFRC}}$ from the 8-10 technically satisfactory measurements made at the optimal jacket pressure for each individual subject were used to calculate the coefficient of repeatability, by first calculating the standard deviation for each subject, and the mean of all the individual observations. The coefficient of repeatability (CR; expressed as %) was calculated as:

$$CR = 100\left[\frac{\sqrt{\sum SD^2}}{n}\right]/\text{mean}$$

where $\sum SD^2 = \text{sum of the squares of the standard deviation for each subject}$

$n = \text{number of subjects}$

$\text{mean} = \text{mean of all observations}$

The CR for healthy male and female infants combined was 13.3%, 8.4% and 7.3% at 1, 6 and 12 months respectively.

4.2.8 Discussion

The baseline measurements of $\dot{V}_{\text{maxFRC}}$ for the healthy subjects, despite the small and selected population (parental atopy), were similar to those few recent reference ranges reported for infants (Hanrahan 1990, Tepper 1993a; figure 4.13). The earlier reference data from Tepper are dissimilar, with the slope of the graph relating $\dot{V}_{\text{maxFRC}}$ to length
being lower (Tepper 1986a). All the reference data show a wide scatter between subjects, but \( \dot{V}_{\text{maxFRC}} \) is repeatable within subjects. There are differences in the populations used for the reference ranges. The Tucson subjects (Tepper 1986a) comprised 125 healthy infants with a *postconceptional age* of 8.5 to 25 months, including 6 preterm infants, of Caucasian and Mexican origin. Relatively few were older than 6 months of age, the mean *postnatal age* being 2.6 months. This probably accounts for the difference between their reference values and the other reported ones. \( \dot{V}_{\text{maxFRC}} \) for the Tucson infants, was significantly lower in boys compared with girls. The reference data from Indianapolis (Tepper 1993a) are from 112 normal, healthy infants, aged 1 to 31 months, and exclude the measurements from 6 infants aged less than a week, as they appeared to be outliers compared with the other infants, who were not neonates. The Boston data are from 72 infants, *postconceptional age* of 37 to 121 weeks, and almost half were non-white. Neither of the last two studies reported any significant difference between male and female flow rates, although amongst the younger children, there was a trend for girls to have higher \( \dot{V}_{\text{maxFRC}} \) at any given length.

Infants with airway obstruction do appear to achieve flow limitation (Allen 1991), but doubt has been cast on the assumption that in healthy infants, flow limitation is achieved by the squeeze technique (ATS-ERS statement 1993). An indirect attempt to assess flow limitation by one group involved superimposing an oscillatory wave on the applied jacket pressure (Ratjen 1989). Flow limitation was demonstrated by increasing the \( P_j \) until the oscillation disappeared from the expiratory flow signal. Ideally, to demonstrate flow limitation in infants, isovolume pressure-flow curves
would need to be generated, a technique which has only recently been described (Flucke 1994). In an important recent study, a small number of infants with no history of respiratory symptoms underwent lung function testing, using a modified "squeeze" technique. In addition to measurements of flow, volume and jacket pressure, oesophageal pressure, using an oesophageal catheter, and mouth pressure were recorded. In order to generate a full flow volume curve, after a brief end-inspiratory pause, the lungs were inflated by a positive pressure applied at the mouth. Transpulmonary pressure was calculated as the difference between mouth and oesophageal pressures. During "squeeze" manoeuvres, at lung volumes below 70% of expired forced vital capacity, once a certain pressure had been achieved, any further increase in transpulmonary pressure (produced by increasing the jacket pressure) did not produce increased forced expiratory flow, indicating that flow limitation had been achieved (Feher 1994).

From occlusion tests at end inspiration, the static pressure transmission to the pleural space with the Hammersmith jacket is 56-80% of the applied pressure (LeSouef 1986). It has been suggested that, for any given Pj, flow rates may be higher if the jacket is wrapped around the infant's chest and abdomen with the arms out (Steinbrugger 1988), in contrast to all the studies described in this thesis, in which the jacket was put on with the arms inside. Despite Steinbrugger's findings, the reference values calculated from the healthy subjects in these studies are similar to others who have used the "arms out" technique (Hanrahan 1990, Tager 1993, Tepper 1993a).

The measurement of $V_{\text{maxFRC}}$ from PEFV curves may be influenced by the upper
airways. The head and neck are positioned with the neck slightly extended to try to avoid glottic closure during the forced expiration. Nasal obstruction, although reducing the peak expiratory flow of the PEFV curve, appears to have little influence on the flow at FRC (Tepper 1993a). The rapid deflation technique, a technique which bypasses the upper airway, has been used by some groups to measure forced expiratory flow in infants (Motoyama 1977, 1987). This technique has a major disadvantage, as it is limited to infants who are intubated, so the subject has to be deeply sedated or anaesthetised. Attempts have been made to look at reflex inspiratory responses to chest compression in unsedated newborn infants (Hoskyns 1987). By using an oesophageal balloon to measure intrathoracic pressure, and applying squeezes at different points during the respiratory cycle, Hoskyns et al showed that reflex inspiratory responses may interfere with the measurement of forced expiratory flow, but the reflex was maximal at low lung volumes. They reported that the jacket inflation took up to 210 msec, which was much longer than in the present study, and might have accounted for some of the reflex inspiratory activity. In the present study, by applying strict criteria to PEFV analysis, and only accepting optimal flow-volume loops, errors induced by other reflexes were minimised.

The end expiratory lung volume in infants is actively maintained (Kosch 1984), and so may vary during a study. If the end expiratory level increases (FRC increases), measured flows will increase, and conversely flows will decrease if FRC decreases. This is likely to be more of a problem when bronchoconstrictor agents or bronchodilator agents are given (see chapter 4.5.3).
Despite the limitations and reservations about the validity of the "squeeze" technique, during recent years many studies have used the technique, and have demonstrated its value as a research tool. Changes in $V_{\text{maxFRC}}^\text{PRC}$ during infancy have helped in the understanding of normal airway growth and development (Tepper 1986a, Hanrahan 1990). Knowledge of normal airway growth and development has enabled $V_{\text{maxFRC}}^\text{PRC}$ to be used to quantify airway obstruction seen in a variety of acute and chronic respiratory disorders in infancy (Godfrey 1983, Tepper 1986b, Beardsmore 1988), and to evaluate various therapeutic interventions (Hiatt 1988, Prendiville 1987a, Kao 1987 & 1989, Tepper 1994). Of the few infant lung function techniques currently available, the "squeeze" technique provides one of the best means of assessing intrathoracic airway function.
4.3 LUNG VOLUME MEASUREMENT - HELIUM DILUTION TECHNIQUE

The helium dilution technique can be used to calculate functional residual capacity (FRC), defined as the volume of gas in communication with the airway opening at end expiration. FRC can be measured in infants by both open circuit (nitrogen washout) and closed circuit techniques (helium dilution). In the helium dilution technique the infant rebreathes from a reservoir which contains a known volume and concentration of the inert gas helium, until there is complete equilibrium and mixing between the infant's lungs and the reservoir. The lung volume can then be calculated when the final concentration of gas is known, provided that the system volume remains constant. These techniques assume complete mixing and equilibration with all the gas contained within the air spaces of the lungs.

4.3.1 Equipment for FRC measurement by helium dilution

A spirometer, with chart recorder, and a helium analyser were required for FRC measurement (figure 4.14). The low dead space (600ml) water filled spirometer (University of Leiden, Holland) incorporated a fan (pump speed 50 ml/sec within an inner cylinder of sodasorb (PK Morgan, Rainham, Kent) to absorb carbon dioxide. A low flow (0 to 100 ml/min) meter (Platon, Basingstoke, Hants) was connected via a valve to an oxygen cylinder. The aluminium rod on top of the spirometer bell was connected to a potentiometer, which allowed volume changes to be recorded. A helium analyser (Morgan md2-FRC, PK Morgan, Rainham, Kent) was incorporated into the circuit, with calcium chloride to absorb any water vapour. The analyser was accurate to 0.01% helium concentration. A facemask (Laerdal size 0/1, Orpington, Kent) was attached to the shutter apparatus. The shutter allowed the infant to breathe
either from room air or from the spirometer circuit, and a further stopcock enabled the spirometer to be flushed with room air whilst the infant was still connected to the facemask. An oxygen analyser (IMI B-D Electrodyne, Becton Dickinson) was included in the circuit, as a method of checking for leaks within the circuit, which could otherwise have been inadvertently compensated for by varying the oxygen input. It also enabled the helium analyser to be adjusted according to the known inspired oxygen within the circuit, as the helium analyser, which detects differences in the thermal conductivity of different gases, unless adjusted, overreads the helium concentration by 0.02% for each one percent that the oxygen concentration exceeds 20.8%. A digital thermometer was incorporated into the circuit, so that measurements could be corrected to BTPS, and a spirit level on the base enabled the spirometer to be adjusted until absolutely horizontal. The helium analyser output and the potentiometer signal were both connected to a chart recorder (Phillips PM 8262) and the computer.

4.3.2 Respiratory analysis computer software program (RASP)

The calculations for measurement of FRC were not incorporated into RASP at the time of the study, but the computer was used as a chart recorder, in order to measure the volumes and helium concentrations with maximum accuracy and resolution.

4.3.3 Calibration

The spirometer was filled with approximately 450 ml of distilled water. The bell was flushed with air and the bell was put in its lowest position before closing the shutter and the stopcock. A known volume of helium was added to the system using a
calibrated syringe. When a constant plateau of helium concentration was obtained, 100 ml of air was introduced into the system, using a calibrated syringe. The new helium concentration was recorded. Further 100 ml aliquots of air were added to the spirometer, recording the helium concentration each time. A 60 g weight was briefly applied to the spirometer bell, to ensure that there was no leak in the system. A graph of volume added against the reciprocal of the helium concentration was plotted, and the intercept on the volume axis was equal to the dead space of the system up to the shutter (figure 4.15). The dead space volume up to the face mask was measured by filling it with water. During the measurement of the dead space of the system, the helium concentration and volume signals were calibrated on the computer and the chart recorder.

4.3.4 Technique

(a) Preparation of the infant

The infant, when asleep, was laid supine, and the oximeter and transcutaneous monitors were applied. A rim of silicone putty (Carters, Bridgend, Mid-Glamorgan) was applied around the mouth and nose, and to the face mask, which was applied to enclose the infant's mouth and nose, with the silicone putty providing an air-tight seal. The shutter was kept closed to the spirometer, so that the infant breathed room air. Having half filled the bell with air, by simultaneously closing the shutter to room air and opening it to the spirometer the infant was allowed to breathe from the spirometer circuit. The oxygen supply was switched on at the low flow meter and adjusted so that the volume trace end expiratory level of the spirometer remained constant, in order to determine the infant's oxygen consumption for the subsequent FRC
measurement (Merth 1991). The shutter was then simultaneously closed to the spirometer and opened to room air, thus removing or "switching" the infant from the spirometer circuit.

(b) Measurement of FRC

The spirometer was flushed with fresh air, and helium was added into the circuit, until the concentration of helium in the circuit was 10% to 12%. Oxygen was then added until the concentration of oxygen in the circuit was approximately 22%. Once the helium concentration had stabilized, the computer and chart recordings were commenced, and the infant was "switched" into the circuit, simultaneously turning on the oxygen supply at the predetermined flow rate, such that the end expiratory level on the chart recorder remained stable. Equilibration was ensured by waiting until the helium concentration was stable (up to six minutes), or there had been minimal change over at least two minutes. The 60 g weight was briefly applied to the bell to ensure no leak and to verify equilibration. The temperature of the spirometer was recorded for BTPS corrections. Three measurements of FRC were made, allowing a washout time at least as long as the equilibration period between measurements. Results were expressed as the mean of technically satisfactory measurements (see section 4.3.4d).

(c) Calculation of FRC

The final helium concentration ($\text{He}_f$) was obtained by extrapolating the final linear portion of the plot of helium concentration against time, back to the point in time at which a decline in helium concentration was first detected (figure 4.16). This compensates for any slow decline in helium concentration due to helium dissolving.
in water and blood and tissues (Merth 1991).

The calculation for FRC is as follows:

\[
FRC (\text{ml}) = \left\{ \left( \frac{[V_{\text{SPIRO}} + V_F] \times [\text{He}_i - \text{He}_e]}{\text{He}_e} - V_{\text{MASK}} \right) \times \text{CorrF} \right\} - V_t
\]

where

- \( V_{\text{SPIRO}} \) = dead space of spirometer (540 to 600 ml, dependent on volume of water added)
- \( V_F \) = filling volume of spirometer bell (ml)
- \( \text{He}_i \) = initial helium concentration (%)
- \( \text{He}_e \) = final helium concentration (%)
- \( V_t \) = infant's tidal volume above FRC when switched into the circuit (ml)
- \( V_{\text{MASK}} \) = additional dead space from shutter to mask (10 to 17 ml, dependent on mask size)
- \( \text{CorrF} \) = correction factor for converting measurements from ATPS to BTPS

\( (d) \) Technical quality of studies

The major problem with determining lung volumes by gas dilution, is ensuring a gas tight system. This was checked in two ways; firstly an oxygen analyser was included in the circuit, so that leaks were not inadvertently compensated for by changing the oxygen input into the system, which could be detected from the analyser reading, and
secondly a weight was briefly applied to the spirometer bell, and when removed, provided that there was no leak, the volume tracing returned to the original end expiratory level (figure 4.17). Any measurements where leaks were detected were excluded from the analysis (figure 4.18), but most leaks, except around the face mask, were detected during calibration, so the system was checked and any leaks made air and helium tight before commencing lung volume measurements. Any measurements where the end expiratory level was unstable were excluded (figure 4.19). These were usually associated with the infant waking, or being in active sleep, so measurements were repeated once the infant was in quiet sleep again.

4.3.5 Reference values of FRC

The apparatus for determining FRC was not fully operational until the cohort study had begun, so few infants had lung volume measurements on their first visit. For that reason there are values from 12 infants at 1 month, 24 infants at 6 months and 23 infants at 12 months, defined as healthy, having had one or less episodes of LRI during the first year of life. Figure 4.20 shows the relationship between length and FRC during the first year of life. The regression equation, for boys and girls combined, for data from 1, 6 and 12 months is:-

\[ \text{FRC (ml)} = 5.27 \text{ (length in cm)} - 196 \]

4.3.6 Repeatability

For the normal, healthy subjects the coefficient of repeatability (see 4.2.7 for definition) was 9.8%, 6.3% and 4.4% at 1, 6 and 12 months respectively, based on 2
or 3 measurements in each infant at each time point.

4.3.7 Discussion

The values of FRC for the healthy subjects in the cohort, despite the small and biased sample, were very similar to published reference ranges for infants (Tepper 1986a, Tepper 1993a; figure 4.21). Values for the Boston infants were dissimilar (Hanrahan 1990). There are differences in the methodology, however. Tepper's group used a larger volume (2 l) spirometer, and waited for the helium concentration to be constant for only 30 seconds, and Hanrahan's group used a 1 l spirometer, and waited for the helium concentration to be stable for at least 20 seconds. Neither group extrapolated the helium concentration back, as no slow decline was detected. This may have been because the measurable decline was so slight, that it could not be detected in 20 to 30 seconds with a relatively large volume spirometer. The likely explanation for the decline in this present study was that the system volume was smaller and therefore more sensitive, so any helium absorption into body tissues would have been more apparent as a greater decline in concentration. The solubility coefficient of helium is very low (the blood/gas partition coefficient is 0.0019), but small volumes are taken up by the blood stream and absorbed into body fat (oil/water partition coefficient 1.7). Hence during exposure of the lung to helium over a few minutes, some helium will be lost from the lung air, and detected by a small, constant drop in helium over time (Merth 1991). It was confirmed that this was due to absorption into body tissues, and not leakage, by measuring the slow decline with the infant breathing from the spirometer, and then without the infant, but with a syringe to mimic respiration. The decline was higher with the infant in the system. Failure to extrapolate back to obtain
the $\text{He}_e$ results in FRC being over estimated, and could explain the relatively large values for the data of Hanrahan (1990).

Measurement of lung volume by helium dilution may not be reliable in the presence of airway obstruction, due to poorly ventilated areas of lung taking much longer to equilibrate. This is seen on the helium dilution curve as an initial rapid mixing phase of the well ventilated areas, followed by a slower decline in helium concentration, which may be difficult to distinguish from the constant decline due to helium absorption into the body tissues. If the extrapolation is inadvertently applied in this situation, the lung volume will be underestimated, as those poorly ventilated areas will have been excluded. Mixing may improve when the weight is applied to the bell, particularly if a sigh is induced. In these studies infants were only studied when free from clinical evidence of bronchoconstriction.

We attempted to quantify equilibration times, as a means of determining airway function by gas mixing (Bates 1950, ATS-ERS statement, 1993). It appeared, however, that the equilibration depended to some extent on the physical properties of the spirometer system, for example the pump and the tubing and their equilibration time constant. It was not possible to discriminate precisely enough between individuals because of the constraints of the system. As equilibration times and mixing indices were beyond the scope of the study, they are not discussed any further in this work.
4.4 TIDAL BREATHING MEASUREMENTS

Classical methods for measuring the mechanical properties of the lungs in infants place great demands on both operator and infant. A method which relied on measurements made during tidal breathing in unsedated infants would represent a great advance. One study observed that in adult patients with a variety of lung diseases, that the pattern of tidal airflow during quiet breathing varied according to the nature of the disease (Morris 1981). Little use was made of these observations because there was no adequate physiological explanation and because in adults simple, well validated alternative techniques were available. The potential value of tidal breathing methods was recently shown in an epidemiological study (Martinez 1988 & 1991) looking at the relationship between lung function in early infancy and subsequent pulmonary outcome. In that study, the ratio between the time to maximal tidal expiratory flow and the total expiratory time \( \frac{t_{pef}}{t_e} \) calculated from tidal breathing flow/time curves early in infancy was shown to predict the development of recurrent wheezing over the subsequent three years of life. The feasibility of using body-surface measurements to determine \( t_{pef}/t_e \) has recently been reported in normal infants (Stick 1992). In the present study, as infant lung function was already being measured by conventional methods, there was an ideal opportunity to evaluate the tidal breathing parameter \( t_{pef}/t_e \) as a measure of lung function, by sequential observations over the first year of life, both in health and in disease and in induced bronchoconstriction (chapters 5 and 6).

4.4.1 Equipment for measurement of tidal breathing parameters

All the equipment required for tidal breathing measurements, namely face mask,
pneumotachograph and silicone putty, was described in section 4.2.1.

4.4.2 Respiratory analysis computer software program (RASP)
The flow signal was digitally integrated with respect to time to give volume. All signals were digitised (100Hz) and stored on computer. Several indices of lung function were calculated during a period of quiet regular tidal breathing, using computerized analysis. These were respiratory frequency (f), $t_{pve}/t_e$, as defined above, and the total respiratory cycle time ($t_{tot}$).

4.4.3 Calibration
The pneumotachograph was calibrated for flow and volume, as described in section 4.2.3.

4.4.4 Technique
(a) Preparation of the infant
Once the infant was sleeping quietly, a rim of silicone putty was applied to the face mask and around the infant's mouth and nose, and the face mask and pneumotachograph were applied over the mouth and nose of the supine infant.

(b) Measurement of $t_{pve}/t_e$
A run of ten breaths was recorded during regular tidal breathing. Runs were collected at 30 second intervals. Results were expressed as the mean of 3-8 runs of 5 sequential regular tidal breaths, collected during behavioural quiet sleep, and chosen from the run of ten breaths, as the most representative, by visual inspection of the time-based flow
and volume signals.

(c) Technical quality of studies

The criteria for satisfactory tidal breathing data was that the infant was in quiet sleep, with a stable end expiratory level (see section 4.2.5 (c)), and regular respiratory rate and tidal volume. During the studies, it was possible to assess this by observing superimposed flow-volume loops on the oscilloscope. It was important to avoid leaks around the face mask. These could be detected by a drift in the volume signals.

4.4.5 Reference values of $t_{pef}/t_e$

The values of $t_{pef}/t_e$ during the first year of life in the healthy infants are shown in figure 4.22 and table 4.1. "Healthy" is defined as having one or less episodes of cough and/or wheeze during the first year of life. Measurements were obtained from 22 infants at 1 month, 16 at 6 months and 13 at 12 months. The frequency of breathing fell and $t_{pef}/t_e$ declined significantly ($p<0.005$) during the first six months of life. No significant change took place between 6 and 12 months. This was in contrast to $\dot{V}_{maxFRC}$, which increased progressively through the first year (shown on table 4.1). $t_{pef}$ tended to lengthen over the first year of life, but this only just reached significance by the age of 12 months ($p=0.05$). $t_{pef}/t_e$ correlated poorly with $\dot{V}_{maxFRC}$ over the first year of life ($p=0.06$; figure 4.23). Although $t_{pef}$ was closely related to $t_{tot}$ (figure 4.24) as of course was $t_e$, because the change in $t_{pef}$ with $t_{tot}$ was proportionately smaller than the change in $t_e$, the ratio $t_{pef}/t_e$ was inversely proportional to $t_{tot}$ during the first year (figure 4.25; $p<0.001$, $r=0.53$). Because of the narrow spread of breathing frequencies, within each individual age-band there was no association between $t_{pef}/t_e$.
and breathing frequency. There was no significant gender difference for $t_{p_{EF}}/t_e$ at any age.

4.4.6 Repeatability

The coefficient of repeatability for $t_{p_{EF}}/t_e$ (see 4.2.7 for definition) was similar at all ages (table 4.1). Compared with $\dot{V}_{\text{maxFRC}}$ and FRC the measurement was more variable within subjects, and this may be one reason for the tidal breathing parameter being relatively insensitive at detecting differences in lung function (see chapters 5 and 6).

4.4.7 Discussion

There are no reference values for $t_{p_{EF}}/t_e$, but these results are similar to data from other studies carried out in sedated infants using face mask measurements. The Tucson group report a value of $t_{p_{EF}}/t_e$ (described as $t_{p_{EF}}/t_e$) of $0.31 \pm 0.09$ (mean ± SD) in 88 infants studied within the first 3 months of life, who had no subsequent LRI during the first year of life (Martinez 1988). They found that, over this age range $t_{p_{EF}}/t_e$ decreased linearly with age. Another group measured $t_{p_{EF}}/t_e$ during the first year of life in 40 healthy babies and found the mean $t_{p_{EF}}/t_e$ to be $0.42$ (SD 0.06) in the neonatal period, 0.29 (SD 0.09) at 6 weeks, and 0.27 (SD 0.07) at 1 year, values almost identical to ours (Stocks 1992). In most of the infants $t_{p_{EF}}/t_e$ was stable beyond the neonatal period. By contrast a Norwegian group studied 19 unsedated newborn infants at a mean postnatal age of 2.3 days, both awake and in natural sleep (Lodrup 1992). Their values for $t_{p_{EF}}/t_e$ are much shorter with a mean of 0.20 when asleep and 0.24 when awake. Their subjects, although all healthy term babies, had rapid respiratory rates of about 70/min, raising the question of the influence of the apparatus as well
as sedation and sleep state on the breathing pattern. For example, breathing through a large dead space or against a high resistance, such as may be imposed by a face mask and pneumotachograph, could produce a change in respiratory frequency and tidal volume (Marsh 1993). Somewhat reassuringly a recent study compared measurements of $t_{pav}/t_e$ obtained by uncalibrated respiratory inductance plethysmography (Respitrace) in unsedated healthy newborn infants, with and without a face mask and pneumotachograph (Stick 1992). There was no significant difference in respiratory frequency or $t_{pav}/t_e$. The effect of sedation has not been reported.

In contrast to adult subjects (Morris 1981), in whom there was a good correlation between $FEV_1$ and $t_{pav}/t_e$, in healthy infants the analysis of tidal expiratory flow patterns was not found to be useful for quantifying airway function, when compared to an independent measurement ($V_{maxFRC}$). The factors which determine $t_{pav}$, $t_e$ and their ratio have not been elucidated. If expiration were completely passive, $t_{pav}$, the interval between the onset of expiration and the achievement of the tidal peak expiratory flow velocity, would be determined by the factors which limit convective acceleration of gas at the airway opening. The inertial properties of the chest wall and lungs and the physical properties (inertance) of the gas in the airways may be relevant determinants. It can be predicted from RC circuit theory that in a passive system, the resistance of the circuit, while modifying the peak flow, will not affect the timing of peak flow. The total duration of expiration ($t_e$) in a passive system is determined simply by the timing of the subsequent inspiration.

Even in healthy, quiet, tidal breathing the process of expiration is not passive and
several active processes come into play. Firstly, the decrement of post-diaphragmatic activity will tend to prolong $t_{\text{per}}$. This can be seen by comparing tidal flow/time patterns with the pattern produced after release of a brief end inspiratory occlusion (during which muscle relaxation is assumed to have developed due to invoking the Hering Breuer reflex), when $t_{\text{per}}$ is close to zero (Mortola 1984) and must clearly be virtually independent of lung mechanics, including the state of laryngeal narrowing. Secondly, two important processes which control the end expiratory lung volume are particularly active in early infancy and exert a major influence on $t_{\text{per}}$: laryngeal braking slows down the rate of emptying of the lungs, thereby tending to prolong the duration of expiration, while increased breathing frequency tends to shorten it. Their combination in the neonatal period leads to a dynamic elevation of end expiratory lung volume (Fisher 1982, Mortola 1984, Kosch 1984).

If the major variable determining the duration of $t_{\text{per}}$ is post-diaphragmatic activity, then this observation implies that as breathing frequency increases, the diaphragmatic contraction is more promptly switched off at the end of inspiration. It was found that $t_{\text{per}}$ taken over the whole of the first year of life was dependent on the frequency of breathing, being shorter at faster rates of breathing. However, the relationship between $t_{\text{per}}$ and $t_{\text{s}}$ was not linear (figure 4.24), as was the relationship between $t_{\text{e}}$ and $t_{\text{tot}}$, so that at higher breathing frequencies (shorter values of $t_{\text{tot}}$) the ratio $t_{\text{per}}/t_{\text{s}}$ was greater. The faster breathing frequencies of neonates would then be the explanation for their greater $t_{\text{per}}/t_{\text{s}}$. When comparing $t_{\text{per}}/t_{\text{s}}$ between groups of subjects, frequency of breathing (or $t_{\text{tot}}$) should be taken into account. It is probably more logical to consider $t_{\text{per}}$ and $t_{\text{s}}$ separately, since they represent the outcome of separate
neuromechanical functions and bear different relationships to breathing frequency.

The relationship between \( t_{\text{per}}/t_e \) and \( \dot{V}_{\text{maxFRC}} \) will be examined further in chapters 5 and 6, looking at various disease states and induced bronchoconstriction during bronchial challenge procedures, in order to assess the usefulness of tidal breathing parameters as measures of lung function in infants. In healthy subjects \( t_{\text{per}}/t_e \) did not appear to be a sensitive measure of pulmonary function, and probably represents the dynamic, neuromuscular response of the infant to mechanical constraints, rather than being a direct measure of lung function.
4.5 BRONCHIAL RESPONSIVENESS

The definition of asthma includes variable airway obstruction. During a bronchial challenge test, it is possible to mimic variable airway obstruction in the laboratory, and quantify the subject's airway sensitivity, or bronchial responsiveness. In order to measure bronchial responsiveness (BR), a bronchoconstricting stimulus is given to the subject, the response to that stimulus is measured and the relationship of stimulus to response is calculated for each individual.

Various different stimuli have been used to measure bronchial responsiveness in infants, including methacholine (Tepper 1987), cold dry air (Geller 1988), histamine (LeSouef 1989, Prendiville 1987b) and carbachol (Gutkowski 1990). Two infant lung function techniques have been used to measure the physiological response. The "squeeze" technique has been used to measure changes in $V_{maxFRC}$ (Tepper 1987, Geller 1988, LeSouef 1989, Prendiville 1987b) and plethysmography to measure changes in specific airway resistance (Gutkowski 1990). A simpler technique has been described by one group, who instead of using complex infant lung function tests, simply used a stethoscope. Bronchial responsiveness was measured by determining the concentration of bronchoconstrictor agent at which wheezing was just audible on auscultation of the chest (Avital 1988). Although in groups of older children this technique has been found to correlate well with the other more conventional spirometric measures of bronchial responsiveness, no comparisons have reported in infants. In this present study BR to increasing doses of aerosolised histamine was assessed from PEFV curves, used to calculate $V_{maxFRC}$. Other methods of assessing BR, using tidal breathing parameters and non-invasive measures of oxygenation were
also evaluated and compared with the standard infant lung function parameter $V_{\text{maxFRC}}$ (chapter 6).

The *relationship* between the stimulus and response is usually expressed by the provoking concentration, the concentration of the bronchoconstricting agent which produces a specified change in some measure of lung function. The specified change depends upon which measure of lung function is used, as it is dependent upon its variability. For example, in adults, bronchial responsiveness is commonly expressed as $PC_{20}$, the concentration of bronchoconstrictor causing the one-second forced expiratory volume ($FEV_1$) to fall by 20% (Cockcroft 1977). A 20% fall is chosen as an appropriate change, as it is greater than the random variation for $FEV_1$ in any individual. The $PC_{20}$ is calculated by linear interpolation from a plot of change in $FEV_1$ against concentration of bronchodilator, with the latter plotted on a logarithmic scale. Other methods of analysing bronchoconstrictor dose-response curves, have been described for the measurement of BR. One method, rather than looking at the concentration producing a certain fall from baseline, which may not occur in normal healthy adults, is to measure the slope of the dose response curve (Cockcroft 1983a). In a study involving a small number of adults the dose-response slope, calculated from the slope of a line extending from the origin to the last point obtained, effectively separated asthmatic from non-asthmatic subjects, and avoided censoring of data (O'Connor 1987). A measurement of BR from the slope can be obtained for every individual undergoing bronchial challenge, rather than just the responders (Peat 1992). This is more relevant in epidemiological studies of adults, where normal subjects will tend to be non-responders, as assessed by $PC_{20}$, but is not such an issue in infant
studies, as most infants exhibit a significant bronchoconstrictor response during the course of a challenge test (LeSouef 1989). Dose-response slopes may be calculated by more sophisticated mathematical methods (Chinn 1993, Sherrill 1993). A "least-squares" slope was found to be preferable to the two-point slope, described above, for epidemiological studies, but offered little advantage over the provoking dose or concentration, other than avoiding censored data (Chinn 1993). Another method of quantifying BR is to use the threshold concentration (Cockcroft 1983b). This takes into account each individual's variability for the measurement concerned. The threshold is defined as the first concentration reached during a challenge procedure which causes the lung function to fall more than two standard deviations below the baseline mean for that individual. One problem with this method, is that the standard deviation for a small number of baseline measurements may not accurately reflect the true standard deviation. The other problem is that as the coefficient of variation varies between subjects, so will the threshold, introducing more bias. PC_{20} was reported to be more reproducible and a better discriminator between asthmatic and normal, when compared with threshold concentration, in a study of adults (Cockcroft 1983b).

4.5.1 Nebulisation and histamine challenge

In the present study, an aerosol of histamine was generated continuously by a Wright jet nebuliser (Aerosol Medical, Colchester, Essex). Using an airflow rate of 8 l/min the nebuliser output was 0.16 ml/min by weighing and aerosol aerodynamic mass median diameter was 1.0-1.5 μm (Juniper 1991). The face mask, pneumotachograph removed, acted as a chamber, into which the aerosol was directed over the mouth and nose of the supine, sleeping infant. Each dose of aerosol was administered for 30
seconds from the nebuliser, which contained 4 ml of solution, with 8 l/min airflow. Histamine solutions were made up by serially diluting the concentrated histamine solution (32mg/ml) with 0.9% saline, a fresh set of solutions being made up for each infant study. After baseline lung function measurements ($V_{maxFRC}$, tidal breathing parameters and FRC), a control aerosol of normal saline was given, followed by doubling concentrations of histamine administered at 5 minute intervals. Commencing with a histamine concentration of 0.25 g/l, the challenge continued until either a 30% fall from baseline $V_{maxFRC}$ had been observed, or the maximum histamine concentration of 32 g/l was reached. Beginning one minute after completion of each nebulisation, six to eight squeeze manoeuvres were then carried out. All technically satisfactory PEFV curves were analysed to give a mean $V_{maxFRC}$ for each dose (see section 4.2.5). The same jacket pressure was used throughout the challenge procedure, after determining the optimal $Pj$ during baseline measurements of $V_{maxFRC}$. The tidal breaths preceding the squeeze were also analysed and the tidal breathing parameter $t_{pef}/t_e$ calculated for each dose (see section 4.4.4). Immediately after completing the histamine challenge and measuring $V_{maxFRC}$, one measurement of lung volume was carried out using the helium dilution technique (see section 4.3.4).

As a safety measure, and also to compare changes in oxygenation during bronchial challenge with changes in pulmonary function, oxygen saturation and transcutaneous oxygen and carbon dioxide tensions were monitored continuously (see section 4.2.4).

4.5.2 Analysis

A computer program was used to calculate $PC_{30}$, the provoking concentration of
histamine producing a 30% fall in $\dot{V}_{\text{maxFRC}}$ from baseline, by linear interpolation from dose-response plots of $\dot{V}_{\text{maxFRC}}$ against log histamine concentration for each subject (Prendiville 1987b; figure 4.26). A 30% fall in $\dot{V}_{\text{maxFRC}}$ was chosen, as this was greater than twice the coefficient of repeatability for $\dot{V}_{\text{maxFRC}}$ (see section 4.2.7). $PC_{30}$ was calculated from the baseline values of $\dot{V}_{\text{maxFRC}}$ rather than the post saline values, as infants showed a variable response to saline - some had no change in $\dot{V}_{\text{maxFRC}}$ from baseline, some showed increased and some decreased $\dot{V}_{\text{maxFRC}}$ after saline. No correction was applied to account for air entrainment (Collis 1990), as for each comparison the subjects were all similar in age and size.

4.5.3 Discussion

As discussed in section 4.2.8, there continues to be debate as to whether or not true maximal flow at functional residual capacity is achieved in healthy infants. If infants achieve flow limitation when bronchoconstricted, for example, during a response to histamine, but are not flow limited during baseline $\dot{V}_{\text{maxFRC}}$ measurements, then BR would be underestimated, with a spuriously high $PC_{30}$. However, recent evidence suggests that this problem does not arise as flow limitation can be achieved in healthy infants during the "squeeze" technique (Feher 1994).

In addition to the problem of flow limitation, the squeeze does not take into account changes in functional residual capacity occurring during bronchial challenge. Indeed the measurement of lung function using PEFV curves and $\dot{V}_{\text{maxFRC}}$ depends upon the stability of the end expiratory level or FRC, which is used as its reference point, for its accuracy. Assessing bronchodilatation and bronchoconstriction using the squeeze
technique can be misleading. If hyperinflation (an increase in FRC) occurs during
histamine induced bronchoconstriction, the response to histamine, as assessed by
changes in $\dot{V}_{\text{max,FRC}}$, will tend to be underestimated. An increase in FRC has been
reported during exacerbations of asthma (Woolcock 1966), and during bronchial
challenge testing in adults (Chadha 1984, Lennox 1985). In a small study of
recurrently wheezy infants undergoing histamine challenge, respiratory inductance
plethysmography (RIP) was used to estimate changes in FRC (Maxwell 1988). Below
the PC$_{30}$, only small increases in FRC were noted, but at PC$_{30}$ larger increases were
detected. These findings are consistent with a study of a group of healthy and
symptomatic adults challenged with methacholine (Pellegrino 1993). They found that
hyperinflation, detected by an increase in total lung capacity, only occurred when the
partial forced expiratory flow curve recorded after methacholine challenge impinged
on the tidal expiratory flow curve recorded before challenge, that is, near flow
limitation. One problem of measuring lung volume by gas dilution, so that maximal
expiratory flow can be referred to absolute FRC, is that due to time constraints
imposed by gas mixing and equilibration, it is not possible to track FRC during
challenge and simultaneously measure forced expiratory flow. In addition, gas
dilution techniques may underestimate FRC in the presence of airflow obstruction, due
to slow and incomplete mixing of the indicator gas within poorly ventilated areas of
lung after induced obstruction, as discussed earlier. Any increases in FRC after
histamine challenge are therefore likely to be real, and this causes problems in
interpreting BR using PEFV curves. Other techniques of measuring lung volumes, for
example thoracic gas volume (TGV) by plethysmography, are equally time consuming
and cumbersome. Potential problems are introduced into both of these methods by the
presence of bronchoconstriction. In contrast to gas dilution techniques, TGV measured by plethysmography, is likely to be overestimated in the presence of airflow obstruction, as mouth pressure is likely to be less than airway pressure, due to failure of equilibration during the occlusion in bronchoconstricted individuals (Godfrey 1986 & 1991).

FRC may also change as a result of the imposed dead space of the equipment. LeSouef (1988) demonstrated a gradual increase in dynamic FRC as a consequence of introducing a dead space (that of the face mask and pneumotachograph), and this can influence the measurement of FRC. As the infant's lung function changes during bronchial challenge, it is likely that the dead space of the measuring apparatus, which remains constant during a study, may have different effects on FRC, depending on the degree of bronchoconstriction.

In contrast to bronchial challenge in older children and adults, infants appear to breathe through their noses rather than their mouths during bronchial challenge. The nose will act as a filter, preventing some of the aerosol reaching the lungs (Salmon 1990). The histamine aerosol deposited in the nose may exert local effects, by stimulating nasal histamine receptors. A recent study compared changes in PEFV curves produced by aerosol inhalation of methacholine with the changes produced by instilling equivalent doses of methacholine liquid into the nares of healthy infants (Tepper 1993b). They found that the changes in the PEFV curve following nasal instillation of methacholine were consistent with an increase in nasal resistance (a decrease in peak flow and flattening of the PEFV curves at higher lung volumes), and
no change in flow around FRC, implying no change in lower airway function. Histamine may have a greater effect on nasal resistance by venodilatation.

Body posture also has an influence on the measurement of BR. All the infants were supine during the present study. A study in adults, looking at the effect of body posture on methacholine responsiveness in nine healthy volunteers and one mild asthmatic (Shardonofsky 1992), showed significantly increased responsiveness in the supine compared with the sitting posture using indices of lung function derived from complete forced expiratory flow-volume curves. When using indices from partial flow-volume curves the difference was not significant. There was little change in baseline measurements on changing position from sitting to supine. These findings suggest that BR measurements are influenced by the interdependence of airway and lung parenchyma. In this infant study, the dose and distribution of the histamine aerosol is likely to be influenced by body position.

Most infants will respond to histamine challenge with a 30% fall in lung function, showing that all have a degree of airway reactivity. There are no "reference" values for PC_{30} in infants, as it would appear that the normal distribution of PC_{30} varies according to the size, and therefore the age, of the infant (Montgomery 1990, LeSouef 1989 & 1992). This is in contrast to the somewhat simplistic approach of some adult studies of BR, in which adults have been described as being hyperresponsive or not (Cockcroft 1977 & 1992). This approach, in which a PC_{20} of 8 mg/ml is chosen as the level of BR which discriminates between hyperresponsiveness and normal, implies that BR is an all or nothing phenomenon, rather than the true situation in which BR
is log-normally distribution across the adult population (Cockcroft 1983a).
Table 4.1  Tidal breathing parameters for healthy infants during the first year of life: median or mean* values (95% confidence interval of median or mean)

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>age (months)*</td>
<td>0.9</td>
<td>6.6</td>
<td>12.6</td>
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<tr>
<td></td>
<td>(0.8-1.1)</td>
<td>(6.3-7.0)</td>
<td>(12.2-13.1)</td>
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<tr>
<td>length (cm)*</td>
<td>54.4</td>
<td>68.7</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>(53.0-55.6)</td>
<td>(67.4-70.1)</td>
<td>(73.7-77.5)</td>
</tr>
<tr>
<td>f (min⁻¹)</td>
<td>62</td>
<td>35**</td>
<td>29**</td>
</tr>
<tr>
<td></td>
<td>(51-67)</td>
<td>(33-41)</td>
<td>(27-43)</td>
</tr>
<tr>
<td>tₚₑᶠ (s)</td>
<td>0.22</td>
<td>0.28</td>
<td>0.28*</td>
</tr>
<tr>
<td></td>
<td>(0.18-0.26)</td>
<td>(0.24-0.31)</td>
<td>(0.26-0.39)</td>
</tr>
<tr>
<td>tₑ (s)</td>
<td>0.55</td>
<td>1.00**</td>
<td>1.20**</td>
</tr>
<tr>
<td></td>
<td>(0.47-0.71)</td>
<td>(0.87-1.14)</td>
<td>(0.81-1.37)</td>
</tr>
<tr>
<td>tₚₑᶠ/tₑ</td>
<td>0.38</td>
<td>0.28**</td>
<td>0.29**</td>
</tr>
<tr>
<td></td>
<td>(0.36-0.43)</td>
<td>(0.26-0.33)</td>
<td>(0.24-0.33)</td>
</tr>
<tr>
<td>CR (%)</td>
<td>14.5</td>
<td>16.7</td>
<td>16.0</td>
</tr>
<tr>
<td>VₑᶠmaxFRC (ml.s⁻¹)</td>
<td>133</td>
<td>200***</td>
<td>331**</td>
</tr>
<tr>
<td></td>
<td>(99-155)</td>
<td>(159-368)</td>
<td>(282-414)</td>
</tr>
</tbody>
</table>

Compared with visit 1 by Wilcoxon matched pairs signed rank test; only paired data were analysed: *p=0.05; **p<0.005; ***p<0.001

CR Coefficient of repeatability of tₚₑᶠ/tₑ (%)
Figure 4.1a Equipment for measuring partial forced expiratory flow volume loops

- Face mask and pneumotachograph
- Pressure port
- Large-bore 3-way tap
- Mechanical, pressure relief valve
- Compressed air 20 l.min⁻¹
- 200l tank

Pressure jacket (inflatable section shaded)
Figure 4.1b Equipment for measuring partial forced expiratory flow volume loops
Figure 4.2 Pressure-flow curve for pneumotachograph

(Hammersmith Hospital low resistance screen pneumotachograph)
Figure 4.3  Pneumotachograph flow calibration

Pneumotachograph → Pressure transducer → Amplifier → Computer

Rotameter → Air
Figure 4.4 Calibration curve of pneumotachograph

(Fleisch size 1 pneumotachograph)
Figure 4.5 Calibration graph for jacket pressure transducer

Pressure transducer output (volts)

Pressure (cmH$_2$O)
Figure 4.6 Measurement of maximal flow at FRC ($\dot{V}_{\text{maxFRC}}$) and jacket pressure ($P_j$) from a flow-volume manoeuvre in an infant aged 6 months.
Figure 4.7 Partial forced expiratory flow-volume curve and the preceding tidal breath from a healthy 6 month infant
Figure 4.8 Flow volume curves illustrating adverse reaction by infants

a) glottic closure

b) irregular breathing pattern
Figure 4.9 $V_{\text{max FRC}}$ against length for healthy infants aged 1, 6 & 12 months

includes multiple measurements from 16 infants
Figure 4.10a $\dot{V}_{\text{max FRC}}$ against length for healthy boys aged 1, 6 & 12 months

Figure 4.10b $\dot{V}_{\text{max FRC}}$ against length for healthy girls aged 1, 6 & 12 months

includes multiple measurements from 16 infants
Figure 4.11 $\dot{V}_{\text{max, FRC}}$ against length for healthy infants aged 1 & 6 months

includes multiple measurements from 16 infants
Figure 4.12a: $V_{\text{max}_{\text{FRC}}}$ against length for healthy boys aged 1 & 6 months

Figure 4.12b: $V_{\text{max}_{\text{FRC}}}$ against length for healthy girls aged 1 & 6 months

includes multiple measurements from 16 infants
Figure 4.13 Reference values for $\dot{V}_{\text{max}}_{\text{FRC}}$ during the first year of life

- Tepper (1986)
- Hanrahan (1990)
- Tepper (1993)
- Clarke (current study)

Current study includes multiple measurements
Figure 4.14a  Equipment for measuring FRC by helium dilution
Figure 4.14b  Equipment for measuring FRC by helium dilution
Figure 4.15 Helium dilution graph for calculating spirometer dead space

Volume added = \(-594 + \frac{5487}{[\text{He}]}\)

Spirometer dead space = 594ml

\[1/\%[\text{He}]\] = reciprocal of % helium concentration
Extrapolation of helium dilution graph to determine the final helium concentration ($He_e$)

$He_i = 9.68$

$He_e = 7.52$
Figure 4.17 Leak detection in spirometer circuit using a weight on the bell

![Graph showing helium concentration, tidal volume, and weight changes over time.](image-url)
Figure 4.18  Leak in spirometer circuit during FRC measurement
Figure 4.19 Unstable end expiratory level during FRC measurement
Figure 4.20 FRC against length for healthy infants aged 1, 6 & 12 months

includes multiple measurements from 11 infants
Figure 4.21 Reference values for FRC during the first year of life

- Tepper (1986)
- Hanrahan (1990)
- Tepper (1993)
- Clarke (current study)

Current study includes multiple measurements
Figure 4.22 $t_{\text{per}} / t_{\text{e}}$ for healthy infants during the first year of life

![Graph showing $t_{\text{per}} / t_{\text{e}}$ vs. age (months) with multiple data points for 16 infants.]

Includes multiple measurements from 16 infants.
Figure 4.23 $t_{pef}/t_e$ against $\dot{V}_{max_{FRC}}$ for healthy infants aged 1, 6 & 12 months

includes multiple measurements from 16 infants
Figure 4.24 \( t_{\text{pef}} \) against \( t_{\text{tot}} \) for healthy infants aged 1, 6 & 12 months

\[ t_{\text{pef}} \, \text{(sec)} \]

\[ (\log \text{scale}) \]

\[ t_{\text{tot}} \, \text{(sec)} \]

Includes multiple measurements from 16 infants
Figure 4.25 $t_{pef}/t_e$ against $t_{tot}$ for healthy infants aged 1, 6 & 12 months

includes multiple measurements from 16 infants
Figure 4.26  Histamine dose response curve

$PC_{30} = 5.8\text{g/l}$
CHAPTER 5 BRONCHIAL RESPONSIVENESS AND LUNG FUNCTION RELATED TO LOWER RESPIRATORY SYMPTOMS

5.1 THE RELATIONSHIP BETWEEN BRONCHIAL RESPONSIVENESS AND LUNG FUNCTION AT 1 MONTH OF AGE AND SUBSEQUENT LOWER RESPIRATORY SYMPTOMS

5.1.1 Introduction

In groups of school children and adults, asthma is associated with increased BR (Cockcroft 1977, Juniper 1981, Woolcock 1987, Peat 1989), although in individuals, variations in BR correlate poorly with the clinical manifestations of asthma (Josephs 1989, 1990, Pattemore 1990) and with airway calibre at the time of testing (Silverman 1972, Wilson 1984). However, the relationship between BR and wheezing in infancy ("infantile asthma") may differ from the situation in older subjects, raising questions about the nature of wheeze and its relationship to asthma in this age group. A large population-based study from Perth, Australia, showed that increased BR in the neonatal period was associated with maternal smoking during pregnancy and parental atopy (Young 1991), but the relationship of neonatal BR to subsequent symptoms has not yet been described in that cohort.

The only physiological predictor of wheeze in infancy is altered neonatal or early infant, premorbid lung function (Martinez 1988 & 1991, Hanrahan 1992, Tager 1993). Whether associated with fetal cigarette smoke exposure (Hanrahan 1992, Tager 1993) or for other as yet unidentified reasons, reduced airway function or altered pattern of breathing were risk factors for recurrent wheezing during infancy or early childhood.

The aim of this study was to test the hypothesis that neonatal lung function and BR predict the subsequent occurrence of recurrent LRI over the first year of life.

5.1.2 Methods

(a) Subjects

The subjects for this study comprised the 54 cohort subjects, together with the nineteen infants recruited in the pilot study, described in chapter 2. Lung function and BR were measured at about 1 month of age, with questionnaire administered at 1, 6 and 12 months of age (see chapters 3 and 4). After the 12 month visit infants were classified according to the presence or absence during the first year of life of two or more episodes of cough or wheezing, or both, associated with an obvious upper respiratory infection (henceforth referred to as lower respiratory illness, LRI). All the physiological data on which this study is based, were collected at the initial study visit at the age of about 1 month, prior to any respiratory symptoms or signs of infection. At the time, no infant had received any medication. Infants were all studied supine and sedated.
(b) *Lung function and histamine challenge*

Measurements of baseline $V_{\text{maxFRC}}$, $t_{p/\text{t}p}$, FRC, $\text{SaO}_2$, and $P_{\text{a}O}_2$ were made before infants underwent histamine challenge to determine $PC_{30}$ (see chapter 4).

(c) *Analysis*

The values of age, length, weight and FRC of the infants with and without LRI were compared using Student's $t$ tests. The gender distribution, ethnic origin and history of maternal smoking in the two groups were compared using a Chi squared test with Yates' correction. $V_{\text{maxFRC}}$ was adjusted to a length of 53cm using the regression equations derived from the healthy subjects in the cohort over the first 6 months of life. For normal male subjects $V_{\text{maxFRC}}$ (ml/s) = 6.97 (length in cm) - 244, and for females $V_{\text{maxFRC}}$ (ml/s) = 8.21 (length in cm) - 278. For female subjects, length-adjusted $V_{\text{maxFRC}}$ was further adjusted for gender from the regression equations (by multiplying by 0.798), in order to group male and female subjects together for comparison. The $V_{\text{maxFRC}}$ data were skewed, and the $PC_{30}$ data censored, so they were compared using the Mann-Whitney U test. Values were expressed as means or medians, with 95% confidence intervals (CI).

5.1.3 Results

(a) *Symptoms*

There were similar numbers of boys and girls in the study (36 and 37 respectively), and birth dates were evenly spread throughout the year. By the age of 1 year, 16 boys and 22 girls had experienced two or more episodes of cough and/or wheezing, of whom twelve boys and eleven girls had wheezed, while the others had only coughed.
Four of the symptomatic infants (3 boys and 1 girl) had intercurrent symptoms of both cough and wheezing, whereas the other infants with LRI only had symptoms during viral upper respiratory tract infections. The values of age, length and weight of the infants in the LRI group were similar to the healthy group (table 5.1). Although proportionally there were twice as many smokers in the symptomatic group compared with the control group, this did not reach statistical significance, possibly because of the small numbers of subjects.

(b) Lung function

For male and female infants grouped together (table 5.2, figure 5.1) there was no significant difference in length- and gender- adjusted $V_{maxFRC}$ before challenge, but when the infants were separated according to gender, symptomatic boys had a significantly lower $V_{maxFRC}$ (table 5.3; figure 5.2). The median difference in length-adjusted $V_{maxFRC}$ in boys was 36.2ml/s (95% CI of the difference 0.7 to 68.6ml/s; p=0.04). When the analysis was repeated, excluding data from the four boys in the LRI group who had coughed but not wheezed, the difference was more marked. The median $V_{maxFRC}$ for the twelve wheezy boys was 60.8ml/s (95% CI 25.4 to 93.6ml/s), with a median difference from healthy infants of 42.0ml/s (95% CI of the difference 8.1 to 79.4 ml/s; p=0.02). This difference was not apparent in girls (table 5.3), nor in boys and girls combined, and analysed according to symptom groups.

For all infants grouped together and for boys alone, there was no significant difference in the tidal breathing parameter $t_{pef}/t_e$ between healthy and symptomatic individuals, but symptomatic girls had significantly longer $t_{pef}/t_e$ than healthy girls (tables 5.2 and
5.3), the mean difference being 0.09 (95% CI of the difference 0.03 to 0.14; p=0.006).

Tidal volumes, corrected for weight, were similar for all groups (tables 5.2 and 5.3).

There was no difference in FRC between healthy and symptomatic infants (table 5.2 and 5.3), but few infants had FRC measurements. Despite the small numbers of FRC measurements, the overall coefficient of repeatability for all infants combined was 8.4%.

(c) Bronchial responsiveness

Eleven infants awoke before completing the histamine challenge, leaving a total of 62, of whom 34 had recurrent LRI. For male and female infants combined, PC\textsubscript{30} did not differ between healthy and symptomatic infants (table 5.2; figure 5.3). When boys and girls were considered separately, PC\textsubscript{30} for symptomatic girls was more than two doubling concentrations lower than for healthy girls. No difference was apparent for the boys (table 5.2; figure 5.4). When the girls with LRI were divided into two groups, 10 who had cough alone, and 10 who had wheeze with or without cough, the median values of PC\textsubscript{30} were 4.3 and 1.4 g/l respectively (95% CI 1.0 to 12.5 and 0.8 to 7.2 respectively). Although not different from each other (p=0.45), both were significantly less than the median value of PC\textsubscript{30} of 11.2g/l (95% CI 6.5 to 29.5) for healthy girls (p=0.05 and p=0.01 respectively).

The data were re-analysed, to look at the strength of the relationship between increased levels of BR and the number of symptomatic episodes, by defining LRI in two different ways. PC\textsubscript{30} was lower in the 21 girls who had one or more episodes of
cough and/or wheeze compared with the eleven asymptomatic girls, the median values of \( PC_{30} \) being 2.6g/l and 13.6g/l respectively (\( p=0.008 \)). When the groups were divided into those who had three or more episodes of cough and/or wheezing and those with two or less episodes, the differences in BR were more marked, with median values of \( PC_{30} \) of 1.6g/l and 12.8g/l respectively (\( p=0.0007 \)). For girls and boys combined together, differences in BR only became significant in comparing the 17 infants who had three or more episodes with the 56 infants who had two or less episodes, median values of \( PC_{30} \) 2.6g/l and 8.5g/l respectively (\( p=0.04 \)). The data were also analysed to look at the relationship between increased levels of BR and wheezy LRI. \( PC_{30} \) was more than two doubling concentrations lower in the 11 girls who had one or more episodes of wheeze (with or without coughing) compared with the 26 other girls, the median values of \( PC_{30} \) being 1.4g/l and 8.3g/l respectively (\( p=0.05 \)). There was no significant difference in BR between healthy and symptomatic boys for any definition of LRI.

5.1.4 Discussion

In this study of the relationship between initial lung function and BR, and subsequent outcome, differences in neonatal lung function and BR were observed between asymptomatic infants and those who had two or more episodes of LRI during the first year of life. For male infants, premorbid lung function measured by \( V_{\text{max,FRC}} \) was reduced but BR was no greater in those who subsequently developed LRI, whether wheezy or not. For females the situation was the opposite: BR was very significantly increased in the female infants who subsequently developed symptoms, but baseline airway function was not impaired. The degree of responsiveness of the girls who
subsequently wheezed was particularly striking. There are some similarities between this study and other published data (Martinez 1988, Tager 1993) in relation to initial lung function, but the observations concerning BR are novel, suggesting for the first time the possibility of a developmental basis for bronchial responsiveness which is independent of lung function and which predicts subsequent wheeze. The differences between boys and girls was particularly striking.

(a) Study population and symptoms

Ethical constraints prevented a truly random population being recruited, so that in this study, infants who had at least one atopic parent were recruited, hoping to increase the chance of subsequent symptoms. Neither the subjects, nor their parents, were tested for atopy, which might have influenced BR (Young, 1991). A difference in the incidence of atopy between the sexes could have accounted for the differences in BR between the two groups. Infants were studied early in life, in order to minimize aeroallergen sensitization and environmental smoke exposure prior to study, although some had been exposed to maternal smoking during fetal life. Cotinine levels were not measured in either the infants or their mothers, and questionnaires were relied on for details of smoking history, so the numbers of smokers may have been underestimated. The small numbers of maternal smokers in this study did not allow the effect of smoking on lung function or BR to be assessed. Previous studies suggest a possible effect on both forced expiratory flows (Hanrahan, 1992) and BR (Young 1991). The distribution of the smokers was weighted towards symptomatic groups in this present study (table 5.1) and could potentially have influenced the initial lung function results. Only a very large study could determine the contribution of smoking
to the relationship between neonatal lung function and subsequent symptoms (Tager, 1993).

Initially LRI was defined as at least two episodes of cough and/or wheeze. Other studies have used different definitions of LRI (Martinez 1988 & 1991, Young, 1991), and found various differences in lung function between healthy and LRI infants (table 5.4). In this present study approximately half of the infants had LRI, defined as *two or more* episodes, whereas in the Tucson study (Martinez 1988) only 29% of their study population had *one or more* episodes of LRI, compared with 61% of the infants studied in Boston (Tager, 1993). One explanation for this apparent discrepancy between these studies could be that in Tucson LRI was diagnosed by a paediatrician, whereas in the present study and that of Tager, the LRI was diagnosed from parental questionnaire. There are likely to be differences in the epidemiology of early childhood respiratory illness between the open, semi-desert Tucson environment and the moist, temperate urban climate of Boston or London. In the Tucson study, Martinez has shown differences between neonatal lung function in those infants who have only one episode of LRI in the first year of life, and those who have either persistent symptoms or late onset symptoms (Martinez, 1991). In redefining LRI (according to the number of episodes) for further analysis of the current data, no further differences in baseline lung function were demonstrated, but there was consistently an association between neonatal BR and subsequent symptoms in the first year of life, which was even more striking in those with multiple episodes. As there were very few infants who wheezed without coughing, it was not possible to analyse the data looking at wheeze independently.
In this study, in contrast to the two studies previously mentioned using different definitions of LRI, more girls than boys had symptoms (44% of boys compared with 59% of girls), but the population was not random.

(b) Lung function

All infants were sedated for this study. The nature of the lung function measurements, which included histamine challenge, meant that the measurements took up to two hours, which without sedation would have been virtually impossible. Even with sedation eleven infants awoke before completing the histamine challenge. Turner and colleagues have looked at the effects of sedation on infant lung function. Other than a slight fall in tidal volume under sedation, they found no other significant difference between measurements made during natural sleep and under sedation with chloral hydrate (Turner, 1990). Another study looking at the effect of sedation on arterial oxygen saturation in infants undergoing pulmonary function testing, suggested that wheezy infants with low baseline oxygen saturation were susceptible to central respiratory depression after 70-100mg/kg chloral hydrate, but did not show any similar effects on infants with clinical stable cystic fibrosis (Mallol, 1988). By monitoring continuously, no clinically significant hypoxaemia was allowed to occur, except transiently during bronchial challenge (see chapter 6.3). At the time of testing, no infant had a history of LRI. Baseline saturations and transcutaneous oxygen and carbon dioxide tensions were similar in those with and without subsequent LRI, so that the subject conditions during testing were unlikely to have biased the results.

Interest in tidal breathing measurements has been stimulated, as a result of the Tucson
study (Martinez, 1988), in which low $t_{pef}/t_e$ ($t_{me}/t_e$ in their abbreviation), predicted subsequent LRI. The sensitivity of the tidal breathing parameter $t_{pef}/t_e$ as a measure of lung function in infants, compared with $V_{maxFRC}$, is examined in chapters 6.1 and 6.2. The finding of a significantly longer $t_{pef}/t_e$ in symptomatic girls in this study was surprising, particularly as in boys no differences in $t_{pef}/t_e$ between healthy infants and those who subsequently developed LRI were detected. In the Tucson study, $t_{pef}/t_e$ was lower in recurrently symptomatic infants (Martinez, 1991), although when looking at males and females separately, only boys with symptoms during the first year of life had significantly lower $t_{pef}/t_e$; in symptomatic girls there was a trend for $t_{pef}/t_e$ to be longer (Martinez, 1988), as in this study. The factors which determine $t_{pef}/t_e$ have not been fully established, as discussed in chapter 4. Tidal volume measurements were not useful in discriminating between infants who remained healthy and those who subsequent had recurrent LRI.

Martinez and colleagues reported that infants with LRI during the first year of life had lower conductance in addition to $t_{pef}/t_e$ in early infancy (Martinez 1988). The effect on $t_{pef}/t_e$ was apparent in males, but not females, whereas FRC was reduced in their subsequently symptomatic female infants, but not in males. They did not show any significant difference in premorbid $V_{maxFRC}$ in the symptomatic infants. However, in subsequent follow-up, initial $V_{maxFRC}$ in those infants who had one or more episodes of wheezing LRI in the first year and at least one other LRI during follow-up, was lower than in infants with no LRI in the first three years of life, or only one LRI confined to the first year, with none subsequently (Martinez 1991).
Tager and colleagues (1993), in a study similar in size to this, found lower pre-illness $\dot{V}_{\text{maxFRC}}$ in infants who developed an LRI during the first year of life, compared with controls. In contrast to the present study these differences in $\dot{V}_{\text{maxFRC}}$ were not confined to males, rather, they found the relationship of antecedent lung function to symptoms to be more pronounced in females. As in the present study, they did not find any difference in FRC between the LRI and normal infants, although the power of the present study was reduced by the small numbers of infants who had FRC measured.

The different results of these cohort studies are probably due to differences in methods, bias in population selection, small study size and differences in definition of LRI. The fact that each study has found that neonatal lung function predicts subsequent symptoms, implies that real risk factors do exist.

A study looking at the effects of antenatal smoking on lung function in the newborn period (Hanrahan 1992), showed reduced levels of $\dot{V}_{\text{maxFRC}}$ in infants born to smoking mothers, without a change in FRC. This would imply that antenatal smoking has somehow either impaired airway growth, or has reduced lung elastic recoil, hence reducing forced expiratory flow. Studies in older children, looking at the effects of passive smoking on lung function, have shown more marked reductions in lung function in boys compared with girls (Murray 1989, Lebowitz 1992, Sherrill 1992, Rona 1993).

Retrospectively, this study design enabled a difference of 35ml/s in length and gender-
adjusted $\dot{V}_{\text{maxFRC}}$ to be detected between the healthy and symptomatic groups, with a power of greater than 80% at a significance level of 5% (Altman 1982). When boys and girls were analysed in separate groups, the power fell to 40% and 20% respectively for $\dot{V}_{\text{maxFRC}}$. In order to achieve an 80% power of detecting the same differences at a 5% significance level, analysing boys and girls separately, it would have needed 35 subjects in each group for $\dot{V}_{\text{maxFRC}}$ analysis i.e. a minimum study group size of 140 children. The small sample size, when analysing boys and girls separately could, therefore, have introduced a type II error into this study, resulting in failure to detect a real difference. This study has important implications for the sample size in future studies of this type. The power to detect differences in FRC between the groups was very low, as only a few of the infants had such measurements.

(c) Bronchial responsiveness

There continues to be debate about the validity of the squeeze technique, as discussed in chapter 4. A recent study looking at healthy and asthmatic adults found that hyperinflation in response to methacholine challenge only occurred when partial forced expiratory flow began to impinge on tidal flow, i.e. at flow limitation (Pellegrino 1993). Forced expiratory flow was lower in subsequently symptomatic males, so by virtue of their relative degree of airway narrowing, their response to histamine-induced bronchial challenge, might be to increase their FRC. Female infants, by contrast, would have more reserve so that airway narrowing could take place in response to histamine challenge before they started to hyperinflate or increase their FRC. Hence a difference between males and females in their FRC response to challenge, could have masked a true difference in PC$_{30}$ between symptomatic males and controls,
secondary to differences in airway size. There would still be a contrast between males and females, since baseline \( \dot{V}_{\text{max FRC}} \) for healthy and symptomatic girls was equivalent.

The levels of BR to histamine which were found in this study were lower (that is \( PC_{30} \) was higher) than those reported in a similar study in Australia (Young 1991) despite the 40% change in \( \dot{V}_{\text{max FRC}} \) used by the Australian group as a response threshold. Differences in methodology, especially the duration of histamine nebulisation and in the type of jet nebuliser used may be the explanation. In that study, which looked at factors which influence BR in early infancy, infants with a family history of asthma or parental smoking were found to have a higher level of BR at one month of age, but baseline lung function assessed by \( \dot{V}_{\text{max FRC}} \) was similar in all groups.

The difference in BR between the healthy and LRI girls is very striking. This cannot be accounted for by a difference in dose of aerosol to the lungs (Collis 1990, LeSouef 1992), as the absolute tidal volumes and respiratory frequency of the healthy and LRI infants were similar, and there was no significant relationship between absolute tidal volume and \( PC_{30} \). The relationship between BR and LRI appears to be "dose dependent", i.e. the more symptomatic episodes, the greater the BR. It is likely that these differences in \( PC_{30} \) represent real differences in BR to histamine. This raises questions concerning the determinants of histamine responsiveness. In addition to histamine acting directly on the bronchial smooth muscle, other mechanisms may be involved in determining non-specific BR, such as neural control of the airways, muscle tone and upper airway or nasal reflexes.
Retrospectively, this study design enabled a difference of 2 doubling concentrations in PC₃₀ to be detected between the healthy and symptomatic groups, with a power of greater than 80% at a significance level of 5%. However, when boys and girls were separated into different groups for analysis, the power fell to 45% and 60% respectively for PC₃₀. In order to achieve the same 80% power at a 5% significance level, a total study group of 50 girls and 50 boys would have been required for PC₃₀ analysis, ie. a minimum study group size of 100 children, assuming similar proportions of symptomatic and healthy infants.

Both neonatal lung function and bronchial responsiveness appear to be risk factors for determining lower respiratory tract symptoms in the first year of life. Gender related differences are apparent. Forced expiratory flow, determined both by airway dynamics and driving pressure, ie. airway calibre and elastic recoil, is important in boys, whereas in girls bronchial responsiveness, rather than airway function, is an important determinant of subsequent LRI in the first year.

(d) Implications

These findings, which showed that neonatal lung function in boys and BR in girls are predictors of recurrent LRI during the first year of life, have important implications. The changes in lung function and BR were apparent at such an early stage in postnatal life that it is unlikely that factors such as inhaled aeroallergens would have had any influence on lung development. The changes were apparent prior to any viral infections, so viral infections do not appear to be the cause of the impaired lung function in boys and increased BR in girls. However, most episodes of wheezing
were associated with viral upper respiratory tract infections, implying that impaired lung function may predispose infants to increased clinical manifestations of viral respiratory infections. The reasons for this are unclear. One group has demonstrated specific IgE to respiratory syncytial virus (RSV-IgE) to be a risk factor for continuing episodic wheeze (Welliver 1986), although the production of RSV-IgE was unrelated both to atopy, measured by skin-prick tests, and bronchial responsiveness in childhood (Welliver 1993). This implies that the recurrent wheezing was not associated with or due to atopy, in contrast to asthma in older children. Indeed, viral infections might offer protection against allergic sensitization of the airways, a hypothesis supported by studies of families in which children with older siblings, and therefore with increased exposure to viruses, have less atopic disease than children of higher birth order (Strachan 1989). There may be other genetic reasons, apart from atopy. The Tucson epidemiological study of childhood respiratory illnesses found that a parental history of respiratory illness in infancy or early childhood, was a risk factor for wheezing LRI (Camilli 1993). This association was present after trying to control for ascertainment bias, and suggests that LRI may have a familial component, and therefore a hereditary basis. It is difficult to distinguish shared environmental factors from the genetic influence in family studies, but a study of adult monozygotic and dizygotic twins, suggested that after adjusting for anthropomorphic differences, much of the measured variability in lung function may be accounted for by genetic influences, thus confirming the importance of genetic factors in the determination of lung function (Redline 1987). The gender related differences found in the present study could be due to hormonal influences on antenatal lung development.
Continued follow-up of these infants, including subsequent testing for atopy, may provide further information about the nature of infant LRI and its relationship to lung function and BR.
5.2 BRONCHIAL RESPONSIVENESS AND LUNG FUNCTION AT 6 MONTHS AND RELATIONSHIP TO LOWER RESPIRATORY SYMPTOMS

5.2.1 Introduction

In a small study from Australia of recurrently wheezy infants, it was shown that, in contrast to older subjects, BR in infancy was independent of the symptom of wheezing, but may be dependent on airway calibre (Stick 1991). The study described in section 5.1 showed gender related differences in lung function and BR, which were associated with infant LRI. By further studying the infants at the age of six months, it was possible to determine whether the gender related neonatal differences in lung function and BR which were risk factors for subsequent LRI (see section 5.1), persist into infancy. Were the differences to become more apparent, by the age of six months, when some infants will have experienced LRI, environmental factors, such as aeroallergen exposure, passive smoking, virus exposure and infection, would be implicated as having significant influences on lung development and function. Alternatively, if BR were found to be unrelated to infant LRI, it would imply that the mechanisms for wheezing LRI and BR in infants are different from the mechanisms of wheezing and asthma in older children and adults.

As discussed in section 5.1, there is an association between increased bronchial responsiveness and asthma in groups of older children and adults (Cockcroft 1977, Juniper 1981, Woolcock 1987, Peat 1989). Although theoretically, reduced prechallenge lung function or airway calibre may produce an apparent increase in BR, because of the relationship between forced expiratory flow rates and airway radius, in practice results of studies have been variable. One group studying children showed
that BR correlates poorly with baseline lung function (Silverman 1972, Wilson 1984), although other studies in children (Burrows 1992) and adults (Cockcroft 1977, Sparrow 1987, Rijcken 1988, Ulrik 1993, Peat 1992) have shown some relationships between baseline lung function and BR. Some of these studies involve population samples, whereas others are mainly asthmatic subjects, and others include smokers and subjects with chronic obstructive pulmonary disease. Alterations in airway calibre may be important in determining the distribution and penetration of the bronchial challenge agent aerosol into the airways, which may in turn affect the measured response.

The aim of this study was to determine whether the airways of infants at age 6 months, who had already experienced one or more episodes of lower respiratory tract illness (wheeze or cough or both) were more responsive to histamine than those of asymptomatic control subjects.

5.2.2 Methods

(a) Subjects

The cohort subjects at the age of 6 months, described in chapter 2, comprised the subjects for this study. Infants were classified according to the presence or absence by the age of six months of one or more episodes of cough or wheezing, or both, (henceforth referred to as lower respiratory illness, LRI), using the questionnaire described in chapter 3. At the time of study at the age of 6 months, all infants had been free of upper and lower respiratory symptoms for at least two weeks. None was receiving any drugs known to affect bronchial responsiveness. Infants were all studied
supine and after sedation, as described above (see section 2.5).

(b) Lung function and histamine challenge

Baseline measurements of baseline $\dot{V}_{\text{maxFRC}}$ and FRC were performed, then infants underwent histamine challenge to determine $PC_{30}$ (see chapter 4). The jacket pressure used for each subject was noted. The shape of the baseline PEFV curves was described qualitatively for each subject, as either concave or convex with respect to the origin.

(c) Analysis

The lengths, weights, ages of the infants with and without LRI were compared using Student's t tests. The gender distribution, ethnic origin and history of maternal smoking in the two groups were compared using a Chi squared test with Yates' correction. $V_{\text{maxPRC}}$ was adjusted to a length of 70 cm using the regression equations derived from the healthy subjects in the cohort over the first 6 months of life (see chapter 5.1.2(c)). For female subjects, length-adjusted $V_{\text{maxFRC}}$ was further adjusted for gender from the regression equations (see chapter 5.1.2(c)), in order to group male and female subjects together for comparison. FRC was adjusted to a length of 70 cm using the regression equations derived from the healthy subjects in the cohort over the first 6 months of life. For normal male subjects $\text{FRC (ml)} = 5.17(\text{length in cm}) - 186$, and for females $\text{FRC (ml/s)} = 4.59(\text{length in cm}) - 159$. For female subjects, length-adjusted FRC was further adjusted for gender from the regression equations (by multiplying by 1.08), in order to group male and female subjects together for comparison.
The $V_{\text{maxFRC}}$, FRC, jacket pressure and coefficient of variation data were skewed, and the PC$_{30}$ data censored, so they were compared using the Mann-Whitney U test. Values were expressed as medians, with 95% confidence intervals (CI) and interquartile ranges. The study design enabled a difference of two doubling concentrations in PC$_{30}$ (boys and girls grouped together), a 120 ml/s difference in length adjusted $V_{\text{maxFRC}}$ (boys and girls compared separately) to be detected between the normal and symptomatic groups, with a power of greater than 80% at a significance level of 5% (Altman 1982).

5.2.3 Results

(a) Subjects

Of the original 54 infants recruited into the cohort study, two had moved away from the area by the age of 6 months, and seven no longer wished to take part in lung function tests, leaving 45 infants for inclusion in this study. There were similar numbers of boys and girls in the study (23/22), but more boys than girls had developed symptoms by the age of 6 months (13/10). Of those with LRI seven symptomatic boys and six symptomatic girls had coughed without any wheeze. Of the five boys and four girls from the original birth cohort of 54 infants who dropped out of the study, two boys and two girls had developed LRI symptoms by six months. The ages and lengths of the infants with LRI and control infants were similar, but the symptomatic male infants were heavier than the normal boys ($p<0.05$). There were no significant differences in the numbers of maternal smokers or non-white subjects between the two groups (table 5.5) or of other family members who smoked. Further analysis into the independent effect of parental smoking was precluded because of the
small number of subjects.

(b)  *Lung function*

For both male and female infants the length-adjusted $\dot{V}_{max\text{FRC}}$ before challenge was significantly lower for the symptomatic group than for the control infants (table 5.6; figure 5.5). The median difference in length-adjusted $\dot{V}_{max\text{FRC}}$ between symptomatic and control infants was 108.0 ml/s for boys (95% CI 45.9 to 251.6 ml/s; p=0.006) and 102.1 ml/s for girls (95% CI 17.8 to 232.2 ml/s; p=0.013). The coefficient of repeatability of $\dot{V}_{max\text{FRC}}$ was slightly greater for symptomatic infants than for control subjects (table 5.6).

The median jacket pressure required to produce flow limitation during the squeeze manoeuvre was significantly lower for the group with lower respiratory tract illness than for the control infants (37 and 46 cmH$_2$O respectively; p<0.05). There was no significant difference between symptomatic subjects and controls in the distribution of the shapes of the PEFV curves prior to the histamine challenge (concave:convex = 16:7 for subjects with LRI and 12:10 for controls; p=0.46).

For boys and girls considered together (table 5.6) and separately FRC did not differ significantly between healthy control and symptomatic infants. For control and LRI boys median length-adjusted FRC was 165.3 ml and 158.7 ml respectively (p=0.20) and for girls 161.0 ml and 161.6 ml respectively (p=0.96).
(c) Bronchial responsiveness

Five infants awoke before completing the histamine challenge. During baseline measurements, one male infant from the group with LRI had flow limited flow volume curves during tidal breathing, precluding histamine challenge. For the remaining infants, (nine control and 11 symptomatic boys; 12 control and seven symptomatic girls), there was no significant difference in $PC_{30}$ between symptomatic infants and normal infants (table 5.6; figure 5.6). There was no correlation between length-adjusted $V_{max,FRC}$ before histamine challenge and $PC_{30}$ for the whole group.

Before histamine challenge, oxygen saturation was greater than 94% for all subjects. During bronchial challenge only four subjects desaturated below 90%, the minimum recorded $SaO_2$ being 83% with spontaneous recovery. The maximum decrease in $P_{e}O_2$ from pre-challenge recordings was 4.2 kPa; in 11 infants $P_{e}O_2$ decreased by more than 2 kPa during challenge. There was no significant change in $P_{e}CO_2$ during histamine challenge.

5.2.4 Discussion

In contrast with reports of an association between BR and wheezing in older subjects (Cockcroft 1977, Juniper 1981, Woolcock 1987, Peat 1989), no association was found between BR to histamine and LRI in 6 month old infants. Baseline lung function, however, was reduced in symptomatic boys and girls, despite their being free of symptoms at the time of study. These findings in children of atopic parents are similar to those reported in a study of a random population sample in Australia, using similar methodology (Stick 1991). The findings in the 6 month infants are slightly
different from the neonatal lung function and BR findings described earlier in this chapter, in which LRI was defined as two or more episodes of cough and/or wheeze in the first year, rather than one or more episodes in the first six months of life. No differences were found in FRC between healthy and symptomatic infants, which is consistent with the findings in the newborn period, although the numbers were somewhat small for statistical analysis.

The configuration of the maximum expiratory flow volume curve is a sensitive index of disturbed pulmonary mechanics, indicating imbalance between peripheral elastic and flow resistive components of the lungs. Minor changes have been interpreted as indicating "small airway disease" (Hyatt 1979). The similarity in the shape of the PEFV curves in the group with LRI and control subjects, despite the lower $V_{\text{maxFRC}}$ in those with LRI, is compatible with a pre-existing anomaly, as shown by the Tucson group (Martinez 1988 & 1991), rather than "lung damage". The configuration of the PEFV curves in infants is, however, at least partly dependent on the external compression applied. Excessive jacket pressures tend to produce increasingly concave curves and a consequent reduction in $V_{\text{maxFRC}}$, referred to as negative pressure dependency of flow (LeSouef 1986). In this present study various jacket pressures were used to determine the optimal pressure at which flow limitation was just achieved.

The levels of BR to histamine which were found were lower (that is PC_{30} was higher) than those reported previously in healthy and symptomatic infants (Prendiville 1987b, Lesouef 1989, Stick 1991). This was likely to be due to differences in methodology.
between studies, as histamine challenge has not been standardised in infant studies.

Various factors may influence infant lung function and BR, as discussed earlier in this chapter. There were insufficient numbers of maternal smokers in this study to look at the effect of maternal smoking on baseline lung function or BR. All of the subjects had a parental history of atopy, though neither the subjects nor their parents were tested for atopy. The numbers were too small to allow BR in boys and girls to be looked at separately, which might have revealed gender related differences, such as was found in the study of BR at 1 month of age.

This study suggests that at 6 months of age, although BR was present in most infants, it did not discriminate between those with and without LRI. LRI symptoms are associated with reduced airway calibre.
5.3 DISCUSSION OF IMPLICATIONS OF THE COHORT STUDY

In young adults, a prospective study has shown that increased BR precedes the development of clinical asthma and is compatible with a genetic basis for it (Hopp 1990). In epidemiological studies of 7 and 11 year old Southampton children, wheeze was not associated with BR in the absence of atopy, but was strongly related to BR when atopy was present (Clifford 1989). A recent study of methacholine hyperresponsiveness in Canadian children aged 11 to 16 years found evidence of increased airway inflammation in currently asthmatic school children compared with asymptomatic children, levels of BR being similar (Pin 1993). This study suggested that inflammation was an important determinant for the clinical expression of asthma. A longitudinal study of 11 year old New Zealand schoolchildren showed that childhood asthma was strongly linked to allergy (Sears 1991). BR correlated significantly with allergy, as determined by the serum IgE concentration, even in some children with no clinical features of atopy. In another New Zealand study, at the age of 7 years the frequency of increased BR amongst atopic individuals was similar to that in non-atopic subjects, but beyond the age of 7 the frequency of increased BR persisted in atopic individuals, declining in those who were non-atopic (Crane 1989). All these studies suggest that at least from the age of 7, BR and atopy and wheeze are closely linked together. However, the relationship between asthma, atopy and BR would appear to be different in infants and young children.

Infantile asthma is essentially a non-atopic illness (Wilson 1989), even among infants of atopic parents (Sporik 1991). At the age of 3 years there was no significant difference in BR between atopic and non-atopic children, all of whom had a history
of severe wheeze (Wilson 1992). This situation seems to persist until the age of 7 years (Crane 1989), although only one study has reported longitudinal data (Sporik 1991). It has been suggested that only those wheezy infants with an atopic predisposition, whose airways become sensitized to aero-allergens in infancy will develop asthma in later childhood (Wilson 1989). Those infants with intercurrent symptoms may be different from those with episodic virus-associated wheezing. It is speculated that they might be the ones who have atopic asthma, and who are likely to continue to have respiratory symptoms beyond infancy. Further follow-up of the cohort of infants, assessment of their atopic status, and further details concerning the pattern of their symptoms will elucidate this hypothesis.

Both neonatal lung function and bronchial responsiveness appear to be risk factors for lower respiratory tract symptoms in the first year of life. Gender related differences are apparent. Forced expiratory flow, determined both by airway dynamics and driving pressure, i.e. airway calibre and elastic recoil, is important in boys, whereas in girls bronchial responsiveness, rather than airway function, is an important determinant of subsequent LRI in the first year. In this cohort study, once LRI had occurred, baseline lung function was impaired compared with healthy controls, but BR became similar between infants with LRI and healthy controls, although the number of subjects in whom BR could be assessed was smaller than during the neonatal period, decreasing the power of the study. The Australian group reported similar lung function and BR findings in a group of wheezy and healthy infants aged between 6 and 20 months (Stick 1991). Both their study and this six month cohort study were too small to consider males and females separately.
The studies of lung function and BR at one and six months of age suggest that impaired lung function in boys and increased BR in girls are prior risk factors for developing LRI, but once LRI has occurred, and symptoms resolved, even though impaired lung function is present in girls and boys, the differences in BR are no longer apparent. As LRIs are usually associated with viral infections, it implies that when infants with reduced lung function develop viral respiratory infections, they develop lower respiratory symptoms, and their lungs suffer further damage, as reflected by the impaired lung function. The situation therefore becomes a vicious circle, in which BR no longer plays a role.
### Table 5.1 Baseline data for cohort at initial visit: mean or median* (range) or number (%) are given

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LRI (n=16)</td>
<td>No LRI (n=20)</td>
</tr>
<tr>
<td>Age* (months)</td>
<td>0.75 (0.30-1.50)</td>
<td>0.88 (0.30-1.30)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.08 (2.95-5.70)</td>
<td>4.06 (2.90-5.25)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>53.6 (48.0-59.0)</td>
<td>53.7 (48.0-59.0)</td>
</tr>
<tr>
<td>Maternal smokers</td>
<td>5 (31)</td>
<td>2 (10)</td>
</tr>
</tbody>
</table>

LRI lower respiratory illness

*p value=significance of difference between subjects with LRI and without LRI

(t test or Chi squared test)
Table 5.2  Early lung function and PC$_{30}$ in relation to subsequent lower respiratory illness (LRI) for boys and girls combined: mean or median values, 95% CI of mean or median, and of the differences

<table>
<thead>
<tr>
<th>LRI</th>
<th>No LRI</th>
<th>p value (CI of difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}_{\text{maxFRC}}$ (ml/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>median</td>
<td>95</td>
<td>108</td>
</tr>
<tr>
<td>95% CI</td>
<td>75-124</td>
<td>86-128</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>mean</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>95% CI</td>
<td>7.7-8.5</td>
<td>8.1-8.8</td>
</tr>
<tr>
<td>$t_{\text{pe}}/t_e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>mean</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.38-0.45</td>
<td>0.36-0.42</td>
</tr>
<tr>
<td>FRC (ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>mean</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>95% CI</td>
<td>90-110</td>
<td>76-101</td>
</tr>
<tr>
<td>PC$_{30}$ (g/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>median</td>
<td>4.3</td>
<td>6.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.6-8.9</td>
<td>4.3-16.2</td>
</tr>
</tbody>
</table>

* adjusted for gender, and to body length of 53cm
p value=significance of difference between subjects with LRI and without LRI (t test or *Mann Whitney U test)
Table 5.3  Early lung function and PC$_{30}$ in relation to subsequent lower respiratory illness (LRI): mean or median values, and 95% CI of mean or median, and of the differences, and coefficient of repeatability (CR).

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LRI</td>
<td>No LRI</td>
</tr>
<tr>
<td></td>
<td>(CI of diff)</td>
<td>(CI of diff)</td>
</tr>
<tr>
<td>( \dot{V}_{\text{max}} \cdot \text{FRC} ) (ml/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>median</td>
<td>64</td>
<td>103</td>
</tr>
<tr>
<td>95% CI</td>
<td>46-88</td>
<td>80-130</td>
</tr>
<tr>
<td>CR (%)</td>
<td>13.7</td>
<td>12.2</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>mean</td>
<td>8.3</td>
<td>8.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>7.9-8.8</td>
<td>8.0-8.8</td>
</tr>
<tr>
<td>CR (%)</td>
<td>9.2</td>
<td>7.9</td>
</tr>
<tr>
<td>( \text{IPE} /L )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>mean</td>
<td>99</td>
<td>89</td>
</tr>
<tr>
<td>95% CI</td>
<td>74-124</td>
<td>68-110</td>
</tr>
<tr>
<td>PC$_{30}$ (g/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>median</td>
<td>7.8</td>
<td>4.3</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.6-26.9</td>
<td>2.4-13.1</td>
</tr>
</tbody>
</table>

* adjusted to body length of 53cm

p value = significance of difference between subjects with LRI and without LRI

(t test or *Mann Whitney U test)

148
Table 5.4  Studies of initial lung function and respiratory outcome in infancy: comparison of LRI definitions and findings.

<table>
<thead>
<tr>
<th>Study</th>
<th>LRI definition</th>
<th>Diagnosed</th>
<th>$\dot{V}_{\text{maxFRC}}$</th>
<th>FRC</th>
<th>Gaw</th>
<th>$t_{\text{perf}}$</th>
<th>BR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>by whom?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martinez et al</td>
<td>a) c only</td>
<td>doctor</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1988)</td>
<td>≥1 episode in 1st yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) w &amp;/or c</td>
<td>doctor</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>$p=0.07$</td>
<td>NS</td>
</tr>
<tr>
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<tr>
<td></td>
<td>≥1 episode in 1st yr</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martinez et al</td>
<td>a) w</td>
<td>doctor</td>
<td>$\downarrow$</td>
<td>$\downarrow$</td>
<td>-</td>
<td>$1^*$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1991)</td>
<td>1 episode in 1st yr only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>or</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>w after 1st yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) w in 1st yr</td>
<td>doctor</td>
<td>$\downarrow$</td>
<td>NS</td>
<td>-</td>
<td>$\uparrow$</td>
<td>-</td>
</tr>
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<td>and</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>w after 1st yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tager et al (1993)</td>
<td>c &amp;/or w</td>
<td>doctor or</td>
<td>NS*</td>
<td>NS</td>
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<tr>
<td></td>
<td>≥1 episode questionnaire</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>c &amp;/or w</td>
<td>questionnaire</td>
<td>$\downarrow$NS?</td>
<td>NS</td>
<td>-</td>
<td>$\uparrow$NS?</td>
<td>NS</td>
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<tr>
<td></td>
<td>≥ 2 episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

c cough; w wheeze; BR bronchial responsiveness

* $\dot{V}_{\text{maxFRC}}/\text{FRC} \downarrow$

* p=0.07 (calculated from table 2 of Martinez 1991)
Table 5.5  Baseline data for cohort at 6 month visit: mean (range) or number (%) are given

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Boys</th>
<th></th>
<th></th>
<th>Girls</th>
<th></th>
<th></th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LRI</td>
<td>No LRI</td>
<td>p value*</td>
<td>LRI</td>
<td>No LRI</td>
<td>p value*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=10)</td>
<td></td>
<td>(n=10)</td>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>6.65</td>
<td>6.45</td>
<td>0.52</td>
<td>6.60</td>
<td>6.21</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5.50-8.00)</td>
<td>(6.00-8.00)</td>
<td></td>
<td>(6.00-8.00)</td>
<td>(6.00-7.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.51</td>
<td>7.74</td>
<td>0.048</td>
<td>7.73</td>
<td>7.61</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.92-10.0)</td>
<td>(6.30-8.74)</td>
<td></td>
<td>(5.59-10.0)</td>
<td>(6.60-10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>69.3</td>
<td>68.5</td>
<td>0.43</td>
<td>67.8</td>
<td>67.7</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(66-74)</td>
<td>(63-72)</td>
<td></td>
<td>(63-70)</td>
<td>(65-73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal smokers</td>
<td>4</td>
<td>1</td>
<td>0.49</td>
<td>2</td>
<td>1</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(31)</td>
<td>(10)</td>
<td></td>
<td>(20)</td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-caucasian</td>
<td>3</td>
<td>1</td>
<td>0.79</td>
<td>2</td>
<td>2</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(10)</td>
<td></td>
<td>(20)</td>
<td>(17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LRI lower respiratory illness

*p value=significance of difference between subjects with LRI and without LRI (t test or Chi squared test).
Table 5.6  Gender and length adjusted $\dot{V}_{\text{maxFRC}}$, FRC and $PC_{30}$ at 6 months of age.

<table>
<thead>
<tr>
<th></th>
<th>LRI</th>
<th>No LRI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}_{\text{maxFRC}}$ (ml/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of subjects</td>
<td>23</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>125.4</td>
<td>214.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>95% CI</td>
<td>85.0 to 164.2</td>
<td>159.4 to 298.0</td>
<td></td>
</tr>
<tr>
<td>CR (%)</td>
<td>12.4</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>FRC (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of subjects</td>
<td>19</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>174.3</td>
<td>167.3</td>
<td>0.47</td>
</tr>
<tr>
<td>95% CI</td>
<td>157.8 to 179.6</td>
<td>162.8 to 193.2</td>
<td></td>
</tr>
<tr>
<td>CR (%)</td>
<td>5.7</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>$PC_{30}$ (g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of subjects</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Median value</td>
<td>10.3</td>
<td>16.5</td>
<td>0.97</td>
</tr>
<tr>
<td>95% CI</td>
<td>2.8 to 23.8</td>
<td>2.4 to 27.9</td>
<td></td>
</tr>
</tbody>
</table>

LRI = lower respiratory tract illness, defined as 1 or more episodes by 6 months of age

CR = coefficient of repeatability

CI = confidence interval
Figure 5.1 Early $\dot{V}_{\text{maxFRC}}$, adjusted for length and gender, for infants classified by their subsequent history into LRI (2 or more episodes of acute lower respiratory illness in the first year) or no LRI (one or less episode).

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers).
Figure 5.2 Early $\dot{V}_{\text{maxFRC}}$, adjusted for length, for boys and girls classified by their subsequent history into LRI (2 or more episodes of acute lower respiratory illness in the first year) or no LRI (one or less episode).

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers).

For boys, the difference was statistically significant ($p=0.04$).
Figure 5.3 Early bronchial responsiveness for boys and girls combined, classified by their subsequent history into LRI (2 or more episodes of acute lower respiratory illness in the first year) or no LRI (one or less episode).

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers).
Figure 5.4 Early bronchial responsiveness for boys and girls classified by their subsequent history into LRI (2 or more episodes of acute lower respiratory illness in the first year) or no LRI (one or less episode).

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers).

For girls, the difference was statistically significant (p=0.008).
Figure 5.5 $V_{\text{maxFRC}}$ at six months of age, adjusted for length, for boys and girls classified by their subsequent history into LRI (1 or more episodes of acute lower respiratory illness by six months of age) or no LRI.

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers). The difference was statistically significant for both boys and girls ($p=0.006$ and $p=0.013$ respectively).
Figure 5.6 Bronchial responsiveness for boys and girls combined, classified by their subsequent history into LRI (1 or more episodes of acute lower respiratory illness by six months of age) or no LRI.

Box and whisker plots show median with 95% confidence interval (notches), interquartile range (ends of box) and range of values (ends of whiskers).
6.1 EVALUATION OF AN INDEX OF TIDAL EXPIRATORY FLOW IN INFANTS IN HEALTH AND DISEASE

6.1.1 Introduction

The measurement of tidal flow together with the analysis of expiratory flow patterns, is a potentially useful tool for assessing lung function in infants. Measurement of airflow during tidal breathing is relatively simple and non-invasive, whereas classical techniques, such as the "squeeze technique" are time-consuming, complicated and require the infant to be sedated. Patterns of tidal expiratory flow including $t_{pef}/t_e$ have been described in chapter 4, in which tidal expiratory flow patterns were reported in healthy infants during the first year of life. The aim of this part of the study was to compare tidal expiratory flow data from these healthy infants with several groups of infants with obstructive airway disease, using an index of forced expiratory flow derived from the squeeze technique, $V_{max FC}$, as an independent means of measuring the severity of airflow obstruction.

6.1.2 Methods

(a) Subjects

The 94 subjects, aged 1 week to 23 months, described in detail in chapter 2, formed three groups. Group 1 comprised the 54 cohort infants, who were studied at 1, 6 and 12 months. By the age of 12 months, 22 of the infants had had one or no LRI, and formed the healthy control group (group 1a), whose results have already been
described in chapter 4. 32 of the cohort infants had a history of recurrent LRI, defined as at least two episodes of cough and/or wheeze (group 1b). Their symptoms were mild and would not normally have occasioned attention at a hospital, although four infants attended the paediatric asthma out-patient clinic. Group 2 comprised 20 children aged 8 to 23 months with infantile asthma recruited from the children's asthma clinic and group 3 comprised the 20 infants aged 2 to 18.5 months with severe, oxygen dependent, chronic lung disease of prematurity (CLD) taking part in a follow-up programme.

Bronchodilator therapy, where used, was stopped at least 24 hours prior to the study. All children had been free of upper respiratory tract symptoms for at least 2 weeks prior to the study. They were sedated for the studies and studied supine (see section 2.5).

(b) Lung function

Measurements of $\dot{V}_{\text{maxFRC}}$ and $t_{\text{pef}}/t_c$ were made (see chapter 4). Those infants with CLD who were oxygen-dependent were given additional oxygen via a funnel attached to the pneumotachograph, in order to maintain an arterial oxygen saturation between 93% and 97%. No correction for altered inspired gas composition was made to the pneumotachograph signals, since the only data which required precisely accurate flows, $\dot{V}_{\text{maxFRC}}$, was measured during air breathing.

(c) Analysis

Comparisons of age and length were made using Student's t tests. Chi squared test
with Yates' correction was used to compare the gender distribution within the different groups. The lung function data were skewed, so comparisons between different groups were made using the Mann Whitney U test, with the Wilcoxon matched pairs test for paired data within groups 1a and 1b. Least squares regression analysis, with log transformation where necessary, was used, with Pearson's correlation coefficient. The study size enabled differences in $t_{pof}/t_e$ between the groups of 0.1 at 1 month and 0.05 after the neonatal period to be detected, with a power of greater than 90% at a significance level of 5% (Altman 1982).

6.1.3 Results

By the age of 6 months, 9 infants had withdrawn from the cohort study, as they had either moved away from the area or they no longer wished to participate in lung function testing. By 12 months, a further 5 had withdrawn. Of the fourteen infants who withdrew from the study, 9 had no symptoms and 5 had recurrent LRI, representing 41% and 16% of the respective groups. There was no difference in $\dot{V}_{maxFRC}$ at 1 month between the study group and those who withdrew.

Comparing healthy infants with those who had the most severe disease, the pattern of tidal expiratory flow was clearly different (figure 6.1). The healthy infant's tidal breathing pattern was sinusoidal, whereas the infant with CLD reached maximum tidal expiratory flow early in the expiratory cycle, and then had a prolonged expiratory phase. No qualitative differences in the flow-time curves were detected between healthy infants and the other clinical groups.
(a) Healthy infants

These results are discussed in chapter 4 and shown in figure 4.22 and table 4.1.

(b) Infants with LRI

There were no significant differences between the LRI and healthy cohort infants with respect to age, gender or mean length or weight at any of the visits (tables 4.1 and 6.1). Although $\dot{V}_{maxFRC}$ was significantly lower at 6 months and 12 months in the LRI group than in healthy infants ($p<0.01$ and $p<0.005$ respectively), the differences between the groups were not reflected in $t_{pes}/t_e$.

(c) Infants with asthma

The 20 infants with asthma were compared with the 13 healthy infants who had lung function tests at 1 year of age, as their ages, weights and lengths were similar (table 6.2). $\dot{V}_{maxFRC}$ was significantly lower ($p<0.005$) in the asthmatic infants, but none of the small differences in tidal breathing parameters were significant.

(d) Infants with CLD

We did not have lung function data for preterm infants without CLD, so that the 20 infants with severe CLD were compared with the 16 healthy infants at a similar mean postnatal age of 6 months. The children with CLD were significantly smaller than the healthy 6 month infants (table 6.2) and their $\dot{V}_{maxFRC}$ was considerably reduced ($p<0.0001$), 15 exhibiting expiratory flow limitation during tidal breathing. Compared with healthy infants, the respiratory frequency was higher ($p<0.005$) and the tidal breathing indices $t_{pes}$, $t_e$, and $t_{pes}/t_e$ were significantly shorter ($p<0.005$) in infants with
t_{per}/t_e did not correlate with $V_{maxFRC}$ either for each group separately, or for the combined data from all the groups.

6.1.4 Discussion

In contrast to adult subjects (Morris 1981), in whom there was a good correlation between FEV$_1$ and t_{per}/t_e, in a range of healthy infants and patients with obstructive lung disease the analysis of expiratory tidal flow patterns could not be used to quantify airway obstruction except under extreme circumstances. In the present study the ratio t_{per}/t_e was an insensitive indicator of airway obstruction compared with $V_{maxFRC}$ and was abnormal (i.e. different from t_{per}/t_e in healthy infants with normal $V_{maxFRC}$) only in the most severe airway obstruction when expiratory flow limitation was present even during tidal breathing. Although infants were not studied during acute airway obstruction (either induced or spontaneous) differences were detected in $V_{maxFRC}$ from the healthy infants for all the post-neonatal groups. Significantly more infants withdrew from the healthy group, compared with the LRI group during follow up, which could have influenced the results. However, the aim of this particular study was to compare t_{per}/t_e with $V_{maxFRC}$, rather than just to determine changes in t_{per}/t_e over the first year of life.

The factors determining t_{per}/t_e have been discussed earlier in chapter 4.
Unlike the reports from Tucson (Martinez 1988 & 1991) in this small cohort it was not possible to confirm that $t_{pef}/t_e$ early in life, prior to any respiratory illness, predicted subsequent wheezing in infancy (column 1 of tables 4.1 and 6.1). In fact, the trend in the present data was in the opposite direction. The more recent Tucson study (Martinez 1991) showed that infants who had recurrent wheezing respiratory illnesses during the first three years of life (group C) had significantly lower $t_{pef}/t_e$ measured prior to any LRI during the first three months of life, than infants who remained healthy (group A), with mean values for $t_{pef}/t_e$ of 24.0 and 29.7 respectively ($p \leq 0.01$). The situation becomes less straightforward when their results are studied more critically. Their other group of infants (group B) consisting of those who had only one wheezy episode in the first year and none in the subsequent two years, together with those who had their first episode of wheezing after the first year, during the second and third years) had a mean $t_{pef}/t_e$ of 34.2. This value is almost 50% higher than the mean value of 24.0 for group C, and is higher than the mean value of 29.7 for their healthy infants (group A). Using the data given in their paper, the difference between $t_{pef}/t_e$ in their healthy group A and group B almost reaches statistical significance ($p=0.07$), but in the opposite direction to the statistically significant difference between groups A and C. In the earlier paper from Tucson, a lower initial $t_{pef}/t_e$ was found in boys who subsequently wheezed in the first year of life, but amongst their female infants, an opposite trend occurred. They do not report results for $t_{pef}/t_e$ beyond three months of age.

Two important factors could account for the difference between the studies: the
infants in the present study all had a parental history of atopy and the population was very small in this present study. A larger study may have detected subtle differences in $t_{pef}/t_e$ between healthy and symptomatic groups.

(b) $t_{pef}/t_e$ and respiratory illness

In the presence of airway obstruction the neuromechanical processes which control the duration of $t_{pef}$ and $t_e$ may be modified. The fact that $t_{pef}/t_e$ was unaltered in infants with mild/moderate airway disease suggests that a ratio of 0.25 to 0.30 represents an optimal value over a wide range of airways obstruction in 6 to 12 month old infants. The reduction in the ratio which was observed in infants with severe airway obstruction and CLD has been reported by others (Morgan 1984). Infants with CLD have been shown to have abnormal pulmonary mechanics, with hyperinflation and decreased compliance (Bryan 1973). They have higher respiratory rates throughout the first year of life, compared with a preterm control group. Another study (Gerhardt 1987) looking at lung function in a group with relatively mild CLD reported tachypnoea during the first year of life, reverting to the normal range after 12 months, but abnormally low compliance throughout the follow up to the age of three years. The reduced $t_{pef}/t_e$ in the infants with severe CLD was due to both a reduction in $t_{pef}$ in relation to breathing frequency, possibly as a result of active expiration or reduced diaphragmatic braking, and to a relative prolongation of $t_e$ (i.e. increased $t_e/t_{tot}$), probably a reflection of their prolonged expiratory time constant.

One possible explanation for the insensitivity of $t_{pef}/t_e$ relates to the shape of the expiratory flow profile (figure 6.1). The rounded shape in healthy infants contrasts
with the peak seen in severe CLD. In order to exaggerate the difference, the data were reanalysed, analysing the expiratory time at a value of expiratory tidal flow, at which the flow rate declined to 95% of its peak value as suggested by J Morris (personal communication; see chapter 6.2). The modified value of t_{pfe}/t_e was no better at discriminating between the different clinical groups.

\( V_{maxFRC} \) in contrast to t_{pfe}/t_e, appeared to provide a satisfactory quantitative measure of airway obstruction. It was sufficiently sensitive to be able to differentiate the clinical groups, all of which had significantly lower median values of \( V_{maxFRC} \) than the healthy infants, the children classified as asthma having lower values than the children with a history of recurrent mild LRI. There was no ideal control group for the infants with severe CLD, but their \( V_{maxFRC} \) was significantly lower than the normal cohort infants at any age.

This study has demonstrated that t_{pfe}/t_e in infancy is not a sensitive measure of pulmonary function in infants with airway disease, and cannot be used as a substitute for conventional tests. The infants in this study were studied during quiet sleep, but were sedated, and flow was recorded via a pneumotachograph and facemask, which introduces an additional dead space and resistance. Both of these factors may influence respiratory patterns, and thereby blunt any differences in tidal breathing parameters which would otherwise be apparent between healthy and symptomatic infants. In a study of newborns during quiet sleep without sedation, t_{pfe}/t_e was measured by respiratory inductance plethysmography, and neither t_{pfe}/t_e nor respiratory rate changed significantly when the infants breathed through a facemask and
pneumotachograph (Stick 1992). Sleep state does influence $t_{pe}/t_{e}$ (Lodrup 1992), but the effect of sedation on tidal breathing parameters has not yet been studied. As discussed in chapter 4, the ratio $t_{pe}/t_{e}$ probably represents the dynamic, neuromuscular response of the infant to mechanical constraints, under the metabolic conditions of quiet supine sleep under which the tests were performed. Tidal expiratory flow measurements in infants were not able to discriminate between healthy infants with normal airway function and those infants with mild degrees of airway obstruction due to disease; the role of tidal breathing indices during drug induced airway obstruction is considered in section 6.2.
6.2 EVALUATION OF TIDAL BREATHING INDICES DURING BRONCHIAL
CHALLENGE IN INFANTS

6.2.1 Introduction

Analysis of patterns of tidal expiratory flow, as described above, did not distinguish
between groups of healthy individuals and those with lower airway obstruction, except
in severe CLD. However, the situation may be different within subjects, and if such
methods were able to detect changes in the degree of airway obstruction, either
spontaneously or as a result of therapy, they would have wide clinical applications.

One study has shown the utility of tidal breathing indices as a measure of acute
airways obstruction during histamine challenge in children (Cutrera 1991). As a
preliminary step in applying tidal breathing methods to clinical monitoring and
therapeutic trials, it is important to establish under controlled conditions which, if any,
indices derived from breathing patterns, reflect changes in airway function measured
by an independent method. The aim of this study was to determine whether \( t_{\text{pef}}/t_e \) and
other data derived from tidal flow measurements, could be used to assess the response
to histamine challenge in infants, using \( V_{\text{maxFRC}} \) obtained from the "squeeze"
technique, as the reference method for quantifying the airway response. In healthy
adults where tidal expiratory flow sometimes forms a plateau rather than a well
defined peak, a revised form of the index where \( t_{\text{pef}} \) was defined as time to the onset
of passive expiration rather than peak expiratory flow, proved to be a more sensitive
measure of acute airflow obstruction (J Morris - personal communication). The
second aim of this study therefore, was to determine whether the revised indices
\( t_{\text{pef}}(a)/t_e \) and \( t_{\text{pef}}(b)/t_e \) (figure 6.2) would better correlate with changes in airways
obstruction in infants during challenge.

6.2.2 Methods

(a) Subjects

Tidal breathing indices were analyzed in 36 studies performed on 27 infants. The infants were recruited from the neonatal cohort. The data reported here relate to tests carried out at 6 and 12 months of age. In half of the studies analyzed, infants responded to histamine challenge with a 30% or greater fall in $\dot{V}_{\text{max FRC}}$. In the other half no significant response to bronchoconstrictor challenge was detected. In each of the two groups (responders and non-responders), 8 of the infants were aged 6 months and 10 were aged 12 months. Nine infants were studied on both occasions: 3 responded and 4 were non-responders on both occasions and two responded differently on each occasion. The studies were randomly selected from the larger cohort to give the greatest number of responders and non-responders in each age group.

(b) Lung Function

Baseline measurements of $\dot{V}_{\text{max FRC}}$ were performed (see chapter 4). The four or five tidal breaths preceding the squeeze were also analyzed (RASP Software, Physiologic Ltd, Newbury, England). Tidal breaths and forced expiratory flows were analysed independently. Only those runs of tidal breathing with a regular end-expiratory level, breathing frequency ($f$) and tidal volume ($V_t$) were included. Inspiratory time ($t_i$), expiratory time ($t_e$), peak tidal expiratory flow (PEF) and the mean tidal expiratory flow ($V_e/t_e$) were recorded together with $f$ and $V_t$. The expiratory time constant ($t_{ea}$) was calculated as the inverse of the slope of a regression line through the latter part
of tidal expiratory flow. The limits of this regression were set at 60 and 90% of volume expired. These limits excluded the early section of expiration around peak tidal flow from the calculation of $t_{ns}$. Breaths were excluded from the analysis if the correlation coefficient of this regression line was less than 0.8, or if the volume expired by peak expiratory flow was greater than 40% of tidal volume. A correlation coefficient of 0.8 was used since this excluded clearly irregular data but included as much of the other data as possible without being overselective. $t_{pef}/t_e$ and the revised indices, time to 95% of maximal expiration before and after the peak divided by the expiratory time ($t_{pef(a)}/t_e$ and $t_{pef(b)}/t_e$ respectively) were also recorded (figure 6.2). Indices were calculated on each individual tidal breath and the mean of 25 to 30 tidal breaths taken.

(c) Histamine challenge

Histamine challenge was carried out as described in chapter 4.

(d) Analysis

Lung function results are presented as mean values and the 95% confidence intervals. Apart from $V_{maxFRC}$, which is reported separately for 6 month and 12 month groups, all the other indices conformed to a normal distribution when the data for the two age groups were combined. The comparisons between baseline measurements of lung function in responders and non-responders and between baseline and post histamine measurements of lung function were made using Student's t tests or Wilcoxon rank sum test as appropriate. The effect of forced expiratory manoeuvres on tidal breathing indices was determined by comparing the first and last tidal runs of the baseline series.
In responders, the post histamine lung function values were those measured after the concentration of histamine causing a 30% or greater fall in $V_{\text{maxFRC}}$ which occurred at a mean histamine concentration of 8.6 g/l. In non-responders, the post histamine lung function was measured after the administration of 8 g/l of histamine solution, which meant that the mean duration of the procedures was very similar for the two groups. Statistical significance was defined as $p<0.05$.

6.2.3 Results

Non-responding infants were slightly heavier than responders at 12 months ($p<0.01$). Otherwise, there was no difference between infants who responded to challenge and the non-responding infants in terms of age, weight and length under baseline conditions (table 6.3). There was no statistically significant difference in $V_{\text{maxFRC}}$ between the 2 groups, nor any difference in the indices of tidal breathing (table 6.4). Baseline $t_{\text{per}}/t_e$ was the same in the two groups (mean 0.26, 95%CI 0.23-0.29). Repeated forced expiratory manoeuvres had no significant effect on tidal indices.

Mean change in $V_{\text{maxFRC}}$ after the inhalation of histamine in responders was -43.3% (mean PC$_{30}$ 8.6 g/l) and in non-responders was +11.8%. There was no statistically significant change in $V_{\text{maxFRC}}$ or in any of the tidal indices in non-responders after bronchoprovocation (table 6.4). In responders, breathing frequency increased ($p<0.01$) and $t_i$ and $t_e$ both decreased ($p<0.05$ and $p<0.005$ respectively) after challenge (table 6.4). There was no change in the ratio of $t/t_e$. The expiratory time constant decreased from 0.61s to 0.51s after challenge ($p<0.05$). Mean tidal expiratory flow rate ($V_t/t_e$) increased after challenge as did peak tidal expiratory flow, though in the latter case
this change did not reach statistical significance ($p=0.06$). There was no change in the indices $t_{pef}/t_e$, $t_{pef(a)}/t_e$ or $t_{pef(b)}/t_e$ after histamine challenge. No consistent change in the shape of the expiratory flow pattern was detectable on visual inspection of time-based curves.

6.2.4 Discussion

Changes in lung function in 6-12 month old infants who responded to bronchial challenge included a decrease in expiratory time constant, and increases in the frequency of breathing and mean tidal expiratory flow rate. The changes suggest that a strategy of active expiration was adopted by the infants in response to acute airflow obstruction as discussed below, or that early and late braking were withdrawn. There were no changes in the tidal breathing indices, $t_{pef}/t_e$, $t_{pef(a)}/t_e$ and $t_{pef(b)}/t_e$. They were insensitive measures of airflow obstruction compared to $\dot{V}_{max}FRC$ and could not be used to assess the response to histamine challenge.

A number of factors which might affect measurements of $t_{pef}/t_e$ in infants have been identified. These include the infants' sleep state, equipment characteristics and the choice of sampling method. Differences have been found in $t_{pef}/t_e$ between awake and to sleeping newborn infants (Lodrup 1992), but the effect of sedation has not been tested. The dead space and resistance imposed by a facemask and pneumotachograph might be expected to alter the pattern of breathing, good agreement between $t_{pef}/t_e$ measured by respiratory inductance plethysmography and by facemask and pneumotachograph in newborns has been shown (Stick 1992). In this present study measurements of $t_{pef}/t_e$ were based on the 4-5 quiet, regular tidal breaths which
preceded the jacket inflations which were performed every 30 seconds. A longer data
collection period may be needed to obtain measurements under steady state conditions.

When measuring $t_m$ from tidal flow volume curves, it is assumed that the latter half
of expiration is completely passive. There are data on muscle activity during
expiration in adults (Morris 1990, Citterio 1981) and in infants (Mortola 1984) to
support this, although defining the point at which tidal expiratory flow becomes
passive without an objective measure of muscle activity is a problem. A second
assumption is that a single exponential fits this portion of expiration. In this analysis,
limits of 60 and 90% of expired tidal volume were set for the regression of tidal
expiratory flow. The fit of the regression line to the data was far better using these
limits than when expiratory flow was regressed through all data points between 40 &
90% of expired volume in a previous analysis (not reported here), because by
commencing analyses at 60% of tidal expiration, the early section of expiration around
peak tidal flow was excluded from the calculation of $t_m$. The data could then be
adequately represented by a single linear regression, as judged by the high correlation
coefficients. However, in spite of a good linear fit, the validity of post histamine $t_m$
measurements in this study, made while infants appeared to be actively exhaling (as
judged by the higher mean expiratory flow rate), must be questioned.

A number of physiological features suggest that the infants either developed active
expiration or lost their laryngeal braking in response to histamine. Not only did the
frequency of breathing increase, but this was accompanied by a greater mean
expiratory flow rate and a shorter expiratory time constant. In the presence of a
marked reduction in forced expiratory flow at low lung volume, this pattern could have been achieved by expiratory effort, or alternatively by decreased diaphragmatic braking. In the first situation the respiratory system was not behaving passively. It may seem surprising that under these circumstances, the expiratory tidal flow volume curve should have had an apparently linear section, since this might be thought to imply a single exponential function for expiratory flow (and volume). It is possible however that reciprocal changes in the effective resistance and compliance of the respiratory system during active expiration could match in such a way that there was no major change in the effective expiratory time constant over the second half of expiration.

Alternative explanations for the short time constant and high peak and mean tidal expiratory flow rates in histamine responders include reduced compliance (i.e. increased elastic recoil) and possibly an increase in FRC. There are no data to support the first of these possibilities, but the second is supported by Maxwell's study (1988).

During expiration, in a completely passive system, changes in resistance will not affect the time to tidal peak flow, only its amplitude. In healthy subjects the rise to peak flow appears to be actively controlled by post-inspiratory activity of the muscles of the chest wall and the diaphragm which may increase the time to tidal peak expiratory flow. In patients with chronic airway obstruction, the cessation of post inspiratory activity has been shown to occur earlier in expiration (Morris 1990), thereby allowing more of the recoil pressure of the lungs and chest wall to drive expiration. The time to maximal expiration will therefore be reduced in these patients. Paradoxically, the
value of tidal PEF may be greater in patients with airway obstruction since PEF will tend to be generated at a lung volume higher in the tidal range.

During histamine challenge, the neuromechanical response represented by $t_{\text{per}}/t_e$ may be modified. Tonic inspiratory activity has been demonstrated throughout expiration (Martin 1980). This would slow the rise to tidal peak expiratory flow. The evidence for this type of activity remains inconclusive (Citterio 1981). It is possible that high doses of histamine are required to evoke this response. Such high doses may cause systemic effects and were not used in the present study. Histamine aerosol may produce an increase in laryngeal resistance. It has been reported that histamine, possibly acting on bronchial irritant receptors in the lungs, produced a reflex reduction in the size of the glottic chink (Higenbottom 1980). If expiratory laryngeal braking had occurred in the subjects in this present study, a fall in PEF and a reduction in $V_e/t_e$ would have been expected. Any alteration in resistance produced by change in laryngeal tone would not be expected to alter $t_{\text{per}}/t_e$ and indeed a recent experiment found no change in $t_{\text{per}}/t_e$ in premature infants pre- and post-extubation (P Seddon, personal communication), confirming that $t_{\text{per}}/t_e$ is independent of direct changes in airways resistance.

Two studies have measured indices of tidal breathing during histamine challenge. J Morris (personal communication) found significant changes in $t_{\text{per}}/t_e$ in adults during challenge when $t_{\text{per}}$ was defined as the time to the onset of passive expiration but not when defined as the time to peak expiratory flow. The explanation for this difference lay in the ill-defined point of peak expiratory flow in many healthy adults compared
with those who have significant baseline airway obstruction. By re-defining \( t_{\text{perf}} \) (equivalent to \( t_{\text{perf}(b)} \) in figure 6.2), differences between healthy and obstructed or baseline and post-challenge values could be exaggerated. In contrast, the revised indices could not be used to assess bronchial challenge in infants in the present study. In children with asthma, histamine challenge produced reductions in FEV\(_1\) of up to 70%, with significant reductions in the index \( dV/V_o \), which is analogous to \( t_{\text{perf}}/t_e \) (Cutrera 1991). However, under baseline conditions, the index was unable to differentiate between children with asthma who were asymptomatic at the time of testing and a control group. This supports the findings in section 6.1, that, in contrast to measures of \( \dot{V}_{\text{max}FRC} \), there was no difference in \( t_{\text{perf}}/t_e \) between healthy infants and those with episodic lower respiratory illness or infantile asthma who were asymptomatic at the time of testing.

The index \( t_{\text{perf}}/t_e \) is an insensitive measure of airflow obstruction and cannot be used to assess the response to histamine challenge in infants under these conditions. Attempts to better define the shape of tidal expiratory flow did not improve the sensitivity of the index. It would be of interest to study awake infants during bronchial challenge, using body-surface measurements to measure \( t_{\text{perf}}/t_e \), thus avoiding the need for sedation. If the study outcome were different, the implication would be that sedation or the face mask had an influence on the factors and reflexes which control tidal breathing.

The pattern of expiratory flow probably represents the outcome of complex interactions of various neuromechanical responses which may change to maintain a
relatively stable pattern of expiratory flow despite wide changes in the level of airway narrowing. The index $t_{exp}/t_e$ alone is inadequate to describe these changes, while other tidal breathing indices simply relate to the increased breathing frequency and tidal flow rate which accompanies a response to histamine challenge.
6.3 OXYGENATION DURING BRONCHIAL CHALLENGE

6.3.1 Introduction

Assessment of BR using PEFV curves to measure $\dot{V}_{\text{maxFRC}}$ requires specialized technical expertise and equipment, in addition to sedation for the infants. A less demanding means of measuring the airway response to bronchial challenge would allow tests to be performed more widely, even under conditions of natural sleep. In asthmatic adults and children, changes in lung function during bronchial challenge with cold air (Eber 1991), methacholine (van Broekhoven 1991, Stewart 1989) and histamine (Stewart 1989), have corresponded closely to changes in transcutaneous oxygen tension ($P_{tcO_2}$). Other studies using various challenges, including nebulised distilled water (Dal Negro 1989), methacholine (Mochizuki 1985 & 1988, Murakami 1990) and histamine (Wilson 1991), have shown that non-asthmatic subjects, both adults and children, respond similarly. To date, change in $P_{tcO_2}$ has been shown to be potentially useful as a measure of bronchial responsiveness to histamine in wheezy infants (Prendiville 1988), but not in healthy infants.

In order to be useful as a technically simple, indirect means of measuring the bronchial response to challenge in infants for epidemiological purposes, changes in $P_{tcO_2}$ must be shown to be equally reliable in healthy and in symptomatic infants. The purpose of this study was to validate changes in $P_{tcO_2}$ by comparison with $\dot{V}_{\text{maxFRC}}$ in a group of symptomatic and asymptomatic infants.
6.3.2 Methods

(a) Subjects

Twenty infants were studied, fifteen were cohort infants and five were asthmatic infants. Six of the cohort infants had no prior respiratory symptoms and formed the control group and the other nine cohort infants had a history of recurrent LRI, defined as at least two episodes of cough and/or wheezing. Any bronchodilator therapy was stopped at least 24 hours prior to the study. All children had been free of upper respiratory tract symptoms for at least 2 weeks prior to the study. Infants were sedated for the study.

(b) Lung function, histamine challenge and monitoring

Measurements of baseline $V_{\text{maxFRC}}$ were made and $P_{\text{eO}_2}$, $P_{\text{eCO}_2}$, and $SaO_2$ recorded before infants underwent histamine challenge to determine $PC_{30}$. After each nebulisation, $P_{\text{eO}_2}$, $P_{\text{eCO}_2}$, and $SaO_2$ were noted every 30 seconds. The changes in $P_{\text{eO}_2}$, $P_{\text{eCO}_2}$ and $SaO_2$ were taken as the maximum deviation from the baseline level, occurring between 2 and 5 minutes after each dose of histamine. The "squeeze" manoeuvre itself was not observed to have any effect on the $P_{\text{eO}_2}$, $P_{\text{eCO}_2}$, or $SaO_2$ (personal observations).

(c) Analysis

The ages, lengths, weights and lung function data of the infants with and without asthma or LRI were compared using Student's t tests. The distribution of baseline $V_{\text{maxFRC}}$ data and of the coefficient of variation data were not normal, so they were compared with the Mann Whitney U test. McNemar's test was used to compare the
gender distribution within the symptomatic and non-symptomatic groups.

In order to group male and female subjects together for comparison of baseline lung function, $V_{\text{maxFRC}}$ was adjusted to the mean length of 72 cm using regression equations and the female data adjusted for gender (see section 5.1.2 c). The study design enabled a change in $P_{te}O_2$ of 2 kPa to be detected during the challenge, with a power of greater than 85% at a significance level of 5%.

The concentrations of histamine producing a 30% fall in $V_{\text{maxFRC}}$ ($PC_{30}\dot{V}_{\text{maxFRC}}$) and a 5% fall in $P_{te}O_2$ ($PC_{5}\dot{P}_{te}O_2$) were calculated by linear interpolation from log dose-response curves for each subject. A change of 5% for $P_{te}O_2$ was chosen, as it was beyond the 95% confidence interval for the individual subjects. Changes were calculated from the mean baseline values for $\dot{V}_{\text{maxFRC}}$ and the minimum baseline value for $P_{te}O_2$. Because of the relatively slow, smooth response time of the $P_{te}O_2$ electrode, this minimum value itself represents a moving-time average value. The reason the mean value of $\dot{V}_{\text{maxFRC}}$ was used, but the lowest value of $P_{te}O_2$ was used, is that during the post-nebulisation period $P_{te}O_2$ fell to a minimum value, returning to the pre-nebulisation value in 5 minutes, whereas $\dot{V}_{\text{maxFRC}}$ did not follow this pattern of response, ie. it was more persistently low.

Logarithmic plots of $PC_{30}\dot{V}_{\text{maxFRC}}$ against $PC_{5}\dot{P}_{te}O_2$ for the two groups were compared by analysis of covariance, fitting the appropriate linear model.
6.3.3 Results

No significant differences existed between the 6 healthy control infants and the 14 infants who had asthma or a history of LRI, with respect to age, length, weight or gender distribution (table 6.5). Comparing the control and symptomatic groups, baseline $P_{w}O_{2}$ was similar, but the symptomatic infants had significantly lower $\dot{V}_{\text{maxFRC}}$ (p=0.003; table 6.5). Because in all subsequent analyses, the two symptomatic groups (LRI and asthma) were indistinguishable, their data were combined. The intrasubject coefficient of variation for baseline measurements of $\dot{V}_{\text{maxFRC}}$ and $P_{w}O_{2}$ was similar for the two groups of subjects. Changes of 30% for $\dot{V}_{\text{maxFRC}}$ and of 5% for $P_{w}O_{2}$ were beyond the 95% confidence intervals for the individual subjects.

Histamine responsiveness as measured by $\dot{V}_{\text{maxFRC}}$ (PC$_{30}$ $\dot{V}_{\text{maxFRC}}$) was similar for the healthy (5.7 g/l) and symptomatic (7.6 g/l) infants (table 6.6). There was, however, a marked difference in responsiveness measured by PC$_{3}$ $P_{w}O_{2}$ (9.8 g/l and 3.2 g/l respectively, p=0.04). The disparity between the responses of control and symptomatic infants is illustrated by the mean data for the final three concentrations of histamine (figure 6.3). A significant change in $P_{w}O_{2}$ was found at the penultimate and final concentrations for the symptomatic group, but only at the final concentration for the healthy infants.

The symptomatic and control infants appear to form two separate populations, due to a difference in the sensitivity of the response as measured by change in $P_{w}O_{2}$ (figure 6.4). The slopes of the linear regressions relating PC$_{30}$ $\dot{V}_{\text{maxFRC}}$ to PC$_{3}$ $P_{w}O_{2}$ were similar for the two groups, but the $P_{w}O_{2}$ response was more sensitive in the symptomatic
group as shown by a significant difference in the intercepts (p=0.05), and by the distribution of the values in figure 6.4. The difference in $PcPC_{O2}$ between the groups was not simply a function of baseline $\dot{V}_{maxFRC}$ for all infants grouped together, or for healthy and symptomatic infants separately. Although the normal infants all had higher baseline $\dot{V}_{maxFRC}$ than did the symptomatic group, within the latter there was no association between baseline $\dot{V}_{maxFRC}$ (allowing for length and sex) and $PcPC_{O2}$.

Pulse oximetry was relatively insensitive in detecting histamine induced bronchoconstriction. In symptomatic infants $SaO_2$ fell significantly (p<0.05) by a mean of 3% at the final histamine concentration, but in the control infants there was no significant change in $SaO_2$ during challenge. Baseline $PcPC_{CO_2}$ was similar in the two groups and did not change significantly from baseline values in either group during the study. No subject developed audible wheezing.

### 6.3.4 Discussion

In contrast to other studies in which normal children and adults have participated (Mochizuki 1985 & 1988, Murakami 1990, Wilson 1991), in the present study healthy normal infants differed from those with a history of LRI in their $PcPC_{O2}$ response to bronchial challenge. The falls in $PcPC_{O2}$ observed during bronchoconstriction with a similar degree of induced decrease in $\dot{V}_{maxFRC}$ were smaller in the healthy subjects than in the LRI group. Consequently reduction in $PcPC_{O2}$ during challenge is a less sensitive indicator of bronchial responsiveness in healthy infants than in infants with a history of LRI.
This difference cannot be explained by differences in histamine concentration, as the 
\( \text{PC}_{30} \dot{V}_{\text{maxFFC}} \) and hence the final concentration of histamine used was the same in the 
two groups. The possibility that the low concentrations of histamine given could have 
had a different effect on skin perfusion in the two groups, and hence on \( P_{E}O_{2} \), cannot 
be excluded, but this seems unlikely. Although the baseline \( P_{E}O_{2} \) in the two groups 
was similar, lung function measured with the squeeze technique was significantly 
impaired in the LRI group, in spite of the fact that all of the infants had been 
symptom-free for at least two weeks prior to study.

An explanation for the difference in response is that the wheezy infants, even prior to 
challenge, were compensating for uneven ventilation by modifying regional perfusion 
in the lungs (Wagner 1978). This fine balance may have been upset by a mild degree 
of induced bronchoconstriction, which in the uncompromised healthy infant did not 
disturb ventilation-perfusion (\( \dot{V}/\dot{Q} \)) balance to the same critical degree. Hence, in the 
control infants, a greater degree of bronchoconstriction was required before a 
detectable deterioration in oxygenation was observed. During methacholine challenge 
it has been shown that there is dissociation between spirometry and gas exchange 

In contrast to the studies in older children, infants were sedated and supine and the 
nebulizer was administered by face mask rather than by a mouth piece. Sedating 
wheezy infants may cause a significant fall in arterial oxygen saturation, in contrast 
to healthy infants or infants with clinically stable cystic fibrosis (Mallol 1988, Jackson 
1991). In the present study \( P_{tc}O_{2} \) was similar at the start of challenge in the two
groups. A recent adult study looked at the effect of body posture on methacholine responsiveness in nine healthy volunteers and one mild asthmatic (Shardonofsky 1992). Subjects showed significantly increased responsiveness in the supine compared with the sitting posture using indices of lung function derived from complete forced expiratory flow-volume curves, but when using indices from partial flow-volume curves the difference was not significant. This effect is unlikely to be important, as all the subjects were evaluated in the supine position.

There is no "gold standard" such as FEV₁ for measuring bronchoconstriction in infants. The squeeze technique provides an assessment of intrathoracic airway function, but there are limitations to the use of the technique in assessing bronchial responsiveness. There is controversy as to whether in healthy infants flow limitation is achieved in partial flow volume curves generated by the "squeeze" technique (Motoyama 1991), although some of these doubts have recently been dispelled (Feher 1994). If, however, baseline flow limitation had been present in the symptomatic group but not in the healthy group, the real degree of bronchoconstriction during challenge in the latter would have been even greater than the measured 30% fall in $\dot{V}_{\text{max}}{FRC}$. Hence the change in $P_{\text{n,O}_2}$ which was found after an apparent 30% fall in $\dot{V}_{\text{max}}{FRC}$ would have overestimated airway responsiveness, rather than the reverse. Therefore, the possible absence of flow limitation in the control subjects during the "squeeze" does not provide an explanation for the findings in this present study.

Measurement of bronchial response from changes in $\dot{V}_{\text{max}}{FRC}$ ignores any change in lung volume or FRC which may occur during induced bronchoconstriction. FRC
increases in some infants during challenge (Maxwell 1988). Since FRC is the reference point for flow measurement in the squeeze technique, bronchial responsiveness as measured by $V_{maxFRC}$ would be underestimated, with a spuriously high $PC_{30}$. A greater degree of hyperinflation in response to histamine challenge in the symptomatic group than in the normal infants could explain some of the apparent discrepancy in $V_{maxFRC}$ and $P_{\tau\tau}O_2$ between the groups and could even hide a real difference in bronchial responsiveness between healthy and wheezy infants. Changes in lung volume during challenge can also influence ventilation-perfusion relationships due to the interdependence of airways and lung parenchyma. In a small study of asthmatic children during histamine challenge (Hedlin 1987), $V/Q$ mismatching and hypoxaemia was demonstrated with one normal and one high $V/Q$ mode, due to hyperinflation compromising regional ventilation and blood flow, in addition to the effect of bronchoconstriction.

There is no evidence that the "squeeze" manoeuvre itself alters $P_{\tau\tau}O_2$. No difference was detected between recordings at the start of the test and after several "squeezes".

Across the 10 month age range in this study, no age-related difference in $P_{\tau\tau}O_2$ response between control and symptomatic infants were detected. In a previous study no age-related differences were seen in asthmatic children over the age range 4-11 years during methacholine challenge (Wilson 1991). Below the age of 4 years, children are generally unable to cooperate with lung function testing and above the age of 2 years it becomes difficult to sedate children adequately for testing, so that in the toddler age range the relationship between $P_{\tau\tau}O_2$ response and other aspects of lung
function during bronchoconstrictor challenge is unknown.

The contrast between normal and symptomatic infants in the effects of inhaled histamine on airway function and gas exchange has a number of important implications. Assuming $V_{\text{max FRC}}$ to be an adequate, direct measure of airway function, changes in $P_{\text{a}}O_2$ are only a very indirect result of changes in airway calibre and cannot be used as a measure of change in airway function. This restriction applies especially to epidemiological projects which involve healthy infants. The age at which these restrictions cease to apply is unknown.

In terms of disturbed physiology, it is surprising that the very mildest wheezy children as well as the clinically asthmatic infants during a symptom-free interval, demonstrated a similar degree of disturbed gas exchange. The nature of the disturbance and clinical significance deserve explanation, using bronchial challenge procedures to model acute asthma. Finally, it must be asked whether, in view of the obvious difference in $P_{\text{a}}O_2$ response between healthy and symptomatic children, the apparent lack of difference in bronchial responsiveness, when measured by $V_{\text{max FRC}}$, is an observation related to the squeeze technique and its limitations.
Table 6.1  Tidal breathing parameters for infants with a history of recurrent lower respiratory illness during the first year of life: median or mean\(^*\) values (95\% confidence interval of median or mean)

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>32</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>age (months(^+))</td>
<td>0.9</td>
<td>6.5</td>
<td>12.5</td>
</tr>
<tr>
<td>((0.7-1.2))</td>
<td>(6.2-6.7)</td>
<td>(12.3-12.8)</td>
<td></td>
</tr>
<tr>
<td>length (cm(^+))</td>
<td>53.1</td>
<td>68.2</td>
<td>76.2</td>
</tr>
<tr>
<td>((52.1-54.1))</td>
<td>(67.4-69.0)</td>
<td>(75.1-77.3)</td>
<td></td>
</tr>
<tr>
<td>(f) (min(^{-1}))</td>
<td>54</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>((51-56))</td>
<td>(33-40)</td>
<td>(30-35)</td>
<td></td>
</tr>
<tr>
<td>(t_{per}) (s)</td>
<td>0.26</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>((0.22-0.30))</td>
<td>(0.20-0.26)</td>
<td>(0.23-0.31)</td>
<td></td>
</tr>
<tr>
<td>(t_{e}) (s)</td>
<td>0.64</td>
<td>0.92</td>
<td>1.07</td>
</tr>
<tr>
<td>((0.59-0.67))</td>
<td>(0.86-1.02)</td>
<td>(1.02-1.19)</td>
<td></td>
</tr>
<tr>
<td>(t_{per}/t_{e})</td>
<td>0.46</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>((0.36-0.49))</td>
<td>(0.21-0.29)</td>
<td>(0.22-0.27)</td>
<td></td>
</tr>
<tr>
<td>CR (%)</td>
<td>13.0</td>
<td>15.2</td>
<td>11.2</td>
</tr>
<tr>
<td>(\dot{V}_{maxFRC}) (ml.s(^{-1}))</td>
<td>121</td>
<td>152(^*)</td>
<td>195(**)</td>
</tr>
<tr>
<td>((83-150))</td>
<td>(94-200)</td>
<td>(136-239)</td>
<td></td>
</tr>
</tbody>
</table>

Mann Whitney U test; compared with healthy cohort at same age:
*p<0.01; **p<0.005.
CR  Coefficient of repeatability of \(t_{per}/t_{e}\) (%)
Table 6.2  Tidal breathing parameters for infants with asthma or severe chronic lung disease (CLD) of prematurity: median or mean* values (95% CI of median or mean)

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>Severe CLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>male:female</td>
<td>11:9</td>
<td>12:8</td>
</tr>
<tr>
<td>age (months)*</td>
<td>13.4 (11.6-15.3)</td>
<td>6 (4.0-10.2)</td>
</tr>
<tr>
<td>length (cm)†</td>
<td>74.7 (72.6-76.8)</td>
<td>56.0 (48.5-62.3)</td>
</tr>
<tr>
<td>f (min⁻¹)</td>
<td>33 (28-36)</td>
<td>57***</td>
</tr>
<tr>
<td>t_{pef} (s)</td>
<td>0.24 (0.21-0.29)</td>
<td>0.11****</td>
</tr>
<tr>
<td>t_e (s)</td>
<td>1.06 (0.86-1.23)</td>
<td>0.64**</td>
</tr>
<tr>
<td>t_{pef}/t_e</td>
<td>0.24 (0.19-0.28)</td>
<td>0.16*</td>
</tr>
<tr>
<td>CR (%)</td>
<td>16.8</td>
<td>15.9</td>
</tr>
<tr>
<td>V_{max,FRC} (ml.s⁻¹)</td>
<td>146* (99-212)</td>
<td>31****</td>
</tr>
</tbody>
</table>

Mann Whitney U test; compared with healthy 1 year infants for asthma group and healthy 6 month infants for CLD group:

*p<0.005; **p<0.001; ***p<0.0005; ****p<0.0001

CR  Coefficient of repeatability of t_{pef}/t_e (%)
Table 6.3  Characteristics of responding and non-responding infants in the study looking at indices of tidal breathing during bronchial challenge: mean (95% confidence interval)

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th></th>
<th>Non-responders</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Age (months)</td>
<td>6.3</td>
<td>12.3</td>
<td>6.5</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>(5.8 - 6.7)</td>
<td>(12.0 - 12.6)</td>
<td>(5.8 - 7.2)</td>
<td>(12.1 - 13.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.2</td>
<td>9.4</td>
<td>8.1</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>(7.0 - 9.5)</td>
<td>(8.6 - 10.3)</td>
<td>(7.0 - 9.2)</td>
<td>(9.7 - 11.4)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>69.3</td>
<td>75.2</td>
<td>67.9</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td>(66.8 - 71.7)</td>
<td>(73.1 - 77.2)</td>
<td>(65.4 - 70.3)</td>
<td>(75.2 - 79.4)</td>
</tr>
</tbody>
</table>

A studied at about 6 months; B studied at about 12 months.
Table 6.4 Pre- and post-histamine lung function in 18 responding and 18 non-responding infants: mean values (95% confidence interval)

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th></th>
<th>Non-responders</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Post-histamine</td>
<td>Base</td>
<td>Post-histamine</td>
</tr>
<tr>
<td>$\dot{V}_{\text{max,RC}}$ (ml.s$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>242.8</td>
<td>139.1</td>
<td>171.9</td>
<td>170.1</td>
</tr>
<tr>
<td></td>
<td>(103.1 - 382.4)</td>
<td>(59.8 - 218.3)</td>
<td>(62.5 - 281.4)</td>
<td>(68.3 - 272.0)</td>
</tr>
<tr>
<td>B</td>
<td>223.1</td>
<td>116.9</td>
<td>289.2</td>
<td>321.0</td>
</tr>
<tr>
<td></td>
<td>(155.8 - 290.4)</td>
<td>(78.2 - 155.7)</td>
<td>(125.7 - 452.6)</td>
<td>(170.3 - 471.6)</td>
</tr>
<tr>
<td>$f$ (min$^{-1}$)</td>
<td>34.0</td>
<td>37.5**</td>
<td>34.4</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>(31.3 - 36.7)</td>
<td>(34.3 - 40.7)</td>
<td>(31.6 - 37.1)</td>
<td>(31.2 - 39.1)</td>
</tr>
<tr>
<td>$V_i$ (ml)</td>
<td>69.2</td>
<td>67.3</td>
<td>70.6</td>
<td>66.6</td>
</tr>
<tr>
<td></td>
<td>(63.4 - 74.9)</td>
<td>(61.5 - 73.2)</td>
<td>(63.0 - 78.2)</td>
<td>(61.0 - 72.3)</td>
</tr>
<tr>
<td>$t_{\text{e}}$ (s)</td>
<td>0.76</td>
<td>0.71*</td>
<td>0.76</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>(0.68 - 0.83)</td>
<td>(0.64 - 0.76)</td>
<td>(0.70 - 0.81)</td>
<td>(0.67 - 0.79)</td>
</tr>
<tr>
<td>$t_{\text{p}}$ (s)</td>
<td>1.05</td>
<td>0.95***</td>
<td>1.03</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>(0.96 - 1.15)</td>
<td>(0.86 - 1.04)</td>
<td>(0.94 - 1.12)</td>
<td>(0.93 - 1.14)</td>
</tr>
<tr>
<td>PEF (ml.s$^{-1}$)</td>
<td>108.9</td>
<td>123.1</td>
<td>112.7</td>
<td>103.5</td>
</tr>
<tr>
<td></td>
<td>(98.3 - 119.4)</td>
<td>(107.2 - 139.0)</td>
<td>(98.4 - 127.0)</td>
<td>(94.7 - 112.4)</td>
</tr>
<tr>
<td>$V/t_{\text{e}}$ (ml.s$^{-1}$)</td>
<td>66.6</td>
<td>72.6*</td>
<td>69.7</td>
<td>65.4</td>
</tr>
<tr>
<td></td>
<td>(61.6 - 71.7)</td>
<td>(66.7 - 78.6)</td>
<td>(62.2 - 77.3)</td>
<td>(59.5 - 71.4)</td>
</tr>
<tr>
<td>$t_{\text{u}}$ (s)</td>
<td>0.61</td>
<td>0.51*</td>
<td>0.50</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>(0.49 - 0.73)</td>
<td>(0.41 - 0.60)</td>
<td>(0.45 - 0.56)</td>
<td>(0.44 - 0.61)</td>
</tr>
<tr>
<td>$t_{\text{w}}/t_{\text{e}}$</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>(0.23 - 0.29)</td>
<td>(0.23 - 0.30)</td>
<td>(0.23 - 0.29)</td>
<td>(0.23 - 0.29)</td>
</tr>
<tr>
<td>$t_{\text{w}}/t_{\text{p}}$</td>
<td>0.20</td>
<td>0.21</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>(0.17 - 0.23)</td>
<td>(0.18 - 0.23)</td>
<td>(0.18 - 0.22)</td>
<td>(0.17 - 0.23)</td>
</tr>
<tr>
<td>$t_{\text{w}}/t_{\text{u}}$</td>
<td>0.32</td>
<td>0.32</td>
<td>0.31</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>(0.28 - 0.35)</td>
<td>(0.29 - 0.36)</td>
<td>(0.28 - 0.34)</td>
<td>(0.28 - 0.36)</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.005; compared with baseline data. A studied at about 6 months; B studied at about 12 months.
Table 6.5 Baseline lung function data in the study of oxygenation during bronchial challenge

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age (mth)</th>
<th>Gender</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>$\dot{V}_{\text{maxFRC}}$ (mls$^{-1}$)</th>
<th>CV $\dot{V}_{\text{maxFRC}}$ (%)</th>
<th>$P_{tcO_2}$ (kPa)</th>
<th>CV $P_{tcO_2}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>F</td>
<td>10.35</td>
<td>74</td>
<td>403</td>
<td>4.89</td>
<td>11.4</td>
<td>0.86</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>F</td>
<td>9.12</td>
<td>74</td>
<td>229</td>
<td>3.23</td>
<td>10.4</td>
<td>1.04</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>F</td>
<td>7.40</td>
<td>67</td>
<td>241</td>
<td>11.84</td>
<td>11.5</td>
<td>0.93</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>M</td>
<td>6.30</td>
<td>69</td>
<td>170</td>
<td>7.49</td>
<td>7.4</td>
<td>2.44</td>
</tr>
<tr>
<td>5</td>
<td>6.5</td>
<td>F</td>
<td>7.06</td>
<td>66.3</td>
<td>542</td>
<td>4.11</td>
<td>12.0</td>
<td>1.14</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>M</td>
<td>8.74</td>
<td>70</td>
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<td>$CV_{V_{\text{maxFRC}}}$ (%)</td>
<td>$P_{O_2}$ (kPa)</td>
<td>$CV_{P_{O_2}}$ (%)</td>
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*Median: 117%*  
95% CI: 92-182, 6.1-10.8, 9.6-10.8, 1.0-1.7

*Adjusted for length and gender

*Significant difference between normal and LRI/asthma: p=0.003.
Table 6.6 Bronchial responsiveness in the study of oxygenation and bronchial challenge

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<th>Subject no</th>
<th>( PC_{30} )</th>
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<th>( PC_{3PtcO_2} ) (g/l)</th>
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* Significant difference between normal and LRI/asthma: p=0.04.
Figure 6.1 Representative examples of tidal flow patterns in two infants.

Allowing for the difference in frequency of breathing, the expiratory flow patterns (below the dotted lines) differ considerably. The healthy infant's breathing pattern is sinusoidal, whereas the infant with CLD reaches maximum tidal expiration early in the expiratory cycle, and then has a prolonged expiratory phase.
Figure 6.2  Two modified indices of the tidal expiratory flow index $t_{pef}/t_{a}$.

Time-based record of tidal expiratory flow from a healthy infant, with expiration below the dashed line. The interval $t_{pef}$ represents the time to maximal (peak) tidal expiratory flow. $t_{pef}(a)$ and $t_{pef}(b)$ represent the time to reach values of flow of 95% of peak tidal flow, preceding and following the peak value respectively.
Figure 6.3 Changes in $P_{tc}O_2$ and in $V_{maxFRC}$ during challenge for healthy (normal) and recurrently symptomatic (LRI and wheezy) infants.

Mean values (SEM) are given for the final 3 concentrations of histamine.

$^*p<0.05; ~ ^{**}p<0.01; ~ ^{***}p<0.0001$ for difference between mean baseline $V_{maxFRC}$ and post challenge value;

$^*p<0.01; ~ ^{**}p<0.0005$ for difference between mean baseline $P_{tc}O_2$ and post challenge value.
Figure 6.4  Relationship between $\text{PC}_{30} \dot{V}_{\text{maxFRC}}$ and $\text{PC}_5 \text{P}_\text{tcO}_2$ with line of identity.

Data for 2 subjects are omitted; one healthy and one wheezy infant did not achieve a 5% fall in $\text{P}_\text{tcO}_2$ and their data could not be analysed in this way.
CHAPTER 7 IMPLICATIONS AND CONCLUSIONS

7.1 TECHNICAL

7.1.1 Future directions in infant lung function testing

The difficulties and demands placed on both the subject and the observer in testing lung function in infants have been discussed in earlier chapters. The techniques used to measure lung function in infants are less well validated than in adults and inherently have greater variability. Because of the need for sedation, it was not possible to repeat measurements of lung function on different study days.

(a) Forced expiratory manoeuvres

Further work needs to be performed to try to confirm that flow limitation is achieved during forced expiratory manoeuvres in infancy. This would require measurements to determine transmission pressure of the applied jacket pressure across the thoracic wall and to demonstrate isovolume pressure-flow relationships. Preliminary data addressing this question are just appearing (Feher 1994).

One method of achieving a reduction in the potential sources of variation of the conventional "squeeze technique", is to start the forced expiration from a higher lung volume, so producing a larger segment of the MEFV curve. Such a method, the raised volume rapid thoracic compression technique (RVRTC), has recently been developed by an Australian group (Turner 1991). By incorporating several electronically operated solenoid valves, a pump and a variable blow-off valve into the apparatus, the lung volume of the infant is raised at end-inspiration, by applying a
preset inflation pressure via the facemask. The brief airway occlusion that takes place, invokes a Hering-Breuer reflex, inducing relaxation of the inspiratory muscles. A "squeeze" is then applied in the usual manner at the same time as the inflation pressure and occlusion are released. This RVRTC technique allows forced expiratory volume-time parameters to be measured, such as forced expiratory flow in 0.75 seconds. Preliminary studies have shown reduced that the variability, measured by the intrasubject coefficient of variation, is less than half that of the conventional "squeeze" technique, which measures $\dot{V}_{\text{maxFRC}}$ (Turner 1993). One explanation for this is that the RVRTC should be independent of small changes in lung volume, as the infant's lung volume is raised to a reproducible volume by a standard inflation pressure, and the measurement of flow is referred to time rather than the infant's functional residual capacity. Another explanation is that the PEFV curve only operates in the variable tidal volume range of the MEFV curve.

Whichever techniques of forced expiratory flow are used, the procedures need to be standardized, and large numbers of normal infants need to be studied longitudinally in order to obtain reference values for the first one to two years of life.

(b) Lung volume measurements

As for other techniques of infant function measurement, there has been no standardization of the technique. Controversies include the optimal ratio of spirometer size to lung volume and the definition of "equilibration" in health and disease. Despite differences in methodology, the published reference data for FRC in infancy are similar, with fairly tight ranges (Tepper 1986a, Hanrahan 1990). Measurements
of gas-mixing may be obtained during measurement of FRC by gas dilution, and give an assessment of lung function in terms of homogeneity of ventilation. These methods, however, are not standardized, and other factors imposed by the measuring equipment are involved in determining equilibration times, including the dead space of the system and gas analyzer response time.

(c) Simpler non-invasive techniques

Respiratory inductance plethysmography is a potentially useful non-invasive technique, which would be simpler to perform in terms of the equipment required, and has the additional benefit of being practicable in unsedated infants, although calibration may be a problem. Parameters of tidal breathing can be obtained by such techniques (Stick 1992), although their application as a measure of lung function has not yet been established, and may be limited, if the findings in this study regarding tidal breathing parameters obtained during sedation via face mask and pneumotachograph (see chapters 4.4, 6.1, 6.2) also apply to freely breathing, unsedated infants. This needs to be established.

7.1.2 Future directions for bronchial responsiveness assessment in infants

The measurement of bronchial responsiveness in infants, as described in this study, relies on the measurement of PEFV curves during histamine challenge. The variability of the "squeeze" technique, together with potential changes in functional residual capacity when bronchoconstriction is induced, may lead to underestimation of bronchial responsiveness (see chapter 4.5). The RVRTC technique, described in section 7.1.1, may provide a more accurate and sensitive method of detecting reduced
lung function during histamine-induced bronchoconstriction (Turner 1993).

Various stimuli have been used to induce bronchoconstriction in infants in order to quantify bronchial responsiveness (see chapter 4.5). The histamine challenge protocol used in this study may not have been optimal. A significant number of infants, particularly older ones, either failed to respond to the maximum histamine concentration of 32g/l nebulized for 30 seconds, or woke during the challenge procedure, prior to any significant response. With hindsight it may have been preferable to quadruple rather than double the concentrations between nebulisations, or double the nebulization period from half to one minute. The latter would have had the disadvantage of lengthening the duration of the challenge procedure. Methacholine offers some advantages over histamine for infant challenge testing, having a more prolonged and cumulative action (Juniper 1978, Cartier 1983), and producing less dose-dependent upper airway irritation and cough (Juniper 1978), which contributed to infants waking before completion of the challenge procedure. Indirect bronchial challenge, using a non-isotonic agent such as hypertonic saline, offers some advantages over histamine and methacholine. Firstly, the use of pharmacological agents is avoided; secondly, bronchoconstriction is induced indirectly by inflammatory changes within the airway, rather than the response being due to a direct stimulus on the bronchial smooth muscle; and thirdly, in asthmatic adults, BR to hypertonic saline related better to the severity of exercise induced asthma symptoms than did BR to either histamine or methacholine (Makker 1993), suggesting that it may be more "physiological". Hypertonic saline challenge has not yet been evaluated in infants.
7.2 CLINICAL APPLICATIONS

The techniques of infant lung function and bronchial responsiveness testing described in this study have some clinical applications. They can be used to investigate the response to therapy in a variety of respiratory diseases in infancy. Response to different therapeutic agents, both symptomatic and prophylactic, have been investigated (Hiatt 1988, Prendiville 1987a, Kao 1987 & 1989 Tepper 1994), and also to different delivery devices (Clarke 1993).

7.3 EPIDEMIOLOGY OF LOWER RESPIRATORY ILLNESS IN INFANCY

In this study some risk factors in the neonatal period for subsequent lower respiratory illness were identified. Reduced lung function for boys, and increased bronchial responsiveness to histamine for girls, were associated with LRI in the first year of life. Larger cohort studies are required to further investigate the epidemiology of infant LRI, and study other possible risk factors. These include in-utero smoke exposure, parental and infant atopic status and allergen exposure in early life. Further studies are needed to investigate viral infections of the respiratory tract in infancy, and in particular, any differences in the immunological response to such viral infections between those infants who wheeze and those infants whose symptoms are confined to the upper respiratory tract (Balfour-Lynn 1993 & 1994).

Long term follow up of the cohort of infants in this study, in terms of respiratory symptoms, atopic symptoms, and tests of atopy, lung function and bronchial responsiveness, will be important in order to study the relationship between infant LRI and asthma at school age.
7.4 MECHANISM OF AIRWAY DISEASE

Lung growth during fetal life may be an important determinant of subsequent airway disease. Studies have shown an association between respiratory illness in early infancy and respiratory symptoms or lung function later in adult life (Britten 1987, Strachan 1990, Shaheen 1994). Another study, following a cohort of low birthweight babies, suggested that despite some remodelling of the lungs after perinatal insults and disturbed fetal lung growth, there is still a remarkable degree of tracking of lung function during infancy and childhood (Chan 1990). These studies imply that the factors which influence lung mechanics are determined during fetal development and over the first few months of life. Data already available from infant studies, suggest that the effects of passive smoking are more important during fetal life than after birth (Tager 1993, Taylor 1987). Late fetal life appears to be a critical time, as cessation of smoking earlier in pregnancy reduces the morbidity in infancy (Ahlsten 1993).

Animal studies during fetal development and early life will help to elucidate these areas further, identify some of the other risk factors involved, discover the histopathological mechanisms involved (Collins 1985 & 1986; Witten 1993), and determine the relationship between lung structure and function.

Another approach to understanding the mechanisms of LRI in infants is to analyse bronchoalveolar lavage fluid. Whilst there are ethical constraints precluding bronchial biopsy in children with LRI and asthma, and in healthy children (although in adults such studies have been performed (Holgate 1992)), it is feasible to obtain bronchoalveolar lavage fluid from infants undergoing anaesthesia for non-respiratory reasons, to study the cellular mechanisms and mediators involved in infant LRI (Grigg
Identification of risk factors for LRI or asthma, may enable preventative measures to be undertaken. In the future, molecular genetic studies may identify the genotype associated with infant LRI, as well as further characterize the genotype associated with asthma and atopy. In older children atopy is associated with asthma. If it were possible to identify in early life those infants who are at risk of developing childhood asthma, either with infant lung function tests or with genetic studies, the exciting possibility of intervention measures, such as allergen avoidance or steroid therapy, aimed at primary or secondary prevention, would open up. There may be a window of opportunity, before atopic sensitization leads to bronchial hyperresponsiveness, in which the natural history of subsequent atopic asthma could be modified with lifelong benefits!


Archer LNJ, Simpson H. Night cough counts and diary card scores in asthma. Arch Dis Child 1985;60:473-5.


ATS-ERS statement. Respiratory mechanics in infants: physiologic evaluation


Beardsmore CS, Godfrey S, Silverman M. Forced expiratory flow volume


Clarke JR, Silverman M. Partial expiratory flow volume curves. In:


Cockcroft DW, Berscheid BA, Murdock KY. Unimodal distribution of


Flucke R, Castile R, Filbrun D, Shani N, McCoy K. Measurement of full


Hanson B, McGue M, Roitman-Johnson B, Segal NL, Bouchard TJ,


Hopp RJ, Townley RG, Biven RE, Bewtra AK, Nair NM. The presence of airway reactivity before the development of asthma. Am Rev Respir Dis 1990;141:2-8.


Hyatt RE. Forced expiration. In: Handbook of Physiology 3. The


Juniper EFJ, Cockcroft DW, Hargrave FE. Histamine and methacholine inhalation tests: tidal breathing method. Laboratory procedure and


LeSouef PN. Validity of methods used to test airway responsiveness in


Makker HK, Holgate ST. Relation of the hypertonic saline responsiveness of the airways to exercise induced asthma symptom severity and to histamine or methacholine reactivity. Thorax 1993;48:142-147.


Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM, GHMA pediatricians. Diminished lung function as a predisposing factor for wheezing


Mochizuki H, Mitsuhashi M, Tokuyama K, Tajima K, Morikawa A, Kroume


Redline S, Tishler PV, Lewitter FI, Tager IB, Munoz A, Speizer FE.


Samet JM, Cushing AH, Lambert WE, Hunt WC, McLaren LC, Young SA, Skipper BJ. Comparability of parent reports of respiratory illnesses with


Tager IB, Hanrahan JP, Tosteson TD, Castile RG, Brown RW, Weiss ST,


Tepper RS, Steffan M. Airway responsiveness in infants: Comparison of


Ulrik CS. Bronchial responsiveness to inhaled histamine in both adults with intrinsic and extrinsic asthma: the importance of prechallenge forced expiratory volume in 1 second. J Allergy Clin Immunol 1993;91:120-6.


Welliver R C, Duffy L. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at


If you are interested in the work we are doing, please tell Sister on the ward. The study is being carried out by Dr Jane Clarke and Mandy Reese who both visit the wards at Queen Charlotte's regularly and Sister can ask either of them to come and discuss the tests with you while you are there.

Alternatively you can phone at no charge by dialling internal number 4316 from here. If you have already been discharged you can telephone the Neonatal Unit - direct line 081-740 3174 and ask for Jane or Mandy.

You are under absolutely no obligation to volunteer for these tests. However we will be pleased to answer any questions you may have concerning your baby and asthma.

We look forward to meeting you.

Dr Jane Clarke & Mandy Reese,
Neonatal Unit,
Hammersmith Hospital,
Du Cane Road, London W12 0HA.
Wheezing and baby asthma is common in the infants and toddlers of parents who have a history of asthma, eczema or hayfever.

Asthma is a chronic disease and the symptoms of breathlessness and wheezing are often ignored in early childhood.

Modern medicines are extremely effective in the treatment of asthma and therefore early diagnosis could avoid unnecessary suffering for the asthmatic child.

In the Neonatal Unit at the Hammersmith Hospital a simple breathing test has been developed which can be performed on newborn babies.

The test is carried out while the baby sleeps and is so gentle that the baby will not even stir while the test is performed. No needles, tubes or painful procedures are involved and so far the test has been safely carried out on over 100 babies.

We would like to use the test on every baby at risk of developing asthma to see if we can predict in newborn babies why some children go on to get asthma while others do not.

If you or your baby’s father have a history of asthma, eczema or hayfever, we would be pleased to perform the breathing test on your baby. The test will reveal whether your baby is likely to be wheezy or develop asthma in childhood.
Dear

We know that up to half of babies whose parents both have asthma, (or those who had this disorder as children) and a smaller number who had eczema or hayfever, will develop wheezing in infancy. Recently, we have made some important discoveries about the nature of infant wheeze and how it might be treated. We do not know why some babies become wheezy, and whether it is possible to predict this.

We would like your baby, , to take part in some breathing tests before he/she is one month old, and again at 6 months and one year. The tests are of a type carried out here on many wheezy infants over the last five years, under light sedation with a simple sleeping medicine and are completely safe. During the tests, which last up to two hours, we will measure the baby's breathing in response to tiny doses of histamine given by nebuliser. This tells us the sensitivity of the bronchial tubes and how this sensitivity changes over the year. You may be present throughout all of the tests.

If, after a full explanation, you are willing to take part in these tests, please sign below.

Thank you very much for your assistance.

Yours sincerely

Dr Jane Clarke
Research Fellow

Signed:

A PART OF HAMMERSMITH HOSPITALS NHS TRUST
APPENDIX III: QUESTIONNAIRE FOR INITIAL VISIT

Infant Cohort Study

INITIAL VISIT

Name: DOB: Hospital no. mother:
Address: Age:
Tel no.: GP:

Birth History

Pregnancy: complications
drugs
Labour: ruptured membranes
drugs

Delivery: normal forceps Em LSCS El LSCS

Indications

Infant: gestation weight

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Neonatal problems: respiratory
other

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Feeding: breast bottle
Drugs:

Social

Maternal smoking: at conception during pregnancy since birth
Housedust Pets Heating
### Family History

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<thead>
<tr>
<th></th>
<th>Age</th>
<th>Asthma</th>
<th>Eczema</th>
<th>Hayfever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siblings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Examination

**Age**

**Weight:** kg  **Height:** cm

<table>
<thead>
<tr>
<th>System</th>
<th>Normal</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>RR</td>
<td>Hyperinflation Recession Cough</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td>Wheeze</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genito-urinary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculo-skeletal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX IV: QUESTIONNAIRE FOR FOLLOW-UP VISITS

Infant Cohort Study
FOLLOW UP VISIT

Name: ____________________________ Date: ____________________________

Age: _______  DOB: _______  Hospital no: _______

Personal History

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
<th>Age at onset</th>
<th>Precipitating factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough day/night</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeze day/night</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eczema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

URTIs: ____________________________

Other illness: ____________________________

Feeds: ____________________________

Drugs: ____________________________

Immunizations: ____________________________

Social History

Smoking: mother father

Housedust

Pets

Heating

Family History

Atopy

Asthma

Examination

Weight: _______ kg  Height: _______ cm

<table>
<thead>
<tr>
<th>System</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>RR</td>
<td>Hyperinflation  Recession Cough Wheeze</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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## APPENDIX V: ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>area at site of flow limitation</td>
</tr>
<tr>
<td>BR</td>
<td>bronchial responsiveness</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CLD</td>
<td>chronic lung disease of prematurity</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CorrF</td>
<td>correction factor for converting measurements from ATPS to BTPS</td>
</tr>
<tr>
<td>CR</td>
<td>coefficient of repeatability</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>d</td>
<td>gas density</td>
</tr>
<tr>
<td>dP&lt;sub&gt;tm&lt;/sub&gt;</td>
<td>transmural pressure</td>
</tr>
<tr>
<td>f</td>
<td>breathing frequency</td>
</tr>
<tr>
<td>FRC</td>
<td>functional residual capacity</td>
</tr>
<tr>
<td>He&lt;sub&gt;f&lt;/sub&gt;</td>
<td>final helium concentration</td>
</tr>
<tr>
<td>He&lt;sub&gt;i&lt;/sub&gt;</td>
<td>initial helium concentration</td>
</tr>
<tr>
<td>LRI</td>
<td>lower respiratory tract illness</td>
</tr>
<tr>
<td>MEFV</td>
<td>maximal forced expiratory flow-volume</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>oxygen</td>
</tr>
<tr>
<td>PC&lt;sub&gt;5&lt;/sub&gt;P&lt;sub&gt;TcO&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;</td>
<td>the provoking concentration of bronchoconstrictor causing a 5% fall in transcutaneous oxygen tension from baseline</td>
</tr>
<tr>
<td>PC&lt;sub&gt;30&lt;/sub&gt;</td>
<td>the provoking concentration of bronchoconstrictor causing a 30% fall in lung function from baseline</td>
</tr>
</tbody>
</table>
the provoking concentration of bronchoconstrictor causing a 30% fall in maximal flow at functional residual capacity from baseline

PEF

peak tidal expiratory flow

PEFV

partial expiratory flow-volume

Pj

jacket pressure

PtcO₂

transcutaneous oxygen tension

PtcO₂

transcutaneous carbon dioxide tension

SaO₂

oxygen saturation

SD

standard deviation

tₜ

total expiratory time

TGV

thoracic gas volume

tᵢ

total inspiratory time

tₚₑᵢᵣ

time to maximal tidal expiratory flow

tₑᵢ

dead space of mask

Vₑᵢᵣ

dead space of spirometer

Vₑᵢᵣ

filling volume of spirometer bell

Vₑᵢᵣ

maximal flow at functional residual capacity

Vₑᵢᵣ

ventilation perfusion

Vₑᵢᵣ

tidal volume circuit (ml)

Vₑᵢᵣ

flow at wave speed

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APPENDIX VI: PERSONAL PUBLICATIONS PERTAINING TO THIS THESIS


