Towards a new method for the measurement of bronchial responsiveness in infancy

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by

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Abstract
Towards a new method for the assessment of bronchial responsiveness in infancy
Dr Isobel Brookes

Neonatal bronchial hyperresponsiveness (BHR) may predict respiratory symptoms in infancy and subsequent decreased lung function. Examination of neonatal BHR, on a larger scale than has previously been possible, would ultimately enable investigation of the relationship between foetal environment and airway function, the role of BHR in airway disease and postnatal lung development, and the contribution of BHR to wheezing phenotypes in infancy.

The aim of this project was to develop a test of bronchial responsiveness suitable for unsedated infants in a domiciliary setting, prior to population studies. Current techniques involve prolonged procedures under sedation in a laboratory.

Resistance by interruption ($R_{int}$) was comprehensively evaluated in the course of this work. Feasibility has been demonstrated in 38 unsedated infants and the entire process of obtaining data from initial approach of 277 families to laboratory success rates examined. For the first time, $R_{int}$ measurements have been attempted in 14 unsedated infants in the community. Preliminary reference values have been generated in 61 infants, by incorporating data from collaboration with others.

$R_{int}$ and another technique applicable in unsedated infants, the high speed interrupter technique (HIT) were compared with the rapid thoracoabdominal compression (RTC) method in the context of a bronchial challenge test with doses of 0.9%, 2%, and 4% saline, carried out in 28 sedated infants with a history of wheezing. Following saline challenge, complex changes in HIT were found, which discount it as a suitable test for bronchial challenge, but have added to theories of the physiology of infant wheezing. Large decreases in $R_{int}$ were demonstrated after saline challenge, which may be explained by the dynamic nature of infant breathing patterns, which were also examined.

In summary, both techniques (HIT and $R_{int}$), originally proposed for use in assessment of BHR in unsedated infants have been excluded from use in this context.

(299 words)
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BR</td>
<td>Bronchial responsiveness</td>
</tr>
<tr>
<td>BHR</td>
<td>Bronchial hyper responsiveness</td>
</tr>
<tr>
<td>cl</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>EEV</td>
<td>Elastic equilibrium volume</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Forced expiratory volume in t seconds</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Forced expiratory flow at x % of FVC</td>
</tr>
<tr>
<td>f&lt;sub&gt;ar,l&lt;/sub&gt;</td>
<td>Frequency of the first antiresonance</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>G&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Airway Conductance</td>
</tr>
<tr>
<td>G&lt;sub&gt;int&lt;/sub&gt;</td>
<td>Interrupter Conductance</td>
</tr>
<tr>
<td>HIT</td>
<td>High-speed interrupter technique</td>
</tr>
<tr>
<td>LAs</td>
<td>Limits of agreement</td>
</tr>
<tr>
<td>MTEF</td>
<td>Mean tidal expiratory flow</td>
</tr>
<tr>
<td>P&lt;sub&gt;ao&lt;/sub&gt;</td>
<td>Pressure at the airway opening</td>
</tr>
<tr>
<td>P&lt;sub&gt;alv&lt;/sub&gt;</td>
<td>Pressure in the alveoli</td>
</tr>
<tr>
<td>PC&lt;sub&gt;30&lt;/sub&gt;</td>
<td>Dose of inhaled stimulus causing a 30% fall in lung function</td>
</tr>
<tr>
<td>PNT</td>
<td>Pneumotachograph</td>
</tr>
<tr>
<td>PTEF</td>
<td>Peak tidal expiratory flow</td>
</tr>
<tr>
<td>R&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Airways resistance</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement (sleep)</td>
</tr>
<tr>
<td>RTC</td>
<td>Rapid thoracoabdominal compression technique</td>
</tr>
<tr>
<td>RVRTC</td>
<td>Raised Volume rapid thoracoabdominal compression technique</td>
</tr>
<tr>
<td>R&lt;sub&gt;int&lt;/sub&gt;</td>
<td>Resistance by interruption</td>
</tr>
<tr>
<td>sR&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>Specific airway resistance</td>
</tr>
<tr>
<td>Sa&lt;sub&gt;O2&lt;/sub&gt;</td>
<td>Oxygen saturation</td>
</tr>
<tr>
<td>SIDS</td>
<td>Sudden Infant Death Syndrome</td>
</tr>
<tr>
<td>T&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Expiratory time</td>
</tr>
<tr>
<td>TGV</td>
<td>Thoracic Gas Volume</td>
</tr>
<tr>
<td>T&lt;sub&gt;rs&lt;/sub&gt;</td>
<td>Time constant of the respiratory system</td>
</tr>
<tr>
<td>T&lt;sub&gt;pfe&lt;/sub&gt;/T&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Ratio of time to peak expiratory flow to expiratory time</td>
</tr>
<tr>
<td>TeO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Transcutaneous oxygen tension</td>
</tr>
<tr>
<td>URTI</td>
<td>Upper respiratory tract infections</td>
</tr>
<tr>
<td>V'*max&lt;sub&gt;FRC&lt;/sub&gt;</td>
<td>Maximal flow at functional residual capacity</td>
</tr>
<tr>
<td>V&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>Z&lt;sub&gt;in&lt;/sub&gt;</td>
<td>Respiratory input impedance</td>
</tr>
</tbody>
</table>
Aims and Objectives

Overall Objective of the work:
To develop a test of bronchial responsiveness suitable for use in unsedated infants in a domiciliary setting.

1. Evaluation of interrupter resistance (R_{int}) in unsedated infants in the laboratory and in the home, including examination of recruitment and success rates.

2. Examination of repeatability of R_{int} to aid interpretation of challenge studies.

3. Development and standardisation of a saline bronchial challenge test in sedated infants (with a history of wheeze), in order to:
   (i) Identify the ideal concentration of saline (0.9%, 2%, or 4%) producing a significant change in lung function (R_{int} or the high speed interrupter technique (HIT)), but not forced flows measured using the rapid thoracoabdominal compression (RTC) technique.
   (ii) Differentiate between R_{int} or HIT as a suitable measurement of the response to saline, applicable to unsedated infants.

4. Generation of reference values for R_{int} in healthy unsedated infants from 4-12 weeks of age, and investigation of the factors related to R_{int} measurements using data from (1) and data from collaboration with colleagues in Bern.
2. Background: Bronchial Responsiveness in Infancy

2.1 Introduction

Bronchial responsiveness, a central concept in asthma, describes the response of the respiratory system to an inhaled stimulus. The classical model is of the interaction between a specific environmental trigger and hyperresponsive airways, leading to airway narrowing, and asthma symptoms. Whether bronchial hyperresponsiveness (BHR) exists as a primary defect from birth, or is acquired as a secondary effect of chemical, infective, or allergic inflammation is unknown, although evidence suggests that all may contribute at different ages. Population studies have suggested that increased bronchial responsiveness and atopy are inherited independently (1). Investigation of neonatal BHR enables the study of the genetic and developmental component, prior to the establishment of postnatal inflammation. Longitudinal studies could then perhaps enable the contribution of early BHR to wheezing throughout childhood and beyond to be examined.

The painstaking studies of bronchial responsiveness in infants over the last fifteen years have challenged previous concepts of airway function in infants, such as the capacity of infants’ airways to respond to $\beta_2$ agonists. The role of genetic and environmental influences on infant BHR, the association with current or future wheezing illnesses, the natural history and potential prognostic value of measuring BHR have all been examined to some extent. However, pharmacological studies have been small scale, limited to a handful of centres worldwide, and very demanding on children, families and research staff (table 2.1).

Developing knowledge of the underlying mechanisms of respiratory disease may provide opportunities for new therapies. For example, in older children with difficult asthma, there has been a move towards treatment directed by knowledge of the “asthma phenotype” (using information about level of BHR, airway inflammation and persistent airflow limitation). Knowledge of airway pathology (obtained via induced sputum or directly via bronchoscopy) is, in some hands, driving therapy. (2).
2.2 The concept of bronchial responsiveness: stimulus and response

Bronchial responsiveness is defined as the (intrathoracic) airway response to a standardized stimulus. Bronchial hyperresponsiveness (BHR) generally refers to an exaggerated degree of airway narrowing, which occurs at a lower threshold dose, or which exhibits a steeper dose response slope (greater reactivity) or exhibits a greater (or unlimited) maximum response (Figure 2.1). Despite this simplistic representation (envisaged by some as a simple “organ bath” dose-response model), the complex interaction between the stimulus and the airway may be modified by numerous factors, related both to the stimulus and the response of the airways. Various stimuli may act in different ways to cause bronchoconstriction via direct action on smooth muscle cells (e.g. methacholine) or indirect action via inflammatory or neural pathways (e.g. hypertonic saline). Different pathological, genetic or developmental factors may contribute to BHR such as underlying inflammation, epithelial damage, and altered smooth muscle cell biology or airway structure. These factors will all be discussed in more detail below, in section 2.3.
2.3 Bronchial Responsiveness in Infants: Current Knowledge

The performance of bronchial challenge tests is highly specialized and therefore limited to research laboratories. Potential clinical applications of the study of infant BHR (including the study of the response to bronchodilators) include improved management of acute wheezy episodes, prognosis for individual infants, rational treatment of persistently wheezing infants, and potential for targeted preventative therapy in those with risk factors for later persistent disease. At present all of these (except perhaps the use of bronchodilators acutely) remain somewhat of a mystery.

Research studies in this field pose a huge undertaking, but provide an opportunity to examine two main areas of interest. Firstly, if neonatal BHR could be quantified reliably in a large cohort of infants who then underwent comprehensive follow-up, it would be possible to determine the risk factors for, and natural history of infant BHR, and to determine whether BHR during infancy is a risk factor for future ill health. If neonatal physiological risk factors ("pre-modelling") are identified, then their relative contributions alongside other factors such as infection or allergy ("re-modelling") to subsequent illness could be studied. As understanding of the mechanisms by which additional factors act increases, new therapies may be developed. It may then be possible to study therapy, in high-risk infants, targeted at preventing other additional factors impacting on eventual disease burden. Physiological risk factors are best identified in cohort studies, which begin at or before birth, and track lung function and symptoms throughout life. Two such studies have been carried out to date, and have provided much new information about neonatal BHR(3;4), but both have relied on cumbersome techniques for measurement of BHR, which have taken place under sedation in a hospital laboratory. Hence, relatively small numbers have been studied with consequent limitations on statistical power.

Targeted preventative therapy would demand greater understanding of the mechanisms that underlie the acquisition of neonatal BHR (and how these interact with additional environmental factors). Neonatal BHR may be heavily influenced by prenatal developmental factors. Genetic risk factors may not be avoidable, but are unlikely to operate in isolation from other prenatal factors such as intrauterine growth and nutrition, premature delivery and exposure to maternal smoking or atopy. Potential mechanisms underlying neonatal BHR will now be examined, before summarizing current knowledge of infant BHR, in both cross sectional and follow up studies.
2.3.1. Potential Mechanisms underlying BHR in infants

It seems logical to suggest that bronchial responsiveness in infancy, particularly in the neonatal period, will be determined by prenatal developmental differences in airway structure and function rather than any post inflammatory changes. Such innate differences, which may result from genetic or intrauterine environmental factors, may explain the increased responsiveness seen in some newborn infants, which may have lifelong implications for lung health. Examination of early BHR in relation to foetal environmental factors such as maternal antenatal smoking, foetal nutrition, and premature delivery and in relation to genetic factors (such as gender) may shed light on the mechanisms that underlie BHR in some neonates.

2.3.1.1 Genetic Risk Factors

The genetic character of BHR is likely to be multifactorial(5), and the particular genes governing infant BHR have yet to be formally investigated. They may well be distinct from those governing lung function, the genetic basis of which in infants is also unknown. Within the field of asthma genetics, there is mounting evidence that genes governing (adult type) BHR are inherited separately from those determining atopy (1;6). Evidence from the Australian birth cohort study suggested that infant and childhood BHR are probably the result of different mechanisms(3). Early BHR may reflect genetic and in utero environmental conditions, with later childhood BHR being influenced greatly by atopic sensitization. If this is shown to be the case then they may well have different genetic risk factors.

If infant BHR does contribute to later asthma then genes known to be associated with that condition are logical targets for initial investigation of such genetic influences, but only if infant BHR can be measured in sufficient numbers of subjects at birth. The current practical difficulties in defining BHR “phenotype” in infants in adequate numbers mean that complex genetic studies are impossible. One hypothesis is that polymorphisms of the beta2-adrenoreceptor (β2AR), might affect airway smooth muscle growth and function during both prenatal and neonatal life, particularly if the infant has been exposed to maternal β2 agonists. In that case likely candidate genes governing neonatal BHR would include such polymorphisms. In both the Australian and British birth cohorts genotyping...
for common polymorphisms of this receptor has been carried out retrospectively. In the Perth cohort, the presence of a least one Arg16 allele of the $\beta_2$AR gene was associated with both neonatal BHR and reduced lung function at 11 yrs(7). In the London cohort there was no affect of genotype on BHR during the neonatal period or at 10yrs(8). However forced expiratory flow (maximal flow at functional residual capacity, $V_{\text{maxFRC}}$) was reduced in those possessing Gln 27 or Arg16 alleles, suggesting a genetic influence on neonatal lung function.

2.3.1.2 Antenatal factors leading to altered neonatal airway structure and function

At present because small numbers of infants have been studied, antenatal factors are difficult to quantify. Impaired foetal nutrition, leading to low birth weight and decreased airway growth has been suggested as a factor predisposing to chronic obstructive pulmonary disease decades later(9), but whether infant BHR may in any way link these two phenomena is completely unknown. Decreased lung function in adulthood after exposure to maternal smoking before and after birth has been demonstrated recently (10).

Bronchial smooth muscle first appears at 6-8 weeks of gestation (the embryonic stage of lung development), and is followed by the formation of the bronchial tree during the pseudo-glandular stage in a process termed lung branching morphogenesis (11). Coinciding with this period is the appearance of spontaneous airway contractions, which are thought to contribute to lung growth. Lung hypoplasia results when the contractions are obliterated pharmacologically (12). After birth there is a particularly rapid increase in the relative amount of bronchiolar smooth muscle, which is increased in infants who have undergone mechanical ventilation (11). Disturbance of the normal patterns of lung development could account for measured differences in lung function in the neonatal period, whether this be altered structure, innervation or cellular function.

In foetal life one of the most important factors in lung development is likely to be exposure to maternal smoking(13). Lung function is impaired in exposed infants(14-16), who are also more likely to develop wheeze before the age of 3 years, independently of family history of asthma, socio-economic factors and birthweight (17). Maternal smoking has also been shown to be associated with increased airway responsiveness in infants, although on further analysis of a larger group of subjects this link was not confirmed (18). The mechanisms that lead to such changes in lung function remain a mystery, but progress in unravelling them has been made using animal models as well as human tissue, (usually
from infants dying of sudden infant death syndrome (SIDS)), and these will be discussed below.

*Changes in airway wall thickness,* will greatly influence the degree of airway narrowing occurring in response to a given amount of smooth muscle shortening. Baseline airway resistance is proportional to the fourth power of the luminal radius, assuming laminar flow conditions and an airway of circular cross section. Therefore a change in the airway lumen diameter (regardless of which component of the wall is increased in thickness) will result in an exponential change in airway resistance. When the airways of infants dying from sudden infant death syndrome were examined, there was a significant increase in airway wall thickness in those whose mothers had smoked heavily (both before and after birth) compared with those whose mothers were non-smokers(19). The differences were in epithelial and inner airway wall regions, rather than smooth muscle thickness. Under conditions of airway narrowing in response to an inhaled stimulus, decrease in diameter for a given amount of smooth muscle shortening will be greater if the airway wall is thicker. In this situation, increased BR can be described as “geometric” in nature.

The degree to which a particular airway narrows when smooth muscle cells contract also depends on the “load” against which the muscle contracts and the “force” with which it contracts.

*Load* consists of a number of forces that act to oppose smooth muscle shortening, the major one being the elastic recoil of the lung parenchyma. Components of the conducting airways (epithelium, connective tissue, and smooth muscle) offer little extrinsic support, and terminal and respiratory bronchioles are likely to be tethered to adjacent interalveolar septa, with additional support from the pulmonary vessels(20). Disruption of the delicate balance between the airway and the lung parenchyma, (so called “uncoupling”) may lead to relatively unopposed smooth muscle contraction and exaggerated bronchoconstriction for the same stimulus, which will amplify any underlying increased narrowing due to co-existing geometric factors. In addition, increased thickness of the airway wall outside the smooth muscle layer may reduce transmission of lung elastic recoil when the smooth muscle contracts. In a guinea pig model, exposure to cigarette smoke in utero resulted in increased mean distance between alveolar attachments to the airway adventitia and increased postnatal airway responsiveness (to acetylcholine) (21) when compared with animals with no exposure, or postnatal exposure only. The area of the airway walls was also increased between exposed and unexposed animals although this did not reach statistical significance. Recently the same group demonstrated an increased
distance between alveolar attachments in the airways of a group of SIDS infants exposed to cigarette smoke in utero (with or without post natal exposure) compared with those without exposure(22). The authors speculated that reduced alveolarisation, perhaps as part of generally reduced somatic growth, may have led to the observed changes, which in turn could result in increased airway responsiveness by decreasing “load”. In addition to smoke exposure, developmental factors may also play a role, and work in rabbits has demonstrated that immature rabbits develop greater maximum increases in pulmonary resistance than mature rabbits(23). In an attempt to unravel the underlying mechanism the same group later demonstrated increased percentage of smooth muscle in the airway wall, a lower proportion of cartilage, as well as fewer alveolar attachments than in the mature animals(24).

Force generated by smooth muscle contraction is likely to be affected by changes in smooth muscle structure and function, which have been cited as important factors in determining the degree to which airways can narrow(25). Differences in smooth muscle cell biochemistry have been implicated in the excessive airway narrowing seen in asthma, and have been shown to occur after sensitisation and allergen challenge(26). Recently, infiltration of the airway smooth muscle by mast cells has been shown in asthma, but not in healthy controls or patients with eosinophilic bronchitis, a condition characterised by a lack of BHR(27). The number of mast cells in the smooth muscle was correlated with the degree of airway hyperresponsiveness. The timing of migration of mast cells during the course of the development of asthma is unknown, so whether mast cells have a role in infant BHR remains unclear. Access to infant airway tissue in order to further investigate these factors is extremely limited.

Even when histological studies are possible, airways are examined at a single time point and after preparation and staining. In life, human airways exist as part of a dynamic system. Recently it has been suggested by Que and co-workers that the configuration of the tracheo-bronchial tree is constantly changing, with the diameter of each of the hundreds of thousands of airways constantly changing around a set point (“homeokinesis”)(28). They measured respiratory impedance for 15 minute periods and demonstrated that, as well as excess narrowing in response to challenge, the variability of respiratory impedance was much higher in asthmatic patients, which was probably due to “unloading” of the airway smooth muscle. They suggest that with increased variability comes the risk of statistically improbable events, such as severe asthma attacks. How this variability may be expressed in young infants and which congenital factors may predispose to it is unclear, but it may well impact on detected levels of BHR.
2.3.1.3 Inflammation modulated by prenatal factors

When markers of atopy (such as cord IgE or skin reactivity to common allergens) are measured in newborns there seems to be little association with neonatal or infant responsiveness(3). The study of markers of inflammation in sputum in attempts to correlate levels of BR and airway inflammation has been widely applied in older children(29), but are currently impractical in infants. Recently, exhaled nitric oxide (eNo), an alternative non-invasive marker of airway inflammation widely used in older children and adults, has been measured in infants. One technique employed a modification of measurement of forced expiratory flow in infants and required the infants to be sedated. The mean level of eNO in the wheezy infants was significantly higher than the level in healthy infants (30). An alternative approach, where measurements were made successfully in unsedated infants during the first weeks of life, demonstrated lower exhaled nitric oxide levels in those infants exposed to antenatal maternal smoking(31), a phenomenon seen in adult smokers. Later work suggested this effect was modified by maternal atopy(32).

Whether differences in exhaled nitric oxide levels in infants reflect active “inflammation” or congenital differences in production or degradation is unclear. The relationship between exhaled nitric oxide, bronchial responsiveness and future symptoms in infants has yet to be explored.

2.3.2. Pioneering Studies

Early studies of bronchial responsiveness established that is was both feasible and safe to demonstrate bronchial responsiveness to a range of stimuli in infants. Initially plethysmographic assessments of \( R_{aw} \) were used to measure the response to inhaled carbachol(33) and nebulised distilled water(34). Following development of the RTC technique change in intrathoracic airway function could be examined more specifically. Wheezy infants developed airway narrowing, detected using RTC, in response to histamine(35), which was reversed using salbutamol. Healthy infants also developed airway narrowing in response to inhaled methacholine, which was also reversible with bronchodilator(36). These and other studies are summarised in table 2.1.

One clear finding from early cross sectional studies was that the level of bronchial responsiveness was not related to baseline airway function, suggesting that geometric
factors were not the sole determinants of the degree of airway narrowing following stimulation(18;36)

2.3.3. Clinical Issues: Airway pharmacology and management of acute episodes of wheeze

Drugs used in the management of asthma in older children and adults often have frustratingly little effect in infants with acute episodes of wheeze, and information from bronchial challenge tests has suggested that in fact bronchodilators could have deleterious effects on lung function in wheezy infants(37). These data complement those demonstrating a lack of a clinical effect of bronchodilators during acute bronchiolitis(38)(although differing definitions of bronchiolitis on each side of the Atlantic complicate interpretation). A recent Cochrane Review concluded that there was no clear benefit of using β2 agonists in the management of recurrent wheeze in the first two years of life, although the evidence was conflicting(39).

In the case of β2 agonists it had been suggested that a lack of sufficient bronchial smooth muscle and β2 receptors in the infant airway explained the lack of clinical response. The detection of a response to inhaled bronchodilator in an early infant bronchial challenge test was important evidence that in fact β2 adrenergic receptors could be found in the infant lower airways(40). In fact when given alone the physiological effects of salbutamol were paradoxical(37), leading to reduced forced expiratory flows in asymptomatic infants with a previous history of wheeze. Loss of smooth muscle tone (and increased airway wall compliance) without any redeeming increase in airway calibre, after administration of a β2 agonist, could explain why forced flows decreased. Ten years later work using high frequency impedance in the context of a methacholine challenge also suggested that changes in airway wall tone might take place during an inhalational challenge, prior to any change in forced flows(41). Congenital (or early acquired) differences in airway wall compliance were proposed as a mechanism for wheezing disorders in infants(42).

Conversely when infants underwent histamine-induced bronchial challenge salbutamol given prior to (40) or after (43) histamine was capable of reversing or speeding the recovery from histamine induced bronchoconstriction. Another study investigated the effect of ipratropium bromide in similar patients(44), and demonstrated no effect on forced expiratory flows, but significantly improved airway resistance during tidal breathing. This may reflect a selective effect on the larger intrathoracic airways.
2.3.4. The role of BHR in lower respiratory tract illness in infancy

The relationship between wheezing in infancy and bronchial responsiveness measured during infancy (following recovery from the acute episode) has been examined in several studies. Generally there is no association between current symptoms of wheeze and current level of bronchial responsiveness, when measured after the neonatal period (45;46), although several studies have shown decreased baseline forced flows in infants with a history of wheeze(45;46;47). It is likely that by the time symptoms have appeared the infant’s respiratory system has been exposed to a number of environmental factors that interact in a complex manner with airway characteristics in the developing lung, some present from birth, (“remodelling” superimposed on “premodelling”).

When measured in a cross sectional study, BHR is increased in the aftermath of an acute respiratory tract infection in infancy (48). Those with low BHR at birth who are recovering from a subsequent wheezy episode induced by an acute viral infection (but destined to never wheeze again) may have transient BHR. Subjects such as these introduce noise and dilute the effect of infants with “congenital BHR” when associations with future illness are sought. In this situation it is likely to be extremely difficult to identify all the factors that may contribute to future wheezing. Adults with symptoms of wheeze with colds were shown to develop a progressive increase in BHR, measured at a maximum of 17 days after an experimentally induced human coronavirus infection(49). At this point symptoms and lung function had all returned to normal, and the time course for resolution of the “post infective” BHR is unknown.

If infants with a history of bronchiolitis are subsequently shown to have increased bronchial responsiveness as in one study (10 months after the illness)(48), this does not necessarily imply a causal relationship. The development of clinical bronchiolitis may merely be a marker of underlying increased bronchial responsiveness, which is expressed as the clinical syndrome of acute bronchiolitis when the infant acquires an RSV infection. Recent follow up of a cohort of infants in whom neonatal bronchial responsiveness was measured did not find BHR in those subsequently developing bronchiolitis(50). In that study however, bronchiolitis was defined as “physician diagnosed bronchiolitis before the second birthday”, which may have included some infants with other conditions such as viral wheeze.

In summary, if the role of congenital BHR with future illness is to be studied measurements must be made as soon as possible after birth, before confounding by environmental factors precludes the deduction of any real causal association.
2.3.5 Longitudinal Studies: Infant BHR as a risk factor in future illness

Bronchial hyperresponsiveness is present in some children and adults prior to the development of asthma symptoms(51), suggesting the existence of a hidden population of individuals with a predisposition to asthma or even chronic obstructive pulmonary disease in later life.

Two birth cohort studies have addressed the hypothesis that differences in lung function and BHR very early in infancy may be risk factors for subsequent airway disease in early childhood or later persistent asthma. So far such cohorts have involved small numbers of infants (a total of 233 infants), with results published up to 11 yrs from one study in Perth, Western Australia, and age 10 yrs from the other in London. In a larger cohort study from Tucson, Arizona it had previously been shown that decreased lung function early in infancy (forced expiration measured using the RTC technique) was associated with transient wheezing before the age 3 years(52). Bronchial responsiveness was not measured in those infants.

In Perth, children who had bronchial hyperresponsiveness to histamine at age one month wheezed more often during the first two years of life (47). When wheezy infants were divided into those developing wheeze during the first or second year, and those with wheeze persisting into the second year, only those with wheeze developing in the second year of life had increased bronchial responsiveness at age one month, relative to non-wheeze. The authors suggested that increased neonatal bronchial responsiveness was a risk factor, not for resolving transient infant wheeze, but more persistent problems, perhaps the first symptoms of “asthma”. Seven percent of the infant cohort developed clinical bronchiolitis (only 1% of the cohort required hospital admission) and there was no difference between the level of bronchial responsiveness between this very small subset and the rest of the cohort(53).

When 95 of the original cohort were studied again at six years (by which time spirometry and an “adult type” histamine challenge were feasible in most children), this hypothesis was confirmed. The level of bronchial responsiveness at one month of age was predictive at age six of a physician diagnosis of asthma, decreased forced expiratory flow in one second (FEV$_1$) and forced vital capacity, and increased lower respiratory tract symptoms. The correlation of neonatal BR with future lung function was low (FEV 1 %
predicted \( r^2 = 0.35 \), FVC % predicted \( r^2 = 0.31 \)(3). The associations were independent of markers of atopy at age one month and six years, and parental history of asthma or smoking. However, bronchial responsiveness at age six was not related to neonatal bronchial responsiveness, but more closely to markers of current atopy. This was the first report to establish that neonatal bronchial responsiveness could be a marker of congenital airway function predisposing to asthma, but that bronchial responsiveness in later childhood was probably based on an independent mechanism.

Further evaluation of the same cohort at age 11 revealed that increased bronchial responsiveness during the neonatal period was associated with future asthma that often resolved (asthma “ever” by 11 yrs), and was not associated with features of atopy. In contrast BHR at age 11 yrs was associated with persistent symptoms and (as at age six) to atopy(54), suggesting that BHR in either infancy or childhood may be an important determinant of wheezing phenotype. Individuals with atopy and BHR at 11 yrs and decreased \( V' \text{maxFRC} \) at one month (rather than BHR at one month) were most likely to have persistent wheeze. These data are in keeping with that of Stein and colleagues who proposed that childhood wheeze could be considered as early nonatopic wheeze and later atopic wheeze(55)

Another group of 73 infants underwent bronchial challenge as neonates in London, and were initially followed to a year of age. In girls an increased level of bronchial responsiveness at one month was predictive of wheezing during the first year of life(4). Interestingly this association in female infants was also found in the Perth two-year follow-up study, suggesting an influence of gender over the interaction between baseline bronchial responsiveness and later illness. In boys there was a trend towards lower baseline forced flows in those who subsequently wheezed. Relative changes in FRC in response to histamine challenge were cited as a possible explanation for this gender difference, with those having lower forced flows elevating their FRC in response to airway narrowing during challenge and thus blunting subsequent ability to detect a response to histamine. Previously the same group had described increases in FRC (using respiratory inductance plethysmography) during histamine challenge in a group of previously wheezy infants(56).

Follow-up of this cohort to 10 years has recently been published(8). In keeping with the findings from Perth, neonatal BHR was significantly related to both reduced lung function (FEV\(_1\)) at 10 yrs and transient (<4 yrs) wheeze. There was no association between neonatal lung function and later lung function or bronchial responsiveness. Such a finding, which is consistent between 2 relatively small cohorts in 2 continents, is unlikely to be
spurious, and points to an association between congenital BHR and early symptoms, but persistently impaired lung function during later childhood.

Cohort studies also have the potential to answer questions about the *natural history of bronchial responsiveness* during infancy and childhood. Because of necessary differences in techniques used to assess bronchial responsiveness in infants, toddlers, and school children comparisons over time are difficult. For example, apparently increased responsiveness seen in infants may merely reflect a relatively large dose of inhaled stimulus(57). One approach is to rank subjects, and compare an individual subject's relative position over time and across different techniques. One interesting study compared BHR between 13yr old girls and 42 yr old women of similar size using identical protocols(58). The teenagers had greater BHR than the adults, suggesting that if size is controlled for, an effect of age persists. Developmental changes in BHR through early life, both physiological and pathological, have yet to be fully described. As a result, whether an infant lies outside population norms for BHR is currently unclear. The prognostic importance of such deviation from normal (gained from longitudinal studies) should remain the goal of clinical physiological studies.
2.4 Methods of measurement of bronchial responsiveness in infancy

2.4.1. General issues

Methods used to investigate BHR in infants have been adapted from those originally used in adult studies. Infants cannot co-operate with forced expiratory manoeuvres or breathe through mouthpieces. Therefore measurements of infant lung function are carried out with the infant lying supine, breathing through a facemask, (probably through the nose), during sleep. The length of the challenge procedure and the nature of the tests have traditionally demanded that the infant is sedated, usually with an oral agent such as chloral hydrate, and in some older studies with rectal thiopentone (33).

The facilities and expertise required to perform infant lung function tests only exist in a handful of institutions in the UK, while only one group has studied infant BHR over the last 20 years(4). The complexities of testing mean that worldwide, relatively small numbers of infants have undergone bronchial challenge tests (table 2.1). Recruitment of infants, where their parents volunteer consent, is extremely time consuming and labour intensive. The infant lung function procedure itself causes little disturbance and parents generally find that the administration of the (often unpleasant tasting) sedative agent is the most distressing part of the entire test. In a recent follow-up questionnaire study 94% of parents were happy to recommend the procedure to other parents (59). Even so, for ethical reasons, it is now not possible in most Western countries to recruit healthy infants for studies requiring sedation. This change in research practice has not been systematically studied and probably varies from centre to centre.

When considering the assessment of bronchial responsiveness in infancy it is logical, as in any age group, to consider the stimulus, response and analysis in turn.
2.4.2. The stimulus

In a standardised bronchial challenge test, carried out in a research laboratory under controlled conditions, the amount of any inhaled agent reaching the lower airways will still vary widely both within and between patients. This is also the case of course, with inhaled drugs given to treat infants. Several subject characteristics influence delivery of a stimulus to the lower airways and these will be discussed below.

Infants are generally considered to be nose breathers, whereas older subjects undergoing challenge tests breathe through a mouthpiece, often with a noseclip. The nose is designed to protect the lower airways by filtering inhaled air and much of the nebulised agent may be deposited in the nasal passages, variably reducing lower airway deposition. However, lack of nasal hair in preadolescent children, and relatively large nasal airways in infants (when compared with total body size) may mean the impact of nasal breathing is less than would be expected\(^60\). Conversely, stimulation of the nasal epithelium may cause enhanced (reflex) bronchoconstriction\(^61\). An infant study comparing inhaled aerosolised methacholine with nasally instilled methacholine found that each had different physiological effects on forced expiratory flow\(^62\). The nasal route produced a change to the partial forced peak expiratory flow consistent with a decrease in large airway flow (presumably due to an increase in nasal resistance) without any change in small airway function, as measured by forced flows at low lung volumes \((V'_{\text{maxPR}})\). Inhaled aerosolised methacholine produced a characteristic fall in small airway flow (figure 2.2). Increase in nasal resistance will influence measurements of overall airway resistance. In addition to the change in resistance, a fall in peak flow, as seen after nasal methacholine instillation, will influence flow at high lung volumes, such as those used to derive measurements of flow and volume during early expiration (figure 2.3) and possibly in the raised volume rapid thoracoabdominal technique.

Infant breathing patterns will in part determine the dose reaching the respiratory system. Collis et al have shown that below approximately 6 months of age infants’ tidal inspiratory flows are usually less than those of the jet nebuliser used in challenge procedures\(^63\). Consequently small babies effectively adjust the inhaled dose automatically in proportion to their inspiratory flow, and therefore their size. Above about six months of age, tidal inspiratory flows usually exceed those of jet nebulisers for most of the inspiration, so that the dose inhaled by any subject is constant, meaning that smaller subjects inhale a relatively large size corrected dose. This could explain the apparently greater levels of bronchial hyperresponsiveness in infants. However, when apparent levels
of bronchial hyperresponsiveness in infants and children were corrected for inspiratory flows, the increased responsiveness seen in the infants disappeared (57). Breathing pattern will also affect deposition of aerosol within the respiratory tract, as well as the total amount of stimulus inhaled. During rapid breathing, turbulence occurs and deposition in the upper airway and major bronchi increases (60). During inhalation of a stimulus by a sleeping infant, a facemask is used, and this alone may affect breathing pattern. Merely applying a facemask (i.e. with no additional dead space) onto the face of a sleeping infant has been shown to alter breathing patterns in response to trigeminal stimulation (64).

The state of the lower airways will also influence deposition of stimulus. A recent review of developmental influences on aerosol delivery (65) concluded that airway calibre is a major influence on the site of deposition of inhaled therapy. Increased airway resistance encourages deposition of aerosol in the central airways (66), and as infant lower airways are obviously of smaller calibre than those of adults (in whom nebulised drug deposition has been studied) the ideal particle size may be lower for infants. Airway function as estimated using forced flows during the rapid thoracoabdominal compression technique (RTC) is greater in infant girls than boys (67), presumably due to their relatively greater airway calibre, suggesting gender may be an additional influence on aerosol delivery. Distribution of ventilation may also be more uneven in infants, since during wakefulness, functional residual capacity is dynamically elevated to avoid airway closure during expiration. The degree of elevation varies with sleep state (68) and in response to inhaled stimuli (56), thus affecting the regional distribution of aerosol in the lungs. During the first year of life the chest wall becomes relatively less compliant, and contributes more to maintaining airway calibre. Obstructive airways disease such as acute bronchiolitis or chronic lung disease of prematurity may cause ventilation inhomogeneities, altering the distribution of inhaled particles and the overall airway response. Protective mechanisms that clear the agent from the airways, such as cough and ciliary function may also affect the degree and duration of any airway narrowing.

In the case of direct stimuli, and some indirect stimuli such as adenosine, receptors must exist in adequate numbers in the airway in order for a response, mediated by contraction of airway smooth muscle, to occur. Early work showing reversibility of histamine or methacholine induced bronchoconstriction with bronchodilators confirmed that functioning β2 receptors existed in the lower airways of wheezy (40) and healthy (36;43) infants. Inflammation may lead to epithelial shedding exposing underlying cells and thus afferent receptors to direct stimulation, which may lead to an exaggerated response to a given stimulus.
In addition to the physiological characteristics of the subject the nature of the stimulus, is important in determining the response. In general inhaled stimuli are divided into direct or indirectly acting agents. Histamine and methacholine act in a pharmacological manner via receptors on smooth muscle, leading to direct bronchoconstriction. Both agents have been used in infants, and shown to cause decreased forced expiratory flows (see table 2.1 for details of these studies)(36). However, in the context of a bronchial challenge test suitable for use in a domiciliary setting, several concerns exist over pharmacological challenges. Despite demonstration of physiological reversibility with bronchodilators, many infants undergoing challenges with histamine or methacholine (for example) develop symptoms of cough and wheeze (see table 2.1). These symptoms imply clinically significant airway obstruction that would be potentially dangerous outside a hospital setting.

Indirectly acting stimuli include physical stimuli such as cold air and exercise and chemical stimuli such as non-isotonic aerosols, (all of which probably act by altering the osmolarity of the airway surface liquid) and agents such as adenosine (which act on specific receptors on mast cells and other inflammatory cells in the airway). They are believed to produce bronchial smooth muscle contraction via a number of intermediate mechanisms, including inflammatory cell activation, vasodilatation, and neural reflex stimulation. Recently it has been suggested that bronchial responsiveness induced by indirectly acting stimuli may be more representative of the airway narrowing that occurs in asthmatic patients in response to exercise or allergen exposure. The mechanisms underlying exercise induced asthma, and bronchoconstriction in response to non-isotonic aerosols, have been extensively investigated and debated(69;70). Non-isotonic aerosols are thought to act by altering airway surface liquid osmolarity(71). Hypotonic (0.3% saline) aerosols have been shown to penetrate further into the peripheries of the lung than those of greater tonicity (4.5% saline)(72), a phenomenon thought to be related to the uptake of water vapour into hypertonic particles towards equilibrium with a humid environment. In general, particles of larger size are deposited in the central airways(73); so inhaled hypertonic stimuli of different concentrations may act at different sites within the airway tree.

In infants bronchial challenges with indirectly acting stimuli have yet to be investigated, although one early study has demonstrated a response to cold air challenge in infants(74). Whether the fall in forced expiratory flow demonstrated in that study was merely a reflex response to cold air on the face or an intrathoracic effect cannot be determined. A group of ventilated preterm infants has been shown to develop increased
airway resistance after a short period of ventilation with air of similar humidity and temperature to that of room air, as opposed to the usual practice of ventilation with warmed, humidified air(75).

In summary, indirect stimuli appear to have several attractions when devising a simple bronchial challenge test for use in unsedated infants in a home setting. Any change in airway function may be more representative of that occurring in acute wheezing illnesses in response to infection or inflammation, and therefore of greater sensitivity in identifying infants at risk of future symptoms. Such a hypothesis assumes that inflammatory cells have a role in symptomatic neonatal BHR. A small dose of non-isotonic saline is likely to be safer than a pharmacological agent and more acceptable to parents, and a single dose approach is vital in an unsedated infant unable to tolerate a multiple dose challenge. However, lung function techniques used to reliably detect the response to such a mild stimulus must be highly sensitive to small changes in airway function. Measurement techniques that are potentially sensitive enough, and also suitable for use in unsedated infants, will now be discussed, along with the previous "gold standard" techniques used in sedated infants in a laboratory setting.
2.4.3. Measurement of the response

In children capable of performing spirometry, (or co-operating with plethysmography) the measurement of response to an inhaled challenge is relatively straightforward, although standardization of equipment and techniques is still vital. In infants the investigator is faced with a multitude of different hurdles. Will the lung function technique wake the infant? Will the infant sleep long enough for the repeated measurements to be made? Is the proposed technique valid and repeatable? Will manoeuvres performed as part of the tests (e.g. lung inflation to raised volume) alter the response, as is the case for “big breath” tests in adults? Are measurements independent of changes in upper airway calibre in nose-breathing infants?

2.4.3.1. Forced expiratory flow

Most studies of bronchial responsiveness in infants have used the rapid thoracoabdominal compression (RTC) technique to assess change in forced expiratory flow after an inhaled stimulus. An inflatable jacket is wrapped around the infant’s chest and upper abdomen and rapidly inflated at end tidal inspiration to produce a partial forced expiratory manoeuvre. During initial measurements jacket pressure is gradually increased until a maximal flow (flow limitation) is obtained, some infants demonstrate a decrease in flow at higher jacket pressures; “negative dependence of flow”.

Maximal flow at functional residual capacity is reported ($V'_{\text{max}_{\text{FRC}}}$) and is thought to reflect intrathoracic airway function. According to wave speed theory, maximal flow through an airway is directly proportional to the airway cross sectional area, and inversely proportional to the airway wall compliance. Any value obtained for $V'_{\text{max}_{\text{FRC}}}$ will be influenced by both factors, and their relative contributions cannot be separated using the RTC technique. Changes in these two factors have been implicated in infants predisposed to wheeze(42).

In addition technical factors such as the jacket pressure, and the proportion of the jacket pressure transmitted to the infant’s chest (which may vary depending on how tightly the jacket is applied), and whether or not the arms are enclosed inside the jacket may also influence the maximum flows produced. Recently, international technical standards for the application of this technique have been published(76). Strict application of a standardized approach (by a group led by one of the authors of the standards) has reduced the previously
much higher quoted variability of $V'_{\text{max}}$ in wheezy children (35) down to a coefficient of variation of 6.3% in healthy infants (77).

Unfortunately in infants FRC is not a fixed volume "landmark", and is dynamically maintained above the passively determined lung volume (sometimes referred to as the elastic equilibrium volume, EEV). Infants may further elevate their FRC during bronchoconstriction, presumably due to an accompanying increased respiratory frequency and increased expiratory time constant (56). A forced flow related to this new, higher FRC would be higher than that at the original FRC. Such changes may mask or lead to an underestimate of changes in forced flow in response to challenge. FRC may also vary with sleep state, degree of sedation, and respiratory rate, adding further "noise" to the results.

A modification of the RTC technique was developed to enable measurements to be made across the entire volume range, the so-called "raised volume technique" (78). The infant's lungs are passively inflated using positive pressure to a raised volume, (although not to total lung capacity) prior to the forced manoeuvre. Several inflations (between three and five) are performed, each followed by a passive expiration as a result of the Hering Breuer reflex. Analysis of flow volume plots of the passive expirations enable the identification of fixed landmarks such as the EEV, to which subsequent forced flows can be related (79). In addition timed volumes during forced expirations from raised volume are obtained, including FEV$_{0.4}$, FEV$_{0.5}$, and FEV$_{0.75}$, values chosen to reflect the fact that, in most infants, expiration lasts far less than one second. Recently it has been demonstrated that small changes (approximately 8%) in the inflation pressures used may significantly affect the timed volumes and flows obtained (80). By increasing the inflation pressure from 2.7kPa to 3.0kPa, FEV$_{0.5}$ increased by a mean of 10%. This finding implies that minor differences between centres in terms of actual inflation pressure delivered at the airway opening could lead to significant bias.

The raised volume technique, although nearer to traditional spirometry in physiological terms, has not been shown to be more sensitive in detecting airway responses to methacholine challenge in infants (81) perhaps because it is likely to be influenced heavily by factors which determine peak flows (which could influence timed volumes) such as nasal calibre (fig 2.3). Studies investigating response to bronchodilators using the raised volume technique have produced differing results. No change in FEV$_{0.75}$ after salbutamol was found in a group of wheezy and healthy infants (82). In a group of infants with acute bronchiolitis, response to bronchodilator was measured using both the tidal and raised volume techniques in order to compare the sensitivity of each technique (83). As a group there was no significant change in pre and post bronchodilator measurements using
either technique. No individual infant demonstrated a significant increase in V'\text{max}_{\text{FRC}} (in some flows decreased after bronchodilator) while 8/14 infants had significantly improved timed expiratory volumes. The authors commented that measurement of FEV\text{0.75} before and after bronchodilator could be used to individualize bronchodilator therapy in infants with acute bronchiolitis, although whether this is a realistic in a clinical setting is debatable. Among a group of 41 normal infants, 6 responded to inhaled albuterol to a significant degree beyond that of placebo in terms of FEF\text{75} (84). However, the change in FEF\text{75} was on average 5.3%, well below that considered significant in clinical tests performed on older children or adults.

In adults and older children active inspiration to TLC or "big breaths", are known to cause bronchodilation in healthy subjects and bronchoconstriction in asthmatics. However, when bronchial responsiveness to methacholine was investigated (in both healthy and wheezy infants, total subjects n=37) using both the RTC and the RVRTC, there was no change in V'\text{max}_{\text{FRC}} measured before or after the six forced inflations, at any of the stages of the challenge procedure(81). Conversely, more recently a significant fall in V'\text{max}_{\text{FRC}} was seen in 29 healthy infants after raised volume manoeuvres, which were carried at a higher inflation pressure than the previous study(85).

2.4.3.2. Resistance measurements

Airway resistance measurements have the disadvantage that they inevitably include the resistance of the upper airway (the nose, pharynx, and larynx) as well as that of the intrathoracic airways. In infants this will also include the resistance of the facemask and any other measurement device (e.g. flowmeter or shutter) attached to it.

Airway resistance (R_{aw}) is measured using an infant plethysmograph, and has been shown to be capable of detecting changes following challenge in infants(33). The technique is very similar to that described for adults, and has the advantage that absolute lung volume can also be measured simultaneously, allowing calculation of a volume corrected (specific) resistance. The infant technique has recently been the subject of comprehensive recommendations, designed to standardise equipment, data acquisition and reporting of results(86). R_{aw} is measured throughout tidal breathing, and enables examination of resistance during inspiration and expiration, which may be altered in different ways depending on where the inhaled stimulus acts(44). The procedure is time consuming and almost always requires sedation, although infants during the first month of life may tolerate measurements in natural sleep after a feed. Certainly the procedure is not
well suited to rapid bronchial challenge test in unsedated infants, and the equipment required is far from portable.

**Resistance by interruption (R_{int})** is obtained by measuring pressure and flow at the airway opening, during tidal breathing, which is briefly interrupted using a shutter device(87). The change in airway opening pressure occurring during the interruption is used to estimate the driving pressure across the airway that was present at the moment of interruption(88). Such a value for pressure is related to flow at the moment of interruption to obtain a value for resistance, various theories exist as to the nature of resistance measured using the technique and these will be discussed in chapter 3.

Equipment used to obtain R_{int} is generally portable and the measurements are relatively simple to obtain, even in toddlers. There has been increasing interest in the technique over recent years, mainly because no active co-operation is required. However, because the values of R_{int} are sensitive to changes in equipment characteristics and interpretation of the raw data, the technique is far from standardized. R_{int} was chosen for evaluation as part of this work in view of a number of factors. Firstly it had been shown to be capable of detecting change during bronchial challenge with methacholine in preschool children (89-91). Secondly, the measurement of R_{int} has recently been shown (by a group with whom we were closely collaborating), to be feasible in healthy unsedated infants in a laboratory setting(92), suggesting a role in bronchial challenge testing in naturally sleeping infants. However, as it had never been applied in an infant bronchial challenge test, the ability of the technique to detect a change in resistance after an inhaled stimulus was unknown before this work was carried out.

**Passive respiratory mechanics** can be measured in infants to obtain estimates of respiratory system resistance and compliance (R_{rs} and C_{rs} respectively). These measurements include contributions from the airways, lungs and chest wall and are based on a single compartment model of the respiratory system. The single occlusion technique employs an occlusion at high lung volume to induce the Hering Breuer reflex, after which a relaxed expiration occurs. Alternatively, the multiple occlusion technique can be employed, and several occlusions performed at different lung volumes about the end expiratory level. Currently published reference data using the technique are centre specific due to a lack of standardisation of equipment and techniques, although quality control issues and acceptance criteria for this technique have been reviewed with a view to improved standardisation(93). The single occlusion technique has been used to detect
changes after methacholine challenge in sedated infants, although changes in both \( C_{rs} \) and \( R_{rs} \) were less sensitive than \( V'_{maxFRC} \) and \( TcO_2(94) \).

2.4.3.3 Impedance measurements (high and low frequency)

Measurement of low frequency input impedance using the forced oscillation technique enables an estimate of airway resistance (as well as lung tissue resistance) to be obtained. Both have been shown to change in infants after methacholine challenge(95). Pressure oscillations are imposed on the respiratory system at the mouth (usually using a loudspeaker), during tidal breathing, and resulting changes in tidal flow at the airway opening recorded. In infants where \( R_{aw} \) is relatively high, the changes in flow used to derive the resistances are highly damped by the upper airways and this makes interpretation difficult. The inclusion of the upper airway component in the measurements may also decrease the sensitivity of the technique, which has been shown to be unreliable in pre-school children when compared with transcutaneous oxygen tension (96). Measurements of airway resistance using this technique therefore are subject to several constraints, which make their use in infant bronchial challenge testing extremely limited.

The interrupter technique was modified to enable the determination of high frequency respiratory impedance, using the high-speed interrupter technique (HIT)(97). This technique does not measure any form of interrupter resistance, but analyses the pressure oscillations ("ringing") occurring immediately after shutter closure. Instead of a loudspeaker a high-speed shutter (which opens and closes 4 times in 124 msec) is used to produce the sudden perturbations that lead to pressure oscillations. The speed at which the shutter closes preserves data at high frequencies, the region where information about airway wall characteristics resides. Pressure oscillations are analysed in order to produce a high frequency impedance spectrum. High frequency impedance measurement in infants has only recently been developed(97), but has been shown to contain anti-resonances, which are related to wave propagation phenomena, and therefore airway wall compliance and airway path length. Airways of different compliance will have different anti-resonances, analogous to the different harmonics produced by wooden or metal organ pipes of identical size. In theory the frequency of these antiresonances will provide semi-quantitative information on airway wall compliance, but they do not measure airway resistance. If BHR in infants is governed by such functional differences in airway mechanics then HIT has the potential to be a useful technique in the investigation of
neonatal BHR. The theoretical background to the technique will be further discussed in chapter 3.

That airway wall compliance may alter in infants during a bronchial challenge test was first suggested by work from Prendiville and co-workers. When salbutamol was given alone, forced flows measured using the RTC technique actually decreased (37), and after small doses of inhaled histamine forced expiratory flows increased (40). These findings suggest that changes in airway wall compliance, (which could be altered by either agent by changes in airway smooth muscle tone) contribute to changes in expiratory flow. For example loss of baseline airway tone after salbutamol would in theory cause an increase in airway wall compliance, and therefore reduce forced expiratory flow, if calibre remained constant. Conversely small doses of histamine could have the opposite effect on forced flows by increasing airway wall tone without an effect on calibre.

Anti-resonances themselves have been shown to change in the early stages of a methacholine challenge (41), prior to any changes in forced expiratory flow. This finding suggested that changes in airway wall tone occurred in response to low doses of inhaled methacholine, before any fall in calibre. Airway wall properties in a group of previously wheezy infants (aged 9-19 months) were shown to be significantly different from a healthy group (who were studied unsedated) (42), suggesting differences in the airway wall structure or function (for example airway smooth muscle “tone”, or inflammatory changes leading to thickening of the various components of the airway wall, ) in asymptomatic wheezy infants. Developmental differences in airway wall mechanics could therefore have a role in the pathogenesis of wheezing disorders. Alternatively, alterations in airway wall mechanics might be a consequence of post-inflammatory remodelling. Whether such differences were present from birth or resulted from remodelling in response to lower respiratory tract infection remains unclear. An important methodological advantage of the HIT technique is that altering the resistance of the upper airway (achieved by occluding one nostril) did not affect the anti-resonant frequency. This suggests that HIT contains information about the lower airways, independent of upper airway calibre or compliance (97).

Because of the sensitivity of the HIT to changes in airway mechanics in response to low doses of methacholine (41) and its feasibility in unsedated infants (42), the technique was chosen for evaluation in the context of a single dose saline challenge. Nevertheless, it was known from the literature that the relationship between changes in airway wall mechanics and the absolute value of the first antiresonance frequency was highly complex, which made it impossible to quantify changes in airway wall mechanics. So far it had been
shown that during a methacholine (or even a saline) challenge changes in airway wall mechanics occurred, but not whether this corresponded to an increase or a decrease in wall compliance. Despite these limitations, we aimed to rapidly and reliably identify changes in airway muscle tone occurring after small doses of saline. Such changes would, in theory, be too small to cause significant change in airway calibre, making a significant change in clinical respiratory status unlikely.

2.4.3.4. Measurements made during tidal breathing

In 1988 a group based in Tuscon, Arizona reported that infants with decreased lung function had an increased risk of wheezy illnesses during the first year of life. One of the measurements that increased the likelihood of later illness was the ratio of time to peak expiratory flow to expiratory time \( T_{\text{pmax}}/T_e \)\(^{(98)}\). As the values contributing to it are simple to measure in infants (a facemask and flowmeter are all that are required for data collection) there was great interest in the potential value of the ratio, including its possible role in assessing response to an inhaled stimulus. Unfortunately, during histamine challenge \( T_{\text{pmax}}/T_e \) was found to be highly insensitive: \( V'_{\text{max}} \text{FRC} \) fell by 40% before any change in the ratio\(^{(99)}\). Another group assessed the ability of several infant lung function tests to detect change during a methacholine challenge\(^{(94)}\). \( V'_{\text{max}} \text{FRC} \) was found to be most sensitive technique, along with tidal peak expiratory flow, which actually increased during challenge in both studies. This phenomenon provides a clue as to why the ratio may be unhelpful in the context of a bronchial challenge test infants, despite its strong relationship to FEV\(_1\) in a histamine challenge in older children\(^{(100)}\). In response to airway obstruction, post-inspiratory braking (which normally acts in infants to prolong early expiration in order to maintain FRC) may decrease, permitting a higher peak flow. If respiratory frequency increases as well, expiratory time will decrease, with little overall change in \( T_{\text{pmax}}/T_e \). Measurements of these tidal breathing indices reflect the complex, ever changing neuro-mechanical response of the infant respiratory system and as such do not correlate directly with airway narrowing. In anaesthetised cats, time to peak tidal expiratory flow has indeed been shown to be highly influenced by inspiratory muscle activity during the early part of expiration, which depends partly on activity of the vagal receptors in the lung\(^{(101)}\).
2.4.3.5 Indirect measurements previously employed in toddlers

The measurement of transcutaneous oxygen tension (TcO$_2$), which requires no subject cooperation, has been used in preschool children (102) and shown to be reliable and repeatable as an outcome measure in bronchial challenge tests (103). Another group demonstrated that falls in TcO$_2$ were most sensitive in detecting change in lung function during histamine challenge in infants (94). The baseline variability of this test was much less than the others, which will contribute to sensitivity.

Work in infants has shown that the reduction in TcO$_2$ during bronchial challenge was a less sensitive index in healthy infants than those with a history of lower respiratory tract illness. This finding may reflect compromised ventilation-perfusion matching in those previously symptomatic infants, which when disturbed by bronchoconstriction was rapidly identifiable by changes in TcO$_2$ (104).

The same group compared the auscultation for wheeze with changes in oxygen tension and found it to be far less sensitive (96), with potentially dangerous falls in TcO$_2$ before wheeze became audible.

2.4.3.6 Measurement of the response: conclusions

Several techniques are available to measure the response to bronchial challenge in infants, and they each measure different physiological components of the response. Several measurements (such as resistance) contain “noise” from the upper airway, and even those measuring small airway function (RTC) contain a mixture of information about airway calibre and airway wall compliance, both of which may change independently of each other following stimulation. If the additional constraints of portable equipment and minimally disturbing techniques suitable for use in unsedated infants are added (as they must be if population studies are to become a reality) then the range of suitable techniques becomes very narrow.

We chose to evaluate R$_{int}$ and HIT, both of which are feasible in unsedated infants, although they measure very different physiological changes after challenge. R$_{int}$ is capable of detecting change in total airway resistance during bronchial challenge in pre-school children, and it was therefore reasonable to expect it to detect change in resistance (including of course that of the nose) after hypertonic saline challenge in infants. In contrast, HIT is sensitive to changes in airway wall compliance, and appears to be independent of upper airway calibre.
2.4.4. Analysis

Bronchial responsiveness describes the stimulus-response relationship, and the analysis of this relationship is not straightforward. The approach to the analysis will be determined by whether single doses of a stimulus (for example during exercise challenge), or multiple doses with repeated lung function measurements are made. In reality, the impact of a stimulus (which as previously discussed may be difficult to quantify in terms of dose and site of action) on a highly complex system is likely to be subject to enormous technical and biological variability.

During those infant bronchial challenge tests that employ a multiple dose procedure, a gradually increasing dose of stimulus is administered, and airway obstruction assessed between doses to seek a threshold effect. The results are analysed in a relatively simplistic way: a “dose-response” model, and a provocative concentration or dose of stimulus (PC$_{30}$) that is required to cause a predetermined fall in lung function (for example a 30% fall in V'$_{max}$FRC) is calculated from a dose/response curve. This is the simplest measure of responsiveness to calculate, and the most commonly used (figure 2.1).

Some subjects may fail to reach the predefined fall in lung function despite maximal doses of the stimulus (according to the particular experimental protocol, although the usual maximal dose is determined by safety). In infants, early waking before the end of the challenge is another common problem. Both scenarios result in incomplete data, and two approaches have been suggested to overcome this. Firstly the dose response curve (based on any previous change prior to waking) can be extrapolated to estimate the PC$_{30}$. An alternative approach to report the slope of the dose response curve as a measure of airway reactivity, and this has been done in some infant studies (48). Generally a normal saline “control” dose is administered prior to the first dose of agonist, and subsequent lung function values related to those obtained “post saline”. Work using the highly sensitive HIT technique had suggested that in some infants, airway wall properties might alter after saline (41). This implies that the use of post saline lung function as a “control” value may be inappropriate, as the lungs have already been altered from their natural state.

Exercise is widely used as a “single dose” challenge procedure in older children. Accompanying changes in airway surface liquid osmolarity in response to hyperventilation during exercise are thought to produce airway narrowing, which can also be induced by breathing dry air, with or without hyperventilation (105). In one study of 42 infants, a single dose challenge of cold, dry air inhaled for 10 minutes was used (74). In unsedated infants with limited time available to perform the entire procedure, a single dose of stimulus is the only realistic approach, and the magnitude of any response must be used to
determine individual responsiveness (rather than investigating the response to gradually increasing doses). We chose to investigate a single dose challenge, using inhaled saline.

Detection of significant change after a stimulus will depend on the repeatability of the measurement. The poorer the repeatability, the larger the change required before it can be considered significant. Therefore, in order to define a threshold for response, measurements of repeatability must first be obtained, i.e. the confidence interval for the difference between two measurements without any intervening stimulus (or after placebo). Such investigations are vital, particularly in sleeping infants, as it is possible that subtle changes in sleep state, breathing pattern and lung volume could influence lung function measured under the same apparent conditions several minutes later.
2.5. Conclusions

Neonatal bronchial responsiveness is an outcome of foetal development and appears from current knowledge to be a contributor to later disease. Studies which monitor groups of newborn subjects throughout childhood and beyond may demonstrate a window of possible therapeutic opportunity by identifying infants who are predisposed, or even predestined, to develop significant symptoms of airway obstruction later in childhood. The elucidation of underlying mechanisms may bring closer the goal of preventative therapy for asthma, to be examined in the context of a clinical trial.

At present these ideals are unlikely to become reality without the power of large studies, and current methods of assessing bronchial responsiveness in infants are impractical for use in population studies. Newer, less invasive measurements of bronchial responsiveness in unsedated infants are required, and the ultimate aim of this thesis was to develop such a method. Otherwise the study of neonatal BHR will remain an expensive hobby with little impact on the care of infants and children with asthma.

The methodology of the three infant lung function techniques employed in this thesis will now be described, followed by an evaluation of $R_{int}$ in unsedated infants. The two techniques that are applicable to unsedated infants ($R_{int}$ and HIT) will then compared with the established method, RTC, in the context of a bronchial challenge with inhaled saline performed in a group of sedated infants with a history of wheeze. Finally, preliminary reference data for $R_{int}$ will be presented, and the relationship between the measurement and various infant factors examined. These data do not relate directly to the initial aim of this thesis, but have a place in the preliminary on going evaluation of this technique in infants.
### 2.6 Tables and Figures

Table 2.1 Summary of all infant bronchial challenge tests worldwide (including bronchodilator responsiveness) in order of year of publication.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Author</th>
<th>Year of publication</th>
<th>Number of infants</th>
<th>Status of infants</th>
<th>Stimulus</th>
<th>Response</th>
<th>Outcome/Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(40)</td>
<td>Prendivile</td>
<td>1987</td>
<td>5</td>
<td>Sedated/Previous wheeze</td>
<td>Histamine, then Salbutamol</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>All infants responded to histamine. Effect abolished by pre treatment with salbutamol.</td>
</tr>
<tr>
<td>(37)</td>
<td>Prendivile</td>
<td>1987</td>
<td>18</td>
<td>Sedated/Previous wheeze</td>
<td>Salbutamol</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>No change in $V^{\text{max}}<em>{\text{FRC}}$ after saline, but a significant decline in $V^{\text{max}}</em>{\text{FRC}}$ after salbutamol.</td>
</tr>
<tr>
<td>(35)</td>
<td>Prendivile</td>
<td>1987</td>
<td>11</td>
<td>Sedated/Previous wheeze</td>
<td>Histamine</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>9/11 responded to histamine Symptomatic cough and wheeze occurred in 6/9</td>
</tr>
<tr>
<td>(44)</td>
<td>Prendivile</td>
<td>1987</td>
<td>17</td>
<td>Sedated/Previous wheeze</td>
<td>Ipratropium Bromide $V^{\text{max}}_{\text{FRC}}$</td>
<td>$sR_{aw}$</td>
<td>Significant reduction in $sR_{aw}$ after treatment with ipratropium bromide (improvement in central and upper airway function) No change in $V^{\text{max}}_{\text{FRC}}$</td>
</tr>
<tr>
<td>(36)</td>
<td>Tepper</td>
<td>1987</td>
<td>10</td>
<td>Sedated/Healthy</td>
<td>Methacholine/ metaproterenol</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>All infants had fall in $V^{\text{max}}_{\text{FRC}}$ by at least 40% Relieved by inhaled metaproterenol No clinical symptoms but fall in $SaO_2$</td>
</tr>
<tr>
<td>(74)</td>
<td>Geller</td>
<td>1988</td>
<td>42</td>
<td>Sedated/Healthy (12 Placebo)</td>
<td>Cold Dry Air</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>Mean fall of 18% in $V^{\text{max}}_{\text{FRC}}$ after cold air v controls</td>
</tr>
<tr>
<td>(106)</td>
<td>Le Souef</td>
<td>1989</td>
<td>12</td>
<td>Sedated/Healthy</td>
<td>Histamine</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>All infants responded (30% fall in $V^{\text{max}}_{\text{FRC}}$) Associated significant fall in $SaO_2$</td>
</tr>
<tr>
<td>(57)</td>
<td>Stick</td>
<td>1990</td>
<td>45</td>
<td>Sedated/Healthy compared Histamine with 30 older non-asthmatic children</td>
<td>Histamine</td>
<td>$V^{\text{max}}_{\text{FRC}}$</td>
<td>Values of $PC_{40}$ (corrected for air entrainment) were not significantly different between infants and older children.</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Challenge</td>
<td>Outcome</td>
<td></td>
<td></td>
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<tr>
<td>-----------</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montgomery 1990</td>
<td>1990</td>
<td>24 Sedated/Healthy</td>
<td>Methacholine</td>
<td>V'\text{max}_{FRC} Decreased sensitivity to methacholine with age (range 4-24months) (no correction for air entrainment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stick 1991</td>
<td>1991</td>
<td>38 Sedated/Previously wheeze Histamine Healthy controls (19)</td>
<td>V'\text{max}_{FRC}</td>
<td>No significant difference in the PC_{40} between the two groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ackerman 1991</td>
<td>1991</td>
<td>28 Sedated/Healthy (14) Cystic Fibrosis(14)</td>
<td>Methacholine</td>
<td>V'\text{max}_{FRC} CF infants had increased methacholine responsiveness compared with healthy controls, after accounting for reduced baseline flows</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young 1991</td>
<td>1991</td>
<td>63 Sedated/healthy/neonates unselected</td>
<td>Histamine</td>
<td>V'\text{max}_{FRC} Airway responsiveness was increased in infants with a family history of asthma or parental smoking (finding not consistent after inclusion of more data)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarke 1992</td>
<td>1992</td>
<td>45 Sedated/healthy (atopic parent) 23 previous LRI 22 previously healthy</td>
<td>Histamine</td>
<td>V'\text{max}<em>{FRC} No significant difference in PC</em>{30} between symptomatic infants and control infants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tepper 1992</td>
<td>1992</td>
<td>42 Sedated Post bronchiolitis (18) Healthy (24)</td>
<td>Methacholine</td>
<td>V'\text{max}_{FRC} Ex-bronchiolitic infants had increased responsiveness, and failed to show age related decline in BHR at 10 months.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tepper 1993</td>
<td>1993</td>
<td>6 Sedated/healthy</td>
<td>Methacholine</td>
<td>V'<em>{\text{max}</em>{FRC}} Forced expiration flow/volume morphology Decreased and PEFV curves became concave in shape, after nasal instillation: decrease in peak flow and flattening of the PEFV curves at higher lung volumes.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Year</td>
<td>Sample Size</td>
<td>Condition</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
| Henderson | 1993 | 40 | Sedated/Healthy | Histamine \nThen salbutamol or placebo  
Infants who received salbutamol had a significantly faster rate of recovery from histamine induced bronchospasm than those who received placebo (saline)  
Similar results were obtained for infants as observed in older subjects for repeatability of challenges and agreement between measures of bronchial responsiveness.  
  
| Stick | 1993 | 23 | Sedated/Healthy (7) CF(5) | Histamine challenge then either Histamine or Methacholine repeat challenge 1 week later  
Younger infants had improved V'\text{max}_{\text{FRC}} after bronchodilator, as did female infants.  
  
| Tepper | 1994 | 34 | Sedated/Mild Acute bronchiolitis | Metaproterenol  
No change in T_{pet}/T_e despite significant fall in V'\text{max}_{\text{FRC}} and significant increases in breathing frequency and mean tidal expiratory flow rate.  
  
| Aston | 1994 | 27 | Sedated/Healthy Infants | Histamine  
Of 42 infants 41 responded to methacholine by a change > or = 2 standard deviations from baseline values. Greatest changes were seen in T_{CO2}, V'\text{max}_{\text{FRC}}, C_{RS}, R_{RS}, Tidalbreathing compared  
  
| Benoist | 1994 | 42 | Sedated/Wheezy | Methacholine  
As a group no index of lung function predicted future wheezing. Boys with subsequent LRI tended to have lower V'\text{max}_{\text{FRC}}. Girls with increased BHR as neonates had increased LRI later.  
  
| Clarke | 1995 | 73 | Sedated/healthy/ neonates (atopic parent) With followup of symptoms to 1 yr. | Histamine  
Agreement between the two methods was good. Bronchoconstriction was not attenuated by the forced inspiration delivered by the raised volume manoeuvre.  
  
| Hayden | 1997 | 11 | Sedated/ previously wheezy(7) healthy (4) | Methacholine  
Raised Volume Forced Expiration (FEV_{0.5}),
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Age</th>
<th>Status</th>
<th>Treatment</th>
<th>Outcome 1</th>
<th>Outcome 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frey 1998</td>
<td>10 Sedated/Previous wheeze</td>
<td>Mch</td>
<td>V'\text{max}_{FRC}</td>
<td>High-frequency input impedance measurements (far,1) were more sensitive to changes in airway wall compliance after methacholine than V'\text{max}_{FRC}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayden 1998</td>
<td>27 Sedated/healthy previous wheeze(22)</td>
<td>Salbutamol</td>
<td>Raised Volume forced Expiration FEV\textsubscript{0.5}, FVC, FEF\textsubscript{75}</td>
<td>Salbutamol produced a significant change in heart rate, but no significant change in any forced expiratory measurements.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayden 1998</td>
<td>22 Sedated/previously wheezy(13) healthy (9)</td>
<td>Salbutamol</td>
<td>Low frequency Forced Oscillation</td>
<td>13% group fall in R\textsubscript{aw} after salbutamol, but not placebo.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modl 2000</td>
<td>17 Acute Bronchiolitis</td>
<td>Salbutamol</td>
<td>RV and Tidal Forced Expiration compared measurements did not differ significantly from baseline measurements. No infant demonstrated a significant increase in V'\text{max}_{FRC}, eight infants had significantly improved timed volumes.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hall 2001</td>
<td>17 Sedated/Healthy</td>
<td>Methacholine</td>
<td>Raised Volume RTC At a provoking concentration of methacholine causing a 15% fall in FEV\textsubscript{0.5} and Forced Oscillation significant changes in airway resistance and inertance were detected.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.1
Change in lung function in response to increasing doses of constrictor stimulus, in normal and hyperresponsive (asthma) subjects.
Bronchial hyperresponsiveness (BHR) refers to an exaggerated degree of airway narrowing, which occurs at a lower threshold dose, or which exhibits a steeper dose response slope (greater reactivity) or exhibits a greater (or unlimited) maximum response.
Special Note

Pages 43 & 44 missing from the original
**Figure 2.2**

A: Aerosol challenge: following methacholine, the peak expiratory flow-volume curves become concave in shape, with decreased forced expiratory flow over the entire tidal volume range.

B: Nasal challenge: following methacholine the peak flows and flows at higher lung volume decrease. The PEFV curve remains convex at lower lung volumes and maximal flow at functional residual capacity remains unchanged.

From reference (62)
Figure 2.3

Peak expiratory flow-volume curves with varying nasal resistance. Note loss of flow at high lung volume with increasing nasal resistance. From reference (62)
3: Methodology

3.1 Introduction

Details of the methods used to apply the three infant lung function technique employed in this thesis will be described in turn. Recruitment, subject characteristics, inclusion criteria, and experimental protocols will be discussed in chapters 4 and 5, as these differed between healthy unsedated (chapter 4) and sedated infants with a history of wheeze (chapter 5).

In addition, pilot data relating to the repeatability of HIT, subsequently used to interpret changes in HIT after inhaled saline, will be presented here.

3.2 Rapid Thoracoabdominal Compression Technique (RTC)

3.2.1. Introduction

The RTC technique was used to assess forced expiratory flow at functional residual capacity \((V'_{\text{max FRC}})\). The theoretical background to this technique has been described in section 2.4.3.1.

This technique was used in a group of 28 sedated babies with a mean age of 9 months with a history of wheeze (see Chapter 5).

3.2.2. Equipment

Flow was recorded using a screen type pneumotachograph (PNT), and pressure in the jacket and at the airway opening with differential pressure transducers (range +/- 100cmH2O), (Jaeger GmgH, Wuerzburg, Germany).

The jacket (Medical Engineering Department, Hammersmith Hospital. London, UK) was positioned to encircle the thorax and upper abdomen, with the arms enclosed. Measurements were made via a Rendell Baker size 2 facemask, with a published effective deadspace of approximately 15ml(112) sealed onto the face using therapeutic putty (Nottingham Rehabilitation, Nottingham, UK.). Data were acquired at 80 Hz, converted from analogue to digital, and displayed using RASP software (Physiologic, Newbury, UK) on a desktop personal computer (IBM 486).
3.2.3. Calibration

Accuracy of flow was confirmed by passing a flow of between 200 and 400 ml.s\(^{-1}\) through a calibrated flowmeter (Rotameter, UK) and the PNT connected in series. An accuracy level of \(\pm 2\%\) was accepted. Pressure transducers were similarly checked for accuracy by applying a known pressure of approximately 20 cmH\(_2\)O using a manometer and comparing this with atmospheric pressure.

3.2.4. Protocol for measurement of \(V'_{maxFRC}\)

The jacket was wrapped around the sleeping infant’s chest and upper body encircling the arms, and held in place with integral Velcro fastenings. The facemask was gently placed over the mouth and nose, using soft putty to obtain an airtight seal. The mask was pressurised by placing a finger briefly (for approximately 1 second) over the expiratory port of the PNT during expiration, if the pressure rose smoothly and a plateau was maintained, the mask was deemed airtight. Flow, volume (integrated from flow) and jacket and mouth pressure were displayed on a monitor using RASP software (Physiologic, Newbury, UK). The jacket was connected via a three way tap to a 50 litre drum, which was pressurized to the desired pressure using medical air and an adjustable blow-off valve.

Once the infant appeared to be in quiet sleep (lack of facial or limb movements, accompanied by regular respiration) measurements of forced expiratory flow were begun. After the infant had taken at least five tidal breaths with a stable end expiratory level (EEL) the jacket was rapidly inflated at end tidal inspiration, by turning the tap by hand, whilst observing the flow and volume traces on the computer screen. The inflation was maintained throughout the forced expiration.

Approximately 5 manoeuvres were performed at increasing jacket pressures, starting at approximately 2.5 kPa. Pressurising the drum to a higher pressure prior to each inflation increased the resulting jacket pressure. Flow volume loops were examined in order to identify the optimum jacket pressure for each infant, which was that producing the maximum flow, without evidence of decreasing flows with increased pressure. A further 5 measurements were then made at this pressure.
3.2.5. Data Analysis

For analysis data were exported as ASCII files to the program “Squeeze” (Imperial College, London), and inspected for technical quality according to recently published standards(76), before obtaining a value for $V'_{\text{maxFRC}}$. In summary, data were only included if (i) the peak expiratory flow occurred within the first 30% of tidal volume (i.e. the jacket had been rapidly inflated correctly at end inspiration), (ii) flow continued past the previously stable EEL, and (iii) there was absence of glottic closure (i.e. rapidly varying flows during expiration which were not repeatable between manoeuvres) particularly during the second 50% of expiration. The highest value for $V'_{\text{maxFRC}}$ from three to five acceptable measurements, within 10% of 10 ml.s$^{-1}$ (whichever was the greater) of each other was used to define baseline expiratory flow, as per the published standards(76).

Variability was expressed as the coefficient of variation ((SD/mean) x100) of those three to five acceptable manoeuvres.
3.3. Resistance by Interruption ($R_{int}$)

3.3.1. Introduction

$R_{int}$ is a minimally disturbing technique to estimate airway resistance that requires a brief interruption to expiratory flow, in order to estimate driving pressure at the point of interruption. Flow and pressure at the airway opening ($P_{ao}$) are recorded. $P_{ao}$ is thought to equilibrate with alveolar pressure ($P_{alv}$) (the driving pressure) during the period of interruption. There are two distinct phases in the $P_{ao}$ trace following interruption, an initial oscillation and a second slow rise to a plateau. Work in animal models has helped elucidate the meaning of these changes (113;114). The initial rise reflects airway resistance, and the second slow rise, more stress relaxation of the respiratory tissues and pendelluft. Estimates of actual $P_{alv}$ at the point of interruption are made by various back-extrapolation procedures. This is then related to flow at the point of interruption to obtain a value for resistance. $R_{int}$ has been applied in young children in bronchial challenge procedures (89-91), but never before in infants during challenge. Prior to the start of this project, there had been no published studies of the use of $R_{int}$ in infants. Since then 2 have appeared, one a methodological paper relating to the feasibility in unsedated infants, the comparison of various analysis techniques of the interrupter curve and the choice of facemask (92), and a second a comparison of $R_{int}$ and resistance measured by passive mechanics(115).

3.3.2. Equipment

Flow was measured using an ultrasonic flow meter, and mouth pressure was measured using a piezo resistive sensor; all channels were sampled at a rate of 200Hz, and displayed on a laptop computer using “Wbreath” software (all measurement equipment and software: Ecomedics, Duernten Switzerland). In the ultrasonic flowmeter flow is determined from the transit time of a pulsed ultrasound signal travelling across the path of gas flow. A shutter was placed proximally between the facemask and the flow meter and contained a slide valve with a reported closure time of <10ms (figure 3.1)

A rigid face mask of the same model and size as that used during RTC measurements was used in the older, sedated infants (chapter 4). In the younger unsedated infants a smaller mask was used. Previous work had shown that use of a compliant walled soft facemask (which is easier to use in unsedated infants) led to significant underestimation of $R_{int}$ (92).
The dead space of the measurement equipment (flow meter, shutter and connector) was 11.8 ml, and the large facemask approximately 15 ml. The deadspace included in the resistance measurements was 19 ml, i.e. the total deadspace of the mask and equipment proximal to the shutter. However, putty placement around and inside the mask would have reduced this deadspace.

The shutter was activated during expiration by a predetermined expiratory flow of 20ml.s\(^{-1}\) in order to interrupt during early expiration at a relatively high lung volume, and therefore to increase the likelihood of activation of the Hering-Breuer reflex, and subsequent respiratory muscle relaxation. The shutter was held closed for 500 msec in order that a passive system could be confirmed by inspecting the pressure/time curve for evidence of active breathing against the shutter (see below). An extended period of interruption allowed exclusion of data with obvious irregularities at any point during the 500msec. A shorter interruption may have led to inclusion of data influenced by active breathing not apparent during the initial period. Such activity could affect the rate of pressure change and produce spurious values for \(R_m\), which assumes a passive system. During relaxed expiration the second half of the pressure change during interruption is strongly influenced by visco-elastic properties of the lung and pendelluft, so we analysed only the first half of the interruption, which relates more to airway resistance.

3.3.3. Calibration
Flow was calibrated with a 100ml calibration syringe (Hans Rudolph Inc, Kansas City, USA). Calibration was considered accurate if the integrated volume was within 2% of the syringe volume. The pressure calibration was checked over the range +/- 20cmH\(_2\)O and recalibrated if necessary. The equipment contained room temperature and atmospheric pressure sensors, the accuracy of which was checked using a thermometer and barometer respectively. Humidity was entered manually, using a humidity monitor. The software made corrections for all three variables.

3.3.4. Protocol
The facemask was placed over the infant’s mouth and nose, and tidal flow volume loops examined to ensure respiration was regular and to confirm the absence of leaks, by absence of volume drift. If the infant had been partially roused by the application of the facemask, this was obvious from a rapid respiratory rate with variable end expiratory level, and the mask was removed. Measurements attempted during such periods of irregular respiration were found to be of poor quality (see below). Interruptions were performed every 4-5 breaths, until approximately 10-15 interruptions had been recorded. After the
operator activated the software the interruption took place automatically during the succeeding expiration, triggered by an expiratory flow of 20ml.s\(^{-1}\). There was a software delay of approximately 40 msec between the sensed flow and the start of shutter closure.

3.3.5. Analysis

Data were inspected offline for technical quality by inspecting the mouth pressure/time plot for a smooth rise during the interruption, indicating a lack of active breathing against the shutter or incomplete relaxation (figure 3.2). Acceptable data were exported as ASCII files and analysed using software routines written by Dr. G. Hall using Matlab Software (Mathworks Inc., USA).

\( R_{\text{int}} \) was calculated by relating the change in \( P_{ao} \) (\( \Delta P_{ao} \)) due to the interruption, by the flow just prior to interruption \( V'_{\text{pre}} \):

\[
R_{\text{int}} = \frac{\Delta P_{ao}}{V'_{\text{pre}}} = \frac{(P_{\text{post}} - P_{\text{pre}})}{V'_{\text{pre}}}
\]

\( T_0 \) was chosen to represent the point at which the valve was half closed, and extrapolations of flow and pressure were made to this point. Estimates of flow at this moment were obtained by forward extrapolation by fitting a polynomial from 150msec to 10 msec prior to the flow minimum and then forward extrapolating this to \( t_0 \). \( T_0 \) was defined as the flow minimum (the point at which expiratory flow began to fall, as the shutter began to close) plus 5 msec, which was half the estimated closure time (see figure 3.2a)

Three previously reported techniques were used to estimate the magnitude of \( P_{\text{post}} \). Two back-extrapolation methods (methods (i) and (ii)) estimated \( P_{ao} \) at \( t_0 \) and the third used different assumptions.

(i) Back extrapolating a fitted smooth curve. A polynomial curve was fitted to the post occlusion pressure data from \( t_{+30} \) to \( t_{+200} \) ms (116). This polynomial was then extrapolated to \( t_0 \) to obtain \( P_{\text{post}} \) and \( R_{\text{int}} \) thus determined (\( R_{\text{pex}} \)) (figure 3.2(c)).

(ii) Linear back extrapolation. A straight line was fitted through two time points, each being the mean of 10 ms of data, centred about \( t_{+30} \) and \( t_{+70} \) ms(87). The line was the extrapolated to \( t_0 \), and \( R_{\text{int}} \) (\( R_{\text{lex}} \)) thus calculated (figure 3.2(d)).
(iii) **End-oscillation pressure.** The mean pressure $t_{15}$ to $t_{25}$ ms was calculated. This was taken to approximate the pressure at the end of the oscillations ($P_{eo}$) and was used to estimate interrupter resistance ($R_{eo}$) (figure 3.2(b)).

The end-interrupter pressure was not used, although it has been reported with short interruption times in schoolchildren and the end interrupter pressure related to flow at the start of the interruption, but this makes no sense in infants, since the pressure after 500 ms of interruption does mainly reflect elastic forces.

The software also calculated the volume of the tidal breath expired prior to interruption, the volume remaining to be expired at interruption, and the ratio of the two volumes.

Within subject variability was expressed as the coefficient of variation ((SD/mean) x100) of the total number of acceptable interruptions obtained.
3.4 High Speed Interrupter Technique (HIT)

3.4.1. Introduction

HIT was used to examine airway wall properties. HIT measures input impedance of the respiratory system ($Z_{in}$). At high frequencies $Z_{in}$ is governed by wave propagation through the respiratory system and provides information about airway wall mechanics (97). Such data are difficult to acquire in infants by alternative impedance methods such as the forced oscillation technique due to relatively high airway resistance and the large gas compliance of the facemask, which reduce the high frequency content of the data.

Pressure oscillations are recorded at the airway opening during sudden high-speed interruptions to flow. The nature of the signal imposed on the respiratory system by the rapid interruptions enables measurement of high frequency impedance ($Z_{in}$) and provides information about airway wall properties.

3.4.2. Equipment:

The equipment used had previously been developed by Dr. Urs Frey (figure 3.4.). The high-speed shutter closes within 1 msec, and remains closed for 14.5 msec. The interruption open-closure cycle is completed in 31 msec. Each time the interruption is triggered the shutter rotates 4 times producing 4 separate interruptions over 124 msec. A photo-optic resistor measures the position of the shutter (open or closed) and ensures that at the end of the interruption the shutter remains open. A perspex tube, the wave tube, (radius 0.5 cm, length 12.7 cm) connects the shutter to the facemask.

The wave tube technique is used to measure $Z_{in}$. Two identical pressure transducers (Eurosensor, Model 33 UK) are mounted in the wall of the tube in two locations 6.7 cm apart. These sample the oscillations that occur in response to flow interruption. $Z_{in}$ is computed by from the two pressure signals, sampled during the four complete cycles (4 x 31 msec=124 msec) of the interrupter at 8258 Hz. The wave tube was connected to the same facemask as used during the $R_{in}$ measurements.
3.4.3. Calibration

The equipment was warmed up for 30 minutes prior to calibration. The offset of both pressure transducers was reset electrically. The gain of both was calibrated using a water manometer over a range of $-10 - +10 \text{cm H}_2\text{O}$. To ensure linearity of the pressure transducers a calibration program was used to display both pressure channels together while exposing both to identical pressures ($-10 - +10 \text{cm H}_2\text{O}$) monitored by a water manometer.

3.4.4. Protocol

The facemask was placed over the infant’s mouth and nose as previously described. During apparent quiet sleep, ten interruptions were performed over a period of 2-3 minutes. The operator manually triggered interruptions during early inspiration, consistent with previous studies using HIT.

3.4.5. Analysis

To process the pressure signals a spectral analysis is performed. A specific mathematical tool, the fast fourier transformation, is employed, using a computer. The pressure signals are decomposed into individual frequency components, and at each frequency the correlation coefficient of input and output is compared to compute the coherence function. This represents the fraction of the output (the pressure signals) that is related to input, i.e. the degree to which the results are influenced by noise. Any points on the impedance spectrum with a coherence of $<0.9$ were discarded.

An impedance spectrum was generated for each subject (figure 3.5). Previous work had identified a particular characteristic of the spectrum, the frequency of the first anti resonance ($f_{ar,1}$), defined as a zero crossing in the imaginary part in the presence of a relative maximum in the real part. The value of $f_{ar,1}$ was obtained for each of the 10 sets of four interruptions for each infant, and the coefficient of variation calculated. For graphical purposes the 10 impedance spectra were averaged.
3.4.6 Repeatability

Repeatability of \( f_{ar,l} \) was examined in eight wheezy infants of mean (SD) weight 9.71 (2.03) kg and length 74.7 (7.55) cm. Ten HIT measurements were obtained, the mask was removed and the putty removed, remodelled and then replaced. A second set of ten measurements was then made. This procedure was designed to mimic remodelling of the putty during the prolonged challenge procedure. Removal and remodelling of the putty was sometimes required because warming from the infant’s faces resulted in alterations in its shape within the mask.

Repeatability was assessed using the approach of Bland and Altman (117) and is shown in figure 3.6. From this figure it can be seen that any change in \( f_{ar,l} \) from baseline +/- 20 Hz can be considered significant. These values were almost identical to values for repeatability (+/- 18Hz) obtained during initial evaluation of the technique (Urs Frey, The High Speed Interrupter technique to measure airway wall mechanics in infants, PhD thesis, University of Leicester 1999, Figure 6.1.6.3, reproduced here as fig3.6b). That value was obtained from a range of baseline \( f_{ar,l} \) from 110Hz to 200Hz.

3.4.7 Discussion

From theory it was known that such an anti resonance as \( f_{ar,l} \) is due to wave propagation (i.e. they are acoustic anti resonances). This means they are related to the inertance of the gas within the airways and the compliance of the airway walls. In infants this was confirmed when it was shown that \( f_{ar,l} \) changed as predicted when infants breathed a mixture of helium and oxygen, i.e. of different inertance than that of room air(97). The same study demonstrated no change in \( f_{ar,l} \) when one nostril was occluded, providing evidence that \( f_{ar,l} \) was not altered by nasal patency and probably reflected the properties of the intra thoracic airways. Subsequent work demonstrated changes in \( f_{ar,l} \) during methacholine challenge in infants(41). Changes took place at very low doses, before any change in \( V'_{maxFRC} \), suggesting that the technique was capable of detecting changes in airway wall compliance. Unfortunately because of the complexities of the analysis, it was not possible to interpret the changes in \( f_{ar,l} \) as increases or decreases in airway wall compliance, but merely as changes (see section 5.4.2.2.). When groups of wheezy and healthy infants were compared, those with wheeze had significantly lower values of \( f_{ar,l} \) than the healthy infants (42), suggesting that differences in airway wall properties, either
congenital or acquired could contribute to wheezing symptoms. For these reasons the outcome of interest in this study was far, 1.
3.5 Tables and Figures
Figure 3.1a Ultrasonic flowmeter, shutter and facemask

Figure 3.1b Unsedated infant during $R_{int}$ measurements
**Figure 3.2** definitions of values of flow and pressure used to calculate \( R_{\text{int}} \)

**Figure a:** Flow trace after interruption. *Definition of \( T_0 \):* the flow minimum (the point at which expiratory flow began to fall, as the shutter began to close) plus 5 msec.

**Figure b:** Representative airway opening pressure (\( P_{\text{ao}} \)) traces following flow interruption in an infant. The initial rapid rise in \( P_{\text{ao}} \), followed by a slower, secondary rise to an end-occlusion plateau can be seen.

**Figure c:** A polynomial curve was fitted to the post occlusion pressure data from \( T_{+30} \) to \( T_{+200} \) ms and back extrapolated to \( T_0 \) to estimate \( P_{\text{post}} \).

**Figure d:** A two-point linear back extrapolation was performed (centred about 30 and 70 ms after \( T_0 \)).

Figure from reference(92)
Figure 3.3 Examples of airway Opening Pressure ($P_{ao}$)/time curves, acceptable and unacceptable for analysis.

- **Active breathing against the shutter**: This curve shows a sudden increase in pressure followed by a gradual decrease, indicating active breathing against the shutter.
- **Incomplete relaxation**: This curve shows an incomplete relaxation of the airway, characterized by a slower decrease in pressure compared to the active breathing curve.

The graphs are labeled with time in milliseconds (msec) and pressure in kilopascals (kPa).
**Figure 3.4** Infant during high-speed interrupter measurements. The clear Perspex wave tube separates the facemask from the high speed shutter.

**Nb:** the infant is wearing the inflatable “squeeze” jacket, which has been loosened after the preceding inflations.
**Figure 3.5** Example of impedance spectrum obtained by high speed interrupter technique for one infant, (mean and standard deviation of 10 measurements). \( f_{ar,1} \) coincides with a relative maximum in the real part and a minimum in the imaginary part.

Frequency of the first antiresonance \( f_{ar,1} \)
Figure 3.6a Bland Altman Plot representing short term repeatability of the first anti-resonance $far_1$. The difference between $far_1$ for the first and second measurement sets are plotted against their mean values for each of 8 infants. The solid line represents the mean difference and the dotted lines the 95% limits of agreement.
Figure 3.6b Bland Altman plot representing repeatability of far,1. The solid line represents the 95% confidence intervals of the mean difference. Subjects were 12 infants with a previous history of wheeze (from PhD thesis Urs Frey, University of Leicester, figure 6.1.6.3.).

4.1 Aims:

1. To examine feasibility of: recruitment (identifying sources of bias in the group eventually studied) and success rates in obtaining good quality data.

2. To describe the accuracy of $R_{\text{int}}$ measurements: within subject variability (variation during a single group of measurements) and short-term repeatability (the limits of agreement of the mean difference) in a group of infants, between two measurements performed on the same occasion at a similar time interval in the same infant.

3. To describe the attitude of parents to participation in infant lung function tests carried out without sedation.

4.2 Background

The aim of this part of the project was to comprehensively evaluate the measurement of resistance by interruption in unsedated infants, with a view to its possible role in a bronchial challenge test suitable for unsedated infants.

Recruitment of infants is a demanding and time consuming process and potential exists at all stages of a study, from the initial approach to parents, to selection of acceptable data for analysis, for the introduction of bias or "sampling error". The final population studied may be far from a "healthy unselected" group of infants.

If $R_{\text{int}}$ was to play a role in a bronchial challenge test in a representative population in unsedated infants information about feasibility could be vital. Infant sleep patterns are unpredictable, and it is unlikely that any test will be feasible in every infant. Historically, feasibility issues are rarely addressed in the literature. For example, the actual number of infants in whom sedation, and subsequent measurements, were attempted in order to obtain data is often not available. Prior to starting this work there was no information available to us about recruitment and success rates in unsedated infants, and so we chose to study this aspect in detail. However, it was suspected that without the requirement for sedation,
recruitment would be "easier". This was based on previous experience on recruitment of infants to previous lung function studies involving sedation.

Prior to the start of this project no studies involving the measurement of $R_{nt}$ in infants had been published. Subsequently, $R_{nt}$ was shown to be possible in unsedated infants (92), but this methodological study contained no information about numbers of infants recruited, or drop out rates in order to obtain measurements in the small number of (14) infants studied.

Only two reports have compared $R_{nt}$ with more established resistance measurements in infants. Airway resistance ($R_{aw}$) measured in an infant plethysmograph was compared with $R_{nt}$ in 13 sedated infants, and published in abstract form (118). Respiratory system resistance ($R_s$) measured by passive mechanics was compared with $R_{nt}$ in 25 sedated infants (115). In the latter study, the authors reported that $R_{nt}$ was only feasible in 25/36 sedated infants, the reason for failure being low tidal peak expiratory flow, insufficient to trigger the $R_{nt}$ shutter. In these two studies $R_s$ values fell between those for $R_{aw}$ (low) and $R_s$ (high), as might be predicted from theory, as chest wall resistance will contribute fully to $R_s$, partly to $R_{nt}$, and not at all to $R_{aw}$.

Repeatability of $R_{nt}$ in unsedated infants has not previously been examined. In 22 sedated infants with severe airway obstruction (who may have had particularly variable lung volumes) $R_{nt}$ was found to be much less repeatable than that seen in older children. However, these data have to be considered cautiously, since between repeated measurements infants underwent a rapid thoracic compression manoeuvre (119).

In naturally sleeping nose-breathing infants, where measurements are made via a facemask, repeatability is expected to be much poorer than in older children. Any change after an intervention is required to be much greater to achieve significance. Estimation of short-term repeatability is required before it is possible to plan or fully interpret intervention studies.

$R_{nt}$ has the important advantage that measurements can be made in much the same way from the neonatal period to early childhood, which may increase its value in studies aiming to track lung function. Measurements are made during tidal breathing, without the need for forced expiratory manoeuvres or passive inhalations to raised lung volume, which may themselves influence lung mechanics. However, $R_{nt}$ measurements in infants must be made via a facemask rather than a mouthpiece and, assuming nasal breathing during quiet sleep, $R_{nt}$ therefore includes the resistance of the nasal passages, as is the case for all measurements of resistance in infants. After infancy, once measurements are possible using a mouthpiece, nasal resistance is no longer included.
Knowledge of expected variability both between subjects in both healthy and disease states (in order to detect differences within a population) and within subjects (in order to determine significant change after intervention, be it therapeutic or experimental) is also important when evaluating a new lung function test. Short-term repeatability describes the similarity between two sets of measurements made on the same occasion, and was important for interpretation of changes after inhaled saline.
4.3 Methods

4.3.1 Recruitment.

A research nurse approached mothers of healthy full term infants on the postnatal wards of the Leicester Royal Infirmary. Visits took place several times a week over a period of 11 months. No infants were excluded on the basis of mode of delivery, ethnic group, or other perinatal factors. Infants admitted to the neonatal unit for any reason were excluded (i.e. those born prematurely or those born at term with medical problems). Written information about the project was provided, and a brief verbal explanation given (appendix 8.1a). Detailed questions about family history of asthma were not asked at this point. Mothers were defined as smokers using information taken from the obstetric notes. Approximately four weeks after initial contact, parents were telephoned and asked whether they wished to take part. If they agreed to take part a second copy of the written information sheet was sent to parents at this stage. Parents were requested to attend the laboratory for measurements, but if this was impossible then the offer was made to perform measurements in the infant's home.

The visit was timed to take place shortly before the infant’s usual daytime nap, normally around the time of a feed. The few days immediately after immunisations were avoided. A further telephone call was made to the infant’s parents on the day before the appointment as a reminder, to exclude respiratory tract illness and to finalise the time of the appointment according to the infant’s sleep pattern over the preceding days. Families were asked to attempt to keep their infant awake on the journey to the laboratory, and to avoid feeding the infant until after arrival at the hospital. Previous experience with unsedated infants in our laboratory had shown both factors to induce sleep prior to arrival, which generally led to less chance of achieving successful measurements. Parents were generally present throughout the tests, and they stayed in the laboratory until the infant fell asleep (or returned to sleep) or for as long as their other commitments allowed. Some parents volunteered to return with their infants on a separate occasion, but the investigating team did not request this routinely. Approval from the Local Ethics Committee was obtained prior to the start of the study (appendix 8.2).
4.3.2. Study Protocol

On arrival at the laboratory a full verbal explanation of the planned measurements was given, before obtaining written consent from parents (appendix 8.3). A questionnaire regarding family history of wheeze, doctor diagnosed asthma, eczema or hay fever in first-degree relatives was obtained. Maternal atopy was defined as a report of current asthma, eczema or hay fever. The infant was fed and, once asleep a pulse oximeter was attached, and heart rate and oxygen saturations monitored throughout the period of sleep for reasons of safety.

Measurements were made during apparent quiet sleep with the infant supine (in a cot or pram), with the head in the midline and neck slightly extended. Quiet sleep was behaviourally determined by observing the infant for regular respirations, and lack of facial or limb movements.

Measurements were made using equipment and protocols as described in section 3.3. As the infants were unsedated particular care was needed when applying the facemask and sealing it onto the face, so as not to rouse the infant. If the infant had been partially roused by the application of the facemask, (despite initially appearing to be in quiet sleep) this was obvious from a rapid respiratory rate with variable end expiratory level, and the mask was removed. Measurements attempted during such periods of irregular respiration were found to further rouse the infant and to be of poor quality (see below). Interruptions were performed every 4-5 breaths, for as long as the infant remained in quiet sleep, or until approximately 10-15 interruptions had been recorded. If at least 4-5 interruptions appeared to be of good quality, then the mask was removed. It was applied again as soon as the infant’s sleep state permitted, in order to repeat the above procedure, to determine short-term repeatability. The time interval between the two sets of measurements was recorded. The short-term repeatability, or variation between two sets of measurements made in the same infant on the same occasion was investigated in order to subsequently evaluate the significance of any changes occurring after saline challenge.

Following their visit we sent each family an anonymised questionnaire regarding their motivation to take part in the study and their experience of having taken part.
4.3.3. Analysis

Visits were defined as successful if at least 4 technically acceptable measurements could be made, regardless of the total number recorded. Repeatability was determined if two separate groups of at least 4 technically acceptable measurements were recorded in the same infant, each after a separate application of the facemask.

Data were excluded prior to analysis if evidence of leak, incomplete relaxation or active breathing was seen when inspecting the airway opening pressure/time (Pao/t) curves for each interruption. R_{int} was calculated using the approach described in section 3.3.5, and 3 values of R_{int} were obtained for each infant, each derived from a different method of estimating Pao at the time of interruption (t_0) and relating this to estimated flow at t_0.

4.3.4 Statistics

Characteristics of the infants in whom measurements were made successfully were compared with those infants in whom measurements had proved unsuccessful. Continuous variables were compared using independent samples t tests, and categorical variables compared with Chi squared tests.

All acceptable interruptions (i.e. at least 4) for each infant were analysed and a mean, standard deviation and coefficient of variation (cv) obtained. Coefficients of variation were used to compare variability between infants. For infants in whom within test repeatability was assessed, the differences between the means of each of the two runs were compared using the approach of Bland and Altman (92), and limits of agreement for each analysis method (twice the standard deviation of the differences between mean paired measurements) were calculated.

4.3.2. Post Visit Questionnaire

A copy of the post visit questionnaire can be found in appendix 8.4. These were mailed to families monthly in batches during the eleven-month period that R_{int} measurements were made in unsedated infants. Parents therefore received their questionnaire a maximum of a month following their participation.
4.4. Results

4.4.1. Recruitment

A total of 277 mothers were approached in hospital and given information about the project. Of the 277 mothers these 186 were contacted by telephone. In 72 cases there was no answer despite several attempts; in 14 the number provided was unobtainable and in 5 the family had no telephone. Of the 186 successfully contacted, 79 (42%) agreed to take part in the study, and appointments were made on a day convenient for the family concerned. Twenty-three families phoned to cancel their appointment or did not attend without giving a reason. In total measurements were attempted on 65 occasions on 56 babies, as some mothers offered to return for a second attempt (figure 4.1). We were not able to take details of maternal or family history of asthma or other atopic conditions at initial contact on the postnatal wards. Therefore it was not possible to the degree of bias introduced at the level of initial contact. Recently published population data for Leicestershire was helpful in estimating bias in those actually taking part (see section 4.5.1).

4.4.2 Subjects

A total of 56 infants were seen on a total of 65 occasions. Forty-seven infants attended the laboratory, 4 returned for a second visit to the lab. Nine infants were visited in their own homes, as were an additional 5 who had already been seen in the laboratory. Mean (SD) age for all infants was 63 (14) days and weight 5.42 (0.88) kg. None had been born prematurely (<37 completed weeks gestation) or required respiratory support or additional oxygen after birth. Infants were not excluded on the basis of ethnic group, and 6 of the 56 were non-caucasian (2 mixed race, 2 Asian (Indian) and 2 Afro-Caribbean).

4.4.3. Success Rates

In 38 of the total 65 (58.5%) occasions (38 of 56 infants, of whom 9 had a second attempt with no infant having successful measurements made on more than one occasion) it was possible to obtain at least 4 acceptable measurements of $R_{int}$. For lab studies the success rate was 31/51 (60%) and for the home visits it was 7/14 (50%). Therefore in 14% (38/277) of families originally approached measurements were made successfully.

Reasons for failure to obtain sufficient data in infants in whom measurements were attempted were complete lack of sleep (n=6), active sleep only (n=5), quiet sleep but intolerance of the facemask (n=3), disturbance of sleep by the sound of the shutter closing...
(n=3), or insufficient number of acceptable interruptions (n=4). The median (range) proportion of interruptions deemed acceptable was 33.1% (8.57-68.8%), in those “successful infants”. Comparisons of infant characteristics in the successful and unsuccessful groups are displayed in table 4.1. The “successful group” had significantly greater birthweight, had a greater proportion of infants with maternal atopy, and a trend towards more maternal asthma.

4.4.4. Variability

Mean CV for $R_{int}$ varied between 12 and 21%, according to analysis method. Tidal volume in the breath prior to the breath during which the interruption occurred was much less variable (CV 7%), as was the relative volume at which the interruption occurred, suggesting that large changes in tidal volume at the point of interruption do not explain the relatively high variability of $R_{int}$ (table 4.2).

4.4.5. Repeatability

Twelve infants slept sufficiently to allow repeated measurements to be made, and there were no significant differences in age, weight, or length between these infants and those in who repeat measurements were not feasible. The time interval between the two runs of interruptions ranged between 1 and 50 minutes (median 7 minutes). This time period was purely determined by the infants’ sleep status being suitable for measurements (for example removal of the mask often marginally disturbed the infant and triggered a change in apparent sleep state (see section 4.3.2)).

Differences between the means of each of the two runs were compared using the approach of Bland and Altman (117), and limits of agreement for each analysis method (twice the standard deviation of the mean differences between paired measurements) were calculated. The limits of agreement for the repeatability of at least 4 technically acceptable measurements of $R_{int}$ ranged between 1.65 and 2.16 kPa.L$^{-1}$.s (between 31% and 60% of mean baseline values) (figure 4.4).
4.4.6. Post Visit Questionnaire

The response rate to the post visit anonymous questionnaire was 68% (38/56). No parent admitted to finding the $R_{int}$ measurements at all distressing. Only 32% of respondents felt that they would have chosen to participate when asked “If the tests had involved the baby having a medicine (in the form of a syrup by mouth) to help them sleep would you still have volunteered?”. Most parents who returned the questionnaire wrote very positive comments about their experience of taking part.
4.5 Discussion

4.5.1. Recruitment:

We approached an unselected group of 277 families from which we hoped to recruit infants to take part in a study involving a single visit to a hospital based infant lung function laboratory for measurement of $R_{in}$ and to estimate short-term repeatability. We attempted initial measurements on 56 infants (20%) and were successful in 38 (14%) (no infants were successful on more than one occasion). The variability for each infant, expressed as coefficient of variation ranged between 4% and 44%, despite careful attention to data quality. Repeatability expressed as limits of agreement ranged between $-1.02 \text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$ and $+1.14 \text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$ and was greatest for $R_{pec}$.

The greatest loss of infants occurred as a result of difficulty in contacting families and of mothers declining to take part after a telephone discussion (figure 4.1). Both factors are heavily reliant on time and facilities available to recruiters, and on their skills in communicating with parents. The facility for recruiters to make calls to families in the evening, and to visit them in their own homes to discuss participation face to face could improve eventual subject numbers. Indeed this is the experience of our laboratory in infant studies in the area of control of breathing. Obviously increased numbers of recruiters, able to work at flexible times, would be required.

Factors that lead parents to volunteer their infants to take part in lung function studies as "healthy volunteers" have not been formally investigated, and may vary year on year depending perhaps on adverse publicity over paediatric research in the media. Differences in public attitudes to infant lung function, and sedation in particular may exist between different countries.

Those parents who have a history of asthma in themselves or their other children may feel particularly motivated to take part. In our study the prevalence of maternal asthma in infants in whom measurements were attempted was 25%. Maternal smoking during pregnancy was reported in 18% of infants. Direct comparisons with those infants whose parents declined to take part in the study are not possible, because detailed family histories were not taken at initial contact. However, a recent large study of our local population found a prevalence of maternal asthma of 15.9%, and of reported smoking during pregnancy of 22.3% (120). A previous study in our laboratory of antenatal recruitment to a study of respiratory control found that the most important factor influencing recruitment was having a partner in employment (121).
In an epidemiological context, factors that distort recruitment will introduce bias. Our cohort included a relatively large proportion of infants at high genetic risk of asthma, and a low proportion of infants exposed to the adverse effects of maternal smoking. Bias may occur at another level, even when families have decided to take part. Infants in whom it was possible to make good quality measurements were significantly larger at birth, and had a greater proportion of mothers with atopy and asthma. It may be that mothers with a history of asthma complied with our instructions to a greater degree to keep their infant awake until reaching the laboratory, resulting in longer periods of sleep during the study period.

Other factors that may introduce bias include social class and ethnicity. We did not collect data on social class, so cannot comment on this directly. Overall 43% of our subjects were breastfed at an average age of 9 weeks, and higher breastfeeding rates are associated with higher social class, indirectly suggestive of higher social class in our infants as the local average for breastfeeding at 6-8 weeks is between 10 and 30% depending on suburb (Leicester City Breast Feeding Audit 2004). Of our cohort of 56 infants, on whom measurements were attempted, 6 (11%) were of non-Caucasian ethnicity, compared with Leicester as a whole where 36% of the population are non-white (Census 2001 - KS06p - Percentage - Ethnic Group).

It is likely that recruitment levels would have been lower, had our study involved sedation, as demonstrated by the responses to our post visit anonymised questionnaire. Ethical constraints now preclude the recruitment of healthy infants to studies involving sedation in many though not all countries. Some groups continue to study sedated healthy infants successfully (80;85).

Of parents who agreed to take part in the study, 28% cancelled their appointments without giving a reason (usually by leaving a message on the project answer phone) or failed to attend the laboratory, or were not at their home when the research team called at an agreed time, despite a phone call from the team within the preceding two days. This phenomenon of has recently been highlighted by paediatric researchers in the field of asthma genetics (122).

4.5.2. Success rates

The only other published study regarding measurement of $R_{int}$ in unsedated infants was a pilot study of methodological issues and did not investigate success rates in
obtaining data. No data on length of time spent waiting for infants to achieve quiet sleep and tolerance of the facemask was given. A study investigating feasibility of $R_{int}$ measurements in awake preschool children reported a success rate of only 12% (1/8) in infants aged less than one year(123).

Success rates may vary with age, and measurement protocol. This will determine study size (power), time and effort needed for recruitment, and generalisability (bias). Parents have different thresholds for the amount of time they are prepared to wait for the infant to go to sleep, and these may vary with social factors such as responsibility for other siblings, work commitments, and parental interest in the project.

We used a rigid facemask that was sealed gently onto the infant’s face using therapeutic putty, as this approach has been shown to reduce measurement error(92). Placing the mask and achieving an adequate seal in a naturally sleeping infant requires skill. The use of a soft mask with an inflatable ring may be easier, but at the expense of reduced accuracy. Other techniques do not expose the infant to the sound of a shutter opening and closing within 5cm of the face, may be more successful, or indeed development of a quieter shutter. In the future, reporting of success rates for different techniques would aid planning of large studies. Recently measurement of $R_{int}$ using the single occlusion technique in 450 healthy unsedated infants been reported (124). A success rate on the first occasion of 69% was found, and failure was most commonly attributed to lack of sleep (16%), followed by failure to relax during interruptions (5%), and marked expiratory braking (4%), broadly similar to our findings.

4.5.3 Variability

We used similar equipment and analysis techniques to those employed by Hall et al (92) although our infants were approximately 4 weeks older. The variability of $R_{int}$ in both groups of infants is similar, and is much greater than for some other infant lung function techniques (table 4.3). For example, measurements of forced flow at functional residual capacity obtained during the rapid thoracoabdominal compression technique had a coefficient of variation of 6.3%, under strictly standardised conditions(77).

Differences in tidal volume, strength of the Hering Breuer reflex, degree of expiratory braking and resistance of the nose and larynx, could all contribute to variations in both estimated flow at the point of interruption and pressure during the interruption. Exclusion of $P_{ao}$ traces with gross irregularities should reduce variability, but this process is subjective. Two infants had $R_{int}$ values three and five times the magnitude of the remaining infants, and these two infants had two of the highest values for variability.
Inspection of the $P_{aw}/t$ curves confirmed excellent quality, but flows were low throughout the preceding tidal expiration, suggesting marked expiratory braking (figure 4.3). There is a software delay after the triggering flow is detected before the shutter closes, and the flow at $t_0$ is estimated by forward extrapolating flow patterns during early expiration prior to shutter closure. Therefore any blunting of the rate of change of flow prior to interruption will exaggerate the effect of low tidal expiratory flows. Relating $P_{aw}$ values to these low flows produced abnormally high values for $R_{int}$ (figure 4.3). Expiratory braking may result from changes in laryngeal calibre or post-inspiratory diaphragmatic activity, both strategies thought to be employed by infants in order to maintain FRC. More subtle differences in expiratory braking, which may be more common in younger, unsedated babies and vary breath to breath and with sleep state, could alter $R_{int}$ values either by altering flow at the point of interruption or by distorting the $P_{aw}/t$ curve with unpredictable effects on the back extrapolation procedure.

4.5.4 Repeatability

The short term or “within occasion repeatability” of $R_{int}$ has been investigated in preschool children (123;125;126). Comparisons of limits of agreement between different age groups (with very different baseline values of $R_{int}$), are facilitated by expressing the limits of agreement as percentages of baseline values. This approach gives some indication of the proportionate change from baseline that would be considered significant after an intervention.

Our limits of agreement for the repeatability of at least 4 technically acceptable measurements of $R_{int}$ performed during the same visit range between 1.65 and 2.16 kPa.L$^{-1}$.s (between 31% and 60% of mean baseline values). The only other published study for infants produced limits of agreement of approximately 4.5kPa.L$^{-1}$.s over a similar range of baseline values, although the effect of the intervening forced expiratory manoeuvres on $R_{int}$ is unknown, but may partly explain it.(119). However, the infants in that study had significant airway obstruction, and this degree of disordered physiology is likely to have contributed to variation in $R_{int}$.

These values are much greater than those reported by groups studying older children. Bridge et al(125) studied 120 preschool children (including healthy and previously wheezy subjects) and found values for repeatability (or limits of agreement) of between 0.13 and 0.24 kPa.L$^{-1}$.s, depending on baseline $R_{int}$ values. Children with higher baseline $R_{int}$ (i.e. younger children) had poorer (i.e. greater absolute values) for repeatability, which expressed as a percentage of baseline $R_{int}$ ranged between 9% and
36%. Lombardi et al(126) investigated repeatability within one minute of both inspiratory (R_{int}) and expiratory (R_{int_e}) R_{int} in children with a mean age of 4.7 years. Values of 0.202 kPa·L^{-1}·s and 0.242 kPa·L^{-1}·s were obtained for repeatability of R_{int,i} and R_{int,e} respectively, or in terms of median baseline R_{int} values, the repeatability is 30% R_{int,i} for and 36% for R_{int,e}.

Important methodological factors may explain why repeatability of R_{int} measured in an infant in quiet sleep is much poorer than in older children. All measurements in infants are made via a facemask, and so include the resistance of facemask and the nose, in addition to the laryngeal and lower airway resistance. Subtle differences in the position of the head and neck (altering upper airway dimensions), and placement of the facemask (including arrangement of the putty) between the two runs of measurements, may result in altered values for R_{int} as alveolar pressure will equalise with mouth pressure over a different volume, leading to change in the magnitude of P_{ao}.

Our infants were studied in apparent quiet sleep. Subtle changes in sleep state, which are not apparent to the investigator, may produce fluctuations in upper airway tone. The stimulus of facemask removal and then repositioning for a second series of measurements may alter both upper airway calibre and compliance, and therefore R_{int}. Altered sleep state may also produce changes in breathing pattern, which could affect the relative volume at which the interruptions occurred.

We used new flow measurement equipment, designed for use in small infants, with a low dead space and resistance, as both factors are known to influence measurements of tidal breathing. However the application of a rim of a facemask alone to the face without additional dead space or resistance has been shown to cause a significant fall in respiratory frequency and an increase in tidal volume (64) in healthy term newborns, which was presumed to be in response to tactile stimulation of the face. These altered breathing patterns persisted for at least five minutes after application of the mask. Although more marked during REM sleep, significant changes still occurred during quiet sleep. Other work has shown that when tidal breathing parameters are examined in two epochs one and five minutes after application of a facemask variability of all tidal breathing parameters derived was significantly higher in the first epoch(127). A bias flow into the facemask was used to virtually eliminate dead space, suggesting that facial stimulation was responsible for the increased variability in tidal breathing in the first two minutes after application. When working with infants in natural sleep (when 42% on infants visiting a laboratory will not sleep well enough for good quality data to be obtained), standardising factors such as length of time after application of facemask until measurements begin is extremely
difficult. In particular, waiting 5 minutes after application of a facemask (so called “acclimatisation”) would drastically reduce success rates even further. Varying degrees of facial stimulation leading to altered end expiratory level, and variable degrees of active expiration may account for the high variability and relatively poor repeatability in naturally sleeping infants.

4.5.5. Post visit questionnaire

Our interest in the attitude of parents taking part in the project was twofold. First to examine the process of recruitment, in particular our communication with families during the recruitment process, including the quality of our parent information leaflets and our verbal discussions with parents. No consistent gaps in information were identified, although one parent stated that the tests had taken much longer than she expected.

Secondly we wished to compare the attitudes of our families to those reported in a similar questionnaire study, which investigated parental attitudes of 81 families after participation in infant lung function tests (for research purposes), including sedation with an oral dose of chloral hydrate. That study had a response rate of 84% (81 families), and of the respondents 59% felt that their infant suffered some distress during the administration of the sedative, and 5% felt that their infant had suffered some distress during the lung function testing procedure(59), 6% felt unable to recommend that other infants took part. In contrast, none of our respondents felt that any distress was involved for them or their baby, and all felt able to recommend to others that they should take part. However, we do not know the views of those who chose not to return their questionnaire, and it is possible that they had less positive experiences of taking part.
4.6 Conclusion

We were able to assess $R_{int}$ reliably in 38 of 56 healthy unsedated infants (where measurements were attempted on 65 occasions) with a mean age of nine weeks. These infants represented 14% of the original cohort of mothers approached. Most potential data was lost by failure to recruit infants after initial contact with parents, rather than failure to obtain data in infants attending the laboratory or visited at home.

Infants in whom measurements were successful had an increased proportion of maternal asthma and atopy, probably as a result of increased commitment to the study. Bias such as this would be important in any future population studies. Even when simple techniques that do not require sedation are used, the population recruited may not be completely “unselected”.

Variability and short term repeatability of $R_{int}$ in unsedated infants appears to be much higher than in older children, and factors, both technical and physiological, contributing to this variability require further investigation.

The proportion of infants in whom repeatability data was obtained was small (12/38), reflecting the difficulties in obtaining good data twice in an unsedated infant. With an intervention between measurements (such as a bronchial challenge) the yield of data is likely to have been even smaller.
### 4.7 Tables and figures

**Table 4.1** Comparison of infant characteristics between successful and unsuccessful occasions (values are mean and SD or n(%))

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Successful Occasions</th>
<th>Unsuccessful Occasions</th>
<th>Difference</th>
<th>Significance of the difference (95% cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of occasions when measurements were attempted</td>
<td>38</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>64 (15)</td>
<td>63 (13)</td>
<td>1.0</td>
<td>p=0.83 (-6.3 ; 7.9)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3570 (537)</td>
<td>3260 (595)</td>
<td>310</td>
<td>p=0.04 (22.2; 597)</td>
</tr>
<tr>
<td>Current weight (g)</td>
<td>5571 (814)</td>
<td>5168 (935)</td>
<td>403</td>
<td>p=0.08 (-52.0; 858)</td>
</tr>
<tr>
<td>Time spent in laboratory (mins) Median (range)</td>
<td>153 (70-390)</td>
<td>135 (60-225)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time spent asleep (mins) Median (range)</td>
<td>39 (3-125)</td>
<td>18 (0-118)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>21 (55%)</td>
<td>15 (55%)</td>
<td>p=0.99</td>
<td></td>
</tr>
<tr>
<td>Maternal smokers</td>
<td>7 (18%)</td>
<td>5 (19%)</td>
<td>p=0.99</td>
<td></td>
</tr>
<tr>
<td>Breastfed</td>
<td>15 (39%)</td>
<td>13 (48%)</td>
<td>p=0.69</td>
<td></td>
</tr>
<tr>
<td>Maternal asthma</td>
<td>12 (31%)</td>
<td>3 (11%)</td>
<td>p=0.05</td>
<td></td>
</tr>
<tr>
<td>Maternal Atopy</td>
<td>25 (66%)</td>
<td>9 (33%)</td>
<td>p=0.01</td>
<td></td>
</tr>
<tr>
<td>Wheeze in first degree relative</td>
<td>25(66%)</td>
<td>13(48%)</td>
<td>p=0.16</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 Absolute values of $R_{int}$, coefficient of variation (CV), and limits of agreement for each of the three analysis methods.

<table>
<thead>
<tr>
<th></th>
<th>$R_{pex}$ (kPa.L$^{-1}$.s)</th>
<th>$R_{ce}$ (kPa.L$^{-1}$.s)</th>
<th>$R_{lex}$ (kPa.L$^{-1}$.s)</th>
<th>Tidal Volume (Vt) of breath prior to interruption (ml)</th>
<th>Proportion of Vt expired at interruption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>3.63 (2.35)</td>
<td>5.12 (2.52)</td>
<td>3.07 (2.12)</td>
<td>43.6 (0.61)</td>
<td>5.8 (1.84)</td>
</tr>
<tr>
<td><strong>Mean (SD) CV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for all infants</td>
<td>18.83 (6.95)</td>
<td>12.33 (5.83)</td>
<td>21.39 (8.76)</td>
<td>6.94 (2.75)</td>
<td>1.12 (0.5)</td>
</tr>
<tr>
<td><strong>Mean difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>between repeated measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.08</td>
<td>-0.04</td>
<td>-0.72</td>
<td>-0.29</td>
</tr>
<tr>
<td><strong>95% limits of agreement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.02, 1.14</td>
<td>-0.72, 0.87</td>
<td>-0.87, 0.78</td>
<td>-5.70, 4.30</td>
<td>-2.193, 1.613</td>
</tr>
</tbody>
</table>

*Coefficient of variation describes the variation within one set of measurements.

**Limits of agreements describe the range of differences between two sets of measurements within the same infant on the same occasion.
Table 4.3 Variability of other infant lung function tests from the literature (References)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Measurement</th>
<th>Coefficient of variation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway Resistance (Plethysmography)</td>
<td>$R_{aw}$</td>
<td>5.9% (128) 18% (129)</td>
</tr>
<tr>
<td>Forced Expiratory flow</td>
<td>$V'_{maxFRC}$</td>
<td>11% (130) 16% (94) 13% (35) 6.4% (77)</td>
</tr>
<tr>
<td>Forced Expiratory Flow and Volumes from Raised volume</td>
<td>FEV$_{0.5}$</td>
<td>3.6% (77) 4.7% (83) 4.8% (77; 131)</td>
</tr>
<tr>
<td>Forc $FEF_{25-75}$</td>
<td>7.8% (77)</td>
<td></td>
</tr>
<tr>
<td>Low Frequency Respiratory Impedance</td>
<td>Airway Resistance</td>
<td>11.3% (132) 6.0% (133)</td>
</tr>
<tr>
<td>High Frequency Respiratory Impedance</td>
<td>$f_{ar,l}$</td>
<td>9% (97)</td>
</tr>
<tr>
<td>Passive mechanics (Single Occlusion Technique)</td>
<td>Respiratory System Resistance (Rrs)</td>
<td>12% (94) 10.9% (134) 11.0% (135) 10.4% (124)</td>
</tr>
<tr>
<td>Lung volume with an ultrasonic flowmeter (unsedated infants)</td>
<td>FRC</td>
<td>5.5% (136)</td>
</tr>
</tbody>
</table>

*Coefficients of variation quoted refer to mean CVs in a group of infants described in the references.
Figure 4.1 Proportion of potential recruits lost at each stage of the recruitment process

Nb: This chart represents the number of infants at each stage of the recruitment process, with ultimately 56 infants attending the lab, and successful measurements made in 38 infants. Although 9 infants were seen on a second occasion, in no infant were measurements made successfully more than once. In total, therefore, successful measurements were made in 38 infants on a total of 65 occasions.
Figure 4.3 Low expiratory flows during interruption, contributing to increased values of $R_{\text{int}}$ (see section 4.5.3.). These plots are from two different infants. The very low expiratory flows manifested on the plot on the figure on the right contribute to high $R_{\text{int}}$ values.

**Interruption during normal expiration**

**Interruption during marked expiratory "braking"**

$R_{\text{pex}} \approx 2.41 \text{kPa.L}^{-1}.\text{s}$

$R_{\text{pex}} \approx 10.32 \text{kPa.L}^{-1}.\text{s}$
Figure 4.4:
Bland Altman Plot representing pilot data of short term repeatability of R_{int} (for each of the three analysis methods, see following page). The difference in mean R_{int} between for the first and second measurement sets are plotted against their mean values for each of 12 infants. The solid line represents the mean difference and the dotted lines the 95% limits of agreement.

Bland Altman Plot of R_{PeX1} and R_{PeX2}

Bland Altman Plot of R_{eo}

86
Bland Altman Plot of \( R_{\text{le}x1} \) and \( R_{\text{le}x2} \)
5. Development and Standardisation of a Saline Bronchial Challenge Test in Sedated Infants

5.1 Introduction

The primary aim of the project was to develop a safe bronchial challenge test applicable to unsedated infants. The purpose of this part of the project was to demonstrate the safety and feasibility of the saline inhalation challenge test in wheezy infants, by identifying a concentration of saline that would cause a change in airway wall properties as measured by the high speed interrupter technique (HIT), prior to changes in forced expiratory flow, as measured by tidal rapid thoracoabdominal compression technique (RTC). Interrupter resistance ($R_{\text{int}}$) was used to examine airway resistance before and after saline.

Previous work had shown specific changes in tidal breathing patterns occurred in infants undergoing bronchial challenge with histamine. In infants responding with a fall in $V_{\max FRC}$ greater than 30%, tidal breathing analysis revealed a fall in expiratory time ($T_e$), an increase in mean tidal expiratory flow (MTEF), an increase in respiratory frequency, and a fall in the respiratory system time constant ($t_{rs}$) (99). The authors interpreted these changes as the infant adopting a strategy of active expiration in the face of lower airways obstruction (as assessed by a fall in $V_{\max FRC}$), in order to maintain a relatively stable pattern of expiratory flow in the face of wide differences in airway obstruction.

We were concerned that changes in calculated $R_{\text{int}}$ might simply reflect similar changes in expiratory breathing patterns by altering the shape of the $P_{aw}/t$ curve during the interruption, or by altering expiratory flow just prior to interruption. For example, a strategy of active expiration would have unpredictable effects on the extrapolation procedures used to calculate $R_{\text{int}}$, which assumes a passive, relaxed system. Therefore we examined features of tidal breathing between interruptions to investigate this phenomenon.
5.2 Methods

5.2.1 Recruitment

Parents of 41 infants, aged 5-12 months, admitted to hospital with acute bronchiolitis or virus induced wheeze were approached directly while resident on the ward with their baby. The project was described in brief and written information was provided (appendix 8.1b). No decision regarding participation was requested at this stage. The parents were contacted via telephone approximately two weeks after discharge from hospital, and a history of at least two episodes of wheeze confirmed with parents. If they had decided to take part then an appointment was arranged for them at a convenient time.

Letters from the consultant in charge of the paediatric admissions unit were sent to 338 parents of babies (aged less than one year) assessed at the hospital for bronchiolitis and/or wheezing over the preceding winter. The letter introduced the study and gave contact information for the research team. Any parent interested in speaking to a member of the research team was asked to reply by freepost or telephone, giving their contact details. On receipt of replies I made contact with parents, to confirm at least two episodes of wheeze, and sent out the same written information about the project. Further contact was made several days later for a decision.

5.2.2 Subjects

All subjects had had at least two episodes of parent reported wheeze, and recruitment via the wards and assessment unit meant a doctor had witnessed at least one of the episodes (i.e. a first episode confirmed by a doctor and a second episode reported by parents during the follow-up recruitment telephone call). Twenty-three infants had suffered from acute bronchiolitis and then subsequent wheezing episodes, the remaining 5 had recurrent wheeze with upper respiratory tract infections (URTI). Any infant who had required ventilation or additional oxygen for >4 days (either as a neonate or subsequently), or had ongoing cardio-respiratory disorders, was excluded. Infants younger than 5 months, or older than 14 months were excluded. As the purpose was to demonstrate safety, younger wheezy infants were not felt to be suitable subjects, and local experience had shown that infants older than 14 months were often unlikely to sleep through a prolonged testing protocol. Ethical approval was obtained prior to the start of the study (appendix 8.2).
5.2.3 Laboratory Visit

Appointments were made at the time of each baby’s usual daytime nap, when the infant had been free from URTI or wheeze for at least 3 weeks. Parents were welcomed to the lab and a full explanation was given about the nature and purpose of the tests to be carried out. Most parents observed all the tests. Written consent was obtained from the parent. A clinical examination was performed to ensure that the baby was not suffering from any respiratory illness or upper airway abnormality, and then the baby was weighed, and baseline oxygen saturation and heart rate measured using a pulse oximeter. A questionnaire regarding pregnancy, birth and respiratory health of the baby, as well as family history of atopic conditions was completed.

As all infants attending the laboratory were well with a normal clinical examination, a dose of 100mg/kg of chloral hydrate by mouth was administered. A cot, sofa and toys were provided to help settle the baby to sleep. Once the baby was asleep the pulse oximeter probe was reattached and the infant moved to the testing area of the lab. One member of the team was responsible for performing the measurements and monitoring the baby and the other for operating the relevant computer. Heart rate and oxygen saturation were continuously monitored throughout the procedure and were recorded on a written chart before each rapid thoracoabdominal compression manoeuvre, before each set of HIT or R<sub>int</sub> measurements, and before and after each dose of saline. An alarm limit of 93% was set on the monitor.

After waking, all infants were observed in the laboratory until they were completely awake, eating, drinking, and playing as normal.
5.2.4 Study Protocol

1. Baseline measurements
   a. RTC (sufficient to obtain at least 3 technically acceptable manoeuvres)
   b. $R_{int}$ measurements (sufficient to obtain at least 4 technically acceptable measurements)
   c. HIT measurements (10 measurements, each consisting of 4 high speed interruptions)

2. 0.9% Saline then:
   a. HIT measurements
   b. $R_{int}$ measurements

3. 2% Saline then:
   a. HIT measurements
   b. $R_{int}$ measurements

4. 4.4% saline then HIT measurements
   a. HIT measurements
   b. $R_{int}$ measurements
   c. RTC measurements

This order of measurements was chosen to minimize time required to change between different pieces of equipment whilst the infant was sleeping. HIT measurements were completed most rapidly and to ensure the maximum data was recorded in the event of the infant waking early, HIT was recorded first after each dose of saline.

Once the infant was sleeping soundly, baseline measurements were recorded for each technique as described in Chapter 3. Although it had been intended to measure RVRTC, this proved impractical in the context of a prolonged protocol, as the nature of the manoeuvres (passive inflations to raised lung volume) increased the risk of waking the infants.
5.2.5 Administration of the Nebulised Saline

Normal saline (0.9%, 154 mmol/L) was supplied in vials from the hospital pharmacy. A sterile 7% (1198 mmol/L) saline solution (Royal Free Hospital Pharmacy Manufacturing Unit, London) was diluted to produce 3 mls each of 2% (342 mmols/L) saline and 4% (684 mmols/L saline). For each concentration, 3 mls of solution was nebulised using a “Pari-Baby” nebuliser driven by a compatible “Pari-Boy” compressor. This particular nebuliser is designed for use in infants in any position, so was ideal for use in a supine, sleeping infant. Each solution was administered for 30 seconds, by placing the nebuliser facemask closely over the infant’s mouth and nose. Between each dose the nebuliser was rinsed with water and dried with paper, as some solution remained in the chamber after 30 seconds.

5.2.6 Analysis

5.2.6.1. $R_{int}$ measurements:

Changes in $R_{int}$, tidal volume ($V_t$), absolute volume above FRC remaining in the lungs at interruption ($V_{int}$) (calculated by subtracting the volume expired prior to the interruption from this tidal volume), and proportion of $V_{int}$ /$V_t$ (%) after inhalation of saline, were deemed significant if they were greater than the 95% limits of agreement for repeatability generated in unsedated babies (section 4.4.5).

In order to determine whether changes in breathing patterns after inhalation of saline could explain changes in $R_{int}$ we examined the characteristics of the expiratory portion of the tidal breath prior to each interrupted breath (interruptions were performed every 4-5 breaths, see section 3.3.4). This breath was the most distant from the previous interruption, and therefore least likely to be affected by it. Expiratory time, peak and mean tidal expiratory flow, time to peak tidal expiratory flow, and respiratory frequency were calculated using the “Add on tool: tidal breathing flow volume loop” component of the Wbreath software (Spiroware version 1.28, Ndd Medizintechnik AG Zurich Switzerland). Changes from baseline for all variables were examined using independent samples t tests (SPSS v 9.0).
5.2.6.2. HIT

Impedance spectra were obtained as described in section 3.4.5, and a value for $\text{far L}$ obtained for each of the ten measurements made at each stage. Changes from baseline in $\text{far L}$ were deemed significant if they lay outside the limits of agreement generated in a separate group of 8 infants (section 3.4.6 and figure 3.6a), which also agreed with a previous repeatability assessment on a group of 13 infants with a history of wheeze (figure 3.6b).

5.2.6.3. $V'_{\text{max FRC}}$

Any change was expressed in units of the standard deviation of 3 acceptable baseline measurements as well as the percentage in $V'_{\text{max FRC}}$, as is the convention in other infant bronchial challenge tests, which seek a threshold fall in flow such as 30% or 40%.
5.3 Results

5.3.1 Recruitment

Of the 41 families approached on the children’s wards to take part, 12 agreed to take part. Of the remainder, 14 families could not be contacted following discharge from hospital, 8 declined to take part, and 7 were ineligible due to lack of a second episode of wheeze (n=5), daily wheeze (n=1), and laryngomalacia (n=1). Of those who agreed that their infant could take part, 2 failed to attend without giving a reason.

Of the 338 letters sent to families of infants seen at the hospital with wheeze, 69 returned the tear off slip to request further information. Table 5.1 details the outcomes for those families.

28 infants (13 boys) were successfully recruited (10/41 from the children’s wards and 18/338 via letter); mean (sd) age in weeks was 41.4 (9.9). Mean (sd) weight was 9.8 (1.5) kg and length 72.5cm (3.8).

5.3.2 Success Rates in obtaining data

Three infants failed to sleep deeply enough for any measurements to be made, despite receiving chloral hydrate. Of the infants who slept, in six cases technical problems with the Rnt equipment, (persistent apparent leak around the facemask, and unexplained malfunction of the shutter) and early wakening by the infant accounted for the lack of complete data. Table 5.2 shows the number of acceptable measurements at each stage of the challenge procedure. Comparisons between baseline and post saline measurements were made only if at least three values for $V'_{maxFRC}$ (within 10mL.s$^{-1}$) or 10% of each other were available, according to recommendations(76), and at least 5 measurements of HIT or at least 4 measurements of $R_{int}$.

5.3.3 Safety

SaO$_2$ remained above 93%, and no infant developed wheeze or respiratory distress. Several infants coughed on waking. No falls in oxygen saturation occurred following administration of nebulised saline of any concentration.
5.3.4 Comparison of Baseline Data

The three measurements represent different physiological properties of the respiratory system, which are not likely to be strongly related to each other. When baseline $V'_{\text{maxFRC}}$, and $R_{\text{int}}$ were compared there was a trend towards an inverse relationship ($R^2 = 0.23$) was demonstrated for each method of obtaining $R_{\text{int}}$ ($R_{\text{ex}}$, $R_{\text{co}}$, and $R_{\text{lex}}$) (figure 5.1). There was no relationship between baseline $V'_{\text{maxFRC}}$ and baseline $far,1$, or between $far,1$ and $R_{\text{int}}$.

Complete data at all stages of the protocol was only obtained in one subject, due to infants' waking prior to completion and therefore it was not possible to compare changes in HIT, $R_{\text{int}}$ and $V'_{\text{maxFRC}}$.

5.3.5 $V'_{\text{maxFRC}}$

Due to the length of the protocol involved, only eight infants had measurements of $V'_{\text{maxFRC}}$ at baseline and after 4% saline. Baseline measurements in all infants demonstrated a wide range of values for $V'_{\text{maxFRC}}$ (15-370 ml.s$^{-1}$), even within this symptomatically homogeneous group of infants aged between 5 and 13 months. From a perspective of safety, only subject 8 developed a fall in $V'_{\text{maxFRC}}$ of more than 40%, from a best recorded value of 55.6 to 29.4 ml.s$^{-1}$. This represents a small change in absolute terms. Five infants had increases in flow following saline. All measurements are shown in table 5.

5.3.6 $R_{\text{int}}$ measurements

Thirteen infants slept for long enough to enable at least four technically acceptable measurements to be made before and after at least one dose of saline. Table 5.2 shows the number of measurements recorded for each technique on each infant at each stage. Five infants developed changes in their $R_{\text{int}}$ values, outside the 95% limits of agreement for repeatability. All of these changes were decreases in resistance. A summary of the results for $R_{\text{int}}$ is in table 5.4.
5.3.7 Tidal breathing

Values for tidal breaths are shown in table 5.4. The most consistent changes in the tidal breathing values were an increase in expiratory time ($T_e$) (with a corresponding fall in the respiratory rate (RR), and a decrease in mean tidal expiratory flow (MTEF), occurring after each dose of saline. Such changes occurred in 3 of the 5 infants with a fall in $R_{int}$ (except subject 8, and subject 22 where there was no fall in MTEF). One or both changes occurred in another 5 infants, and occurred after 0.9% saline prior to the detectable change in $R_{int}$ that occurred after subsequent doses of saline. There were inconsistent changes in peak tidal expiratory flow, and time to peak tidal expiratory flow.

5.3.8 HIT

Twenty-three infants slept for long enough for good quality data to be obtained at baseline and after at least one dose of saline (table 5.5). Mean baseline $f_{ar,1}$ was 181 Hz (SD 44Hz). In our infants baseline $f_{ar,1}$ was inversely correlated with length in a linear fashion, (figure 5.2), $R^2=0.14$, p=0.07. (An exponential decay model did not result in an improved correlation). The absolute value of baseline $f_{ar,1}$ will be dependent on airway length, and thus on the infants body size(137).

Increases and decreases in $f_{ar,1}$ occurred after saline challenge, outside the limits of agreement for 2 sets of repeated measurements in the same infant (section 3.3.6). This suggests that a true change in airway mechanics was occurring. Eight infants developed significant falls in $f_{ar,1}$, in 7 $f_{ar,1}$ increased, in 7 $f_{ar,1}$ was unchanged, and one infant had increases at 0.9% and 2%, then a fall at 4%.

From theory it is thought that the direction of change in $f_{ar,1}$, occurring as a result in change in airway wall compliance, could depend on baseline conditions of the airway (the relationship between baseline $f_{ar,1}$ and the baseline mechanical wall resonant frequency)(137). Infants were therefore classified as non-, up- or down-responders (by the presence of any significant response during the challenge) and the relationship between their responder status and baseline $f_{ar,1}$ examined. Infants with an increase in $f_{ar,1}$ had significantly lower baseline $f_{ar,1}$ than those with a decrease (2 unrelated samples non parametric test SPSS v 9.0, p =0.005). Those infants with a fall in $f_{ar,1}$ had a trend towards a higher $f_{ar,1}$ than those with no change (2 unrelated samples non parametric test SPSS v 9.0, p =0.053). Those with no change were not significantly different from those with an increase, in terms of baseline $f_{ar,1}$. This finding may simply reflect regression to the mean, and will be discussed below.
When baseline \( \text{far,1} \) is plotted against absolute change for the whole group we see a nonlinear relationship (figure 5.3), which is best described by a sigmoid 4 parameter model (\( R^2 = 0.56, \ p = 0.001 \)). Baseline \( \text{far,1} \) was weakly correlated with length (\( R^2 = 0.14, \ p = 0.07 \)) (figure 5.2), the relationship between length and change in \( \text{far,1} \) was also investigated (see figure 5.4), and a linear relationship was demonstrated \( R^2 = 0.24, \ p = 0.02 \). Multivariate linear regression with change in \( \text{far,1} \) as the dependant variable and baseline \( \text{far,1} \) and length as predictors revealed that only baseline \( \text{far,1} \) had an independent effect on change in \( \text{far,1} \).
5.4 Discussion

We aimed to detect physiological changes in response to inhaled saline in sedated infants with a history of wheeze. Ultimately we aimed to develop a bronchial challenge procedure that was applicable to healthy, unsedated infants. As a result it was necessary to investigate minimally disturbing physiological techniques suitable for use in naturally sleeping infants. Currently the RTC technique is the gold standard for measuring change in intra-thoracic airway function during bronchial challenge, but we believed that HIT was capable of detecting minor changes in airway mechanics, prior to any change in forced flow(41). As \( R_{nt} \) is a noninvasive technique to measure airway resistance, we evaluated whether it could detect changes after inhaled saline, as we had demonstrated its feasibility in unsedated infants.

Prior to this study, HIT had been applied in the context of a methacholine challenge in 10 wheezy infants(41). Before this study took place, no published studies of \( R_{nt} \) in infants were available and the technique had certainly never been applied in the context of an infant bronchial challenge test.

The challenge procedure proved lengthy and often infants woke even before the first dose of saline had been administered, as obtaining quality measurements using the three separate techniques was more time consuming than originally anticipated. Three of the total of twenty-eight infants failed to sleep despite receiving standard doses of chloral hydrate solution. In order to minimize disturbance and thereby to increase the yield of HIT and \( R_{nt} \) data after saline, RTC measurements were only made at baseline and after the final dose of saline. The problems we faced in obtaining large amounts of data serve to highlight the difficulties involved in applying conventional multiple dose bronchial challenge procedures to sleeping infants. Our choice over the order in which measurements were made was also influenced by concerns over early waking of infants. In order to maximize the amount of data obtained we performed RTC measurements prior to the tidal techniques at baseline and then performed post 4% saline RTC measurements at the very end. It is impossible to comment on whether the measurement of forced tidal expiration may have influenced subsequent \( R_{nt} \) and HIT measurements, as making a second set of baseline measurements would have limited the time available to make post challenge assessments.
5.4.1 Changes in $V'_{\text{maxFRC}}$

In the eight infants in whom measurements were made at baseline and after saline, no infant developed a severe fall in $V'_{\text{maxFRC}}$. Decreases in $V'_{\text{maxFRC}}$ of 30-40% are routinely induced in traditional infant bronchial challenge tests with histamine or methacholine. One of the eight infants with post saline measurements developed a fall in maximal flow in this region (47%) but this was from a baseline flow of 56ml.s$^{-1}$, where a relatively small absolute decrease in flow will be proportionally greater.

This is evidence that saline challenge in infants, albeit using very small doses of saline, is safe even in infants with a history of recurrent wheeze. However interpretation of changes in $V'_{\text{maxFRC}}$ after any challenge are limited by possible changes in FRC. Infants may elevate FRC in response to airway obstruction during a bronchial challenge, in order to improve expiratory flow by breathing at a relatively higher lung volume. As a result the true fall in $V'_{\text{maxFRC}}$ (related at both time points to the original FRC) may be underestimated.

Changes in FRC are not the only potential complicating factor when interpreting changes in $V'_{\text{maxFRC}}$. Flow limitation (as measured using RTC), is dependant on airway calibre and an inverse function of airway wall compliance, so that any change in measured forced flow after an inhaled stimulus could be a result of changes in either or both factors. This phenomenon was demonstrated during methacholine challenge, when at low concentrations, prior to any change in forced flows, significant changes in airway wall properties occurred(41).

The fact that only one of the eight infants reaching that stage had a fall >40% in $V'_{\text{maxFRC}}$ after inhaled 4% saline suggests that this stimulus was very mild. It is unlikely that a real change in airway diameter was occurring, and that any change that did occur could be compensated for by alteration in upper airway calibre and respiratory rate, for example.
5.4.2 HIT

5.4.2.1 Baseline measurements in a group of wheezy infants

High frequency input impedance measurements made in the group of infants with a history of wheeze aged 5-12 months were very close to those reported previously (42). When 23 wheezy and 19 healthy infants were compared in a previous study (42), f_{ar,1} was significantly less in wheezy infants than in healthy infants, (mean (SD) f_{ar,1} 176 (14) Hz and 212 (21) Hz respectively. Both groups were similar in age and size, so developmental differences in airway path length (which in theory is inversely related to f_{ar,1}) were not thought to be responsible for the differences.

Mean (SD) baseline f_{ar,1} in our 23 infants was 181 (44) Hz, close to the previously reported values and significantly different from the healthy infants mentioned above, (95% confidence interval for the difference between the means 8.9-56.5 Hz, p=0.008, unpaired t test). The mean (SD) body length of the original reported group of healthy infants 71.0cm (7.2) was close to that of the current wheezy group 72.9cm(4.0). This demonstrates once again that wheezy infants have significantly lower f_{ar,1} than healthy infants of a similar size. f_{ar,1} is an acoustic anti-resonance, and therefore is related to wave propagation phenomena, which in turn depend on airway wall compliance, airway path-length and density of gas within the airway. By comparing infants of similar size (i.e. with similar airway lengths) breathing room air, the implication is that differences in airway wall mechanics exist between healthy and currently asymptomatic infants with a history of wheeze. Airway wall properties are thought to play a role in flow limitation and this data adds weight to the hypothesis that changes in airway mechanics (compliance) due to developmental differences in airway structure could contribute to airway collapsibility, flow limitation and hence wheezing.

In our infants baseline f_{ar,1} was inversely correlated with length (figure 5.2). This finding fits with the acoustic anti- resonance model, where f_{ar,1} is inversely related to airway path length.
5.4.2.2. Changes after saline challenge

Any bronchial challenge procedure suitable for use in very young infants requires a mild stimulus safe for use in such a population, i.e. with a very small physiological effect. It follows that the technique used to measure any response must be both feasible in unsedated infants, and sensitive to small changes in airway wall properties and/or calibre. Evidence from a previous study suggested that HIT could fulfill these criteria(41). Changes in airway wall properties, detected by a change in $f_{ar,1}$ were demonstrated previously in 10 infants (41) at concentrations of methacholine, too low to cause any change in forced expiratory flow ($V'_{maxFRC}$). One infant even had an increase in $f_{ar,1}$ after inhaling normal saline, implying a change in airway wall compliance, prior to the first dose of methacholine. We had hoped to show that HIT was sensitive enough to demonstrate changes after normal, 2% or 4% saline.

To further evaluate the potential role for HIT in bronchial challenge procedures, it is necessary to consider our results, (and their validity), their physiological interpretation, the mechanisms by which saline may produce such physiological changes, and their clinical interpretation.

Significant changes in $f_{ar,1}$ (both increases and decreases) occurred in 16 of our 23 infants for who paired data at baseline and after at least one dose of saline were available. The changes were outside those expected during two repeated runs of measurements within the same infant and the same sleep period. Previously we had identified the expected difference between 2 repeated groups of HIT measurements, without any intervening inhalation. Between each run we removed the facemask and rearranged the putty, in order to identify any potential affect of inadvertent change in facemask volume on the second group of measurements. These results after bronchial challenge suggest that inhaled saline produced changes in HIT outside the expected technical variability produced by removing the mask and then reapplying it to repeat the measurements. Our estimates for repeatability were derived from a group of infants with baseline $f_{ar,1}$ between 142 and 200Hz, and agreed with a previous group of 12 infants with baseline $f_{ar,1}$ between 110Hz and 200 Hz.(see fig 3.6). If follows that HIT may be capable of detecting changes in airway wall mechanics in response to small doses saline as well as small doses of methacholine as reported previously(41). However, it should be noted that the intervening time between repeat measurements was less during repeatability assessments described in chapter 3 than during the challenge procedure described in this chapter. Regression to the mean as an explanation for the changes cannot be fully excluded, in particular as extreme changes...
(decreases) occurred in infants with baseline $\Delta r,l$ above 200Hz, where we have not assessed repeatability.

*Physiological interpretation* of these changes demands examination of previous work on oscillatory airway mechanics and the complex physics of traveling pressure waves in an elastic tube. Due to the non linear nature of the relationship between the frequency of a traveling pressure wave and its wave propagation velocity within the elastic tube, a change in airway wall compliance can have different effects on $\Delta r,l$. $\Delta r,l$ can either increase, decrease or not change at all during a change in airway wall compliance e.g. that induced by a challenge test. Similar changes (i.e. in both directions) occurred in previous work in schoolchildren where the frequency of postocclusional pressure transients (relative maximum in the power spectrum $f_{FS}$) was examined after rapid flow interruption during bronchial challenge testing with carbachol (a muscarinic agonist thought to act directly on smooth muscle cells) (138). The direction of change was related to the baseline value for $f_{FS}$. In contrast, the 10 previously reported infants had increases in $\Delta r,l$ following methacholine challenge(41). In the current study, a range of changes, both up and down, occurred: $\Delta r,l$ fell in 8 infants, increased in 7, and in 7 was unchanged. One infant had increases at 0.9% and 2%, then a fall at 4%.

How can these various findings been interpreted? According to an acoustic anti-resonance model, in response to a given change in airway wall properties, changes in $\Delta r,l$ in either direction are possible. The direction and magnitude of the change in $\Delta r,l$ will be influenced by underlying (baseline) airway wall properties, in particular the value of $\Delta r,l$ relative to the underlying resonant frequency of the airway. The relationship between the frequency of the propagating pressure wave and the airway wall properties (or the wave propagation speed) is highly non linear(137),(139) and is represented in figure 5.5. The position of $\Delta r,l$ relative to the resonant frequency of the airway wall will influence whether, for a given change in airway wall compliance, $\Delta r,l$ will increase or decrease. Consider, for instance, an airway with underlying characteristics (i.e. it is relatively long and/or compliant) such that the airway anti-resonant frequency lies in sector 1 of figure 5.6. When the airway wall compliance changes from C-C' during a bronchial challenge the wave propagation velocity (and therefore $\Delta r,l$) increases. In sector 2, which corresponds to a shorter airway, for the same change in compliance, a fall in $\Delta r,l$ would occur. Intermediate between sectors one and two is a region of no change. The infants in this study appear to exhibit all these conditions. It is the relationship of $\Delta r,l$ to the resonant frequency that determines the direction of change. If $\Delta r,l$ is close to the resonant
frequency, then it may be unchanged, despite a real alteration in airway mechanics. The fact that in this study infants had changes in either direction enables us to estimate the average resonant frequency of the airway wall in our infants, by using the intercept of our sigmoid regression curve in figure 5.3.

In summary we have seen that changes in $f_{ar,1}$ are dependant on baseline characteristics of the airway. We cannot quantify the change in compliance occurring in response to saline challenge, or even necessarily discriminate between a change and no change. In addition, we have no data on the repeatability of these changes. The complexities of the analysis of high frequency impedance do not lend themselves to use in a traditional bronchial challenge test, but provide new information about airway physiology in infants. These will be discussed in Chapter 7.

**Hypertonic saline** is an "indirect" stimulus, producing changes in airway calibre by a variety of different mechanisms, which have been extensively investigated in older patients. As a result of changes in osmolarity, inflammatory mediators are thought to be released from mast and epithelial cells leading to reflex bronchoconstriction and increased microvascular permeability(70). The effect of such changes on airway wall compliance, as opposed to calibre, is unclear. The reason for this is that the flow limitation that may occur during a forced expiration is determined by both airway calibre and airway wall compliance. Such changes cannot be investigated individually without a technique (such as HIT, used alongside RTC) capable of isolating airway wall compliance. The underlying mechanism for a change in wall mechanics in response to saline could be dependant on changes in smooth muscle “tone”, oedema, or vascular changes. The doses of saline used in older patients have generally been much higher (4.5% saline for gradually increasing periods of time in a multiple dose bronchial challenge procedure) than the doses we used(105). The relatively tiny doses used in our infants probably explain the lack of a convincing change in $V'_{maxFRC}$, (although data is unfortunately limited due to early waking of infants) but were sufficient enough to cause real changes in HIT.

**Clinical implications** of changes in airway mechanics in response to relatively mild stimulus (which is known to act indirectly) are several. Small changes in airway mechanics may be more representative of changes occurring in the infant airway in response to infection or inflammation. Although “small”, changes in airway properties could contribute to flow limitation in some infants, particularly those who are flow limited at baseline due to congenitally small airways.
Nebulised saline is widely used in clinical practice both alone, and as diluents for other drugs. We suggest that real changes in airway wall mechanics may occur in response to a relatively mild stimulus, without changes in forced expiratory flow, or any clinical signs of respiratory distress. It seems unlikely that such changes would cause clinically important changes in respiratory status.

5.4.3 $R_{int}$

Our finding, in five of thirteen infants, of a decrease in resistance measured by the interrupter technique after inhalation of saline was unexpected. We were careful to exclude interruptions of low technical quality, such as those affected by leak or active breathing against the shutter. Significant changes were defined in terms of the limits of agreement for the means of two groups of measurements. This approach was valid because, although repeatability was assessed in a separate population of younger, unsedated babies from those undergoing bronchial challenge with saline, there were no significant differences in variability (assessed by the 95% confidence interval of the mean coefficient of variation) between sedated wheezy infants and unsedated healthy babies.

Mean falls for each analysis method were 42%, 34%, and 41% for $R_{pex}$, $R_{ex}$, and $R_{lex}$ respectively. There were no consistent differences in baseline $V'_{maxFRC}$ or HIT between those infants with or without a change in $R_{int}$.

Resistance of flow through the airways is influenced mainly by airway calibre. Resistance to laminar flow is inversely proportional to the fourth power of the radius of a straight, smooth circular tube, for turbulent flow the increase in resistance for a given change in calibre is even greater. As lung volume changes during the breathing cycle the parenchyma exerts varying elastic forces on the airways, causing fluctuations in airway calibre. The relationship between lung volume and airway resistance is non-linear, so small changes in lung volume at which measurements are made may have large effects on airway resistance. This relationship exists in adults, but in infants the situation is more complex. Young infants modulate expiratory flow in order to maintain FRC above the elastic equilibrium volume (the point at which elastic outward forces of the chest wall producing recoil perfectly balance the inward recoil of the lungs). Post inspiratory diaphragmatic activity and laryngeal braking achieve modulation of flow. Values of $R_{int}$ include both laryngeal resistance (and nasal) as well as that of the lower airways. Therefore, even if measurements are made at constant lung volume, alterations in upper airway calibre could influence resistance values.
Several potential explanations therefore exist for our observed fall in resistance in five of thirteen infants receiving at least one dose of saline. These will be addressed in turn.

5.4.3.1. Lung volume increased after saline.

Measurements of absolute lung volume at the point of interruption were not made in this study, although the volume remaining to be expired when the interruption took place was derived ($V_{int}$). Infants with a significant decrease in resistance had no change in $V_{int}$, suggesting no change in the relative volume of interruption. One of the five infants with a fall in resistance had an increase in tidal volume ($V_t$), the remainder had a fall in $V_t$. Although lung volumes at interruption before and after inhaled saline were not measured, it is feasible that sedation led to an increase in absolute lung volume (at the time of interruption) as repeated measurements were made. Studies investigating resistance measured during unsedated and sedated sleep, as well as those investigating the effect of sleep state are useful in examining this hypothesis.

Lung volume is probably affected by sleep stage, and chloral hydrate is thought to decrease the proportion of time that is spent in REM sleep(140). The effect of chloral hydrate on respiratory resistance, as measured by the single occlusion technique, was investigated in one study(141). No studies at the present time have examined the effect of chloral hydrate on resistance measured by other means, such as $R_{int}$ or $R_{aw}$ measured in a plethysmograph. Ideally, in order to compare measurements during natural and sedated sleep, paired measurements should be made within the same infant. Techniques must therefore be applicable to both unsedated and sedated infants. Turner and colleagues investigated the effect of sedation with chloral hydrate on tidal volume, respiratory rate, respiratory system compliance and passive expiratory conductance, $V'_{maxFRC}$, and thoracic gas volume, measured with a plethysmograph (TGV) (141). The only significant change was a small, but significant fall in tidal volume. Tepper and colleagues demonstrated a significantly lower respiratory rate in a group of sedated infants compared with an age and size matched group who slept spontaneously through infant lung function tests(130). Jackson and colleagues compared paired measurements of respiratory rate, heart rate and oxygen saturation in 10 infants, first during natural night time sleep and then after sedation with triclofos sodium prior to elective surgery(142). They demonstrated a statistically significant increase in respiratory rate after sedation (mean 1.9 breaths per minute, 95% c.l. 1.3-3.7), but no other changes.
Other studies have examined differences between REM and non REM sleep. Respiratory mechanics using the single occlusion technique were studied in 13 sedated infants with mild to moderate bronchiolitis (incorporating electrophysiological sleep stage monitoring) (143). During non REM sleep an elevated end expiratory level was seen, but no change in tidal volume, suggesting increased dynamic elevation of FRC during this sleep stage. Compliance of the respiratory system was significantly lower during REM, but there was no detectable change in Rrs. This finding confirmed previous work that suggested that infants in REM have a relatively lowered FRC (68). Although we were careful not to make measurements during periods of obvious active sleep, we did not perform full electrophysiological sleep staging. It is possible that some infants, sedated with chloral hydrate, but initially with an absolute lung volume consistent with REM type sleep, had a progressively elevated EEL as successive measurements were made. Our infants visited the lab at the time that they would have had a daytime nap, so sleep during baseline measurements could have had more “active” characteristics, with chloral hydrate having its full effect on sleep state, and lung volume towards the end of the protocol.

No studies have investigated the effect of time course from administration of sedative to serial measurements of airway resistance, or any other respiratory function test, so it is impossible to exclude the possibility that the decreases in resistance that we found merely reflected changes in level of sedation and were not the result of the challenge procedure.

5.4.3.2. A true increase in airway calibre occurred after saline.

For a “true” fall in resistance to occur, airway calibre must increase. In theory, this change could have occurred at any point from the airway opening to the lower airways. Infants are generally regarded as obligate nose breathers, and several studies have demonstrated that the nose contributes approximately 50% of the value of airway resistance(144;145). However, healthy newborn infants have been shown to breathe through either the mouth or the nose during sleep(146). The switch from one to the other occurred spontaneously during both quiet sleep and active sleep. Oral breathing was also seen in response to complete nasal occlusion. Relatively large falls in airway resistance, as seen in our subjects, could be explained by a switch to mouth breathing, perhaps in response to the effects of aerosolized saline on the nose. This highlights the limitations of
using airway resistance measurements (which incorporate nasal resistance) as opposed to the measurement of $V'_{\text{maxFRC}}$.

The effects of inhaled saline, either directly via the epithelium or indirectly via deposition of a cold aerosol on the face, on these structures and their relative resistances is unclear, and separating the effect of nasal and lower airway calibre during an infant bronchial challenge is extremely difficult. The overall airway response to an inhaled stimulus is a result of changes throughout the respiratory system, from the nose to the smallest airways.

The mechanism of action of inhaled hypertonic saline is likely to be a change in the osmolarity of the airway surface liquid, which mimics that which takes place during hyperventilation during exercise(71). As a result of changes in osmolarity, inflammatory mediators are thought to be released from mast and epithelial cells leading to reflex bronchoconstriction and increased microvascular permeability(70;147). In infants, it has been shown that less than 2% of dose of a nebulised drug reaches the lower airways(148). It seems highly unlikely that a dose as low as 0.9% saline, administered for 30 seconds, could produce an increase in airway calibre and a fall in airway resistance in the order of 40%, by acting on the lower airways alone. The only possible mechanism would be that of a change in airway wall tone sufficient to increase airway calibre. As resistance is indirectly proportional to the fourth power of the radius, then a relatively small change in the radius has a relatively greater effect on resistance. RTC measurements were only made after saline in eight infants, and in none of these were there large increases in $V'_{\text{maxFRC}}$, which would confirm significant increases in lower airway calibre.

Stimulation of receptors in regions of the respiratory tract other than the intrapulmonary airways can lead to reflex bronchoconstriction or, in some circumstances, bronchodilation. In our infants it is likely that the vast majority of the hypertonic aerosol was deposited in the upper airways, particularly the nose. Mechanical or chemical stimulation of various “irritant” receptors throughout the respiratory tract (the pharynx, larynx, and tracheo-bronchial tree) usually leads to bronchoconstriction. However the action of irritants in the nose is more complex, and may actually cause bronchodilation (149). In fact, the nose should be considered as an organ of respiratory control, as it gives rise to a number of reflexes acting on other areas of the respiratory tract. For example, nasal irritation can also cause reflex laryngeal constriction and increased secretion in the lower airways(61). Cold stimuli in the nose also lead to bronchoconstriction. In older subjects, exercise or hyperpnoea leads to an increase in nasal patency, probably mediated via vascular changes(150;151). Whether changes in the osmolarity of the airway surface
liquid play any role is unclear. However, direct instillation of hypertonic saline into the nostrils of healthy adults has been shown to reduce the volume of the anterior nasal cavity and the minimum cross-sectional area of the nose, as measured using acoustic rhinometry (152). The mechanism was neurogenic glandular secretion (relieved by blowing the nose), rather than vasodilatation or altered vascular permeability as the effect was the same when a vasoconstrictor, oxymetazoline, was given prior to the saline. It is therefore feasible that changes in osmolarity of the nasal mucosa could cause reflex changes in nasal, laryngeal or lower airway resistance.

Normal (isotonic saline) drops are sometimes used in infants with nasal obstruction secondary to acute upper respiratory infection (URTI), where their action may simply be to wash away or soften troublesome secretions. The infants reported here were all free from URTI at the time of testing, so this mechanism also seems unlikely.

Chloral hydrate could act to alter respiratory resistance by an effect on upper airway calibre. An electrophysiological study carried out in animals demonstrated an effect of depression of genioglossus activity, a muscle that draws the tongue forward from the pharynx and therefore contributes greatly to upper airway stability(153). The genioglossus was selectively affected when compared with the diaphragm. During REM sleep the dilating muscles of the upper airway are thought to be less well coordinated with the thoracic muscles, and as a result during REM upper airway resistance may be higher(154). Subsequently, if measurements are made during quiet sleep following the deepening of sedation, resistance could appear to fall.

5.4.3.3 Airway obstruction led to delayed equilibration and underestimation of $P_{\text{post}}$

$R_{\text{int}}$ assumes that, post interruption, pressure at the airway opening reflects alveolar pressure. Our infants already had airway obstruction at baseline, reflected in their relatively low values for $V'_{\text{max FRC}}$. If the saline stimulus had been sufficient to cause airway obstruction, be that at the nasal, laryngeal or extra or intra-thoracic airway level, a reduction in the amplitude of the initial pressure oscillation ("damping" secondary to increased resistance(138)) and a decreased rate of rise subsequently, could both contribute to a lower estimated value for $P_{\text{post}}$. If this is the case, then it may be that $R_{\text{int}}$ may be an unsatisfactory method for assessment of airway resistance in infants who have airway obstruction. Underestimation of $R_{\text{int}}$ compared with $R_{\text{aw}}$ in children with significant airway obstruction has been documented in older children(123;155).
5.4.3.4. Differences in breathing pattern leading to artefactual changes in $R_{int}$ or changes in tidal breathing obscured changes in $R_{int}$.

Changes in tidal breathing patterns occurred in most infants after challenge with saline. The most consistent changes were an increase in expiratory time, accompanied by a fall in mean tidal expiratory flow (table 5.4). This change in breathing pattern may have occurred in response to a genuine fall in resistance, or merely be an effect of sedation. Such changes imply increased expiratory braking, which may be occurring in order to maintain end expiratory level in the face of decreased resistance. These changes contrasts markedly with changes seen during a histamine challenge(99), where an active expiratory pattern was seen with a fall in expiratory time, increase in respiratory frequency, decrease in the time constant of the respiratory system, and increase in mean and peak tidal expiratory flows. An increase in respiratory rate was also seen in another group of infants undergoing methacholine challenge(94).

If changes in breathing pattern do occur as a result of sedation, how might they influence $R_{int}$? Increased braking mediated by laryngeal narrowing could slow the rate of equilibration between mouth and alveolar pressure after occlusion, with unpredictable effects on the back extrapolation procedures.

Differences in both $P_{ao}$ and flow just prior to interruption could affect the measured value of $R_{int}$. One of our sedated infants (and several unsedated younger babies (see section 4.5.3) had very low flows during the preceding tidal expiration, suggesting marked expiratory braking (figure 4.3). The value of flow used to calculate $R_{int}$ is an estimate of the flow that would have been present at $t_0$ had the shutter not closed. $t_0$ is defined, as previously discussed, as the flow minimum (i.e. the moment at which the shutter begins to obstruct expiratory flow, which then starts to decrease) plus 5 msec, which is half the estimated shutter closure time. Flow during early expiration is forward extrapolated using two points 150 and 10 msec prior to flow minimum, past the actual decrease, to estimate flow at $t_0$. Therefore any blunting of the rate of change of flow prior to interruption will exaggerate the effect of low tidal expiratory flows. Relating $P_{ao}$ values to these low flow rates produced abnormally high values for $R_{int}$ (figure 4.3). Although the situation of this infant was extreme, all our wheezy infants appeared to have a more active strategy at baseline, which may reflect their relatively poor baseline lung function (the majority of infants had a value of $V'_\text{maxFRC}$ below the third centile for their length (67)). Decreases in expiratory braking, leading to greater flow during early expiration, could have been responsible for subsequent apparent decreases in $R_{int}$, although peak tidal and mean
expiratory flow did not change consistently with $R_{int}$. The infant whose flows are shown in figure 4.3 is an extreme example, but it seems possible that subtle increases in expiratory flows could be at least in part responsible for subsequent decreases in $R_{int}$. Unfortunately the software used to analyse the raw data and obtain values for $R_{int}$ does not supply the individual values for pressure and flow used to calculate $R_{int}$, so at present it is impossible to separate effects of erroneous values for $P_{ao}$ or flow on the $R_{int}$ values obtained. Any effect of differences in tidal expiratory flow patterns on $P_{ao}/t$ traces may be too subtle to be easily excluded by simple examination of $P_{ao}/t$ traces for gross irregularities. In future studies, a more complete method for analyzing $R_{int}$ in infants with highly variable expiratory flows should allow examination of raw values for flow and $P_{ao}$.

In conclusion, we saw significant changes in some infants in some tidal breathing parameters, which have a theoretical potential to influence the measurement of $R_{int}$. However there was no clear pattern between altered breathing patterns and changes in $R_{int}$, probably reflecting the difficulties of applying a technique which assumes a passive relaxed system to obtain a single value for resistance from a highly complex system of infant breathing patterns, with additional factors of sedation and a mild inhaled stimulus.

5.5 Conclusion

The aim of this part of the project was to investigate the role of HIT and $R_{int}$ measurements in detecting physiological changes in response to inhaled saline, and compare these with the current "gold standard" technique in sedated infants, RTC. From our data we have generated further evidence that HIT is capable of detecting small changes in airway mechanics in response to a mild stimulus. Earlier data suggested HIT was a highly sensitive technique for measurement of airway mechanics during methacholine challenge, where changes in flow after methacholine were also demonstrated. However, because of the complex nature of impedance data and their relationship to wave propagation in the airways HIT does not lend itself to use in a bronchial challenge test where the stimulus is milder and acts indirectly. Despite this, our data have contributed to further developments in the understanding of the physiological basis of wheezing in infants (see chapter 7).

Although the physiological basis of $R_{int}$ appears to be much more simple, its ability to detect changes in small airway function is limited by large baseline variability, even when strict quality control criteria are adhered to. Despite this large variability we were able to detect falls in $R_{int}$ following saline challenge in wheezy babies under sedation. The
true nature of these apparent falls remains unclear, but seems most likely to be a result of the fact that $R_{int}$ measures resistance of the entire airway, from nose (or mouth) to intrathoracic airways. Changes at any level will be reflected in $R_{int}$. A separate issue is the dynamic nature of infant breathing patterns and the potential for these to complicate interpretation of apparent changes in $R_{int}$. $R_{int}$ assumes a passive relaxed system, and in wheezy babies with significant baseline airway obstruction (as assessed by $V'_{maxFRC}$) this assumption may be inappropriate.

To summarize, neither of the techniques investigated appear to be suitable for use in a bronchial challenge test in unsedated infants, which demands a mild, safe stimulus.
### 5.6 Tables and Figures

**Table 5.1** Outcome of 69 infants whose mothers requested further information after receiving a letter sent to parents of all infants seen with wheeze at the Leicester Royal Infirmary October 2000-March 2001.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreed to participate, and took part</td>
<td>18</td>
</tr>
<tr>
<td>Agreed to participate, did not attend (no reason given)</td>
<td>1</td>
</tr>
<tr>
<td>Agreed to participate, subsequently cancelled</td>
<td>3</td>
</tr>
<tr>
<td>Declined after hearing details about the project</td>
<td>18</td>
</tr>
<tr>
<td>Attempts to contact family failed</td>
<td>15</td>
</tr>
</tbody>
</table>

**Infants Ineligible:**

- Outside age range                                        | 4
- No second episode of wheeze                              | 6
- Chronic wheeze, several admissions, never wheeze free for weeks | 1
- Diagnosed with laryngomalacia                             | 1
- Required Ventilation during admission for bronchiolitis   | 1
- Down's Syndrome                                           | 1

**Total**                                                | 69
Table 5.2 Number of technically acceptable measurements made at each stage in each infant.

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Baseline Measurements</th>
<th>Post 0.9% measurements</th>
<th>Post 2% measurements</th>
<th>Post 4% measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT C</td>
<td>HIT R_int</td>
<td>HIT R_int</td>
<td>HIT R_int</td>
</tr>
<tr>
<td>1</td>
<td>3 0 15</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 5 7</td>
<td>4 X</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 t</td>
<td>t</td>
<td>5</td>
<td>X</td>
</tr>
<tr>
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<td>3 4 5</td>
<td>2 5</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Infant failed to sleep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 7 x</td>
<td>x</td>
<td>x</td>
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Key
- t technical problem with equipment
- * infant intolerant of squeezes
- # 0.9% saline omitted for speed
- x data poor quality
- + infant woke before measurements made
Special Note

Page 114 missing from the original
Table 5.3 $V'_{\text{maxFRC}}$ at baseline and after saline in those infants sleeping long enough for post saline data to be obtained.

| Subject ID | Pre saline | Post saline | Change in SDscore | %change in $V'_{\text{maxFRC}}$ \\
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<td>167 (2.04)</td>
<td>220 (10.6)</td>
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Table 5.4 Rint and tidal breathing values (95% cl of the mean) for each infant for each stage that data were obtained. Significant decreases are shown in red and increases in blue.

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<td>102 (95-110)</td>
<td>0.184 (0.154-0.214)</td>
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<td>121 (112-130)</td>
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The table contains the following measurements:

- **Rpex**: Resistance elastance
- **Reo**: Resistance effective
- **Rlin**: Resistance linear
- **Vt**: Volume
- **%vol**: Percentage volume
- **Te**: Time
- **PTEF**: Partial thromboplastin time
- **tPTEF**: Thrombin time
- **RR**: Resistance ratio
- **MTEF**: Mean thrombin time factor

Each row represents a subject with their respective measurements in parentheses.
Table 5.5 Change in \( f_{\text{AR,1}} \) from baseline for each infant. Significant decreases are shown in red and increases in blue.

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Figure 5.1 Baseline $V_{\text{maxFRC}}$ (best of at least three values within 10% or 10ml.s$^{-1}$) vs Baseline $R_{\text{int}}$ for 13 subjects. A trend towards a significant linear regression was found for each method of calculating $R_{\text{int}}$, values for $R^2$ and the p value are shown in the accompanying table.

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Figure 5.2 Baseline far,1 v length ($R^2$ 0.14, $p=0.07$)
Figure 5.3 Relationship between baseline $f_{ar,1}$ and (absolute) change in $f_{ar,1}$ after any dose of saline. A significant relationship was found ($R^2=0.56$, $p=0.001$).
Figure 5.4 Infant length and change in far,1 after inhaled saline. Linear regression $R^2=0.24$, $p=0.07$
Figure 5.5 Wave propagation velocity as a function of frequency of the travelling pressure wave in a more (C) and less (C') compliant tube (from 139)

Consider an airway with underlying characteristics (relatively long and/or complaint) such that the airway resonant frequency lies in sector 1, when the airway wall compliance changes from C-C' during a bronchial challenge the wave propagation velocity (and therefore $f_{ar,1}$) increases. In sector 2, which corresponds to a shorter airway, for the same change in compliance, a fall in $f_{ar,1}$ would occur. Intermediate between sectors one and two is a region of no change.
6: Reference values for interrupter resistance in healthy unsedated infants in the first three months of life

6.1 Aim

To generate reference values for $R_{int}$ in this age group.

To investigate the relationship between $R_{int}$ and length, age, weight, maternal smoking during pregnancy, gender, family history of asthma, and gestational age at birth.

6.2 Background

6.2.1. General Issues

Lung function measurements are subject to variability, both within and between patients even in the absence of disease. Technical variability results from differences in equipment and procedures, and biological variability exists both within an individual (diurnal and seasonal differences in older patients and sleep state related differences in infants) and between individuals (size, age, race, sex, and environmental factors). In generating reference values the aim should be to minimise technical variation and account for biological variation.

Reference values are generated to summarize the distribution of lung function indices in specific populations, in order to predict expected ("normal") values and their confidence intervals for particular subjects. In addition to the development of a reference range of data within a particular population, characterization of the variables, which are correlated with a particular lung function test, enables identification of the most useful predictor or predictors (age, weight, height etc) on which to base a prediction equation.

In adults and older children, many regression equations have been formulated which use an independent biological variable, such as height, to predict the dependent variable, such as $FEV_1$. Some infant reference values exist, particularly for established techniques such as the rapid thoracoabdominal compression technique (RTC), for which sex specific prediction equations have recently been generated using data collected in 459 healthy infants in three centres(67). The expected deviation from a single predicted value (the "z score") was also described. This is a far more logical approach than describing a "percentage of predicted value" when interpreting lung function tests, as it takes into account the variability of the measurement. This is particularly important in infants.
Prior to this, infant lung function reference data were usually based on small numbers of subjects and were generally specific to the subjects and equipment used in the particular laboratory in which they were obtained. It should always be borne in mind that reference values for any index of lung function merely represent data pertaining to a sample of the population. Consideration must therefore be given to the characteristics of the reference population and their relationship to any future study population. In nose breathing infants studied during sleep, via a facemask, the potential for variation may well be greater than in older children. This variation must then be accounted for in reference values. The purpose for which the reference values are to be used should also be considered, for example, diagnosis (deviation of subject values from “normal”), monitoring of treatment response or research purposes, which may be many. A single reference set may not be suitable for all purposes, and selection bias may lead to misinterpretation of values that deviate from normal.

6.2.3. Reference values for $R_{int}$

Despite the difficulties we encountered in the use of $R_{int}$ in a bronchial challenge test in infants, we were able to show that measurements were feasible in unsedated infants, including in the domiciliary setting (Chapter 4). Equipment specifically designed for neonates and small infants (with low deadspace and equipment resistance), was easy to use and relatively portable. This equipment is likely to become more popular and reference values for healthy infants would be of use for those requiring comparative data. Measurements are made via a facemask during sleep and under conditions of dynamic control of lung volume peculiar to infancy, which invalidate any extrapolation of values obtained in preschool children to estimate infant data.

$R_{int}$ has been measured in groups of children from age 2 years upwards, using several different types of equipment and data analysis, and as a result equipment-specific reference values are required. Several reports containing reference values for $R_{int}$ measured using the Micro Medical equipment in children have been published over the past 4 years in 236 British Children aged 2-10yrs(156), 108 Dutch preschool children (123) and 284 Italian preschool children (126). Some infants under two years of age were included in the Dutch study, but these values were not included in reference data as measurements were made using a facemask of unknown compliance. An alternative system manufactured by Jaeger, which uses different algorithms to calculate pressure and flow from which resistance is calculated, has been used to generate further reference values. Each group
investigated factors predictive of $R_{\text{int}}$. Lombardi et al found that only height was significantly and independently correlated with $R_{\text{int}}$ measured during expiration, when height, age and weight were investigated as independent variables using multiple linear regression. Similarly in the Dutch children standing height was the best predictor of $R_{\text{int}}$ in a linear model, (during either inspiration or expiration) age and weight not contributing significantly to the model. Conversely in British children (of heterogeneous ethnic origin), there was no significant effect of height once age had been allowed for, and therefore it was recommended that $R_{\text{int}}$ measurements be standardised against age. In none of these three groups was there any significant effect of gender on $R_{\text{int}}$, and in London ethnicity was investigated and was not significantly related to $R_{\text{int}}$. There was no identifiable effect of exposure to parental smoking on $R_{\text{int}}$ in the 2 studies of older children discussed above(123;126).

In infants airway resistance may be influenced differently, for example by maturational changes in respiratory physiology as well as airway calibre. Airway growth during infancy is described as dysanaptic, with a relatively greater increase in the number of alveoli (and therefore lung volume) over growth of the airways, summarised recently in (65). Values of $R_{aw}$ in infants are known to decrease rapidly during the first year of life with the inverse relationship between resistance and length being curvilinear (157). Specific conductance ($sG_{aw}$), the reciprocal of resistance ($G_{aw}$) related to plethysmographic FRC, was recommended as a more constant parameter during growth after 40 weeks corrected post conceptional age. This is a convenient approach as both $R_{aw}$ and FRC can be measured simultaneously in a plethysmograph.

In infants, any effect of exposure to parental smoking may be more apparent when measurements are made in early life, prior to the additive influence of other factors such as infection or chronic inflammation. Antenatal smoking has been shown to lead to decreased forced flows measured using the RTC technique, with data from a recent meta-analysis suggesting flows are reduced on average by approximately 20% in infants whose mothers smoke(13). Airways resistance ($R_{aw}$) has also been shown to be increased after prenatal maternal smoking, measured before 13 weeks, although there was no difference in specific conductance (corrected for lung size), suggesting the increased airway resistance was at least partially mediated through changes in somatic growth and lung size(158).

We set out to investigate factors influencing $R_{\text{int}}$ measured during the first 3 months on life in a group of healthy unsedated babies. Because of the practical challenges involved in obtaining data in this group we combined data from two centres, the Department of
Child Health, University of Leicester UK, and the University Hospital of Bern, Switzerland. Both centres used identical equipment and analysis methods.
6.3. Method

Before beginning measurements in Leicester, I spent 2 weeks in Bern becoming familiar with all aspects of the technique and regular communication between both centres continued for the duration of the two year project. Visits by members of each team to both laboratories aimed to ensure that technical and analytical methods remained identical as far as possible. My responsibilities were for physiological measurements and data analysis in Leicester and collation of data from Leicester and Bern.

6.3.1. Bern

Mothers were recruited during the antenatal period to a prospective study of neonatal lung function and subsequent symptoms. A history of active maternal smoking during pregnancy and diagnosed asthma in first-degree relatives was obtained at recruitment. There were no exclusion criteria such as mode of delivery, birthweight, family history of asthma, or ethnic origin. Infants were excluded if they required respiratory support for more than three days postnatally, severe malformations or known diseases, maternal drug abuse, severe maternal disease, or severe difficulties with communication. Following delivery at term (greater than 36 completed weeks gestation), mothers were contacted by phone and invited to attend the laboratory with their infants at an approximate age of four weeks. No infant had respiratory symptoms prior to testing.

$R_{\text{int}}$ measurements were made during natural sleep, along with other measurements as part of a larger study. Data were subject to the quality control measures described previously (section 3.3.5.) and then analysed using software identical to that described previously. Infants were included if at least four acceptable measurements were made.

6.3.2. Leicester

Infants were recruited as previously described by approaching mothers on the postnatal wards of the Leicester Royal Infirmary (see section 4.3.1). Smoking during pregnancy was confirmed from the maternal antenatal records. A history of diagnosed asthma in first-degree relatives was obtained during the laboratory visit. Data were obtained and analysed as described in chapter 4. Weight and length were measured and verified by two researchers, experienced in obtaining accurate measurements in infants. Again, there were no exclusion criteria based on mode of delivery, family history of asthma, or ethnic origin. A history of mild respiratory symptoms since discharge from hospital was not an exclusion criterion, but as the infants were very young (and recruitment took place in the spring and summer months) this was rarely encountered.
R\textsubscript{int} data from the older, wheezy infants described in chapter 5 were not included as they were measured under sedation and were a selected group with previous symptoms requiring hospital treatment.

6.3.3. Analysis

R\textsubscript{int} estimated using linear back extrapolation (R\textsubscript{tex}) was used to generate the reference values (section 3.2.5). This form of R\textsubscript{int} was chosen because it is the most common method used to estimate R\textsubscript{int} in older children, and the method reported in the vast majority of published studies related to R\textsubscript{int}. Age was the actual number of days since delivery. The relationship between R\textsubscript{int} and length, weight, and age was investigated using simple (univariate) and multiple (multivariate) linear regressions. The influence of gender and maternal smoking during pregnancy was also investigated by adding this to one of the multiple linear regression models. Differences in continuous variables, such as age, length and weight, between the groups measured in each centre were examined using independent samples t tests. SPSS version 9 was used throughout.
6.4. Results

Full \( R_{int} \) and demographic data were available in a total of 61 infants, of which 26 were studied in Bern. The raw data of three infants (one from Bern) demonstrated very low expiratory flows similar to the phenomenon described in section 4.5.3 (figure 4.3), reflected in the fact that their values for \( R_{int} \) were at least twice the mean of the infants as a group, and these infants were excluded.

6.4.1. Differences between the groups

Infant characteristics for each centre are shown in table 6.1. There was no significant difference in gestational age at birth, as infants born before 36 weeks completed gestation were excluded from both groups (the actual range of gestational age at birth for the group was 37.0-42.3 weeks). Infants in Bern were significantly younger by a mean of 28 days, (95% confidence interval 34-22 days) (figure 6.1) and this was reflected in significantly lower length and weight in the Swiss infants. Infants were almost entirely Caucasian in origin, with only two of the Leicester infants non-Caucasian (one mixed race; one Afro-Caribbean). The relative distributions of \( R_{int} \) against age within the two centres are shown in figure 6.1.

6.4.2. Factors related to \( R_{int} \) measurements

Simple linear regression relating \( R_{int} \) to length, age, and weight showed that all three variables were significantly related to \( R_{int} \) (table 6.2). The greatest correlation with \( R_{int} \) was seen for age. Graphical representation of linear regression of age and \( R_{int} \) with 95% prediction intervals is shown in figure 6.2. Extrapolation of this model implies that at approximately 140 days of age \( R_{int} \) would equal zero (and by 80 days the lower limit of normal would be zero), suggesting that a curvilinear model may be more appropriate (figure 6.3). Scatter plots of \( R_{int} \) and postnatal age were generated as an initial stage in investigating any potential effect of gender (figure 6.4) and maternal smoking (figure 6.5).

Multiple linear regression was used to investigate the relationship between \( R_{int} \), postnatal age, length, weight and centre at which measurements were made. The possibility of a centre effect (in addition to that attributable to size) was investigated by including centre (at which measurements were made) as a separate variable. Variables related to infant size, such as length, weight and age, are likely to be closely related to each other. If several variables are included in the same multiple regression model the results may be
confounded by such co-linearity, because the different variables explain the same variation. The highest correlation between the variables was between weight and length (correlation co-efficient 0.79). In order to fully interpret the multiple linear regression models, correlations between height, weight and length were investigated and are displayed in table 6.3. In addition, it was important to control for body size when investigating a possible "centre effect" as infants at each centre were of significantly different size (table 6.1).

The results of 3 multiple linear regression models are summarised in table 6.4. Standardised beta coefficients are presented, to compare the relative contributions of variables expressed in different units. From table 6.4 it can be seen that, in a model that included length, age and centre a significant effect of both length and centre remained even after controlling for weight, in that infants seen in Bern had higher values for \( R_{int} \).

Examination of the residual plots confirmed that the model was appropriate as the residuals were reasonably close to normality and had a constant variability across the range. If weight is added to the model based on length, age, and centre \( R^2 \) is almost identical, reflecting the relatively high correlation between length and weight.

A third model shown in table 6.4 was used to further investigate the effect of smoking, gender, family history of asthma, gestational age at birth and centre on \( R_{int} \), from which it can be seen that only maternal smoking during pregnancy and centre had a significant influence on \( R_{int} \).

The most appropriate non-linear model for \( R_{int} \) and postnatal age was found to be an exponential decay model, shown in figure 6.5, for which \( R^2 = 0.35 \).

\[
R_{int} (\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}) = 2.26 + 8.577e^{-0.0433 \times \text{age (days)}}
\]

Although the relationship between resistance and length in infancy is curvilinear (157), airway conductance (\( G_{aw} \)) in infants is known to be linearly correlated with body size (159;160). Therefore values of \( R_{int} \) were converted into "\( G_{int} \)" (conductance derived from interrupter resistance) by obtaining the reciprocal of the mean value of \( R_{int} \) for each infant. Values of lung volume at interruption are not available for the interrupter resistance obtained in this project so \( sG_{aw} \) cannot be obtained. Simple regressions with length, age, and weight are shown in table 6.5. As with \( R_{int} \) all 3 variables were significantly related to \( G_{int} \), with the best correlation being with age (\( R^2 = 0.209 \)), displayed in figure 6.6.

The results of the three multiple regression models using \( G_{int} \) rather than \( R_{int} \) are summarised in table 6.6. The effect of centre was investigated for \( G_{int} \) as for \( R_{int} \) resulting
in the same finding that only centre and maternal smoking had significant independent effects on $G_{int}$. 
6.5. Discussion

We have developed preliminary reference values for $R_{\text{int}}$ and $G_{\text{int}}$ in unsedated infants drawn from two populations of term infants between 18 and 116 days of age. Although studied in different laboratories, both groups of researchers were in close contact for the period of the study and carried out measurements using the same protocols. However, we found that the centre at which measurements had been made had a highly significant effect on $R_{\text{int}}$. Length also both contributed significantly and independently to $R_{\text{int}}$ in models relating to body size. When other factors were introduced into the model, only maternal smoking during pregnancy and centre were significantly associated with $R_{\text{int}}$, after controlling for length, age, family history of asthma, gender and gestational age at birth. This was a cross-sectional study in which all subjects were studied on single occasions, so we have no information on longitudinal changes in individual infants.

Ideally populations used to derive reference data should be large, and the sample size of 61 infants is relatively small. This sample size reflects the many challenges involved in working with unsedated infants, in whom we have demonstrated a success rate of 56% (of infants in whom measurements were attempted) in obtaining data (chapter 4). It also highlights the differences in measurements made at different centres on different populations.

6.5.1 Other Reference Data in Infants

There is a relative lack of large published studies of reference data for other techniques for measuring (airway or respiratory system) resistance in infants, reflecting the fact that such techniques have not been standardized in the past.

A recent paper setting standards for the measurement of plethysmographic airway resistance ($R_{\text{aw}}$) acknowledged this lack of data and recommended caution in the application of older published reference data for $R_{\text{aw}}$ (86). Such data are likely to be highly specific for the population studied and the equipment used. The range of values that would be expected for $R_{\text{aw}}$ in our group of infants can be estimated (using 5 published prediction equations(161) applied to the range of our infants' lengths and weights) at approximately 1.63-2.76 kPa.L$^{-1}$.s, compared with our range of $R_{\text{int}}$ values of 1.11-5.97 (mean 3.42) kPa.L$^{-1}$.s. In general, our values for $R_{\text{int}}$ are higher than estimated $R_{\text{aw}}$, in keeping with findings in adults(87), older children(162) and sedated infants(118). Four of our infants had particularly low values for $R_{\text{int}}$, below values expected for $R_{\text{aw}}$, and there was no clear
reason for this finding. However, extreme caution must be applied when attempting to estimate values for $R_{aw}$ in our group of infants, using historical data from different populations.

Similarly, several relatively small studies have reported values for resistance measurements derived from passive mechanics, all of which are laboratory specific. A recently published study of 328 infants using the single occlusion technique (according to recently published standards(93)) found a range of 7.95 (2.61) kPa.L$^{-1}$.s mean (SD) in infants with a mean age of 4 weeks(124). These values are notably higher than ours for $R_{int}$, mean (SD) for the group 3.42 (1.30), or even the younger Swiss infants alone (mean (SD) 4.38(0.98)). $R_{int}$ and $R_{es}$ have been compared directly in 25 sedated wheezy infants, and $R_{int}$ found to be markedly lower that $R_{es}$ by a mean of 1kPa.L$^{-1}$.s(115). This is compatible with theoretical knowledge of $R_{int}$. The initial pressure change (obscured by oscillations) reflects equilibration between $P_{ao}$ and $P_{alv}$. The slow rise of $P_{ao}$ to a plateau, reflects visco-elastic properties of the lungs and pendelluft (equilibration between disparate lung compartments)(114). Hence values for $R_{int}$ contain information about both airway resistance and a contribution from the lung and chest wall, and its magnitude should lie intermediate between that of $R_{aw}$ and $R_{es}$.

A large multicentre collaboration recently published reference data for $V'_{maxFRC}$ based on 459 healthy infants measured on 654 test occasions (67). As discussed in chapter 3, $V'_{maxFRC}$ (if measured at flow limitation) reflects intrathoracic airway function and is not directly comparable in physiological terms with $R_{int}$. In the above study multiple regression analysis revealed that body length and age were equally powerful when predicting $V'_{maxFRC}$, with each variable explaining about half the variation in flow, and both remained significant when combined in a multiple regression model. Age may be easier to measure but may be misleading, as the authors point out, if the infant being studied has a chronic disease with adverse effects on growth. $V'_{maxFRC}$ was approximately 20% higher in girls, such that separate, sex-specific, prediction equations were required. A subset of the infants used to generate these reference values were preterm infants, who also had respiratory system resistance measured at the time of discharge from hospital, with girls tending to have lower resistance then boys(163). These findings suggest that infant girls may have relatively greater intrathoracic airway calibre compared with boys.

$R_{int}$ is affected by the resistance of the entire airway from the airway opening (mouth and/or nose) to small intrathoracic airways. The upper airway is subject to fixed geometric differences in calibre as a result of alterations in growth, variable effects due to the nasal or sleep cycle, in addition to the differential effects of diseases (such as acute
viral infection) on the nose and the lower airways. The influences of variable upper airway calibre and dynamic control of lung volume during sleep may influence $R_{int}$ to a greater degree than $V'_{\max_FRC}$, which is independent of wide variation in upper airway calibre.

Several groups have generated reference values for $R_{int}$ for toddlers and children breathing through mouthpieces. Most recently 236 children between 2.7 and 10 yrs of age from several different ethnic backgrounds were studied in London(156). Although on simple linear regression analysis both height and age were significantly related to $R_{int}$, when the variables were combined in a multiple regression only age was significantly and independently related. Gender or ethnicity did not have any significant effect. Age has advantages for use in predicting lung function as it is easily measured in community settings, and the authors recommended it as the most accurate predictor of $R_{int}$ in their heterogeneous population.

Lombardi and co-workers studied 284 Italian children aged 3-6.4 yrs and measured $R_{int}$ in both expiration and inspiration(126). For expiratory $R_{int}$, age height and weight were all significantly related in individual simple linear regressions, but only contributed between 6 and 14% of the variation in $R_{int}$. In a model containing all three variables, only height was significantly related once the other variables had been controlled for, and the overall contribution of the model to variation was only 15%.

Similar results were found when 108 Dutch children aged 2-7yrs were studied as part of a larger investigation that included some data on reference values(123). Height was the only significant predictor of $R_{int}$ once age, weight or gender had been controlled for, with the model contributing 40% of the variation in $R_{int}$. In the London cohort measurements were made with two different models of interrupter systems manufactured by the same company, one of which was used in the Italian group and the other in the Dutch children. It seems unlikely therefore that major differences in equipment or data analysis can explain the differences in the optimal variable used to predict $R_{int}$.

Absolute values of $R_{int}$ in preschool children (for example of height one metre) are approximately of 1.00 kPa.L-1.s(123;126;156). Back extrapolations using those three groups would suggest that our infants should have values for $R_{int}$ between1.5-2.0 (+/-0.6) kPa.L-1.s, (actual values for the group were a mean (SD) of 3.42 (1.30) kPa.L-1.s). If anything, our values of $R_{int}$ are higher than the preschool data would suggest. However, as infants are measured using a facemask during sleep, using different equipment, software and analysis techniques this difference could easily be explained by physiological or biological differences in measurement. The flaws involved when back extrapolating childhood data to infancy are well recognized and support the need for specific infant data.
Other published infant values for $R_{int}$ (mean (SD)) in infants aged 4 weeks age group were 4.68 (0.29) kPa.l\(^{-1}\).s(92), and in older wheezy infants 2.94 (0.68) kPa.l\(^{-1}\).s(115), in keeping with our results.

An exponential decay model best described the relationship between $R_{int}$ and postnatal age, reflecting the underlying curvilinear relationship between the two variables. Maturational changes in dynamic control of lung volume and airway calibre occur over the first weeks and months of life. Therefore age may influence $R_{int}$ independently of actual airway geometry, which is thought to be directly related to somatic growth and therefore length. However, reference data or prediction equations based on non-linear models are more cumbersome to use in practice and for this reason the relationship between $G_{int}$ and the various predictor variables were explored. Age was found to be the only independent predictor in a variety of multiple regression models, simplifying the prediction of expected values for $G_{int}$.

Each of the models accounted for a maximum of 52% of the variation in interrupter resistance (or conductance) between the infants, similar to the best fit for studies of older children(123) The remaining variation may be due to technical factors such as variations in shutter closure time, or biological variation such as subtle irregularities in the mouth pressure/time curves produced (Section 3.2), secondary to alterations in upper airway calibre, post inspiratory braking, or active expiration against the shutter.

Although $R_{ex}$ had been used for reasons of ease of comparison with other data, the final model was also examined using $R_{co}$ and $R_{pex}$, but the findings were the same, in that the only significant independent contributors to $R_{int}$ were maternal smoking and the centre in which measurements were made.

Regressions over a relatively narrow age range as this are not ideal, but are constrained by feasibility of measurements in unsedated infants. As infants age they tend to sleep less predictably during daytime hours. Certainly more data in infants aged 3-6 months would be valuable in interpreting these models, which are currently based on preliminary data.
6.5.3. The Centre Effect

An important finding in this study was that the centre in which the measurements were made, even after controlling for body size, had a highly significant effect. This difference may relate to population, technical, or analysis related factors. Populations in different countries may be different in terms of ethnicity, genetics, and socioeconomic background all of which may impact on lung function. Despite close collaboration between centres, absolute consistency between groups cannot be guaranteed (particularly when new equipment and software is being used during a process of evaluation) and could contribute to the observed bias. Possible explanations for technical variation include subtle differences between groups in terms of shutter closure time(164) or compliance of the facemask (92)both of which are known to introduce error into the measurement. Selection of appropriate data for analysis is also subjective, and different observers may have subtly different thresholds for acceptance.

6.5.4 The Effect of Maternal Smoking

Many studies have found evidence of reduced lung function during the first year of life in infants whose mothers smoked during and after pregnancy, and these have recently been reviewed(13). Difficulty exists in separating the effects of antenatal versus postnatal exposure to the toxic effects of tobacco, as many mothers continue to smoke after pregnancy. Lung function measured prior to discharge from hospital after birth will reflect prenatal rather than antenatal exposure. Hoo et al measured $V'_{\text{max}} F_{\text{RC}}$ and $T_{\text{PTEF}}/T_{\text{E}}$ in 108 infants born at a mean gestation of 33 weeks, prior to their discharge from the neonatal unit. Those exposed to antenatal smoking had reduced $V'_{\text{max}} F_{\text{RC}}$ and $T_{\text{PTEF}}/T_{\text{E}}$, but after controlling for gender, ethnicity, size, age and socio-economic status only $T_{\text{PTEF}}/T_{\text{E}}$ remained significantly diminished (165). Stick et al (166) and Carlsen et al (167) have both demonstrated reduction in $T_{\text{PTEF}}/T_{\text{E}}$ in newborns after antenatal smoking. This ratio is reflects complex interactions between lung mechanics and neurological control of breathing, which itself is known to be affected by prenatal smoke exposure(168). Changes in $T_{\text{PTEF}}/T_{\text{E}}$ may simply reflect alterations in control of breathing rather than mechanical changes in the lung. The of rapid change in lung volumes and expiratory braking with lengthening of $T_{\text{E}}$ which occur during the first days of life (169) could obscure changes due to altered lung mechanics as a result of prenatal smoke exposure.
Airways resistance ($R_{aw}$) was reduced in healthy infants aged less than 13 weeks in those exposed to prenatal smoking (158). There was no significant reduction in specific conductance in smoke exposed infants, suggesting that the alteration in airway calibre was mediated, at least in part, by changes in body and lung size. At follow-up at one year, decreased $sG_{aw}$ was significantly associated with decreased $sG_{aw}$ in early infancy and maternal smoking, a persistent adverse effect of prenatal smoke exposure on lung function that tracked through the first year of life (170). Specific conductance cannot be calculated for our data, as lung volumes were not measured.

An increase in airway resistance probably reflects a change in airway calibre, which might reflect increased thickness of the components of the airway wall, a relative lack of distending pressure, increased secretions or bronchoconstriction. There are several potential mechanisms by which prenatal smoking may have produce such changes, which are measurable using $R_{int}$. Among infants dying of SIDS, those exposed to prenatal and postnatal smoke had increased airway wall thickness, which the authors suggest might contribute to exaggerated airway narrowing (19). The same group found the distance between alveolar attachments around intra-parenchymal airways was greater in infants exposed to prenatal smoking or pre and postnatal smoking, but not in infants exposed only to postnatal smoking (22). Alveolar attachments anchor conducting airways, and a reduction in their number could reduce the distending pressure that opposes airway narrowing resulting in a reduction in luminal size. All the infants studied had died of SIDS, and in life the pulmonary arteries (attached to the airway adventitia) also offer considerable support to the airways (20).

There is no doubt that smoking during pregnancy adversely affects infant health, and there is increasing evidence that these adverse effects are at least partially explained by reduced lung function in early infancy (13). Diminished lung function in adulthood in those exposed to smoking before and after pregnancy has also been reported (10), which is particularly concerning as impaired lung function in adult life is a major indicator of all cause mortality in adults (171). Our finding of the independent adverse effect of maternal smoking after controlling for other important possible confounding factors, despite a relatively small sample size, provides further evidence of the detrimental effects of prenatal smoking.
6.6 Conclusions

In this section pilot data on reference values for interrupter resistance and conductance in unsedated infants age 2-16 weeks have been presented. Maternal smoking in pregnancy was found to have an adverse effect on $R_{int}$ after controlling for age, size and other factors that may influence lung function in early infancy, such as family history of asthma, gender, and gestational age at birth. However, a large and significant effect attributable to the centre in which measurements were made was also found. The equipment used to obtain the data, an ultrasonic flow meter and shutter (Ecomedics, Duernnten, Switzerland) are now commercially available and being used in several infant lung function laboratories around the world, although ongoing independent evaluation of the equipment continues. Strict standardization of equipment, measurement protocols, data quality control and analysis are required before data sets can be combined. Future collaborations between these centres could produce reference data from larger numbers of infants and further characterise the predictors of this measurement in unsedated infants, such as ethnicity and socio-economic status. When large amounts of data are available, it can then be expressed as z scores, makes possible meaningful comparisons of measurements made at different ages (for example in infancy as compared with the preschool years). With that information longitudinal studies (for example of change after an intervention) become feasible.
### 6.7 Tables and figures

**Table 6.1** Characteristics of infants studied in each centre

<table>
<thead>
<tr>
<th></th>
<th>Bern</th>
<th>Leicester</th>
<th>95% cl of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants (boys)</td>
<td>26(21)</td>
<td>35(19)</td>
<td></td>
</tr>
<tr>
<td>Infants exposed to antenatal maternal smoking</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gestational Age at birth (weeks)</td>
<td>40.0(1.06)</td>
<td>40.0(1.10)</td>
<td>(-0.69; 0.475)</td>
</tr>
<tr>
<td>Mean age (days) (95% cl)</td>
<td>36.0 (6.9)</td>
<td>63.94(14.10)</td>
<td>(-35.0; -21.8)</td>
</tr>
<tr>
<td>Mean weight (g) (sd)</td>
<td>4661 (510)</td>
<td>5530 (733)</td>
<td>(-1394; -716)</td>
</tr>
<tr>
<td>Mean length (cm) (sd)</td>
<td>56.5 (1.77)</td>
<td>57.7(2.27)</td>
<td>(-2.79; -0.613)</td>
</tr>
</tbody>
</table>

* Significantly less than the Leicester Group
Special Note

Page 144 missing from the original
Table 6.2 Simple linear regression of $R_{int}$ on length and age.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (a)</th>
<th>SE of coefficient</th>
<th>Intercept (b)</th>
<th>P value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>-0.247</td>
<td>0.071</td>
<td>17.51</td>
<td>&lt;0.001</td>
<td>0.155</td>
</tr>
<tr>
<td>Age (days)</td>
<td>-0.0367</td>
<td>0.008</td>
<td>5.333</td>
<td>&lt;0.001</td>
<td>0.246</td>
</tr>
<tr>
<td>Weight (g)</td>
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<td>0.000</td>
<td>7.067</td>
<td>&lt;0.001</td>
<td>0.169</td>
</tr>
</tbody>
</table>
Special Note

Page 146 missing from the original
Table 6.3 Correlations between age, length and weight. Values are Pearson correlation coefficients.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>0.344</td>
<td>0.786</td>
</tr>
<tr>
<td></td>
<td>p=0.007</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Weight</td>
<td>0.557</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
### Table 6.4 Summary of multiple linear regression models for $R_{nt}$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised Beta coefficient</th>
<th>$P$ value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length and age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>-0.226</td>
<td>0.035</td>
<td>0.45</td>
</tr>
<tr>
<td>Age</td>
<td>-0.01</td>
<td>0.947</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>0.573</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Length, age, and weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>-0.426</td>
<td>0.011</td>
<td>0.44</td>
</tr>
<tr>
<td>Age</td>
<td>-0.026</td>
<td>0.869</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>-0.307</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>0.658</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Length, age, maternal smoking, family history of asthma, gender, gestational age at birth, and centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>-0.166</td>
<td>0.173</td>
<td>0.52</td>
</tr>
<tr>
<td>Age</td>
<td>-0.066</td>
<td>0.681</td>
<td></td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>0.241</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Family History of Asthma</td>
<td>0.046</td>
<td>0.652</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.017</td>
<td>0.883</td>
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</tr>
<tr>
<td>Gestational age at birth</td>
<td>-0.095</td>
<td>0.327</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>0.574</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

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Table 6.5 Simple linear regression of $G_{int}$ on length, age, and weight

<table>
<thead>
<tr>
<th></th>
<th>Coefficient ($a$)</th>
<th>SE of coefficient</th>
<th>Intercept ($b$)</th>
<th>$R^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>0.023</td>
<td>0.008</td>
<td>-0.952</td>
<td>0.148</td>
<td>0.009</td>
</tr>
<tr>
<td>Age (days)</td>
<td>0.004</td>
<td>0.001</td>
<td>0.146</td>
<td>0.209</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.00007</td>
<td>0.000</td>
<td>0.003</td>
<td>0.122</td>
<td>0.001</td>
</tr>
</tbody>
</table>
### Table 6.6 Summary of Multiple Regression Prediction Models for $G_{int}$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardised Beta coefficient</th>
<th>$P$ value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length, age, and centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.154</td>
<td>0.175</td>
<td>0.38</td>
</tr>
<tr>
<td>Age</td>
<td>0.037</td>
<td>0.826</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>-0.572</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Length, age, weight and centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.374</td>
<td>0.034</td>
<td>0.40</td>
</tr>
<tr>
<td>Age</td>
<td>0.003</td>
<td>0.986</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.337</td>
<td>0.103</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>-0.66</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Length, age, maternal smoking, family history of asthma, gender, gestational age at birth and centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.555</td>
<td>0.555</td>
<td>0.45</td>
</tr>
<tr>
<td>Age</td>
<td>0.052</td>
<td>0.761</td>
<td></td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-0.260</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>-0.139</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>-0.055</td>
<td>0.660</td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>0.010</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>Bern infant</td>
<td>-0.609</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

150
Figure 6.1 $R_{in}$ according to age for all infants (infants identified according to centre).
Figure 6.2 Simple linear regression of $R_{int}$ and age ($R^2 = 0.25$).

(The solid line indicates the regression line and the dashed lines the 95\% prediction intervals.)
Figure 6.3 Exponential Decay model of $R_{int}$ and age ($R^2=0.35$)
Figure 6.4. Mean $R_{int}$ according to age for each gender
Figure 6.5 Mean $R_{\text{int}}$ ($R_{\text{lex}}$) according to age for all infants.

(infants identified with respect to antenatal smoking.)
Figure 6.6 Simple linear regression of $G_{int}$ and age.

(The solid line indicates the regression line and the dashed lines the 95% prediction intervals.)
7. Conclusions

7.1 The requirement for measurement of BR in unsedated infants

The original aim of this thesis was to develop a method for safe and rapid measurement of bronchial responsiveness in unsedated infants. This aim sprung from work using traditional infant bronchial challenge tests (i.e. those carried out under sedation in a hospital infant lung function laboratory), which suggested that the level of neonatal responsiveness was associated with respiratory health in infancy (4).

Subsequently neonatal BHR was found to be associated with early transient symptoms and decreased lung function at school age in both the London group of infants referred to above, and a Western Australian birth cohort (7;8;172) Such a consistent finding, across 2 relatively small cohorts in 2 continents, is unlikely to be spurious, and points to an association between congenital BHR and persistently impaired lung function during later childhood.

Against a backdrop of evidence for the role of early life BHR in future respiratory health, the need for large-scale population studies has increased. Only epidemiological scale studies are capable of unravelling the nature of this "congenital" BHR: what factors predispose to it, by which mechanisms do those factors act, what prognostic value do they have, and are they amenable to therapeutic intervention before or after birth? In 2006 it is unlikely that ethical approval would be granted for similar studies of a group of unselected healthy infants to undergo traditional bronchial challenge procedures under sedation. By their nature such procedures limit the numbers of infants that can be studied. Even without ethical barriers, a healthy "unselected" group of infants is unlikely to be recruited to a study involving laboratory based tests. Parents motivated to take part in such an involved procedure may well have higher levels of asthma and atopy themselves, introducing sample bias at the very first stage of a study. Lung function techniques feasible in unsedated infants are being evaluated, increasing the feasibility of larger scale studies. If larger numbers of infants are to be comprehensively studied in infancy and through childhood then techniques suitable for use in the home are required, in order to increase recruitment by minimising disruption for families.
7.2 Conclusions from this work

This work aimed to establish a technique that was suitable for use in unsedated infants, was acceptable to parents, and relatively simple to perform. Ultimately we envisaged translating our technique to multi-centre studies of large numbers of infants. This extremely ambitious goal has not been fulfilled by the work carried out as described in this thesis, but useful practical and physiological information has been obtained.

7.2.1 General Issues: Unsedated Infants

Prior to this project there had been many reports of lung function measurements in unsedated infants. Historically, some measurements of forced expiration (163;165) and plethysmography (157) had been attempted in infants less than 4 weeks old in natural sleep, without sedation. Other techniques, such as passive mechanics (124;173;174), measurement of FRC by inert gas washout (136;175;176), and tidal breathing analysis (166;167;177;178) have also been applied in unsedated infants. However, no information on success rates has been published. Therefore, before even considering the use of Rmt in any future infant bronchial challenge test, we examined our success rates in recruiting infants and obtaining data. Such data are of great importance in planning large-scale future studies.

After approaching 277 mothers, over a period of 11 months, we were able to obtain good quality measurements in 38 out of 56 infants attending the lab or seen at home. Nine mothers offered to return for another attempt and therefore measurements were attempted on a total of 65 occasions, with no infant having successful measurements made on more than one occasion. This represented a success rate of 58% (38 occasions from the total of 65 occasions) of measurements attempted. The average period of time spent in the laboratory for all infants was in excess of two and a half hours (section 4.5.2). The greatest loss of potential infant subjects was related to recruitment issues (fig 4.2). Working with unsedated infants is incredibly time consuming and requires dedicated teams with excellent communication and technical skills. We also visited 14 infants in their own homes, as our equipment was relatively portable. As far as is known, this is the first time that any infant lung mechanics technique (except measurements of respiratory pattern) has been evaluated in a home setting. Although our work involved relatively small numbers of infants, we did not encounter spectacularly improved success rates in the home setting.

Twelve of the total of 38 unsedated infants in whom measurements were made (during 65 attempts at measurement) slept long enough for a second set of measurements to be made, in order for repeatability to be investigated. The proportion of infants sleeping for long enough to obtain 2 reliable sets of measurements was rather small. With an
intervening dose of nebulised saline, it is likely to have been even smaller. From our experience, it appears that the likelihood of infants sleeping through the giving of inhaled stimulus, in addition to the acquisition of good quality data at baseline and following the challenge is extremely low, suggesting that a single dose inhaled saline challenge test for use in sedated infants is not feasible.

7.2.3 New information about $R_{\text{int}}$

$R_{\text{int}}$ has many attractions as a measurement technique for use in bronchial challenge tests. It had previously been shown to be sensitive to changes in airway resistance in preschool children undergoing bronchial challenge(89). We, along with others, have shown it to be correlated with other more invasive measurements of infant lung function (section 5.3.4) (115;118). New equipment specifically designed for use with small infants is now available, which can be dismantled and transported to subjects’ homes successfully (section 4.3.3). We have demonstrated that measurements can be made in some unsedated infants both in laboratory and home settings.

We have shown that measurements are more variable than other infant lung function techniques, and $R_{\text{int}}$ itself when measured in older children. When repeatability was assessed, we found it to be far poorer than in older children. This increased variability is not unexpected when we consider that infant measurements are made during sleep, breathing through a facemask (usually through the nose), and most importantly under conditions of dynamic control of lung volume and upper airway calibre.

Secondly we have demonstrated large falls in interrupter resistance after small doses of 0.9% and 2% saline. Whether true changes in lower airway resistance occurred in these infants is unknown. Reflex changes in breathing patterns or glottic calibre, invalidating the passive conditions necessary for the technique, may explain these unexpected changes (section 5.4.3). That exaggerated or erroneous changes in $R_{\text{int}}$ occur after the mildest of challenges complicates any use of $R_{\text{int}}$ in bronchial challenge tests, and particularly precludes the technique from use in a single dose challenge that we originally envisaged. Our data are also useful in interpreting for example, any subsequent studies of bronchodilator responsiveness in infants, and should prevent the misinterpretation of large falls in resistance as a direct effect of bronchodilation.

We have also shown that maternal antenatal smoking has an independent effect on $R_{\text{int}}$ measured during early infancy, with no significant influence contributed by length,
gender, family history of asthma, or gestational age at birth. That maternal smoking has an adverse effect on infant health, particularly respiratory health, is widely accepted, and thought to be mediated by adverse effects on lung growth and development measurable using infant lung function techniques(13). Unfortunately, because gathering of reference data for Rint was not a primary aim of this project we did not measure infant cotinine levels, which limits our exploration of the effect of maternal smoking (such as the investigation of a possible dose response effect, and correlation with reported smoking and actual exposure). Because of the well recognised adverse effect of maternal smoking on infant lung function, it could be argued that routine collection of infant cotinine levels (either urinary or salivary) has a place in any infant lung function research project, especially during evaluation of newer techniques.

During this phase we also showed that there were significant differences in measurements made in two centres, even after correction for body size. This difference could be related to differences in the populations studied, technical differences in the equipment at each centre, or in data selection for analysis. In retrospect, further attention to the process of selection of acceptable data would have been useful. For example, inter and intra-observer differences in selection of acceptable Pao2/t curves have not been examined, and to what degree these contribute to variability within patients, and between centres are unknown. Gross abnormalities in curve morphology are easy to identify, but more subtle differences are open to individual interpretation. Whether this represents quality control or bias towards exclusion of infants who manifest, for example, expiratory braking is debatable. Where technical acceptability ends and pathophysiology begins is not at all clear in this situation, and further investigation of inter- and intra-observer error would be very useful.

7.2.4 New information regarding airway wall mechanics

Prior to this project HIT was a novel technique that had only been applied by one group of investigators. We have confirmed previous findings that airway wall mechanics are altered in infants with a history of wheeze, even when clinically well.

In contrast to previous work using HIT during a methacholine challenge in infants where fAR,1 increased during the challenge, we saw both increases and decreases in fAR,1. Such heterogeneous changes had been seen in comparable work in older children(138), and can be expected from theory if subjects with a range of fAR,1 relative to the airway resonant
frequency are studied. Infants in this study incorporated a wider range of baseline values of $f_{ar,1}$ (104-287 Hz), than those undergoing methacholine challenge previously (85-218 Hz) (figure 6.1b).

As discussed in section 5.4.2, according to a simplified acoustic anti-resonance model of a single floppy tube, in response to a given change in airway wall properties, changes in $f_{ar,1}$ in either direction are possible. The relationship of $f_{ar,1}$ relative to the resonant frequency that determines the direction of change. If $f_{ar,1}$ is close to the resonant frequency, then it will be appear to have no change, despite a real alteration in airway mechanics (figure 5.5). We have combined baseline and post challenge data from both the current study and the previously published work(41) and plotted baseline $f_{ar,1}$ against change after challenge (fig 7.1). The curve best fitted to the data remained a sigmoid 4-parameter model, as when the saline challenged infants alone were plotted. The intercept of the regression curve (which in theory corresponds to the resonant frequency of the airway wall) is approximately 200 Hz. In a recent study the mean frequency of wheeze recorded in infants aged 7-10 months and 201 Hz in inspiration(179). These two findings support the hypothesis that wheeze is heard as a result of airways resonating at flow limitation(180). By measuring a change in $f_{ar,1}$ over a range of baseline values we are able to estimate the resonant frequency of the airway wall, which lies in the region of no change in $f_{ar,1}$ after challenge. This estimate of 200 Hz is very similar to the frequency of wheeze recorded in infants of a similar age. A major concern though remains, that the changes in HIT after bronchial challenge seen represent regression to the mean, and only by making repeated measurements at baseline (in each infant with an intervening removal of the mask) before challenge could this be confidently excluded. Repeatability data however were examined in an attempt to address this issue and were found to be consistent between this project and previous work (figure 3.6a and 3.6b). In ideal world further measurements in a larger number of infants would be helpful in addressing this unresolved question.

The complexities of the interpretation of HIT preclude it from use in conventional infant bronchial challenge tests. This information has only been gained by attempting to apply the technique in a second, larger group of infants. The consistent nature of the changes in $f_{ar,1}$ during methacholine challenge now seems to be a result of a relatively narrow range of baseline $f_{ar,1}$. The original attraction of a technique capable of selectively examining airway wall properties over airway calibre remains, but only if HIT is performed in conjunction with more direct measures, such as forced expiration.
Any future role for the HIT technique will probably be in examining whether differences in airway wall mechanics in wheezy infants during the first year of life exist immediately after birth or are acquired subsequently. We see no role for HIT in determining bronchial responsiveness.

7.3 Alternative approaches to the assessment of BHR in infants

At the inception of this project, $R_{im}$ and HIT were felt to be suitable techniques for use in the development of a method for the assessment of bronchial responsiveness in unsedated infants. This however, has proven to not be the case. During the course of this work, serious flaws have been identified in both techniques, which preclude them from use in such a test. In retrospect, further study of each technique, prior to the start of this study would have been of great benefit, particularly with response to repeatability, or estimation of the expected difference between groups of measurements performed on the same occasion a matter of minutes apart. Otherwise interpretation of changes after a challenge procedure is fraught with difficulty.

Lung function tests in unsedated infants hold much promise and are satisfying to obtain, as data relates to the natural situation, and there is no administration of unpleasant tasting sedative, with potential risks to the infant. However, the necessary degree of dedication and commitment by parents and members of the research team cannot be underestimated. After completing this project, it appears that the feasibility of performing reliable challenge tests in unsedated infants is extremely low. The limits of length of sleep, which must include a period of stable quiet sleep suitable for the performance of lung function tests, and the "stimulus", which is likely to stimulate arousal or at least a change in sleep state, at present seem to be insurmountable. Standardisation of a challenge protocol with respect to timing of measurements after inhalation of a stimulus is fraught with risks of early waking and then long waits for the infant to resettle.

In such work as this there is a conflict between using standardized techniques, some of which have useful multicentre normal values, (but are only really applicable in a hospital laboratory setting with sedated infants and therefore limited in application and potentially open to increased bias in recruits) and newer approaches which although exciting, and potentially of wider application, suffer from lack of standardisation and
potential misinterpretation. The amount of training necessary to achieve competence in the peculiarities of measuring lung function in infants cannot be underestimated.

If a challenge test is not practicable in large studies, then what alternative approaches exist? Could measurements of the inherent variability of lung function yield information about the stability of the respiratory system? In this way the tendency of a lung function measurement to change after an inhaled environmental stimulus could perhaps be estimated without the need for a stimulus at all.

It has been acknowledged that fluctuations or irregularities in physiological measurements carry information rather than simply reflecting technical or biological noise(181). Such information may be sensitive to physiological or pathological changes, as seen with breathing patterns in term and preterm infants, where the statistical properties of inter-breath intervals showed maturational changes with age (182).

Recently, a novel approach to predict the risk of worsening airflow obstruction showed that increased variability of peak expiratory flows in an asthmatic population augmented the risk of unstable airway function(183). It has been suggested that the characterization of fluctuations in airway function provides a basis for objective risk prediction of asthma episodes and for evaluation of therapy. Rather than challenging the respiratory system in a conventional way, perhaps measurement of variability of measurements in the neonatal period may be a more productive avenue of enquiry. Mathematical analysis of the timing of respiratory symptoms in the first year of life has been examined using a model in which episodes (of lower respiratory tract symptoms) are triggered in the same way as “avalanches”(184). The model takes into account the branching nature of the airway tree, inflammation and remodelling and contraction of airway smooth muscle in response to inhaled stimuli. After a build up of stimuli a small additional stimulus is sufficient to generate a disproportionate cellular response, which is ultimately reflected in clinical symptoms.

One of our aims in identifying infants with congenital airway dysfunction manifested as early BHR, was ultimately to facilitate intervention studies designed to alter the progression of wheezing disorders. In the evolution of asthma, the development of the immune system is thought to act in a complex manner with lung development(185). The finding that neonatal BHR was associated with subsequent decreased lung function in two birth cohorts(7;8;172), raises the possibility that those newborns with exaggerated airway narrowing in response to inhaled stimuli may be most at risk of subsequent remodeling of the airways, which is thought to result from immune mediated mechanisms leading to inflammation and ultimately tissue damage.
Interventions that may alter immune mediated inflammation in early life include new pharmacological agents designed to alter the type 2 T helper cell dominated response that predominates at the foeto-maternal interface. This response remains active in those with a family history of atopy, and is associated with early respiratory symptoms(185). An alternative strategy, that of allergen avoidance, has been examined in a randomized controlled trial, part of the National Asthma Campaign Manchester Asthma and Allergy study(186). Families were recruited antenatally and stratified according to parental allergy and pet ownership. Those at high risk (both parents with atopy) underwent interventions (including allergen impermeable bedding covers and removal of carpets from infants’ rooms) designed to reduce their exposure to house dust mite allergen. Subsequently, high-risk infants randomized to such environmental manipulation had a lower relative risk of respiratory symptoms in the first 12 months of life(187). Lung function was examined for the first time at 3 years of age in 503 subjects (although measurements had been attempted in 803 children, a success rate of 62%), in the form of specific airway resistance (sRaw)(188). Children who had experienced episodes of wheeze had higher values for sRaw. Among those without a history of wheeze, those with atopy, as well as those without atopy but with parental atopy also had significantly higher values for sRaw than nonatopic children, or those without parental atopy. Had baseline neonatal lung function tests been feasible in such numbers of families, it would have been fascinating to investigate possible inherent differences in lung function which may modify the response to intervention in infancy and resulting lung function in the pre school years.

7.4 Conclusion

The measurement of infant lung function (including bronchial responsiveness) offers an exciting opportunity to examine the role of many factor implicated in early infant respiratory health as well as that in later childhood, and to prospectively identify infants at high risk for future illness. Ultimately the role of such studies is to set the scene for evaluation of interventions designed to reduce the burden of illness of infants, their families, and the wider community. Small groups working in isolation cannot reach this goal, and ongoing collaboration and standardisation of the new and exciting techniques applicable to larger numbers of unsedated infants is vital. The importance of such painstaking “background” work cannot be underestimated, as without it the application of the techniques to intervention studies will be fraught with problems.
7.5 Tables and figures

**Figure 7.1a:** Baseline $\text{far,1}$ v. change in $\text{far,1}$ for all infants undergoing challenge with saline or methacholine.
Figure 7.1b Baseline $f_{ar,1}$ vs change in $f_{ar,1}$ for all infants undergoing challenge with saline or methacholine, with infants undergoing saline challenge separate from those undergoing methacholine challenge.

change in $f_{ar,1}$ vs baseline $f_{ar,1}$ (Saline and Metacholine)

- • baseline $f_{ar,1}$ vs absolute change in $f_{ar,1}$ after saline
- ○ baseline $f_{ar,1}$ vs absolute change in $f_{ar,1}$ after methacholine
8.1a parent information sheet (unsedated babies)

Congratulations on the birth of your new baby!

We would like to invite you and your baby to take part in a study we are involved in at the Department of Child Health, when your baby is about six weeks old.

It is called "The Assessment of Bronchial Responsiveness in Infancy", and is funded by the National Asthma Campaign.

We perform simple breathing tests during sleep on babies who are about two months old, which we hope will help us understand more about the airways of babies when they are very young, before they have had any colds or wheezes. Nothing painful is involved for the baby. Before deciding whether you would like to take part, you will have lots of questions, some of which we have tried to answer below:

1. **Why have we chosen to do this study?**

Wheeze in babies and young children is very common. In some cases it can lead to asthma. One feature of asthma is that the airways (breathing tubes) are very sensitive and readily become narrow in response to certain things such as house dust, dogs and cats, and exercise. If we can measure the sensitivity of the airways we can begin to look at the things which contribute to wheeze and asthma, and (eventually) to ways of preventing them. Measurements made when babies are only a few weeks old will give us an idea of what the airways are like very early in life, before any signs of wheezing have developed.

Until now we have only been able to measure the sensitivity of the airways in the laboratory. The laboratory tests take some time and, although they are not painful, all require that the baby has a sleeping medicine beforehand. We are developing a new test, which is so simple and quick so that it can be done in the home, during a daytime nap. The current stage of this involves seeing healthy babies in the laboratory, without any sleeping syrup, so that we can see how reliable the new test is. The new tests have already been carried out in lots of babies in this laboratory.

2. **What will be involved if my baby takes part in this study?**

You will be asked to bring your baby to the laboratory at Leicester Royal Infirmary when it is convenient for you, and at a time when your baby would generally have a nap. Your baby will be weighed and examined and we will complete a short questionnaire. We will measure the level of oxygen in the blood (this is done with a small probe taped on to the foot, so no blood samples are needed and no needles are used). S/he will be allowed to settle to sleep whenever s/he is ready.

We will be making a new type of measurement. The baby breathes through a facemask around the nose and mouth. On the end of the facemask is a small hand-held motor which, when triggered, operates a shutter very rapidly during the course of a breath. This produces small vibrations that are scarcely noticeable. We would expect the baby to sleep soundly throughout.

After we have made all the measurements for the first time, we will repeat the measurements to see if there have been any changes in the test. This will enable us to collect information on the measurement in a healthy baby. This will, in turn, help us to assess babies with wheeze or other chest conditions.

3. **Will information obtained in the study be confidential?**

All information will be kept confidential. Details stored on computer will be anonymised (i.e. no names or other means of identification will be used). Results of the study may be presented or published for scientific purposes. In all cases, neither your baby's name nor identity will be disclosed and confidentiality will be maintained.

As a matter of routine, we will inform your GP of your baby's participation in the study.

4. **What if my baby is harmed by the study?**

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs.
5. Will I receive out-of-pocket expenses for taking part in this study?

We will pay travel expenses, including petrol and car parking, or a taxi fare if this is needed.

6. What happens if I do not wish my baby to participate in this study or I want to withdraw him/her from the study?

If you do not wish your baby to participate in this study or if you wish to withdraw him/her from the study you may do so without justifying your decision and his/her future treatment will not be affected.

7. What happens now?

We will contact you when your baby is about six weeks old to answer any further questions you have, and to see if you would like to take part. Our number is 0116 258 6769

Jenny Westaway Research Health Visitor
Isobel Brookes Paediatric Research Doctor

Principal Investigator: Dr Caroline Beardsmore
You may contact Dr Beardsmore by telephone (0116 252 5811)
8.1 b (parent information sheet sedated babies)
The Assessment of Bronchial Responsiveness in Infancy

Information Sheet for Parents

Principal Investigator: Dr Caroline Beardsmore
You may contact Dr Beardsmore by telephone (0116 252 5811)

This study is sponsored by The National Asthma Campaign

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

Wheezeing in babies and young children is very common. In some cases it can lead to asthma. One feature of asthma is that the airways (breathing tubes) are very sensitive and readily become narrow in response to certain triggers such as house dust mite, dogs and cats and exercise. If we can measure the sensitivity of the airways we can begin to look at the factors which contribute to wheezing and asthma and (eventually) to ways of preventing them.

At present we can only measure the sensitivity of the airways in the laboratory, which limits the number of babies we can study. The laboratory tests take some time and, although the tests are not painful, all require that the baby is given some sleeping medicine by mouth (mild sedation) beforehand. What we hope to do is develop a test which is simple and quick so that it can be done in the home, without the need for sleeping medicine. The first stage of this involves seeing babies in the laboratory so that we can compare the existing tests with newer tests.

2. **What will be involved if my baby takes part in this study?**

You will be asked to bring your baby to the laboratory at Leicester Royal Infirmary when it is convenient for you, and at a time when your baby would generally have a nap. If your baby takes certain chest medicines (e.g. Salbutamol (Ventolin) or Bricanyl) we will ask you to stop these for 24 hours beforehand, if the baby can manage without them. (However, if you feel that s/he needs to use these, or if the baby gets wheezy, then you should give them as usual). When you come to the laboratory your baby will be weighed and examined and we will complete a short questionnaire. We will measure the level of oxygen in the blood (this is done with a small probe taped on to the foot, so no blood samples are needed and no needles are used). S/he will be given some sleeping medicine (by mouth) and allowed to settle to sleep.

The baby will be wrapped in an inflatable jacket which extends from the shoulders to the thighs, and s/he will breathe through a facemask positioned around the nose and mouth. This has a small device on the end which does not interfere with breathing but measures how much air goes in and out with each breath and how fast. At the end of a normal breath in, we inflate the jacket, which gives a squeeze to the chest and tummy. This causes the baby to breathe out rapidly and we can see how much ‘puff’ the baby has. The measurement is commonly called ‘the squeeze’. We repeat the squeeze several times to identify how much we need to inflate the jacket to give the biggest blow. This does not disturb the babies, who generally sleep soundly throughout. We then repeat the squeeze six more times, except that before inflating the jacket we give the baby an extra big breath in. This is done by passing extra air through the facemask in a carefully controlled manner.

After these measurements (which are standard in many laboratories) we will move on to the two newer types of measurements. Again, these both involve the baby breathing through a facemask around the nose and mouth. On the end of the facemask is a small hand-held motor which, when triggered, operates a camera shutter very rapidly several times during the course of a breath out. This produces small vibrations which are scarcely noticeable. For one set of measurements we introduce a small perspex tube between the facemask and the motor where we measure pressure changes, but the procedure for the baby is identical for both measurements.

After we have made all the measurements once, we will give the baby a mist of salty water to breathe through a facemask for 30 seconds. The measurements will then be repeated to see if there have been any changes, before giving a more concentrated mist to breathe. We plan to give up to three different concentrations, and we expect that one of them will produce small changes in the newer measurements, but not in the squeeze. This will enable us to say which concentration of mist is most suitable and which measurement is most sensitive.
Any effect of the mist wears off very quickly and you will be able to take your baby home straight afterwards.

3. Will the information obtained in the study be confidential?

The results of the routine measurements from your baby will be made available to your GP and to the hospital doctors responsible for his/her medical care.

All information collected for the purposes of the research will be kept confidential. Details stored on computer will be anonymous (i.e. no names or other means of identification will be used). Results of the study may be presented or published for scientific purposes. In all cases, neither your baby’s name nor identity will be disclosed and confidentiality will be maintained.

4. What if my baby is harmed by the study?

Medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs.

5. Will I receive out-of-pocket expenses for taking part in this study?

We will pay travel and parking expenses.

6. What happens if I do not wish my baby to participate in this study or I want to withdraw him/her from the study?

If you do not wish your baby to participate in this study or if you wish to withdraw him/her from the study you may do so without justifying your decision and his/her future treatment will not be affected.

7. What happens now?

Thank you for taking the time to read this information sheet. If you would like to discuss the project or if you have any questions, please feel free to contact either Dr. Beardsmore, Dr. Brookes or Claire Reid at the University of Leicester, Dept. of Child Health.

Dr. Isobel Brookes
Research Fellow
Telephone: 0116 258 5691
Email: ib26@le.ac.uk

Claire Reid
Research Technician
Telephone: 0116 258 6769
Email: cer15@le.ac.uk
Appendix 8.2

Documentation of ethics approval

Dr Caroline Beardsmore
Paediatrics
Leicester Royal Infirmary NHS Trust

Dear Dr Beardsmore

RE: Project Number: 01436  [Please quote this number in all correspondence]
The development of a simple bronchostrictor challenge test for infants

We have now been notified by the Ethical Committee that this project has been given ethical approval (please see the attached letter from the Ethical Committee).

Since all other aspects of your LRI R+D notification are complete, on behalf of Dr Howlett, I now have pleasure in confirming full approval of the project at the Leicester Royal Infirmary NHS Trust.

This approval means that you are fully authorised to proceed with the project, using all the resources which you have declared in your notification form.

The project is also now covered by Trust Indemnity, except for those aspects already covered by external indemnity (e.g. ABPI in the case of most drug studies).

We will be requesting annual and final reports on the progress of this project, both on behalf of the Trust and on behalf of the Ethical Committee.

In the meantime, in order to keep our records up to date, could you please notify the Research Office if there are any significant changes to the start or end dates, protocol, funding or costs of the project.

I look forward to the opportunity of reading the published results of your study in due course.

Yours sincerely,

Dr Nichola Seare
Research and Development Business Manager
8.3 Parent consent form

Consent Form for Parents

The Assessment of Bronchial Responsiveness in Infancy

Principal Investigator: Dr Caroline Beardsmore

This form should be read in conjunction with the Information Sheet for Parents

I agree to my baby taking part in the above study as described in the Information Sheet for Parents.

I understand that I may withdraw my baby from the study at any time without justifying my decision and without any effect on his/her normal care and medical management.

I understand that all information concerning my baby will be treated as confidential.

I understand medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs.

I have read the Information Sheet for Parents on the above study and have had the opportunity to discuss the details with ........................................ and ask any questions. The nature and the purpose of the tests to be undertaken have been explained to me and I understand what will be required if my baby takes part in the study.

Signature of parent ..........................................................Date.............................................

Name (In Block letters)..................................................................................

Name of infant (In block Letters)..................................................................

I confirm I have explained the nature of the tests, as detailed in the Information Sheet for Parents, in terms, which in my judgement are suited to the understanding of the parent.

Signature of Investigator ..................................................Date.................................

Name (IN BLOCK LETTERS).............................................................................
Appendix 8.4  The Assessment of Bronchial Responsiveness in Infancy
Follow up questionnaire

Please tick the appropriate box, and feel free to write down any comments you have.

1. Were any of the following reasons important in your decision to take part?
   Family history of asthma in you or your partner ~
   Family history of asthma in the baby’s older brothers or sisters ~
   Other chest problems in family members ~
   Other reason .................................................................................................

2. If the tests had involved the baby having a medicine (in the form of a syrup by mouth) to help them sleep would you still have volunteered?
   Yes ~
   No ~
   Don’t know ~ Comments .....................................................................................

3. Did the information sheet you received before visiting the lab explain clearly what was going to happen?
   Yes ~
   No ~
   Not sure Comments ..............................................................................................

4. Was your visit to the lab as you had expected
   Yes ~
   No ~ (in what way) ..............................................................................................

5. Was there anything else we could have explained to you better or told you about before the visit?
   .................................................................................................................................

6. Did you find anything about the tests unpleasant or distressing in any way, (even if it seems trivial)
   Yes ~
   No ~ ........................................................................................................................

7. If we had offered to come to your home to do the measurements, would this have been:
   More convenient for you ~ .....................................................................................
   Less convenient for you ~ .....................................................................................

8. If the study had involved several visits (to your home) during your baby’s first year, would you still have taken part
   Yes ~
   No ~ ........................................................................................................................
   Don’t know ~ .........................................................................................................

9. If another parent asked you whether they should let their baby take part in the study what would you say
   Yes ~
   No ~
   Please comment .....................................................................................................

Thank you again for your interest in the project
Please return this questionnaire in the freepost envelope provided

Your responses will remain anonymous, but feel free to give your name if you wish. Further comments can be added to the other side of this page.
9. Bibliography


[38] Kellner JD, Ohlsson A, Gadomski AM, Wang EE. Bronchodilators for bronchiolitis. Cochrane Database of Systematic Reviews 2000; 2(CD001266,).


