The high speed interrupter technique to measure airway wall mechanics in infants

PhD Thesis

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Urs Frey, MD
Previous studies have demonstrated that airway wall mechanics seem to play a crucial role in the development of flow limitation in the airways and in wheezing disorders. Airway wall compliance is higher in young infants than in adults and may play a much more important role in wheezing disorders in infants. High frequency impedance measurements derived from oscillatory mechanics in adults and animal models allow inferences on airway wall mechanics. Thus, the overall aim of this work was to develop a non-invasive way of measuring high frequency input impedance in infants which enables airway wall mechanics to be studied in vivo during normal tidal breathing. The established forced oscillation technique and the novel high speed interrupter technique were used to measure high frequency input impedance in infants. After the technical development and validation of the techniques, the variability and reproducibility of the measurements were determined to be similar to other lung function techniques for this age group. Using both techniques, high frequency input impedance showed particular features, the so called anti-resonant frequencies. Similar to human adults, in these infants anti-resonances are also related to wave propagation phenomena but not to tissue properties. Measurements during bronchial challenge with methacholine showed that airway wall compliance crucially determines the wave propagation velocity in the infants airways.

In order to determine whether airway wall mechanics are different in infants with wheezing disorders, high frequency Zin was examined in wheezing infants in comparison to healthy infants. Infants with wheezing disorders had lower anti-resonant frequencies even when they were asymptomatic, indicating differences in airway wall mechanics. Such structural changes may be due to developmental differences, differences in airway smooth muscle function or may be induced by airway inflammation and airway remodelling.
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The content of this thesis is original and elaborated by myself under the supervision of Prof. M. Silverman (Dept. of Child Health Leicester University), with advice from Prof. Evans (Dept. of Medical Physics, Leicester University).

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Supervisors (formal and informal):

Prof. M. Silverman, Head, Dept. of Child Health, Leicester University, PO Box 65, Leicester LE2 7LX, UK

Prof. Evans, Head, Dept. of Medical Physics, Leicester University, Leicester LE2 7LX, UK

Prof. A.C. Jackson Dept. of Biomedical Engineering, Boston University, 44. Cummington Street, 02215 Boston, MA

Collaborators and assistants:

Dr. C. Beardsmore, Dept. of Child Health, Leicester University, PO Box 65, Leicester LE2 7LX, UK (head of infants lung function lab)

Dr. K. Makkonen, Dept. of Child Health, Leicester University, PO Box 65, Leicester LE2 7LX, UK (for help in study 6.5)

Ms. T. Wellman, Dept. of Child Health, Leicester University, PO Box 65, Leicester LE2 7LX, UK (for technical help)

Ms. W. Newcombe, Dept. of Child Health, Leicester University, PO Box 65, Leicester LE2 7LX, UK (for help with patient recruitment)

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Prof. R. Kraemer, Head Dept. of Paediatrics, University Hospital of Berne, 3010 Inselspital, Berne, Switzerland (long time mentor and supervisor)

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3. ABBREVIATIONS

lung function techniques:

forced oscillation technique FOT
high speed interrupter technique HIT
rapid thoracic compression technique RTC

impedance features:

respiratory input impedance Zin
real part of Zin Zinre
imaginary part of Zin Zinim
first resonant frequency f1r
first anti-resonant frequency f1ar
second resonant frequency f2r
second anti-resonant frequency f2ar
real part of Zin at f1r Zinre (f1r)
real part of Zin at f1ar Zinre (f1ar)
real part of Zin at f2r Zinre (f2r)
real part of Zin at f2ar Zinre (f2ar)
imaginary part of Zin Zinim
autopowerspectrum input Gxx
autopower spectrum output Gyy
crosspower spectrum Gyx
coherence function γ²

wave propagation related parameters

wave propagation velocity ν
Moens-Korteweg wave speed νMK
gas or fluid of density ρ
dynamic viscosity η
mean gas velocity $V$
Womersley number $\alpha$
Reynolds number $Re$
airway cross-sectional area $A$
airway wall compliance $C_{aww}$
airway wall elastance $E_{aww}$
airway wall resistance $R_{aww}$
airway wall resonant frequency $f_{aww}$
maximal flow (flow limitation) $\dot{V}_{\text{max}}$
maximal flow at FRC (flow limitation) $\dot{V}_{\text{max,FRC}}$
functional residual capacity (end-expiratory level) $FRC$
bulk modulus of the gas $K_B$
complex propagation coefficient $\Gamma=\alpha+j\beta$
ratio of specific heats $\eta$

wave tube technique
characteristic impedance of the wave tube $Z_c$
propagation coefficient of the tube $\Gamma$

modelling parameters (DuBois model)
airway resistance $R_{aw}$
airway inertance $I_{aw}$
tissue resistance $R_{ti}$
tissue compliance $C_{ti}$
tissue inertance $I_{ti}$
gas compression compliance $C_g$
3. BACKGROUND

3.1. Clinical background

3.1.1. Wheezing disorders in infants

Childhood respiratory disease remains an important cause of mortality and morbidity, accounting for one-third of general practitioner consultations, one-fifth of hospital admissions and one-twentieth of deaths at all ages in childhood in England and Wales. In the first 5 years of life, two-thirds of respiratory deaths are due to lower respiratory tract infections. A further dimension of the public health importance childhood respiratory disease lies in its potential contribution to chronic respiratory disorders in adult life (Shaheen et al. 1994). There is a growing body of evidence from epidemiological studies (e.g. recently summarised by Silverman 1995, or Dezateux and Stocks 1997) confirming a link between childhood lower respiratory illness (LRI) and wheezing and the development of adult chronic respiratory disease (Reid 1969, Burrows et al. 1977, Barker et al. 1991, Shaheen et al. 1994). The nature of this link, the biological mechanisms which mediate it, and the genetic and environmental factors which influence its expression have been the focus of a considerable research effort in recent years.

3.1.2. Predictors of wheezing

One concept evoked to explain this association is that of ‘programming’ - the permanent alteration of the structure and function of organs and tissues by factors operating during sensitive periods in fetal or early postnatal life (Shaheen et al. 1994). Factors implicated in ‘programming’ of the respiratory system include fetal nutrition (Shaheen et al. 1994, Schwartz et al. 1990, Rona et al. 1993, Lewis et al. 1995, Godfrey et al. 1994), fetal exposure to maternal smoking during pregnancy (Lewis et al. 1995, Taylor et al. 1987, Evans et al. 1992), and exposure to environmental allergen (Sporik et al. 1990, Strachan 1995) or viral respiratory infections (Martinez 1994, 1995, Cogswell et al. 1982, Holt 1995, Strachan et al. 1994) during infancy. The issue of a genetic predisposition to asthma or atopy is also pertinent (Strachan 1994, Holt 1995) and it is likely that, for childhood asthma at least, outcome is determined by a combination of familial and environmental factors (Sherman et al. 1990). Some of these factors are also potential ‘programmers’ of the developing immune system. It has been suggested that exposure to viral infections in early infancy in those who are genetically susceptible to atopic disorders may favour dominance of TH-1 lymphocytes and reduce the expression of atopic disorders as mediated by competing TH-2 lymphocyte
populations (Holt 1995, Martinez 1994). From these experiences it becomes obvious that developmental aspects of the lung and particularly of the airways may play a crucial role for the wheezing disorders in infants. Most striking are studies looking at the influence of antenatal smoking on airway function at birth and subsequent wheezing disorders. It has been suggested that infants from mothers who smoked during pregnancy achieve lower maximal flows through their airways than healthy infants and that this flow limitation is related to subsequent wheezing disorders in the first three years of life (e.g. Martinez et al. 1988). Thus airway function seems to be an important 'programmer' for wheezing disorders in infants, and worthwhile studying in more detail.

3.1.3. Response to inhalation therapy

Some of these predisposing factors or 'programmers', and particularly the airway structure function-relationship, may also influence the action of therapeutic agents in wheezing disorders. While broncho-dilators are capable of reversing broncho-constriction due to smooth muscle activation, they might have more complex or even deleterious effects in cases with developmental disturbances of airway mechanics. Pharmacological studies have shown that broncho-dilators might not only affect airway diameter in infants but might increase flow limitation by removing airway smooth muscle tone and therefore increasing airway wall compliance (Prendiville et al. 1987). Particularly in infants with severe disturbance of airway wall mechanics such as infants with tracheomalacia, broncho-dilators can have adverse effects (Panitch et al. 1992).

Therefore, both epidemiological and pharmacological evidence stress the important role of developmental aspects of the airway in the aetiology of wheezing disorders.
3.2. Physiological background

3.2.1. Airway development in early infancy

As with other body tissues and organs, fetal and early postnatal life is a period of rapid growth and development of the respiratory system (Stocks 1995). Bronchial development and airway branching are mainly complete by the 16th week of gestation and, thereafter, airways increase in size and complexity only (Hislop 1995). True alveoli do not begin to develop until about 28 weeks gestation and increase rapidly in number, size and complexity during the first 3-4 years of life (Hislop 1995). In a full term infant, lung volume doubles by 6 months and triples by 1 year. These different growth patterns of the airways and parenchyma (i.e. disynaptic growth) during fetal and early life result in airways that are relatively large in relation to lung volume at birth, when airway conductance may be higher than at 2-3 months of age (Stocks 1995). The extent to which gender differences in these growth patterns contribute to the increased incidence and severity of respiratory illnesses observed in boys is unclear. Although some remodelling of the lung may occur during the first year of life following prenatal and perinatal insults, there appears to be considerable tracking of respiratory function from the end of the first year of life to late childhood (Stocks 1995).

The somatic growth that occurs during the first year of life is accompanied by major developmental changes in respiratory physiology (Stocks 1995), including changes in the shape compliance and deformability of the rib cage. The highly compliant chest wall of the newborn infant gradually stiffens during the first year of life. Infants also modulate expiratory flow in order to dynamically elevate functional residual capacity (FRC) above the level passively determined by the outward recoil of the chest wall and the inward recoil of the lung, an important strategy to establish and maintain an adequate lung volume in the presence of a highly compliant chest wall. Transition to a more relaxed pattern of expiratory flow occurs between 6-12 months of age. Another consequence of a highly compliant chest wall is an increased tendency of the peripheral airways to close during tidal breathing in early infancy, which results in impaired gas exchange in the dependent parts of the lung. This, together with the small absolute size of the airways, increases susceptibility to airway obstruction in the infant and young child (Stocks 1995).

While there is clear evidence that airway responsiveness is present from birth, the contribution of pre-existing alterations in airway geometry and lung mechanics or pathologic changes such as airway oedema and mucus hyper-secretion to wheezing LRIs may be of greater importance. Smooth muscle tone is modulated by a balance between slowly adapting receptors, which normally evoke smooth muscle relaxation, and rapidly adapting receptors and pulmonary C
fibres, which normally promote cough and broncho-constriction. In newborn infants, the coupling between smooth muscle and slowly adapting receptors is influenced by the mechanical properties of the cartilage of the large airways, elastic recoil of the lung-chest wall system, as well as the relatively reduced number of rapidly adapting receptors. Any given increase in airway resistance may reflect differing combinations of airway smooth muscle shortening and relative thickness of the airway wall. Thus, airway inflammation will increase and potentiate the effect of smooth muscle shortening on airway resistance, although the overall effect may vary with airway generation, being counteracted in the small airways by elastic lung recoil. Thus, in infants, the balance between central airway wall compliance, peripheral airway resistance and lung recoil may differ from that of older children and adults, resulting in different patterns of airflow limitation.

3.2.2. Classical explanations of the mechanism of flow limitation and wheezing

Gas flow into and out of the lungs occurs along pressure gradients. During inspiration alveolar pressure is lowered below atmospheric pressure, due to the actions of the inspiratory muscles expanding the thorax. This results in a pressure gradient between the mouth and the alveoli and gas flows into the lungs along that gradient. As the pressure surrounding the intrathoracic airways is essentially alveolar pressure, a pressure gradient also exists across the airway wall, which tends to expand the airways during inspiration. The lungs are inflated to a volume above the elastic equilibrium volume of the respiratory system, at the end of inspiration when the inspiratory muscles relax. At this volume, the inward recoil of the lungs and chest wall produces a positive alveolar pressure, relative to atmospheric pressure, and provides the driving force necessary to produce expiration. Contraction of expiratory muscles can contribute to this driving pressure. The airway transmural pressure gradient is reversed and the intrathoracic airways tend to narrow during expiration. The higher the expiratory driving pressure, the greater the transmural pressure and hence the greater the tendency of the intrathoracic airways to narrow.

During forced expiration, expiratory flow is independent of the driving pressure over most of the expired vital capacity, once a threshold value of driving pressure is exceeded (Fry et al. 1960). This phenomenon is known as expiratory flow limitation. The mechanism for expiratory flow limitation is complex (Macklem et al. 1986). In fluid dynamic terms, a system cannot carry a greater flow than the flow for which fluid velocity equals wave speed at some point in the system. The wave speed is the speed at which a small disturbance travels in a compliant tube filled with fluid. The speed of a pressure wave is determined by the elasticity
of the wall of the tube and the density of the fluid within. In the arteries this is the speed at which the pulse propagates. In the airway the speed is higher than this, mainly because the fluid density (i.e. gas density) is lower. The wave speed or wave propagation velocity ($v$) in a compliant tube with an area $A$ that depends on a lateral pressure $P$, filled with a fluid of density $\rho$ is given by (Dawson et al. 1977 (equations (1)-(3))):

$$v = (A \cdot dP/\rho \cdot dA)^{0.5}$$

$$v = (A/\rho \cdot C_{aww})^{0.5}$$

(1) $C_{aww} = \rho \cdot v^2 / A$

where $C_{aww} = dP/dA$ is the slope of the pressure-area curve for the airway, an expression of airway wall elasticity. Maximal flow ($V_{\text{max}}$) is the product of the fluid velocity at wave speed and airway area,

(2) $V_{\text{max}} = v \cdot A$

(3) $V_{\text{max}} = (A/\rho \cdot C_{aww})^{0.5} \cdot A$ (fig. 3.5.1.)

Flow limitation occurs in the airways when actual flow equals $V_{\text{max}}$ within the airway tree. At high lung volumes the flow limiting site in the human airways is typically in the second and third generations. As lung volume decreases, airway calibre decreases, the flow-limiting site moves peripherally and $V_{\text{max}}$ decreases. At low lung volumes the density dependence of maximal flow is small and the viscosity dependence is large and becomes the predominant mechanism limiting expiratory flow.

Flow limitation in a compliant tube is accompanied by flutter of the walls at the site of flow limitation (Webster et al. 1985). This flutter occurs to balance the energy in the system, as the
driving pressure in excess of that required to produce $V_{\text{max}}$ is dissipated in causing wall flutter. In the presence of airway obstruction, this flutter may become large enough to generate sound, heard as wheezing. Thus expiratory wheezing is a sign of expiratory flow limitation (Gavriely et al. 1987) Note that wheezing always implies flow limitation, but flow limitation can occur without producing a wheeze. In children with mild asthma, wheezes may be heard during forced expiration (including during a cough) but may be absent during tidal breathing. This implies that flow limitation is occurring during forced expiration but not during tidal breathing. As the severity of the asthma increases and airway narrowing worsens, flow limitation may occur during tidal expiration and wheezes may be heard during tidal breathing.

Flow limitation is one of the physiological key mechanism for the occurrence of wheezing in the airways (Gavriely et al. 1987). There is no wheezing without flow limitation. The importance of airway wall compliance for flow limitation in infants has been emphasised in morphological studies showing that airway wall compliance in newborns is much higher than in adults leading to more flow limitation (Buthani et al. 1981, Deoras et al. 1989, 1991(2x), Koslo et al. 1986, McFawn 1997, Panitch et al. 1989, 1992, 1998, Penn et al. 1988 (2x), 1989, Shaffer 1989) (details see section 3.2.5.). Animal work in lamb suggests that this is due to developmental differences in tracheal cartilage composition but also differences in airway smooth muscle tone and longitudinal tension. It has also been suggested that therapeutic interventions in infants – e.g. artificial ventilation in preterm infants – may alter the airway wall structure function relationship.

All this evidence suggests that it may not be sufficient to measure flow limitation in the airways but that more detailed information concerning the separate influences of airway wall mechanics and airway diameter might be important in order to understand the pathophysiology of wheezing disorders in infants. Until now it has only been possible to measure flow limitation in infants using forced expiratory manoeuvres by the rapid thoracic compression technique. These techniques do not allow the contribution of airway diameter and airway wall mechanics to be assessed.
3.2.3. Measuring flow limitation using the rapid thoracic compression technique

Forced expiratory manoeuvres, as used to stress the respiratory system in lung function testing, are not natural phenomena. Forced expiration can also be measured in infants using the rapid thoracic compression technique (RTC) (e.g. Clarke et al. 1992). The RTC technique produces forced expiratory flows by suddenly applying a pressure to the thorax and abdomen at the end of a tidal inspiration, using an inflatable thoraco-abdominal jacket connected to a positive pressure reservoir. Flow is measured at the mouth with an appropriately sized pneumotachograph attached to a mask sealed around the infant's nose and mouth and a flow-volume curve constructed. Prior to the RTC manoeuvre, a reproducible FRC is established from at least three tidal breaths. RTC, initiated at end-inspiration, then produces a partial expiratory flow volume curve, with exhalation continuing to a volume below the previous FRC. RTC manoeuvres are performed with increasing jacket pressures until the pressure that produces the highest expiratory flows is determined. The maximal flow occurring at the previously established tidal FRC (\( V_{\text{maxFRC}} \)) is reported (fig. 3.5.2.). Use of the RTC has led to major advances in the understanding of the normal growth and development of the respiratory system and of respiratory diseases (e.g. Tepper et al. 1986). However, the RTC technique has not proved to be the key to intrathoracic airway function which it initially promised to be. The utility of measurements of forced expiration rely on expiratory flow limitation being achieved. While this may be the case with the RTC technique in infants with airway obstruction, it is still uncertain that flow limitation is achieved in healthy infants. Furthermore, FRC is notoriously variable in infants, even over short time periods. This leads to substantial variability in the values of \( V_{\text{maxFRC}} \).

3.2.4. Maximal expiratory flows, airway wall compliance and wheezing disorders

Maximum flows achieved during forced expiration are directly related to airway cross-sectional area (calibre) and inversely related to airway wall compliance (Dawson et al. 1977), so that the technique cannot determine the contribution of these two properties to overall airway function. For instance, broncho-dilator agents can cause both an increase in airway calibre and, by removing airway smooth muscle tone, an increase in airway wall compliance. Their effect on maximum expiratory flow will be determined by both the baseline conditions of airway calibre and airway wall compliance and the relative changes induced, since an increase in calibre and airway wall compliance will have opposite effects on maximum flow rates. Indeed, many physiological studies have consistently failed to demonstrate a broncho-dilator response following single doses of inhaled sympathomimetics, yet clinical studies have shown that some
infants appear to 'benefit' from inhaled broncho-dilators in that they breathe more easily, have less wheezing and can be discharged from hospital earlier than similar infants not treated with broncho-dilators. It has even been reported that the RTC can therefore sometimes lead to paradoxical results when the effect of inhaled broncho-dilators is tested in infants (Prendiville et al 1987).

3.2.5 Measurements of airway wall compliance

For this reason there has been an increasing need for a technique which can measure airway wall mechanics independently of airway calibre. Until now there have only been invasive ways of measuring airway wall mechanics. Several investigators have determined airway wall elasticity from excised airways at various stages of lung development.

*Airway wall compliance in immature airways.* Immature tracheae are highly compliant structures (*Penn et al. 1988 (2x)*). Exhibiting extreme collapsibility under both static (compliance) and dynamic (pressure-flow) conditions. High airway compliance has been documented in both the premature lamb (*Penn et al. 1988 (1), Buthani et al. 1981, Panitch et al. 1987*) and the premature rabbit (*Buthani et al. 1981*) as well as in airways excised post-mortem from infants of low birth weight. Pressure-flow relationships in these airways have demonstrated a strong influence of compression forces on tracheal resistance, not unlike those observed in Starling resistors (*Penn et al. 1988 (1)). Although differences in methodology preclude direct comparison of pressure-flow data between different species, it is likely that airway collapsibility under dynamic conditions is also a function of developmental age (*Penn et a 1988 (1)).*

The functional implications of high airway compliance can be profound. Collapse in distal airways results in gas trapping and poor gas exchange. Dynamic compression during forced expiration increases total airway resistance and can increase the work of breathing in the already compromised neonate. Flow limitation is also more likely to occur in the premature infant whose highly compliant flow-limiting airway segment exhibits a low wave speed and consequently allows a low maximum flow (*Dawson and Elliot, 1977*). In addition, high airway compliance may predispose premature infants to airway damage consequent to the vigorous ventilatory support they frequently require. Pressure-induced deformation, acquired tracheomegaly and bronchopulmonary dysplasia all may result in part from chronic mechanical ventilation. Furthermore, mechanical ventilation has been shown to increase the collapsing compliance of immature airways (*Buthani et al. 1981*), potentially exacerbating all of the above-mentioned complications.
Effect of altering airway compliance. Numerous investigators have demonstrated that altering the mechanical properties of airways can effect maximum expiratory flow (MEF) and resistance to airflow (Olsen et al. 1967, Pride et al. 1967, Blank et al. 1969, Hyatt et al. 1975, Buthani et al. 1986, McCormack et al. 1986). Jones et al. (1975) altered airway mechanics in both excised and intact canine lungs through a variety of methods and found MEF to depend upon the mechanical properties of the compressed segment of airway. Blank et al. (1969) prevented tracheal compression by splinting intact canine tracheae and increased MEF values. Rabbit tracheae treated with the proteolytic enzyme papain showed an increase in in-vivo collapsing compliance and produced increased lung resistance and decreased MEF values at all lung volumes (McCormack et al. 1986).

Airway smooth muscle function and airway wall mechanics. In an attempt to define the role of airway smooth muscle in influencing airway mechanics/function, Coburn et al. (1972) found that smooth muscle contraction induced by acetylcholine decreased compliance in the excised adult canine trachea and attenuated the increase in airway resistance observed when compressive pressures were applied. Knudson and Knudson (1975) reported similar findings and also provided photographic evidence of decreased tracheal collapsibility consequent to trachealis contraction. Bethanecol-induced trachealis contraction in an in vivo newborn lamb model (Buthani et al. 1986) produced results qualitatively similar to those observed in the adult canine. Penn et al. (1988) confirmed the functional role of tracheal smooth muscle in the premature lamb. They found that the reduction in tracheal collapsibility was reflected by a reduction in airway resistance at all flow values and compressive pressures. In human infants the same phenomenon has been indirectly described (Prendiville et al 1987). They pretreated infants with salbutamol, a broncho-dilator which potentially removes airway smooth muscle tone. They then observed that at low doses of methacholine maximal expiratory flows (at FRC) through the airways improved. They also speculated that this might be an effect of changes in baseline airway smooth muscle tone.

Regulation of baseline smooth muscle tone. Little is known how baseline smooth muscle tone is regulated. The human airways are innervated via efferent and afferent autonomic nerves, which regulate many aspects of airway function. It has been suggested that neural control of the airways may be abnormal in asthmatic patients, and that neurogenic mechanisms may contribute to the pathogenesis and pathophysiology of asthma. The parasympathetic nervous system is the dominant neuronal pathway in the control of airway smooth muscle tone.
Stimulation of cholinergic nerves causes bronchoconstriction, mucus secretion, and bronchial vasodilation. Although abnormalities of the cholinergic innervation have been suggested in asthma, thus far the evidence for cholinergic dysfunction in asthmatic subjects is not convincing. Sympathetic nerves may control tracheobronchial blood vessels, but no innervation of human airway smooth muscle has been demonstrated. Beta-adrenergic receptors, however, are abundantly expressed on human airway smooth muscle and activation of these receptors causes bronchodilation. The physiological role of beta-adrenergic receptors is unclear and their function seems normal in asthmatic patients. Inhibitory nonadrenergic noncholinergic (NANC) nerves, containing vasoactive intestinal peptide and nitric oxide (NO), may be the only neural bronchodilator pathways in human airways. Although a dysfunction of inhibitory NANC nerves has been proposed in asthma, thus far no differences in inhibitory NANC responses have been found between asthmatics and healthy subjects. Excitatory NANC nerves, extensively studied in animal airways, have also been detected in human airways. In animal studies, stimulation of excitatory NANC nerves causes bronchoconstriction, mucus secretion, vascular hyperpermeability, cough, and vasodilation, a process called 'neurogenic inflammation'. Recent studies have demonstrated an interaction between the excitatory NANC nervous system and inflammatory cells. Neuropeptides may influence the recruitment, proliferation, and activation of leukocytes. On the other hand, inflammatory cells may modulate the neuronal phenotype and function. The functional relevance of the excitatory NANC nervous system and its interaction with the immune system in asthma still remains to be elucidated, however the potential role of the NANC mediator nitric oxide (NO) for the regulation of baseline airway smooth muscle tone has been discussed (e.g. Gaston et al. 1994, Van der Velden et al. 1999).

Alterations in tracheal longitudinal tension. The influence on longitudinal tension on tracheal patency or tracheal compliance has been recently investigated. The effect of altering airway longitudinal tension on upper airway collapsibility was suggested by Wilson et al. (1980), when they examined the role of transmural pressure and neck posture on the patency of the upper airway in infantile cadavers. Neck extension was found to make the airway less susceptible to collapse, whereas neck flexion had the opposite effect. Thus, in vivo measurements of airway wall mechanics strictly rely on standardised neck position. Changes of MEF resulting from varying degrees of neck flexion in healthy adult humans were determined by Melissinos and Mead (1977). MEF at high lung volumes was found to increase with neck hyperextension. This finding was attributed to increases in tracheal longitudinal...
tension. This assertion was supported by MEF-volume curves obtained from anaesthetised dogs; measured increases in tracheal longitudinal tension resulted in an increased MEF. Alterations in the mechanical properties of the central airway consequent to central airway stretching have also been suggested as a mechanism explaining the positive-effort dependence of MEF in an interesting study by Allen et al. 1987. Subjects who exhibited early rib cage emptying (as opposed to early abdominal contribution) tended to have positive effort dependence. These subjects also showed clear increases in initial flow values during MEF efforts when asked to accomplish early rib cage emptying. The authors attributed these findings to alterations in tracheal traction related to the relative position of the diaphragm. Early abdominal forcing would tend to elevate the diaphragm and push up on the lung reducing central airway tension. During early rib cage emptying, the relatively shortened diaphragm would help to maintain longitudinal tension on the central airways and to produce the observed alterations in flow. Penn et al. (1988(2)) quantified the decrease in compliance of immature tracheae consequent to tracheal stretch in vitro. The concomitant alterations in pressure-flow characteristics are consistent with the findings of the aforementioned studies. They also found a large effect of longitudinal stretch on tracheal compliance and flow resistance.

In conclusion. Previous work concludes that airway wall compliance in the infantile airways is increased which leads to increased instability and to greater flow limitation. Longitudinal tension of the airways can be influenced by the head position and the relative position of the diaphragm. Longitudinal tension influences airway wall compliance. Furthermore, bronchial reactivity is influenced by the mechanical properties of the airway walls at baseline. Airway wall compliance at baseline is influenced by baseline airway smooth muscle tone, which is determined by neuro-regulatory processes and may be influenced by pro-inflammatory cytokines (via the effect of induced NO).

These in vitro technique are an interesting approach to study structure function relationship of the airways during development, however it may not fully reflect the in vivo situation where airway wall tension is markedly influenced by the elastic recoil of the surrounding tissue. Two approaches have been used to overcome this problem. Firstly, some investigators have used invasive pressure transducers to measure airway wall compliance (Martin et al. 1958). However, this is an invasive approach and can not ethically be used in infants. Secondly, Panitch et al. (1998) have proposed a bronchoscopic method of the in vivo assessment and quantification of airway wall collapsibility in newborn lamb. However, up to now there is no
non-invasive technique available which provides a direct or indirect measure of airway wall mechanics in spontaneously breathing infants.
3.3. New concepts of measuring airway wall mechanics

A non-invasive method to measure airways wall mechanics depends on respiratory input impedance measurements using forced oscillatory techniques. In this section these techniques will be reviewed and their ability to measure airway wall mechanics discussed.

3.3.1. Measurement of respiratory impedance

Oscillatory respiratory mechanics is the study of the structural and mechanical properties of the respiratory system as deduced from its mechanical responses to small time-varying forces. The first series of studies employing small-amplitude external oscillations at high frequencies was made by DuBois and co-workers in the early 1950s (e.g. DuBois et al. 1956). They obtained frequency-response curves from 1 to 15-20 Hz in healthy humans by applying sinusoidal pressure variations both at the chest and at the mouth while measuring flow at the mouth. They also measured chest and abdomen surface displacements and observed that the chest wall did not behave homogeneously at high frequencies. Using simple modelling they derived values from their measurements for total respiratory resistance and the first estimates of respiratory inertance.

Respiration physiologists were not the only people to be interested in lung impedance at high frequencies. As early as 1958 an acoustician, Van den Berg, obtained input impedance data from 3 to 2,000 Hz from dog and human cadavers. In order to interpret his data he also built a sophisticated electrical analogue of the airways and of the tissues (Van den Berg et al. 1960). Clinical applications of the method required the development of pressure generators that were easier to handle than those that physiologists had previously used. This was achieved by Ferris et al. (1964), who described loudspeaker-based systems that permitted application of pressure variations at the chest and mouth. The first clinical studies concerned obese subjects, patients with chronic obstructive lung disease, patients with various lung or cardiac disorders, and children. The 1970s were marked by various technical and methodological improvements that permitted further applications. Up to that time it had been difficult to obtain amplitude ratios and phase angles from the original recordings until special-purpose signal analysers and analog and digital computers made the data immediately available. They permitted processing a greater amount of information and improved the quality of the measurements. On the other hand, computers made it possible to analyse the response to non-sinusoidal signals and pulses (e.g. Fredberg et al. 1978), random noise (Michaelson et al. 1975), and regularly recurring
impulses (Landser et al. 1976) that could substantially reduce the time necessary to explore a
given frequency range.

During that period, measurements of impedance at one or at a few frequencies progressively
became a routine tool to estimate total respiratory resistance. The method requires very little
co-operation and appeared particularly useful in children. In addition to experimental work,
several theoretical studies led to better understanding of how frequency responses were
influenced by various factors, including gas velocity profiles, airway geometry and branching,
and airway wall properties (Dawson et al. 1972, Fredberg et al. 1978(2), 1979).

3.3.2. Respiratory impedance below 100 Hz

Studies using Zin to measure lung function in animals or adults have made measurements
either at low frequencies (f $<$ $\sim$ 2 Hz) (e.g. Hantos et al. 1986, Lutchen et al. 1993) or at
higher frequencies (f $>$ $\sim$2 Hz) (e.g. Jackson et al. 1987, Lutchen et al. 1987, Jackson et al.
1989, Chalker et al. 1992, Jackson et al. 1993, Habib et al. 1994 (2x)). Zin at low frequencies
is largely a function of the viscoelastic properties of the tissues and airway resistance (Raw).
At higher frequencies, Zin is similarly influenced by Raw, influenced less by tissue properties
and influenced more by airway wall properties.

Zin data have been analysed either using systems identification techniques (e.g.
by considering changes in specific features of the Zin spectra (Desager et al. 1991, Chalker et
al. 1992). For example, in adults the level of airway obstruction is directly related to the
amount of negative frequency dependence in the real part of Zin (Zin$_{re}$) and the frequency of
the resonance (where the Zin$_{re}$ is a relative minimum and the imaginary part Zin$_{im}$ crosses
zero) Unlike feature analysis, systems identification techniques provide estimates of
physiological parameters which is accomplished by fitting a model to the Zin data. One such
model, the DuBois 6-element model (Dubois et al. 1956) provides separate estimates of
airway and tissue resistance (Raw, Rti), as well as thoracic gas volume (gas compression
compliance: Cg).
3.3.3. High frequency respiratory input impedance (Zin) above 100 Hz

However, the DuBois 6-element model can be used only if the Zin data include an anti-resonance that is related to the issue inertance (Iti) and the alveolar gas compression compliance (Cg). There is such an anti-resonance in dogs (Jackson et al. 1987, Lutchen et al. 1987, Jackson et al. 1991) and rabbits but not in adult humans (Jackson et al. 1989). Instead, the anti-resonances in adults are due to wave-propagation phenomena and are thus related to inertance of the gas within the airways and the compliance of the airway walls (Jackson et al. 1989). Since the anti-resonances are wave propagation related, estimates of Raw and Vtg are not possible in human adults but inferences about airway wall properties are possible (Jackson et al. 1989, Chalker et al. 1992).

In order to understand the relationship between high frequency Zin, flow limitation and airway wall mechanics, one has to understand the physics of propagation waves in compliant tubes. The following sections elucidate the relationship between airflow limitation, airway wall mechanics, wave propagation and anti-resonance phenomena in compliant airways.

3.3.4. Measurement of high frequency impedance using the wave tube technique

The forced oscillation technique is used to calculate respiratory impedance by measuring oscillatory pressures and the resulting oscillatory flows at the mouth. When measuring high frequency impedance the physical characteristics of the pneumotachograph influence the high frequency impedance data. Thus, another technique based on two pressure transducers and a rigid tube has been developed. This wave tube technique is based on the transmission line theory of oscillatory flows in rigid tubes.

Following , the fundamental work of Rayleigh (1945), Brown (1962) developed a one-dimensional model including the effects of varying velocity profile and heat transfer on the spatial distribution of fluid pressure. In this analysis the tube wall was assumed to be a perfect thermal conductor. Benade (1968) used a similar approach. Franken et al. (1981) wondered if the assumption of perfect thermal conduction in the tube wall did not introduce large errors in the estimation of the respiratory impedance. Therefore, they developed a line model, describing the tube as a pneumatic transmission line and taking into account the axial pressure losses, the gas compressibility, and the thermal conductivity of the tube wall. They verified the quality of this model by measuring velocity profiles at the end of an open tube at various frequencies.
Based on this theory, the characteristic impedance ($Z_c$) of a rigid tube can be determined. How can now this theory be used to calculate the load impedance of a tube? While $Z_c$ of the tube is constant, the pressure – pressure ratio of two pressure transducers connected at both ends of the tube depends on the load impedance of the tube ($Z_{in}$). With use of transmission line theory it can be shown that the pressure ratio ($P_0/P_1$) for a rigid tube of length $L$ terminated with an arbitrary load impedance $Z_{in}$ is given by (Benade et al. 1968, Franken et al. 1981)

$$P_0/P_1 = \left[ \cosh(\Gamma L) + Z_c/Z_{in} \sinh(\Gamma L) \right]^{-1}$$

To be able to theoretically calculate the pressure ratios at the two ends of a tube (of length $L$) terminated with an arbitrary load impedance ($Z_{in}$), one must know the propagation coefficient ($\Gamma$), the characteristic impedance $Z_c$, and $Z_{in}$. The $\Gamma$ and $Z_c$ can be calculated from theoretical considerations (Benade et al. 1968, Franken et al. 1981). Note, that if $Z_{in}$ is infinite, which is just the close-end termination case, the second term in the denominator approaches zero and (compare also section 3.3.9.)

$$P_0/P_1 = \left[ \cosh(\Gamma L) \right]^{-1}$$

Solving equation (4) for $Z_{in}$, $Z_{in}$ is given by:

$$Z_{in} = Z_c \sinh(\Gamma L) / \left[ (P_1/P_0) - \cosh(\Gamma L) \right]$$

The ratio of $P_1/P_0$ was estimated from the cross power spectra of $P_0 P_1$ and the auto power spectrum of $P_1$. The method for measuring $Z_{in}$ by the forced oscillation technique (+ wave tube technique) and its validation up to 2000 Hz has been described elsewhere (Jackson et al., 1993). The calculation of auto power spectrum and cross power spectrum is based on work of Michaelson et al. (1975):
3.3.5. Principles of power spectral analysis and coherence functions (*Michaelson et al. 1975*)

For a simple system with an input \( x(t) \) and an output \( y(t) \), the transfer function (H) can be obtained as follows:

\[
H = \frac{S_y}{S_x}
\]

Whereas \( S_y \) is the Fourier transform of the output function and \( S_x \) is the Fourier transform of the input function. If \( x(t) \) is a random pressure function and \( y(t) \) is flow, the \( H \) can be found at all frequencies and the impedance \( Z = \frac{1}{H} \). Because of synchronisation problems and statistical considerations related to the randomness of the input signal, the computation of \( H \) and \( Z \) by this method is not practical (*Richards 1967, Roth 1971*). Therefore the concept of power spectra is used to calculate \( Z \). For the variables in the above system \( S_x \) and \( S_y \) can be used to calculate three power spectra. The input power spectrum is:

\[
G_{xx} = S_x S_x^* = (A_x + j B_x)(A_x - j B_x) = A_x^2 + B_x^2
\]

Where \( S_x^* \) is the complex conjugate of \( S_x \). The product \( (A_x^2 + B_x^2) \) no longer contains any phase information. Similarly the output power spectrum is:

\[
G_{yy} = S_y S_y^* = A_y^2 + B_y^2
\]

The cross power spectrum is defined as

\[
G_{yx} = S_y S_x^* = (A_y A_x + B_y B_x) j(B_y A_x - B_x A_y)
\]

and preserves the phase relationship between the input and the output signals and can be used to compute the impedance magnitude as follows:

\[
|Z| = \frac{G_{xx}}{|G_{yx}|}
\]
The phase angle is given by

\[
\Psi = -\tan^{-1} \left( \frac{(B_y A_x - B_x A_y)}{(A_y A_x + B_y B_x)} \right)
\]

The advantage of computing the transfer function or impedance in this way is the rapidity with both magnitude and phase can be obtained over a wide range of frequencies. Further, the computation of \( Z \) does not depend on the character of \( x(t) \), since \( Z \) is obtained as a ratio of the input and cross power spectra. This is particularly important for the high speed interrupter technique as the input is not a Gaussian white noise signal but rather a step function (Michaelson et al. 1975).

The principles outlined above apply to a one input/one output linear system not contaminated by extraneous noise. To apply spectral analysis in biological systems where these ideal conditions are unlikely, a method of evaluating differences between the input and output, which may be caused by factors other than the transfer characteristics of the system is necessary. The coherence function \( \gamma^2 \) provides this need. \( \gamma^2 \) is defined (Roth, 1971) as follows:

\[
\gamma^2 = \frac{|G_{yx}|^2}{G_{xx}G_{yy}}
\]

\( \gamma^2 \) is a number between 0 and 1 similar to a correlation coefficient and provides an index of (i) causality between the input and output of a linear system. The value of \( \gamma^2 \) is less than 1 if the output is (ii) the result of more than one input, if the (iii) system is non-linear, or the system is (iv) contaminated by extraneous noise. Using \( \gamma^2 \) confidence limits for spectral estimates can be obtained. This is particularly important for the high speed interrupter technique.
3.3.6. Interaction between uni-directional flow and oscillatory flow

The airway tree is a complex structure of tubes. The flow properties within these airways are highly complex. In a simple tube, the analysis of the behaviour of pressure drop as a function of flow rate is based on the thickness of the fluid boundary layer. The outer edge of this layer of fluid is attached to the wall and the inner edge travels with the main stream. The thickness of the boundary layer is between two extremes: 1) extending all the way to the centre of the tube if velocities are so low than viscous forces completely dominate inertial forces (Poiseuille, or parabolic, flow) and 2) diminishing to zero thickness as inertial forces dominate viscous forces (turbulent flow). Between these two extreme conditions, two other flow conditions may exist: 1) laminar but non-Poiseuille flow and 2) transitional (nonlaminar), but not fully turbulent, flow.

Because the oscillatory frequency is far beyond the normal physiological range and the amplitude is extremely small compared with spontaneous breathing, the respiratory system is thought to respond passively and linearly to forced oscillations. This assumption appears specious, if one considers the typically nonlinear aerodynamic behaviour of the spontaneous breathing flow due to, e.g., developing flow effects at bifurcations and turbulence in main bronchi (Pedley TJ and Drazen JM, 1986). Indeed, if oscillatory and constant flows were to physically interact, the above linearity assumption would become questionable. The frequency response of the respiratory system, e.g., the frequency dependence of respiratory impedance, might then become dependent on flow rate of spontaneous breathing. The problem of the interaction between oscillatory and steady unidirectional flows has already been studied for laminar steady-flow conditions by Dorkin et al. (1982) and Franken et al. (1981). Dorkin et al. (1982) measured the oscillatory resistance (the real part of impedance) from 2 to 64 Hz in rigid tubes and in airway casts. In the absence of unidirectional flow, frequency dependence of resistance was observed. In the presence of unidirectional flow, oscillatory resistance at low frequency was independent of frequency and determined by the magnitude of the unidirectional flow. Oscillatory resistance at high frequency was frequency dependent but still influenced by the magnitude of the unidirectional flow. Their results indicate that the presence of unidirectional flow alters the oscillatory resistance of tubes, presumably by changing the shape of the boundary layer. They were able to quantify the flow conditions. In a rigid tube airway model the oscillatory resistance was higher that the calculated Poiseuille resistance of a tube if the dimensionless Womersley number $\alpha = r \frac{\omega}{\sqrt{v}} > 2.5$. $r$ is the radius of the tube, $\omega = 2\pi f$ is the frequency of the oscillation and $v$ is the gas kinematic viscosity (ratio of dynamic viscosity to density). $\alpha$ reflects relative effects of inertial to viscous forces and characterises oscillatory flow much as the Reynolds number characterises uni-directional flow.
Since flow is likely to be turbulent in the large airways. The interaction between oscillatory
flow and unidirectional flows has also been investigated under turbulent flow conditions
(Louis B and Isabey D 1993). These investigators have demonstrated that high frequency
oscillatory flows, as used in this work, are not likely to be affected by turbulent flow in the
airways, however for lower frequencies this will be the case.

From both of these papers, it becomes obvious that uni-directional flow might interact with
oscillatory flows, particularly if the uni-directional flow is turbulent and oscillatory flow is
used at low frequencies. In the last 20 years, most work in oscillatory mechanics has been
performed during spontaneous tidal breathing. Very few investigators have performed
oscillatory measurements during apnea (e.g. Sly et al. 1996).

3.3.7. Relationship between airway wall mechanics and wave propagation velocity

The ideas of waves in the airway and wave speeds are familiar to investigators of flow
limitation during forced expiration (Dawson et al. 1977). The flow-limiting wave speed in the
airways is much smaller than the speed of sound waves in free space. Surprisingly both of
these wave speeds are relevant to the propagation of pressure and flow oscillations in the
airways as studied in oscillation mechanics. Wave speeds are readily derived from the
physical properties of the working gas and airway walls. Guelke and Bunn (1981) have
measured the speed at which energy and information waves advance along the trachea (fig.
3.5.3). Although the details of this picture are likely to depend on airway size and airway wall
properties, some general principles may be deduced.

There are at least three distinct frequency ranges of physiological interest. At low frequencies
(<20 Hz) the wave speed is very nearly frequency invariant. In this low-frequency range,
airway wall inertance and airway wall resistance play no role; gas compressibility, being far
smaller than airway distensibility, is also of little consequence. As a result, waves in this
frequency range propagate by virtue of inertia in the gas coupled to compliance of the airway
walls (Eaww^1). In this case the waves are called Moens Korteweg waves and the propagation
wave speed becomes the Moens-Korteweg wave speed (MacDonald 1974)

(14) \( v_{MK} = A \cdot E_{aww}/\rho \)

where Eaww is the combined elastance \( (\delta p/\delta A) \) of the wall and supporting structures, \( \rho \) is gas
density, and \( A \) is tube cross-sectional area. Moens-Korteweg waves are precisely the type that
arises in blood flow in the great blood vessels. Of greater significance to respiration
physiologists, Moens-Korteweg waves are also relevant to the wave speed concept of maximal expiratory flow limitation (Dawson et al. 1977), wherein maximal expiration flow ($V_{\text{max}}$) is the product of airway area and wave speed (compare section 3.2.2)

(15) \[ V_{\text{max}} = v_{MK} \cdot A \]

Because $v_{MK}$ is given by Equation 5, and the specific wall elastance $E_{\text{aww}}$ is $A(\delta p/\delta A)$, this yields the familiar formula given by Dawson et al. (1977)

(16) \[ V_{\text{max}}^2 = (A^3/p)(\delta p/\delta A) \]

Flow limitation occurs when information of downstream pressure variations cannot propagate upstream; information propagating upstream at wave speed is brought to a standstill by equal and opposite downstream convectional flow speed. If flow is independent of downstream pressure, flow limitation is said to have occurred.

Proceeding to higher frequencies the wave speed increases by nearly an order of magnitude over the next two frequency decades (20-2,000 Hz). In this range, airway wall resistance ($R_{\text{aww}}$) and airway wall inertance ($I_{\text{aww}}$) impede the shunt pathway accommodating airway wall distensions. The wall elastance, in comparison, begins to play less of a role. The dynamics in this range become relatively complicated and include resonance of the airway walls. The smoothness of the curve is attributable in large part to the dominant influence of airway wall resistance. The wave speed in this range has been inferred also by a variety of indirect means (Fredberg 1978(2), Ishizaka et al. 1976, Van Den Berg et al. 1960), by independent measures of $E_{\text{aww}}$ (e.g., Martin et al. 1958) and $R_{\text{aww}}$ (Bobbaers et al. 1978), and by estimates of $I_{\text{aww}}$.

At frequencies above 1-2 kHz the wave speed attains another frequency-invariant plateau. At these frequencies the dominant effect of airway wall inertance virtually freezes all wall motions. Because airway walls are unable to distend as a result of their inertia, waves in the airway propagate by virtue of gas inertia coupled to gas compliance, which is precisely the nature of sound waves in free space. Rice (1980) has confirmed that high-frequency disturbances in the airway do propagate at the speed of sound in free space.
3.3.8. Wave propagation velocity and Zin

The effect of subglottic impedance on vocal cord vibration during speech motivated the earliest studies of respiratory system impedance at high frequencies (100-10,000 Hz). Van den Berg (Van den Berg 1960) had expected that the lung, because it was highly dissipative and constructed of numerous small airways, would absorb all sound waves incident at the trachea in a fashion analogous to an anechoic chamber. To his surprise he found in measurements of human and dog cadavers that pronounced reflections occurred with corresponding well-defined peaks (anti-resonances) and dips (resonances) in respiratory system impedance. He modelled the airways and the very small shunt impedance accorded by alveolar gas compressibility at high frequencies as a uniform tube open to atmosphere at an alveolar end, much like an organ pipe.

The impedance over the range of frequencies between 100 and 10,000 Hz has been measured in humans and in dogs by a variety of methods (Fredberg et al 1978 (1), Jackson et al. 1991, 1993; Habib et al 1994 (2)) with generally similar results. A series of impedance peaks and dips is observed corresponding to standing waves in the airways, much like organ pipe resonances and overtones.

Based on a variety of distributed-parameter modelling methods (see 3.7.7.) in symmetrical and asymmetrical airway branching morphologies (Fredberg 1978 (1), Jackson et al. 1993, Habib 1994 (1,2)), it is generally concluded that the resonant frequencies do not correspond well to a rigid-walled airway model, but rather that the resonant frequencies are shifted upward by airway wall dynamic responses. The magnitudes of the impedance peaks also are controlled by dynamic distensions of the central airway walls over much of this frequency range (<2000Hz). Impedance measured at the airway opening becomes increasingly insensitive to chest wall and lung tissue properties with increasing frequency because the shunt pathway through alveolar gas compression uncouples central airway gas motions from chest wall motions.

3.3.9. Wave propagation velocity and anti-resonances in compliant tubes

In order to understand wave propagation in compliant tubes we start off using the experiences from a rigid wall tube. As described in section 3.2.2, during a maximal expiratory effort, flow is limited to \( V_{\text{max}} \) given by:
where $A$ is cross-sectional area, $\rho$ is gas density, and $C_{aww}$ is the airway wall compliance. This equation tells us that $V_{max}$ is directly related to $A^{3/2}$ and inversely related to $C_{aww}^{0.5}$. This also means that if we were to make a pressure disturbance in a tube, that pressure wave would propagate with a maximal velocity of $V_{max}$. Thus, to obtain information about the airway wall compliance, we might want to measure the propagation velocity ($v$). There are several different ways of measuring $v$.

**$P_i/P_o$ in an occluded tube.** For an occluded tube of length $L$, the ratio of the pressures measured at the inlet ($P_i$) and at the closed end ($P_o$), is given by,

$$P_i/P_o = \cosh(\Gamma \cdot L)$$

where $\Gamma$ is the complex propagation coefficient defined by,

$$\Gamma = (Z \cdot Y)^{0.5} = \alpha + j\beta$$

where $Z$ is the impedance to axial flow and $Y$ is the admittance to radial flow. The equivalent circuit for an elemental length of **rigid walled tube** is

\[
\begin{array}{c}
R_g \quad I_g \\
\text{axial} \\
C_g \\
\text{radial}
\end{array}
\]

Whereas $R_g$ is the gas resistance, $I_g$ is the gas inertance, and $C_g$ is gas compression compliance. If we assume that viscous losses ($R_g$) are negligible, and $\omega$ is the angular frequency, $Z = j\omega I_g$ (transaxial) and $Y = j\omega C_g$ (radial), thus,

$$\Gamma = j\omega (I_g \cdot C_g)^{0.5}$$
and the wave propagation velocity is

\[ v = \omega / \beta = 1/(I_g \cdot C_g)^{0.5} \]  

In a rigid tube filled with a compressible gas, \( I_g \) per unit length is just \( \rho / A \), and gas compliance per unit length is \( A / K_B \) here \( K_B \) is the bulk modulus of the gas. In the case of air, the adiabatic bulk modulus is used which is given by \( p_0 \eta \) where \( \eta \) is the ratio of specific heats. Making these substitutions, we find that

\[ v = (p_0 \eta / \rho)^{0.5} \]  

For room air, \( p = 1.0132 \times 10^5 \) kPa, and \( \rho \) is 1.2047 kg/m\(^3\), we find that \( v \) is 340 m/s which is just the speed of sound in air.

The equivalent circuit for a compliant walled tube is,

The impedance to axial flow is again \( Z = j \omega I_g \) (transaxial), but the admittance to radial flow is and \( Y = j \omega (C_g + C_{aww}) \) (radial), where \( C_g \ll C_{aww} \), then

\[ \Gamma = j \omega (I_g \cdot C_{aww})^{0.5} \text{ (from (18))} \]  

and

\[ v = \omega / \beta = 1/(I_g \cdot C_{aww})^{0.5} \text{ (from (19))} \]
Note that equation (22) is equivalent to equation (3) since $I_g = \rho / A$, and $V_{\text{max}} = v \cdot A$ (see (2)). In equation (22) $v$ is frequency independent. However, this can not be the case in ‘real’ compliant walls. The equivalent circuit for a ‘real’ compliant walled tube such as an airway is more complex and minimally,

Thus a real non-rigid airway wall includes a mass term and a viscous loss term. *Guelke and Bunn (1981)* were the first to report propagation velocities as a function of frequency in non-rigid walled tubes (dog tracheae). They found that $v$ was frequency dependent; less than the free-field speed of sound at low frequencies, greater than the free-field speed of sound at mid-frequencies, and asymptotically approaching the free-field speed of sound at high frequencies.

To consider what happens to the resonant behaviour of the lung we could as a first approximation assume that it behaves like a simple tube with constant cross-sectional area. Such a tube that is oscillated at $x=0$ and is closed (i.e., is terminated by a infinite impedance) at $x=L$ will have anti-resonances at,

$$ (23) \quad \text{far},n = (2n-1)/4 \cdot v / L, \text{ with } n = 1, 2, 3..., \text{results in } \text{far},2 = 3 \cdot \text{far},1 \text{ and } \text{far},3 = 5 \cdot \text{far},1... $$

In a tube that is driven at $x=0$ and is open (i.e., is terminated by a negligible impedance) at $x=L$, the anti-resonances will occur at

$$ (24) \quad \text{far},n = n v / L, \text{ with } n = 1, 2, 3..., \text{results in } \text{far},2 = 2 \cdot \text{far},1 \text{ and } \text{far},3 = 3 \cdot \text{far},1... $$

Thus, the frequencies at which the anti-resonances occur are directly related to the propagation velocity, $v$, and inversely related to the pathlength. Where will the anti-resonances occur in a
compliant walled tube? This is not easy to answer because \( \text{far,} n \), depends on the length of the tube but the propagation velocity is not length dependent but is frequency dependent. Thus, it is possible that in a non-rigid tube low frequencies, which are travelling relatively slowly, will resonate at a relatively low frequency while higher frequencies will resonate at a higher frequency because they are propagating at higher velocities. Thus, there could be multiple 'first anti-resonances', or multiple higher order anti-resonances. What happens when the length of a non-rigid walled tube is unchanged but there is a change in the wall compliance? Again, this is not easy to answer. To provide some insight into this question, let's consider the following figure (fig. 3.5.4.). If the length of the tube is such that its first resonance occurs in region I (i.e. it is relatively long), then an increase in compliance would result in a decrease in \( v \) and a decrease in \( \text{far,} 1 \). However, if the tube was shorter such that its \( \text{far,} 1 \) occurred somewhere in region II, then an increase in compliance would result in an increase in \( v \) and an increase in \( \text{far,} 1 \). Finally, if the tube was even shorter and its \( \text{far,} 1 \) occurred in region III, an increase in compliance would result in a decrease in \( v \) and a decrease in \( \text{far,} 1 \).

3.3.10. The anti-resonant frequency in compliant airways

Let's now transpose these theoretical considerations into the situation in airways. There are three factors that determine the anti-resonant frequency in a cylindrical tube: 1) the tube length 2) the speed of sound, or velocity of wave propagation and 3) whether the tube is open or closed ended, more generally speaking the boundary condition at the distal end.

First let us consider the speed of sound. There are two factors that influence the wave propagation velocity in tubes: tube diameter and wall rigidity. In large-diameter rigid tubes waves will propagate at the free-field speed of sound, independent of tube diameter. In rigid tubes with diameters <0.4 cm, wave propagation velocities with room air are decreased by >5% (Benade, 1968). In the Horsfield et al. (1982) airway model, airways distal to the 27th order have diameters <0.4 cm in human adults. In the terminal airways where the diameter is 0.08 cm, the wave propagation velocity is 21,400 cm/s or 62% of the free-field speed of sound. Thus in airways between the 26th and first order (terminal airways), wave propagation velocities are significantly reduced. A reduction in the wave propagation velocities in these distal airways would cause them to resonate at a lower frequency.

The second factor that influences propagation velocities is tube wall compliance. In compliant tubes, the velocity of wave propagation is a function of the wall properties as well as the properties of the gas within the tube. Guelke and Bunn (1981) measured propagation velocities in an excised dog trachea (fig. 3.5.3.) and found that there was a transverse wall
resonance ($f_{swv}$) at 150 Hz. They developed a model for the acoustic behaviour of compliant wall tubes that could predict propagation velocities that correlated well with their measurements.

The third condition affecting the length-resonant frequency relationship is the boundary condition at the distal end. We have thus far considered only the two extreme conditions, that is, where the distal end is either open or closed. If the respiratory system is behaving like an open-ended tube, this would imply that the chest wall and lung tissues have a negligible impedance at these frequencies. In the other extreme, that is, the chest wall and lung tissue impedance is infinite, the system would behave as though it is closed ended. If the tissue impedance has some value between zero and infinity, then the airway resonance would occur at a frequency higher than if the tissue impedance was zero and at a frequency lower than if the tissue impedance was infinite. In this case, the open-ended equivalent length would be less than the actual airway length and the closed-end equivalent length would be greater than the actual airway length.

To summarise, any phenomenon that reduces wave propagation velocity will result in the effective length being less than the actual length. Conversely, any phenomenon that increases wave propagation velocity would result in the effective length being greater than the actual length. There are two factors that reduce wave propagation velocity. First, velocities in the peripheral airways predicted to be reduced because of their small diameters. Second, velocities will also be decreased for frequencies below the wall transverse resonance provided the airway walls are not rigid. There is only one phenomenon that results in an increase in wave propagation velocity (and concomitantly the equivalent length being greater than the actual length) and that is in cases where the airway walls are compliant and the wall impedance is dominated by their inerance and this will occur for frequencies above the wall resonant frequency $f_{swv}$. A factor that does not affect propagation velocity but does affect the equivalent length estimate is the terminal, or tissue, impedance. There is evidence that the respiratory system behaves more like an open-ended system than like a closed-ended system. \textit{(Jackson et al.1989, Van den Berg 1960). Initial application of a simple open-ended cylinder representation of the airways provided estimates of an effective length that are somewhat less than published airway path lengths. The effective length estimate is, however, a function of 1) airway wall properties, 2) airway length, 3) possibly, the impedance of the terminal elements i.e. lung and chest wall tissues). These results suggest further studies to determine the propagation velocities in human airways and whether the equivalent length is function of tissue impedance. However, data from \textit{Jackson et al.1989} suggest, that high frequency Zin may provide information about \textit{airway length and/or airway wall properties}.}
3.3.11. Modelling of high frequency Zin data (distributed parameter models)

The models discussed in section 3.3.3 (Lumped parameter models, e.g. DuBois model) deal with pressures and flows at specific system boundaries or nodes such as the airway opening, alveolar spaces, and body surface. In these models the morphological characteristics of airways are bypassed a priori by assignment of extensive dynamic variables. These variables include airway inerance and resistance, which lump together the entire spatial extent of the upper airway and tracheo-bronchial tree. Lumped-parameter modelling methods such as this become awkward in at least three circumstances when:

1) spatial distribution of oscillatory pressures and flows within the upper and lower airways are to be investigated,

2) specific physical properties of the airway tree (airway sizes, wall properties, and branching) are to be taken into account and

3) oscillatory frequencies become high enough that wavelike phenomena occur in the airways, similar to standing waves in an organ pipe. In these circumstances spatially distributed models (see section 3.3.7) become preferable to lumped-parameter models.

A spatially distributed model is one in which spatial dimensions, co-ordinates and branching topology are dealt with explicitly. Pressures and flows are computed not only at convenient nodes such as alveolar pressure or airway opening pressure but also at every position in every airway in between. Even parenchyma itself is no longer described by a single alveolar pressure but rather by pressure that could be different from point to point throughout. An airway of length $L$ may be divided into infinitesimal segments of length $dx$. The equivalent Circuit for such a segment incorporates airway inerance ($I_{aw}$) and viscous resistance ($R_{aw}$) as series elements. Shunt elements can be subdivided into the gas compressibility pathway, consisting of gas compressibility ($C_g$) and gas thermal conductance ($G_t$), and the wall distension pathway, consisting of airway wall elastance ($E_{aww}$), inerance ($I_{aww}$), and resistance ($R_{aww}$). The impedances, pressures, and flows of these small segments $dx$ must be marched along airways between bifurcations and combined among airways at bifurcations. Jackson et al. (1993) and others have proposed computational schemes (system identification techniques) to execute the repeated application of these equations (which describe the mechanical properties of a segment $dx$) for a relatively general class of asymmetrically branching models of the tracheo-bronchial tree (Horsfield et al. 1982). Based on these models they have been able to calculate means of serial distribution of airway resistance as well as airway wall mechanical properties. However, such models or anatomical data are currently not available,
thus Zin information on airway wall mechanics can at the moment only be assessed qualitatively and not quantitatively.

3.3.12. Respiratory impedance in infants

There was increasing interest in using respiratory input impedance measurements (Zin) as a pulmonary function test in infants. Zin measurements can be performed within seconds during tidal breathing, and do often not disturb the infant during natural unsedated sleep. Few studies have reported Zin measurements in infants (Marchal et al. 1988 (2x), Desager et al. 1991, Sly et al. 1996, Jackson et al. 1996). Sly et al. (1996) reported Zin measurements in healthy infants at low frequencies (0.2 - 20 Hz) during reflex induced apnoea. Desager et al. (1991) and Marchal et al. (1988) reported Zin between 6 and 48 Hz during tidal breathing in wheezy and healthy infants, respectively. Finally, Jackson et al. (1996) measured Zin between 20 and 256 Hz in healthy infants. However, in none of these studies were the Zin measurements made following induced airway constriction or dilation, whereby the change in airway mechanics was verified by a reference lung function technique. It has only recently been shown that there is an anti-resonance in healthy infants (at approx. 120 Hz) (Jackson et al. 1996). Even though, it was not clearly understood what phenomena contribute to this anti-resonance in infants, preliminary results indicate that it is related to the total respiratory system inertance (Irs) and at least partly due to the gas compression compliance in the face mask (Sly et al. 1996). A lumped parameter model was suggested (Jackson et al. 1996) for analysing infant Zin data which according to computer simulations results in an estimate of total respiratory system resistance (Rrs) that may be related to airway calibre. However, up to now it was not clear whether high frequency Zin data in infants can be modelled using lumped parameter (DuBois) models as in dogs or whether wave propagation phenomenon play an important role as in human adults. It the latter case these high frequency impedance measurements in infants would infer information regarding airway wall mechanics.
3.4. Conclusions from previous work and implications for aim of thesis

In summary, previous literature has demonstrated the airway wall mechanics seems to play a crucial role in the development of flow limitation in the airways and in wheezing disorders. There is little known about its role in wheezing disorders in infants, despite the fact that airway wall compliance seems to be higher in young infants than in adults and may play an much more important role in wheezing in these small human beings. It might be crucial for the understanding of the physiology of the developing airways and of the effect of drugs which not only alter airway diameter but also airway wall tone and therefore airway wall compliance. From previous work in adults and animal models, high frequency impedance measurements derived from oscillatory mechanics might provide new insight into the understanding of the role of airway wall mechanics. High frequency respiratory impedance is governed by wave propagation phenomena. Wave propagation phenomena are the basics for flow limitation in compliant airways. In sufficiently large airways they are little determined by airway diameter, but more by airway wall mechanics and by the boundary conditions of standing waves in the airways. Up to now there is little evidence of high frequency impedance and frequency dependence of wave propagation in the infant airways. If one standardised the boundary conditions of the airways, one might be able to derive information on airway wall mechanics and/or mean effective airway pathlength in infants. Standardising the boundary can be achieved if physiological experiments (during bronchial challenge tests) in which airway pathlength is not expected to change, or if cross-sectional data of healthy and wheezing infants of the same age groups are compared.
3.5. Figures and Tables

Figure 3.5.1.: Simulation of equation 3. Maximal flow through a compliant tube is crucially dependant on airway diameter as well as airway wall compliance.

Figure 3.5.2.: Setup of the rapid thoracic compression technique to measure forced expiratory flows in infants.

Figure 3.5.3.: The relationship between the frequency of a propagation wave in a compliant airway and the wave propagation velocity (from Guelke and Bunn 1981).

Figure 3.5.4.: The relationship between the frequency of a propagation wave in a compliant airway and it’s wave propagation velocity can be parted into three zones I,II, III (see text) dependant on the airway wall resonant frequency $f_{aww}$ (adapted from Bioengineering Course BE 760, Boston University Boston, MA, US). The solid and the dotted line represent changes of the wave propagation velocity if the wall compliance increases from C to C'.
Figure 3.5.1: 

![Graph showing relationship between max flow (V_max), airway wall compliance, and tube area.

- The x-axis represents tube area, ranging from 0 to 20.
- The y-axis represents airway wall compliance, ranging from 0 to 20.
- The z-axis represents V_max, ranging from 0 to 300.

The graph illustrates the non-linear relationship between V_max, airway wall compliance, and tube area.
**Figure 3.5.2**

**Flow** = \( \frac{dV}{dt} \)

- Jacket Pressure
- Inflatable Jacket
Figure 3.5.4:

- Phase Velocity, m/s
- Frequency, Hz

- $c = 0.04$
- $c' = 0.08$

- I: $f_c < f_c$
- II: $f_c > f_c$
- III: $f_c < f_c$
4. AIMS AND HYPOTHESIS

Since non-invasive conventional infant lung function techniques have failed to separate the influence of airway diameter and airway wall compliance on flow limitation, it has so far been impossible to demonstrate the importance of airway wall elasticity in wheezing disorders in infants. Therefore the aims of this thesis can be defined as follows:

4.1. Can airway wall compliance been measured non invasively in infants?

The overall aim of this work was to develop a non-invasive lung function technique which enables the study airway wall mechanics in vivo during normal tidal breathing in infants. This overall aim had to be split into the technical development and the validation of the techniques as follows:

4.1.1. Technical development of the forced oscillation technique for infants (see 6.1)
4.1.2. Technical development of the high speed interrupter technique for infants (see 6.2, 6.3)
4.1.3. Variability, repeatability of high frequency impedance in infants (see 6.1, 6.3)
4.1.4. Comparison between forced oscillation and high speed interrupter technique (see 6.2)
4.1.5. Analysis of the nature of high frequency impedance data in human infants (see 6.3)

4.2. Do airway wall mechanics contribute to flow limitation during bronchial challenge test in infants?

After having established a technique sensitive to changes in airway wall mechanics, we aimed to assess whether airway wall mechanics change during bronchial challenge with methacholine and whether these changes contribute to flow limitation in the airways in vivo in infants. If so, not only factors contributing to smooth muscle constriction but also factors contributing to alterations in airway wall mechanics are determinants of flow limitation and therefore wheezing in infants. (see 6.1 and 6.3).
4.3. Are airway wall mechanics different in wheezy infants in comparison to healthy infants of similar age?

Finally, we aimed to test whether infants with wheezing disorders show differences in airway wall mechanics in comparison to their healthy controls, in an interval when they are asymptomatic. If true, this would be highly suggestive that infants prone to wheezing disorders had structural changes of airway walls or the surrounding tissue leading to changes in airway wall mechanics. (see 6.5)
5. METHODS

5.1. General technical aspects of measurement techniques

5.1.1. The rapid thoracic compression technique

The technical aspects of the rapid thoracic compression technique have previously been described (e.g. Clarke et al. 1992). Briefly, as described in section 3.2.3.. The RTC technique produces forced expiratory flows by suddenly applying a pressure to the thorax and abdomen at the end of a tidal inspiration, using an inflatable thoraco-abdominal jacket connected to a positive pressure reservoir of 50 L (MEDICAL ENGINEERING DEPARTMENT, ROYAL POSTGRADUATE MEDICAL SCHOOL, HAMMERSMITH HOSPITAL LONDON) with arms in.

This reservoir is necessary to maintain a relatively constant pressure during the period of thoracic compression approximately for 3-4 seconds. The release of pressure into the jacket was manually triggered at the beginning of expiration which was detected on a two channel oscilloscope showing flow and volume on a X-Y plot. Flow was measured using a face mask (RENDELL BAKER SOUCEK, size 1, AMBU INTERNATIONAL) and FLEISCH NO 1 pneumotachograph (VALIDYNE MP45, NORTHRIDGE, CA). The linearity was estimated to be accurate within 2%. The flow signals were AD-converted and assessed using RASP-software (PHYSIOLOGIC, NEWBURY, UK).

Prior to the RTC manoeuvre, a reproducible FRC is established from at least three to five tidal breaths. RTC, initiated at end-inspiration, then produces a partial expiratory flow volume curve, with exhalation continuing to a volume below the previous FRC. RTC manoeuvres were performed with increasing jacket pressures in steps of 0.5 kPa, starting at a pressure of 3 kPa, until the pressure that produces the highest expiratory flows was determined. This pressure was then used for all subsequent studies on that child. The mean and standard deviation of 10 technically satisfactory maximal flows at FRC (\(V_{\text{maxFRC}}\)) was determined.

5.1.2. Respiratory impedance measurements by forced oscillations

The data acquisition and measurement system is based on a standard technique that has been described elsewhere (Dorkin et al. 1982). However instead of measuring flow using a pneumotachometer, oscillatory flow is measured using the wave tube technique described below. Firstly, in the 'classical technique' the loudspeaker plethysmograph functions both as the oscillatory flow generator and the detector by which the impedance and resistance are measured. The loudspeaker plethysmograph is separated into two chambers by the
loudspeaker. The volume of the reference chamber (fig. 5.5.1.) is measured by water displacement, and the compliance of the equivalent gas volume is calculated. The speaker movement generates either gas compression or rarification in this chamber, developing pressure changes proportional to the volume of the speaker excursion. From this plethysmographic relationship of pressure and compliance, the volume of speaker displacement per unit time (flow) can be determined at each frequency. The outlet of the test chamber (fig. 5.5.1.) can either be closed or connected to an experimental load. When the outlet is closed, speaker movement produces gas compression and rarification in the test chamber as it does in the reference chamber. The volume changes in the two chambers are equal in magnitude but 180° out of phase.

When an experimental load such as a subject is connected to the test chamber, speaker displacement causes both gas compression and rarification in the test chamber as well as gas flow into the respiratory tract of the subject. Pressure measured in the test chamber under these conditions is less than that measured when the experimental outlet is closed and the patient is disconnected. The decrease in test chamber pressure is proportional to the volume of gas flowing from the test chamber into the respiratory tract.

Input impedance is defined as

\[
Z_{in} = \frac{(P_{ao} - P_{bs})}{V_{in}} = \frac{P_{tr}}{V_{in}}
\]

where trans-respiratory pressure (Ptr) is the difference between pressure at the airway opening (Pao) and pressure at the body surface (Pbs, which is atmospheric pressure), and Vin is the airflow into the subject. From this we can then use the ratio of pressure developed in the test chamber to the flow exiting as the measured input impedance of the subject's respiratory system. Thus, from measurements of pressures in the two chambers, flow into the airway opening is calculated and from this the oscillatory impedance, resistance, and reactance of the respiratory system can be computed. This plethysmographic method avoids the frequency response problems often associated with the use of pneumotachometers for oscillatory flow measurements at high frequencies.

**Signal processing.** A computer-generated sinusoidal function is established at the minimum frequency (8 Hz) and increased in steps of 4 Hz over the range of frequencies desired. The signal is amplified, converted into an oscillatory pressure by the loudspeaker plethysmograph,
and directed into the subject. The resultant pressure signals in the reference and test chambers of the plethysmograph are then detected by pressure. The signals are then amplified, band-pass filtered between 1 and 315 Hz, and returned to the computer where impedance is computed from the pressure magnitude and the phase relationship between pressure and flow. From these, the real and imaginary parts of impedance are determined.

5.1.3. Forced oscillation technique for infants

We aimed to apply this technique for use in infants (see 6.1), which requires adaptation regarding the application of the forced oscillation via a face mask to the mouth. Furthermore we needed to adapt to new unknown pressure and flow conditions in the infant. Due to small airway and lung size, we theoretically expected a relatively high respiratory impedance in infants. If the infant respiratory system is forced with a given oscillatory pressure signal the resulting oscillatory flow amplitude will be low which potentially leads to insufficient signal to noise ratios.

Let's firstly consider the problem in measuring respiratory input impedance with the influence of the upper airways and the influence of the face mask. Gas volume and to a certain extent also tissue compliance act like a shunt impedance. This impedance is mainly determined by the gas compression compliance. The tissue compliance will influence the lower frequency contents of the Zin spectrum but will become less important with increasing frequency. This shunt impedance is particularly important if airway resistance is particularly high. This issue cannot be fully resolved. However, in order to minimise the influence of shunt compliance of the upper airways, one has to minimise and standardise the gas volume in the face mask. This was done this by filling the face mask with putty (see 5.3.3, 5.3.4.) and reducing and standardising the face mask volume to 6-7 ml. Furthermore the head position was standardised as proposed by Desager et al. (1991).
5.1.4. Development of the high speed interrupter technique

The conventional interrupter technique (VonNeergaard and Wirz, 1927) is based on the assumption that following a rapid interruption of flow at the airway opening ($V_{ao}$) there is an equilibration between alveolar pressure ($P_{alv}$) and airway opening pressure ($P_{ao}$). The interrupter resistance $R_{int}$ can be obtained by dividing the change in $P_{ao}$ following interruption by the flow immediately preceding the interruption. We showed that the classical technique was easy to use in children because it is non-invasive and needs little co-operation (Wirz et al. 1993, Frey et al. 1995 (1), 1997(2)). However, this conventional analysis often underestimates lung resistance in children, especially in the presence of severe airway obstruction, because pressure equilibration is often not achieved. In previous work (Frey et al. 1995 (1,2), 1997(2)) we aimed to find a method to analyse the interrupter curve in a way which did not depend on complete pressure equilibration, and which focused on the damped mouth pressure oscillations that occur shortly after the flow interruption (fig. 5.5.2.). We used a standard shutter and wrote software in order to sample the pressure changes at high resolution. We showed that the damping properties of these postocclusional oscillatory pressure transients are correlated with airway resistance in children (Frey et al. 1995 (1)) and that they change during bronchial challenge with broncho-constrictors (Frey et al. 1997 (2)).

In a next step we aimed to determine whether these postocclusional pressure transients were correlated with airway wall properties. We measured their frequency by spectral analysis and found resonant frequencies around 70 Hz (Frey et al. 1995 (1,2), 1997(2)), or around 150 Hz if the length of the mouthpiece was reduced (Frey et al. 1995 (2)). As mentioned above, Jackson et al. (1989) found an anti-resonance phenomenon around 180 Hz using the forced oscillation technique in human adults. We demonstrated similarities between these postocclusional pressure transients and the anti-resonance phenomenon found by Jackson et al.(1989). This has important implications, because this first anti-resonant frequency is therefore dependent on the airway path length, as well as on gas density and on the airway wall compliance (Jackson et al. 1989).

After showing that the frequency of the postocclusional pressure transients are correlated to airway wall properties, we wanted to demonstrate that it was possible to measure changes in airway obstruction and changes to airway wall properties non-invasively in vivo in children (Frey et al. 1995 (1), 1997(2)). In 60 healthy and asthmatic children we showed that the damping properties of postocclusional pressure transients changed with increasing airway
obstruction during carbachol challenge and that their frequency, which is a function of airway wall compliance, also changed during carbachol challenge test (Frey et al. 1997(2)).

High frequency input impedance measurements using the interrupter technique. After these empirical and qualitative findings we wanted to find a way to quantify changes in airway wall properties. This turned out to be difficult because the absolute value of the frequency of the oscillatory transients depended on the shutter speed, and the length of the mouthpiece (Frey et al. 1995 (2)) since we only assessed a single pressure signal. In order to eliminate these problems, we wondered whether it might be possible to create a new physiological concept and measure high frequency respiratory input impedance (Zin) using the interrupter technique by not only measuring the postocclusional pressure transients but also the resulting oscillatory flow transients. The potential advantage of measuring Zin using the interrupter technique is that the device has a small dead space, the shunt impedance is lower and the flow amplitudes at higher frequencies are better, leading to a better signal to noise ratio (see 6.2.).

Furthermore, the interrupter technique might be advantageous because during airflow interruption, there is no uni-directional flow component which could potentially interact with the oscillatory flow measurements (see 3.3.6). The high speed interrupter technique might be useful for measurements of Zin during spontaneous breathing, since during airflow interruption there is no unilateral flow component but only an oscillatory flow within the airways.

In order to measure high frequency respiratory impedance, it is optimal if the forcing function contains a sufficient amount of energy at high frequencies. The faster the flow interruption is the higher is the energy content of the flow power spectrum. It is maximal if the interrupted airflow signal would resemble a true mathematical flow step-function. To boost the high frequency content of the forcing function we developed a particularly fast airflow interrupter, which we called high speed interrupter technique (HIT).

This high speed interrupter was then combined with the wave tube technique (described in section 3.3.4.) enabling the measurement of oscillatory pressure and flow using two pressure transducers.

Mechanical aspects of the interrupter valve. The high speed interrupter device consists of a rapidly turning slide valve. The HIT is based on rapid multiple interruptions of airflow. If flow interruption were to occur instantaneously, the input flow signal applied to the lungs would be a step function, the frequency (f) content of which varies as 1/f. If the interruption occurs less rapidly the amplitude of the flow at higher frequencies is reduced.
This interrupter is driven by a stepper motor (start speed 1200 degrees/s, maximal speed 7500 degrees/s, ramp time 13 ms). The shutter consists of a rotating blade which closes the airway opening within 1 ms, remains closed for 15.5 ms, then opens for another 15.5 ms. Thus the complete interruption-closure cycle occurs once every 31 ms. The motor is controlled by a digital-to-analog converter (MODEL AT-MIO-16, NATIONAL INSTRUMENTS, TX, USA). The position of the shutter valve (open or closed) was measured by a photo-optic resistor to ensure that the shutter was reopened after an interruption so that the subject was able to breath between the measurements. Whenever the interrupter was enabled, it triggered only at inspiratory flow rates of less than 0.1 Ls\(^{-1}\) producing 5 separate interruptions.

**Measurement of pressure changes.** These interruptions caused post-occlusional damped pressure transients within the wave tube. The calibrated pressures (see section 5.2.3.) were measured at two locations 6.7 cm apart in infants (see sections 6.3-6.5), 12 cm apart in adults (see section 6.2) along the tube between the shutter and the mouthpiece. The electrical output of these transducers was band pass filtered (8-2000 Hz) and analog/digital converted at 8258 Hz (Model AT-MIO-16, National Instruments). A representative example of the pressure \(P_1\) and \(P_0\) as well as the delta pressure (\(P_1-P_0\)) are illustrated in Figure 5.5.3. Peak pressures of less than 1.5 cm H\(_2\)O and more that 10 cm H\(_2\)O were not accepted because of possible signal to noise ration problems and linearity problems (see section 5.1.5 and 5.1.6. for full details). Power density spectra of both pressure channels are illustrated in figure 5.5.4. It can be seen that the power density spectrum of \(P_1\) and \(P_0\) are close to a \(1/f\) amplitude distribution. The calculation of impedance from \(P_1\) and \(P_0\) is described in the sections on wave tube technique (section 5.1.5.).

5.1.5. The wave tube technique for use in infants

The principle of the wave tube technique have been described in section 3.3.4. So far, the wave tube technique has not been implemented in infants. Apart from the fact that we can measure impedance up to higher frequencies (see 3.3.4), we profit from the fact that there is no pneumotachograph within the system, which ensures a low dead space. We derived the dimensions of the wave tube from previous work in dogs with approximately similar lung size (Habib et al, 1994). We then optimised the wave tube based on the following criteria: (i) low resistance, (ii) sufficient pressure drop between \(P_0\) and \(P_1\), (iii) low dead space (iv) low Reynolds number, (v) no abrupt changes between shutter and wave tube, and (vi) no tubing between pressure transducer and wave tube. We optimised the wave tube to have a length of 13.3 cm and a radius of 0.5 cm. The pressure transducer \(P_1\) was placed 3.3 cm from the facemask, \(P_0\) was placed 10 cm from the face mask. Including the interrupter device and the
connector, which had identical internal radius of 0.5 cm, the total length was 14.5 cm from the valve to the face mask. We will discuss (i)-(vi) based on these dimensions.

(i) Maximal equipment dead space for infants has been defined in recent guidelines for infant lung function equipment (Frey et al. 1999 (2)) worked out by the ERS-ATS task force on standardisation of infant lung function equipment (ERS: European Respiratory Society, ATS American Thoracic Society). These guidelines recommend equipment resistance for infants and young children to be less than 0.5 kPa L^{-1} s at 300 mL s^{-1} because higher resistances change the breathing pattern of the infants. Our equipment (interrupter valve and wave tube) had a resistance of 0.12 kPa L^{-1} s at 300 mL s^{-1}. We also plotted the Poiseuille Resistance ($R_{poi}$) of the wave tube as a function of radius to find the optimal radius. (figure 5.5.5.).

(ii) The optimal distance between $P_0$ and $P_1$ determined the impedance of the wave tube between $P_0$ and $P_1$. Since this impedance is frequency dependent we determined the optimal distance empirically by measuring known load impedances. If the distance were too short the signal to noise ratio, particularly of the flow, would be insufficient and coherence would decrease below acceptable levels. Regarding maximal length of the wave tube, it has to be considered that the half-wave resonance of the open end wave tube should be substantially higher than the highest frequency at which $Z_{in}$ is estimated. This is important, because at frequencies around this half wave resonance, two pressure nodes would fall on the location of the transducers. We have chosen a values that were close to the dimensions proposed by Habib et al. (1994).

(iii) Infants have a tidal volume of 5-10 mL/kg body weight. Large dead space volume causes CO$_2$ re-breathing and disturbance of the breathing pattern. The ERS/ATS guidelines recommend a maximal dead space of 1.5-2 mL kg$^{-1}$ for a device that is used intermittently. We have calculated the dead space of the equipment as a function of radius (figure 5.5.5.).

dead space. Our wave tube had a volume of 20 mL corresponding to the tolerated dead space of infants of more that 1 year of age. If we used the technique in infants under 1 year, we had to frequently take the face mask off the face to avoid re-breathing. Lower dead space was not compatible with the other requirements of the equipment.

(iv) The Reynolds number of the wave tube characterises the DC flow behaviour of the tube. Reynolds numbers (Re) > 2000-2300 would indicate turbulent flow in the wave tube. The Reynolds number is defined as:

\[
Re = \frac{\delta \times V \times 2\pi r}{\eta}
\]

Where $\delta$ is the density of air, $V$ the mean velocity and $\eta$ the dynamic viscosity of air. We plotted the Reynolds number of the wave tube as a function of the radius of the tube, for flow
expected during tidal breathing in infants (<100 mL/s) (figure 5.5.5.). The Reynolds number for a flow of 100 mL/s was 836 (not accounting for entrance effects). For a wave tube with a radius of 0.5 cm, we also calculated the Reynolds number as a function of flow (figure 5.5.6.). The aspect ratio was 28 and the entrance length was 4.5 cm. Both values are small and both effects could disturb the flow pattern in the wave tube. Thus, in order to test whether the unidirectional flow components influence the impedance measurements, we firstly connected a known load resistance to the wave tube and measured $P_1$ and $P_0$ at flows from 33 mL/s to 167 mL/s. Flow was feed into the wave tube from its distal end. This resistor has been validated in previous work (Frey et al. 1999 (3)). This particular resistor was made of pressed metal powder forming a screen resistor with nearly linear flow properties over the flow range mentioned above. Figure 5.5.7. shows $P_0$ and $P_1$ as a function of flow under two conditions: the wave tube alone and the wave tube connected to the interrupter device. It can be seen that $P_0$ and $P_1$ measure identical pressures under all flow conditions. If there was large turbulence, $P_0$ and $P_1$ would not read the same values. We examined the same situation, during additional oscillatory flow. We measured high frequency impedance of this resistor screen under at various flow ranges (33, 67, 100, 133, 167 mL/s). In figure 5.5.8. $Z_{in}$ from 32 to 1000 Hz are plotted for each flow. It can be seen that uni-directional flow did not influence the impedance measurements. We also used a 30 cm tube as a load impedance and measured $f_{ar,1}$ which occurred at the predicted value of 282 Hz at all flows. This very interesting finding indicates that $Z_{in}$ measurements using the HIT are robust to flow. This is in contrast to the work of Dorkin et al. (1982) where they showed that superimposed oscillatory flow is influenced by unidirectional flows in tubes if the Womersley number $\alpha > 2.5$ (see section 3.3.6). Our findings can only be explained by the fact that during airflow interruption there is no DC-flow component but only oscillatory flow within the tube. This might be an additional advantage of the HIT, that DC-flow components play no role, which would make the technique even more suitable for tidal flow measurements.

(v, vi) To keep the flow in the measurement device as steady as possible, the connector and the interrupter were built such that the internal diameter of the tube was always 0.5cm and had no discontinuities. Furthermore, we built the pressure transducers directly into the tube walls. Tubing between the pressure transducers and the wave tube drastically decreased the coherence of the measurements. Similar behaviour was found when the pressure transducers reached into the lumen of the wave tube.
5.1.6. The role of the coherence function in the high speed interrupter technique

The coherence function $\gamma^2$ (see section 3.3.5.) is an number between 0 and 1 similar to a correlation coefficient and provides an index of (i) causality between the input and output of a linear system. The value of $\gamma^2$ is less than 1 if the output is (ii) the result of more than one input, is the (iii) system is non-linear, or the system is (iv) contaminated by extraneous noise. Using $\gamma^2$ confidence limits for spectral estimates can be obtained. This is particularly important for the high speed interrupter technique:

(i) The amplitudes in the power spectrum of a step function decrease as a function of $1/f$ with increasing frequency. Thus, the signal to noise ratio of the pressure measurements worsens with increasing frequency. Subsequently, $\gamma^2$ is decreased at high frequencies and provides an excellent estimate quality control of the measurements. Furthermore, if the maximal amplitude of the pressure in the wave tube was $<1.5 \, \text{cm H}_2\text{O}$, signal to noise ratio was low and the coherence decreased below 0.9.

(ii) Since the lowest frequency measured by the high speed interrupter technique was 32 Hz, there is no potential contamination of the impedance spectrum by cardiac noise, as the maximal heart rate in infants is $<3$ Hz. However, secretions in the upper airways or snoring caused vibrations between 10 and 60 Hz with a subsequent rapid decrease in the coherence function below 0.9. While such vibrations were clinically often not detectable, the decrease in coherence provided a reliable means of detecting these artefacts.

(iii) Non-linear behaviour of the load impedance (the respiratory system) is a particularly delicate problem if the high speed interrupter technique is used. Non-linear behaviour can be induced if the respiratory system is forced (input function) by high amplitude pressures. This is less of a problem with the forced oscillation technique, because the technique controls the input energy at each frequency by using a pseudo-random noise input pressure signal. However, this may be the case with the interrupter technique where the inspiratory flow and the impedance of the respiratory system determine the pressures in the wave tube at every frequency. By keeping the pressure amplitudes at each frequency sufficiently low, non-linear behaviour of the respiratory system should be avoided. For the high speed interrupter technique we had to limit the flows to $<100 \, \text{mL/s}$ (see Reynolds numbers in section 5.1.5.) and pressures to $<10 \, \text{cm H}_2\text{O}$ in the wave tube. These values have been derived from empirical observations while at higher flows or pressures respectively the coherence decreased $<0.9$. Non-linearities occur predominantly around anti-resonances where abrupt changes in the impedance spectrum of the respiratory system occur. We particularly found decreased
coherence values around anti-resonances. Again the coherence function provided an excellent means for data inclusion criteria (coherence >0.9).

(iv) Extraneous noise came mostly from vibrations or electrical noise from the stepper motor driving the slide valve. By using co-axial cables for the pressure transducers, we were able to significantly reduce the electrical noise levels. However, it was more difficult to dampen the vibrations of the motor. Since the interrupter was constructed such that the slide valve had a low mass, acceleration and stopping of the valve caused only little vibration. However, if the system was not hand-held but connected to a rigid supporting arm, vibrations were amplified by resonances of these supporting structures. In this case extraneous noise levels were high and coherence decreased significantly.

In summary, the coherence function is an important marker of the quality of the data and non-linear behaviour of the respiratory system due to high pressure or high unsteady flow. It should always be used with the interrupter technique.
5.1.7. Analysis of the nature of high frequency impedance data in human infants

From high frequency impedance measurements in human adults and in dogs there are two possibly explanations for the occurrence of anti-resonances in the Zin spectrum. They either occur due to the lumped interaction of lung tissue inertance and gas compression compliance as in dogs or they are due to wave propagation phenomena as in human adults (Jackson et al. 1989). In the first case information regarding airway and tissue resistance can be derived from these data using lumped parameter models. In the second case anti-resonances contain information of airway wall mechanics. Since the nature of anti-resonances is not yet resolved in infants we try to elucidate high frequency Zin data by performing Zin measurements using gases of different densities as proposed by Jackson et el (1989) (see 6.3). Furthermore we model high frequency Zin data in infants using system identification techniques based on lumped DuBois models (see 6.1).
5.2. Calibration of the measurement devices

5.2.1. The rapid thoracic compression technique

The linearity of the VALIDYNE pressure transducers (NORTHRIDGE, CA) was tested on a monthly basis and estimated to be accurate within 2%. Prior to each measurement, after warming up of the equipment for 30 minutes, the offset of pressure and flow transducers were reset electrically. The gain of the pressure transducers in the jacket was calibrated using a water manometer. The working range was set to 0 – 100 cm H₂O (0-10 kPa). The FLEISCH No 1 pneumotachograph (VALIDYNE MP45, NORTHRIDGE, CA) was calibrated using a 'mechanical flow meter'. After each set of measurements the calibrations were checked whether there have been changes in gain or offset during the measurements.

5.2.2. The forced oscillation technique

High frequency respiratory impedance was measured using the wave tube technique in combination with the forced oscillation device. Therefore both oscillatory pressure and flow were calculated using two pressure transducers (P₁ and P₀), which had to be calibrated. Prior to each measurement, after warming up the equipment for 30 minutes, the offset of both pressure transducers (EUROSENSOR, MODEL 33, UK) was reset electrically. The gain of the pressure transducers in the jacket was calibrated using a water manometer. The working range was set from -10 to 10 cm H₂O (-1 to 1 kPa). To optimise the AD-board range, we have set the gain of the pressure transducers to 0.1 kPa = 1 Volt. In order to match frequency response of two pressure transducers a loudspeaker plethysmograph was used (see section 5.1.2.). P₁ and P₀ were matched (within ± 2%) in frequency domain from 32 to 1500 Hz.

5.2.3. The high speed interrupter technique

High frequency respiratory impedance was measured using the wave tube technique in combination with the high speed interrupter device (see 5.1.4 and 5.1.5.). Both oscillatory pressure and flow were calculated using two calibrated pressure transducers (P₁ and P₀). This made the calibration of the HIT technique relatively easy. We had only to ensure that the two pressure transducer were linear and matched in gain and offset, and ensure that the frequency response of both pressure transducers was similar.

**Linearity.** The equipment allowed the sampling of the pressure channels in DC-mode for calibration and in AC-mode for frequency response check and measurements. For offset, gain and linearity check, we switched the pressures into DC mode. Prior to each measurement,
after warming up the equipment for 30 minutes, the offset of both pressure transducers (EUROSENSOR, MODEL 33, UK) was reset electrically. The gain of the pressure transducers in the jacket was calibrated using a water manometer. The working range was set from -10 to 10 cm H2O (-1 to 1 kPa). To optimise the AD-board range, we have set the gain of the pressure transducers to 0.1 kPa = 1 Volt. The linearity of both solid state piezo-resistive pressure transducers (EUROSENSOR, MODEL 33, UK) was tested prior to each measurement by exposing both pressure transducers to identical pressures monitored by a water manometer (from -0.1 to 1 kPa in steps of 0.01 kPa). Using a special calibration program (Labview for Windows) both pressure channels were displayed in the same graph, and discrepancies in linearity were detected with a high degree of sensitivity. A representative linearity check is shown in figure 5.5.9.

Frequency response of the pressure transducers was checked by a newly developed calibration device (figure 5.5.10.). Constant flow of 0.05 L/s was blown through the interrupter device and through a calibration tube of 12 cm length and 0.5 cm radius. There were no edges or abrupt changes in the internal diameter, so that there was little risk of turbulence. The two pressure transducers were placed into the calibration tube wall, opposite each other, and were therefore exposed to the same pressure. Five interruptions were performed and the pressure-pressure ratio of P1 and P0 was calculated. Figure 5.5.11. shows a pressure-pressure ratio (see sections 3.3.4 and 3.3.5) of a representative calibration. The calibration was accepted if the P1/P0 ratio was within 2% (real part: range 0.98-1.02) from 32 to 1500 Hz.
5.3. Subjects

5.3.1. Inclusion criteria

The study was performed in infants and young children (aged 36-81 weeks) with a history of episodic or recurrent cough or wheeze, who had been referred from the outpatient clinic for lung function tests for clinical purposes. Infants with other specific diseases and infants with upper respiratory tract infection within the previous 3 weeks were not included in the study. Similarly measurements were done in healthy infants of similar age. Details of study groups are specified in section 6.

5.3.2. Ethics, consent

All the lung function measurements were approved by the Ethics Committees of the Royal Postgraduate Medical School, Hammersmith Hospital, London or the Leicestershire Health Authority, Leicester, UK where the measurements were performed. Written consent was obtained from parents.

5.3.3. Preparation, sedation of subjects

Most infants and toddlers were sedated using a single oral dose of triclofos sodium up to 150 mg/kg. Other infants were studied during natural sleep. Lung function was measured during periods of regular quiet breathing in the supine position. The head position was standardised as described by Desager et al. (1991), but we did not tape the mouth for safety reasons. The head position was strictly standardised and not changed during the measurement procedure. The head was put into midline position. We did not allow the head to be turned more that 45° to the lateral side. Furthermore, the head was place onto a plane surface or we allowed a neck hyperextension of max -20°. The latter condition corresponded to an elevation of the shoulders of maximal 2 cm. We strictly omitted any neck flexion because of possible longitudinal tension of the trachea, as described in detail in section 3.2.5.

A firm face mask which covered the nose and the mouth was applied using a putty ring (THERAPEUTIC PUTTY, CARTER’S, BRIDGEND, UK). The dead space in the face mask was reduced by partially filling it with putty. For 19 subjects of the subjects it was possible to reliably measure the residual face mask dead space by water displacement. The mean dead space volume (SD) was 6.12 (SD1.7) ml, which is equal to about 2-5% of the total lung volume.
5.3.4 Safety aspect

Electrical safety of the prototype lung function equipment was tested by the Hammersmith Hospital Medical Physics Department and the Medical Physics Department of Leicester University. To optimise electrical safety, the prototype lung function devices were separated from the power supply by an isolation transformer which was built at the Dept. of Medical Physics, Leicester University. The hand held equipment was only used by well trained supervised technicians in a hospital environment. Written manuals of the equipment were used to calibrate and to perform the measurements. All measurements were performed in the presence of a well trained medical doctor. During all measurements transcutaneous PO$_2$ (TMC3, RADIOMETER, COPENHAGEN) and transcutaneous saturation S$_{tc}$O$_2$ (BIOX 3740, OMEDA, OMAHA, NE) were observed.

5.4. Data handling

All measured data were encoded using a study code. The study code was stored in a database located at the lung function lab and only accessed via a password though the study crew. Data were doubly backed up using a photo-optical disk drive. These photo-optical disk drives were locked in the infant lung function lab.
5.5 Figures and Tables

Figure 5.5.1:
Setup of the forced oscillation technique, the patient is connected to the experimental outlet via a face mask.

Figure 5.5.2:
In the conventional interrupter technique, the expiratory airflow is interrupted for 100 ms and airway opening pressure is measured. From preceding flow and the measured mouth pressure drop a Newtonian interrupter resistance can be calculated.

Figure 5.5.3:
(a) Pressure recordings (P₁ and P₀) of an impedance measurement in a healthy human subject as a function of time. (b) Pressure difference (P₁ - P₀) in the wave tube as a function of time.

Figure 5.5.4:
Normalised power spectral density of the two pressure measurements (corresponding to figure 5.5.3.) (P₁ (dotted line) and P₀ (solid line)) as a function of frequency. It can be seen that both pressure channels have similar power spectra, however, prior to normalisation, P₁ had a higher amplitude than P₀ because P₁ is closer to the patient.

Figure 5.5.5:
Simulation of the Poiseuille resistance (Rpoi) in a wave tube of 14.5 cm length as a function of radius (corresponds to setup used for infants). Similarly the Reynolds number (at a flow of 100 mL/s) and the dead space were simulated as a function of radius. During quiet tidal breathing, the flow of infants does not exceed 100 mL/s. It can be seen that a radius of 0.5 cm is optimal in terms of dead space, tube resistance and Reynolds number.

Figure 5.5.6:
Reynolds number of the wave tube (radius 0.5 cm) as a function of flow. It can be seen that critical Reynolds numbers (>2000) occur at flows > 240 mL/s, which are higher than normal tidal breathing flows in infants.

Figure 5.5.7:
A known load resistance (metal powder screen resistor) was connected to the wave tube and the pressures P₁ and P₀ were measured at flow rates ranging from 33 to 167 mL/s. Both pressure transducers measured identical values, whether or not the interrupter valve was connected between the flow source and the wave tube. The leak behaviour of the interrupter
device caused a decrease of both pressures. However, this is not relevant for the impedance measurements.

**Figure 5.5.8:**

Impedance measurements of a known load impedance (screen resistor see figure 5.5.7.) between 32 and 1000 Hz at flow rates of 33 to 167 mL/s. Between 600 and 1000 Hz, impedance showed large variability due to the physical properties of the test device. In this frequency range the coherence values decreased <0.95 at single frequency points which were excluded from the measurements. It can be seen that there was no systematic difference in impedance between the five measurements at different flow rates.

**Figure 5.5.9:** Representative linearity check of the two pressure transducers; $P_1$ is plotted versus $P_0$.

**Figure 5.5.10:**

In order to calibrate and compare frequency response of the two pressure transducers, we designed a calibration device, in which both pressure transducers are exposed to the same pressure step function induced by the high speed interrupter device.

**Figure 5.5.11:** Pressure pressure ratio of $P_1/P_0$ as a function of frequency measured with the calibration device (figure 5.5.10.). It can be seen that at all frequencies between 32 and 1500 Hz the real part was close to 1 (within 0.98-1.02).
Figure 5.5.1:
Figure 5.5.2:

- **INTERRUPTION**
- post-occlusional oscillatory pressure transients
- stress adaptation, Pendelluft
- initial rapid pressure change

Airway opening pressure (pao) (cm H$_2$O)

Time (ms)
Figure 5.5.3a:

- Pressure $P_0$ (cmH$_2$O/L·s)

- Pressure $P_1$ (cmH$_2$O/L·s)

Time (s)
Figure 5.5.3b:
Figure 5.5.4:
Figure 5.5.5:

Reynolds number at 100mL/s flow

Rpoi

deadspace

radius (m)
Reynolds number

Figure 5.5.6:

A graph showing the relationship between Reynolds number and flow (mL/s). The graph is a straight line indicating a linear relationship. The x-axis represents flow (mL/s) ranging from 0 to 400, and the y-axis represents Reynolds number ranging from 0 to 3500.
Figure 5.5.7:

- $p_0$ with interrupter valve
- $p_1$ with interrupter valve
- $p_0$ without interrupter valve
- $p_1$ without interrupter valve

Pressure (cmH$_2$O/L^-1s) vs. Flow (mL/s)
**Figure 5.5.8:**

- **Real part of $Z$ (cmH$_2$O L$^{-1}$ s)**

- **Imaginary part of $Z$ (cmH$_2$O L$^{-1}$ s)**

Graphs showing the variation of real and imaginary parts of $Z$ with frequency for different flow rates: 33 mL/s, 67 mL/s, 100 mL/s, 133 mL/s, and 167 mL/s.
Figure 5.5.9:

A graph showing the relationship between pressure $P_0$ (cm H$_2$O) and pressure $P_1$ (cm H$_2$O). The graph is a straight line with points plotted at various values. The x-axis represents $P_0$ and the y-axis represents $P_1$. The range of values for $P_0$ is from -10 to 10 cm H$_2$O, and for $P_1$ is from -10 to 10 cm H$_2$O.
Figure 5.5.10:

- Constant calibration flow of 0.05 L/s
- Stepper motor
- Photo-optic resistor
- Slide valve
- Open outlet
- Calibration tube
- Pressure transducers

$P_0$ $P_1$
Figure 5.5.11:

Graph showing the real part and imaginary part of the ratio $(P_1 - P_2)$ against frequency (Hz). The real part starts around 1.00 and remains relatively constant, while the imaginary part decreases linearly from 0.00 to -0.10 as the frequency increases from 0 to 1600 Hz.
6. SPECIFIC ASPECTS OF MEASUREMENT TECHNIQUES, PHYSIOLOGICAL AND CLINICAL APPLICATION


6.1.1. Abstract

Measurements of respiratory input impedance (Zin) in infants using forced oscillations at the airway opening up to 256 Hz have been shown to include a first anti-resonance (ar,1). We wondered whether features derived from high frequency Zin change during methacholine-induced airway obstruction in infants, whether these changes could be explained by a lumped parameter model as in dogs (providing a value for respiratory resistance (Rrs)) or whether they behave similarly to Zin data in human adults which are related to wave propagation in the airways. In 13 infants (age 58 ± 19 weeks) Zin(ω) was assessed at baseline and in 9 infants after methacholine challenge using a provocation dose defined by > 30% fall in VmaxFRC (rapid thoracic compression technique). Following methacholine challenge VmaxFRC decreased significantly (p<0.0005), the frequency at which ar,1 occurred (far,1) increased significantly (p=0.0007), the relative maximum in the real part at far,1 (Zinre(far,1)) increased significantly (p=0.02) whereas Rrs did not change. We conclude, that in infants ar,1 is highly sensitive to changes in lung mechanics. While ar,1 cannot be explained based on a simple lumped parameter model, it is more likely due to wave propagation phenomena as in human adults. In either case, far,1 potentially contains information about airway wall compliance, which is important for the understanding of flow limitation in infant wheezing disorders.
6.1.2. Introduction

In this first study we investigated the use of the forced oscillation technique for high frequency impedance measurements in infants. Few studies have reported Zin measurements in infants (Marchal et al. 1988 (2x), Desager et al. 1991, Sly et al. 1996, Jackson et al. 1996). Sly et al. (1996) reported Zin measurements in healthy infants at low frequencies (0.2 - 20 Hz) during reflex induced apnoea. Desager et al. (1991) and Marchal et al. (1988) reported Zin between 6 and 48 Hz during tidal breathing in wheezy and healthy infants, respectively. Finally, Jackson et al. (1996) measured Zin between 20 and 256 Hz in healthy infants. However, in none of these studies were the Zin measurements made following induced airway constriction or dilation, whereby the change in airway mechanics was verified by a reference lung function technique.

This study aimed to demonstrate that changes in Zin measured over a wide range of frequencies, were sensitive to alterations in airway mechanics in infants. We used methacholine challenge to induce changes in airway mechanics and verified these by a reference lung function technique. Secondly, we wished to determine whether the induced changes in Zin were similar to those found in adults with airway obstruction. This would imply that similar analysis techniques could be applied to Zin data from infants in order to extract relevant physiological parameters using system identification techniques.

Unlike feature analysis, systems identification techniques provide estimates of physiological parameters which is accomplished by fitting a model to the Zin data. One such model, the DuBois 6-element model (Dubois et al. 1956) (Fig. 6.1.6.1a) provides separate estimates of airway and tissue resistance (Raw, Rti), as well as thoracic gas volume (Vtg). However, this model can be used only if the Zin data include an anti-resonance that is related to the issue inertance (Iti) and the alveolar gas compression compliance (Cg). There is such an anti-resonance in dogs (Jackson et al. 1987, Lutchen et al. 1987, Jackson et al. 1991) and rabbits but not in adult humans (Jackson et al. 1989). Instead, the anti-resonances in adults are due to wave-propagation phenomena and are thus related to inertance of the gas within the airways and the compliance of the airway walls (Jackson et al. 1989). Since the anti-resonances are wave propagation related, estimates of Raw and Vtg are not possible in human adults but inferences about airway wall properties are possible (Jackson et al. 1989, Chalker et al. 1992). It has only recently been shown that there is an anti-resonance in healthy infants (at approx. 120 Hz) (Jackson et al. 1996). Even though, it is not clearly understood what phenomena contribute to this anti-resonance in infants, preliminary results indicate that it is related to the
total respiratory system inertance (Irs) and at least partly due to the gas compression compliance in the face mask (Sly et al. 1996). A model was suggested (Jackson et al. 1996) (Fig 6.1.6.1b) for analysing infant Zin data which according to computer simulations results in an estimate of total respiratory system resistance (Rrs) that may be related to airway calibre.

Based on these theoretical considerations in section 3.3.2 and 3.3.3, the goals stated above can be described in more detail. We were interested in determining whether induced airway obstruction resulted in increased frequency dependence in the real part of Zin and changes in the frequency of the resonance and/or anti-resonance. Questions related to model analysis were whether the method suggested by Jackson and co-workers (Jackson et al. 1996) provides an estimate of Rrs that is a sensitive indicator of changes in airway calibre.
6.1.3 Methods

Subjects. The study was performed in 13 infants and young children (6 girls, 7 boys) (age 26 to 149 weeks) with episodic or persistent cough or wheeze, who had been referred from the outpatient clinic for lung function tests (Table 6.1.7.1). Infants with other specific diseases and infants with upper respiratory tract infection within the previous 3 weeks were not included in the study. The patients were sedated using a maximum dose of 150 mg/kg triclofos sodium and lung function was measured during behaviourally defined quiet sleep. The protocol was approved by the Ethics Committee of the Royal Postgraduate Medical School, Hammersmith Hospital, London, where the study was carried out. Written consent was obtained from the patient's parents.

Forced oscillation technique for infants (FOT). Zin was measured using loud-speaker generated, low amplitude pressure oscillations applied to the airway opening via a latex face mask (RENDELL BAKER SOUCEK, SIZE 1, AMBU INTERNATIONAL). The setup and its calibration has been described previously (Clarke et al. 1994). A pseudo-random noise signal containing frequencies between 2 and 256 Hz in 2 Hz increments, was generated by computer and output via a digital-to-analog converter. Zin was measured using the wave tube technique described in detail in section 3.3.4 and 5.1.5. P₁ and P₀ were measured with piezo-resistive solid state pressure transducers that were matched within ± 2% in magnitude and ± 2° of phase (EUROSENSOR, Model 33, UK). The electrical output of these transducers was band pass filtered (8-2000 Hz, 4th order Butterworth filter), amplified, and analog/digital converted at 2048 Hz (METRABYTE Model DAS 16/16F, US). The ratio of P₁ /P₀ was estimated from the cross power spectra of P₀P₁ and the auto power spectra of P₁. The coherence function was computed using the method of Michaelson et al. (Michaelson et al. 1975). The wave-tube method of measuring Zin has the advantage since flow is not measured directly but is inferred by two pressure measurements made by transducers whose frequency response is good to high frequencies. When flow is measured using a pneumotachometer care must be taken to compensate for the frequency response characteristics of the pneumotachometer and there is an upper frequency limit where this can be done accurately (Renzi et al. 1990). The wave-tube technique does, however, have the disadvantage since accurate and reliable measurements at low frequencies become difficult. With the particular tube used in this study, we were unable to measure Zin reliably below 16 Hz (Franken et al. 1981).

The rapid thoracic compression technique (RTC). The RTC has been described in the sections 3.2.3., 5.1.1. and 5.2.1. From the 10 forced expiratory maneuvers we calculated the mean and
standard deviation of $V_{\text{max}}^{\text{FRC}}$ and related the values to the reference values of Tepper et al. (1986).

**Experimental protocol.** Measurements were made with the infant in the supine position. The general measurement conditions and the monitoring were described in the sections 5.3.3. and 5.3.4. The head position was not changed between measurements using the different techniques or subsequent methacholine challenge. In order to achieve a complete seal and to reduce gas volume, the face mask was filled with putty (THERAPEUTIC PUTTY, CARTERS, BRIDGEND, UK). During quiet regular tidal breathing, 2 sets of 8 $Z_{\text{in}}(\omega)$ measurements were performed using the forced oscillation technique, each set required 5 sec. The second set was taken in order to estimate the reproducibility of the $Z_{\text{in}}$ measurements. Thereafter, the inflatable jacket was wrapped around the infants chest and 10 forced expirations were performed at baseline.

**Methacholine challenge test:** In 9 of the 13 infants a methacholine challenge test was performed as described previously (Clarke et al. 1994) using cumulative doses of methacholine (saline, 0.5, 1, 2, 4, 8, 16, 32 and 64 g/l). At each provocation dose, 5 $V_{\text{max}}^{\text{FRC}}$ were performed and averaged. Once a provocation dose resulted in a decrease of $V_{\text{max}}^{\text{FRC}}$ of 30% (i.e., the PC30), no higher concentrations of methacholine were given and a final set of $Z_{\text{in}}$ measurements was taken. $Z_{\text{in}}$ was not measured at intermediate steps. As already stated, head position and face mask were not changed during, nor between the tests. We kept the sequence of measurements constant (FOT-FOT-RTC-Challenge-RTC-FOT), because putting and removing the squeeze jacket can disturb the child. Using this sequence we had to put on the jacket once and remove it once.

**Data analysis of spectral features.** Four parameters from the $Z_{\text{in}}$ spectra related to airway obstruction were analysed: the resonant ($f_r, 1$) and the anti-resonant frequencies ($f_{ar}, 1$), the relative maximum in the real part at $f_{ar}, 1$ ($Z_{\text{in},re}(f_{ar}, 1)$) and the frequency dependence of $Z_{\text{in},re}$ at $f < f_r, 1$. The latter parameter was quantified by the slope $\alpha$ of a linear regression between 16 Hz and 30 Hz as well as between 16 Hz and $f_r, 1$ ($\alpha_{30}$ and $\alpha_{f_r, 1}$, respectively). Each of these 4 parameters was assessed from the mean of 8 single runs. The short term repeatability of these 4 parameters was estimated by the standard error of the mean of the differences between the first and second parameter derived from the two sets of baseline $Z_{\text{in}}$ measurements. The reproducible parameters were displayed in Bland Altman plots. To estimate the change in the parameters derived from $Z_{\text{in}}$ during the bronchial challenge tests,
we compared the first measurement set at baseline to the post challenge set using paired t-tests.

**Data analysis using systems identification technique.** The $Z_{\text{in}}(\omega)$ data were analysed using a lumped 4-element model (Fig. 6.1.6.1b) as proposed by Jackson et al. (1996). The parameters in this model were estimated by minimising the following performance index, P.I., given by:

\[
P.I. = \frac{\sum_{i=1}^{n} |Z_{\text{rd}}(f_i) - Z_{\text{rs,m}}(f_i, \Theta)|^2}{|Z_{\text{rd}}(f_i, \Theta)|^2}
\]

where $n$ = the number of data points, $Z_{\text{rs,d}} = Z_{\text{in}}$ data at a given frequency $f$ and $Z_{\text{rs,m}}$ = model predicted $Z_{\text{in}}$ data at a given frequency using the model parameters. The model parameter of particular interest as a possible index of airway calibre was $R_{\text{rs}}$ (fig. 6.1.6.1b). Similarly, we calculated short term repeatability of $R_{\text{rs}}$ using the standard error of the mean differences and we estimated the change of $R_{\text{rs}}$ after bronchial challenge using a paired t-test.
6.1.4. Results

Reference lung function technique. At baseline the mean (± SD) $V_{\text{maxFRC}}$ was found to be $196 ± 93 \text{ mLs}^{-1}$ which corresponds to $64 ± 27\%$ of predicted values (Tepper et al. 1993). In the subgroup of 9 infants who had a challenge test, $V_{\text{maxFRC}}$ decreased significantly from $234 ± 97 \text{ mLs}^{-1}$ to $125 ± 69 \text{ mLs}^{-1}$ ($p<0.0005$) confirming that a significant change in airway mechanics was induced by the methacholine challenge test.

Description of Zin data. In all subjects the coherence was $>0.95$ within the frequency range 16 to 256 Hz. At baseline $Z_{\text{in}}(\omega)$ spectra in all 13 infants showed a first resonant frequency ($f_{r,1}$) at $37 ± 9$ Hz and a first anti-resonant frequency ($f_{ar,1}$) at $155 ± 28$ Hz (Fig. 6.1.6.2). The relative maximum of $Z_{\text{inre}}$ at $f_{ar,1}$ ($Z_{\text{inre}}(f_{ar,1})$) was found to be $51 ± 10 \text{ cm H}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}$. Most of the infants showed a certain degree of frequency dependence of resistance at frequencies below $f_{r,1}$. The frequency dependence of resistance was $-0.25 ± 0.35 \text{ cm H}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s Hz}^{-1}$ between 16 and 30 Hz and $-0.21 ± 0.27 \text{ cm H}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s Hz}^{-1}$ and between 16 Hz and $f_{r,1}$.

The standard error of the mean differences in the spectral features for $f_{r,1}, f_{ar,1}$ $Z_{\text{inre}}(f_{ar,1}) \alpha f_{r,1}$ and $\alpha 30\text{Hz}$ as a measure of short term repeatability were $3.03 \text{ Hz}$, $2.64 \text{ Hz}$, $1.78 \text{ cm H}_2\text{O}$ L$^{-1}$ s, 0.07 and 0.09, respectively. Bland Altman plots are only presented (Fig. 6.1.6.3) for the parameters $f_{ar,1}$ and ($Z_{\text{inre}}(f_{ar,1})$), which we found to be sensitive to changes in airway mechanics (see below). These findings indicate a relatively good short term repeatability for all parameters. The repeatability of $Z_{\text{inre}}(f_{ar,1})$ in a single subject (Fig. 6.1.6.3*) was poor. This subject was an infant with recurrent wheeze since birth, and who suffered from oligohydramnios antenatally and who was suspected of having lung hypoplasia.

Lumped 4- element model. An example of an impedance spectrum from 16 to 256 Hz in one infant and the fit of the 4-element model are shown in figure 6.1.6.4. In fitting the Zin data with this model, data at frequencies where the real part of Zin had a negative frequency dependence were eliminated ($f < 27 ± 9$ Hz). In one infant Zin data at $f > 220$ Hz could not be followed by the model, so were not used in the system identification process. In all other infants, the model fitted the data reasonably well up to 256 Hz. From the first set of Zin measurements for the whole group, the mean Rrs was found to be $20.6 ± 4.7 \text{ cm H}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}$, $C_{rs} 1.87 \times 10^{-3} ± 2.42 \times 10^{-3} \text{ L cm H}_2\text{O}^{-1}$, $I_{rs} 0.025 ± 4.5 \times 10^{-3} \text{ H}_2\text{O}\cdot\text{L}^{-1}\cdot\text{s}^2$ and the face mask
shunt compliance $C_m$ was $2.2 \times 10^{-5} \pm 1.1 \times 10^{-5}$ L cm H$_2$O$^{-1}$ (equivalent to a face mask dead space volume of 21 mL). The standard error of the mean of the differences between $R_{rs}$ from the first and the second $Z_{in}$ measurement was found to be 2.32 cm H$_2$O L$^{-1}$ s.

*Changes of $Z_{in}$ and RTC derived parameters during methacholine challenge.* At baseline, there was a tendency for $f_{r,1}$ to be higher and $f_{ar,1}$ to be lower if $V_{maxFRC}$ was low, however the correlations were not significant. There was no correlation between $\alpha$, $R_{rs}$ and $V_{maxFRC}$ under baseline conditions for the group of 13.

A representative example of $Z_{in}$ at baseline and after methacholine challenge is shown in figure 6.1.6.5 for one subject. Both frequency ($f_{ar,1}$) and the relative maximum in the real part ($Z_{inre}(f_{ar,1})$) of $Z_{in}$ increased following methacholine challenge. On average $f_{ar,1}$ increased significantly ($t=5.34$, $p = 0.007$) from 148 ± 26 to 203 ± 15 Hz (Fig. 6.1.6.6). $Z_{inre}(f_{ar,1})$ also changed significantly for the whole group ($t = 3.0791$, $p = 0.015$) from 51 ± 10 to 70 ± 15 cm H$_2$O L$^{-1}$ s, but as seen in figure 6.1.6.6, $Z_{inre}(f_{ar,1})$ did not change in 3 of the infants.

The frequency dependence of resistance represented by $\alpha_{30Hz}$ (post challenge: -0.27 ± 0.34, $p=0.28$) and $\alpha_{f_{r,1}}$ (post challenge: -0.22 ± 0.23, $p=0.34$) as well as the resonant frequency $f_{r,1}$ (post challenge 38 ± 8 Hz, $p=0.77$) showed no significant change during methacholine challenge. None of the respiratory system parameters ($R_{rs}$, $C_{rs}$, $I_{rs}$) derived from the system identification technique using the 4-element parameter model changed significantly following methacholine ($t = 0.05$, $p = 0.95$) (Fig. 6.1.6.7).
6.1.5. Discussion

Zin measurements are typically reported over one of two frequency ranges; a low frequency range \((f < \text{about 2-10 Hz})\) where tissue properties dominate the impedance spectrum \((\text{Sly et al. 1996, Hantos et al. 1986, Lutchen et al. 1993})\) and a higher frequency range where tissue properties become less important and airway calibre as well as airway wall properties become more influential \((\text{Jackson et al. 1987, 1989, 1991, 1993, Lutchen et al. 1987, Habib 1994 (2x)})\). Zin measurements at the low frequency range are potentially useful as a means of measuring the mechanical properties of the respiratory tissues, lung and chest wall. Furthermore as argued by \text{Sly et al. (1996)} parameters derived from Zin measurements at these lower frequencies may be more pertinent to conditions of natural breathing. However, the goal of the current study was not to investigate the behaviour of the infant respiratory system under conditions of natural breathing but to test the hypothesis that Zin measurements well above 2 Hz are sensitive to changes in airway calibre and to changes in airway wall properties in infants. Zin measurements have been reported in infants \((\text{Marchal 1988 (2x), Desager et al 1991})\) but not to high enough frequencies to reveal an anti-resonance where Zin is thought to be sensitive to airway wall properties. It has also not been shown that Zin is sensitive to changes in airway mechanics by comparing values before and after induced bronchial obstruction, which was verified with an independent lung function measurement. We report here for the first time Zin in infants to frequencies that include an anti-resonance during baseline conditions and following broncho-constriction induced by methacholine.

Comparisons of our results with other studies reporting Zin in infants are not straightforward because of differences in technique, frequency range and patient population. However, our Zin measurements are qualitatively similar to those reported in the literature. In most of our subjects, the real part showed negative frequency dependence at frequencies below \(f_{r,1}\). \text{Desager et al. (1991)} and \text{Marchal et al. (1988)} found similar frequency dependence but \text{Jackson et al. (1996)} did not. These discrepancies could be due to differences in the patient populations since frequency dependence was seen in the studies which included subjects with wheezing disorders \((\text{Marchal et al. 1988, Desager et al. 1991})\) whereas \text{Jackson et al. (1996)} found no frequency dependence in the real part in healthy infants. \text{Marchal et al. (1988)} found frequency dependence of the real part below \(f_{r,1}\) in both healthy and wheezy infants, but it was more prominent in wheezy infants \((\text{Marchal et al. 1988})\). These findings are consistent with findings in adult patients with airway obstruction \((\text{VanNoord et al. 1991})\) in comparison to healthy adults. The frequency dependence of resistance in adults is thought to be due to parallel inhomogeneity or to non-rigid behaviour of the upper, or central airways.
The results by Marchal et al. (1988(1)) might be explained by recent computer predictions by Jackson et al. (1996) suggesting that since infantile airways are so compliant the real part could be frequency dependent even in healthy infants. Our mean \( f_r,1 \) (37±9 Hz) was slightly lower than the \( f_r,1 \) reported by Jackson et al. (1996) and Desager et al. (1991). The frequency of the first anti-resonance (155± 28 Hz) was significantly higher than the one reported by Jackson et al. (1996) (113 ± 10 Hz), presumably because our measurements were made in infants with wheezing disorders, and theirs were made in healthy subjects. This would be consistent with our findings that \( f_a,1 \) increased after airway obstruction (discussed in more detail below), as well as the findings of Chalker et al. (1992) who reported that \( f_a,1 \) was higher in adults with chronic airway obstruction compared to healthy individuals, possibly as an expression of altered airway wall properties.

Model fitting using system identification techniques. The lumped 4-element model (as well as the 6-element model of DuBois) is not capable of fitting the negative frequency dependence of the real part of \( Z_{in} \) for frequencies below \( f_r,1 \). The 4-element model is however able to follow the \( Z_{in} \) data between about 16 and 256 Hz which includes the resonance and anti-resonance providing estimates for \( R_{rs}, C_{rs}, \) and \( I_{rs} \). We therefore tested the hypothesis whether this might be true in infants, as proposed by Jackson et al.(1996). As expected the lumped 4-element was not capable of fitting the negative frequency dependence of the real part of \( Z_{in} \) for frequencies below \( f_r,1 \) in our data but it was able to follow the \( Z_{in} \) data between about 16 and 256 Hz which includes the resonance and anti-resonance (e.g. Fig 6.1.6.4). The mean baseline value for \( R_{rs} \) (20.6 ± 4.7 cmH\(_2\)O-L\(^{-1}\cdot \)s) was similar to the value reported by Desager et al (1991) in a patient population similar to ours, but slightly higher than the values reported in healthy infants by Marchal et al. (1) (17.4 ± 5.3 cmH\(_2\)O-L\(^{-1}\cdot \)s) and by Jackson et al. (5) (15.6 ± 3.6 cmH\(_2\)O-L\(^{-1}\cdot \)s) consistent with studies in adults (VanNoord et al. 1991). Our baseline values for \( C_{rs} \) (1.87 ± 2.42 10\(^{-3}\) L-cmH\(_2\)O-L\(^{-1}\)) were between the values reported by Marchal et al. (1988(1)) (4.95 ± 8.26 10\(^{-3}\) L-cmH\(_2\)O-L\(^{-1}\)) and by Jackson et al. (5) (1.03 ± 0.58 10\(^{-3}\) L-cm H\(_2\)O-L\(^{-1}\)) in healthy infants. This would be consistent with the age distribution of the patients in the different studies. However, one would expect much higher values for \( C_{rs} \) (1-2 10\(^{-3}\) L-cm H\(_2\)O-L\(^{-1}\) per kg body weight under quasi static conditions or at tidal breathing frequencies). One contribution to this difference could be the frequency dependent decrease of \( C_{rs} \). However, as shown by Jackson et al. (1987) from modelling studies, the imaginary part of \( Z_{in} \) even for frequencies surrounding \( f_r,1 \) is significantly influenced by \( C_{aw} \) and \( C_{g} \). As a consequence, the \( C_{rs} \) extracted from the \( Z_{in} \) data using the 4-element model would be a
complex function of all three compliances (Cti, Caw and Cg). Although we find similar values of Rrs and Crs under baseline conditions to other research groups, of more importance is whether parameters derived from the 4-element model are sensitive to changes in airway mechanics during induced airway obstruction.

**Behaviour of the maximal expiratory flows (RTC) and Zin during induced airway obstruction.**

In comparison to the reference values of Tepper et al. (1986) our baseline values for \( \dot{V}_{\text{maxFRC}} \) showed mild airway obstruction at baseline and significant airway obstruction following methacholine challenge. Despite a significant decrease in \( \dot{V}_{\text{maxFRC}} \) following methacholine challenge, there was no significant change in \( \dot{f}_t \) or the frequency dependence of the real part of Zin (\( \alpha \)). It appears there is no simple parameter in the Zin spectrum between 16 and approximately 100 Hz (below the first anti-resonance) which is as sensitive to changes in airway mechanics as \( \dot{V}_{\text{maxFRC}} \).

However, at higher frequencies (>100 Hz) we found that the anti-resonance frequency (\( \text{far}_1 \)) and the relative maximum in the real part at \( \text{far}_1 \) (\( Z_{\text{re}}(\text{far}_1) \)) of the first anti-resonance were highly sensitive to changes in airway mechanics, as demonstrated independently by the decrease in \( \dot{V}_{\text{maxFRC}} \). As mentioned above, at these higher frequencies, Zin is influenced by Raw, less influenced by tissue properties and more influenced by airway wall properties. We conclude that in infants Caw significantly influences Zin at frequencies surrounding the \( \text{far}_1 \). Since the 4-element model assumes that all of the shunt compliance is assigned to the face mask, it is thus not able to explain the changes in Zin during methacholine challenge and as a consequence the resulting respiratory resistance (Rrs) does not reflect the changes in airway resistance during induced airway obstruction. We suggest that more complex models that include the separate face mask gas compression as well as the airway wall compliance might be needed to interpret Zin over this frequency range in infants. However, the strong correlation between \( \text{far}_1 \) and \( \dot{V}_{\text{maxFRC}} \) during the methacholine challenge is evidence that \( \text{far}_1 \) is, at least in part due to wave-propagation phenomena and is thus related to inertance of the gas within the airways and the compliance of the airway walls similar to the \( \text{far}_1 \) in adults (Jackson et al. 1989). If the \( \text{far}_1 \), and its higher harmonics are wave propagation related this means that Zin at these frequencies is a function of airway geometry (lengths and diameters), gas density and wall mechanical properties (Jackson et al. 1989, 1993, Chalker et al. 1992, Habib et al. 1994(2x), Guelke et al. 1981). This concept has been experimentally verified in dogs (Jackson et al. 1991) and in human adults (Jackson et al. 1989) where it has been shown
that \( f_{ar,l} \) increased as a function of gas density when a gas (\( \text{He}_02 \)) with lower density was inhaled.

Although the high frequency Zin data in infants seem to behave similarly to those in adults, extraction of physiologically relevant parameters using distributed parameter models is not possible as it is in adults and in dogs (Jackson et al. 1993, Habib et al. 1994 (2x)). Instead, changes in the frequencies of the anti-resonances provide empirical information related to changes in airway compliance as is the case with \( V_{maxFRC} \). Before distributed parameter models can be used in infants more research is needed. Firstly, it will be necessary to incorporate the effect of the upper airways. The situation in infants is particularly complex because measurements have to be during nasal breathing through a face mask. Nevertheless, the fact that the anti-resonance changed in both frequency and amplitude during methacholine challenge supports the conclusion that high frequency Zin is sensitive to intra-thoracic airway mechanics. Although methacholine can theoretically affect nasal pathway diameter (vasodilatation and mucus secretion (Baraniuk et al. 1992)), we are certain that we induced a change in intra-thoracic airway mechanics in our experimental setup, because \( V_{maxFRC} \) is mainly a function of intra-thoracic airway function (Tepper et al. 1993). In one of our subjects we measured Zin before and after occlusion of one nostril, which did not change \( f_{ar,l} \) and \( Zin_{re}(f_{ar,l}) \) (Fig. 6.1.6.8). However, the influence of upper airway geometry on high frequency Zin needs to be studied in greater detail. Secondly, measurements of Zin to frequencies higher than 256 Hz must be made in order to include the second, and hopefully the third anti-resonance. Thirdly, detailed anatomical models of the infant bronchial tree, similar to the Horsfield model which is used for the distributed parameter models (Jackson et al. 1993, Habib et al. 1994(2x)) in adults and dogs are needed.

In summary: We have shown that high frequency Zin in infants contains an anti-resonance whose frequency is repeatable and changes significantly during induced airway obstruction. The frequency of the first anti-resonance is higher following airway obstruction. This behaviour is consistent with the findings in human adults (Chalker et al. 1992). High frequency Zin data might therefore be useful to assess changes in airway mechanics in a non-invasive manner in infants. Since measurements only take a few seconds, measurements are often possible in unsedated infants during natural sleep (unpublished observations). This makes the technique very useful for the rapid assessment of changes in airway mechanics during bronchial challenge. We have shown that the 4-element model cannot explain high frequency Zin data, probably because airway wall compliance becomes increasingly important at higher frequencies. We suggest that, as in human adults, changes in frequency \( f_{ar,l} \) during
airway obstruction might be explained by changes in wave propagation velocity in airways with changing wall compliance. We further suggest, that the combined measurements of $\dot{V}_{\text{maxFRC}}$ and $\varphi_{1}$ from Zin could provide additional information about changes in airway calibre versus changes in airway wall compliance. For example, if $\dot{V}_{\text{maxFRC}}$ were to decrease while $\varphi_{1}$ remained constant this would imply a change in airway calibre but not change in airway wall compliance.
6.1.6. Figures

Figure 6.1.6.1:

a) 6 element model (DuBois et al. 1956), b) 4 element model (Jackson et al. 1996): Cm: face mask shunt compliance, Rrs: respiratory resistance, Crs: respiratory compliance, Irs: respiratory inertance.

Figure 6.1.6.2:

Impedance spectra (mean and standard deviation for all 13 infants) from 16 to 256 Hz. The mean resonant frequency $f_{r,1}$ was $37\pm9$ Hz and the first anti-resonant occurred at $(155\pm28$ Hz).

Figure 6.1.6.3:

Bland Altman plot representing short term repeatability of the first anti-resonance $ar,1$: The difference in frequency $f_{ar,1}$ and amplitude $Z_{inre}(f_{ar,1})$ derived from the first and second measurement sets are presented as a function of their mean values. The solid lines represent the 95% confidence intervals of the mean differences.

Figure 6.1.6.4:

Mean impedance spectrum of a set of 8 measurements. In a single subject, the first resonance occurs at 34 Hz and the first anti-resonance occurs at 200 Hz. The 4-element model fit is represented by a solid line ($Cm = 2.20 \times 10^{-5}$ L·cmH$20^{-1}$, respiratory system resistance $Rrs = 17.13$ cmH$20$·L$^{-1}$·s, compliance $Crs = 1.19 \times 10^{-3}$ L·cmH$20$·s$^{-1}$ and inertance $Irs = 2.2 \times 10^{-5}$ cmH$20$·L$^{-1}$·s$^{-2}$).
Figure 6.1.6.5: Representative measurement sets (mean (SD) of 8 Zin runs) in a single subject assessed at baseline (closed circles) and after methacholine challenge (open circles). The frequency far,1 and amplitude Zin_{re}(far,1) of the first anti-resonance increased during the challenge (see text).

Figure 6.1.6.6: The frequency far,1 \( (t = -5.34, p = 0.007) \) from 148 ± 26 to 203 ± 15 Hz and Zin_{re}(far,1) increased significantly for the whole group of 9 infants after methacholine challenge \( (t = -3.0791, p = 0.015) \) from 51 ± 10 to 70 ± 15 cm H\textsubscript{2}O-L\textsuperscript{-1}·s

Figure 6.1.6.7: Rrs derived from system identification of Zin(\omega) using a 4 element model in comparison to the V maxFRC following methacholine challenge. The resistance Rrs did not change during methacholine challenge test. (see text).

Figure 6.1.6.8: Example of a set of measurements (mean and SD) assessed before (closed circles) and after occlusion of one nostril, mimicking an increased nasal resistance. In a single subject the occurrence of ar,1 was not change significantly influenced by the occlusion of a nostril, indicating that upper airway resistance cannot be a very important determinant of the anti-resonance and that these high frequency Zin data are essentially not influenced by nasal patency.
Figure 6.1.6.1:

a)

(Zaw: Raw, Caw, Iaw) (Zti: Rti, Cti, Iti)

![Diagram a)

b)

Airway opening

![Diagram b)
Figure 6.1.6.3:

\[\text{far, 1 (2nd)} - \text{far, 1 (first)} \quad (\text{Hz})\]

\[\text{mean (far, 1)} \quad (\text{Hz})\]

\[\text{Zin}_{\text{re}}(\text{far, 1}) \quad (\text{2nd}) - \text{Zin}_{\text{re}}(\text{far, 1}) \quad (\text{first}) \quad (\text{cm H}_2\text{O L}^{-1} \text{ s})\]

\[\text{mean (Zin}_{\text{re}}(\text{far, 1})) \quad (\text{cm H}_2\text{O L}^{-1} \text{ s})\]
Figure 6.1.6.6:

Before and after methacholine challenge

Before and after methacholine challenge
Figure 6.1.6.7: Before and after methacholine challenge.

- $R_s$ (cm H$_2$O L$^{-1}$ s$^{-1}$)
- $V_{maxFRC}$ (mL s$^{-1}$)

Before and after methacholine challenge.
Figure 6.1.6.8:
### Table 6.1.7.1. Physical characteristics of subjects:

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PCA: postconceptional age, PNA: postnatal age

6.2.1 Abstract

Respiratory input impedance ($Z_{in}$) over a wide range of frequencies has shown to be useful to determine airway (Raw) and tissue resistance (Rti) in dogs or to determine airway wall properties in human adults. $Z_{in}$ measurements are non-invasive and therefore potentially useful to determine airway mechanics in infants. However, accurate measurements of $Z_{in}$ at these frequencies using forced oscillatory techniques in infants are difficult because of their relatively high airway resistance and large upper shunt compliance (face mask). If pseudo-random noise pressure oscillations are applied at the airway opening (forced oscillation techniques (FOT)), the power of the resulting flow decreases $1/f$ with frequency. We studied whether high frequency respiratory impedance can be measured using rapid flow interruption (High speed interrupter technique (HIT)) in which we expect the flow amplitude in the respiratory system to be higher than in the FOT. We compared $Z_{in}$ measured by high speed interrupter technique (HIT) with $Z_{in}$ measured by the forced oscillation technique (FOT) in a dried dog lung and in 5 healthy adult subjects. The impedance was calculated from two pressure signals measured between the mouth and the high speed interruption valve (HIT). The impedance could be assessed from 32 to 800 Hz. Its real part at low frequency as well as the frequency and amplitude of the first and second acoustic resonance, measured by FOT and measured by HIT were not significantly different. Particularly at frequencies > 400 Hz, the power spectrum of oscillatory flow showed 100 times higher amplitudes using the HIT in comparison with FOT. In conclusion, the high speed interrupter technique enables the measurement of high frequency $Z_{in}$ data from 32 to 800 Hz with particularly high flow amplitudes and therefore better signal to noise ratio. This is particularly important in systems with high airway resistance i.e. in infants, when measurements have to be performed through a face mask.
6.2.2. Introduction

Input impedance (Zin) of the respiratory system is routinely measured at frequencies between 2 and 32 Hz. However, Zin to higher frequencies has been shown to contain additional information about the mechanical properties of the respiratory system. Zin at frequencies between 2 and approximately 120 Hz can be used to separate airway (Raw) and tissue resistance (Rti) as well as to estimate thoracic gas volume (TGV) in dogs (Jackson et al. 1987) but not in adult humans (Jackson et al. 1989). Zin in adult humans to even higher frequencies (256 Hz) has been shown to contain information about airway wall properties (Habib et al. 1994). It is not yet known whether high frequency Zin measurements in human infants can provide information about Raw, Rti, and TGV or about airway wall properties. But, accurate measurements of Zin at these frequencies using forced oscillatory techniques (FOT) in infants are difficult because of low signal-to-noise ratios due to several causes (Jackson et al. 1994). First, the energy content of the forcing function in the forced oscillatory technique decreases as 1/f (see section 5.1.5). Second, measurements in infants must be made through a face mask that acts as a shunt compliance which further degrades the energy content of the flow into the infant's respiratory system as frequency increases. An alternative broad spectrum signal that could be used to excite the respiratory system is rapid airway interruption. Rapidly interrupting the flow at the airway opening causes a step change in flow whose frequency content, like the forced oscillatory technique, decreases as 1/f. However, the magnitude of the flow forcing function is expected to be much larger in the HIT compared to the FOT (see section 5.1.4). In this methodological paper we studied whether high frequency Zin can be measured during airflow interruption. We performed these measurements in a dried dog lung model and in healthy adults in comparison to Zin measurements using the FOT, where the FOT has been proven to reliably measure high frequency Zin (Jackson et al. 1989).
6.2.3 Methods

**Measurement system (Fig. 6.2.6.1).** The high speed interrupter technique has been described in the sections 5.1.4. and 5.2.3. The shutter mechanism was connected to the mouthpiece through a tube of 0.6 cm radius and 30 cm length.

Zin was measured using the wave tube technique described in the sections 3.3.4 and 5.1.5. Briefly, in these measurements in adults pressures are measured at two locations (12 cm apart) along the tube between the shutter and the mouthpiece. The proximal pressure transducer (P₁) was placed 5 cm and the distal pressure transducer (P₀) 17 cm from the airway opening (mouthpiece). The pressures P₁ and P₀ were measured with Microswitch (Model 164) transducers that were matched to within ± 2% in magnitude and ± 2° of phase. The electrical output of these transducers was band pass filtered (8-2000 Hz) and analog/digital converted at 8258 Hz (Model AT-MIO-16, National Instruments). Data were stored during 4 complete cycles of the interrupter (4 times 31 ms = 124 ms, with a sampling rate of 8258 Hz = 1024 points). The ratio of P₁ / P₀ was estimated from the cross power spectra of P₀P₁ and the auto power spectrum of P₁. The method for measuring Zin by the forced oscillation technique and its validation up to 2000 Hz has been described elsewhere (Jackson et al. 1996). Briefly, Zin(oh) was measured using loud-speaker generated, low amplitude pressure oscillations applied to the airway opening. A pseudo-random noise signal containing frequencies between 32 and 800 Hz in 8 Hz increments, was generated by computer and output via a digital-to-analog converter. Zin was measured using the same wave tube technique described above.

The quality of the measurements for both the interrupter technique and the forced oscillation technique was assessed by computing the coherence function according to Michaelson et al. (1974).

**Experimental protocol.** Zin of a dried dog lung was measured by HIT and the FOT and compared at each frequency. Since a dried dog lung is minimally permeable, the airflow interruption was performed at a flow rate of 0.1 L/s. Zin was also measured using the FOT and using the HIT in 4 healthy non smoking adult volunteers whose biometric data are given in table 6.2.7.1. Informed consent was obtained and the study was approved by the Institutional Review Board at Boston University, where this work was carried out. The measurements in the human subjects were made as follows: during tidal breathing the airflow interruption was triggered at a inspiratory flow rate of 0.1 L/s during 10 respiratory cycles. The subjects were seated, wearing nose clips, and they supported their cheeks by their hands.
The mean and standard deviation (SD) as well as the coherence of the 10 measurements were calculated.
6.2.4. Results

Figure 6.2.6.2 shows the Zin and coherence of the dried dog lung measured by the HIT and by the FOT. A paired t-test (p<0.01) was used to determine those frequencies where there was a significant difference between impedances measured by the two different techniques. For nearly all frequencies the differences were insignificant except at those frequencies surrounding the anti-resonances and between 160 Hz and 290 Hz in the imaginary part. Coherence was > 0.95 for all frequencies.

Figures 6.2.6.3-4 show the mean ± SD and coherence of the Zin measured by the FOT and by the HIT in 5 healthy subjects (2 examples). Apart from single frequency points close to the anti-resonances and at very high frequencies, the coherence of the measurements were > 0.95 between 32 and 800 Hz in both techniques. In all 5 subjects there were two dominant anti-resonance both using the HIT (first anti-resonance far,1 at 266 ± 39 Hz, second anti-resonance far,2 at 653 ± 104 Hz) and FOT (255 ± 36 Hz, 650 ± 114 Hz). Regarding the group of 5 subjects, the frequencies and the amplitudes of the anti-resonances were not significantly different in both techniques (table 6.2.7.2). As a means of comparing the variability of the two techniques, we computed the average standard deviation for all frequencies in all 5 subjects. The average standard deviation in the real part measured by HIT was 3.59 ± 1.61 and by FOT 1.37 ± 1.01 cm H\textsubscript{2}O/L/s, which was not significantly different (paired t-test, p > 0.05) In the imaginary part the averaged standard deviation was significantly different between the HIT (4.03 ± 1.13) and the FOT (0.99 ± 0.62), p < 0.05.
6.2.5. Discussion

Zin data over a wide range of frequencies (2 < f < 100 Hz) has been shown to be useful in providing separate estimates of airway (Raw) and tissue resistance (Rti) (Lutchen et al. 1987). However, to obtain reliable estimates of Raw and Rti one must obtain data up to the first anti-resonance (at ~ 80 Hz in dogs). This anti-resonance in dogs has been shown to be due to the interaction of gas compression compliance (Cg) and tissue inertance (Iti). In dogs, there is a second anti-resonance at ~ 180 Hz which is an acoustic anti-resonance that is primarily dependent on airway path length and airway wall properties. Unlike dogs, adult humans do not a Cg-Iti related anti-resonance but their first anti-resonance (at ~ 180 Hz) is acoustic related. As a consequence, Zin data in adult humans cannot be used to estimate Raw and Rti.

Zin are particularly attractive for measuring lung function in infants since they are non-invasive, can be performed during tidal breathing, and have the potential of providing estimates of Raw and Rti. However, Zin measurements made by the FOT in infants are problematic because of the shunting of face mask dead-space and measurements to high enough frequencies which include an anti-resonance cannot be reliably made (Jackson et al. 1994) using the forced oscillation technique. Infants have relatively high airway resistance in comparison to adults. Therefore, if measurements are made through a face mask, very little flow goes into the respiratory system and more flow goes into the parallel shunt impedance of the face mask. Since the energy content of the forcing function decreases 1/f in the forced oscillation technique, we expect particularly low flows at higher frequencies and even lower in the presence of a parallel shunt impedance (gas in the face mask). But to measure high frequency respiratory impedance accurately we need high oscillatory flow amplitudes at higher frequencies.

We were therefore looking for a alternative broad spectral forcing function, that allows us to control the flow power and to put more energy into the respiratory system. The high speed interrupter technique provides a signal that could be used to excite the respiratory system. The high speed interrupter technique was derived from the standard interrupter technique introduced by Neergaard and Wirz (1927) and modified by several other authors. It has been shown by Bates et al. (1988) that the value of the standard interrupter technique is limited because it only enables the measurement of a Newtonian resistance of airways and tissue. It was proposed that the initial pressure change reflects airway resistance only, but the modelling of the interrupter curve is often difficult because the technique is based on the complete pressure equilibration between alveolar and mouth pressure (Pao). The latter is
often not achieved in patients with severe airway obstruction or in small children with generally higher airway resistance. However, it has been shown by Shepard (1962) and by Jackson et al (1974) that damped pressure oscillations occur after rapid airflow interruption. Romero et al (1990) found in dogs that an anti-resonance in the power spectrum of Pao occurred at ~80 Hz and a gas density dependent anti-resonance occurred at 180 Hz. They speculated that these anti-resonances corresponded to the Cg-Iti-tissue resonance (80 Hz) and the first acoustic resonance (180 Hz) described by Jackson et al. (1987). Frey et al. (1995(1,2)) found in humans only a single resonance which was after correction of the mouth-piece length close to 180 Hz, and speculated that it corresponds to the acoustic anti-resonance in the Zin spectrum found by Jackson et al. (1993). Based on these previous studies in dogs and humans, it appears that similar information can be measured by the forced oscillation technique and by the interrupter technique. In fact, if the exact flow and Pao could be measured before and during interruption, Zin could be computed, which has not been done so far. Unfortunately, it is very difficult to measure flow reliably above 200 Hz. However, by measuring two pressures and invoking the tube technique allows for the first time to calculate not only Newtonian resistance but the complex Zin over a wide range of frequencies. The difference between the FOT and the interrupter technique consists mainly in the nature of the forcing signal. Whereas the FOT uses a pseudo-random noise with has in most cases equal energy at each frequency, the interrupter technique uses a pseudo-step function with a 1/frequency distribution of the applied energy. The faster the airflow interruption the more the input signals corresponds to a step function and the more high frequency contents with a good coherence will be found in the impedance spectrum. Therefore we constructed a high speed interrupter technique (HIT), which enables an airflow interruption of less than 1 ms.

Rapidly interrupting the flow at the airway opening causes a step change in flow whose frequency content, like the forced oscillatory technique, decreases as 1/f. The interrupter is basically a flow generator, therefore the magnitude of the flow forcing function is much larger in the HIT compared to the FOT. This is demonstrated in figure 6.2.6.5, which shows the power spectrum of pressure and flow signal in subject 5 using the FOT and the HIT. In both techniques the power of pressure and flow signal decrease with frequency, but the HIT technique provides flow signals with two orders of magnitude higher energy at higher frequencies than the forced oscillation technique. Furthermore, the decrease of the flow power with frequency is slower in the HIT than in the FOT. This effect can even be seen more distinctly in the power spectrum of pressure and flow signals measured in a 12 month old infant through a face mask (Figure 6.2.6.3). While the power of the flow signal drops
immediately at frequencies higher than 400 Hz, the power of the flow signal forced by the HIT is much higher above 400 Hz. We showed therefore that the new high speed interrupter technique enables the excitement of higher flow amplitudes at high frequencies than the forced oscillation technique in a simple way. High oscillatory flows could theoretically be produced by the forced oscillatory technique if very large loudspeakers were used and very high pressures applied, but this seems unpractical in a clinical setting, specially in the paediatric field where dead space of measurement tools should be minimised. The question remains whether the large flow amplitudes produced by the HIT cause non-linear behaviour of the respiratory system. Since the step function produced by the HIT is represented by continuous spectrum in frequency domain, non linear behaviour will cause a drop in the coherence function. Figure 6.2.6.5 and 6 show that the coherence did not drop in these subjects. We explored this possibility of introducing non-linear behaviour by performing flow interruption at high flows. We found that at flows higher than 100 mL/s the coherence drops and we assume that therefore the respiratory system starts to behave in a non linear way. In this respect, we hypothesise that the HIT will also be superior to the FOT during measurements in infants, because flow limitation and therefore non-linear behaviour is frequently seen in infants because of the relatively high airway wall compliance.

Using the HIT it was possible to measure $Z_{in}$ from 32 to 800 Hz with a coherence of >0.95 in a dried dog lung and in humans. In humans the coherence function dropped around anti-resonances and at frequencies close to 800 Hz in both techniques. The comparison of impedance measurements in a dried dog lung done by FOT and HIT showed a congruence of the spectra over a large range. At frequencies smaller than 32 Hz the HIT shows a large standard deviation and therefore the data below 32 Hz are not reliable. Theoretically, this could be due to the tube technique. It has been shown (Franken et al. 1981), that the length of the tube limits the frequency range over which reliable data can be obtained. However, this can not be the only reason, because the tube technique was also used in the FOT. The other reason could be the consecutive interruptions with a interruption time of 31 ms, which gives a interruption frequency of 32 Hz. Thus there is little, if any, energy below 32 Hz using the HIT. The frequencies below 32 Hz are probably less represented in the impedance spectra assessed by HIT. However, the interrupter time could be changed in order to investigate lower frequency ranges. In humans, the correlation between the measurements using the FOT and the HIT was not as good as in the dog model. Since the coherence function was good in these measurements, non linear effect cannot be a major reason for the differences measured by these two techniques. As discussed above a major difference between the two technique is the amount of energy that has been put into the system, and therefore the accuracy of the high
frequency impedance measurements is different. A further difference may be the lung volume at which the measurements were performed. As shown by Frey et al. (1995(1)), the oscillatory pressure transients after airflow interruption are sensitive to the change in lung volume. The airflow interruptions were performed at the beginning of a breath, and the lung volume might have slightly changed from breath to breath. This effect might also be responsible for the larger variability in the HIT in the imaginary part if the impedance spectrum. However, the major factor that contributed to the larger variability of $Z_{in}$ using HIT in comparison to the FOT is mainly due to the shorter data sampling period. A short sampling period, however, can be advantageous if future measurements will be performed in infants with limited co-operation. The total measurement time of less than 0.15 sec, prevents reflex muscle activity. A last reason for variability and differences between the two techniques might be the role of the upper airways, which might behave differently in case pressure waves are applied externally via a loudspeaker or whether flow produced by the respiratory muscles is interrupted. For example, if the glottis is closed there will still be flow measured if the FOT is used (shunting into the upper airways), but there will be no flow if the HIT is used. Regarding the entire group of subjects, there was no significant difference between the FOT and HIT concerning the real part at low frequencies (32 - 96 Hz) and the real and imaginary part of the anti-resonance frequencies. Anti-resonances were found at ~ 260 Hz and at ~ 650 Hz in both techniques. Only in the work of Habib (1994(1,2)) and Jackson et al. (1993) the tube technique was used, and they found acoustic resonances higher than we found in this paper. They measured the subglottal $Z_{in}$ in intubated patients, which reduces the mean airway path length and therefore increases the resonant frequency. They pointed out that high frequency $Z_{in}$ over the range of at least 2 acoustic resonances can be used to assess information concerning serial distribution of airway resistance into central and peripheral airway resistance using the Horsfield model (Habib et al. 1994, Jackson et al. 1993). This may be a further advantage for impedance measurements in infants, in which the peripheral airway parts play an important role.

In summary, in comparison to the forced oscillation technique the high speed interrupter technique enables the measurement of high frequency $Z_{in}$ data from 32 to 800 Hz. Impedance data in this frequency range potentially enables the non-invasive measurements of resistive and elastic properties of the airways. The forcing function derived from the HIT excites higher flow amplitudes at high frequencies, and makes therefore high frequency respiratory impedance measurements more accurate because of better signal to noise ratio. This is particularly true in systems with high airway resistance i.e. in infants, when measurements have to be performed through a face mask. Further advantage for use in infants consists in
the easy use and cleaning of the device, the small dead space, the short measurements period, and the fact that the forcing function in the HIT provides a continuous spectrum which causes a drop in coherence function if the system behaves non-linear. The HIT-device can potentially be implemented into ventilatory circuits, where ventilatory pressures up to 30 cm H$_2$O are present. Under these conditions the loudspeaker in the FOT would easily reach its physical limitations, whereas the forcing function in the HIT will not be damped by high pressures in the respiratory system.
6.2.6. Figures

Figure 6.2.6.1:
High speed interrupter setup

Figure 6.2.6.2:
Impedance spectra (mean, standard deviation and coherence of 10 measurements) measured by forced oscillation technique (dotted line) and high speed interrupter technique (solid line) in a dried dog lung model. The circles indicate whether the two techniques were not significantly different at a specific the frequency.

Figure 6.2.6.3-4:
Impedance spectra (mean, standard deviation and coherence of 10 measurements) measured by forced oscillation technique (dotted line) and high speed interrupter technique (solid line) in 2 healthy subjects.

Figure 6.2.6.5:
Power spectrum of pressure and flow assessed using the HIT in comparison with FOT in healthy adult subject #5 (figure 7). All signals were assessed using the same pressure transducers and amplifiers. Note that the power of the flow is 100 times higher using the HIT and decreases less with increasing frequency than using the FOT. Open squares symbols = power spectrum of pressure (HIT), closed squares symbols = power spectrum of resulting flow (HIT), Open circle symbols = power spectrum of pressure (FOT), closed circle symbols = power spectrum of resulting flow (FOT).

Figure 6.2.6.6:
Power spectrum of pressure and flow assessed using the HIT in comparison with FOT in a 12 month old infant. Airway opening pressures are measured through a facemask. Note that the power of the oscillatory flow drops above ~400 Hz if FOT is used to measure Zin but not if HIT is used. Open squares symbols = power spectrum of pressure (HIT), closed squares symbols = power spectrum of resulting flow (HIT), Open circle symbols = power spectrum of pressure (FOT), closed circle symbols = power spectrum of resulting flow (FOT). Coherence: solid line HIT, dotted line FOT.
Figure 6.2.6.1

- dried dog lung
- subjects

Pressure transducers:
- PI
- PO

Open outlet

Slide valve

Stepper motor
Figure 6.2.6.2:
Power spectrum \( \left( P_1 (\text{cm H}_2\text{O})^2, \text{flow (L/s)}^2 \right) \)
### 6.2.7. Tables

**Table 6.2.7.1. Physical characteristics of subjects**

<table>
<thead>
<tr>
<th>Subj.</th>
<th>Age, yr.</th>
<th>Sex</th>
<th>Ht, cm</th>
<th>Wt, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>M</td>
<td>178</td>
<td>75</td>
</tr>
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<td>2</td>
<td>25</td>
<td>M</td>
<td>172</td>
<td>74</td>
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<td>3*</td>
<td>28</td>
<td>M</td>
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<td>81</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>M</td>
<td>171</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>F</td>
<td>163</td>
<td>50</td>
</tr>
</tbody>
</table>

* Ex smoker
Table 6.2.7.2: Comparison of the impedance measurements using high speed interrupter technique (HIT) and forced oscillation technique (FOT)

<table>
<thead>
<tr>
<th>Nr.</th>
<th>( Z_{\text{in, real}} )</th>
<th>far,1</th>
<th>( Z_{\text{in, real}} )</th>
<th>far,2</th>
<th>( Z_{\text{in, real}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cmH₂O/L/s) (32-96 Hz)</td>
<td>mean freq.</td>
<td>(cmH₂O/L/s) (far,1)</td>
<td>mean freq.</td>
<td>(cmH₂O/L/s) (far,2)</td>
</tr>
<tr>
<td>HIT</td>
<td>1</td>
<td>4.0 280 25.4 624 11.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.3 304 28.6 704 13.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.5 264 31.1 738 26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.2 280 38.1 712 29.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.1 200 21.9 483 26.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOT</td>
<td>1</td>
<td>3.2 276 30.9 599 9.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.1 296 32.6 688 27.4</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>4.1 248 22.8 744 12.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.4 256 45.7 744 38.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4 200 17.7 477 26.1</td>
<td></td>
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</tr>
</tbody>
</table>

\( p(\text{paired}) \) 0.29 0.06 0.78 0.86 0.81

\( Z_{\text{in, re}}(32-96Hz) \): mean real part of \( Z_{\text{in}} \) at the frequencies 32 - 96 Hz, \( \text{far,1} \): first anti-resonance, \( \text{far,2} \): second anti-resonance, \( Z_{\text{in, re}}(\text{far,1}) \) real part of \( Z_{\text{in}} \) at first anti-resonance. \( Z_{\text{in, re}}(\text{far,2}) \) real part of \( Z_{\text{in}} \) at second anti-resonance, \( p(\text{paired}) \): probability derived from paired t-test (compares HIT vs FOT).

6.3.1. Abstract

High frequency input impedance (Zin) measurements including anti-resonances provide useful non-invasive information on airway geometry and especially airway wall mechanics in the canine and human adult respiratory system. A knowledge of airway wall mechanics would be particularly important in understanding flow limitation phenomena in infants. High frequency Zin has not been measured in infants above 256 Hz, because the high impedance of the infantile respiratory system would be expected to result in low amplitudes of oscillatory flow at higher frequencies. The aim of this study was to develop a technique to measure of high frequency Zin in infants, and to elucidate the nature of the anti-resonance phenomena in the Zin spectrum in infants. We measured Zin from 32 to 900 Hz during rapid airflow interruption by the high speed interrupter technique (HIT) in 18 infants (age 24 to 149 wks) with wheezing disorders. The HIT enables the excitement of higher flow amplitudes at high frequencies using an pseudo-step forcing function (J. Appl. Physiol. 82:1018-1023, 1997). In all infants Zin showed a mean (SD) first anti-resonance (far,1) of 172 (35) Hz (real part of Zin at far,1 (Zin\text{re}(far,1)): 4.9 (1.1) kPa L^{-1}s) and in 5 infants a second anti-resonance (far,2) of 564 (51) Hz (Zin\text{re}(far,2): 2.0 (0.7) kPa L^{-1}s). The anti-resonances were found to be related to wave propagation in the airways (acoustic anti-resonances), because they increased by a factor of ~2 when HeO2 was inhaled. This implies that far,1 and its harmonics are a function of airway wall compliance. far,1 and far,2 might therefore be helpful in understanding flow limitation in wheezing disorders in infants, because flow limitation is not only related to airway diameter but also to airway wall compliance.
6.3.2. Introduction

Even though one can infer changes in airway wall properties from Zin at frequencies that include only the first anti-resonance (Jackson et al. 1996, Frey et al. 1998 (1,2)), Zin is needed at frequencies that include additional anti-resonances are needed before quantitative estimates of airway wall properties can be obtained (Jackson et al. 1993, Habib et al. 1994(1,2)). Measurements of Zin with loudspeaker-generated forced oscillations applied at the airway opening are problematic because infant airways are small and the respiratory system has a relatively high impedance. Therefore if pressure waves are applied at the airway opening, the amplitude of the resulting flow will be small, and the signal to noise ratio will be low. This is particularly true for higher frequencies because of capacitative shunting into the gas compression compliance of the loudspeaker dead-space.

To overcome these problems we developed a new technique, the high speed interrupter technique (HIT) (Frey et al. 1997(2)), which uses a pseudo-step-flow forcing function and enables the excitement of much higher flow amplitudes at high frequencies than the FOT.

The aim of the current study was to determine whether it is technically possible to measure coherent high frequency Zin in infants using the HIT in infants and whether the baseline variability of the anti-resonance features is similar to the variability of a reference lung function technique such as the RTC. Furthermore we aimed to determine whether these anti-resonances were related to wave propagation phenomena in the airways as in adults, which would imply that the frequency of the first anti-resonance was a function of airway wall properties independent of airway calibre. Finally, we aimed to measure whether patency of the upper airways might influence high frequency Zin.
6.3.3. Methods

Subjects. The main study (part 1) was performed in 18 infants and young children (11 girls, 7 boys, age 24 to 149 wks) with a history of episodic or recurrent wheeze, who had been referred from the outpatient clinic for lung function tests as part of their clinical investigation (Table 6.3.7.1). Physiological and technical aspects (part 2 and 3) were studied in a separate group of 6 infants (3 boys and 3 girls, aged 1 to 18 month). Infants with other severe diseases and infants with upper respiratory tract infection within the previous 3 weeks were not included in the study. The infants and toddlers were sedated using maximum oral dose of 150 mg/kg triclofos sodium and lung function was measured during behaviourally defined quiet sleep. The additional HIT measurements were approved by the Ethics Committee of the Royal Postgraduate Medical School, Hammersmith Hospital, London. Written consent was obtained from parents.

Study design. The study was performed in 3 parts. In part 1, in 18 infants we determined whether it was technically possible to measure high frequency impedance. In these infants we determined the variability (10 measurements) of the frequency and relative maxima in the real part at the anti-resonances, and compared it to the variability of the maximal flow at FRC (V\textsubscript{maxFRC}) by the RTC. In part 2, in 3 additional infants we made measurements of Z\textsubscript{in} breathing a gas mixture of 21% oxygen and 79% helium (Heliox: He\textsubscript{02}) to elucidate the physiological basis of the anti-resonances. If the anti-resonance in infants were related to wave propagation phenomena, then the frequency at which this anti-resonance occurred would increase as a function of gas density. In a gas of lower density wave propagation velocity would be faster and the frequency of the anti-resonance in a tube would be higher. In part 3, we tested the hypothesis that the upper airways significantly influenced the anti-resonances by measuring 5 sets of HIT measurements in 3 infants before and after occluding one nostril.

High speed interrupter technique for infants. The principle of the HIT technique (Fig. 6.3.6.1) has been described in the sections 5.1.4. and 5.2.3. The shutter mechanism was connected to the mouthpiece by a tube of 1 cm internal diameter and 14 cm in length. Z\textsubscript{in} was measured using the wave tube technique described in detail in section 3.3.4. and 5.1.5. We chose a RENDELL BAKER SOUCEK face mask (SIZE 1, AMBU INTERNATIONAL, BATH, AVON) because its dead space was small. In the process of study design we tried different face masks and studied their effect on high frequency Z\textsubscript{in} data. The dead space in the face mask was minimised to 10 - 15 mL by filling it as much as possible with putty (THERAPEUTIC PUTTY, CARTER'S, BRIDGEND, UK). The wave tube pressures P\textsubscript{1} and P\textsubscript{0} were measured with piezo-resistive pressure transducers that were
matched within < 2% in magnitude and < 2° of phase (EUROSENSOR, MODEL 33, UK). The electrical output of these transducers was band pass filtered (8-2000 Hz) and analog/digital converted at 8258 Hz (MODEL AT-MIO-16, NATIONAL INSTRUMENTS, TX, USA). Data were stored during 5 complete cycles of the interrupter (5 times 31 ms = 155 ms, with a sampling rate of 8258 Hz). The ratio of P\textsubscript{1}/P\textsubscript{0} was estimated from the cross power spectra of P\textsubscript{0}P\textsubscript{1} and the auto power spectra of P\textsubscript{1} (Michaelson et al 1975). The length of the FFT window was 1024 points. Control of the interrupter shutter, data acquisition, and computation of the cross and auto power spectra and their ratio were done using LABVIEW FOR WINDOWS (NATIONAL INSTRUMENTS, TX, US).

**The thoracic compression technique.** Partial expiratory flows at FRC (V\textsubscript{maxFRC}) were measured using the rapid thoracic compression technique (RTC) described in the sections 3.2.3 5.1.1. and 5.2.1. After a series of RTC measurements to determine the optimal jacket pressure, 10 RTC measurements were performed at end tidal inspiration using this optimal jacket pressure. The mean value of all technically satisfactory values was determined. The general measurement conditions and the monitoring were described in the sections 5.3.3. and 5.3.4. The head position was not changed between measurements using different techniques. For both techniques we used the same face mask and putty filling.

**Part 1: Description and repeatability of high frequency impedance phenomena.** In order to test short term repeatability within a period of 10 - 15 minutes, 10 sets of Z\textsubscript{in} measurements were performed during quiet regular tidal breathing. Each measurement took 0.15 sec at the beginning of inspiration. Airflow interruptions which did not show peak pressure changes of at least 0.15 kPa were not accepted. None of the infants was disturbed in sleep by the measurements, no symptoms occurred, and pO\textsubscript{2} and SaO\textsubscript{2} remained stable. Thereafter, the inflatable jacket was wrapped around the infants chest and 10 forced expirations were performed. From each of the 10 sets of 5 airflow interruptions Z\textsubscript{in} and coherence γ was calculated. The measurement of 1 set of 5 airflow interruptions took 0.2 seconds; the 10 sets were assessed during a period of 10 - 15 min to determine short term repeatability. Only Z\textsubscript{in} data with coherence > 0.90 were accepted. If the coherence of the whole set of measurements was low the set were rejected. If the coherence of a few data points was below 0.9, these particular frequency points were rejected and excluded from the spectrum. From the sets which fulfilled the criteria mentioned above (number n), the mean and standard deviation of the Zin
was calculated from 32 Hz to the maximal frequency with $\gamma > 0.90$ ($f_{\text{max}}\gamma$). The $Z_{in}$ was presented by complex numbers (real and imaginary part): Short term repeatability of frequency ($f_{ar,1}$) and the relative maximum in the real part at $f_{ar,1}$ ($Z_{in}(f_{ar,1})$) at the anti-resonances were expressed by their coefficient of variation (CV).

From the 10 forced expiratory manoeuvres we calculated the mean and standard deviation of $V^*_{\text{maxFRC}}$ as well as is percent predicted (for age) based on the reference values of Tepper et al (1986). Short term repeatability $V^*_{\text{maxFRC}}$ was expressed by the coefficient of variation (CV). The differences in CV between the different parameters were compared using a non-parametric Wilcoxon test.

**Part 2: Measurements during Heliox ($\text{HeO}_2$) breathing.** We collected 5 sets of $Z_{in}$ measurements in 3 infants (aged 1, 9 and 12 months) during air breathing and during humidified Heliox ($\text{HeO}_2$) breathing after 8-10 minutes of equilibration.

**Part 3: Influence of nasal patency on $Z_{in}$.** In another 3 infants (aged 12, 12 and 18 months) we examined the possibility that high frequency $Z_{in}$ data were influenced by the upper airway patency. In each of these three infants, we performed 5 sets of $Z_{in}$ measurements before and after the occlusion of one nostril by gentle pressure over the alae nasae and by putting THERAPEUTIC PUTTY inside the face mask. During this 30 second manoeuvre $\text{SaO}_2$ was stable in all infants. With this manoeuvre we altered the resistive and inertive properties of the upper airways, without any direct alteration of the intrathoracic airway function.
6.3.4 Results

Representative examples of Zin data in two subjects (CJ and JA) are shown in Figure 6.3.6.2. All subjects showed similar high frequency Zin. The real part of Zin at 32 Hz was between 1.34 and 3.54 (mean 2.34 ± 0.64) kPa L \(^{-1}\) s. In all but 4 of the subjects we found a first resonance above 32 Hz (\(f_{ar,1}\)) at 53 ± 14 Hz and in all subjects a second resonance \(f_{ar,2}\) at 762 ± 158 Hz. All subjects showed a well defined anti-resonances (\(f_{ar,1}\)) at 172 ± 35 Hz. In 3 subjects the first anti-resonance was split up into 2 distinct maxima but only one zero crossing in the imaginary part (HJ: 140/209 Hz, MC: 113/185 Hz, DL: 86/200 Hz). In 5 of the infants (as in JA, Fig. 6.3.6.2) there was a relative maximum in the real part of Zin at a frequency \(f_{ar,2}\) = 690 ± 180 Hz that was associated with relative maximum in the imaginary part but the imaginary part did not cross zero as it does in a well defined anti-resonance. At 1003 ± 9.67 Hz (\(f_{ar,3}\)) we found a well defined anti-resonance in all subjects. However, this anti-resonance varied within subjects, when the setup or the face mask were changed (as discussed in more detail below). We therefore only report Zin measurements up to 900 Hz (Fig. 6.3.6.3). The short term repeatability of the first and second anti-resonance is given by the coefficient of variation (CV; Table 6.3.7.2). The CV of \(f_{ar,1}\) (9.7 ± 5.4%), \(f_{ar,2}\) (7.1 ± 2.7%) and \(V_{maxFRC}\) (6.4 ± 2.4%) in these subjects were not significantly different (p > 0.01), whereas the CV of \(Zin_{re}(f_{ar,1})\) (13 ± 5.9%) and \(Zin_{re}(f_{ar,2})\) (23.7 ± 18%) were higher than the CV of \(V_{maxFRC}\) (p < 0.01).

The technical reliability of the HIT is described by the number of measurement sets (\(n\) out of 10) with coherence (\(\gamma\)) > 0.90 and the maximal frequency up to which Zin could be measured with \(\gamma\) > 0.9 (\(f_{max,\gamma}\)) (Table 6.3.7.3). In all infants the coherence was > 0.9 between 32 Hz and 1395 ± 49 Hz except for a few single frequency points usually around 350 and 900 Hz. These frequency points were excluded from analysis.

Part 2: Measurements during Heliox (He\(_0\)\(_2\)) breathing. When a gas of lower density (He\(_0\)\(_2\)) was inhaled, \(f_{ar,1}\) increased from 193 Hz to 403 Hz (ratio 2.1) in subject CT (Fig. 6.3.6.4), from 112 Hz to 274 Hz (ratio 2.4) in subject CC and from 209 to 314 Hz (Ratio 1.5) in subject CJ. These results are close to the theoretical value of 1.93 and provide evidence that the first anti-resonance is a phenomenon which is related to wave propagation velocity within the airways.

Part 3: Influence of nasal patency on Zin. Occluding one nostril had no significant effect on the first anti-resonance in all three infants (Fig. 6.3.6.5). The differences in \(f_{ar,1}\) and \(Zin_{re}(f_{ar,1})\)
before and after occlusion for the three subjects were 0, 0, 8 Hz and (-0.5), 0.5, and 0.22 kPa L⁻¹ s respectively.
6.3.5 Discussion

We addressed two problems, firstly whether high frequency input impedance in infants could be measured by the non invasive high speed interrupter technique (HIT), and secondly whether high frequency $Z_{in}$ data could provide information about intrathoracic airway mechanics. For this purpose we had to elucidate the nature of the anti-resonances in infants. It has recently been demonstrated in human adults that the frequency of these anti-resonances contains information about airway wall properties (Jackson et al. 1993, 1989, Habib et al. 1994(1,2), Dorkin et al. 1988). The possibility of measuring airway wall mechanics in vivo in infants would thus help to elucidate flow limitation phenomena in wheezing disorders in infants, since flow limitation is not only influenced by the airway diameter but also by airway wall compliance (Dawson et al. 1977).

The widely used rapid thoracic compression technique is not able to distinguish between the effects of airway diameter and airway wall compliance on airflow limitation. Measurement of airway wall properties would help to answer the question whether only developmental differences in airway size (Martinez et al. 1988) or possibly also developmental differences in airway wall compliance might predispose to wheezing disorders in infancy. It could also explain why broncho-dilators can have paradoxical effects in infants (Prendiville et al. 1987), bearing in mind that they have the potential to change both airway diameter as well as airway wall compliance by changing airway smooth muscle tone.

Information about airway wall properties in adults has been extracted from measurements of $Z_{in}$ from 5 to 320 Hz (Jackson et al. 1989) and in more detail from 8 to 2000 Hz in adults (Habib et al. 1994(2)). In infants $Z_{in}$ data have never been measured in the frequency range above 256 Hz, and the nature of anti-resonance phenomena has never been elucidated. Measurement of $Z_{in}$ at these high frequencies was expected to be difficult, because infant airways are small and the system has a relatively high impedance. Therefore if pressure waves are applied at the airway opening, as in the forced oscillation technique (FOT), the amplitude of the resulting flow will be small, and the signal to noise ratio will be low. This is particularly true for higher frequencies because of capacitative shunting into the gas compression compliance of the loudspeaker (FOT) (Frey et al. 1997(2)). To overcome these problems we developed a new technique, the high speed interrupter technique (Frey et al. 1997(2)), which uses a pseudo-step-flow forcing function and enables the excitement of much higher flow amplitudes at high frequencies than the FOT.

The interrupter technique was invented by Neergaard and Wirz (1927) and has been modified many times (Mead et al. 1954, Jackson et al. 1973, 1974, Bates et al. 1988, 1989). The value of the standard interrupter technique is limited because it only permits the measurement of a
Newtonian lung resistance (Bates et al. 1988, 1989). But changes in airway opening pressure after airflow interruption can be analysed in more detail. It has been shown by Jackson et al. (1973, 1974) that highly damped pressure oscillations occur after rapid airflow interruption. Romero et al. (1990) showed in dogs that an anti-resonance in the power spectrum of the airway opening pressure occurs at ~80 Hz and a gas density dependent anti-resonance occurred at 180 Hz. They speculated that these anti-resonances correspond to the Cg-Iti-tissue resonance (80 Hz) and the first acoustic resonance (180 Hz) described by Jackson et al. (1991) in the Zin spectrum. We found only a single anti-resonance in human adults which after correction for the mouthpiece length was close to 180 Hz (32, 33), and we speculated in analogy that it corresponds to the wave propagation-related anti-resonance in the Zin spectrum found by Jackson et al. (1989).

Recently we showed in a dog lung and in human adults that it is possible to measure high frequency Zin using the new high speed interrupter technique (HIT) (Frey et al. 1997(2)), when not only the postocclusional pressure transients but also postocclusional oscillatory flow transients are measured.

**Measurement of Zin in infants using the HIT** from 32 to ~1300 Hz was technically possible with a coherence of > 0.9 using a pseudostep flow forcing function generated by the high speed interrupter technique. Two resonances and one anti-resonance was found in all the infants, whereas a second anti-resonance was detectable only in 5 of the subjects. At frequencies over 1000 Hz a third anti-resonance occurred which was dependent on the equipment in particular the face mask. The frequency and the real part at this third anti-resonance changed when the setup, the head position and the face mask were varied within the same patient. Furthermore, despite matching the pressure transducers we also expected inaccuracies at about 1000-1100 Hz, because the resonant frequency of the solid state pressure transducers was expected to be in this frequency range. For these reasons, we therefore only report Zin data up to 900 Hz.

**Physiological interpretation of Zin data in infants below 100 Hz.** We found the first resonance frequency \( f_{r,1} \) below 32 Hz in 4 subjects and at 53 ± 14 Hz in the others, which is slightly higher than in our previous study (20) (37 ± 9 Hz), but similar to the studies of Desager et al. (1991) and Jackson et al. (1996). Marchal et al. (1988) did not find a resonance in their study, probably because their measurements were not made to high enough frequencies. Marchal et al. (1989) found \( f_{r,1} \) at lower frequencies when they used the head generator technique, pointing to the effects of extrathoracic airway walls on \( f_{r,1} \). Also, Sly et al. (1996) found \( f_{r,1} \) to be at much lower frequencies. This might be explained by the fact that they performed measurements under conditions of raised lung volume and induced relaxation (Hering Breuer reflex) in healthy infants. The geometry of the face mask might also have been different in their study.
In our study, \( f_{r,1} \) was inversely related to postnatal age; the older the child the lower the first resonance. Based on the DuBois model (1956) \( f_{r,1} \) is given by:

\[
f_{r,1} = \frac{1}{2} \cdot [C_t (I_{law} + I_{ti})]^{0.5}
\]

where \( C_t \) is the tissue compliance, \( I_{law} \) the airway inertance and \( I_{ti} \) the tissue inertance. This dependence on compliance would explain the inverse correlation between \( f_{r,1} \) and age. The frequency dependence of the real part of \( Z_{in} \) below \( f_{r,1} \) was difficult to assess with the HIT because the lowest frequency we measured was 32 Hz. However, this frequency dependence of resistance in infants is influenced not only by parallel inhomogeneity but also by non-rigid behaviour of the upper, or central airways (Jackson et al. 1996). Recent computer predictions by Jackson et al. (1996) suggest that since infant airways are so compliant, the real part could be frequency dependent even in healthy infants, as observed by Marchal et al. (1988). Using the forced oscillation technique we have demonstrated that this frequency dependence of resistance as well as \( f_{r,1} \) changed in a very complex and unsystematic manner during induced airway obstruction (Frey et al. 1998(3)).

**Physiological interpretation of \( Z_{in} \) above 100 Hz in infants.** The most prominent features in the \( Z_{in} \) above 100 Hz are the anti-resonances. We found a first anti-resonance in all infants and a second anti-resonance in 5 infants. The frequencies of both the first and second anti-resonance showed a coefficient of variation similar to \( V_{maxFRC} \) from the widely used rapid thoracic compression technique, whereas the relative maxima at the anti-resonant frequencies showed a slightly higher variability. The frequency and relative maxima in the real part were not related to age. However the patients were not healthy and so a final conclusion on age dependence cannot be drawn. The first anti-resonance occurred at ~ 170 Hz which is similar to the findings in adults (Jackson et al. 1989) and to our previous study (Frey et al. 1998(1)), but higher that reported in the study of Jackson et al (1996) in healthy infants. The fact that we found higher anti-resonant frequencies in infants with wheezing would be consistent with observations in adults with obstructive pulmonary disease (Chalker et al. 1992). However, while we found a tendency for \( V_{ar,1} \) to be higher with decreasing \( V_{maxFRC} \) and therefore increasing airway obstruction, this correlation was not significant. To understand this we have to know more about the nature of the anti-resonances in infants, since in wheezy infants the airways might be very different from the airways of patients with COPD.

Our current findings support mainly the hypothesis that the anti-resonances are caused by wave propagation phenomena in the airways, as in human adults. Theoretically the fact that the anti-resonant frequency changed when a gas of lower density (He\(_0\)) was inhaled could be explained
either by the interaction of gas compression compliance (\(C_g\)) and tissue intertance (\(I_{ti}\)) (DuBois model), or by the influence the average molecular weight of the gas mixture (gas density) on the wave propagation velocity. In the first case according to the DuBois model, \(f_{ar,1}\) would be inversely related to \(C_g\) and \(I_{ti}\). It should not be affected by a gas of different density; \(C_g\) would change provided the ratio of specific heats of the two different gases are different and the compressions in the alveoli are adiabatic. The differences in specific heat between air (1.4) and \(\text{HeO}_2\) (1.56) would result in an 11% increase of \(C_g\) and a 5% decrease in \(f_{ar,1}\) during \(\text{HeO}_2\) breathing. Similarly, if the anti-resonance were purely related to the gas compression compliance in the face mask (\(C_m\)), \(f_{ar,1}\) would change, but not by a factor 2. In the second case, if the anti-resonance is wave propagation related, \(f_{ar,1}\) is proportional to the wave propagation velocity (\(v\)). For example in a rigid tube \(f_{ar,1}\) would be \(v/4L\), where \(L\) is the length of the tube. In a rigid tube \(v\) is only dependent on gas density and not on diameter, in a compliant tube \(v\) is a function of gas density and wall compliance (Guelke et al 1981). However, airway wall compliance did not change during the \(\text{HeO}_2\) experiment. The ratio of \(v_{\text{HeO}_2}\) (64.5 ms\(^{-1}\)) and \(v_{\text{air}}\) (33.3 ms\(^{-1}\)) is 1.93. In our study \(f_{ar,1}\) increased by a factor \(\sim 2\) following \(\text{HeO}_2\) breathing, which makes it likely that the anti-resonance is related to wave propagation in the airways and not to tissue properties. In this case anti-resonances are often referred as acoustic anti-resonances (Jackson et al. 1989).

Assuming a very simple acoustic airway model consisting of a single open rigid airway, the harmonics (\(f_{ar,2}, f_{ar,3}, f_{ar,4} \ldots\)) of \(f_{ar,1}\) would occur at multiples of 3, 5, 7 .. times \(f_{ar,1}\). In 4 of 5 subjects in which a second anti-resonance \(f_{ar,2}\) occurred, we found it to occur at a multiple of \(\sim 3\) (resp. 5 in subject DL) of \(f_{ar,1}\), which would support the hypothesis. However, \(f_{ar,1}\) of 171 Hz would correspond to a mean airway path length of 48.7 cm, which seems too long for an infant. But in compliant tubes, \(f_{ar,1}\) depends not only on airway path length and gas density, but also on the compliance of the tube. The relationship between the wave propagation velocity in a non-rigid tube and the wall compliance is highly complex and dependent on the frequency of the travelling pressure wave (Guelke et al. 1981). This might lead to two phenomena, firstly \(f_{ar,1}\) is dependent on airway wall compliance and secondly the ratio \(f_{ar,2}/f_{ar,1}\) might not simply be a harmonic function.

This new evidence that the anti-resonances are dependent on wave propagation is interesting from a developmental point of view since high frequency \(Z_{in}\) data of human infants correspond more closely to \(Z_{in}\) in human adults (Dorkin et al. 1988, Jackson et al. 1989) that to \(Z_{in}\) of animals (Jackson et al. 1991) with lungs of similar size to infant lungs. However, wave propagation related anti-resonances in human adults are much sharper and narrower. We can think of two possible explanations. In wheezy infants we expect parallel inhomogeneities. Since
far,1 corresponds to a mean airway pathlength, we hypothesise that we would expect to see a bundle of anti-resonances which are close together and might originate from different parallel segments. An alternative explanation would be that multiple peaks could occur because of the frequency dependence of wave propagation velocity in a compliant tube (Guelke et al. 1981). Both hypotheses would also explain the occurrence of multiple peaks around far,1 in 3 of the subjects at baseline.

After having elucidated the origin of the anti-resonance phenomena, the question remains whether the high frequency input impedance was largely determined by intrathoracic airway geometry or whether the extrathoracic airway significantly influenced the high frequency Zin. In three subjects, after occlusion of one nostril the high frequency Zin did not change significantly, providing reassuring evidence that upper airway resistance had little influence on the anti-resonances. The influence of extrathoracic airway wall compliance on Zin data at frequencies below 100 Hz has been pointed out by several authors (e.g. Marchal 1989, Jackson et al. 1996). The current data support the hypothesis that the anti-resonances occurring above 100 Hz are related to wave propagation phenomena, and hence that its frequencies are related to airway wall properties. We must assume, that pressure waves are propagated along the entire airway pathlength from the airway opening to the alveolar space. The compliance of the upper airway may therefore partly influence wave propagation velocity and the high frequency impedance spectrum of the respiratory system. We minimised the influence of the upper airway in our measurement setting by filling the face mask with putty and stabilising the cheeks by holding the face mask firmly in place. In order to determine the relative contribution of extra- and intrathoracic airway wall compliance on far,1 one must selectively change one or the other. Since airway smooth muscle is the major determinant of intrathoracic airway wall compliance and cholinergic receptors in the extrathoracic airway are only located in vessels and glands (Baranuik et al. 1992), we propose that methacholine challenge might help to elucidate the situation. We report this in the accompanying paper.

**Summary and clinical importance of the findings.** We have developed a new non-invasive lung function technique, the high speed interrupter technique (HIT), which enables the non invasive measurement of high frequency Zin in infants from 32 to 1300 Hz. Measurements can be performed without the co-operation of the patient within a few seconds. At frequencies between 100 and 900 Hz we found two anti-resonances. At around 1000 Hz we found a 3rd anti-resonance, which was artefactual and depended on the setup and face mask. The first anti-resonance was related to wave propagation velocity in the airways, similar to the situation in human adults. This implies that the frequency of the first anti-resonance is a function of airway
wall compliance independent of airway diameter. The frequencies of these anti-resonances show a similar variability to standard lung function parameters in this age group. We showed that the anti-resonance was not significantly affected when nasal patency was altered by occluding one nostril. Since $f_{ar,1}$ is a function of airway wall compliance and not of airway diameter, it can be used to elucidate developmental differences of airway wall compliance in the first year of life and their consequences for wheezing disorders, and to explore the actions of therapeutic agents which affect airway function.
6.3.6. Figures

Figure 6.3.6.1:
Using the non-invasive high speed interrupter technique (HIT) high frequency input impedance can be measured using a fast rotating interrupter valve which generates a pseudo-step flow function. The resulting pressure and flow oscillations can be assessed using the wave tube technique (section 3.3.4. and 5.1.5.).

Figure 6.3.6.2:
Mean impedance spectrum, standard deviation (bars) and coherence from 10 Zin measurements assessed by the high speed interrupter technique in two representative infants (CJ: closed circles, JA: open circles). Both infants showed a first and second resonance frequency \( (f_r,1; f_r,2) \), and a first anti-resonance \( (f_{ar,1}) \). Subject JA showed a second anti-resonance \( (f_{ar,2}) \). A third anti-resonance \( (f_{ar,3}) \) at \( \sim 1000 \) Hz occurred in both infants, but was dependent on the setup and the type of face mask (artefactual). Apart from single frequency points, which were excluded from the spectrum, the coherence was \( > 0.9 \) from 32 to \( \sim 1300 \) Hz.
Figure 6.3.6.3:

Impedance spectra (mean and standard deviation for all 18 infants) from 32 to 900 Hz, showing the first resonant frequency $f_{r,1}$ ($53\pm14$ Hz) and the first anti-resonance ($172\pm35$ Hz) frequency $f_{ar,1}$ and the second resonance $f_{r,2}$ ($762\pm158$ Hz).

Figure 6.3.6.4:

High frequency $Z_{in}$ measurements (mean and SD of 10 measurements) during room air (circles) and He$\text{O}_2$ breathing (squares) in one infant. The anti-resonances $f_{ar,1}$ shifted from 193 Hz to 403 Hz when breathing gas of low density ($\text{He}_2$).

Figure 6.3.6.5:

Example of mean and SD of 5 high frequency $Z_{in}$ measurements in 3 infants (circles, squares, triangles) before (open symbols) and after (closed symbols) occlusion of one nostril, indicating that the anti-resonance in the $Z_{in}$ measurements was not significantly influenced by altering the upper airway patency.
Figure 6.3.6.4:

![Graph showing real and imaginary parts of the system response for air and HeO₂ as a function of frequency. The x-axis represents frequency in Hz, ranging from 128 to 896, and the y-axes represent real and imaginary parts in kPa L⁻¹ s⁻¹.]
Table 6.3.7.1. Physical characteristics of subjects. GA: gestational age, PNA: postnatal age, \( V_{\text{maxFRC}} \): maximal flow at FRC (RCT), predicted values from (Tepper et al. 1986).

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Table 6.3.7.2: The Zin anti-resonances (HIT) were described by the frequencies \( f_{ar,1}, f_{ar,2} \) [Hz] and the relative maxima in the real part at \( f_{ar,1} \) and \( f_{ar,2} \) respectively \( (\text{Zin}_{re}(f_{ar,1}), \text{Zin}_{re}(f_{ar,2}) \) [kPa L\(^{-1}\) s\(^{-1}\)]. \( V_{\text{maxFRC}} \) [mL s\(^{-1}\)] is the maximal expiratory flow at FRC (RCT).

Mean and coefficient of variation (CV) of 10 measurements. (* see discussion).

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Table 6.3.7.3: Technical quality of the high frequency impedance measurements using the HIT. From 10 measurements (inclusion criteria: pressure change >0.15 kPa) $n$ measurements showed a coherence > 0.9 up to a maximal frequency ($f_{\text{max}}$).

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| m    | 9.3 | 1395            |
| SD   | 1   | 49              |

6.4.1 Abstract

High frequency Zin measurements (Zin) provide useful non-invasive information on airway geometry and specially airway wall mechanics in the canine and human adult respiratory system. Using the high speed interrupter technique (HIT) we have shown that it is possible to measure high frequency Zin in infants up to 900 Hz including 2 anti-resonances, which are known to related to wave propagation velocity. This implies that the first anti-resonant frequency (far,1) is a function of airway wall compliance. Since airway wall mechanics are particularly important for the flow limitation phenomena, we wondered whether airway wall properties were important for the occurrence of flow limitation during methacholine (mch) challenge in infants. We measured Zin from 32 to 900 Hz and the forced expiratory flow ($V_{\text{max,FRC}}$) in 10 infants (aged 36 to 81 wks) with wheezing disorders. Following methacholine challenge far,1 increased significantly at very low doses of mch before a fall in $V_{\text{max,FRC}}$ occurred. We conclude, that high frequency Zin are sensitive to changes in airway mechanics at lower doses than the forced expiratory flows. Moreover, we have shown that airway wall properties influence flow limitation in infants, suggesting that developmental differences of the airway wall might contribute to the risk for wheezing in infants and could explain some of the paradoxical effects of broncho-dilators.
6.4.2. Introduction

For the understanding of the physiological development of airway geometry and airway wall stability in the first years of life and more importantly for the understanding of wheezing disorders and drug action in infancy, there was a need for a non-invasive lung function technique which provides a measure of airway wall properties independent of airway calibre. We have introduced such a new method - the high speed interrupter technique (Frey et al. 1997(2)) - which enables the non-invasive and rapid (0.2 sec) measurement of high frequency input impedance (Zin) in infants (Frey et al. 1998(2)). The high frequency Zin contain two anti-resonances, which have shown to be related to wave propagation velocity of pressure waves within the airways (acoustic anti-resonances). This implies that the frequency at which these anti-resonances occur is therefore only dependent on gas density, airway pathlength and airway wall properties. This technique potentially enables us to study the hypothesis that in infants both airway wall properties and airway calibre contribute to flow limitation.

The aim of the current study is to demonstrate that high frequency Zin data are sensitive to changes in airway mechanics to show the usefulness of the high speed interrupter technique. Furthermore to demonstrate the importance of airway wall properties for flow limitation and wheezing in infants, we wondered whether the first acoustic anti-resonance as an indirect measure of airway wall compliance will be changed already at low doses of methacholine before flow limitation occurs, as hypothesised by Prendiville et al. (1987).
6.4.3 Methods

Study subjects. The study was performed in 10 infants and young children (3 girls, 7 boys, aged 36 to 81 wks) with a history of episodic or recurrent cough or wheeze, who had been referred from the outpatient clinic for routine lung function tests (Table 6.4.7.1). Infants with other severe diseases and infants with upper respiratory tract infection within the previous 3 weeks were not included in the study. The infants and toddlers were sedated using maximum oral dose of 150 mg/kg triclofos sodium and lung function was measured during behaviourally defined quiet sleep. The additional HIT measurements were approved by the Ethics Committee of the Royal Postgraduate Medical School, Hammersmith Hospital, London. Written consent was obtained from the parents.

Study design. In these 10 infants we performed 10 baseline measurements of $V_{max}^{FRC}$ as an expression of flow limitation using the rapid thoracic compression technique (RTC), as well as 10 high speed interrupter measurements (HIT) featuring the first and second anti-resonances, of which the frequency ($f_{ar,1}, f_{ar,2}$) is known to be an indirect measure of airway wall compliance. We then slowly administered methacholine in doubling doses and performed both 5 HIT and 5 RTC measurements until $V_{max}^{FRC}$ decreased by more than 30%. To demonstrate that HIT is sensitive to airway mechanics in individual subjects we compared baseline measurements with post-methacholine measurements of the frequencies ($f_{ar,1}, f_{ar,2}$) and relative maxima in the real part ($Z_{in_{re}}(f_{ar,1}), Z_{in_{re}}(f_{ar,2})$) of these anti-resonances in the high frequency input $Z_{in}$ of individual subjects. To quantify the time course effect of airway wall mechanics (e.g., $f_{ar,1}$) in comparison to flow limitation ($V_{max}^{FRC}$) during the methacholine challenge, we calculated the standard deviation scores of each parameter derived from HIT and RTC at each provocation dose.

High speed interrupter technique for infants. The principle of the HIT technique (Figure 6.4.6.1) has been described elsewhere (Frey et al. 1997(2), 1998(2)) and in section 5.1.4. The shutter mechanism was connected to the mouthpiece by a tube of 1 cm internal diameter and 14 cm in length. $Z_{in}$ was measured using the wave tube technique described in detail elsewhere (Franken et al. 1981) and in the sections 3.3.4 and 5.1.5. Briefly, in this technique pressures are measured at two locations (6.7 cm apart) along the tube between the shutter mechanism and the mouthpiece. The first pressure transducer ($P_1$) was placed 3.3 cm and the second pressure
transducer \((P_0)\) 10 cm from the airway opening (face mask). The electrical output of these pressure transducers was band pass filtered (8-2000 Hz) and analog/digital converted at 8258 Hz (Model AT-MIO-16, National Instruments, TX, USA). Data were stored during 5 complete cycles of the interrupter (5 times 31 ms = 155 ms, with a sampling rate of 8258 Hz). The ratio of \(P_1/P_0\) was estimated from the cross power spectra of \(P_0P_1\) and the auto power spectra of \(P_1\). This analysis technique calculates impedance independently of the input function (Michaelson. 1975). The length of the FFT window was 1024 points, the impedance data were averaged from a moving FFT window. Control of the interrupter shutter, data acquisition, and computation of the cross and auto power spectra and their ratio were done using Labview for Windows (National Instruments, TX, US).

**The squeeze jacket technique.** The rapid thoracic compression technique has been described in the sections 3.2.3., 5.1.1. and 5.2.1.

**Experimental protocol.** The infants were in the supine position. The monitoring of the subjects has been described in section 5.3.4. The dead space in the face mask was minimised to 10 - 15 ml. At baseline, 10 sets of \(Z_{\text{in}}\) measurements were performed during quiet regular tidal breathing. Each measurement took 0.15 sec at the beginning of inspiration. Impedance measurements with a coherence below 0.9 were not accepted. None of the infants was disturbed in sleep by the measurements. Thereafter, the inflatable jacket was loosely wrapped around the infants chest and 10 forced expirations were performed.

The methacholine challenge test was performed as described previously (Clarke et al. 1994) using cumulative doses of methacholine (saline, 0.5, 1, 2, 4, 8, 16, 32 and 64 g/l). At each provocation dose, 5 sets of \(Z_{\text{in}}\) measurements (HIT) and then 5 RTC were performed, using the same jacket pressure throughout each test. The provocation test was terminated after a decrease of \(\dot{V}_{\text{max,FRC}}\) of 30 % (PD) has been found. In one infant who showed a marked improvement of \(V_{\text{max,FRC}}\) after saline, PD was defined from the post-saline measurement. Head position and face mask were not altered during the tests.

Parameter extracted from \(Z_{\text{in}}\) features were the frequencies \((\omega_{\text{r},1}, \omega_{\text{r},2})\) and amplitudes \((Z_{\text{in,}\text{re}}(\omega_{\text{r},1}), Z_{\text{in,}\text{re}}(\omega_{\text{r},2}))\) of the anti-resonances defined by an zero crossing in the imaginary part and a relative maximum in the real part of the impedance spectrum. From previous work (Frey et al. 1998 (1,2)) we know that mainly these anti-resonances were expected to change
systematically during methacholine challenge, and therefore only focused on the description of these features. The significance of the change in $\omega_r,1$ and $\text{Zin}_r(\omega_r,1)$ in individual subjects following methacholine challenge test was determined by t-test. For the whole group, differences in the values of $V_{\text{max FRC}}, \omega_r,1$ and $\text{Zin}_r(\omega_r,1)$ before and after methacholine challenge were described using a paired t-test, since the differences data showed nearly normal distributions.

**Standard deviation score analysis.** In order to investigate whether the frequency $\omega_r,1$ and the relative maximum in the real part ($\text{Zin}_r(\omega_r,1)$ of the first anti-resonance already change at lower doses of methacholine than $V_{\text{max FRC}}$, we expressed the change of $\omega_r,1$ $\text{Zin}_r(\omega_r,1)$ and $V_{\text{max FRC}}$ at each provocation dose by their change in standard deviation scores from the mean. E.g. the standard deviation score for the parameter $\omega_r,1$ at a provocation dose PD was calculated as follows:

$$\text{SDS}(\omega_r,1, \text{PD}) = \frac{\text{mean } \omega_r,1 \text{ at PD} - \text{mean } \omega_r,1 \text{ at baseline}}{\text{SD of } \omega_r,1 \text{ at baseline}}.$$  

Since we wanted to demonstrate that $\omega_r,1$ and/or $\text{Zin}_r(\omega_r,1)$ change at lower doses of methacholine than $V_{\text{max FRC}}$, this statistical approach is valid because the coefficient of variation (CV) for $V_{\text{max FRC}}$ is equal or smaller than the CV of $\omega_r,1$ and $\text{Zin}_r(\omega_r,1)$ at baseline. Any statistical error would favour $V_{\text{max FRC}}$. Provocation dose levels were expressed as fractions ($\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1) of the final provocation dose (PD) at which $V_{\text{max FRC}}$ had decreased at least 30% from baseline.
6.4.4. Results

A representative example of $Z_{\text{in}}$ at baseline and after methacholine challenge is shown in figure 6.4.6.1. Both the frequency $\omega_{\text{r,1}}$ of the first anti-resonance as well as the relative maximum in the real part of $Z_{\text{in}}$ at $\omega_{\text{r,1}}$ ($Z_{\text{inre}}(\omega_{\text{r,1}})$) increased following methacholine challenge. For the whole group of 10 infants, $\omega_{\text{r,1}}$ and $Z_{\text{inre}}(\omega_{\text{r,1}})$ increased significantly ($p = 0.0025$ and $p = 0.005$ respectively) (Fig. 6.4.6.2); $V_{\text{maxFRC}}$ decreased significantly from $233.6 \pm 96.5$ ml/s to $125.4 \pm 68.5$ ml/s ($p<0.0005$) confirming that a significant change in airway mechanics was induced by the methacholine challenge test. Considering individual responses, $\omega_{\text{r,1}}$ increased significantly following methacholine in 9 of 10 infants and $Z_{\text{inre}}(\omega_{\text{r,1}})$ increased significantly in 8 of the infants (table 6.4.7.3).

A second anti-resonance was found in all subjects following methacholine challenge leading to a relative maximum in the real part at about 600 occurred in all subjects (between 554 and 834 Hz). The frequency of this second anti-resonance ($\omega_{\text{r,2}}$) occurred at 3 times $\omega_{\text{r,1}}$ in all but one subject (dl) where $\omega_{\text{r,2}}$ occurred at $\sim 5 \times \omega_{\text{r,1}}$. $\omega_{\text{r,2}}$ and $Z_{\text{inre}}(\omega_{\text{r,2}})$ did not significantly change in 4 of the 5 subjects where second anti-resonances were already detectable at baseline. In one subject (dl) $\omega_{\text{r,2}}$ changed significantly. However, it is possible that in this subject the second anti-resonance phenomenon after methacholine ($\omega_{\text{r,2}} = 5 \times \omega_{\text{r,1}}$) did not correspond to the second anti-resonance phenomenon at baseline ($\omega_{\text{r,2}} = 7 \times \omega_{\text{r,1}}$).

The averaged standard deviations scores at each provocation level for 8 of the subjects are shown in figure 6.4.6.3. In these subjects $V_{\text{maxFRC}}$ did not change at low doses of methacholine ($1/8$, $1/4$ PD) but increases suddenly and very rapidly at higher methacholine levels ($1/2$ PD, PD), whereas $\omega_{\text{r,1}}$ and $Z_{\text{inre}}(\omega_{\text{r,1}})$ change already significantly at very low doses ($1/8$ PD) of methacholine, but then remain relatively stable. There is also a moderate change of $\omega_{\text{r,1}}$ and $Z_{\text{inre}}(\omega_{\text{r,1}})$ after saline inhalation. In two of the subjects (AE, MC) Expressed by negative standard deviation scores (indicating increase in flow), $V_{\text{maxFRC}}$ did significantly improve from baseline after saline and at low doses of methacholine (figure 6.4.6.4). In parallel to this phenomenon $\omega_{\text{r,1}}$ and $Z_{\text{inre}}(\omega_{\text{r,1}})$ changed several SDS at low doses of methacholine in these subjects. In subject AE (Figure 6.4.6.4a), a term infants with episodic wheeze, this corresponds to an increase of frequency from 118 to 225 Hz (190%) and $Z_{\text{inre}}(\omega_{\text{r,1}})$ from 26 to 86 cm H20 L-1 s (330%), indicating this is not only a pure expression of a small baseline SD. In the other subject (MC) (Figure 6.4.6.4b) who has been born prematurely, $\omega_{\text{r,1}}$ decreased initially and then raised with increasing dose of methacholine, whereas $Z_{\text{inre}}(\omega_{\text{r,1}})$ increased at low doses of methacholine already over 200%. 
6.4.5. Discussion

Developmental differences have shown to be predisposing for the subsequent development of wheezing disorders in infancy. Martinez et al. (1988) described that infants who subsequently developed wheezing disorders have had already marked flow limitation at birth. It is known that flow limitation and the occurrence of wheeze are related (Fry et al. 1960). Martinez et al. (1988) postulated that infants with wheeze had already smaller airway diameter at birth. This implies that these developmental differences in the airway mechanics might be important how these infants respond to viral infections. These studies have been performed using maximal expiratory flow volume curves measured by the rapid thoracic compression technique (RTC) which cannot distinguish between the effects of airway calibre and airway wall properties onto flow limitation.

We have addressed the problem whether information about intra-thoracic airway mechanics and specially airway wall mechanics can be measured independent of airway calibre during bronchial challenge test with a unspecific broncho-constrictor such as methacholine using the new non invasive high speed interrupter technique (Frey et al., 1997(2), 1998(2)). If we find evidence that airway wall properties change during broncho-constriction in infants, the hypothesis that flow limitation at birth in infants who subsequently developed wheezing disorders, might not be purely an expression of developmentally smaller airway, but that at least in some of these infants the airway wall structure might be developmentally different. As shown by Panitch et al. (1992) anatomical and physiological differences in the sheep model would support this hypothesis. They demonstrated that in very young infantile sheep airway wall compliance is much higher than later on in life.

Airway wall properties have never been measured in vivo in human infants. It has been shown in dogs and human adults that high frequency respiratory input impedance measurements contain information about airway wall compliance (Jackson et al. 1987, 1991, 1989, 1993, Lutchen et al. 1987, Habib et al. 1994 (1,2,)). In dogs Jackson et al. (1991) found an anti-resonance at ~ 80 Hz which was related to the lumped interaction between tissue inertance and gas compression compliance and a anti-resonance at ~180 Hz, which was dependent on the density of the inhaled gas. The latter anti-resonance was exclusively found in Zin of human adults. These anti-resonances in adults are due to wave-propagation phenomena and are thus related to inertance of the gas within the airways and the compliance of the airway walls (Jackson et al., 1989) as well as on the length of the airways. High frequency impedance data including wave propagation related anti-resonances (often referred as acoustic anti-resonances) can be analysed using system identification techniques based on the Horsfield-model in dogs (Jackson et al.
1993) and in human adults (Habib et al. 1994). These distributed parameter model can provide information regarding the serial distribution of airway resistance. However, these models cannot currently be applied to infants high frequency $Z_{in}$ data because detailed anatomical information about the branching of the infant bronchial tree is currently not available. Nevertheless, the frequency of the first anti-resonance is only related to airway pathlength, gas density and airway wall compliance. Feature analysis of $f_{ar,1}$ can therefore provide indirect information regarding airway wall properties. This has been shown in adult patients with chronic obstructive lung disease, where $f_{ar,1}$ was increased possibly as an effect of altered airway wall properties (Chalker et al. 1992).

Using the forced oscillation technique Jackson et al. (Jackson et al. 1994) has shown that it is possible to measure high frequency $Z_{in}$ in infants up to 256 Hz, and we have demonstrated that in this frequency range $Z_{in}$ is sensitive to changes in airway mechanics (Frey et al. 1998(1)). With the new high speed interrupter technique (Frey et al. 1997(2)) we were able to measure $Z_{in}$ from 32 to 900 Hz and we have demonstrated that 2 anti-resonance occurred which increased as a function of gas density supporting the concept that these anti-resonances are wave propagation related, similar to the anti-resonances in human adults (Frey et al 1998(2)).

**High frequency $Z_{in}$ measurements in infants during methacholine challenge test.** Since anatomical models such as the Horsfield model in adults are currently not available for the infantile bronchial tree, high frequency $Z_{in}$ data can currently only be described by the analysis of particular features such as the frequency and the amplitude (relative maximum in the real part) of the anti-resonances. We found that frequency and amplitude of the first anti-resonance increase significantly in the group of 10 infants during methacholine challenge test. Furthermore we found that following methacholine challenge, a second anti-resonance was detectable in all the infants, whereas it was only present at baseline in 5 of the subjects. We have therefore shown that high frequency $Z_{in}$ data are sensitive to changing airway mechanics and have validated the usefulness of the high speed interrupter technique.

Since the anti-resonances have been shown to be acoustic in nature, the frequency $f_{ar,1}$ is only a function of mean airway pathlength, and wave propagation velocity which is a function of gas density and airway wall compliance. Assuming the change in frequency would be only due to changing airway pathlength, an increase of 200% in $f_{ar,1}$ (as seen in some of the infants) would cause a shortening of the mean airway pathlength of 50% which seems very unlikely. It is therefore much more likely that airway wall properties have changed due to an increase in smooth muscle tone. Changing airway wall compliance caused an alteration in the propagation
velocity of the pressure waves and changed the first acoustic anti-resonant frequency. We have therefore demonstrated that during methacholine challenge test airway wall properties are very likely to change.

Furthermore, standard deviation score analysis showed that $f_{ar,1}$ changed already at much lower doses of methacholine than $V_{\text{maxFRC}}$ but remained then relatively constant at higher doses of methacholine when $V_{\text{maxFRC}}$ continued to decrease. This indicated that the high speed interrupter technique is much more sensitive than the rapid thoracic compression technique but not at higher provocation doses where the first anti-resonant frequency reached a plateau. The latter fact would furthermore support the hypothesis that the change in $f_{ar,1}$ is mainly determined by changing airway wall properties rather than changing airway pathlength. If the pathlength would change this would probably be related to narrowing or closing airway, in which case we would expect a further decrease during broncho-constriction with higher doses of methacholine.

We hypothesise that at low doses of methacholine the airways increase their smooth muscle tone leading to an increase in $f_{ar,1}$ but potentially facilitates the propagation of flow in the airways, on the other hand beginning broncho-constriction inhibits flow propagation in the airways leading to the summoned effect and results in no change in $V_{\text{maxFRC}}$. At higher doses of methacholine airway wall properties might not change any more (expressed by an plateau in $f_{ar,1}$), but then the effect of broncho-constriction mainly determines flow limitation. If this hypothesis is true, this has important physiological implications, because the quantitative effect of an unspecific broncho-constricting agent will be determined by the airway wall compliance at baseline. We could think of two possible scenarios. Firstly, if the airway wall compliance at baseline is decreased by e.g. increase in wall thickness caused by an chronic inflammatory process, the response to an unspecific broncho-constrictr will be enhanced. Secondly, if the airway wall compliance at baseline is very high (e.g. as shown in very young infants (Panitch et al, 1993), the maximal flow might increase at low doses of a methacholine. We have seen this second scenario in two of the infants in our study. Subject MC who has been born at 30 weeks gestational age, and in subject AE , who only wheezes with upper respiratory tract infections, $V_{\text{maxFRC}}$ improved following low doses of methacholine and subsequently decreased at higher doses of methacholine. In these two subjects we found that $f_{ar,1}$ changed much more than in the other infants, suggesting differences in airway wall compliance.

Two questions remained, firstly why does subject MC first decrease its anti-resonant frequency at low doses of methacholine and secondly why do some infants show effects of saline onto $f_{ar,1}$ ?
To answer the first question, we have to consider the frequency dependence of wave propagation velocity in a compliant tube (Guelke et al. 1981). Wave propagation velocity in a compliant tube depends on the frequency of the travelling pressure wave. This relationship is very complex and the effect of changing airway wall compliance on wave propagation velocity varies dependent on the relationship between frequency of the travelling pressure wave and the resonant frequency of the airway wall. Theoretically the first anti-resonant frequency can decrease with decreasing airway wall compliance, if the airway wall resonance is very different in this infants in comparison to the other infants. We have seen a similar effect when we examined postocclusional pressure transients after flow interruption in school children (Frey et al. 1997(1)). To answer the second question, 0.9% saline is not completely 'physiological'. The Na+ and Cl− content are 150 mmol/L and the pH is not 7. Dependent on the delivery device the solution might become slightly hypertonic when administered. It could therefore well be that the HIT is sensitive enough to detect effect of saline onto the airways. Another possibility which we have ruled out is the fact that the forced expiratory manoeuvres performed between baseline Zin measurements and saline Zin measurements could have altered the properties of the airways. We tested this hypothesis in 4 infants, in which we have performed 10 HIT measurements before and after 10 $V_{max}^{FRC}$ manoeuvres at baseline. The second set was performed even with a loosely fitted squeeze jacket in place. We found small differences in $Z_{in}^{re}(far,1)$ in two of the infants and no differences in $far,1$. however these differences were not systematic, $Z_{in}^{re}(far,1)$ increased slightly in one subject and decreased in the other following rapid thoracic compression.

Why $Z_{in}^{re}(far,1)$ increased during methacholine challenge is currently difficult to explain. Previous studies have shown that $Z_{in}^{re}(far,1)$ changed significantly during histamine challenge in tracheotomised (Habib et al. 1984). However, the situation in dogs is very different from the situation in infants where measurements have to be performed through the upper airways including the face mask. Until detailed anatomical models of the infant bronchial tree are available which will allow to develop distributed parameter models to analyse high frequency Zin, the change in $Z_{in}^{re}(far,1)$ has to considered as an empirical finding derived from feature analysis.

**Summary and clinical implications of the findings.** We have shown that during bronchial challenge high frequency respiratory input impedance Zin measured by the high speed interrupter technique is more sensitive to changes in airway mechanics than $V_{max}^{FRC}$ at low doses of methacholine. With high speed interrupter technique (HIT) high frequency Zin data can be assessed non invasively within seconds at bedside even in unsedated infants (unpublished...
observations). These makes the technique potentially very useful for clinical studies of changes in airway mechanics to monitor inhalation therapy as well as to assess bronchial hyperactivity in larger cohorts for research purposes.

Specially interesting is the fact that \( f_{ar,1} \) is a function of airway wall compliance. We found indirect evidence that airway wall compliance changes during challenge with a unspecific broncho-constrictor. This supports the hypothesis of Prendiville et al. (1987) that airway wall compliance has potentially an important role in flow limitation and wheezing in infants. We have therefore to question whether developmental differences in flow limitation in infants who subsequently develop wheezing disorders are purely caused by smaller airways or whether developmental differences in airway wall structure and therefore wall compliance might also be a possible mechanism that makes these infants more prone to develop wheezing disorders. Based on our findings, we proposed a hypothetical model how airway wall properties and airway narrowing might interact during challenge with a unspecific broncho-constrictor in infants. We hypothesise that at low doses of methacholine the airways increase their smooth muscle tone leading to an increase in \( f_{ar,1} \) but potentially facilitates the propagation of flow in the airways, on the other hand beginning broncho-constriction inhibits flow propagation in the airways leading to the summoned effect and results in no change in \( V_{max}^{FRC} \). At higher doses of methacholine airway wall properties might not change any more (expressed by an plateau in \( f_{ar,1} \)), but then the effect of broncho-constriction mainly determines flow limitation. This potentially implies the quantitative effect of an unspecific broncho-constricting agent will be determined by the airway wall compliance at baseline, which could vary because of developmental differences, but also because of increase in wall thickness caused by an chronic inflammatory processes, in which case the effect of a broncho-constricting agent could be enhanced. However, on the other hand developmental differences (e.g. in premature infants) could also result in a high airway wall compliance. In this group of infants airway wall compliance might cause flow limitation and wheezing even in the absence of airway obstruction. Moreover it implies that broncho-dilators has to be considered carefully in these group of infants bearing in mind that they cause broncho-dilation but also remove the airway smooth muscle tone, which is obviously important for airway stability. High frequency Zin measurements might be useful in future research to detect groups of infants with developmental differences in airway wall compliance and might help to understand why broncho-dilators can have paradoxical effects in some infants.
6.4.6. Figures

Figure 6.4.6.1:

Representative example of the high frequency impedance spectra before (mean ad SD of 10 measurements: closed circles) and after methacholine challenge (mean and SD of 5 measurements: open circles). In this subject the amplitude (Zin\textsubscript{ref}(far,1)) and frequency (far,1) of the first anti-resonance (ar,1) increased and a second anti-resonance (ar,2) which has not been present at baseline developed after methacholine challenge at about 520 Hz.

Figure 6.4.6.2:

The mean amplitude (Zin\textsubscript{ref}(far,1)) and frequency (far,1) of the first anti-resonance (ar,1) increased significantly (p<0.005) following methacholine challenge (defined by a decrease of $V_{\text{maxFRC}}$ of > 30 %.)

Figure 6.4.6.3:

Standard deviation scores (SDS) was calculated for each parameter (far,1: frequency of the first anti-resonance (open circles) and its relative maximum at far,1 (=Zin\textsubscript{ref}(far,1)) (closed triangles), as well as $V_{\text{maxFRC}}$ (closed squares)) at each provocation dose. In 8 infants, these SDS were averaged (+standard error) and plotted against the provocation doses of methacholine, expressed as a fraction of the provocation dose (PD) at which $V_{\text{maxFRC}}$ had changed > 30%. far,1 and Zin\textsubscript{ref}(far,1) did change significantly at much lower provocation doses than $V_{\text{maxFRC}}$. A change in far,1 must reflect changes in airway wall properties at low doses of methacholine when flow limitation does not yet occur, however, at higher doses of methacholine it remained relatively stable, while $V_{\text{maxFRC}}$ further decreased.

Figure 6.4.6.4:

Standard deviation scores (SDS) was calculated for each parameter (far,1: frequency of the first anti-resonance (open circles) and its relative maximum at far,1 (=Zin\textsubscript{ref}(far,1)) (closed triangles), as well as $V_{\text{maxFRC}}$ (closed squares)) at each provocation dose in subject AE (a) and MC (b). In both subjects AE with episodic wheeze and MC with wheeze shortly after birth following
prematurity, did show an improvement of $V_{\text{max}}^{\text{FRC}}$ following saline and low doses of methacholine accompanied by an unusually large change in $f_{\text{ar,1}}$ and $Z_{\text{in,}(f_{\text{ar,1}})}$. We hypothesise that these infants had developmentally different airway wall compliance, so that a small increase in airway smooth muscle tone might have improved the flow properties of the airways.
Figure 6.4.6.1:

![Graph showing the real and imaginary parts of the admittance vs. frequency for two different models, ar,1 and ar,2.](image)

- Real part (cmH2O L^-1 s)
- Imag part (cmH2O L^-1 s)
- Frequency (Hz)
Figure 6.4.6.2:

Before and after methacholine challenge
Figure 6.4.6.3:

Change from baseline in SDS

Methacholine Challenge

Baseline saline 1/8 PD 1/4 PD 1/2 PD PD
6.4.7 Tables

Table 6.4.7.1. Physical characteristics of subjects

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Table 6.4.7.3a: measurements following methacholine challenge

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6.5. Alterations in airway wall properties in infants with a history of wheezing disorders.


6.5.1. Abstract

Both airway diameter but also airway wall mechanics (compliance) are important determinants of flow limitation and wheezing. Using the high speed interrupter technique (HIT) we have previously measured input impedance (Zin) in infants at frequencies of up to 900 Hz, including anti-resonant phenomena which are known to related to wave propagation velocity and shown that the frequency \( f_{ar,1} \), at which the first anti-resonance occurs, is a function of airway wall compliance. We aimed to determine whether \( f_{ar,1} \) (and thus airway wall compliance) was different in infants with a history of wheezing disorders. We compared 24 asymptomatic infants (aged 36 to 81 weeks) with a history of wheezing with an age-matched group of 18 healthy controls. We found that \( f_{ar,1} \) was significantly lower in infants with wheezing disorders than in the control group (\( p<0.005 \)), implying differences in airway wall compliance, even when they were clinically asymptomatic. Developmental differences in airway wall mechanics may be important in the pathogenesis of wheezing disorders or alterations in airway wall mechanics might be a consequence of post-inflammatory remodelling.
6.5.2 Introduction

Developmental differences in airway wall properties rather than airway calibre (Martinez et al. 1988, Clarke et al. 1992, Tager et al. 1993, Brown et al. 1995) could be at least partly responsible for the association between excessive flow limitation and subsequent wheezing disorders in infants. The implication of this hypothesis is that a technique to measure airway wall properties may enhance our understanding of airway stability in the developing lung and of often unpredictable responses to bronchoactive drugs.

The aim of this study was to use the high speed interrupter technique (HIT) to determine whether $f_{aw}$ was different in asymptomatic infants with a history of wheezing disorders in comparison to age matched healthy controls. If true, not only airway narrowing but also alterations in airway wall compliance might be associated with wheezing disorders in infants. This has not previously been demonstrated in vivo in human infants.
6.5.3. Methods

Study subjects. The study was performed in 23 infants and young children (aged 36-81 weeks) with a history of episodic or recurrent cough or wheeze, who had been referred from the outpatient clinic for lung function tests for clinical purposes (Table 6.5.7.1). 16 infants suffered from recurrent wheeze both with and without upper respiratory tract infections (URTI) whereas 7 infants only had a history of episodic wheeze with URTI. Infants with other specific diseases and infants with upper respiratory tract infection within the previous 3 weeks were not included in the study. Similarly measurements were done in 19 healthy infants of similar age. Twenty-five of the 42 infants and toddlers were sedated using a single oral dose of triclofos sodium up to 150 mg/kg. Other infants were studied during natural sleep. Lung function was measured during periods of regular quiet breathing in the supine position. The HIT measurements were approved by the Ethics Committees of the Royal Postgraduate Medical School, Hammersmith Hospital, London and of the Leicestershire Health Authority, Leicester, UK where the measurements were performed. Written consent was obtained from parents.

Experimental protocol. The principles and technical details of the HIT technique have been described previously (Frey et al. 1997(2), 1998(2,3)). Briefly, high frequency respiratory input impedance was measured using a propeller valve that rapidly occluded the airway opening several times within a period of 0.15 seconds at the beginning of inspiration without disturbing the infant. The resulting pressure and flow oscillations were measured using the wave tube technique (sections 3.3.4. and 5.1.5.). Measurement conditions and monitoring were described in sections 5.3.3. and 5.3.4. The dead space in the face mask was reduced by partially filling it with putty. For 19 subjects of the subjects it was possible to reliably measure the residual face mask dead space by water displacement. The mean dead space volume (SD) was 6.12 (SD1.7) ml. There was no difference in deadspace volume between healthy and wheezy infants in this subgroup.

In all infants we performed 7-10 Zin measurements (1 set) during quiet regular tidal breathing. In 10 healthy infants we performed 2 sets of measurements on two different days within the same week to test repeatability. Impedance measurements with a coherence below 0.9 were not accepted (Frey et al. 1998(2)). From the impedance spectrum, we extracted the frequency of the first anti-resonance (far,1), defined by an zero crossing in the imaginary part in the presence of a relative maximum in the real part of the impedance spectrum. Since this report concerns airway wall properties, we do not report other aspects of the Zin spectrum.
Data analysis. In all infants we calculated the mean and standard deviation of the sets of far, l. For the repeatability test in 10 infants, we determined the 95% confidence intervals (CI) of the differences of the corresponding paired mean values of far, l. We then compared the two measurement sets by using a paired t-test. The mean age, weight and far, l values for the infants in the wheezing group and the healthy control group were compared by t-tests, and the group gender distribution was compared using $\chi^2$-tests.
6.5.4 Results

The healthy and the wheezy group were not significantly different in age, weight and gender. Since we were not allowed to sedate the healthy infants, the distribution of sedated to non-sedated infants was unbalanced in the two groups (table 6.5.7.1).

We were able to detect anti-resonant frequencies in all subjects. A representative example of the mean (SD) /Zin spectrum for one subject is shown in figure 6.5.6.1. Repeat values of /far,1 regressed to the group mean but there was no systematic difference between the two sets of repeated measurements in 10 healthy infants: first set: mean (95% CI) /far,1 = 220 Hz (9.5), second set: /far,1 = 215 Hz (27.6), (paired t-test: p = 0.68). However, the 95% CI for the differences of sets observations of /far,1 was high at ± 27.3 Hz, indicating marked day to day variability.

The mean (95% CI) first anti-resonance in wheezy infants occurred at a lower frequency (175.7 (+14.1) Hz, p<0.005) than in the healthy control group (212.1 (+21.1)Hz) (table 6.5.7.1, figure 6.5.6.2). When divided into subgroups of wheezers, the values of /far,1 for 16 recurrent wheezers (179.6 (+18.0) Hz) and 7 episodic wheezers (166.6 (+28.1) Hz were significantly different from the healthy infants (p<0.05 and p<0.02) respectively, but not from each other.
Developmental differences in airway function are associated with subsequent development of wheezing disorders in infancy ((Tepper al. 1986, Martinez et al. 1988, Clarke et al. 1992, Tager et al. 1993, Brown et al. 1995). Lower values of $V_{\text{maxFRC}}$ measured by the rapid thoracic compression technique (RTC) in early infancy have been interpreted as due to smaller airway calibre (Martinez et al. 1988, Clarke et al. 1992, Tager et al. 1993, Brown et al. 1995). But during flow limitation, no analysis of maximal expiratory flow volume curves can distinguish between the effects of airway calibre and airway wall compliance on flow limitation. It might be especially important to distinguish these effects in infants since, as has been demonstrated in the lamb by Panitch et al. (1992), that airway wall compliance is much higher than in later life and may lead to greater airway collapsibility. In order to understand the developmental basis for wheezing disorders, it is thus important to devise a technique featuring parameters which are related to airway wall compliance independently of airway calibre. We have developed such a technique, based on the measurement of high frequency respiratory impedance ($Z_{\text{in}}$) in infants using the high-speed interrupter technique (HIT).

High frequency respiratory impedance is influenced by the properties of airway walls as shown in dogs (Jackson et al. 1987, 1993) human adults (Habib et al. 1994(1,2), Jackson et al. 1989) and infants (Jackson et al. 1996, Frey et al. 1998 (1-3)). In human adults and infants a particular feature of the high frequency Zin, the anti-resonance is related not to tissue properties of the lung but to wave propagation phenomena in the airways (Frey et al. 1998(2), Jackson et al. 1989). This implies that the frequency at which the first anti-resonance occurs ($f_{\text{ar,l}}$) is a function of the factors determining wave propagation phenomena and the boundary conditions of the airways, that means the fact whether the airways behave like an open or a close system at the distal end. Wave propagation velocity in a complaint tube is related to gas density, airway path-length and airway wall compliance and to a minor extend to airway diameter in very small tubes.

The influence of airway diameter on wave propagation velocity can be described as follows as described in section 3.3.7-9. In large-diameter rigid tubes waves will propagate at the free-field speed of sound, independent of tube diameter. In rigid tubes with diameters $<0.4$ cm, wave propagation velocities with room air are decreased by $>5\%$ (Benade, 1959). In the Horsfield et al. (1982) airway model, airways distal to the 27th order have diameters $<0.4$ cm in human adults. In the terminal airways where the diameter is 0.08 cm, the wave propagation velocity is 21,400 cm/s or 62% or the free-field speed of sound. Thus in airways between the 26th and first order (terminal airways), wave propagation velocities are significantly reduced. A
reduction in the wave propagation velocities in these distal airways would cause them to resonate at a lower frequency.

However, the influence of airway wall compliance is much more important in infants. We have previously demonstrated in infants that far,1 increases following methacholine challenge (Frey et al. 1998(3)). This could hardly been explained by changes in path-length or gas density or decreasing airway diameter, and must therefore have been to be due to change in airway wall compliance, perhaps due to increasing airway smooth muscle tone during the stepwise challenge procedure. If the effect of airway diameter on far,1 were important, far,1 would decrease and not increase as seen during the methacholine challenge. It is also important to note that far,1 is independent of wide changes in upper airway calibre (Frey et al. 1998(2)).

We aimed to determine whether far,1, hence airway wall compliance, was different in asymptomatic infants with a known history of wheezing and in healthy children. We found that far,1 was significantly lower in wheezy infants than in age matched healthy controls. Both subgroups, the recurrent wheezers as well as the infants who only wheezed episodically with viral respiratory tract infections, had lower mean values of far,1. However, far,1 was not different between recurrent and episodic wheezers. These data suggest, that infants with wheezing disorders have differences in airway wall compliance, even when they are asymptomatic at the time of measurement. It is unlikely that differences in far,1 could be explained by changes in airway path-length since age and size were not significantly different between groups. From the current data it cannot be concluded whether the lower values of far,1 correspond to lower or higher airway wall compliance, since the interaction between far,1 and wave propagation is highly complex (Guelke et al. 1981). We can only conclude that it is different. We hypothesise that these differences might be either acquired due to post-viral inflammatory changes in wall mechanics or due to developmental differences in wall mechanics. If they are acquired we can conclude that they even persist in the absence of obvious wheezing symptoms.

Our findings contrast with those of Chalker et al in adults (Chalker et al. 1992), who found far,1 to be higher in chronic obstructive pulmonary disease (COPD). They also hypothesised that airway wall compliance might be different in this group of patients. Again, this hypothesis is based on the fact that a decrease of airway diameter would result in an decrease in wave propagation velocity and therefore a decrease in far,1. Thus an increase in far,1 is likely to be an expression of changing airway wall compliance. We can think of two possible explanations for the increase in far,1 in COPD, in contrast to a decrease in infants with wheezing disorders. Firstly, the underlying pathology of COPD might be very different from that in wheezy infants.
An alternative explanation is the complex relationship between anti-resonant frequency, wave propagation velocity and the resonance frequency of the airway wall (Guelke et al. 1981). Dependent on whether $f_{ar,1}$ is higher or lower than the wall resonant frequency, alterations in wall compliance could lead to an increase or decrease in $f_{ar,1}$ (Guelke et al. 1981). In adults this relationship might differ from that in infants and might also explain why methacholine challenge resulted in an increase in $f_{ar,1}$ in individual subjects (Frey et al. 1998(1,3)) whereas as a group, wheezy infants have lower $f_{ar,1}$ than healthy controls at baseline.

**Limits of the method.** To improve readability we have only focused on $f_{ar,1}$. For this parameter, we have a theoretical model of its physiological meaning which allows us to test our initial hypothesis. We have not described features of the Zin spectrum between 32 and 100 Hz, since in previous work we have not found the frequency range to be closely related to changes in airway mechanics in infants (Frey et al. 1998(1,3)). Similarly, although the relative maximum in the real part of Zin at $f_{ar,1}$ is sensitive to changes in airway mechanics (Frey et al. 1998(1,3)), its physiological meaning in infants is so far unresolved, and we have not focused on it in this paper.

In previous work we have reported that short term variability of $f_{ar,1}$ (coefficient of variation of 10 measurements) was between 5 and 10% in sedated wheezy infants (Frey et al. 1998(2)). The current study showed that $f_{ar,1}$ varies more widely than this from day to day in healthy infants. However, variation was random and was unlikely to be relevant to this cross-sectional study. It indicated that the technique may not be especially useful for longitudinal measurements in individual subjects, for instance for clinical purposes.

Since previous work has shown that the characteristics of the face mask can influence $f_{ar,1}$ (Jackson et al., 1994, Frey et al. 1998(1-3)), great care was taken to minimise its volume and standardise the application of the facemask. This could be especially crucial in small newborn infants with a short airway path-length, since theoretically the facemask deadspace will have a significant effect on $f_{ar,1}$ by increasing parallel impedance (Jackson et al., 1994). The face mask volume was small (mean 6.1 ml). Even more important was the standardisation of the procedures, which reduced the variation in the face mask deadspace between subjects (group SD = 1.9 ml) and which thus avoided systematic bias between healthy and wheezy infants.

While both groups were not significantly different in age and gender, more measurements were made during natural sleep than during sedated sleep in the healthy group. Sedated or not, we only performed measurements during quiet sleep, as judged by regular breathing, and the absence of rapid eye or limb movements. While this only partly resolves the issue, one must
consider that in infants the sleep stage is very difficult to classify, whether or not sedation is used. Furthermore, the influence of sleep stage and sedation on lung mechanics in infants is not yet fully resolved.

Summary and hypothesis for future research. There was a significant difference in high frequency respiratory input impedance (Zin) between infants with wheezing disorders who were asymptomatic at the time of measurement and age matched controls. This was true for a particular feature of the Zin, the first anti-resonant frequency $f_{ar,1}$ which is known to be related to wave propagation velocity in the airways and to airway wall compliance. However, there was no significant difference in $f_{ar,1}$ between recurrent and episodic wheezers. Although Zin measurements give only indirect information on airway wall compliance, they can be assessed non-invasively in vivo, which is a major advantage since airway mechanics is crucially influenced by such factors as the elastic recoil of the surrounding tissue which exerts its effect only on airways in situ. This is therefore the first evidence that airway wall compliance in vivo is different in infants suffering from wheezing disorders, even when they are asymptomatic. Moreover, it allows the interpretation of forced expiratory flow volume curves by the RTC technique, to be more fully interpreted in infants.

Our findings have important implications for the study of airway structure-function relationships in wheezing disorders in infants. In future, studies of both airway calibre airway wall structure and mechanics will be needed. The current study design does not allow to distinguish whether these changes in airway wall compliance are acquired (e.g. by inflammatory remodelling) or whether they pre-existed the onset of wheeze. Prospective studies, commencing shortly after birth, before inflammatory injury occurs, will help to distinguish between (fetal) developmental determinants of airway function and postnatal remodelling.
6.5.6 Figures

Figure 6.5.6.1:
Example of the high-frequency impedance spectrum from a single infant (mean and SD of 10 measurements). The anti-resonant frequency ($f_{ar,1}$) is defined by the relative maximum in the real part ($Z_{in}(f_{ar,1})$) in the presence of a zero crossing in the imaginary part.

Figure 6.5.6.2:
Group mean and 95% confidence intervals (CI) of the averaged $Z_{in}$ spectra for the infants with wheezing disorders (circles) and the healthy infants (triangles). The frequency ($f_{ar,1}$) was significantly lower in wheezing infants than in healthy infants ($p<0.005$).
Figure 6.5.6.1:

- **Coherence**
- Imag. part (kPa \( \cdot \) L\(^{-1} \cdot \) s)
- Real part (kPa \( \cdot \) L\(^{-1} \cdot \) s)

Frequency (Hz)

200
400
600
800

0
1
2
3
4
5
6
7
8
9
10
12
### Table 6.5.7.1: Biometric data.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gender</th>
<th>Sedation</th>
<th>Age (±SD) (weeks)</th>
<th>Weight (±SD) (kg)</th>
<th>mean fαr,1 (±95%CI) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>19</td>
<td>7/12</td>
<td>18/0</td>
<td>45.4 ± 22.8</td>
<td>8.9 ± 2.5</td>
<td>212.1 (±21.1)</td>
</tr>
<tr>
<td>Wheezy:</td>
<td>23</td>
<td>15/8</td>
<td>7/16</td>
<td>55.4 ± 26.6</td>
<td>10.1 ± 1.8</td>
<td>175.7** (±14.1)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>16</td>
<td>9/5</td>
<td>4/12</td>
<td>56.2 ± 27.3</td>
<td>10.5 ± 1.9</td>
<td>179.6* (±18.0)</td>
</tr>
<tr>
<td>Episodic</td>
<td>7</td>
<td>4/3</td>
<td>3/4</td>
<td>53.5 ± 26.8</td>
<td>10.4 ± 1.8</td>
<td>166.6* (±28.1)</td>
</tr>
</tbody>
</table>

The groups of wheezy infants were not significantly different from the healthy group in age and weight and gender. m: male, f: female, ns: natural sleep, ss: sedated sleep, * p<0.05, **p<0.005
7. CONCLUSIONS

7.1. Synthesis

7.1.1. Airway physiology in healthy infants

In healthy infants, the somatic growth that occurs during the first year of life is accompanied by major developmental changes in respiratory physiology, including changes in the shape compliance and deformability of the rib cage. The highly compliant chest wall of the newborn infant gradually stiffens during the first year of life. Infants also modulate expiratory flow in order to dynamically elevate functional residual capacity above the level passively determined by the outward recoil of the chest wall and the inward recoil of the lung, an important strategy to establish and maintain an adequate lung volume in the presence of a highly compliant chest wall. Transition to a more relaxed pattern of expiratory flow occurs between 6-12 months of age. Another consequence of a highly compliant chest wall is an increased tendency of the peripheral airways to close during tidal breathing in early infancy. This dynamic interaction between elastic recoil of the lung tissue and the airway patency is furthermore enhanced by the increased airway wall compliance in infants in comparison to adults. Several morphological studies showed that airway wall compliance in newborns is much higher than in adults leading to more flow limitation. While, there are marked differences between species, animal work in lamb suggests that this is due to developmental differences in tracheal cartilage composition but also differences in airway smooth muscle tone and longitudinal tension. Smooth muscle tone is modulated by a balance between slowly adapting receptors, which normally evoke smooth muscle relaxation, and rapidly adapting receptors, which normally promote cough and broncho-constriction. A important local mediator of neuro-muscular interaction is nitric oxide (NO). Recently, it has been emphasised that the baseline airway smooth muscle tone may be predominantly directed by local concentrations of nitric oxide (NO) (e.g. Gaston et al. 1994, Van der Velden et al. 1999) (see also 7.2.8.). In summary, airway wall compliance in infants is regulated by both airway smooth muscle tone as well as differences in the composition of fibres and cartilage in the walls.

7.1.2. Airway wall mechanics in inflammatory airway disease and wheezing disorders

In inflammatory infant airway disease, these physiological differences enhance the effect of the disease process in comparison to adults. Airway inflammation will increase and potentate the effect of smooth muscle shortening on airway resistance, because the coupling between smooth muscle and slowly adapting receptors is influenced by the mechanical properties of the cartilage of the large airways, elastic recoil of the lung-chest wall system, as well as the
relatively reduced number of rapidly adapting receptors. Any given increase in airway resistance may reflect differing combinations of altered baseline airway wall compliance, airway smooth muscle shortening and relative thickness of the airway walls due to inflammation, resulting in different patterns of airflow limitation. Baseline airway wall compliance will be determined by baseline airway smooth muscle tone, which is influenced by local concentrations of NO. NO is not only produced by the constitutive nitric oxide synthesis (cNOS) but also by the inducible nitric oxide synthesis (iNOS), which is present in epithelial cells as well as monocytes and macrophages, and which can be stimulated by pro-inflammatory cytokines. Thus, during airway inflammation baseline smooth muscle tone might be altered. While there is clear evidence that airway responsiveness is present from birth, the contribution of pre-existing alterations in airway wall mechanics and airway geometry to wheezing LRIs in the infant and young child is not known.

7.1.3. Impact of airway wall mechanics on airway pharmacology

Pharmacological studies have stressed the importance of airway wall compliance in wheezing disorders. While broncho-dilators are capable of reversing broncho-constriction due to smooth muscle activation, they may have more complex or even deleterious effects in cases with developmental disturbances of airway mechanics. Pharmacological studies have shown that broncho-dilators might not only affect airway diameter in infants but might increase flow limitation by removing airway smooth muscle tone and therefore increasing airway wall compliance.

7.1.4. Non invasive estimate of airway wall compliance (aim 4.1)

Previous literature has demonstrated that airway wall mechanics play a crucial role in the development of flow limitation in the airways and in wheezing disorders, however, there has been no method to assess airway wall compliance non-invasively in the quietly breathing infant. A indirect estimate of airway wall compliance could be derived from high frequency respiratory impedance measurements. We demonstrated that such measurements were possible even in unsedated infants during natural sleep. As described above, airway wall compliance is influenced not only by fibres and cartilage of the walls, but also dynamically by the baseline smooth muscle tone and by elastic recoil of the surrounding tissue and thus lung volume. Classical measurement of airway wall compliance in excised airways will not measure the contribution of the in vivo baseline smooth muscle tone and the effect of the surrounding tissue. The work in this thesis presents for the first time a method able to estimate non-invasively an estimate related to airway wall compliance in infants in vivo.
From previous work in adults and animal models, it is known that high frequency impedance measurements derived from oscillatory mechanics are governed by wave propagation phenomena. Wave propagation phenomena represent the basic principles influencing flow limitation phenomena. Wave propagation is affected little by airway diameter in large airways, but more by airway wall mechanics and by the boundary conditions of standing waves in the airways. Up to now there are little data concerning high frequency impedance and frequency dependence of wave propagation in infant airways. In this thesis we developed oscillatory lung function techniques that allow the non invasive assessment of high frequency respiratory input impedance in infants. The \textit{forced oscillation technique for use in infants} and newly the \textit{high speed interrupter technique} are both capable of measuring high frequency respiratory input impedance in infants.

7.1.5. Technical implications of high frequency Zin measurements in infants

The forced oscillation has been described previously, but this is the first time that it has been used at frequencies up to 256 Hz in infants during a methacholine challenge test (aim 4.1.1). The FOT has the advantage that the pseudo-random input signal does not induce non-linear behaviour of the respiratory system, as the amount of energy in the input pressure signal at each frequency is well defined. However, the technique has the disadvantage that the loudspeaker pressure generator has got a large dead space volume which acts as a parallel shunt impedance particularly at high frequencies and in the presence of the relatively large impedance of the infant respiratory system. This shunt impedance causes small oscillatory flows in the respiratory system and consequently signal to noise problems. A second problem consisting of the interaction of oscillatory flows and DC-flows in the respiratory system during tidal breathing also exists (see also 7.2.3.). In order to eliminate these problems, we created a new physiological concept and measured high frequency respiratory input impedance (Zin) using the interrupter technique (aim 4.1.2). The high speed interrupter technique measured the high frequency Zin in healthy human adults similar to the forced oscillation technique (aim 4.1.4), however, in infants the high speed interrupter technique has potential advantages. The potential advantage of measuring Zin using the interrupter technique is that the device has a small dead space, the shunt impedance is lower and the flow amplitudes at higher frequencies are better, leading to a better signal to noise ratio. In order to measure high frequency respiratory impedance, it is optimal for the forcing function to contain a sufficient amount of energy at high frequencies. The faster the flow interruption is, the higher the energy content of the flow power spectrum. To boost the high frequency content of the forcing function we developed a particularly fast airflow interrupter, which we called the
high speed interrupter technique (HIT). A second potential advantage of the HIT is the fact that during airflow interruption there is no DC-flow but only oscillatory flow. Thus potentially the HIT is capable of measuring respiratory impedance which is not influenced by unidirectional flow. However, this has to be tested in detail in future studies. The HIT has also disadvantages. The non-linear behaviour of the load impedance (the respiratory system) is a particularly delicate problem when the high speed interrupter technique is used. The non-linear behaviour is induced if the respiratory system is forced (input function) by high amplitude pressures. In the interrupter technique the inspiratory flow and the impedance of the respiratory system determine the pressures in the wave tube at every frequency. For the high speed interrupter technique we had to limit the maximal flows and pressures in the wave tube. Such non-linearities occur predominantly around anti-resonances where abrupt changes in the impedance of the respiratory system occur. This phenomenon was confirmed by decreased coherence values around anti-resonances. A further potential problem is the signal to noise ratio due to vibrations of the mechanical valve closure (see also 7.2.2.), however contamination of the signal by extraneous noise was well detectable by a decrease in coherence function. We conclude that the coherence function provides an excellent means for measurement quality criteria and should strictly be used with the HIT (coherence > 0.9-0.95).

7.1.6. Physiological implications of high frequency Zin measurements in infants

We have shown that high frequency Zin in infants contains anti-resonances whose frequency is repeatable (aim 4.1.3). In most infants we only found a first anti-resonance, and in some infants we found a second anti-resonance approximately three times higher than the first anti-resonance. Our findings support the hypothesis that anti-resonances are caused by wave propagation phenomena in the airways, as found in human adults (aim 4.1.5). Similar to published experimental work in human adults, we also found that the anti-resonant frequency increased by a factor two when a gas of lower density (He02) was inhaled. This could not be explained either by the lumped interaction of gas compression compliance (Cg) and tissue intertance (Iti) (DuBois model). By using system identification techniques, we have shown that a lumped lung model cannot explain high frequency Zin data, probably due to airway wall compliance becoming increasingly important at higher frequencies. These findings are essential, as the first anti-resonant frequency is therefore related to airway path length and wave propagation which is a function of airway wall compliance.
7.1.7. Contribution of airway wall mechanics to bronchial reactivity in infants (aim 4.2.)

As discussed above, bronchial reactivity is influenced by the particularities exhibited by infants respiratory physiology. We found that during methacholine challenge high frequency Zin changed significantly. In particular the first anti-resonant frequency increased in all infants.

During methacholine challenge a decrease in diameter as well as a change in airway wall compliance is expected to occur. Broncho-constriction exhibiting a decrease in airway diameter would decrease the wave propagation velocity and therefore the first anti-resonant frequency. However, during the methacholine challenge we did not find a decrease but rather an increase in the first anti-resonant frequency, indicating a major change in airway wall compliance during the methacholine challenge.

These results support the hypothesis that airway wall compliance plays an important role in the observed flow limitation in infants. We found indirect evidence that airway wall compliance changes during challenge with a unspecific broncho-constrictor. Based on our findings, we conclude that differences in flow limitation in infants who subsequently develop wheezing disorders may not be purely caused by smaller airways but by differences in airwall compliance.

Based on our findings, we proposed a hypothetical model how airway wall properties and airway narrowing may interact during a challenge with an unspecific broncho-constrictor in infants. We hypothesise that at low doses of methacholine the airways increase their smooth muscle tone leading to an increase in $f_{ar}$, but potentially facilitate the propagation of flow in the airways, on the other hand, beginning broncho-constriction inhibits flow propagation in the airways leading to the summoned effect and results in no change in forced expiratory flows ($V_{max}^{FRC}$). At higher doses of methacholine airway wall properties might not change any more, but then the effect of broncho-constriction mainly determines flow limitation.

This potentially implies the quantitative effect of an unspecific broncho-constricting agent will be determined by the airway wall compliance at baseline, which could vary because of developmental differences, but also because of increase in wall thickness caused by an chronic inflammatory processes, in which case the effect of the broncho-constricting agent could be enhanced. However, on the other hand developmental differences (e.g. in premature infants) could also result in a high airway wall compliance. In this group of infants airway wall compliance might cause flow limitation and wheezing even in the absence of airway obstruction. Moreover it implies that broncho-dilators has to be considered carefully in these group of infants.
bearing in mind that they cause broncho-dilation but also remove the airway smooth muscle
tone, which is obviously important for airway stability. High frequency Zin measurements may
be useful in future research to detect groups of infants with developmental differences in airway
wall compliance and may help to understand why broncho-dilators can have paradoxical effects
in some infants.
7.1.8. Airway wall mechanics in disease (aim 4.3.)

Since airway wall mechanics seem to play an important role in bronchial reactivity in infants, the question arises whether or not infants prone to wheezing disorders have particular airway mechanical properties. Airway wall mechanics could be altered either by developmental differences or by acquired post-inflammatory remodelling of the airway walls with differences in fibre or cartilage content. In addition, the presence of a chronic inflammation will increase the levels of NO in the airways and may alter baseline airway smooth muscle tone. We investigated high frequency impedance measurements in age matched groups of healthy infants and infants with wheezing disorders, which were asymptomatic at the time of the measurements. In cross-sectional measurements, the frequency of this anti-resonance was significantly reduced in infants with a history of wheezing disorders in comparison to healthy infants.

Since the boundary conditions and the effective mean airway path length are likely to be similar in age matched groups of infants, the data support the hypothesis that airway wall mechanics are different in these groups of infants. Theoretically, broncho-constriction could also reduce wave-propagation velocity and decrease the first anti-resonant frequency in the airways. However, our results of the bronchial provocation test with methacholine suggest, that the influence of the airway wall mechanics on $f_{ar,1}$ is much larger than the effect of a reduction of airway calibre during the challenge, because changes in $f_{ar,1}$ occurred very early during the challenge test. Despite a significant decrease in maximal flow in these airways (as an expression of increasing broncho-constriction) at higher doses, there was no further change in $f_{ar,1}$ at these higher doses (plateau). These data support the hypothesis that airway wall mechanical properties in infants prone to wheezing are different from the healthy control group.

These findings are important and give new insight into the structure function relationship of airways of infants who are prone to wheezing.
7.2. Implications for future research

Future research needs to address issues arisen from this thesis. They are either related to current technical limitations (7.2.1., 7.2.2., 7.2.4.) and physiological limitations (7.2.4., 7.2.5) or potential applications (7.2.3., 7.2.6.-7.2.9.).

7.2.1. Technical improvement of the forced oscillation technique

As previously described, the FOT has the disadvantage that the loudspeaker pressure generator has got a large dead space volume, which acts as a parallel shunt impedance, particularly at high frequencies and in the presence of a the relatively large impedance of the infant respiratory system. This shunt impedance causes small oscillatory flows in the respiratory system and consequently signal to noise problems. In order to overcome these problems, high energy, low amplitude, low dead space pressure generators need to be used in the measurement of high frequency impedance data. In preliminary high frequency impedance measurements in neonatal rabbits with a extremely high load impedance, we have used compression drivers (usually used in megaphones) and were able to generate impedance data from 500 to 2000 Hz with a coherence above 0.95. While these compression drivers are useful for Zin measurements in very small animals, they are not suitable for measurements in infants since their performance under 500 Hz is limited.

7.2.2. Technical improvement of the high speed interrupter technique

One of the big disadvantages of the current high speed interrupter device is the stepper motor which drives the interrupter blade. This motor causes extraneous noise due to mechanical vibrations, decreasing the coherence of the data, particularly when the device is mounted to a supporting arm structure. We have developed a second generation prototype based on to magnetic pistons which open and close the airway opening within 2-3 ms. This device has no motor vibrations but produces an acoustic noise when the magnets return into their default position. This noise wakes up the infants. We currently try to dampen this noise. However, the final version will be a combination of the two principles. We will return to the system of a turning blade, which is driven by a fast electro-magnetic field.

A further improvement consists of the automated adjustment of flows and pressures, so that high pressures and flows are omitted by improved trigger mechanisms.
7.2.3 Technical implications of the new high speed interrupter technique

Because the oscillatory frequency is far beyond the normal physiological range and the amplitude is extremely small compared with spontaneous breathing, the respiratory system is thought to respond passively and linearly to forced oscillations. This assumption appears specious, if one considers the typically nonlinear aerodynamic behaviour of the spontaneous breathing flow due to, e.g., developing flow effects at bifurcations and turbulence in main bronchi (Pedley TJ and Drazen JM, 1986). Indeed, if oscillatory and constant flows were to physically interact, the above linearity assumption would become questionable. The frequency response of the respiratory system, e.g., the frequency dependence of respiratory impedance, might then become dependent on the flow generated during spontaneous breathing. The problem of the interaction between oscillatory and steady unidirectional flows has already been studied for laminar steady-flow conditions by Dorkin et al. (1982) and Franken et al. (1981). Surprisingly, this problem has also been considered for turbulent flow conditions (Louis B and Isabey D 1993) since breathing flow is not necessarily maintained in the laminar regimen. Especially in trachea and main bronchi spontaneous breathing flow is likely to be turbulent and quasi-steady. These investigators have demonstrated that high frequency oscillatory flows, as used in this work, are not likely to be affected by turbulent flow in the airways, however for lower frequencies this will be the case. Some investigators (Sly et al 1996) have performed forced oscillation measurements in apnea, however the majority of oscillatory measurements at lower frequencies have been performed during spontaneous breathing. While using the interrupter technique the DC-flow component during airflow interruption is zero by definition. During interruption there is only oscillatory flow in the airways. Zin measurements using the interrupter technique might therefore be less influenced by turbulent DC-flow. Future studies demonstrating that this is the case, need to be conducted.

7.2.4 Distributed parameter models

The main physiological limitation of the high frequency Zin measurements is that Zin only provide indirect determination of airway wall mechanics resulting in semi-quantitative inferences of airway wall mechanics in infants. So far, there is no direct way of calculating airway wall compliance from high frequency impedance data in infants.

The analysis of anti-resonant frequencies is based on simplified models of the airways including the upper airways. Thus limiting the conclusions which can be drawn concerning airway wall properties. These models discussed in sections 3.3.2-3. and 6.1. (Lumped
parameter models, e.g. DuBois model) deal with pressures and flows at specific system boundaries or nodes such as the airway opening, alveolar spaces, and body surface. In these models the morphological characteristics of airways are bypassed a priori by assignment of extensive dynamic variables. These variables include airway inertance and resistance, which lump together the entire spatial extent of the upper airway and tracheo-bronchial tree. To calculate airway wall compliance more sophisticated lung models are needed. A spatially distributed model is one in which spatial dimensions, co-ordinates and branching topology are dealt with explicitly. Pressures and flows are computed not only at convenient nodes such as alveolar pressure or airway opening pressure but also at every position in every airway in between. Even parenchyma itself is no longer described by a single alveolar pressure but rather by pressure that could be different from point to point throughout. An airway of length L may be divided into infinitesimal segments of length dx. The equivalent circuit for such a segment incorporates airway inertance (law) and viscous resistance (Raw) as series elements. Shunt elements can be subdivided into the gas compressibility pathway, consisting of gas compressibility (Cg) and gas thermal conductance (Gt), and the wall distension pathway, consisting of airway wall elastance (Eaww), inertance (Iaww), and resistance (Raww). The impedances, pressures, and flows of these small segments dx must be marched along airways between bifurcations and combined among airways at bifurcations. In the adult lung proposed computational schemes (system identification techniques) have been proposed to execute the repeated application of these equations (which describe the mechanical properties of a segment dx) for a relatively general class of asymmetrically branching models of the tracheo-bronchial tree (Horsfield et al. 1982). Based on these models they have been able to calculate means of serial distribution of airway resistance as well as airway wall mechanical properties. However, such models or anatomical data in infants are currently not available, thus Zin information on airway wall mechanics can at the moment only be assessed qualitatively and not quantitatively. In future research more sophisticated distributed parameter models need to be used in order to quantitatively calculate airway wall impedance. However, in order to use these models, more detailed anatomical data of the infant bronchial tree have to be established. Only when such models are available, can the effect of the boundary conditions of the airways (effects of terminal alveolar space + tissue compliance) as well as the serial distribution of the airway wall diameter be taken into account. We believe the current research is valid, based on currently available models and anatomical data, but has to be confirmed in the future using more detailed analysis.
7.2.5. The influence of the upper airways

A further limitation is the influence of the upper airways, which always contribute to the input impedance measurements in infants. In order to eliminate the influence of the upper airways, high frequency pressure oscillations need to be applied either at the chest wall (transfer impedance technique) or a head generator needs to be used (Marchal et al. 1989). We consider the latter technique as disturbing and potentially dangerous, since the acoustic load to the head will reach levels of approximately 80 dB, which could endanger the infants' hearing. It might be more sensible to measure high frequency respiratory transfer impedance in infants. Pressure oscillations are applied at the chest and the resulting oscillatory airflow is measured at the mouth which is open to atmosphere. Thus the parallel shunt impedance of the cheeks is minimised. We have recently demonstrated that using this technique high frequency transfer impedance measurements from 4 to 256 Hz can be made in infants (Jackson et al. 1999).

7.2.6. Pathogenesis of wheezing disorders, smooth muscle function

Our findings suggest that airway wall mechanics might play an important role in the pathogenesis of wheezing disorders. We have demonstrated that infants with a history of wheezing who were asymptomatic at the time of measurements have differences in high frequency Zin which is consistent with differences in airway wall mechanics. These data cannot distinguish whether the mechanism or structural basics are acquired (e.g. by post-inflammatory remodelling) or developmentally different. Particularly interesting is the fact that tension of the smooth muscle itself contributes to airway wall compliance, thus there may be developmental or acquired differences in smooth muscle function. Future research needs to examine infants prospectively shortly after birth and to identify those infants who are going to subsequently develop recurrent wheezing. We have currently set up a prospective study where we recruit healthy infants antenatally, measure their lung function including high frequency Zin at 6 weeks of age and prospectively determine whether or not they are going to develop wheezing disorders. If infants with subsequent wheezing disorders show differences in Zin at 4-6 weeks of age, we might find evidence that alterations in airway wall mechanics are due to developmental differences.
7.2.7. Effect of salbutamol on airway wall mechanics

The combination of the rapid thoracic compression technique and the high speed interrupter technique allow investigations into whether airway wall mechanics may be altered by salbutamol, a widely used bronchodilator. This is particularly interesting in infants with already altered airway wall mechanics due to developmental disturbances of airway growth. In these infants removing airway smooth muscle tone may lead to increased airway collapsibility and flow limitation. High frequency Zin measurements might be useful to identify those infants susceptible to this potential side effect of salbutamol.

7.2.8. Baseline smooth muscle tone and nitric oxide (NO).

There has been recent evidence that smooth muscle cells themselves change their mechanical properties, if they are exposed to asthma related pro-inflammatory cytokines and growth factors (Hakonarson et al., 1999). There is increasing evidence that local concentrations of NO may be important in the maintenance of airway smooth muscle tone, however, there has been no work demonstrating a direct relationship between NO concentrations and airway wall mechanics (e.g. Gaston et al. 1994, Van der Velden et al. 1999). So far NO measurements during tidal breathing were not possible in infants because of slow NO analysers. We have started to measure tidal NO changes in natural sleep using a rapid NO analyser (40 Hz) with an accuracy of 1 ppb. Currently we are unaware of studies relating tidal changes in NO and airway wall mechanics and are comparing these NO measurements and high frequency Zin measurements in naturally sleeping infants.

7.2.9. Baseline variability of airway wall mechanics

Very young infants modulate expiratory flow in order to dynamically elevate functional residual capacity above the level passively determined by the outward recoil of the chest wall and the inward recoil of the lung. Thus airway wall mechanics might constantly be adapted by regulatory mechanism. The nature of such regulatory mechanism is unclear. Previous own work has for example shown that inter-breath intervals are not randomly distributed but follow a statistical power law (Frey et al. 1998 (4), editorial by Bruce et al. 1998) revealing feed back mechanism in the control of breathing. Similar statistical properties may be found if far,1 is measured breath by breath during long traces of tidal breathing. We are currently setting up a study looking at long range correlations in breath by breath far,1 measurements in spontaneously breathing subjects.
In conclusion, the high speed interrupter technique opens up a large variety of potential applications which may contribute to the understanding of the physiological mechanism which determine airway wall mechanics in infants.
8. BIBLIOGRAPHY


Fredberg JJ, Hornig A. Mechanical response of the lung at high frequencies. *J Biomech Eng* 1978; 100: 57-60. (2)

Fredberg, JJ, Mead J. Impedance of intrathoracic airway models during low frequency periodic flow. *J Appl Physiol: Respirat Environ Exercise Physiol* 1979; 47: 347-351.


Habib RH, Chalker RB, Suki B, Jackson AC. Airway geometry and wall mechanical properties estimated from subglottal input impedance in humans. *J Appl Physiol* 1994; 77: 441-51. (1)


