Influence of low birthweight for gestational age on airway function during early infancy

A thesis by

Sook-Yuen Lum

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Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit
Institute of Child Health
University College London Medical School
University of London

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Abstract

Background

There is increasing evidence from epidemiological studies suggesting an association between abnormalities of fetal growth and diseases in adult life. Low birth weight has been identified as an independent risk factor for wheezing in early childhood and it has been suggested that some of the clinico-pathological findings in sudden infant death syndrome (SIDS) may be related to intrauterine growth retardation. However, the physiological mechanisms underlying these associations have not been elucidated.

Aim

The aim of this study was to test the hypothesis that, relative to infants of normal birthweight, those of low birthweight for gestational age have impaired airway function shortly after birth.

Method

Healthy infants ≥ 35 weeks gestation, of small (SGA) and appropriate (AGA) birthweight for gestational age, who were and were not exposed to maternal smoking in pregnancy were recruited shortly after birth during 1998 - 2000. Respiratory function was assessed in 79 SGA and 104 AGA infants prior to any lower respiratory illness, by measuring forced expiratory flow during tidal breathing and raised lung volume at a mean postnatal age of six weeks. Fetal and postnatal exposure to tobacco smoke was assessed from maternal report. This was compared with cotinine levels in infant urine and maternal saliva obtained at the time of respiratory function test.

Findings

SGA infants were significantly shorter and lighter than AGA infants at time of test. In univariate analysis, lung volume as reflected by forced vital capacity (FVC), forced expired volume in 0.4 seconds (FEV_{0.4}), and maximal expiratory flow at 25% vital capacity (MEF_{25}), were significantly diminished in infants born SGA compared to AGA, but there was no difference in maximal flow at functional residual capacity (V'_{maxFRC}). After adjusting for body size, maternal smoking status and postnatal age,
FVC and FEV_{0.4} remained significantly reduced in SGA infants. When analysis was restricted to those not exposed to maternal smoking, this reduction appeared to be mediated primarily through the reduction in body size of SGA infants at time of test. Both parameters of peripheral airway function, MEF_{25} and \( V'_{\text{maxFRC}} \) were significantly lower in boys than girls. \( V'_{\text{maxFRC}} \) but not MEF_{25} was significantly lower among infants whose mothers smoked.

**Interpretation**

The findings of this study suggests that airway function is diminished in SGA infants at birth and that this diminution precedes any lower respiratory tract illness. While this appears to be mediated primarily through the reductions in body size, it could contribute to the increased incidence of respiratory morbidity observed in such children during early life.
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<td></td>
<td>Area</td>
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<td>AGA</td>
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<td>Appropriate for gestational age</td>
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<td>APH</td>
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<td>Antepartum haemorrhage</td>
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<td>bpm</td>
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<td>breaths per minute</td>
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<td>CF</td>
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<td>Cystic fibrosis</td>
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<td>CGF</td>
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<td>CI</td>
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<td>Confidence Interval</td>
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<td>$C_{rs}$</td>
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<td>dP$_{tm}$</td>
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<td>DI</td>
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<td>EDD</td>
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<td>IUGR</td>
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<td>Peak expiratory flow</td>
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<tr>
<td>PEFV</td>
<td></td>
<td>Partial expiratory flow-volume</td>
</tr>
<tr>
<td>PIH</td>
<td></td>
<td>Pregnancy induced hypertension</td>
</tr>
<tr>
<td>PNT</td>
<td></td>
<td>Pneumotachometer</td>
</tr>
<tr>
<td>PROM</td>
<td></td>
<td>Premature rupture of membranes</td>
</tr>
<tr>
<td>Abbreviations/symbol</td>
<td>Unit</td>
<td>Description</td>
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<tr>
<td>----------------------</td>
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<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PTEF</td>
<td>mL.s⁻¹</td>
<td>Peak tidal expiratory flow</td>
</tr>
<tr>
<td>RASP</td>
<td></td>
<td>Respiratory analysis program</td>
</tr>
<tr>
<td>RR</td>
<td>bpm</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>RTC</td>
<td></td>
<td>Rapid thoraco-abdominal compression</td>
</tr>
<tr>
<td>RVRTC</td>
<td></td>
<td>Raised volume RTC</td>
</tr>
<tr>
<td>RV</td>
<td>mL</td>
<td>Residual volume</td>
</tr>
<tr>
<td>SaO₂</td>
<td>%</td>
<td>Oxygen saturation</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SGA</td>
<td></td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SIDS</td>
<td></td>
<td>Sudden infant death syndrome</td>
</tr>
<tr>
<td>τ</td>
<td>s</td>
<td>Time constant</td>
</tr>
<tr>
<td>τₚₛ</td>
<td>s</td>
<td>Respiratory system time constant</td>
</tr>
<tr>
<td>tₑ</td>
<td>s</td>
<td>Expiratory time</td>
</tr>
<tr>
<td>tᵢ</td>
<td>s</td>
<td>Inspiratory time</td>
</tr>
<tr>
<td>t_FE</td>
<td>s</td>
<td>Duration of forced expiration</td>
</tr>
<tr>
<td>t_PTIIF</td>
<td>s</td>
<td>Time to peak tidal inspiratory flow</td>
</tr>
<tr>
<td>t_PTEF</td>
<td>s</td>
<td>Time to peak tidal expiratory flow</td>
</tr>
<tr>
<td>t_PTEF: tₑ</td>
<td></td>
<td>Tidal expiratory flow ratio</td>
</tr>
<tr>
<td>TLC</td>
<td>mL</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>URI</td>
<td></td>
<td>Upper respiratory illness</td>
</tr>
<tr>
<td>V</td>
<td>mL</td>
<td>Volume</td>
</tr>
<tr>
<td>V'</td>
<td>mL.s⁻¹</td>
<td>Flow</td>
</tr>
<tr>
<td>Vₜ</td>
<td>mL</td>
<td>Tidal volume</td>
</tr>
<tr>
<td>Vₜ_maxFRC</td>
<td>mL.s⁻¹</td>
<td>Maximal expiratory flow at FRC</td>
</tr>
</tbody>
</table>
# Glossary of terms used in this study

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appropriate for gestational age (AGA) infant</td>
<td>Infant whose birthweight lies between the 20\text{th} and 95\text{th} centile for gestational age.</td>
</tr>
<tr>
<td>Small for gestational age (SGA) infant</td>
<td>Infants whose birthweight is \leq 10\text{th} centile for gestational age.</td>
</tr>
<tr>
<td>Ponderal Index (PI)</td>
<td>((\text{Birthweight [g]} / \text{length}^3 [\text{cm}]) \times 100)</td>
</tr>
<tr>
<td>Compliance ((C))</td>
<td>A measure of distensibility, i.e. change in volume per unit change in pressure: (C = \text{volume} / \text{pressure} \times (\text{mL.kPa}^{-1})).</td>
</tr>
<tr>
<td>Elastance</td>
<td>A measure of its lung stiffness i.e. a reciprocal of compliance, (\text{pressure/volume})</td>
</tr>
<tr>
<td>Resistance ((R))</td>
<td>A measure of pressure required to move gas at a flow of one litre per second: (R = \text{pressure} / \text{flow} \times (\text{kPa.L}^{-1}.s))</td>
</tr>
<tr>
<td>Airway conductance</td>
<td>Reciprocal of airway resistance.</td>
</tr>
<tr>
<td>Specific airway conductance</td>
<td>Airway conductance / Functional residual capacity.</td>
</tr>
<tr>
<td>Jacket pressure transmission</td>
<td>Change in pressure at airway opening (measured during brief airway occlusion) following inflation of the jacket expressed as % of applied jacket pressure.</td>
</tr>
<tr>
<td>Coefficient of variation ((CV))</td>
<td>((\text{Standard deviation} / \text{Mean}) \times 100)</td>
</tr>
<tr>
<td>Odds Ratio ((OR))</td>
<td>A ratio of odds, i.e. number of individuals with the outcome / number of individuals without the outcome.</td>
</tr>
<tr>
<td>P-value</td>
<td>A measure of the strength of evidence against the null hypothesis.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Random error – Type I</td>
<td>Falsely concluding that the null hypothesis is false when in fact it is true. The p-value gives a measure of how likely this is to have occurred.</td>
</tr>
<tr>
<td>Random error – Type II</td>
<td>Falsely concluding that the null hypothesis is true when in fact it is false. This type of error commonly occurs where samples are small and the study is under-powered.</td>
</tr>
<tr>
<td>Relative risk</td>
<td>Relative risk (RR) is a ratio of risk probabilities.</td>
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<tr>
<td></td>
<td>RR = number of individual with outcome (e.g. lung cancer) [a] / total number of individuals in this group (e.g. smokers) [a+b] / number of individuals with the outcome [c] / number of cases in a second group (e.g. non-smokers) [c+d], i.e. RR between groups = (a/[a+b]) / (c/[c+d]).</td>
</tr>
</tbody>
</table>
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I wish to thank all my friends and colleagues who have been very patient and supportive during the writing of this thesis.

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To my two supervisors, Professor Janet Stocks and Dr Carol Dezateux, I am truly grateful for their unfailing support, encouragement and guidance.
Peer reviewed publications associated with this study


General Introduction

There is increasing evidence that small for gestational age infants are at increased risk of respiratory morbidity and death during the first year of life. However, the physiological mechanisms underlying these associations remain unclear. The aim of this study was to examine the hypothesis that, relative to infants of normal birthweight, infants of low birthweight for gestational age have diminished airway function shortly after birth. This thesis contains:

- A literature review pertaining to issues relating to the identification of the infant with restricted growth in-utero, and the relevance of low birthweight for impaired respiratory function and increased respiratory morbidity during later life.

- Chapter 2 contains a review of the growth and development of the respiratory system during the intra-uterine and postnatal periods and the potential effect on airway function when development is compromised due to adverse intra-uterine conditions.

- An overview of the study design with a detailed description of equipment and methodology used in this study is presented in chapter 3. Data collection, calculation of respiratory parameters and statistical methods are also described. Studies assessing potential methodological factors that may influence results obtained from the raised volume technique used in this study are also presented.

- Infant characteristics and lung function results are presented in chapter 4 and the possible influence of birthweight status, sex and maternal smoking status on airway function are investigated. Methodological issues such as validation and analytical aspects of the raised volume thoraco-abdominal compression (RVRTC) technique are also discussed.

- A discussion of the potential implications of these results and their relationship to previous publications is presented in the final chapter, together with a critical appraisal of the current study design and suggestions for future research in this area.

- The thesis concludes with a list of references and appendices including parental information sheets, questionnaires and publications arising from this study.
1 Literature review: Influence of low birthweight for gestational age on respiratory morbidity and mortality
1.1 Introduction

The clinical importance of fetal growth restriction was first reported by Lubchenco et al in 1963, who recognized that perinatal mortality and morbidity were increased among infants whose birthweights fell at or below the 10th percentile for gestational age (Lubchenco et al. 1963). This observation was subsequently confirmed by others (Battaglia and Lubchenco, 1967; Lin and Santolaya-Forgas, 1998). Since then, a number of risk factors for low birth weight have been identified of which the most important are smoking, maternal nutritional deprivation and low social status (Kramer et al. 1990; Mellis and Woolcock, 1992; Emanuel et al. 1992; Lieberman et al. 1994; Godfrey et al. 1996; Power and Hertzman, 1997; Das et al. 1998; Kramer, 1998). Other risk factors such as pre-eclampsia, alcohol and drug use in pregnancy and fetal nutrition have also been recognized (Brooke et al. 1989; Bakketeig et al. 1993; Harding, 1995; Kim et al. 1996; Sekhon and Thurlbeck, 1996).

Low birth weight has been identified as an independent risk factor for wheezing in early childhood (Brooke et al. 1995; Dezateux et al. 1999). There is also increasing evidence from epidemiological studies, suggesting an association between abnormalities of fetal growth and diseases in adult life (Barker et al. 1991; Shaheen et al. 1994; Barker, 1995; Stein et al. 1997). In studies investigating the relationship between fetal growth, childhood respiratory disease and adult lung function, Barker has suggested that a history of pneumonia in infancy may reflect small peripheral airways, present from birth, but that impaired airway function in adults may be related to lower birth weight independent of an adverse postnatal environment (Barker et al. 1991; Shaheen et al. 1994; Stein et al. 1997). Barker proposed a long term effect of an adverse intrauterine environment on lung development during the period of critical growth, namely a permanent reduction in lung size and DNA content (Barker et al. 1991).

In recent years, there has been a considerable research effort addressing the nature of this link, the biological mechanisms mediating it, and the potential role of genetic and environmental factors. However, many of the underlying processes remain unclear. In particular, there is little information regarding the biological processes
during infancy that might mediate an association between low birthweight and airway function at birth and airway development during infancy, a critical period of growth and development of the respiratory system.

This chapter will briefly review the issues relating to the identification of the infant with restricted growth in utero, and the relevance of low birthweight for impaired respiratory function and increased respiratory symptoms during childhood and adult life.

1.2 Prediction and the identification of the growth restricted infant

In England and Wales, 7.4% of all infants weigh less than 2.5 kg at birth and are classified as low birth weight (Office for National Statistics, 1997).

1.2.1 Definition

The term ‘low birth weight’ is usually used to refer to all infants whose weight at birth is less than 2,500 g. However, birthweight is governed by two main considerations, namely, the duration of gestation and the intrauterine growth rate. Consequently, low birthweight may be due to a short gestation (prematurity), intrauterine growth restriction (IUGR) or a combination of these two factors.

Traditionally, IUGR has been defined by size at birth. The terms IUGR and Small for Gestational Age (SGA) are often used interchangeably but they are not synonymous. IUGR is defined as a ‘result of a persistent suppression of growth potential in response to a reduction in substrate supply, infective or toxic insults’ (Mahadevan et al. 1994). In contrast, SGA is a ‘statistical definition of size when birthweight lies below an arbitrary centile for gestational age on a standard birthweight chart’ (Mahadevan et al. 1994).

Clinically, SGA infants are generally defined as those whose birthweight is less than the 10th percentile for gestational age. However, infants who are growth restricted may have a birthweight that is apparently ‘appropriate’ for gestational age and conversely, infants who are SGA may be small but appropriately grown.
Nevertheless, SGA is often used as a proxy for IUGR. During recent years, it has become well recognized that not all SGA infants are truly growth retarded or malnourished. Some infants are constitutionally small and their size at birth is determined by maternal ethnic group, parity, weight and height (Kramer, 1987; Freeman et al. 1995; Johnson, 1995; Chin et al. 1996; de Jong et al. 1998). While in most epidemiological studies, the $10^{th}$ percentile is used to explore risk related to low birthweight for gestational age, Seeds et al have recently shown that fetuses with weight at delivery between the $10^{th}$ and $15^{th}$ percentiles for gestational age were also at increased risk for fetal death (odds ratio: 1.9) and recommended that the $15^{th}$ percentile be used as the cut-off point (Seeds and Peng, 1998).

1.2.1.1 Assessment of gestational age

Accurate dating of pregnancy is essential for the assessment of fetal growth and size. In 1812, Naegele formulated the rule, still currently in use, for the calculation of the expected date of delivery (EDD). He suggested that gestational age be calculated by adding nine months and seven days to the first day of the last normal menstrual period (LMP). This depends on a woman being certain of her menstrual history and having regular periods. Over the past twenty years, increasing emphasis has been placed on the use of ultrasound technology for the assessment of fetal age using fetal biometry (Chervenak et al. 1998; Gardosi and Geirsson, 1998). Chervenak et al. compared fetal biometry based on their own gestational age prediction equation with that reported previously (Chervenak et al. 1998). They concluded that the biparietal diameter was the best predictor of gestational age during pregnancy with accuracy further improved by the addition of fetal abdominal circumference and fetal femoral length. Several studies have since suggested the use of ultrasound as the first choice for dating pregnancies, not just to correct menstrual dates when there is discrepancy but in preference to menstrual dates (Tunon et al. 1996; Leeson and Aziz, 1997; Gardosi and Geirsson, 1998).

1.2.1.2 Assessment of fetal growth and size

IUGR is associated with stillbirth, prematurity, perinatal morbidity and decreased fetal reserve during labour (Buck et al. 1989; Bonatz et al. 1997; Divon et al. 1998;
Levy et al. 1998; McIntire et al. 1999). Hence, adequate surveillance of fetal size and growth is one of the most important tasks for obstetricians, midwives and general practitioners. However, antenatal recognition remains difficult (Chang and Cheng, 1994; Irion et al. 1998; Chauhan et al. 1998). In a confidential review of perinatal death conducted in South-East Thames Region from 1988 to 1991, only 25% of 1,662 babies born SGA and who died had been identified antenatally (de Courcy-Wheeler et al. 1995).

With the current organization of maternity services, women who are considered to be at low risk of obstetric complications receive antenatal care from midwives and general practitioners in the community setting. In many hospital units, these women are seen at mid-trimester and, if the pregnancy progresses well, are not asked to return until in spontaneous labour or when their pregnancy exceeds 40 weeks gestation. Thus, in the community setting, the general prediction of fetal weight is by clinical palpation and serial symphysis-fundal height measurements, ideally, by the same observer (Bailey et al. 1989; Neilson, 1999a). However, the performance of clinical palpation and symphysis-fundal height measurements as a screening test for growth retardation is poor (Woo et al. 1985; Jensen and Larsen, 1991; Scichilone et al. 1999). Although widely used, the rule of thumb of ‘weeks gestation = symphysis-fundal height in cm’ is a frequent source of error as it is applied irrespective of maternal size and fat stores, parity, liquor volume and head engagement. A systematic review to assess the effectiveness of the routine use of symphysis-fundal height measurements during antenatal care on pregnancy outcome when compared to abdominal palpation alone proved inconclusive as only one trial was identified (Neilson, 1999a).

When there is a suggestion of reduced fetal growth antenatally, the woman may be referred for serial ultrasound assessment of fetal anthropometry to estimate fetal weight and fetal growth. In recent years, Doppler ultrasound has been increasingly used to monitor flow velocity waveforms in the umbilical and utero-placental arteries in an effort to improve obstetric care and fetal outcome (Chang and Cheng, 1994; Harrington et al. 1997; Irion et al. 1998). In a systematic review of trials evaluating Doppler ultrasound, Neilson and Alfirevic (1998) reported that, among high risk pregnancies, admission to hospital during pregnancy, elective delivery and induction
of labour were all reduced in those randomized to Doppler. However, there was little evidence to confirm or refute a reduction in fetal distress or Caesarean section during labour. It was concluded that a larger trial was required to provide definitive evidence of effectiveness (Neilson and Alfrevic, 1998).

There are two ways in which antenatal ascertainment of growth retardation may be unsatisfactory. First, failure to identify those pregnancies, who at delivery are SGA may be due to measurement error, e.g. during symphysis-fundal height measurements, clinical palpation or estimation of fetal weight (Hepburn and Rosenberg, 1986). Second, the method used may incorrectly identify a constitutionally small infant as SGA or growth retarded in pregnancy, this error being increased the more frequently measurements using ultrasound are made (Mongelli et al. 1998). Furthermore, ultrasonically estimated fetal weight as part of routine antenatal care was found to have overestimated birth weights of infants who were less than 2,500 g while underestimating the birth weights of those who were equal to or greater than 2,500 g (Bistoletti, 1986; Sherman et al. 1998).

Many clinicians advocate routine ultrasound screening during pregnancy for more accurate calculation of gestational age and to detect fetal growth disorders and congenital abnormalities. Routine ultrasound in early pregnancy appears to enable better gestational age assessment and earlier detection of multiple pregnancies (Neilson, 1999b). However, while routine scanning is associated with a reduced incidence of induction of labour for apparent post-term pregnancy, it has not been shown to improve fetal outcome (Neilson, 1999b). Similarly, evidence that routine ultrasound in late pregnancy reduces perinatal mortality or morbidity is lacking (Bakketeig et al. 1984; Newnham et al. 1993; Ewigman et al. 1993).

Thus SGA infants are not reliably predicted antenatally either by clinical or sonographic methods.

1.2.1.3 Methods available for the identification of the growth restricted infant

Birthweight centile charts based on populations with different inclusion criteria have been developed and are used to identify SGA infants (Freeman et al. 1995; Ariyuki
et al. 1995). Centile charts published by the Child Growth Foundation (CGF), were constructed from cross sectional data from the Cambridge Infant Growth Study and Whittington Birth Data Study (161 boys/139 girls, aged 35 weeks to term) based on corrected gestational age (i.e. corrected according to the expected date of delivery by ultrasound if date by last menstrual period differs by more than one week), birth weight and sex (Freeman et al. 1995). Gestation was calculated from the last menstrual period (LMP) and confirmed by ultrasound. All births with confirmed gestational age were included and no other exclusion criteria were applied. However, there were relatively few infants of less than 37 weeks gestation in this group. Thus, there will be greater uncertainty in estimates of birth centiles for preterm infants from these centile charts. Furthermore, while the resolution of the paper chart is adequate for the estimation of birth centiles up to 40 weeks gestation, it is harder to use reliably for infants delivered after this gestation due to the smaller scale of the chart. At later gestations, i.e. after 40 weeks, the Child Growth Foundation Excel computer program allows greater precision and is preferred (Freeman et al. 1995).

In 1993, Wilcox et al developed a computer model or customized ‘growth program’ which incorporated a number of factors affecting birthweight, such as maternal height, booking weight, parity, ethnic group, sex and gestational age, to predict ‘ideal’ birthweight and to calculate an individualized birthweight centile (Wilcox et al. 1993). All births from two provincial teaching hospitals were included. Data were only excluded if they were impossible values such as maternal weight less than 35 kg or maternal height less than 1.35 m or more than 2 m. It has been argued that these centiles predict fetal size rather than fetal growth as proposed, on the basis that cross sectional rather than longitudinal data were used (Altman and Chitty, 1994). At present, this method is not in widespread clinical use, hindered, amongst other things, by incomplete routine recording of required data such as maternal height and ethnic group.

Whichever method is used to identify the growth restricted infant, it is well recognized that perinatal morbidity and mortality increase with decreasing body size (Kramer, 1987; Lin and Santolaya-Forgas, 1998; McIntire et al. 1999). However, this relationship is not a dichotomy but a graded risk (whether it is the 3rd, 10th or 15th...
birthweight percentile) depending on the exposure to risk factors such as smoking and poor socio-economic status (Kramer, 1987).

1.2.1.4 Anthropometric measurements associated with SGA infants

As most SGA infants are not diagnosed antenatally, the majority is identified postnatally from birth weight centiles for gestational age (de Courcy-Wheeler et al. 1995). Anthropometric measurements, such as skinfold thickness, mid-arm circumference to head circumference ratio (MAC/HC), chest circumference and ponderal index [i.e. (birthweight (g) / length³ (cm)) *100], may also be used to measure neonatal nutritional status (Miller, 1995; Drossou et al. 1995).

Gruenwald distinguished two patterns of growth retardation, which gave an indication of when the insult is likely to have occurred (Gruenwald, 1963):

Symmetrical IUGR, in which infants have normal body proportions (normal ponderal index) and where an insult is considered to have occurred early in the antenatal period, during the period of general organ growth. Congenital abnormalities, uterine infections and toxic insults (tobacco, alcohol and drugs) are the main associated conditions (Leger et al. 1997);

Asymmetrical IUGR, where abdominal girth and fat stores are reduced but head circumference is not, owing to the brain sparing effect. These infants have a lower weight in relation to body length (low ponderal index) due to growth faltering during the period of fat deposition in late pregnancy. This is usually associated with pathology of later onset such as placental insufficiency and pre-eclampsia or pregnancy induced hypertension (Bakketeig and Hoffman, 1983).

However, this distinction was challenged by Kramer and colleagues who compared body proportions among infants with and without IUGR but of similar birth weights in a cohort of 8719 infants with concordant menstrual dating and early ultrasonographic estimates of gestational age. Infants with IUGR had slightly but significantly greater body length and head circumference, and increased variability in body proportions, but there was no evidence of the bimodality that would
characterize two distinct (symmetrical vs. asymmetrical) subtypes (Kramer et al. 1989). In a subsequent study, Kramer et al. confirmed their original findings and concluded that the variation in body proportions among infants of a given birth weight remains largely unexplained and that while this may be partially attributable to measurement error (particularly of body length), much of it probably represents true biological variation (Kramer et al. 1990).

1.3 Morbidity and mortality associated with growth restriction

Fetal growth retardation is associated with stillbirth, prematurity, perinatal morbidity and decreased fetal reserve during labour (Miller, 1995; Vik et al. 1996; Leger et al. 1997; Bonatz et al. 1997; Magowan et al. 1998). Over the last decade, several studies have also found a strong association between fetal growth retardation and sudden infant death syndrome (Oyen et al. 1995; Friedman et al. 1995; Schellscheidt et al. 1998). In addition there is evidence to support the hypothesis that alterations in airway function can be detected shortly after birth in infants who are at increased risk of wheezing and impaired airway function in later childhood (Martinez et al. 1991)(Dezateux and Stocks, 1997). It has been hypothesized that adult diseases are mediated by altered fetal nutritional status (Barker and Fall, 1993).

1.3.1 Effects on health in infancy and childhood

Infants who are SGA were previously thought to have accelerated pulmonary maturation and a reduced risk of respiratory distress syndrome relative to their appropriately grown counterparts because gestational age had not been taken into account (Dahms et al. 1974; Gould et al. 1977). However, this concept of accelerated pulmonary maturity has been challenged (Tyson et al. 1995) and it is now recognized that, when compared with AGA infants of the same gestational age, sex and race, SGA infants are at increased risk of death and respiratory illness (Tyson et al. 1995; Vik et al. 1996). The relative risk for respiratory distress syndrome and respiratory failure associated with being SGA after correcting for gestational age (odds ratio [OR], 1.94; 95% confidence interval [95% CI], 1.19 – 3.17) was found to be of similar magnitude to that associated with male sex (OR, 1.99; 95% CI, 1.21 – 3.26) or white race (OR, 1.88; 95% CI, 1.10 – 3.22) (Tyson et
al. 1995). This is corroborated by recent findings from our laboratory showing significant reductions in airway function during early infancy in preterm boys and white infants (Stocks et al. 1997; Hoo et al. 1998). However, there are no similar studies examining the effects of SGA on airway function in infancy (Dezateux and Stocks, 1997).

SGA infants are more likely to be admitted to hospital during their first year of life and these admissions are mainly due to respiratory tract infections (Vik et al. 1996). In a systematic review by Strachan and Cook, hospital admissions due to respiratory infections were significantly associated with maternal smoking at the time of conception, rather than postnatal exposure (Strachan and Cook, 1997). Maternal smoking is also strongly associated with SGA births, thus the association between SGA and respiratory infections may be confounded by maternal smoking. Low birth weight has also been significantly associated with wheezing in children up to 5 years of age (Lewis et al. 1995) and reduced FEV₁ by < 2% in later childhood (Rona et al. 1993).

1.3.2 Effect on somatic growth

Catch-up growth in children born SGA has been of great clinical interest to both obstetricians and paediatricians. In short term follow up studies, SGA infants experience extremely fast catch-up growth for weight during the first three months of life, while catch-up growth for height is more gradual (Wennergren et al. 1995; Leger et al. 1997). These findings were corroborated by Kalberg and Albertsson-Wikland in a large population based study (n = 3650) which showed that the vast majority of healthy, full term, singleton SGA infants achieve catch-up growth (weight and height) during the first two years of life. SGA children who do not show postnatal catch-up growth and remain short at 2 years of age have a higher risk of short stature (less than 2 standard deviation scores below the mean) in later life (Karlberg and Albertsson-Wikland, 1995). Furthermore, it has been suggested that shortness is associated with cardiovascular and respiratory morbidity and mortality (Leon et al. 1995; Davey et al. 2000) which may reflect a failure to reach their growth potential and a marker of pre-/postnatal undernutrition.
1.4 Association between SGA and Sudden Infant Death Syndrome (SIDS)

It has been suggested that Sudden Infant Death syndrome (SIDS) is preceded by poor postnatal weight gain (Emery et al. 1985). However, evidence from recent studies suggests that adverse intrauterine conditions may be more important since it is birthweight rather than growth velocity in early postnatal life that is reduced in infants who die suddenly and unexpectedly (Brooks et al. 1994; Williams et al. 1996; Blair et al. 1997).

It has been proposed that some of the clinico-pathological findings in SIDS may be related to IUGR, such as history of malnutrition; atrophy of subcutaneous fat and thymus, perinatal asphyxia (Haas et al. 1993). There is some evidence that SGA infants had increased risk of respiratory distress syndrome and respiratory failure or death (Tyson et al. 1995) and were more often admitted to hospital than non-SGA infants (Vik et al. 1996). It has also been hypothesized that sudden death in infancy may result from a final episode of progressive peripheral bronchial occlusion and occurs in infants with pre-existing diminished airway function (Martinez, 1991). Thus, minor alterations in lung structural development during fetal life could have marked postnatal consequences, as fetal insults may also exert effect on postnatal growth, leading to critical disturbances in airway calibre in response to subsequent respiratory infections and resulting in severe, and potentially fatal, respiratory compromise.

1.5 SGA and its association with adult lung function and diseases in adult life

There has been increasing evidence that an adverse intrauterine environment may have important long term consequences and that some failures of adaptation are not corrected with the passage of time or further experience (Dawes, 1990). The question of whether there might be other aspects of early development which may have long term effects upon the individual has been investigated extensively by Barker and colleagues, who suggest that defective perinatal growth may be associated with many aspects of adult ill health (Barker, 1990; Barker, 1995; Barker, 1995; Barker, 1997).
1.5.1 Fetal origin hypothesis

The fetal origin hypothesis proposed by Barker et al. states that fetal undernutrition in middle to late gestation, leads to disproportionate fetal growth, and is associated with coronary heart disease in later life, mediated by fetal 'programming' (Barker, 1995). This hypothesis has been supported by evidence from numerous animal experiments, which suggest that poor nutrition and other influences affecting development during a critical period of early life may permanently change the structure and physiology of a range of organs and tissues (Harding, 1995; Sekhon and Thurlbeck, 1996; Cherukupalli et al. 1997). This phenomenon has been described as 'programming', where there are critical periods for the development of different organs and tissues and maturation of these organs or tissues is not achieved during these 'critical windows of time'. It is suggested that this failure of maturation is to some extent irreversible (Barker, 1995).

This hypothesis has been explored in studies of elderly adults for whom records of birth measurements and infant growth have been located (Barker, 1990; Barker et al. 1991; Barker, 1995). Follow up studies have been possible in Hertfordshire, Preston and Sheffield, where detailed measurements on all newborn infants have been kept since the early 1900s (Barker, 1990; Barker, 1995; Barker, 1997). These studies suggest that impaired fetal growth is a risk factor for coronary heart disease and also for other associated conditions such as non-insulin dependent diabetes, hypertension, altered lipid metabolism and disordered blood coagulation (Barker, 1995). Furthermore, from the studies in North Hertfordshire, Barker et al also postulated that early under-nutrition results in impaired development of molecular and cellular repair mechanisms which may affect all tissues but which are most critical in later life (Sayer et al. 1997).

This hypothesis however, has been challenged. A physiological situation that can be used to explore the proposed associations, is that of multiple births, where infants are generally of lower birth weight than singletons. Contrary to projections from the Barker hypothesis, mortality from ischaemic heart disease was not higher among twins than the general population though the shorter twin in a twin pair was more likely to die of heart disease than the taller (Vagero and Leon, 1994). Furthermore,
documented evidence of the effect of under-nutrition on the outcome of pregnancy during the Dutch Hunger Winter of 1944–5 showed that birthweight of children born following exposure in the third trimester averaged 9% below that of controls. There were no significant effects on fetal parameters for women exposed only during the first and second trimesters (Keirse, 1993). Other factors such as social class of origin and social and biological events that evolve over time, have not been adequately considered (Power and Hertzman, 1997).

In the original three key data sets cited by Barker, length of gestation was not recorded because at the time it was not reliably known. As a result, no distinction was made between low birth weight infants who were preterm or who were small for gestational age. Similarly, low socio-economic status, which is associated with a higher incidence of prematurity and of infants who are small for gestational age was not controlled for as a potential confounding factor in Barker’s original analyses (Joseph and Kramer, 1996; Wilson, 1999). Subsequently, Koupilova et al. have found that the strong inverse associations between birthweight and blood pressure among 50-year old Swedish men are unlikely to be explained by confounding with socio-economic circumstances at birth or in adult life (Koupilova et al. 1997).

Lucas and colleagues (Lucas et al. 1999) suggested that while the fetal origin hypothesis is plausible, evidence cited for it is flawed due to misinterpretation and inappropriate analysis of growth data. They proposed that when size in early life is related to later health outcomes only after adjustment for current size, it is probably the change in size (i.e. weight) between the two measurement periods (postnatal centile crossing) rather than fetal biology that is implicated.

There is also evidence that social and biological pathways are linked with early life and adult health status. Power and Hertzman presented evidence from the 1958 British Birth Cohort emphasizing the role and cumulative effect of life events along developmental trajectories. They explored the distribution of social and biological risk factors according to social class at birth. Strong associations with health in later life are observed according to social class of origin for factors such as birthweight, childhood material circumstances, height, educational attainment and smoking behaviour (Power and Hertzman, 1997).
1.5.2 Effect on adult lung function

The implications of low birthweight for respiratory health in later life have received increasing attention with evidence from a number of studies suggesting that impaired airway function in adult life is a major clinical indicator of mortality risk in both men and women (Hole et al. 1996). In the Hertfordshire study, Barker found that low birth weight was associated with reduced adult lung function. Risk of death from chronic obstructive airway disease fell with increasing birthweight and weight at one year (Barker et al. 1991). At age 59-70 years, mean forced expiratory volume at one minute (FEV<sub>1</sub>) adjusted for height and age, rose by 0.06 L (95% CI 0.02 to 0.09) with each pound (454 g) increase in birth weight, independent of smoking habit and social class. Bronchitis or pneumonia in infancy was associated with a 0.17 L (95% CI 0.02 to 0.32) reduction in adult FEV<sub>1</sub>, after adjustment for birthweight, smoking habit and social class, while whooping cough in infancy was associated with a 0.22 L (95% CI 0.02 to 0.42) reduction (Barker et al. 1991). Thus from these figures, relative to postnatal respiratory illness, low birthweight seems to have a lesser, albeit significant, effect on adult lung function. Similar findings were observed in a study in India where mean FEV<sub>1</sub> fell with decreasing birthweight in both men and women. FEV<sub>1</sub> and FVC were lower in men who smoked, but the associations with size at birth were independent of smoking (Stein et al. 1997).

In a study examining the association between pneumonia in early childhood and impaired lung function in late adult life, mean FEV<sub>1</sub> and FVC, adjusted for age, height and smoking were significantly diminished in men but not in women (Shaheen et al. 1994). It was suggested that pneumonia in infancy was simply a marker of small airways, present from birth. However, in a subsequent longitudinal study of 239 adults who were 60-70 years of age, the authors suggested that the deficits in FEV<sub>1</sub> and FVC associated with pneumonia and bronchitis in the first two years of life were consistent with a causal relationship. After adjusting for age, sex, height and smoking, no association between birthweight and lung function was found (Shaheen et al. 1998).

Similar investigations were carried out in two national British cohorts. Evidence from the 1946 birth cohort study showed that lower respiratory illness in the first two
years of life was a significant risk factor for adult chronic obstructive pulmonary disease. Birthweight, overcrowded home circumstances at age two years and a parental history of bronchitis were each independently associated with reduced peak expiratory flow rate at age 36 years, even after adjusting for smoking, level of education and social circumstances in adult life (Mann et al. 1992; Wadsworth and Kuh, 1997). Incidence and prognosis of asthma and wheezing illness and their relation to perinatal factors were investigated in the 1958 cohort from birth to age 33. The incidence of wheezing during childhood was strongly and independently associated with pneumonia, hay fever and eczema (p<0.001) and incidence from age 17 to 33 was also strongly associated with a history of hay fever. However, the independent associations of asthma at age 7, with maternal age, parity, gestational age, birth weight and birthweight for gestation were found to be non significant. Subjects with a history of wheezing illness in childhood were also shown to retain a risk of wheezing in adult life, above that of their healthy peers (Strachan et al. 1996).

1.6 Summary

In England and Wales, 7.4% of all infants born are of low birth weight, i.e. they weigh less than 2,500 g at birth. The implications of low birth weight for respiratory health during infancy, childhood and later life have received increasing attention. Evidence from a number of studies has suggested that:

- Low birth weight is an independent risk factor for wheezing in early childhood and impaired airway function in 5-11 year olds but not asthma.

- Low birth weight is associated with diminished airway function but not respiratory symptoms in adult life.

- Hospital admissions for respiratory tract infections are increased among SGA infants during the first year of life. Maternal smoking in pregnancy is also strongly associated with hospital admissions for respiratory illness and SGA births. Therefore, smoking may be an important confounding factor between SGA infants and respiratory illness during the first year of life.
• Some of the clinico-pathological findings in SIDS may be related to IUGR and infants who are SGA are at increased risk of both respiratory infection and death during the first year of life.

However, the physiological mechanisms underlying these associations remain unclear and information is lacking regarding the effect of low birthweight for gestational age on airway function at birth and airway development during infancy, a critical period of growth and development of the respiratory system. Similarly, when size in early life is related to later health outcomes, there is continuing controversy as to whether this is partly or wholly explained by postnatal rather than prenatal factors. While a number of studies have shown that impaired airway function in term infants precedes and predicts wheezing in early childhood (Martinez et al. 1991; Dezateux et al. 1999; Dezateux et al. 2001; Turner et al. 2002), none have addressed the issue of whether airway function measured shortly after birth is impaired in infants of low birthweight for gestational age. Hence, research is needed in infants to enhance understanding of the mechanisms linking low birthweight and respiratory problems in infancy and early childhood. Specifically research is needed to examine whether lung growth and development during fetal and early life is impaired among infants who are SGA, and whether this is independent of maternal smoking in pregnancy.
2 Growth and development of the respiratory system
2.1 Introduction

The respiratory system consists of the airways that conduct the air to the alveoli, respiratory units specialising in gas exchange between air and blood, and the respiratory pump, the chest structures responsible for moving the air in and out of the lungs. The lung does not fulfil its postnatal function during intra-uterine life although it has to function efficiently immediately after birth. Hence, the lung at birth is not a miniature version of the adult lung, and structures within the lung differ in the time pattern of differentiation and rate at which they develop.

This chapter will briefly review the growth and development of the respiratory system during the intra-uterine and postnatal period and the potential effect on airway function when development is compromised due to adverse intra-uterine conditions.

2.2 Early lung development

Embryologically, the lung is derived from the gut. Human lung development starts in the fourth week of gestation, as a ventral outpouching from the foregut of the embryo. It is from this endoderm that the lining epithelium of the whole respiratory system, including airways and alveoli, is formed (Hislop and Reid, 1974). Following this embryonic period, four overlapping phases of lung development are recognised (Jeffery and Hislop, 1995) and this is mirrored by development of pulmonary vasculature:

- Pseudoglandular phase (5 to about 17 weeks’ gestation): during which the pre-acinar branching pattern of airways and blood vessels is established. In humans, the division of intra-segmental airways is fastest (especially in the right lung) between 10-14 weeks’ gestation, by which time 70% of the airway generations present at birth have formed (Bucher and Reid, 1961). By 11-16 weeks’ gestation, differentiation of a ciliated epithelium is apparent as the bronchial tubes increase significantly in number, mesenchyme differentiates into cartilage and bronchial smooth muscle and formation of a ciliated cell border is initiated. All pre-acinar airway branching is complete by the end of this phase.
• Canalicular phase (16 to 26 weeks' gestation): when vascularization of peripheral mesenchyme rapidly increases and the respiratory portion of the lung begins to develop, also known as alveolarisation. This is characterised by the transformation of distal non-respiratory bronchioles (including terminal bronchioles) into primitive respiratory bronchioles (Murray, 1986a). Pre-acinar airways increase in diameter and length with an increase in the amount of cartilage, gland and muscle in the airway wall (Hislop, 1995). Pulmonary epithelium differentiates into type I and type II cells, which allows for surfactant secretion and formation of the first areas with what will become a thin air-blood barrier (Burri, 1999).

• Saccular phase (from about 24 to 36 weeks): when additional respiratory airways develop and the future respiratory units differentiate. The respiratory units are the acini, each of which comprise a single terminal bronchiole and its subsequent divisions of respiratory bronchioles, alveolar ducts and alveoli. During this phase, a network of elastic fibres is deposited throughout the interstitium forming small crests, which subdivide the saccules. This forms the foundation for alveolar development (Burri, 1999). There is progressive thinning of the epithelium and protrusion of additional capillaries into the lining layer. This increases the total surface area for gas exchange until, by approximately the 28th week, the air-blood membrane is sufficiently mature to support life (Murray, 1986a).

• Alveolar phase (from about 36 weeks to term and continuing for the first three years of infancy): Just before birth, primitive alveoli can be detected in the wall of the saccules although true alveoli do not develop until after birth. Though there is considerable variation in the number of alveoli at birth, it is estimated there are approximately 150 million alveoli present by term, constituting a third to half the adult number (Hislop, 1995).

• Pulmonary vasculature: The development of pulmonary arteries and veins is closely related to that of the bronchial tree. By the end of the pseudoglandular phase, all preacinar artery branches, including those that will supply the alveoli, are present. The pulmonary veins grow at the same time as the arteries. However, they do not accompany the airways and arteries but lie in the intersegmental plane. Around 24 weeks of gestation, a network of capillaries line
each saccule and as the interstitial mesenchyme between each saccule thins, these lie close together as a double capillary network between two epithelial layers. By the end of the canalicular phase, the air to blood barrier is thin enough to support gas exchange (Hislop, 1995). In later fetal life, the walls of the alveoli become thinner and have only a single capillary network (Zeltner et al. 1986). The intra-acinar arteries develop accompanying respiratory airways and alveoli. These vessels grow in size and length, the main branches increasing more rapidly during fetal life and infancy (0-18 months of age) than childhood (Jeffrey, 1998).

2.3 Fetal lung function

Although the fetal lung is not used as a gas exchange organ, there are three special features that are essential for normal fetal lung development:

2.4 Secretion of lung liquid

A unique function of the lung in-utero is the constant secretion by the alveolar epithelium of a relatively large volume of liquid (4-6 mL/hr/kg in the human fetus) by active transport of chloride ions (Hislop, 1995). Once formed, lung liquid moves up the tracheo-bronchial system to the mouth where it is swallowed or added to the amniotic fluid (Harding and Albuquerque, 1999).

Fetal lung liquid plays a crucial role in the growth and development of the lungs by maintaining them in a distended state. The implications of disordered fetal lung liquid for lung growth will be discussed in more detail in section 2.7.1. Its secretion also keeps the lungs free of amniotic fluid and clears the airway lumen of cellular debris and mucus (Wigglesworth, 1987; Harding and Hooper, 1996). The switch from secretion to absorption of lung liquid occurs abruptly during labour and is triggered by a large rise in adrenaline in fetal blood. Adrenaline and arginine vasopressin play an important role in suppressing fetal lung liquid secretion and inducing liquid re-absorption by activating sodium channels (Olver et al. 1986; Hislop, 1995). Two-thirds of the fluid is absorbed prior to delivery (90% via the circulation and 10% via the lymphatic system), while the remaining third is partially displaced during passage through the birth canal and absorbed after birth (Duncan, 1999). Hence, successful transition from intra-uterine to extra-uterine life is
dependent upon clearance of this liquid from the fetal lungs at the time of birth, so that the lungs may function effectively as an organ of gas exchange. The failure to completely clear lung liquid, such as may occur during emergency or elective Caesarean Section, may predispose the infant to transient tachypnoea soon after delivery (Dani et al. 1999).

2.5 Fetal breathing

The human fetus is known to undergo periods of episodic breathing movements as early as eight weeks of gestation, and the extent of breathing movements correlates with gestational age and compliance of the fetal lung (Holm et al. 1997). In animal studies, fetal breathing movements have been shown to be an important factor in maintaining lung expansion (Alcorn et al. 1977; Harding, 1994). During episodes of fetal breathing movements, the resistance of the upper respiratory tract is reduced due to rhythmic laryngeal dilation, and lung liquid flows from the lungs at a greater rate than during intervening periods of fetal ‘apnoea’ when the resistance of the upper respiratory tract is increased due to the sustained constriction of the larynx (Harding et al. 1986). Hence it has been suggested that the ability of fetal breathing to maintain lung liquid volume is the principal means by which fetal breathing facilitates lung growth, rather than the small variations in lung dimensions that fetal breathing movements induce (Harding and Albuquerque, 1999).

2.6 Synthesis of surfactant

At about the 24th week of gestation, alveolar type II cells are a major source of pulmonary surfactant, a phospholipid that lines lung alveoli and has unique biophysical and biological features such as surface tension lowering capacity. The presence of adrenaline during labour also triggers the release of surfactant, while surfactant synthesis can be accelerated by exposure to thyroid and glucocorticoid hormones (Robertson et al. 1992). Thus, neonatal respiratory distress syndrome is likely to develop if the production of surfactant is faulty or delayed or if the baby is born prematurely before adequate amounts have been synthesised and released (Kohlendorfer et al. 1998).
2.7 Factors influencing intrauterine lung growth

Intrauterine lung growth is affected by a large number of factors, including respiratory movements, fetal lung liquid, thoracic volume, maternal hypoxia, hypoglycaemia, alcohol, nicotine, glucocorticoids, sex hormones, insulin and several growth factors (Thurlbeck, 1975; Inselman et al. 1985; Moessinger et al. 1990; Simmons et al. 1992; Hooper and Harding, 1995; Jaskoll et al. 1996; Ji et al. 1998; Harding and Albuquerque, 1999). Furthermore, maternal malnutrition is known to inhibit animal lung growth (Kalenga et al. 1999). Thus, intrauterine lung development is very sensitive to external stimuli. It is possible that the wide range of alveolar numbers at birth is a mere reflection of this sensitivity (Merkus et al. 1996).

Of the numerous factors implicated, a more detailed review on the mechanical factors influencing prenatal lung growth, the effect of maternal nutritional deprivation, antenatal glucocorticoids and maternal smoking on lung growth and function will be presented.

2.7.1 Mechanical factors influencing fetal lung growth

Since the development of the pre-acinar airways and blood vessels is complete halfway through fetal life, disease or mechanical interference at this stage may have an irrevocable effect (Wohl, 1995). When later interference in fetal development occurs, the multiplication and differentiation of alveoli is affected, but since these continue to multiply during childhood, some adjustment may occur (Harding, 1994).

There is increasing evidence that, in the fetus, the mean level of lung expansion (i.e. luminal volume) plays an important role in determining the rate of lung growth (Harding and Hooper, 1996; Nardo et al. 1998). Thus, intra-uterine abnormalities that cause a prolonged reduction in the mean level of fetal lung expansion could lead to impaired lung development such as pulmonary hypoplasia, defined as lungs that are sufficiently restricted in growth that their ability to exchange respiratory gases is impaired (Harding and Albuquerque, 1999).
The capacity of the thoracic cage has considerable impact on lung growth. Thus, in fetuses with congenital diaphragmatic hernia, restriction in early fetal life leads to a reduction in airway number (Hislop, 1995). Similarly, the effect of oligohydramnios, in fetuses with renal agenesis, renal dysplasia or those with prolonged leakage of amniotic fluid, is more severe when present early in pregnancy (Thibeault et al. 1985; Moessinger et al. 1986; Higuchi et al. 1991; Thompson et al. 1992; Roberts and Mitchell, 1995). In addition to having a reduced number of smaller airways (bronchioli), fewer alveoli and reduced total surface area for gas exchange, the air-blood interface may be thicker than expected, have reduced elastin formation and an attenuated pulmonary vascular bed in severely hypoplastic lungs, all of which can cause varying degrees of respiratory compromise in the newborn (Harding and Albuquerque, 1999).

Lung fluid may also, theoretically, be influenced by acquired loss of amniotic fluid, as occurs following amniocentesis. Amniotic fluid analysis is commonly used to diagnose genetic or neural tube defects during mid trimester. In an assessment of the hazards of amniocentesis for the Medical Research Council, United Kingdom in 1978, the working party reported an increased prevalence (1.3% in matched subjects versus 0.4% in controls) of unexplained respiratory distress at birth after amniocentesis (Working Party on Amniocentesis, 1978). Since then, it has been suggested that invasive antenatal procedures such as amniocentesis may have an adverse effect on perinatal lung function (Vyas et al. 1982; Hislop et al. 1984; Tabor et al. 1986; Grant et al. 1991; Yüksel et al. 1997; Greenough et al. 1997). In a randomised controlled trial comparing 4606 women, Tabor and colleagues reported a small but significantly increased risk of respiratory distress syndrome (Relative risk: 2.1; 95% Confidence limits (CI): 1.1 – 4.1) and pneumonia (Relative risk: 2.5; 95% CI: 1.1 – 6.3) in infants after amniocentesis. Vyas et al. (1982) reported diminished crying vital capacity in 10 infants born to mothers who had amniocentesis, suggesting that lung volume may be reduced as a result. In a later study, Yuksel et al. (1997) reported that, compared with controls, airway resistance was increased among infants following early amniocentesis and / or CVS. This same group of investigators also reported that these invasive first trimester procedures were associated with a significant increase in respiratory symptoms during early infancy and also a significant elevation of their mean FRC (Greenough et al. 1997).
In animal studies on the effects of amniocentesis, structural changes, such as a significant reduction in the generations of respiratory bronchioli as well as in the total number of alveoli, were observed in newborn monkeys exposed to amniocentesis (Hislop et al. 1984). However, lung volumes in the amniocentesis cases were not as low as might be expected as the reduced alveolar number was accompanied by the increased alveolar size. It has been suggested that the lungs of Macaca fascicularis resemble those of humans more than those from sheep, pigs or rats. Therefore it is possible that some of the changes seen in monkey lungs are also present in human infants after maternal amniocentesis. However, in a study of 354 women who underwent mid-trimester genetic amniocentesis and their matched controls, there was no evidence that infants exposed to maternal amniocentesis had a higher prevalence of neonatal respiratory complications (Hunter, 1987).

2.7.2 Influence of pre-natal nutrition on lung development

Over the last two decades, the potential influence of inadequate nutrition on lung structure and function has been extensively explored. Knowledge of nutrition-related pulmonary changes is based mainly on animal studies.

2.7.2.1 Animal studies

Fetal lung metabolism increases dramatically near term when glucose oxidation serves as the most important source of energy fuel (Hamosh et al. 1981). When rat fetuses were exposed to prolonged periods of nutritional deprivation during critical periods of growth, Simmons et al. demonstrated that glucose transport was spared in the brain but diminished in the fetal rat lungs, thus possibly affecting lung growth (Simmons et al. 1992).

Evidence has also been reported that pre-natal under-nutrition is associated with delayed cytodifferentiation of epithelial cells, decrease in lung phospholipid content and negatively interferes with pulmonary surfactant production (Faridy, 1975; Lin and Lechner, 1991; Guarner et al. 1992). The effects of pre-natal underfeeding on the formation of terminal air spaces varies with both animal species and timing of malnutrition and this has led to much contradictory evidence in the literature (Curle
and Adamson, 1978; Lin and Lechner, 1991). Morphometric analyses do, however, reveal that prenatally starved animals have a significantly reduced volume and surface area of parenchymal components as adults, even though catch up growth occurs once normal feeding is resumed (Kalenga et al. 1999). In a study to investigate the effects of intrauterine deprivation on aspects of structural development of the trachea and lungs of fetal sheep, Rees et al. found abnormal development of the trachea, particularly affecting the mucosal and submucosal layers. There was frequently a lack of a ciliated border on epithelial cells in the mucosal layer and a reduction in the extent of the folds usually characteristic of this layer in near term fetal sheep (Rees et al. 1991). Furthermore, in a recent study, near term sheep fetuses in whom IUGR was induced by umbilico-placental embolization were shown to have a thicker air-blood barrier (Harding et al. 2000) and lower total DNA content than controls (Cock et al. 2001). However, pulmonary DNA concentration (per g of lung weight) in the IUGR group was elevated compared to controls (Cock et al. 2001). The authors proposed that as pulmonary DNA concentration decreases during normal gestation, the higher pulmonary DNA concentration in the IUGR group suggests that the lungs in these fetuses were structurally immature.

Dietary lipids are also critical for fetal lung development as fatty acids play an essential role in the structural and functional dynamics of fetal lung cells towards maturation (Nelson et al. 1980). During early lung development, lipids play an important role in protecting against oxidant-induced lung injury. Pregnant rats fed with diets enriched with polyunsaturated fatty acids have been shown to have better tolerance for hyperoxia as newborns (Sosenko et al. 1988).

There is increasing evidence that Vitamin A (retinol) and its metabolite, retinoic acid, are major factors involved in differentiation and in the maturation of the lungs (Chytil, 1992). In his review, Chytil reported that Vitamin A is involved with pulmonary gene expression and that lack of this dietary micronutrient causes keratinizing squamous metaplasia of the bronchopulmonary tree that can be reversed by refeeding the animal with retinol. In addition, Vitamin A is an essential factor in the regulation of type II pneumocyte proliferation (Zachman, 1995). Conversely, prenatal hypervitaminosis A has been shown to cause thickening of septal walls with
atelectasis in fetal lung parenchyma (Kalenga et al. 1999). Similarly, trace elements such as selenium are involved in fetal and neonatal lung development and deficiency of this element may result in lung growth retardation with septal attenuation (Kim et al. 1991).

### 2.7.2.2 Human observations

Currently, there is little information on the influence of specific nutrients or micronutrients on human fetal lungs or infant airway development, relative to animal data presented earlier. One form of fetal malnutrition is intrauterine growth restriction, resulting in infants who are small for gestational age.

Post-mortem studies of the newborn infants who are underweight have shown that their lungs are lighter than expected for body weight although alveolar development appeared appropriate for gestational age (Gruenwald, 1963). In a study undertaken of people born around the time of the Dutch famine in 1944-5 to determine the effects of maternal malnutrition, the prevalence of obstructive airways disease was increased in people exposed to famine in early and mid gestation (Lopuhaa et al. 2000). It has also been suggested that fetal malnutrition may impede growth of conducting airways. Barker et al reported that FEV₁ was significantly reduced in adults aged 59-70 years whose birth weight was < 2500 g, even after adjusting for age, height, smoking habits and social class (Barker et al. 1991). Impaired airway function in relation to low birth weight was also reported in adult Indians (Stein et al. 1997). These data suggest that maternal and fetal nutrition may influence fetal lung growth and development. However, in humans it is not known how much of the gas exchange compartment is affected structurally and functionally by pre-natal under-nutrition.

A recent study by Mathews et al. examined the influence of smoking status and age on nutrient intakes of 774 women during pregnancy (Mathews et al. 2000). The authors reported that pregnant smokers had poorer intakes of most micronutrients than non-smokers. Although some antioxidants such as zinc were consumed in similar amounts by smokers and non-smokers of comparable age and education, the apparently greater requirement for antioxidants among smokers means that their poor
intakes may have greater biological implications. In addition, older women had higher intakes of most nutrients than younger women. Thus young smokers are at risk of poor nutritional status during pregnancy (Mathews et al. 2000). There is however, currently no evidence that antioxidant supplementation in normal pregnancies is beneficial as placental and infant birthweights were not associated with the intake of any micronutrient during early or late pregnancy (Mathews et al. 2000).

In a recent prospective Aberdeen study, Devereux and colleagues demonstrated that

in vitro T helper cell proliferative responses of cord blood mononuclear cells (CBMC) to allergenic stimuli from a sample of 223 neonates, representative of children born to a cohort of 2000 pregnant women, were positively associated with established epidemiological risk factors for asthma and atopic disease, including history of atopy and maternal smoking (Devereux et al. 2002). In addition, the CBMC-proliferative responses were negatively associated with maternal dietary intake of vitamin E (Devereux et al. 2002), the reduced intake of which is being increasingly associated with atopic disease in children and adults (Bodner et al. 1999; Hijazi et al. 2000). Hence, the demonstration of an association between maternal dietary intake of vitamin E and the development of the fetal immune system suggests that manipulation of maternal diet during pregnancy should be further investigated.

2.7.3 Influence of antenatal glucocorticoids

The use of corticosteroids, specifically dexamethasone and betamethasone, for the prevention of respiratory distress syndrome (RDS) in infants of women who experience preterm labour has been investigated extensively since it was first reported in 1972 (Liggins and Howie, 1972). Randomised controlled trials, case-control studies and meta-analyses have shown that these steroids, when administered antenatally, accelerate lung maturation, and hence are effective in reducing the incidence of RDS and in lowering morbidity and mortality in preterm infants (Wong et al. 1982; Wiebicke et al. 1988; Ryan and Finer, 1995; Pinkerton et al. 1995). Animal models have shown that the functional effects of corticosteroids are primarily mediated by rapid alveolisation i.e., accelerated thinning of the alveolar wall.
thickness, and an increase in aerated parenchyma (Pinkerton et al. 1995; Massaro and Massaro, 1996) rather than by increased production of alveolar surfactant, though maturation of surfactant producing type II pneumocytes is also accelerated (Adamson and King, 1988; Vyas and Kotecha, 1997). However, Beck et al. have demonstrated in a study using Rhesus monkeys that the betamethasone-treated fetuses had increased collagen and decreased elastin concentrations compared to controls (Beck et al. 1981). The functional effect of changes in the collagen/elastin content of lung tissue is complex but is thought to contribute to the suppression of the formation of secondary septa, hence a decrease in the number of alveoli and lung growth (Schellenberg et al. 1987). In a follow-up study of 15 infants of very low birthweight (<1500 g), seven of whom had been exposed to antenatal dexamethasone, no differences were found with respect to thoracic gas volume, dynamic pulmonary compliance or airway resistance during the first year of life (Wong et al. 1982). A later study on 20 school age children (nine were exposed to antenatal dexamethasone), also showed no differences with respect to vital capacity, functional residual capacity by helium dilution, residual volume or total lung capacity between the two groups, suggesting that, if antenatal dexamethasone initially inhibits lung growth in humans, catch-up growth may have occurred postnatally (Wiebicke et al. 1988). However, the study numbers are very small and so do not have sufficient power to detect differences between the groups. Previous studies have also suggested there may be different rates of lung maturation for males and females and hence sex differences in the response to the glucocorticoid induction of this process (Papageorgiou et al. 1981; Torday et al. 1981).

In addition to concerns that antenatal dexamethasone is associated with diminished birthweight (French et al. 1999; Bloom et al. 2001) and head circumference (French et al. 1999; Shelton et al. 2001) in infants, the effect of multidose antenatal corticosteroids on maternal and infant outcomes has been under increasing scrutiny. Repeated courses of maternal betamethasone given to fetal lambs have resulted in progressive improvements in postnatal lung function in prematurely delivered lambs, but this was accompanied by a decrease in body weights of the newborns and a persistent suppression of cortisol and epinephrine postnatally, particularly after the fourth dose of betamethasone (Ikegami et al. 1997). French et al reported a significant reduction in birthweight by as much as 9% (p = 0.01) and approximately
4% (p < 0.01) reduction in head circumference in cases in which ≥ 3 courses were given (French et al. 1999). A systematic review on the risks and benefits of multiple courses of antenatal steroids, for which only well-designed animal studies were included, suggested that risks included multiple adverse consequences such as decrease in birth and lung weights and brain growth restriction (Walfisch et al. 2001). However, in a randomised controlled trial (n = 414 human fetuses), the authors reported that prolonged antenatal betamethasone therapy was not associated with higher risks of antenatal maternal fever, chorioamnionitis, reduced birthweight, neonatal adrenal suppression, neonatal sepsis or neonatal death (Thorpe et al. 2001). Nevertheless, the National Institutes of Health Consensus Development Panel in their latest conference statement recommended the use of a single course of antenatal corticosteroids and that repeat courses should be reserved for patients enrolled in clinical trials (National Institutes of Health Consensus Development Panel, 2001).

2.7.4 Influence of maternal smoking on prenatal lung development

Carbon monoxide and nicotine are two of the major components in cigarette smoke and the adverse effects of these toxins on the fetus have been studied by a number of authors (Mochizuki et al. 1984; Koren, 1995; Aubard Y and Magne I, 2000).

Carbon monoxide has an affinity for haemoglobin 250 times greater than oxygen. It readily dissolves in the plasma thereby perturbing oxygen exchange in the tissues (Aubard Y and Magne I, 2000). The dissolved carbon monoxide in maternal plasma crosses the placental barrier by passive diffusion and as fetal haemoglobin has a higher affinity for carbon monoxide than adult haemoglobin, the unborn child is exposed to higher risk from this gas than the mother. In pregnant smokers, the concentration of carboxyhaemoglobin in the blood is about 3%, while the level in the blood of a newborn infant of a non-smoking mother is 2%, which increases to 6–9% if the mother is a smoker (Aubard Y and Magne I, 2000). Thus chronic exposure to carbon monoxide during pregnancy and post delivery may produce significant growth restriction as a result of the adverse effects on oxygenation by carboxyhaemoglobin (Lambers and Clark, 1996).
Nicotine is a weak base, and as fetal blood is more acidic than maternal blood, the alkaloid is more ionised in the fetal circulation, creating a driving force of movement from mother to fetus (Koren, 1995). Thus, nicotine readily gains access to the fetal compartment via the placenta, with fetal concentrations generally 15% higher than maternal levels (Koren, 1995; Lambers and Clark, 1996). In a recent study, Jauniaux et al. reported that cotinine, a derivative of nicotine, was found in coelomic and amniotic fluid as early as seven weeks' gestation in both active and passive smokers (Jauniaux et al. 1999). In addition, cotinine levels from newborn infants' first urine were shown to be significantly higher than those not exposed to in-utero smoke (Etzel et al. 1985) and were comparable with levels found in active adult smokers (Hoo et al. 1998). Thus, throughout gestation, the fetus is exposed to increasing concentrations of nicotine through maternal blood and via gastrointestinal and skin absorption of nicotine from the amniotic fluid (Koren, 1995). The physiological effects of nicotine on fetal growth appear to be primarily vasoconstrictive effects on the uterine and potentially the umbilical artery, hence causing abnormal placental development (Genbacev et al. 2000) and impaired placental function through reduction in placental blood flow (Lambers and Clark, 1996).

The potential effects of smoking during pregnancy on lung and airway development may include structural alterations, interference with ventilatory response to hypoxia and alterations to the developing immune system (Collins et al. 1985; Milerad et al. 1995; Ji et al. 1998; Wisborg et al. 1999). Animal studies have shown that maternal cigarette smoke exposure during pregnancy is characterised by fetal growth retardation and lung hypoplasia with decreased airspaces and a reduction in the length of elastin in the saccule walls (Collins et al. 1985; Sekhon et al. 1999). Maritz and Thomas reported alveolar fenestrations, blebbing and rupturing of the blood-air barrier in the alveolar epithelial cells of smoke exposed neonatal rats (Maritz et al. 1993). Sekhon et al. reported that prenatal nicotine increases α7 nicotinic receptor expression in the developing lung of rhesus monkeys and that the increased collagen deposition around airways was stimulated by the interaction of nicotine with the α7 nicotinic cholinergic receptor-bearing fibroblasts (Sekhon et al. 1999). In addition, absolute and specific (per 100g body weight) lung weight were significantly lower (16% and 14%) respectively in the nicotine-exposed group compared to the controls, suggesting that prenatal nicotine exposure decreases lung
growth. Peak tidal expiratory flow and FEV\(_{0.2}\) was significantly lower, but pulmonary resistance and specific pulmonary resistance (corrected for lung volume) were significantly increased in monkeys exposed to prenatal nicotine (Sekhon et al. 2001). Furthermore, maternal exposure to environmental smoke was found to alter the normal development of the Clara cell in the fetal rat lung and to increase the size of neuroepithelial bodies in the fetal lung (Chen et al. 1987; Ji et al. 1998). Thus it has been suggested that these changes may alter airway defence in early life and contribute to the pathophysiology of sudden infant death syndrome (SIDS) (Nicholl and O'Cathain, 1992; Cutz et al. 1996).

Milerad and colleagues also suggested that nicotine alters peripheral chemoreceptor oxygen sensitivity and affects central processing of the chemoreceptor input. Hence they hypothesised that the observed associations between parental smoking and SIDS is mediated by adverse effects of nicotine on central control of breathing (Milerad et al. 1995). Similar findings were reported by Lewis and Bosque who observed that infants of mothers who smoked during pregnancy have deficient hypoxic awakening responses, which may contribute to the increased risk of SIDS in such infants (Lewis and Bosque, 1995). Recently, Hubbard et al. suggested that infants exposed to in-utero smoke have an altered arousal response (Hubbard et al. 2000) but Poole and colleagues did not find an independent effect of maternal smoking on respiratory control (Poole et al. 2000).

The association of maternal smoking during pregnancy with increased childhood respiratory illnesses has been well documented (Strachan and Cook, 1997; Strachan and Cook, 1998). Infant lung function studies have shown changes in the pattern of breathing and diminished airflow in infants whose mothers smoked in pregnancy (Hanrahan et al. 1992; Brown et al. 1995). Martinez et al. also reported an increased incidence of asthma in children of smoking mothers and observed that children of lower socio-economic status may be at considerable risk of developing asthma if their mothers smoked more than 10 cigarettes per day (Martinez et al. 1992). Thus it has been hypothesised that maternal smoking in pregnancy may cause a reduction in airway size as well as alterations in the growth and maturation of the mechanical properties of the respiratory system in the newborn. In a study of preterm infants, these changes are evident at least seven weeks prior to their expected
date of delivery, suggesting that the adverse effects of prenatal exposure to tobacco are not limited to the last weeks of pregnancy (Hoo et al. 1998). Furthermore, among term infants, specific airway conductance during end expiration was significantly diminished at one year of age in those exposed to maternal smoking (Dezateux et al. 2000).

2.8 Postnatal lung growth

Although birth represents a radical environmental change for the lung, alterations induced by the birth process tend to be more functional than structural in nature, such that lung development transits smoothly from the prenatal to postnatal period.

At birth, although already functional, the lung is structurally still in an immature condition. Alveoli in newborns are fewer in number and less complex in anatomic detail than in adults. This dissimilarity has led some morphologists to call them ‘saccules’ rather than alveoli (Dunnill, 1962). It has been estimated that there are about 150 million alveoli present at birth compared with approximately 300 million in adults (Hislop et al. 1986).

Following delivery at term, the airspaces present are smooth-walled transitory ducts and saccules with primitive type septa that are thick and contain a double capillary network (Burri, 1984). Between the sixth and eighth postnatal week true alveoli develop rapidly (Boyden, 1967). The respiratory bronchioli elongate and alveoli bulge from the areas of flattened epithelium. Saccules and transitional ducts are converted into alveolar ducts by their lengthening and by deepening of the primitive alveoli in their walls (Hislop and Reid, 1974). In the human lung, rapid alveolisation occurs during the last few weeks of the prenatal period and the first 5 to 6 months of postnatal life. However, a slower phase must be assumed to last up to the age of 2 years or even older (Thurlbeck, 1982). Morphometric studies reveal that the human lung volume increases about 23 fold between birth and adulthood. During the same period, the alveolar and capillary surface areas expand about 12 fold and the capillary volume 35 fold (Burri, 1999). During the first few years of life, there is dysanaptic growth as airways and lung parenchyma develop disproportionately in size. This is because the conducting airways are complete in number at birth and
increase only in size, whereas alveoli increase both in size and in number (Mead, 1980a; Sherrill et al. 1989; Sherrill et al. 1990).

During the first 18 months of life, as new alveoli form and enlarge, the majority of the new blood vessels develop at the periphery of the lung. The small pulmonary arteries reach adult wall thickness within the first few days of life while the larger vessels take up to three months. Beyond this period there is an increase in airway lumen diameter, wall thickness and smooth muscle cell diameter as arteries increase in size as lung volume increases (Hislop, 1995).

Although both collagen and elastin are important in airway development and branching, the interstitium of the lung contains little collagen and elastin during late gestation and at birth. This may contribute to the ease with which pneumothoraces develop in premature lungs.

Elastin is the primary elastic vertebrate protein responsible for passive recoil as the lungs undergo repeated cycles of expansion and contraction. Together with collagen fibres, it provides a primary force for expiration (Mariani and Pierce, 1999). Elastin, which appears to be closely related to the development of alveoli and lung collagen, increases during early postnatal life (Wohl, 1998). The pre- and post-natal development of saccules/alveoli by septation is closely related to the elastic tissue network in the lung parenchyma. Each new septum begins as a crest that is demarcated by an elastic fibre (Hislop and Reid, 1974). Hence a lack of elastin may result in decreased alveolarisation.

2.8.1 Postnatal airway growth and function

At birth, the basic formation of cartilaginous airways is complete and additional division does not occur. Growth of each segment of the conducting airways appears to occur in a symmetric fashion, both in length and in diameter, until growth of the thorax ceases (Hislop and Reid, 1974). During the first year of life there is rapid increase in bronchial smooth muscle especially in the bronchioli. It has been suggested that this rapid increase is related to the change to air breathing and that wall structures of the healthy lung increase with age in proportion to airway
diameter. However, infants born prematurely or those requiring artificial ventilation following delivery have increased muscle in peripheral airways (Hislop and Haworth, 1989). Recent findings by Elliot and colleagues have also shown that, among infants who succumbed to SIDS, the inner airway wall thickness was greater in the larger airways of those whose mothers smoked > 20 cigarettes per day when compared to those infants not exposed to maternal smoking (Elliot et al. 1998). The authors suggest that the increased airway wall thickness may contribute to exaggerated airway narrowing which may explain the diminished airway function observed in infants of smoking mothers. In asthmatics, airway walls are generally thicker than normal due to the increased amounts of connective tissue and muscle, hence influencing airway calibre (James et al. 1989), which could have a similar effect.

Conducting airways perform many functions in addition to gas conduction e.g. warming, humidification and cleansing of inhaled air of potentially harmful dust particles and micro-organisms. In addition, they have secretory functions. They dilate and contract passively in response to influences such as lung inflation and actively in response to a variety of humoral and chemical stimuli mediated by the epithelium, smooth muscles, glands, nerves and cells (Jeffery, 1995). In humans, four cell types comprise the surface epithelium of the conducting airways, namely ciliated, goblet, indeterminate and basal cells. In the terminal bronchioles, Clara cells are also found (Jeffery and Hislop, 1995).

Ciliated cells, the dominant cells of the epithelial layer, are present throughout all conducting airways. The co-ordinated, sweeping motion of the cilia provides the force that impels the superficial layer of secretions along its journey from peripheral airways into the pharynx and is an important natural defence mechanism for removing inhaled particles deposited within the lung (Murray, 1986b). In disease there may be widespread loss of cilia, particularly at sites of airway branching where air-borne pollutants often impact (Jeffery, 1995).

The main function of the goblet cell is the secretion of the correct amount of mucus with the optimal viscoelastic profile, which is important for the maintenance of mucociliary clearance (Jeffery, 1995). The number of goblet cells increases with
diseases such as chronic bronchitis or following inhalation of tobacco smoke. The 
basal cell is considered to be the major stem cell from which the more superficial 
mucus-secreting or goblet cells and ciliated cells derive. The function of the Clara 
cell is as yet undetermined. However it acts as the principal stem cell of small 
airways where basal and mucous cells are normally sparse and both ciliated and 
mucous cells may develop from the Clara cell subsequent to its division and 
differentiation (Jeffrey, 1998).

2.9 Factors influencing postnatal lung growth and function

Several environmental factors known to influence intrauterine lung growth as 
discussed in section 2.7 also affect post-natal lung growth. These factors include 
smoking (Section 2.7.4), growth factors, drugs such as glucocorticoids (Section 
2.7.3), oxygen tension and infection. In addition, other factors such as sex, ethnic 
group and family history of asthma and atopy may also have an important role. In 
this section, the influence of sex, ethnic group and nutrition are briefly reviewed.

2.9.1 Influence of sex

Numerous studies have suggested that airway function is diminished and respiratory 
ilness is more prevalent among boys than girls during both infancy and childhood 
(Taussig et al. 1981; Hanrahan et al. 1990; Rona and Chinn, 1993; Gold et al. 
1994; Hibbert et al. 1995; Stocks et al. 1997; Hoo et al. 1998). Furthermore, it has 
been proposed that this sex difference in respiratory morbidity is directly related to 
differences in airway structure in early infancy as significantly more muscle was 
found in airways of male infants (McKay, 2000). Recent evidence also suggests that 
after maximal inhalation, girls have higher flows than boys at age 11 (Marotti et al. 
2001). However, by adulthood, boys have larger airways relative to lung size than 
girls. This enhanced airway growth in adolescent males may partly explain the 
marked clinical improvement in males with respiratory disease as they become adults 
(Martin et al. 1988; Hibbert et al. 1995).
2.9.2 Influence of ethnic group

Neonatal mortality is lower among Afro-Caribbean infants of low birth weight who are also less likely to develop respiratory distress syndrome than white infants of similar gestational age (Collins and David, 1990; Greenberg et al. 1993). This suggests that the respiratory system is either more mature or that airway function is enhanced in black preterm infants (Stocks et al. 1994a). As infants are preferential nose breathers, another reason for this difference may be attributed to differences in airway anatomy. The lower total airway resistance found in Afro-Caribbean infants when compared with Caucasian infants of similar age and weight was accounted for in part by their lower nasal resistance (Stocks and Godfrey, 1977; Stocks and Godfrey, 1978). While lung volume and forced expiratory flows based on values predicted from standing height tend to be lower in Afro-Caribbean adults and older children, no discrepancies are observed when respiratory function is related to sitting height (Pool and Greenough, 1989; American Thoracic Society, 1991), suggesting that differences primarily reflect ethnic variation in trunk:length ratio.

2.9.3 Influence of nutritional deprivation on postnatal lung growth

In most mammalian species, the lung parenchyma still undergoes several structural and functional changes such as the formation of alveoli, maturation of the capillary network and the accumulation of connective tissues during the early neonatal period. Therefore neonatal starvation at the time of alveolar multiplication may reduce the rate of cell division and lung growth (Hislop, 1995). Animal studies undertaken to investigate the consequences of protein restriction on the rat lung during early life have shown that pups from protein restricted dams had lower lung volumes, though specific lung volumes were increased (Kalenga et al. 1995). Furthermore, in a previous study by Kalenga and Eeckhout, it was noted that these rat lungs had a lower recoil pressure and that elastin concentration was greatly reduced following protein deprivation during the first three weeks of life (Kalenga and Eeckhout, 1989). The decrease in lung recoil pressure is thought to be related to the lowered elastin concentration. However there is contradictory and conflicting evidence due to the different effects according to the timing and severity of these insults (Kalenga et al. 1999). Another common finding was the enlargement of the terminal air spaces such
that alveolar surface area was reduced, with the subsequent emergence of an emphysematous pattern. However the exact mechanism governing these changes is unclear (Sahebjami and MacGee, 1983; Kerr et al. 1985).

Thus human malnutrition in late fetal life and infancy may have an important effect on lung size (Gaultier, 1991). A typical example in which nutrition plays a critical role in postnatal lung remodelling is the tiny premature infant. Due to lung immaturity, poor nutritional intake, increased energy expenditure and poor intestinal absorption, these infants undergo periods of inadequate nutrition with resulting growth failure. It is now recognised that inadequate post-natal nutrition plays an important role in the development of bronchopulmonary dysplasia (BPD) (Skelton and Chetcuti, 1996).

Characteristically, lung parenchyma of long-standing BPD presents an emphysematous-like aspect and is associated with alteration in the structural biochemistry, mainly in lung elastin. There is speculation that, in infants prone to BPD, a first phase of increased elastolysis is followed by decreased elastosynthesis, possibly because of inadequate nutrition (Sosenko and Frank, 1991). Similar evidence has been presented by Zemel et al. on children with cystic fibrosis. The authors observed that growth and nutritional status are associated with changes in FEV₁% predicted in these children and suggested that nutritional intervention may slow the decline in pulmonary function in children with cystic fibrosis (Zemel et al. 2000).

In developing countries, malnutrition is the most important cause of morbidity and mortality among pre-school children and the still restructuring lung may be sensitive to various nutritional deficiencies and hence at increased risk for pulmonary infections (Kalenga et al. 1999). However, little is known about the association of malnutrition and lung growth in this age group. While decreased peak flows in relation to poor nutrition have been repeatedly shown in both children and adults who do not have respiratory disease, interpretation of these results is confounded by the fact that respiratory muscle weakness due to poor nutrition probably reduces the patient's capacity to perform forced expiration (Primhak and Coates, 1988; Ong et al. 1998).
2.10 Relevance of current literature to IUGR

While IUGR may be caused by maternal (e.g. poor nutrition, pregnancy-induced hypertension, or smoking), placental (e.g. abruptio placenta or gross placental structural abnormalities) or fetal (e.g. karyotypic abnormalities or multiple gestation) factors, approximately one-third of IUGRs are due to genetic causes and two-thirds are related to the intrauterine environment (Wollmann, 1998). Fetal, neonatal and perinatal mortality are increased in SGA compared to AGA infants (Miller, 1995; Friedman et al. 1995; Vik et al. 1996; Bonatz et al. 1997; Oyen et al. 1997) and evidence from both epidemiological studies and data from animal and cellular studies indicates that diminished lung growth and development may also accompany impaired somatic growth during pre and postnatal life.

On the influence of pre-natal under-nutrition on lung development, animal studies (Section 2.4.2.1) have shown that glucose transport is diminished in fetal lungs due to the brain sparing effect, thus possibly explaining why the lungs of newborn human infants who were underweight were lighter than expected for body weight in post-mortem studies (Section 2.4.2.2). In addition, other effects of under-nutrition observed in animal studies, such as the lack of a ciliated border on epithelial cells in the mucosal layer of the trachea, a thicker air-blood barrier and thickening of septal walls with atelectasis in fetal lung parenchyma or septal attenuation, may be similar in the human fetus thereby providing a possible mechanism underlying the increased respiratory morbidity and mortality associated with growth restricted infants.

Studies investigating the influence of antenatal steroids on lung development in animal models have shown that the functional effects of corticosteroids are primarily mediated by rapid alveolisation and increase in aerated parenchyma (Section 2.4.3). While the use of corticosteroids for the prevention of RDS in infants of women who experienced preterm labour has been effective, repeated use of antenatal steroids in high risk pregnancies, such as severe intrauterine growth restriction or recurrent mid-trimester abortions which may necessitate preterm delivery, is increasingly common (Goldenberg and Wright, 2001). In animal studies, risks associated with the use of multiple courses of antenatal corticosteroids include decrease in birth and lung weights and brain growth restriction. However, evidence in human pregnancies
remains controversial, possibly due to the multiple factors affecting fetal growth and the difficulty in controlling for these factors during analyses. Nevertheless, it is possible that when multiple courses of antenatal steroids are administered to a mother with a severely growth-restricted fetus, it may further compromise fetal growth and development in-utero.

In its 1999 consultation, the World Health Organisation concurred with other reviewing bodies concerning the effects of maternal smoking on birthweight, lower respiratory illnesses and lung function in children and the increased risk for SIDS (WHO, 1999). Infants with parents who smoke have an increased risk of lower respiratory tract illness, including a significantly increased frequency of bronchitis and pneumonia during the first year of life (Strachan and Cook, 1997). However, the nature of the common lower respiratory tract illnesses of infancy remains a subject of uncertainty and debate. While many appear to be triggered by viral infections, there is evidence of premorbid susceptibility related to lung function abnormalities detectable shortly from birth (Dezateux and Stocks, 1997).

Currently, there are very limited data regarding the influence of intrauterine growth retardation on airway function in infants. To date, there has only been one study by Dahms et al., which was based on nine SGA infants, whose gestational age ranged from 33 – 41 weeks (Dahms et al. 1974). However, this study was small and gestational age was determined from physical appearance and neurologic characteristics, which are less accurate than sonographic assessments. The current study was therefore established to examine the hypothesis that airway function is impaired in infants who are of low birthweight for gestational age.
3 Study design and Methods
3.1 Study design

3.1.1 Hypothesis and aims of study

This study was established to investigate whether airway function and development are impaired shortly after birth among infants with low birthweight for gestational age compared with those of normal birthweight. The null hypothesis is that airway function is not impaired in infants with low birthweight for gestational age compared to normal birthweight infants. Infants of both smokers and non-smokers were recruited to this study, as maternal smoking during pregnancy is known to be a major risk factor for both reduced airway function and being SGA.

The specific aims of the study are:

- to determine whether, relative to infants of appropriate weight, infants of low birthweight for gestational age have impaired airway function, as assessed by measurements of forced expiratory flow and tidal breathing parameters shortly after birth, and

- to determine the effects of maternal smoking shortly after birth, to examine any interactions between maternal smoking and birth status on airway function, and to establish the relative effect of such exposure on parameters derived from the partial and raised volume technique within the same infant.

3.1.2 Overview of study design

Healthy infants ≥ 35 weeks gestation, of low and appropriate birthweight for gestational age, who were and were not exposed to maternal smoking in pregnancy were recruited shortly after birth. Respiratory function was assessed by measuring forced expiratory flow during tidal breathing and at raised lung volume. These measurements were undertaken at four to twelve weeks postnatal age (PNA). Fetal and postnatal exposure to tobacco smoke was assessed from maternal report. This was compared with cotinine levels in infant urine and maternal saliva obtained at the time of respiratory function test.
3.1.3 Case definition

3.1.3.1 Determination of estimated date of delivery

In order to ensure accurate dating of the pregnancy, only infants of women who had received antenatal care and a dating scan before the 20th week of pregnancy were included in the study. The estimated date of delivery, calculated from the first day of the last menstrual period, was generally used unless there was a discrepancy of more than two weeks on the basis of the scan findings. In such case, the estimated date of delivery was revised. If the date of the last menstrual period was unknown, the estimated date of delivery was based on the scan dates.

3.1.3.2 Assessment of birthweight for gestational age

Infants were routinely weighed at birth by the midwife. Gestational age at birth was calculated from the estimated date of delivery (Section 3.1.3.1). This information was used to derive a birthweight for gestational age centile and standard deviation score using an Excel macro, based on the Child Growth Foundation (CGF) algorithm (Freeman et al. 1995).

As the optimal method of classifying infants according to their birthweight centiles remains unclear, whenever additional background information such as mother’s height and booking weight were available in the mother’s obstetric records, an individualised birthweight centile was also calculated using the Gestation Related Optimal Weight (GROW) program (Wilcox et al. 1993).

3.1.3.3 Small for gestational age (SGA)

Infants with birthweight at or below the 10th centile according to the CGF or the GROW program were identified as small for gestational age (SGA). However, as there was a systematic difference in birthweight centiles calculated using these two algorithms (Lum et al. 1999), infants who were between the 10th and 15th centile according to CGF but were at or below the 10th centile according to GROW were also classified as SGA.
3.1.3.4 Appropriate for gestational age (AGA)

Infants whose birthweight centile fell between the 20th and 95th centile according to the CGF program were recruited as controls.

3.1.4 Study population

3.1.4.1 Inclusion criteria

Infants were included in this study if they:

- were healthy, singleton infants of ≥ 35 weeks gestation.
- had a white European mother.
- required no or minimal resuscitation at birth.
- were classified as SGA or AGA according to criteria given in Sections 3.1.3.3 and 3.1.3.4.

3.1.4.2 Exclusion criteria

Infants were excluded if:

- their mothers had gestational diabetes or ruptured membranes for more than one week prior to delivery.
- they had respiratory disease at birth requiring ventilation and/or supplemental oxygen.
- they had known congenital or neuromuscular disorders.
- they had lower respiratory illness prior to initial respiratory function test.
3.1.4.3 Maternal smoking classification

In this study, a smoking mother was defined as one who continued to smoke after eight weeks gestation, irrespective of the number of cigarettes smoked. Maternally reported smoking was validated by cotinine assay of maternal saliva and a sample of the infant’s urine on the day of test.

3.1.5 Recruitment

Potentially eligible infants were identified from the Labour Ward birth register, obstetric and neonatal records at the Homerton Hospital, and whenever possible invited to participate in the study while their mothers remained on the maternity wards. Mothers were approached and details of the study were explained. They were also given an information leaflet about the respiratory function test (Appendix A) and encouraged to ask questions about the project. Due to the short length of stay in hospital post delivery (24 - 48 hours), parents often requested time to consider the issue at home. They were contacted by telephone a few days later to seek consent.

Mothers who were transferred home before contact could be made on the ward were sent an introductory letter inviting them to participate in the study, together with an information leaflet about the respiratory function test. This was followed by a telephone call a few days later to discuss the project further and seek consent. Those who did not have a telephone were sent a letter and an information leaflet with a stamped addressed envelope for their reply slip, which registered their interest to join the study. If necessary, a home visit was made to ensure that the parent/s had a good understanding of the project and their baby’s role in the study before consent was sought.

Mothers with a history of depression or with social difficulties (e.g. siblings in care or children on ‘at risk’ register) were not recruited. The wishes of the parents were respected if they did not want their baby to participate in the study.
3.1.6 Neonatal anthropometry

Neonatal anthropometry was not routinely undertaken by midwives and junior medical staff. As part of this study, we attempted to introduce routine selected anthropometric measurements, prior to discharge, of infants delivered at the Homerton Hospital during the study period. Junior medical staff were trained to measure occipito-frontal, chest and mid arm circumferences (Dangerfield and Taylor, 1983). Birth weight was measured shortly after birth, to the nearest 10 g, using Seca electronic scales. Midwives were also trained to measure crown-heel length using a calibrated infant stadiometer. (Traditionally at Homerton Hospital NHS Trust, infant lengths had been measured using paper tape.) Validation checks of anthropometric measurements were made by two researchers at three to six monthly intervals during the initial period following this change in practice and training updates were offered to junior medical staff and midwives to ensure accuracy of data.

- Occipito-frontal circumference

The occipito-frontal circumference (OFC) was measured by the paediatric Senior House Officer at the baby's first examination, usually performed within 24 hours of birth (United Nations, 1986).

- Chest circumference

The baby's chest circumference was measured at the level of the fourth intercostal space while the baby was not crying. This procedure was repeated at least twice, and the result was reported as the mean of two measurements within 0.5 cm of each other (Dangerfield and Taylor, 1983).

- Mid arm circumference

The length of the upper arm was measured from the head of humerus to the olecranon process (elbow). The mid-point of this distance was identified and the mid-arm circumference was measured at this point. This procedure was repeated at least twice and the result was reported as the mean of two measurements within 0.5 cm of each other (United Nations, 1986).
Body length measurement

Crown-heel length was measured by two people. One gently held the baby’s head in the mid-line central position, ensuring that the crown was touching the plate at the top of the stadiometer, whilst the other gently depressed the baby’s knees to fully extend the legs. The footplate was then positioned firmly against the soles of the baby’s feet. The length was read off the counter once the footplate was locked into position. This procedure was repeated at least twice and results reported as the mean of two measurements within 0.5 cm of each other (United Nations, 1986).

3.1.7 Background information

Once mothers were recruited to the study, information on clinical, medical and social factors including smoking habits during pregnancy, details of previous pregnancies (gestational age, birthweight, sex of infants and inter-pregnancy interval); medical problems, e.g. essential hypertension; and obstetric problems such as pre-eclampsia, infection, antepartum haemorrhage or anaemia were extracted from the obstetric notes. Similarly, the records of relevant antenatal investigations including amniocentesis, chorionic villi sampling, ultrasound assessment of growth, liquor volume and any measurements of uterine and fetal blood flow and distribution were also obtained (Appendix B).

3.1.8 Ethical approval and parental consent

Ethical approval for this study was granted by the East London and The City Health Authority Research Ethics Committee and the Institute of Child Health Research Ethics Committee and written informed consent to participate was obtained from one or both parents prior to the measurements (Appendix C and Appendix D).
3.1.9 Sample size and power of study

Five groups of infants were proposed:

a) infants whose birthweight was at or below the 10th centile for gestational age on either the CGF or the GROW programs, whose mothers did not report smoking during pregnancy;

b) infants whose birthweight was at or below the 10th centile for gestational age and whose mothers reported smoking during pregnancy;

c) and d) infants whose birthweight was between 20-95th centile and whose mothers did and did not report smoking during pregnancy respectively.

e) infants with prenatal diagnosis of growth restriction in utero.

A sample size of 40 infants in each group was required to provide 80% power to detect a 10% difference in adjusted estimates of forced expired flows and volumes between birthweight groups, significant at the 5% level.

3.2 Equipment

3.2.1 Introduction

All data were collected using the Respiratory Analysis Software Program (RASP, version 7, Physio Logic LTD, Newbury, Berkshire). The RASP system was semi-automated and was used for the collection of airway function data which were then exported as .DAT/ASCII files for analyses in the Squeeze program, a software package developed in collaboration with the Imperial College of Science, Technology and Medicine (Dixon and Stocks, 1997).

3.2.2 Essential equipment

The following equipment was checked to ensure they were in working order before each respiratory function test.
• Resuscitation equipment, oxygen supply and suction apparatus.

• CO$_2$SMO end tidal carbon dioxide (ETCO$_2$) / oxygen saturation (SpO$_2$) monitor with oximeter flexiprobe for continuous monitoring of arterial oxygen saturation during each study.

3.2.3 Equipment used

Tidal breathing parameters, partial forced expiratory flow using the rapid thoraco-abdominal compression (RTC) during tidal breathing and full forced expiratory flows using the raised volume RTC were recorded using the Respiratory Analysis Software Program (Figure 3.1).

The equipment included (see Appendix G for manufacturer’s details):

• a personal computer (Elonex PC 486) with Analog Devices RTI 815 A-D converters to digitise analog outputs of the transducers

• differential pressure transducers

• $\pm 0.2 \text{ kPa (Flow, } V')$

• $\pm 5 \text{ kPa (airway opening pressure, } P_{ao})$

• $\pm 10 \text{ kPa (Jacket pressure, } P_j)$

• amplifiers and integrators for the pressure transducers (Junction box, Biomedical Engineering Department, Great Ormond Street Hospital, London)

• Hans Rudolph 0-10 L.min$^{-1}$ and 0-35 L.min$^{-1}$ screen pneumotachometers (PNTs)

• Pneumotachometer Heater Control

• 10 and 100 mL calibrated syringes (Hans Rudolph)

• plastic square bladder, 16 cm x 16 cm (Hannover, Germany)

• fabric jacket (Columbus, Ohio, USA)

• 100 L air reservoir tank with large bore three way tap and tubing (Biomedical Engineering Department, Great Ormond Street Hospital, London)

• pressure regulator
• firm translucent vinyl tubing (Sims Portex), 3 mm internal diameter, to connect pressure transducers to the PNT ports ($V^*$ and $P_{ao}$) and to the port proximal to the RTC bladder.

• Neopuff Infant Resuscitator (Fisher and Paykel)

• clear tubing to connect the tank to the wall air supply

• Digitron, P200 Manometer

3.2.4 Other equipment

• various sizes of clear Rendell-Baker, Soucek face masks

• silicone therapeutic putty to create an airtight seal between mask and face

• neck roll for supporting the neck and head
3.3 **Equipment assessment**

The equipment was assembled and the performance of the system as a whole assessed for safety, linearity, frequency response and resistance of the apparatus (Hoo, 1997).

A circuit diagram of all the equipment is shown in Figure 3.1.

**Figure 3.1  Schematic diagram of equipment for lung function test**

![Diagram showing the equipment setup](image)

Definition of abbreviations: ETCO₂: end tidal carbon dioxide; SpO₂: oxygen saturation; Pₐo: airway opening pressure; Pₗ: jacket pressure; PNT: pneumotachometer; RTC: rapid thoraco-abdominal compression.
3.4 Equipment calibration

Equipment was calibrated with respect to measurements of \( V', P_{so} \) and \( P_j \) on a daily basis before each respiratory function test. Measurements were performed in the Research Room on the Special Care Baby Unit at Homerton Hospital.

3.4.1 Preparation of equipment

The computer unit was turned on and the RASP program loaded. The Hans Rudolph PNT, its heating source, and the amplifiers were switched on at least half an hour before calibration and measurement.

The two clear tubes attached to the Furness pressure transducer (± 0.2 kPa) were connected to the relevant pressure ports on the PNT to measure \( V' \). One tube from the (± 5kPa) pressure transducer was connected to the \( P_{so} \) port on the PNT, whereas the remaining tube was left unattached as a reference to atmospheric pressure. Similarly, jacket pressure was recorded by attaching one of the tubes from the \( P_j \) transducer (± 10 kPa) to the pressure port situated on the proximal portion of the rigid connector from the RTC air reservoir tank to the jacket. The second tube was left open as reference to atmospheric pressure. Care was taken to ensure that the tubes attached to either side of each of these differential transducers were of equal proportions (Sly and Davis, 1996).

3.4.2 Flow calibration

Prior to \( V' \) calibration, a draught-free environment was ensured by closing the door and window in the Research Room. To maintain accuracy over the range of signals obtained, the stability and full-scale deflections were checked for any offset from zero in the RASP configuration menu, before known set points were applied to the transducer.

A Hans Rudolph PNT with a linear range, appropriate for the age and size of the infant to be tested (Table 3.2) was connected to a source of air supply via a calibrated
rotameter. A zero reference point was established when there was no flow through the PNT. A high reference point was obtained when a flow of 100 mL.s\(^{-1}\) (6 L.min\(^{-1}\)) or 50 mL.s\(^{-1}\) (3 L.min\(^{-1}\)) was passed through the PNT with a linear range of 0-35 L.min\(^{-1}\) or 0-10 L.min\(^{-1}\), respectively.

To validate the calibration factors, a series of flow signals, which encompassed the entire range of flow to be measured, was delivered and checked. This was achieved by recording the flow for approximately 5 seconds at 0, 50, 100 mL.s\(^{-1}\) for the smaller PNT (0-10 L.min\(^{-1}\)) and an additional recording of 150 and 200 mL.s\(^{-1}\) for the larger PNT (0-35 L.min\(^{-1}\)). Each section of recorded flow at these different rates was checked for accuracy and linearity against known values. If the signals were within ± 2% of the delivered values, a note was made regarding the signals and then saved as part of the study file.

### 3.4.3 Volume check

As \(V'\) is the first time derivative of volume, the \(V'\) calibration was further validated by applying a known volume to the assembled PNT, using a Hans Rudolph calibrated syringe. Following a recording of approximately 5 seconds of zero flow, a 40 mL volume was injected and then withdrawn through the calibrated PNT (0-35 L.min\(^{-1}\)) at a frequency approximating that of the respiratory rate of the infant to be studied. A 10 mL calibrated syringe was used when checking the small PNT (0-10 L.min\(^{-1}\)). After recording 6-8 of these signals, the amplitude was checked by the placement of the cursors in the peaks and troughs of the 'inspiratory' and 'expiratory' phase of the volume signals. Validation was accepted and saved if the measured signals were within ± 2% of the known value used.

### 3.4.4 Calibration of pressure at airway opening (\(P_{ao}\))

The \(P_{ao}\) signal was calibrated by delivering low and high set points to the Furness differential transducer (range ± 4.910 kPa or ± 50 cmH\(_2\)O). One of the two transducer tubings was connected to a digital manometer, Digitron (Digitron Instrumentation Ltd., Herts, England) via a 3-way tap while the other tubing was opened to the atmosphere. A zero reference point was recorded when the 3-way tap
was opened to the atmosphere. To establish a high reference point, a 5-mL syringe was attached to the 3-way tap and a pressure of 2 kPa was delivered to the differential transducer via the manometer.

This was subsequently validated and the accuracy of the calibration assessed by delivery of known pressure signals using a 5-mL syringe via the manometer. Recordings of approximately 5 seconds duration at zero or 0 kPa, 1 kPa, 3 kPa and 4 kPa were made. The displayed signals were checked for accuracy by placing the cursors over the respective portions of the pressure signal. The calibration check was accepted and saved if the values were within ± 2% of the known signals.

3.4.5 Calibration of jacket pressure (Pj)

Similarly, $P_j$ was calibrated as for $P_{ao}$. One of the tubes attached to the Furness differential transducer ($P_j$) was connected to the Digitron via a 3-way tap while the other tube was opened to the atmosphere. A zero reference point was set when the 3-way tap was left opened to the atmosphere. To establish a high reference point, a 5-mL syringe was attached to the 3-way tap and a pressure of 5 kPa was delivered via the manometer to the differential transducer and the value recorded.

The calibration was assessed by delivering known pressure signals i.e. 0 kPa, 3 kPa, 6 kPa and 10 kPa to the transducer via the manometer using a 5-mL syringe as described in section 3.4.4. The calibration check was accepted and saved if the values were within ± 2% of the known signals.

Once the $P'$, $P_{ao}$ and $P_j$ had been satisfactorily calibrated, the calibration factors were saved in a specific RASP profile, i.e. FEXP737.PRF. This profile was used to collect tidal breathing parameters and forced expiratory flows. A record of the calibration factors was printed and kept as part of each infant’s study record.
3.5 Other equipment check

3.5.1 Neopuff Infant Resuscitator check

The Neopuff system, which allows the setting of a predetermined pressure for the delivery of positive airway pressure, was connected to the capnogram and Y connector. The pressure on the neopuff system was set to deliver a positive pressure of approximately 3.0 kPa using a flow of 10-12 L.min⁻¹ of air for healthy term infants.

Figure 3.2 Diagram of Neopuff and connectors

3.5.2 Air reservoir tank and water column for the measurement of RTC

The air reservoir tank, which has a capacity of 100 L, was connected to the wall compressed air supply. The flow outlet from the tank could be adjusted using a pressure regulator and the three-way tap was connected to wide bore tube (diameter 3 cm) to allow for rapid inflation of the RTC bladder. Both the pressure regulator and three-way tap were checked prior to each study to ensure they were in working order.

As a safety feature to ensure the air pressure within the air tank did not exceed 10 kPa, one end of a "blow off" tube, was attached to the RTC air reservoir tank, and the other end was immersed in a water column to a depth of 100 cm.
3.6 Preparation of the Infant for Respiratory Function Test

3.6.1 Introduction

Soon after arrival, the infant was clinically examined to ensure their physical well being and that administration of sedation was not contra-indicated. All respiratory function test measurements were performed during consecutive periods of behaviourally determined quiet sleep following a feed. A mild sedative, chloral hydrate was administered to enable the infant to sleep over a sufficient period of time for completion of data collection. Within the respiratory laboratory, full resuscitation equipment, oxygen and suction apparatus were available and checked daily prior to each test. Medical cover was provided at all times by the neonatal team at the Homerton Hospital.

The baby was lightly dressed so as not to restrict respiratory movements. The ambient room temperature was maintained at 22-25°C using the hospital’s continuing monitoring system, which controls all services within the hospital, since small changes in body temperature could affect cardio-respiratory behaviour and sleep state. A quiet environment with dimmed lighting and careful handling of the baby also ensured minimal disturbance. Parents were invited to stay during all respiratory function measurements.

3.6.2 Written consent

Discussion with the parents about the respiratory measurements had usually occurred over the telephone prior to the appointment. The procedure was explained to the parent/s again on arrival to the Laboratory when written informed consent was obtained (Appendix E). Three copies of the consent were completed. One was given to the parent/s, the second was kept with the infant’s records while the third was sent to the GP when consent was requested to access the infant’s medical records, at a time when the infant was a year of age.
3.6.3 Anthropometric measurements

The infant's weight, crown-heel length, crown-rump length, occipito-frontal circumference, mid arm circumference and chest circumference were measured at the time of test as described in Section 3.1.6.

Maternal height and weight were measured with the Leicester Portable Measure and Soehnle scales respectively. The mother's reported birthweight was also documented if known for later analysis.

3.6.4 Sedation

As lung function was measured at 4-12 weeks postnatal age, infants were unlikely to stay asleep naturally throughout the proceedings. Thus, chloral hydrate sedation, at a dose of 60 mg.kg\(^{-1}\), was given orally.

To facilitate rapid onset of sleep, parents were advised to try and keep the infant awake as long as possible before arriving for the respiratory function measurements and not to feed until after the sedation had been administered. Sleep normally followed within the hour.

3.6.5 Monitoring of oxygen saturation and heart rate

The cardio-respiratory system of the sedated infant was monitored with pulse oximetry using the CO\(_2\)SMO monitor throughout the study via an oximeter flexiprobe attached to the lateral border of the foot with microfoam surgical tape or a velcro band.

As the raised lung volume manoeuvre was a relatively new test performed in the laboratory, initially, end tidal carbon dioxide (ETCO\(_2\)) was monitored via the capnogram during these manoeuvres. However, as the ETCO\(_2\) level was stable and always remained within the normal limits of 4 to 6 kPa (Brouillette, 1992) during passive inflations, monitoring was discontinued to minimise apparatus dead space.
and resistance and hence reduce the infant’s work of breathing during the test. Oxygen saturation (SpO₂) and heart rate were monitored continuously.

3.6.6 Sleep state

Sleep state itself has a profound influence on respiratory patterns. Respiratory rate and minute ventilation are greater during active than quiet sleep (Hathorn, 1974) and the increased variability of all respiratory parameters during active sleep can lead to problems with interpretation (Gaultier et al., 1996). Furthermore, infants are more likely to be disturbed by application of a mask or shutter closure during active sleep. Measurements were therefore obtained during behaviourally determined quiet sleep (Table 3.1) when the posture was stable, respiration regular and in the absence of eye movements (Prechtl, 1974).

Table 3.1 Classification of sleep state by the behavioural criteria of Prechtl (1974)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Active Sleep</th>
<th>Quiet Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rapid and/or slow eye movements</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2 Facial grimaces</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 Frequent small body movements</td>
<td>+</td>
<td>Stable posture</td>
</tr>
<tr>
<td>4 Startles every 2-3 minutes</td>
<td>+</td>
<td>Isolated startles only</td>
</tr>
<tr>
<td>5 Irregular respiration</td>
<td>+</td>
<td>Regular respiration</td>
</tr>
</tbody>
</table>

3.6.7 Position of the infant

All measurements including forced expiratory manoeuvres were carried out with the infant supine, with a slightly extended neck and the head supported in the midline with small fluid-filled bags. Neck position was carefully adjusted to avoid potential problems such as glottic closure during the procedure.

3.6.8 Application of face mask

A thin ring of therapeutic silicone putty was placed round the rim of a transparent facemask of an appropriate size for the infant. The facemask, attached to the
pneumotachometer (PNT) and recording apparatus, was then placed over the infant’s nose and mouth once s/he was in quiet sleep. By gently moulding or pressing the putty onto the face, an airtight seal was made between the mask and face. Close observation was carried out throughout the procedure, via the clear facemask, to ensure that the putty did not occlude the mouth and nostrils.

3.6.9   Test occlusion

Data recording commenced once the infant was breathing quietly through the PNT. After recording 5-10 regular tidal breaths with a stable end expiratory level (EEL), a test occlusion was performed at end inspiration for a period of one tidal breath, to assess whether there was any airflow around the mask (Figure 3.3). The mask had achieved a good seal with no leaks in the measuring circuit if the EEL re-established its original level after release of the occlusion, there was no fall in $P_{\text{ao}}$, and no flow was observed during the occlusion (Stocks et al. 1987).

Figure 3.3   Time based trace showing satisfactory test occlusion

![Diagram of flow, volume, and airway opening pressure with occlusion marker and end expiratory level](image_url)
If a leak was suspected i.e. when ‘step up’ in the EEL baseline or a decay in $P_{ao}$ was observed after the test occlusion (Figure 3.4), the mask was reapplied and the procedure repeated.

**Figure 3.4 Leak around facemask during a test occlusion**

3.7 Measurement of infant respiratory function

Air flow ($V'$) was measured using a PNT attached to a differential pressure transducer, digitised and sampled at 200 Hz (Analog Devices RT1-815) and digitally integrated to yield volume. The start and end of expiration were defined as the zero crossing of flow during inspiration and expiration, respectively. Zero flow crossings were estimated using sample to sample linear interpolation. This strategy ensured that any expiratory pause was incorporated into expiratory time. An adjustable scan period (usually set to 0.3 sec) prevented identification of false troughs and peaks. Peak expiratory flow was taken as the first sample at which maximum flow was recorded.

The size of PNT and facemask used was dependent on the size of the infant as summarised in Table 3.2.

**Table 3.2 Guide to use of various sizes of PNTs and face masks**

<table>
<thead>
<tr>
<th>Infant's Test weight</th>
<th>Size of Hans Rudolph PNT used</th>
<th>Size of face mask used</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3 kg</td>
<td>0-10 L. min$^{-1}$</td>
<td>0 or 1</td>
</tr>
<tr>
<td>&gt; 3 kg</td>
<td>0-35 L. min$^{-1}$</td>
<td>1 or 2</td>
</tr>
</tbody>
</table>
3.7.1 Tidal breathing

The study commenced with the measurement of the infant’s tidal breathing pattern. As soon as the infant was breathing quietly and regularly through the PNT, data for the analysis of tidal breathing parameters were collected in discrete 30-60 sec epochs.

Parameters measured were tidal volume ($V_T$), respiratory rate (RR), total expiratory time ($t_E$), time from onset of expiration to peak tidal expiratory flow ($t_{PTEF}$) and the time taken to reach peak expiratory flow as a proportion of total expiratory time ($t_{PTEF:t_E}$). These tidal breathing indices provide useful information regarding the control of respiration in the developing infant (Dezateux et al. 1994; Stocks et al. 1994b; Stick, 1996; van der Ent et al. 1997).

3.7.1.1 Tidal volume ($V_T$)

During unloaded quiet sleep in mammals, tidal volume ($V_T$) has been found to be a constant across species, being approximately 6-8 mL.kg$^{-1}$ when standardised for body weight (Mortola, 1987; Hanrahan et al. 1990; Stocks et al. 1994b). Factors that determine $V_T$ are the duration of inspiration and mean inspiratory flow, which reflects respiratory centre drive (von Euler, 1986).

3.7.1.2 Respiratory rate (RR)

Respiratory rate (RR) is the number of breaths that occur per minute and is a global index of respiratory function. The RR changes with age, being most rapid during the neonatal period and decreasing by 6–12 weeks postnatal age for term infants (Stick, 1996; Gagliardi and Rusconi, 1997). Together with tidal volume, the respiratory rate determines minute ventilation, defined as RR x tidal volume.

3.7.1.3 Expiratory time ($t_E$)

The expiratory time is the time in seconds taken from the point of zero flow at the end of inspiration to the next point of zero flow at the end of expiration. There is
usually a brief pause before the onset of inspiration and this is added to the expiratory time.

During passive expiration, in healthy adults and older children, the functional residual capacity (FRC), i.e. the volume of gas remaining in the lung at end expiration usually coincides with the elastic equilibrium volume (EEV). The EEV is the volume at which the outward recoil of the chest wall balances the inward recoil of the lungs so that the net recoil pressure is zero. In the presence of a completely relaxed expiration, the expiratory time ($t_E$) is dependent on the passive time constant of expiration ($\tau_p$). However, in infants, FRC is often elevated above EEV (Kosch et al. 1988; Dezateux et al. 1991) and under these circumstances, the effective time constant of the respiratory system is usually longer than during passive expiration, as discussed below.

The mechanisms by which infants modify expiratory flow are:

- the braking activity of the larynx/glottic closure which increases resistance and lengthens the effective time constant and
- the persistence of post inspiratory muscle activity until late in expiration which decreases the driving pressure and also results in a lengthening of the effective time constant (Mortola, 1987; Kosch et al. 1988).

However, a short $t_E$ coupled with a rapid respiratory rate also plays a part in maintaining the lung volume (Mortola, 1987; Kosch et al. 1988).

3.7.1.3.1 Respiratory system time constant ($\tau_{rs}$)

A time constant ($\tau$) is defined as the time taken for a system to deflate to 37% of its original volume following an inflation. During passive expiration and assuming a single compartment model, lung emptying follows an exponential decay such that it takes five $\tau$ for complete emptying to occur.
### Table 3.3 Theoretical pattern of lung emptying under passive conditions with a single expiratory time constant

<table>
<thead>
<tr>
<th>( \tau_{rs} )</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>% expired volume</td>
<td>63</td>
<td>86</td>
<td>95</td>
<td>98</td>
<td>99.6</td>
</tr>
<tr>
<td>% volume remaining</td>
<td>37</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

As \( \tau_{rs} = \text{Resistance} \times \text{Compliance of the respiratory system} \), this has important clinical implications. For example, stiff lungs (low compliance) with a low resistance will have a short \( \tau_{rs} \) and will empty and fill rapidly, while lungs with normal or high compliance or a high resistance will have a longer \( \tau \) and will empty and fill more slowly.

#### 3.7.1.3.2 Dynamic elevation of lung volume

Since three \( \tau \) are required to achieve 95% lung emptying (Table 3.3), the combination of a rapid respiratory rate (short expiratory time) and a long \( \tau \) is likely to result in dynamic elevation of end-expiratory lung volume and the development of intrinsic positive end expiratory pressure. This is observed in many healthy young infants where the \( \tau_{rs} \) may be prolonged due to laryngeal braking and/or post inspirational diaphragmatic activity (Kosch et al. 1988; Stocks et al. 1994b). When combined with a relatively rapid respiratory rate, the end expiratory level (EEL) is seen to be dynamically elevated above the passive functional residual capacity (FRC).

**Figure 3.5** Time based trace showing dynamic elevation of end-expiratory lung volume

![Time based trace showing dynamic elevation of end-expiratory lung volume](image)

The change in end expiratory level on a time based trace during periods of prolonged expiration.
3.7.1.4 *Time from the onset of expiration to peak tidal expiratory flow (t_{PTEF})*

In a relaxed system, the relative time at which peak tidal expiratory flow (PTEF) occurs during passive expiration is dependent on the elastic recoil pressure of the respiratory system ($P_{el}$) which provides the driving force to overcome its flow-resistive pressures. Hence, as $P_{el}$ is highest at end inspiration when the airways are also well distended, PTEF should theoretically be observed almost instantaneously at onset of expiration. This pattern however, is rarely observed, demonstrating that some braking of expiratory flow is a normal feature of tidal breathing in infants (Stocks et al. 1994b).

3.7.1.5 *Time taken to reach peak tidal expiratory flow as a proportion of total expiratory time (t_{PTEF:t_E})*

t_{PTEF:t_E}, the tidal expiratory flow ratio is a measure of the extent to which the infant modifies expiratory flow, possibly in response to underlying respiratory mechanics. In newborn infants, laryngeal and post-inspiratory diaphragmatic braking, accompanied by late onset of peak expiratory flow, result in a higher $t_{PTEF:t_E}$ which helps to maintain a dynamically elevated FRC and to maximise gas exchange (Kosch et al. 1988). It has also been reported to be significantly related to indices of airway function in adults and children (Morris and Lane, 1981; Cutrera et al. 1991), but in infants, $t_{PTEF:t_E}$ explained only a small proportion of the variance in specific airway conductance (Dezateux et al. 1993). Diminished $t_{PTEF:t_E}$ measured shortly after birth has been shown to be predictive of subsequent wheezing in boys during the first 3 years of life (Martinez et al. 1988; Martinez et al. 1991). However, it has been suggested that the low values in $t_{PTEF:t_E}$ observed in adults with airway obstruction reflect alterations in the control of breathing in response to underlying mechanics rather than airway size (Morris and Lane, 1981; van der Ent et al. 1998).
Figure 3.6  Time based trace of tidal breath showing definitions of parameters

Inspiration  Expiration

Time

Volume

Flow

Time

\[ V_T \]

\[ t_i \quad t_e \quad t_{tot} \]

\[ t_{PTIF} \quad t_{PTEF} \]
3.8 **Forced expiratory manoeuvres**

Forced expiratory parameters are achieved in infants by applying thoraco-abdominal pressure at end inspiration. The aim is to assess airway function by achieving expiratory flow limitation.

It has been suggested from previous research, that maximum expiratory flow (MEF) on the descending portion of the maximum expiratory flow-volume (MEFV) curve is independent of effort and reflects airway calibre upstream, from the periphery to the airway segment subjected to flow limitation (Mead et al. 1967; Dawson and Elliot, 1977). Flow limitation can be seen to have occurred when there is no increase in expiratory flow with further increases in transpulmonary pressure, once a certain critical level has been reached. This concept is widely accepted and three theories have been postulated to explain the mechanics of flow limitation:

- equal pressure point theory (Mead et al. 1967)
- Starling resistor theory (Permutt et al. 1962)
- choke point or wave speed theory (Dawson and Elliot, 1977)

Expiratory flow is limited because the airways are compliant and their calibre is dependent on the transmural pressure, i.e. the pressure difference between the inside and outside of the airway. As this pressure difference is dependent on lung volume and flow, transmural pressure is positive at end inspiration when there is no flow and the airways are expanded. However, with increasing expiratory flow at any given lung volume, there is a fall in pressure within the airways due to a resistive pressure drop. The higher the flow, and the narrower the airways, the greater the pressure drop within the airways. By contrast, the more force that is applied outside the airways (either voluntarily or by applied thoraco-abdominal compression) during a forced expiratory manoeuvre, the greater the pressure outside the airways.

Airflow is said to be limited when an application of additional force cannot produce higher flows, i.e. when flow becomes independent of effort. The narrower the airways, the higher the resistance, the greater the pressure drop, such that flow limitation occurs at progressively higher lung volumes.
Theoretically, during expiration, measured flow is proportional to the elastic recoil of the lung and inversely proportional to the airway resistance (Permutt et al. 1962). In wave speed theory, airway specific elastance and gas density are also important determinants of maximal flow. A summary of factors determining wave speed airflow limitation is shown in Figure 3.7.

For this study, airway function was assessed using both partial and full flow-volume curves, by applying external thoraco-abdominal pressure at the end of a normal tidal inspiration, and after inflating the lungs to a preset pressure of approximately 3 kPa, respectively.
Figure 3.7  Factors determining wave-speed airflow limitation

\[
\text{WAVE SPEED FLOW} = (1/6)^{1/2} \cdot (dP_{\text{tm}}/dA)^{1/2} \cdot A^{3/2}
\]

Factors determining wave-speed airflow limitation (adapted from (Wohl, 1991)). Those that change with growth are marked with an asterix. \( P_{p}: \) pleural pressure; \( P_{\text{tm}}: \) transmural pressure (intrabronchial pressure-pleural pressure); \( P_{\text{alv}}: \) alveolar pressure; \( P_{b}: \) intrabronchial pressure.
3.8.1 Tidal Rapid Thoraco-abdominal Compression (RTC)

The rapid thoraco-abdominal compression technique (RTC) was first used in infants in 1978 (Adler and Wohl, 1978) and was later modified in 1982 by the addition of an inflatable jacket (Taussig et al. 1982). Since then it has been widely used for the assessment of peripheral airway function in both healthy and diseased infants.

With this technique, partial forced expiratory flows are produced by the sudden application of a compressive pressure to the thorax and abdomen at end tidal inspiration. This is usually referred to as the “squeeze” or RTC technique. In infants, peripheral airway function is assessed by achieving expiratory flow limitation using this technique (American Thoracic Society/European Respiratory Society, 1993; Le Souëf et al. 1996).

3.8.2 The inflatable compression jacket (Squeeze jacket)

The squeeze jacket was secured round the infant, encircling the chest and abdomen, and an inflatable plastic pouch with a wide bore connector, placed over the infant’s chest. The arms were left outside the jacket. The jacket extends to the highest point of the axilla and inferiorly to the symphysis pubis. When securing the jacket, a space equivalent to two adult fingers breadth under the jacket was ensured to allow for chest expansion during inspiration (Sly et al. 2000).

The RTC air reservoir tank was connected to the wall air supply and airflow to the air reservoir tank was maintained at 12 L.min\(^{-1}\). The large bore, rigid connecting tube with the three way tap attached to the air reservoir tank was connected to the inflatable plastic pouch secured under the jacket while one of the clear 3 mm vinyl tubes attached to the jacket pressure transducer was connected to the pressure port on the proximal arm of the rigid tube from the air reservoir tank. The second tube was left open as reference to atmospheric pressure.
3.8.3 The standard RTC manoeuvre

After 5 to 10 regular breaths had been recorded, the jacket was inflated at end inspiration. Jacket inflation was held and released at end expiration. Jacket pressure ($P_j$) commenced at 3 kPa (30 cmH$_2$O) and was increased in increments of 0.5 to 1 kPa until flow at functional residual capacity (FRC) had reached a reproducible maximum (i.e. $V'_{\text{maxFRC}}$ at optimal $P_j$) and/or higher jacket pressures were causing a reduction in flow (flow limitation reached).

Parameters analysed from these partial expiratory flow volume (PEFV) curves include maximal forced expiratory flows at FRC ($V'_{\text{maxFRC}}$) and the shape of the expiratory flow curve.

3.8.3.1 $V'_{\text{maxFRC}}$

$V'_{\text{maxFRC}}$ is thought to reflect peripheral airway function that is relatively uninfluenced by the resistance of the upper airways and is a valuable measure of intrathoracic airway function in infants. However, the measurement of $V'_{\text{maxFRC}}$ relies on a stable FRC which in infants is often dynamically determined and elevated (Section 3.7.1.3.2). The shift in FRC may be due to changes in sleep state or addition of apparatus dead space (Stick et al. 1991) and is a reason for the high variability of $V'_{\text{maxFRC}}$ (Henschen and Stocks, 1999). The mean of the three highest, technically satisfactory, flows at FRC was calculated and reported. $V'_{\text{maxFRC}}$ SD
score was also calculated based on prediction models, incorporating age, sex and length at test (Hoo et al. 2001).

**Figure 3.9** Time based trace showing a tidal rapid thoraco-abdominal compression (RTC) manoeuvre

![Diagram showing tidal rapid thoraco-abdominal compression](image)

- $V^r$: Inspiration
- $V$: Volume
- $P_J$: Jacket inflation
- 1 sec
3.8.3.2 *Shape of the expiratory flow curve.*

The shape of the expiratory portion of the PEFV curves from healthy infants is usually convex (Figure 3.10) whereas in infants with airway obstruction, there is a marked reduction in \( V'_{\text{maxFRC}} \) and concavity of the flow-volume curves (Figure 3.11d) (Le Souëf et al. 1988). Common problems which contribute to the high variability in \( V'_{\text{maxFRC}} \) include unstable end expiratory level due to irregular respiration (Figure 3.11a), glottic closure and early inspiration. Late glottic closure would overestimate \( V'_{\text{maxFRC}} \) (Figure 3.11b) while early inspiration would underestimate \( V'_{\text{maxFRC}} \) (Figure 3.11c).
Figure 3.11  Partial expiratory flow volume curves illustrating common problems

a) Irregular respiration: Unstable end expiratory level

b) Glottic closure

c) Early inspiration

d) Tidal flow limitation
3.8.4 **Transmission Pressure.**

The amount of pressure transmitted from the jacket to the pleural space may be variable, dependent on how tightly the jacket is applied and on the infant’s respiratory compliance. Hence it was necessary to assess the transmission of jacket pressure to assist data interpretation and quality control.

After recording at least 5 regular tidal breaths, an occlusion was performed at end-tidal inspiration. When a brief plateau was observed on the $P_{\text{ao}}$ signal, the jacket was inflated and held until a second plateau was observed on the $P_{\text{ao}}$ signal. Airway occlusion and jacket pressure were then released (Figure 3.12). Three measures of static jacket transmission pressure were made.

**Figure 3.12  Diagram showing time based trace of jacket transmission**

When an occlusion is performed during the procedure, it is assumed that pressure equilibrates within the lungs and thus the pressure at the airway opening equals alveolar pressure. Providing the occlusion also induces muscle relaxation, under conditions of no air flow (no resistive pressure losses), $P_{\text{ao}}$ (i.e. P1 on diagram) also reflects the elastic recoil pressure of the respiratory system at the occluded volume (i.e. lung plus chest wall: which is zero at EEV).
When the jacket is inflated, provided the infant remains relaxed, makes no inspiratory effort and there is no leak, a second plateau, P2 is obtained. The pressure difference P2 - P1, reflects the pressure which has been transmitted to the intrathoracic structures as a result of jacket inflation, i.e. \( P_{\text{aoj}} = P2 - P1 \). This should be in the order of 2 kPa to achieve flow limitation, except in infants with peripheral airway obstruction when lower pressures may be required and where application of higher pressures may result in negative flow dependence. The relative efficiency of jacket pressure transmission can be assessed by \( \left( \frac{P_{\text{aoj}}}{P1} \right) \times 100 \). This is generally approximately 50% (range 30-75%).

3.8.5 Raised Lung Volume RTC

Though \( V'_{\text{maxFRC}} \) is a valuable measure of intrathoracic airway function in infants, its value is reliant on a stable FRC which in infants is often dynamically determined and elevated. Another potential disadvantage of the tidal technique especially in healthy infants, is not knowing whether flow limitation has been reached.

In adults, the use of the full lung volume range for assessing forced expiration has been found to produce more useful information than the use of the tidal range. Forced expiratory volume in 1 second (FEV₁) is the most useful parameter, achieving good reproducibility, as forced expiration is mainly flow limited.

During the last few years, raised lung volume RTC assessments in non-intubated infants have been reported (Tepper and Reister, 1992; Flucke et al. 1994; Turner et al. 1995; Feher et al. 1996; Henschen and Stocks, 1999) where the infant’s lungs are passively inflated towards total lung capacity (TLC) before applying the thoraco-abdominal compression pressure. This enables full forced expiration manoeuvres to be obtained in infants as in adults, providing data comparable to that obtained in older subjects.
The raised volume RTC (RVRTC) has several advantages:

Firstly, lung volume is standardised across infants using predetermined inflation pressures (i.e. the driving pressure is standardised). Hence comparisons can be made between raised volume breaths in the same infant or between different infants.

Secondly, by applying several augmented breaths prior to forcing expiration problems due to early inspiratory efforts are minimised. This is because:

i) the lung inflation invokes the Hering-Breuer Inflation Reflex (HBIR) and thus prolongs expiratory time,

ii) small decreases in PaCO\textsubscript{2} may result in the chemoreceptor drive being temporarily switched off, thereby enhancing the HBIR and minimising respiratory muscle activity.

Thirdly, the majority of the forced expiration is flow limited and therefore the resultant flows and volumes should be more reproducible using this technique. Forced expired volume in a given time (FEV\textsubscript{t}), which is considered to be one of the most useful respiratory function parameters in older children and adults, can be then measured in infants.

3.8.5.1 Raised lung volume

Lung volume was raised by inflation to a positive airway pressure of 2.75 to 3.0 kPa using the Neopuff system. After regular tidal breaths were observed, the assembled unit (Figure 3.2) with (capnogram), ‘Y’ or ‘T’ connector and Neopuff system set to 3.0 kPa was connected to the PNT. To avoid adding more load to the infant’s work of breathing (due to the added dead space of the assembled unit), augmented tidal breaths were initiated at the start of the next inspiration. Repeated occlusions of the expiratory side of the ‘Y’ or ‘T’ piece at a frequency approximating the infant’s respiratory rate resulted in passive inflations and deflations of the respiratory system.
3.8.5.2 Raised volume RTC manoeuvre

The lowest jacket pressure required to achieve $V'_{\text{maxFRC}}$ (Section 3.8.3) was used during raised volume manoeuvres with the airway pressure set to 3.0 kPa. Repeat occlusions of the expiratory side of the ‘Y’/‘T’ connector at a frequency approximating the infant’s respiratory rate, resulted in inflations and passive deflations of the respiratory system. In order to induce respiratory muscle relaxation and ensure a constant inflation volume, inflations were held until a plateau was observed on both the airway pressure and volume traces. Once relaxation had been achieved, the jacket was inflated at the end of a passive inflation causing forced expiration from raised lung volume. The jacket pressure was released after forced expiration has been completed (Figure 3.14). To minimise the infant’s work of breathing during spontaneous respiration, the assembled unit was removed immediately after passive inflations ceased. The manoeuvre was repeated until three successful raised volume RTC flow volume curves were obtained. The jacket was removed once the protocol for all respiratory function had been completed. A flow-volume loop showing forced expiration generated using the raised volume RTC technique is shown in Figure 3.15.
Figure 3.14  Time based trace showing a Raised Volume RTC manoeuvre

Figure 3.15  Raised Volume RTC flow volume curve
3.9  **Data analysis and criteria for acceptability of data**

For analysis, all RASP data were exported as ASCII (.DAT) files. Analog signals were digitised at 200 Hz and analysed using a software package developed in collaboration with Imperial College of Science, Technology and Medicine.

Criteria for acceptability of forced expiratory flow volume curves were: regular tidal volume ($V_t$) and EEL for the tidal RTC or regular relaxed inflations for the raised volume RTC, jacket inflation initiated within 100 ms of end inspiration with jacket inflation time less than 100 ms and peak expiratory flow being achieved before 30% of inspired volume had been expired, expiration proceeding beyond the previous EEL, a smooth flow volume curve without significant glottic closure or flow transients, especially during the last half of expiration, and no evidence of leaks.

With respect to the raised volume RTC curves, the 'best' curve was defined as the technically acceptable curve with the highest sum of FVC and FEV$_{0.4}$ (Le Souëf et al. 1996). Criteria for acceptance of the data included the fact that both FVC and FEV$_{0.4}$ from the 'best' curve should be within 10% of those from the next best manoeuvre, recorded under the same measurement conditions.

3.9.1  **Parameters**

Parameters analysed include forced vital capacity (FVC), duration of forced expiration ($t_{FE}$), maximal expiratory flow at fixed proportions of FVC (MEF$_{50}$), and forced expiratory volume at specific time during expiration (FEV$_t$).

3.9.1.1  **Forced vital capacity (FVC)**

FVC is the difference in the volume between the beginning of the raised volume RTC manoeuvre and the 'residual volume' (RV) at the end of the manoeuvre. The rate of expiration can be measured at fixed proportions of FVC e.g. maximal expiratory flow measured at 50%, 25% and 10% of FVC. It is recognised that FVC will be dependent on the applied inflation pressure (which differs between centres) and that during the RVRTC in infants, there is no guarantee that either total lung
capacity (TLC) or residual volume (RV) will be reached. Consequently, during infant studies, the term FVCp is sometimes used with the p denoting inflation pressure e.g. FVC\textsubscript{3.0}.

### 3.9.1.2 Forced expiratory volume (FEV)

Airway function can also be determined by examining the volume forcibly exhaled at timed intervals (FEV\textsubscript{i}). In adults and older children, FEV\textsubscript{i} (i.e. the volume expired in one second) is the most useful respiratory function parameter. However, in infants, due to the rapid respiratory rate, FEV\textsubscript{0.4}, FEV\textsubscript{0.5} and FEV\textsubscript{0.75} are used instead (Figure 3.16). It is thought that the FEV\textsubscript{i} parameters may correlate better with the size of the infant and be more discriminatory in their ability to distinguish between normal and abnormal airways than $V'_{\text{maxFRC}}$ (Turner et al. 1995). However, much further work is required before the relative sensitivity and specificity of these various parameters can be determined.

**Figure 3.16 Measurement of FEV\textsubscript{i} from full forced expiration**
Figure 3.17  Examples showing problems encountered with Raised Volume RTC curves

a) Late inflations, infant not relaxed

b) Glottic closure

c) Early inspiration: infant not relaxed

Patho-physiology: evidence of tidal flow limitation
3.9.1.3 Maximal expiratory flow (MEF)

Maximal expiratory flow was measured at 25%, and 15% (i.e. MEF$_{25}$ and MEF$_{15}$) of forced vital capacity (Figure 3.18) with positive inflation pressure of 3.0 kPa (FVC$_{3.0}$). In general, if flow limitation has been reached, MEF$_{50}$ will reflect more central airway function, whereas MEF$_{15/25}$ reflects more peripheral airway function. However, in practice, due to the difficulty in obtaining perfect manual timing during the manoeuvre, flow limitation is not always achieved at MEF$_{50}$.

Figure 3.18 Maximal expiratory flow volume curve showing MEF measured at 50 and 25%

3.9.2 Assessment of Raised Volume RTC parameters

Recent international collaboration has led to recommendations for standardisation of the most commonly used tests of infant lung function, including the RTC technique (Stocks et al. 1996; Frey et al. 2000; Sly et al. 2000). However, despite increasing interest in and use of the RVRTC technique, there is as yet no consensus regarding analysis when performing this technique (Gappa, 1999; Allen and Gappa, 2000). In addition, it is unclear as to what the parameters measured by the RVRTC technique actually represent in infancy, or which are the most appropriate parameters to report for specific research or clinical applications. Thus, a study looking at analytical issues such as assessing the feasibility of calculating a range of timed forced expiratory volumes (FEV$_t$) and their relationships to FVC; examining the
relationship between MEF₂₅, MEF₁₅ and \( V'_{\text{maxFRC}} \); and also assessing the within-subject variability of \( V'_{\text{maxFRC}} \) and that of various parameters derived from the RVRTC technique was performed.

### 3.9.2.1 Data analysis

Respiratory function data were analysed as described in Section 3.9. The variability for both volume (FVC and FEV₁) and flow parameters (\( V'_{\text{maxFRC}} \) and MEF₁₅) was assessed from the within-subject coefficient of variation \([CV = (SD/\text{Mean}) \times 100]\) (Hutchison et al. 1981). For FEV₁, the number of infants in whom each parameter could be obtained was calculated.

The relationship between the different FEV₁ and MEF₁₅ parameters was examined using least squares linear regression analysis and that between \( V'_{\text{maxFRC}} \) and MEF₁₅ and MEF₂₅ was assessed by Bland and Altman analysis (Bland and Altman, 1986).

### 3.10 Validation of the Raised Volume RTC technique

Potential methodological factors which may influence results obtained include: the pre-set pressure used to inflate the lungs, the tightness of fit and efficiency with which pressure is transmitted from the jacket to the intra-thoracic airways, the method used to assess flow limitation and the optimal jacket pressure applied. Methodological studies were conducted in any healthy infant in whom the RVRTC technique was used, to assess the potential impact of some of these factors as discussed below.

#### 3.10.1 Influence of inflation pressure on RVRTC parameters

One of the major methodological differences in the use of the RVRTC technique has been the different inflation pressure \( (P_{\text{inf}}) \) used in various centres. Some investigators have obtained measurements after raising lung volume to 2 kPa (20 cm H₂O) (Henschen et al. 1998; Hayden et al. 1998), while others have used \( P_{\text{inf}} \) of 3 kPa (Modi et al. 1999; Jones et al. 2000). In addition, when first introducing any new lung function technique there is usually a steep learning curve, before any degree
of standardisation can be introduced. Even when adhering to a pre-set pressure, many factors may influence the precise pressure and volume delivered to the infant. Failure to appreciate that the pre-set pressure would not be reached rapidly enough in infants with larger lung volumes at the relatively low bias flow used through the Neopuff system, unless the cheeks were supported and inspiratory time was increased, meant that the actual inflation pressure delivered to the breath immediately prior to forced expiration was in fact less than the pressure set. While results will obviously not be comparable if inflation pressures vary from two and three kPa between centres, the effect of subtle variations in $P_{inf}$, such as may occur within and between infants studied at any one centre, was unknown at the inception of this study.

3.10.1.1 Aim of study

The aim of this methodological study was to assess the effect of small differences in $P_{inf}$ on parameters derived from the RVRTC technique.

3.10.1.2 Method

Healthy full term infants were recruited to the main study and respiratory function tests were carried out as detailed in Section 3.8.5.2. Paired measurements were obtained from 32 infants.

Airway function was assessed from forced expiratory flow-volume (FEFV) curves obtained at raised lung volume (Section 3.8.5). The inflation pressure was pre-set to either 2.7 or 3.0 kPa, and the order of application of the inflation pressures was randomised. At least 3 technically satisfactory FEFV curves (Section 3.9, (Le Souëf et al. 1996)) were obtained at each inflation pressure in each infant.

Within each infant, an identical jacket pressure was used during both sets of measurements. This was selected as the jacket pressure above which no further increase in $V'_{maxFRC}$ was achieved. The extent to which pressure was transmitted from the jacket to intra-thoracic airways was assessed by performing a brief airway occlusion at end tidal inspiration immediately prior to jacket inflation and the
subsequent change in pressure at the airway opening was measured (Section 3.8.4), which in this methodological study was on average 2.3 kPa (SD 0.8).

3.10.1.3 Data and statistical analysis

Technically acceptable manoeuvres (Le Souëf et al. 1996) were analysed using previously validated software (Dixon and Stocks, 1997) to calculate FVC, FEV\textsubscript{0.4} and MEF\textsubscript{25}. Within subject comparisons were made using the best curve obtained at each inflation pressure. The ‘best’ curve was defined as the technically acceptable curve with the highest sum of FVC and FEV\textsubscript{0.4} (Le Souëf et al. 1996) (Section 3.9). The $P_{\text{inf}}$ delivered to the infant was taken as the mean pressure at the airway opening during the plateau immediately prior to jacket inflation to force expiration. Statistical analysis of data was performed using paired t-test, SPSS version 8.0 for Windows.

3.10.2 Influence of jacket placement on RVRTC parameters

Another factor, which may influence results from the RVRTC technique, is the tightness of jacket fit and the efficiency with which pressure is transmitted from the jacket to the intra-thoracic airways. The aim of this methodological study was to assess the influence of jacket placement on parameters derived from the RVRTC technique.

3.10.2.1 Method

Recruitment to the methodological study and respiratory function tests were performed in 20 infants as described in Section 3.8.5.2.

The RTC Jacket was wrapped snugly, according to recent recommendations for standard RTC manoeuvre (Sly et al. 2000), (i.e. with space to insert 2 adult fingers between the jacket and sternum) or loosely wrapped around the infant’s torso (i.e. allowing 4 adult fingers breadth between jacket and sternum). At least 3 technically satisfactory FEFV curves (Section 3.9) were obtained at each jacket placement in each infant.
Within each infant, an identical $P_{inf}$ and jacket pressure was used during both sets of measurements. The optimal jacket pressure used was that above which no further increase in $V'_{maxFRC}$ was achieved. Jacket transmission pressure was also assessed as described in Section 3.8.4. Passive respiratory system compliance ($C_{rs}$) was assessed using the relaxed augmented breaths prior to expiratory forcing and calculated using multiple linear regression as previously described (Hoo et al. 2001).

Data and statistical analysis were as previously described (section 3.10.1.3).

3.10.3 Influence of jacket pressure on RVRTC parameters

In order to assess the influence of jacket pressure on RVRTC parameters, paired measurements of airway function at raised lung volume, using 'optimal' and higher jacket pressure (~1 kPa above 'optimal' pressure) were obtained in 14 infants. Within each infant, an identical $P_{inf}$ and jacket placement was used during both sets of measurements. Jacket pressure transmission was assessed as described above. Data and statistical analysis were as previously described (section 3.10.1.3).

3.11 Other background information required

Other information relating to a family history in the infant's first degree relatives of respiratory illness and smoking habits was obtained from the parent/s at the time of the lung function test. Maternal age on leaving full time education, and parental current/previous occupation were recorded so that the family's socio-economic status could be determined (Appendix B).

3.12 Cotinine assay

Cotinine is the major metabolite of nicotine and has a half-life of 20-24 hours. To confirm maternally reported smoking habits as recorded by the midwife at the 'booking' visit, and the infant's exposure to tobacco smoke at time of test, the mother was asked to report her smoking status and habit during pregnancy and her current smoking status and passive smoking exposure since delivery (Appendix B).
Maternal saliva and infant urine samples were collected for cotinine assay at time of test.

2-3 cotton wool balls were carefully placed near the genital region in the nappy to facilitate urine collection from the infant. Once the infant had passed urine, the saturated cotton wool balls were placed in the barrel of a 20-mL syringe. The plunger was replaced to squeeze the urine sample into 2 aliquots. One aliquot was for cotinine assay while the second aliquot was used as a back-up specimen.

For the collection of the maternal saliva sample, the mother was requested to place a cotton dental roll in the hollow of her cheek for 15-20 min. The dental roll was then removed by the mother using a latex glove and placed in the barrel of a syringe. The use of a glove was to prevent any contamination of nicotine from her fingers onto the sample. The saliva was squeezed out from the syringe into a 5-mL plain bottle.

All samples were clearly labelled and stored in the freezer at a temperature of -20°C as soon as possible following collection. The samples were later sent for cotinine assay using gas liquid chromatography at the ABS Laboratory, Medical Toxicology Unit, London.

3.13 Post sedation advice

On completion of the study, infants were only allowed home if fully rousable and able to take a feed. Parents were advised that the infant was likely to remain drowsy for several hours following sedation. A written record of the type of sedation used was given to the parents together with names and telephone number of the research team in case of queries or concerns. A telephone call to the parents was made the following day to confirm the well being of the infant and in addition, an audit of the parents’ response to the study performed.

3.14 Post study calibration check

At the end of each study, to ascertain that the calibration factors in the RASP system were still accurate, known signals encompassing the entire range of flow, volume and pressure were recorded and saved as part of the infant’s data. (Section 3.4).
Once the calibration checks were completed, the utility sheet, generated by RASP software, which listed details of all the epochs of data collected, was printed and kept as part of the study record.

3.15 Data handling and backup

Each infant recruited to the study was given a sequential number, with a prefix specific to the project and a suffix denoting the test occasion, e.g. 0102901 would read as follows: ‘01’ as the project number, ‘029’ as the infant number and ‘01’ as the test occasion.

All data collected during each infant study were backed up onto 2 zip disks. Each disk was clearly labelled with the infant study number. One disk was kept in a fire-safe box at the Homerton Hospital, and the second disk was used as a transfer disk to enable a second set of data to be saved and stored at the Institute of Child Health.

3.16 Data analysis and quality control

Data were exported, using RASP software, in ASCII format, and then analysed using the ‘Squeeze’ program (Dixon and Stocks, 1997). To maintain quality control of the data analysis, every analysed dataset was reviewed by an experienced member of the research team who was blinded to the status of the infant, i.e. whether the subject was an SGA or AGA infant. To further validate and to ensure minimal inter-observer bias during analysis, one in every 10 sets of data collected were cross-analysed and checked for concordance between the results.

3.17 Data entry and checking

The infant’s background information and test details (Appendix B) were manually entered on the Project specific relational database (Microsoft Access 97). Results of the respiratory function tests were analysed, checked for quality and then directly exported to this database. The data entered were double-checked by two people at a later date.
3.18 Statistical methods

Data were checked for normality and transformed when necessary. Comparisons of group characteristics and respiratory function between the groups were performed using t tests, chi-square or exact tests as appropriate (StatXact version 4.01). The extent to which airway function at 4-12 weeks postnatal age is independently associated with low birthweight for gestational age was examined using multiple linear regression, before and after allowing for pre- and postnatal exposure to tobacco (SPSS for Windows version 8.0.2). Analyses were also undertaken for all infants as a group and separately for those not exposed to maternal smoking in pregnancy or postnatally and for SGA infants who were ≤ 3rd birthweight centile. The relationship between CGF and GROW algorithms and between $V'_{\text{maxFRC}}$ and MEF$_{15}$ was assessed using methods described by Bland and Altman (Bland and Altman, 1986).

3.19 Equipment cleaning

After every test occasion, all equipment was cleaned as per protocol. Equipment such as PNTs, plastic connectors and mask were initially washed with soapy water and when dried, were soaked in 96% ethanol for 10 minutes. The ETCO$_2$ sensor and PNT mount were cleaned with alcowipes. All surfaces such as the plethysmograph, stadiometer, changing mat etc. were cleaned with soapy water.
4 Results
4.1 Introduction

The influence of birthweight on airway function in early infancy was examined by measuring respiratory function in infants within the first three months of life prior to any lower respiratory tract infection (See Methods). This chapter will present airway function results and their association with birthweight, sex of infant, maternal smoking status and other potential confounding factors such as social class of parents, family history of asthma and other obstetric factors. Results from the studies undertaken to validate methodological and analytical aspects of the RVRTC technique are also presented.

4.2 Recruitment and subject accrual

Over a three-year period (1998 – 2000), attempts were made to contact parents of 1,318 potentially eligible infants born at Homerton and University College Hospitals, London (Section 3.1.4.1.). Initially, recruitment to the study was conducted by face-to-face interview on the wards. However, due to the short length of stay of mothers post-delivery and the time consuming nature of recruiting by this method, after the first six months, all subsequent recruitment was initiated by postal contact with parents. Despite repeated telephone calls (up to four times) it was impossible to establish any contact with 505 of these families before the infant was two and a half months of age. Of the 813 parents contacted, 283 (35%) agreed to participate. The major reasons given for not wishing to participate were unwillingness to take part in research studies, the need to sedate the baby and lack of time (Figure 4.1).

Of the 283 infants whose parents agreed to take part (129 SGA and 154 AGA), 26 had to be excluded prior to testing due to having had either a lower respiratory illness or repeated upper respiratory illnesses such that they exceeded the upper age limit for lung function measurements in this study (Figure 4.1). A further 57 infants did not attend, because their parents either had no time to participate or changed their mind (Figure 4.1).

Lung function measurements were therefore attempted in 200 of these 283 infants (71%). Successful lung function measurements were achieved in 79 of the 86 SGA
infants and 104 of the 114 AGA infants who attended for lung function tests. Lung function measurements were unsuccessful or incomplete in seven SGA and 10 AGA infants (17/200 [9%]) due to poor quality data or the inability to complete the study protocol. Thus, lung function measurements from 183 infants (79 SGA and 104 AGA), i.e. 23% of the eligible population who had been contacted, form the basis of this thesis (Figure 4.1).
Figure 4.1  Recruitment and subject accrual

Total approached
1318

Unable to contact 505
Declined participation & reasons:
♦ Research – 254
♦ Sedation – 106
♦ Time constraints - 170

Agreed participation 283

129 SGA

154 AGA

Reasons for default: (n= 43)
♦ LRI (exclude) – 5
♦ Repeated URI -10
♦ Time constraints - 14
♦ Changed mind - 14

86 SGA
attended LFT
Successful LFT
79 SGA

114 AGA
attended LFT
Successful LFT
104 AGA

Reasons for default: (n= 40)
♦ LRI (exclude) – 4
♦ Repeated URI – 7
♦ Time constraints - 16
♦ Changed mind - 13

Definition of abbreviations: LRI = lower respiratory illness; URI = upper respiratory illness; LFT = lung function test; SGA = small for gestational age; AGA = appropriate for gestational age.
4.3 Comparison of birthweight centiles using CGF and GROW algorithms

Since the optimal method of identifying SGA infants remains unclear, two methods to classify infants' size at birth were used, namely the Child Growth Foundation (CGF) (Freeman et al. 1995) and Gestation Related Optimal Growth (GROW) (Sanderson et al. 1994) algorithms. Figure 4.2 shows a scatter plot of birthweight centiles calculated by the CGF and GROW algorithms for 178 infants with successful lung function measurements (missing data from five infants preclude the calculation of GROW centiles - Table 4.1). Each data point represents results from one infant and the line of identity is shown. Data points above the line of identity indicate that centiles estimated from the CGF algorithms were greater than those estimated from the GROW algorithms, whereas data points below the line indicate that centiles estimated from GROW, were greater than CGF.

Overall, centiles based on CGF algorithms were greater than those estimated by GROW for both SGA and AGA infants.

Figure 4.2 Comparison of birthweight centiles using CGF and GROW algorithms
Table 4.1 Birthweight centile classification according to CGF and GROW algorithms

<table>
<thead>
<tr>
<th></th>
<th>GROW</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 10</td>
<td>&gt; 10 to 15</td>
<td>&gt; 15 ≤ 95</td>
<td>Total</td>
</tr>
<tr>
<td>CGF ≤ 10</td>
<td>60</td>
<td>4</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>CGF &gt; 10 to 15</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>CGF 20-95</td>
<td>5</td>
<td>95</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>9</td>
<td>95</td>
<td>178*</td>
</tr>
</tbody>
</table>

* missing data from five infants, hence unable to calculate GROW centile.

SGA: CGF centile - 1.4; AGA: CGF centile - 33.1, 34.7, 21.6 and 21.1.

Of the 74 SGA infants, 64 were ≤ 10th centile by CGF classification, 74 by GROW and 60 by both (Table 4.1, Figure 4.3). In four SGA infants, birthweight was between 10th – 15th centile according to the GROW algorithm, but were ≤ 10th centile according to the CGF algorithm, while in 14 infants, birthweight was between 10th – 15th according to CGF but were ≤ 10th centile by GROW. Birthweight was above the 20th centile by CGF classification in all 100 AGA infants, but in five infants, birthweight fell between 12th – 15th centile when classified by GROW (Table 4.1, Figure 4.2).

Figure 4.3 Comparison of birthweight centiles using CGF and GROW algorithms (expanded scale showing SGA infants only)
In order to examine the possible relationship between centiles estimated by CGF and GROW algorithms, a scatter plot of the mean difference between the methods used was plotted against the average centile obtained using both methods (Bland and Altman, 1999). As the 'true' birthweight centile for each infant is unknown, the mean of the two measurements was taken as the best estimate. Figure 4.4 and Figure 4.5 shows the level of agreement between CGF and GROW algorithms in the calculation of birthweight centiles for SGA and AGA infants respectively. Among SGA infants (Figure 4.4), birthweight classification was on average 3 centiles higher according to CGF than GROW (95% CI of the mean difference, CGF - GROW: 2.2, 3.7) and the limits of agreement showed that 95% of the differences observed were between -3.5 and 9.4 centiles (mean difference ± 1.96 SD). In order to determine how precise these limits were, 95% CI of the limits were also calculated. For the lower limit of agreement, the 95% CI was -2.2 to -4.8 and for the upper limit of agreement, 8.2 to 10.7.
Figure 4.4  Measuring agreement between CGF and GROW algorithms in SGA infants

Figure 4.5  Measuring agreement between CGF and GROW algorithms in AGA infants
However, the scatter of the mean difference between CGF and GROW classification was observed to be wider for higher birthweight centiles. Birthweight classification among the AGA infants was on average 5.3 centiles higher according to CGF than GROW (95% CI of the difference: 3.1, 7.4) and the limits of agreement (-16.2 to 26.7) was wider with 95% CI for the lower limit of agreement: -12.5, -19.9 and for upper limit of agreement: 23.0, 30.4 (Figure 4.5).

In summary, of the 78 SGA infants recruited, 14 would not have been identified using the CGF algorithm only, while four would have been missed if the GROW algorithm was used alone (Table 4.1). There was reasonable agreement between these two methods of birthweight classification, but there was a systematic difference in that the GROW algorithm tended to assign infants to a lower birthweight centile than the CGF algorithm. Nevertheless for the purposes of this study, it was unlikely that any AGA infant was included among those classified as SGA.
### Table 4.2 Group characteristics at birth according to birthweight status

<table>
<thead>
<tr>
<th></th>
<th>SGA</th>
<th>AGA</th>
<th>95% CI of Difference: SGA-AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>79</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.9(1.5)</td>
<td>39.8(1.4)</td>
<td>-0.5, 0.3</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
<td>2.7(0.3)</td>
<td>3.5(0.4)</td>
<td>-0.9, -0.6</td>
</tr>
<tr>
<td>Birthweight SD score (CGF)</td>
<td>-1.7(0.4)</td>
<td>0.06(0.6)</td>
<td>-1.9, -1.6</td>
</tr>
<tr>
<td>Birthweight centile (CGF)</td>
<td>6.3(3.8)</td>
<td>51.2(19.3)</td>
<td>-49.2, -41.5</td>
</tr>
<tr>
<td>Birthweight centile (GROW)</td>
<td>3.4(3.5)</td>
<td>47.3(23.3)</td>
<td>-48.6, -39.3</td>
</tr>
<tr>
<td>Crown-heel length (cm)</td>
<td>49.1(3.0)</td>
<td>52.1(2.8)</td>
<td>-3.9, -2.1</td>
</tr>
<tr>
<td>Crown-heel length SD score (CGF)</td>
<td>-0.8(1.3)</td>
<td>0.9(1.3)</td>
<td>-2.1, -1.3</td>
</tr>
<tr>
<td>Crown-heel length centile (CGF)</td>
<td>30.7(30.0)</td>
<td>70.7(26.7)</td>
<td>-48.8, -31.3</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.0(1.6)</td>
<td>34.6(1.3)</td>
<td>-2.0, -1.1</td>
</tr>
<tr>
<td>Head circumference SD score (CGF)</td>
<td>-1.4(1.1)</td>
<td>-0.2(1)</td>
<td>-1.5, -0.9</td>
</tr>
<tr>
<td>Boys</td>
<td>43 (54%)</td>
<td>55 (53%)</td>
<td>-13%, 16%</td>
</tr>
<tr>
<td>Any maternal smoking after 8 w gestation</td>
<td>39 (49%)</td>
<td>39 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>Firstborn</td>
<td>51 (65%)</td>
<td>67 (64%)</td>
<td>-14%, 14%</td>
</tr>
<tr>
<td>Ever breastfed</td>
<td>41 (52%)</td>
<td>52 (50%)</td>
<td>-12%, 16%</td>
</tr>
<tr>
<td>Maternal age at delivery (yr)</td>
<td>31.8(5.4)</td>
<td>33.0(5.6)</td>
<td>-2.8, 0.5</td>
</tr>
<tr>
<td>Maternal age at completion of full-time education (yr)</td>
<td>19.8(3.5)</td>
<td>20.7(3.5)</td>
<td>-1.9, 0.2</td>
</tr>
<tr>
<td>Maternal weight at booking (kg)</td>
<td>59.4(10.5)</td>
<td>64.2(11.5)</td>
<td>-8.0, -1.5 **</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>162.1(6.6)</td>
<td>164.5(6.6)</td>
<td>-4.3, -0.4</td>
</tr>
<tr>
<td>Mothers in non-manual occupation</td>
<td>54 (70%)</td>
<td>81 (81%)</td>
<td>-24%, 2%</td>
</tr>
<tr>
<td>First degree family history of asthma</td>
<td>21 (27%)</td>
<td>30 (29%)</td>
<td>-15%, 11%</td>
</tr>
</tbody>
</table>

1 Data shown as mean (SD) for continuous and n (%) for categorical variables.

* p < 0.05; ** p < 0.01

**Definition of symbols**

SGA (n) | AGA (n)
---|---
§ 78  | 100
¶ 68  | 97
† 69  | 98
‡ 79  | 100
# 77  | 103
β 77  | 100

---

119
The characteristics of 79 SGA and 104 AGA infants are summarised in Table 4.2. Mean gestational age was similar and differences with respect to birthweight, birth length and head circumference, reflect the selection criteria used. There was a similar distribution of boys and firstborn between the SGA and AGA infants, and of the proportion of infants who were ever breast-fed.

While there were no significant differences between the groups with respect to maternal age at delivery, maternal age at completion of full time education or the percentage of mothers in non-manual occupations, mothers of SGA infants were significantly shorter and lighter when compared to mothers of AGA infants. Mean self reported maternal birthweight for mothers of SGA infants was also significantly lower when compared to mothers of AGA infants (mean [95% CI of the difference]: 3.0 vs. 3.3 kg [-0.46, -0.05]; p = 0.016). In addition, of those infants with siblings (n = 65), a significantly higher proportion of SGA infants had an SGA sibling than their appropriately grown counterparts (SGA:AGA with SGA siblings: 11/28 (39%) vs. 6/37 (16%); 95%CI of the difference: 1%, 45%; p = 0.04).

A positive family history of asthma was reported in a similar proportion of SGA and AGA infants, and in 11 (14%) SGA and 13 (13%) AGA infants, the mother was one of the affected family members. A higher proportion of SGA infants was born to mothers who smoked, though this difference was not significant (Table 4.2).
4.4.1 Obstetric history

Of the 79 SGA infants studied, poor fetal growth was suspected antenatally in 22 (28%), of whom 18 (23% of all SGA infants) were \( \leq 5^{th} \) centile according to the GROW algorithm while 14 (18%) were \( \leq 5^{th} \) centile according to the CGF algorithm. However, two AGA infants had also been suspected of poor fetal growth from antenatal ultrasonography.

Uterine artery notches were observed antenatally in two SGA pregnancies while one SGA infant who was electively delivered at 35 weeks gestation was observed to have reduced umbilical artery end diastolic blood flow. These three SGA infants had birthweights \( \leq 3^{rd} \) centile according to both CGF and GROW algorithms. The proportion of SGA and AGA pregnancies with antenatal procedures such as chorionic villus sampling and amniocentesis was similar but low (Table 4.3). Similarly, complications of pregnancy and labour such as antepartum haemorrhage, pregnancy induced hypertension, prolonged rupture of membranes (< 72 hours from delivery) and meconium staining of liquor in labour were evenly distributed between the two groups, as were the proportions of infants delivered by Caesarean section or whose mothers were given dexamethasone antenatally (Table 4.3).

<table>
<thead>
<tr>
<th>Table 4.3</th>
<th>Antenatal procedures performed and obstetric complications in SGA/AGA pregnancies$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA ( (n = 79) )</td>
</tr>
<tr>
<td>Chorionic villus sampling</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Prolonged rupture of membranes ((&lt; 72) hr)</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Pregnancy induced hypertension</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Antenatal dexamethasone</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Meconium stained liquor</td>
<td>13 (16%)</td>
</tr>
<tr>
<td>Non-vaginal delivery</td>
<td>22 (28%)</td>
</tr>
</tbody>
</table>

$^1$Data shown as n (%).
4.4.2 Description of smoking status

For the purposes of this study, we defined a smoking mother as one who smoked at any time after eight weeks of gestation, irrespective of the number of cigarettes smoked.

4.4.3 Influence of maternal smoking on group characteristics

Maternal and infant characteristics at birth according to maternal smoking status are presented in Table 4.4. Of the 183 infants, 78 (43%) were born to mothers who smoked and there was a similar distribution of boys in both groups. However, more SGA infants were born to mothers who smoked (49% vs. 38%; p = 0.11) and this may in part account for the significant difference in birthweight when compared with infants not exposed to maternal smoking. Women who smoked in pregnancy were more likely to be multiparous (p = 0.05) and less likely to breast feed their infants (p = 0.001). In addition, mothers who smoked were on average 2.5 years younger at delivery (p = 0.004), were more likely to have left full time education at a younger age (p < 0.001) and to have a manual occupation (p < 0.001). A positive family history of asthma was reported in a higher proportion of infants whose mothers smoked but this was not significant (Table 4.4). Maternal asthma was reported in 11 (14%) of mothers who did and 13 (12%) of mothers who did not smoke.
Table 4.4  Group characteristics at birth according to maternal smoking status

<table>
<thead>
<tr>
<th></th>
<th>Smoking</th>
<th>Non-smoking</th>
<th>95% CI of Difference: Smoking – Non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>78</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.7 (1.5)</td>
<td>39.9 (1.4)</td>
<td>-0.7, 0.2</td>
</tr>
<tr>
<td>Birthweight (kg)</td>
<td>3.0 (0.6)</td>
<td>3.2 (0.5)</td>
<td>-0.35, -0.04 *</td>
</tr>
<tr>
<td>Birthweight SD score (CGF)</td>
<td>-0.88 (0.99)</td>
<td>-0.53 (0.97)</td>
<td>-0.63, -0.05 *</td>
</tr>
<tr>
<td>Birthweight centile (CGF)</td>
<td>26.9 (25.0)</td>
<td>35.8 (27.8)</td>
<td>-16.8, -1.0 *</td>
</tr>
<tr>
<td>Birthweight centile (GROW)</td>
<td>21.9 (25.2)</td>
<td>32.5 (29.3)</td>
<td>-18.9, -2.5 *</td>
</tr>
<tr>
<td>Crown-heel length (cm)</td>
<td>50.5 (3.4)</td>
<td>51.2 (3.1)</td>
<td>-1.8, 0.2</td>
</tr>
<tr>
<td>Crown-heel length SD score (CGF)</td>
<td>-0.01 (1.56)</td>
<td>0.34 (1.55)</td>
<td>-0.83, 0.13</td>
</tr>
<tr>
<td>Crown-heel length centile (CGF)</td>
<td>51.3 (34.8)</td>
<td>56.4 (33.9)</td>
<td>-15.8, 5.5</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.9 (1.7)</td>
<td>33.9 (1.5)</td>
<td>-0.6, 0.4</td>
</tr>
<tr>
<td>Head circumference SD score (CGF)</td>
<td>-0.63 (1.16)</td>
<td>-0.69 (1.20)</td>
<td>-0.31, 0.42</td>
</tr>
<tr>
<td>Boys (%)</td>
<td>40 (51%)</td>
<td>58 (55%)</td>
<td>-19%, 11%</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>39 (49%)</td>
<td>40 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>Firstborn (%)</td>
<td>44 (56%)</td>
<td>74 (70%)</td>
<td>-28%, 0% *</td>
</tr>
<tr>
<td>Ever breast-fed (%)</td>
<td>24 (31%)</td>
<td>69 (66%)</td>
<td>-49%, -21% ***</td>
</tr>
<tr>
<td>Maternal age at delivery (yr)</td>
<td>31.0 (6.4)</td>
<td>33.5 (4.5)</td>
<td>-4.2, -0.8 **</td>
</tr>
<tr>
<td>Maternal age at completion of full-time education (yr)</td>
<td>18.5 (3.3)</td>
<td>21.6 (3.1)</td>
<td>-4.1, -2.2 ***</td>
</tr>
<tr>
<td>Maternal weight at booking (kg)</td>
<td>60.8 (11.1)</td>
<td>63.1 (11.4)</td>
<td>-5.6, 1.0</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>162.4 (7.3)</td>
<td>164.4 (6.0)</td>
<td>-3.95, -0.02 *</td>
</tr>
<tr>
<td>Mothers in non-manual occupation (%)</td>
<td>41 (56%)</td>
<td>94 (90%)</td>
<td>-47%, -22% ***</td>
</tr>
<tr>
<td>First degree family history of asthma</td>
<td>25 (32%)</td>
<td>26 (25%)</td>
<td>-6%, 21%</td>
</tr>
</tbody>
</table>

1 Data shown as mean (SD) for continuous and n (%) for categorical variables.

*** p < 0.001; ** p < 0.01; * p < 0.05

Definition of symbols

<table>
<thead>
<tr>
<th></th>
<th>SGA (n)</th>
<th>AGA (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>§</td>
<td>77</td>
<td>102</td>
</tr>
<tr>
<td>¶</td>
<td>71</td>
<td>94</td>
</tr>
<tr>
<td>‡</td>
<td>72</td>
<td>95</td>
</tr>
<tr>
<td>†</td>
<td>77</td>
<td>101</td>
</tr>
<tr>
<td>#</td>
<td>77</td>
<td>103</td>
</tr>
<tr>
<td>β</td>
<td>73</td>
<td>104</td>
</tr>
</tbody>
</table>
4.4.4 Assessment of maternal and infant exposure to tobacco smoke

Maternal reports of smoking were validated by cotinine assay of maternal saliva and infant urine samples obtained at time of test. Generally, there was good concordance between maternal reports of smoking and cotinine assay of the saliva and urine samples. However, five infants (2 AGA and 3 SGA) were re-classified into the smoking category as maternal salivary cotinine concentrations ranged from 20.8 to 434.6 ng.mL\(^{-1}\) and were consistent with values from active smokers (> 15 ng.mL\(^{-1}\)) (Figure 4.6) (McNeill et al. 1987; Jarvis et al. 2000). Thus, following re-classification of the five infants into the smoking category, cotinine levels were negligible in the non-smoking group while higher levels were observed in the smoking category in both saliva and urine samples (Table 4.5). Seven mothers who reported smoking after eight weeks' gestation and/or postnatally and who were therefore classified as 'smokers', were found to have salivary cotinine ≤ 2 ng.mL\(^{-1}\), suggesting they were 'light' smokers (Figure 4.6).

Table 4.5 Summary of cotinine results according to validated maternal smoking status\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal salivary cotinine(^1)</td>
<td>0.2 (0.1 – 0.7)</td>
<td>198.1 (77.2 – 357.5)</td>
</tr>
<tr>
<td>Infant urinary cotinine(^1)</td>
<td>1.5 (0.6 – 2.5)</td>
<td>11.9 (5.6 – 30.8)</td>
</tr>
</tbody>
</table>

\(^1\)Data shown as median (inter-quartile range) ng.mL\(^{-1}\)

\(^1\) n = 100; n = 74 respectively

\(^1\) n = 89; n = 66 respectively.
Figure 4.6  Cotinine levels of maternal saliva and infant urine classified according to validated maternal smoking status

Note: Logarithmic scales; only three mother-infant pairs of the five re-classified are shown as two infant urine samples were unavailable. Only five of the seven light smokers are shown, for the same reason.

While maternal reports of smoking were broadly reliable, there was considerable overlap in infant cotinine values between infants who were and were not exposed to maternal smoking. In addition, from the wide range of infant urinary cotinine levels observed among infants not exposed to maternal smoking, it was clear that there were other significant sources of tobacco smoke exposure even at this young age (Figure 4.6).
Table 4.6  Cotinine levels according to SGA/AGA classification and maternal smoking status

<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th></th>
<th>Smoking</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA</td>
<td>AGA</td>
<td>SGA</td>
<td>AGA</td>
</tr>
<tr>
<td>Maternal salivary cotinine $^1$</td>
<td>0.3 (0.1 – 0.7)</td>
<td>0.1 (0.1 – 0.6)</td>
<td>226.2 (97.7 – 435.75)</td>
<td>182.0 (31.4 – 305.25)</td>
</tr>
<tr>
<td>Infant urinary cotinine $^1$</td>
<td>1.5 (0.8 – 2.4)</td>
<td>1.6 (0.4 – 2.5)</td>
<td>15.3 (7.0 – 30.8)</td>
<td>9.2 (4.2 – 31.3)</td>
</tr>
</tbody>
</table>

$^1$Data shown as median (inter-quartile range) ng.mL$^{-1}$

$^\dagger$ n = 38; n = 62; n = 38; n = 36 respectively;

$^\ddagger$ n = 32; n = 57; n = 35; n = 31 respectively.

When cotinine concentration was compared according to birthweight classification and smoking status, salivary and urine cotinine levels were similar in the two non-smoking groups. However, levels observed in the SGA infants exposed to maternal smoking tended to be higher when compared to the AGA smoking group, though this difference was not significant.
Figure 4.7 Maternal salivary cotinine levels according to number of cigarettes smoked

Note: Logarithmic scale; Definition of abbreviation: P/N = postnatal; Maternal salivary cotinine not available for 3 mothers who stopped smoking by 20 weeks gestation but continued to smoke postnatally and 2 mothers who stopped smoking by 20 weeks gestation, hence only 7 and 4 data points respectively are shown.

Figure 4.7 shows maternal salivary cotinine level according to self reported smoking habit at time of test. A wide range of cotinine levels (from 0.7 – 722 ng.mL\(^{-1}\)) was observed among those who reportedly smoked ≤ five cigarettes per day. Thus while maternal report of smoking status was broadly reliable with only 5/110 (4.5%) self reported non-smokers probably being active smokers, maternal report of the amount smoked correlated poorly with the biochemical assay of exposure. Additionally, Figure 4.7 shows that, above 4 cigarettes per day, the number of cigarettes smoked tended to be rounded to multiples of five.
4.4.4.1 Association between infant cotinine levels and method of feeding

Figure 4.8 Cotinine levels according to method of infant feeding at time of test

![Graph showing relationship between maternal salivary cotinine and infant urinary cotinine.]

In Figure 4.8 infant’s tobacco smoke exposure, as reflected by maternal salivary and infant urinary cotinine levels, was classified according to maternal report of infant feeding method. Among mothers who did not smoke, as represented by most of the data points to the left of the cotinine cut-off level for maternal saliva (15 ng.mL⁻¹), infant cotinine levels were varied but there was no clear pattern according to feeding method. By contrast, among mothers who smoked, as represented by all the data points to the right of this threshold, infant urinary cotinine was highest among infants who were breast-fed and lowest in those who were bottle-fed, with those who were breast and bottle-fed having intermediate values.

Infant and maternal cotinine levels from mothers who smoked are presented in Table 4.7 according to infant feeding method at time of test. Women who bottle-fed their
infants were more likely to smoke > 10 cigarettes per day. Hence, mean salivary cotinine levels were also higher in the mothers who bottle-fed their infants (Table 4.7). In contrast, infant urinary cotinine levels were, on average, higher in the breast-fed group, probably reflecting additional ingestion of cotinine via breast milk.

Table 4.7 Cotinine levels from mothers who smoked and infant feeding method at time of test

<table>
<thead>
<tr>
<th>Infant feeding method</th>
<th>Breast (n = 24)</th>
<th>Breast/Bottle (n = 15)</th>
<th>Bottle (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked &gt; 10 cigarettes/day</td>
<td>5 (21%)</td>
<td>3 (20%)</td>
<td>15 (38%)</td>
</tr>
<tr>
<td>Maternal salivary cotinine</td>
<td>146 (35 – 218)</td>
<td>217 (102 – 372)</td>
<td>253 (89 – 391)</td>
</tr>
<tr>
<td>Infant urinary cotinine</td>
<td>32 (8 – 118)</td>
<td>17 (9 – 32)</td>
<td>8 (5 – 16)</td>
</tr>
</tbody>
</table>

Data shown as median (inter-quartile range) ng.mL$^{-1}$ for continuous and n (%) for categorical variables.

Thus, among infants whose mothers smoked, median infant urinary cotinine was four times higher in infants who were exclusively breast fed, relative to those infants fed only by bottle. By comparison, median infant urinary cotinine level was only twice as high in those who were breast and bottle-fed.
4.4.4.2 Association between cotinine levels and sources of tobacco smoke exposure

Table 4.8 Infant urinary cotinine levels according to maternal and household exposure

<table>
<thead>
<tr>
<th></th>
<th>No exposure (n = 56)</th>
<th>Household exposure only (n = 23)</th>
<th>Mother exposure only (n = 15)</th>
<th>Mother &amp; household exposure (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant urinary cotinine</td>
<td>1.4 (0.6 – 2.5)</td>
<td>1.8 (0.8 – 4.5)</td>
<td>8.4 (3.7 – 24.1)</td>
<td>15.3 (6.9 – 32.6)</td>
</tr>
</tbody>
</table>

1Data shown as median (inter-quartile range) ng.mL.

In order to assess the influence of other sources of tobacco smoke exposure in the infant, infant urinary cotinine levels were calculated according to maternal smoking and other household exposure (Table 4.8). Infant urinary cotinine levels were negligible in the group with no exposure and minimally elevated in the 23 infants who were exposed to other household smokers (mother being a non-smoker). By contrast, for infants who were exposed to maternal and other household smokers, there was a more marked increase in their urinary cotinine levels when compared to those who were only exposed maternal smoking.

Table 4.9 Cotinine levels according to maternal and household exposure

<table>
<thead>
<tr>
<th></th>
<th>No exposure (n = 56)</th>
<th>Household exposure only (n = 23)</th>
<th>Mother exposure only (n = 15)</th>
<th>Mother &amp; household exposure (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant urinary cotinine</td>
<td>1.4 (0.6 – 2.5)</td>
<td>1.8 (0.8 – 4.5)</td>
<td>8.4 (3.7 – 24.1)</td>
<td>15.3 (6.9 – 32.6)</td>
</tr>
<tr>
<td>Maternal salivary cotinine</td>
<td>0.1 (0.1 – 0.6)</td>
<td>0.4 (0.1 – 1.2)</td>
<td>132.6 (21 – 343)</td>
<td>266.3 (158 – 391)</td>
</tr>
<tr>
<td>Smoked &gt; 10 cigarettes per day</td>
<td>4 (29%)</td>
<td></td>
<td>19 (42%)</td>
<td></td>
</tr>
</tbody>
</table>

1Data shown as median (inter-quartile range) ng.mL. for continuous variables and n (%) for categorical variable.

The same pattern was observed for maternal salivary cotinine, suggesting that mothers who are active smokers may be exposed to passive smoke exposure from other household members as well. However, some of the differences noted may be due to the fact that mothers who smoked and lived with other smokers within the same household tended to smoke more than those who lived with a non-smoker. In households where the mother is the only smoker, the median number of reported
cigarettes smoked was three per day (range 1 – 20) and in households where there was at least one other smoker (excluding the mother), the median number of cigarettes smoked by the mother was reported to be 10 per day (range 1 – 30).

Thus, relative to infants who were not exposed to tobacco smoke, median infant urinary cotinine levels were on average six times higher among infants who were exposed to maternal smoking only and 11 times higher when exposed to maternal and other household smokers.

4.5 Group characteristics at test

At the time of test, both SGA and AGA infants were just over six weeks postnatal age (mean [SD] SGA:AGA: 6.2 wk [2.5] vs. 6.1 wk [2.1]) but the SGA infants remained significantly shorter, lighter and had smaller head, chest and mid arm circumference than their controls (Table 4.10).

Group characteristics at test according to maternal smoking status are summarised in Table 4.11. Infants of mothers who smoked remained significantly lighter but not shorter for their age, although absolute weights were similar to those not exposed to maternal smoking. Table 4.12 shows group characteristics at test according to both birthweight classification and smoking status as a subgroup. SGA infants whose mothers smoked tended to be lighter and smaller than SGA infants whose mothers did not, but this was not evident in AGA infants. While differences in test weight, length and head circumference between smoking and non-smoking SGA infants were not significant, SGA infants of smoking mothers had significantly smaller chest (mean difference [95% CI], 1.6 cm [0.4, 2.8]; p=0.01) and mid arm circumference (0.7 cm [0.03, 1.3]; p=0.04) when compared to SGA infants not exposed to maternal smoking. However, there was a preponderance of girls in the SGA smoking subgroup, which may account for some of the differences seen. There was a marked discrepancy in sex distribution among SGA infants who were exposed and not exposed to maternal smoking. At time of test, SGA infants born to mothers who smoked were on average one week younger than SGA infants not exposed to maternal smoking. This may account for the weight difference as well as chest and mid arm circumferences observed between these two subgroups Group
characteristics at test according to maternal smoking status and birthweight classification as a subgroup are presented in Appendix H.

Table 4.10  Group characteristics at test according to birthweight status\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>SGA (n = 79)</th>
<th>AGA (n = 104)</th>
<th>95% CI of Difference: SGA-AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>43 (54%)</td>
<td>55 (53%)</td>
<td>-13%, 16%</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>39 (49%)</td>
<td>39 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>Age (wk)(^4)</td>
<td>6.2 (2.5)</td>
<td>6.1 (2.1)</td>
<td>-0.6, 0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.1 (0.8)</td>
<td>4.8 (0.7)</td>
<td>-0.9, -0.4 (^*)</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>-1.1 (0.9)</td>
<td>0.05 (0.9)</td>
<td>-1.4, -0.9 (^***)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>54.0 (2.9)</td>
<td>56.5 (2.8)</td>
<td>-3.3, -1.7 (^***)</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.8 (0.9)</td>
<td>0.5 (0.9)</td>
<td>-1.5, -1.0 (^***)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>37.8 (1.7)</td>
<td>39.1 (1.6)</td>
<td>-1.7, -0.7 (^***)</td>
</tr>
<tr>
<td>Head circumference SD score</td>
<td>-0.2 (0.9)</td>
<td>0.8 (1.0)</td>
<td>-1.3, -0.7 (^***)</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>37.0 (2.8)</td>
<td>39.1 (2.1)</td>
<td>-2.8, -1.4 (^***)</td>
</tr>
<tr>
<td>Mid arm circumference (cm)</td>
<td>11.8 (1.5)</td>
<td>12.4 (1.3)</td>
<td>-1.0, -0.2 (^*)</td>
</tr>
</tbody>
</table>

\(^1\)Data shown as mean (SD) for continuous and n (%) for category variables. SD scores were calculated using CGF algorithms.  \(^*\) age after expected date of delivery  
\(^*\) ** p < 0.01;  \(^***\) ** p < 0.001
Table 4.11  Group characteristics at test according to maternal smoking status

<table>
<thead>
<tr>
<th></th>
<th>Smoking (n = 78)</th>
<th>Non-smoking (n = 105)</th>
<th>95% CI of Difference: Smoking – Non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>39 (50%)</td>
<td>40 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>Boys</td>
<td>40 (51%)</td>
<td>58 (55%)</td>
<td>-19%, 11%</td>
</tr>
<tr>
<td>Age (wk)$^\dagger$</td>
<td>6.0 (2.1)</td>
<td>6.2 (2.4)</td>
<td>-0.9, 0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.4 (0.9)</td>
<td>4.6 (0.8)</td>
<td>-0.5, 0.01</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>-0.65 (1.11)</td>
<td>-0.31 (1.00)</td>
<td>-0.65, -0.04 *</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>55.0 (3.3)</td>
<td>55.8 (2.8)</td>
<td>-1.6, 0.2</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.20 (1.13)</td>
<td>0.07 (1.07)</td>
<td>-0.59, 0.06</td>
</tr>
</tbody>
</table>

$^1$Data shown as mean (SD) for continuous and n (%) for category variables. SD scores were calculated using CGF algorithms. $^\dagger$ age after expected date of delivery

* p < 0.05
### Table 4.12  Group characteristics at test according to birthweight and smoking status

<table>
<thead>
<tr>
<th></th>
<th>SGA</th>
<th>AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoking (n = 39)</td>
<td>Non-smoking (n = 40)</td>
</tr>
<tr>
<td>Boys</td>
<td>15 (38%)</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Age (wk)†</td>
<td>5.7 (2.3)</td>
<td>6.7 (2.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.9 (0.8)</td>
<td>4.3 (0.8)</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>-1.30 (0.94)</td>
<td>-0.95 (0.78)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>53.4 (3.2)</td>
<td>54.6 (2.4)</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.82 (1.04)</td>
<td>-0.72 (0.64)</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>37.5 (1.8)</td>
<td>38.2 (1.6)</td>
</tr>
<tr>
<td>Head circumference SD score</td>
<td>-0.18 (1.06)</td>
<td>-0.19 (0.82)</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>36.2 (2.8)</td>
<td>37.8 (2.5)</td>
</tr>
<tr>
<td>Mid arm circumference (cm)</td>
<td>11.4 (1.5)</td>
<td>12.1 (1.4)</td>
</tr>
</tbody>
</table>

1 Data shown as mean (SD) for continuous and n (%) for categorical variables. SD scores were calculated using CGF algorithms.

† age after expected date of delivery

* p < 0.05;  *** p < 0.001
Table 4.13  Group characteristics at test according to sex

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 98)</th>
<th>Girls (n = 85)</th>
<th>95% CI of Difference: Boys – Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>43 (44%)</td>
<td>36 (42%)</td>
<td>-13%, 16%</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>40 (41%)</td>
<td>38 (45%)</td>
<td>-18%, 10%</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>6.4 (2.4)</td>
<td>5.8 (2.1)</td>
<td>-0.04, 1.28</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.7 (0.9)</td>
<td>4.2 (0.6)</td>
<td>0.2, 0.7 ***</td>
</tr>
<tr>
<td>Weight SD score</td>
<td>-0.4, (1.1)</td>
<td>-0.5 (0.9)</td>
<td>-0.3, 0.4</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>56.0 (3.2)</td>
<td>54.8 (2.7)</td>
<td>0.4, 2.1 **</td>
</tr>
<tr>
<td>Length SD score</td>
<td>-0.1 (1.1)</td>
<td>0.02 (1.0)</td>
<td>-0.5, 0.2</td>
</tr>
</tbody>
</table>

Data shown as mean (SD) for continuous and n (%) for category variables. SD scores were calculated using CGF algorithms. * age after expected date of delivery. ** p < 0.01; *** p < 0.001

Group characteristics at test according to sex (98 boys and 85 girls) are summarised in Table 4.13. There was a similar proportion of boys and girls who were SGA, and whose mothers smoked during pregnancy and / or postnatally. Boys were tested on average 0.6 week later than girls. In terms of absolute weight and crown-heel length at test, boys were heavier and longer than girls but these differences were not significant when expressed as SD scores.
4.6 Lung function results

4.6.1 Introduction

The main outcome measures of airway function in infants were obtained using forced expiratory manoeuvres. Parameters selected from the raised volume RTC technique were forced vital capacity (FVC), timed volume parameters such as FEV$_{0.4}$ and FEV$_{0.5}$, thought to reflect the integrated output from both central and peripheral airways, and maximal flow at 15% and 25% of vital capacity (MEF$_{15}$ and MEF$_{25}$) which are thought to reflect peripheral airway function in infants. $V'_{\text{max-FRC}}$ obtained from partial forced expiratory flow volume curves using tidal RTC manoeuvres, also thought to reflect peripheral airway function is also presented. Other measures presented in this section are the tidal breathing parameters which include respiratory rate, tidal volume and the tidal expiratory flow ratio ($t_{\text{PTF:E}}$) (Section 3.7.1). These results will be presented initially according to birthweight classification, maternal smoking status and infant sex in univariate analyses. Multivariate analyses of factors significantly associated with central and peripheral airway function are then presented. The association of obstetric complications with airway function is briefly explored. This section concludes with analyses of analytical and methodological aspects, which could potentially influence lung function measurements. Since the most appropriate parameters to report from the raised volume technique have yet to be established, analytical aspects of the technique will also be presented.

4.6.2 Association of birthweight and maternal smoking status with airway function

As maternal smoking has clearly been demonstrated to be associated with low birthweight (Peacock et al. 1991; Lieberman et al. 1994; Horta et al. 1997) as well as diminished airway function in infants (Hanrahan et al. 1992; Hoo et al. 1998; Dezateux et al. 1999; Dezateux et al. 2001), respiratory function was analysed according to birthweight (Table 4.14) and maternal smoking status (Table 4.16).
Table 4.14  Respiratory function results according to birthweight status

<table>
<thead>
<tr>
<th></th>
<th>SGA</th>
<th>AGA</th>
<th>95% CI of Difference: SGA - AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 79)</td>
<td>(n = 104)</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>43 (54%)</td>
<td>55 (53%)</td>
<td>-13%, 16%</td>
</tr>
<tr>
<td>Any maternal smoking</td>
<td>39 (49%)</td>
<td>39 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>after 8 w gestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (wk)</td>
<td>6.2 (2.5)</td>
<td>6.1 (2.1)</td>
<td>-0.6, 0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.1 (0.8)</td>
<td>4.8 (0.7)</td>
<td>-0.9, -0.4 ***</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>54.0 (2.9)</td>
<td>56.5 (2.8)</td>
<td>-3.3, -1.7 ***</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>118 (31)</td>
<td>143 (34)</td>
<td>-35, -16 ***</td>
</tr>
<tr>
<td>FEV0.4 (mL)</td>
<td>104 (26)†</td>
<td>124 (28)</td>
<td>-28, -12 ***</td>
</tr>
<tr>
<td>FEV0.5 (mL)</td>
<td>110 (28)‡</td>
<td>133 (30)§</td>
<td>-32, -14 ***</td>
</tr>
<tr>
<td>MEF25 (mL.s⁻¹)</td>
<td>168 (64)</td>
<td>197 (69)</td>
<td>-49, -9 **</td>
</tr>
<tr>
<td>MEF15 (mL.s⁻¹)</td>
<td>102 (39)</td>
<td>119 (45)</td>
<td>-30, -5 **</td>
</tr>
<tr>
<td>FEV0.4/FVC</td>
<td>0.88 (0.06)†</td>
<td>0.87 (0.06)</td>
<td>-0.01, 0.02</td>
</tr>
<tr>
<td>V'_{maxFRC} (mL.s⁻¹)</td>
<td>129 (67)</td>
<td>138 (73)§</td>
<td>-29, 13</td>
</tr>
<tr>
<td>V'_{maxFRC} SD score</td>
<td>-0.03 (0.99)</td>
<td>-0.13 (1.09)§</td>
<td>-0.21, 0.41</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>47 (9)</td>
<td>46 (10)</td>
<td>-2, 4</td>
</tr>
<tr>
<td>V₁ (mL)</td>
<td>35 (8)</td>
<td>41 (8)</td>
<td>-8, -4 ***</td>
</tr>
<tr>
<td>V₇/kg (mL)</td>
<td>8.6 (1.4)</td>
<td>8.8 (1.5)</td>
<td>-0.6, 0.3</td>
</tr>
<tr>
<td>Ṯ₁ₑ/PEF: Ṯₑ</td>
<td>0.34 (0.10)</td>
<td>0.34 (0.10)</td>
<td>-0.03, 0.03</td>
</tr>
</tbody>
</table>

|                          |                   |                   |                                 |

† n = 78; † n = 73; † n = 99; † n = 102

** p < 0.01; *** p < 0.001

Note: V'_{maxFRC} SD score was calculated based on prediction models, incorporating age and length at test (Hoo et al. 2001).

In univariate analysis (Table 4.14), FVC was on average 25 mL (95% CI: 16, 35 mL) lower in SGA than AGA infants (p < 0.001). Similarly, FEV0.4 was 20 mL (12, 28 mL) (p < 0.001) and MEF25 29 mL.s⁻¹ (9, 49 mL.s⁻¹) lower in SGA than AGA infants (p<0.01). However, there was no significant difference between SGA and AGA infants in V'_{maxFRC}, or in the FEV0.4:FVC ratio.

It may be argued that some of the infants classified as being ≤ 10th birthweight centile may be constitutional small rather than small for gestational age. Hence, respiratory
function results from infants whose birthweight were ≤ 3rd centile according to either CGF or GROW algorithm were analysed separately and these are presented in Table 4.15.

Table 4.15  Respiratory function results comparing SGA (≤ 3rd birthweight centile) and AGA infants

<table>
<thead>
<tr>
<th></th>
<th>SGA (BW centile ≤ 3)</th>
<th>AGA (n = 104)</th>
<th>95% CI of Difference: SGA – AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>29 (56%)</td>
<td>55 (53%)</td>
<td>-14%, 19%</td>
</tr>
<tr>
<td>Any maternal smoking after 8 w gestation</td>
<td>25 (48%)</td>
<td>39 (38%)</td>
<td>-6%, 27%</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.0 (0.8)</td>
<td>4.8 (0.7)</td>
<td>-1.0, -0.5 ***</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>53.5 (2.9)</td>
<td>56.5 (2.8)</td>
<td>-4.0, -2.1 ***</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>112 (30)</td>
<td>143 (34)</td>
<td>-42, -20 ***</td>
</tr>
<tr>
<td>FEV0.4 (mL)</td>
<td>99 (24)†</td>
<td>124 (28)</td>
<td>-34, -16 ***</td>
</tr>
<tr>
<td>FEV0.5 (mL)</td>
<td>105 (27)‡</td>
<td>133 (30)§</td>
<td>-38, -18 ***</td>
</tr>
<tr>
<td>MEF25 (mL·s⁻¹)</td>
<td>167 (61)</td>
<td>197 (69)</td>
<td>-52, -7 *</td>
</tr>
<tr>
<td>MEF15 (mL·s⁻¹)</td>
<td>100 (38)</td>
<td>119 (45)</td>
<td>-33, -5 **</td>
</tr>
<tr>
<td>FEV0.4/FVC</td>
<td>0.88 (0.06) †</td>
<td>0.87 (0.06)</td>
<td>-0.01, 0.03</td>
</tr>
<tr>
<td>VᵐaxFRC (mL·s⁻¹)</td>
<td>133 (68)</td>
<td>138 (73)‡</td>
<td>-29, 19</td>
</tr>
<tr>
<td>VᵐaxFRC SD score</td>
<td>0.07 (0.99)</td>
<td>-0.13 (1.09)</td>
<td>-0.15, 0.56</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>47 (9)</td>
<td>46 (10)</td>
<td>-2, 4</td>
</tr>
<tr>
<td>VT (mL)</td>
<td>34 (8)</td>
<td>41 (8)</td>
<td>-10, -5 ***</td>
</tr>
<tr>
<td>VT/kg (mL)</td>
<td>8.7 (1.7)</td>
<td>8.8 (1.5)</td>
<td>-0.6, 0.5</td>
</tr>
<tr>
<td>tPTEF: tE</td>
<td>0.35 (0.09)</td>
<td>0.34 (0.10)</td>
<td>-0.03, 0.04</td>
</tr>
</tbody>
</table>

1 Data shown as mean (SD) for continuous and n (%) for categorical variables.
† n = 51; ‡ n = 47; § n = 99;  $ n = 102
* p < 0.05; ** p < 0.01; *** p < 0.001

Findings however were not changed when analyses were restricted to only those SGA infants whose birthweight was ≤ 3rd centile (Table 4.15).
<table>
<thead>
<tr>
<th>Smoking (n = 78)</th>
<th>Non-smoking (n = 105)</th>
<th>95% CI of Difference: Smoking – Non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys 40 (51%)</td>
<td>58 (55%)</td>
<td>-19%, 11%</td>
</tr>
<tr>
<td>SGA 39 (50%)</td>
<td>40 (38%)</td>
<td>-3%, 26%</td>
</tr>
<tr>
<td>Age (wk) 6.0 (2.1)</td>
<td>6.2 (2.4)</td>
<td>-0.9, 0.4</td>
</tr>
<tr>
<td>Weight (kg) 4.4 (0.9)</td>
<td>4.6 (0.8)</td>
<td>-0.5, 0.01</td>
</tr>
<tr>
<td>Length (cm) 55.0 (3.3)</td>
<td>55.8 (2.8)</td>
<td>-1.6, 0.2</td>
</tr>
<tr>
<td>FVC (mL) 126 (36)</td>
<td>137 (33)</td>
<td>-21, -0.3*</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.4&lt;/sub&gt; (mL) 110 (31)</td>
<td>120 (26)†</td>
<td>-19, -2*</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt; (mL) 117 (33)</td>
<td>129 (28)</td>
<td>-21, -3*</td>
</tr>
<tr>
<td>MEF&lt;sub&gt;25&lt;/sub&gt; (mL.s&lt;sup&gt;−1&lt;/sup&gt;) 175 (68)</td>
<td>192 (68)</td>
<td>-37, 3</td>
</tr>
<tr>
<td>MEF&lt;sub&gt;15&lt;/sub&gt; (mL.s&lt;sup&gt;−1&lt;/sup&gt;) 105 (45)</td>
<td>116 (42)</td>
<td>-24, 2</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.4&lt;/sub&gt;/FVC 0.87 (0.07)</td>
<td>0.88 (0.06)†</td>
<td>-0.02, 0.01</td>
</tr>
<tr>
<td>V&lt;sub&gt;maxFRC&lt;/sub&gt; (mL.s&lt;sup&gt;−1&lt;/sup&gt;) 122 (68)‡</td>
<td>143 (71)§</td>
<td>-42, -0.3*</td>
</tr>
<tr>
<td>V&lt;sub&gt;maxFRC&lt;/sub&gt; SD score -0.3 (1)</td>
<td>0.03 (1)</td>
<td>-0.6, 0.02</td>
</tr>
<tr>
<td>Respiratory rate (bpm) 47 (10)</td>
<td>46 (9)</td>
<td>-2, 4</td>
</tr>
<tr>
<td>V&lt;sub&gt;T&lt;/sub&gt; (mL) 37.5 (9.2)</td>
<td>39.9 (7.5)</td>
<td>-4.90, -0.02*</td>
</tr>
<tr>
<td>V&lt;sub&gt;T/kg&lt;/sub&gt; (mL) 8.6 (1.3)</td>
<td>8.8 (1.6)</td>
<td>-0.6, 0.3</td>
</tr>
<tr>
<td>t&lt;sub&gt;PTF&lt;/sub&gt;– t&lt;sub&gt;E&lt;/sub&gt; 0.33 (0.09)</td>
<td>0.35 (0.10)</td>
<td>-0.053, 0.003</td>
</tr>
</tbody>
</table>

When infants were classified according to maternal smoking status (Table 4.16) FVC, FEV<sub>0.4</sub> and V'<sub>maxFRC</sub> were significantly reduced in those infants whose mothers smoked. FVC was on average, diminished by 11 mL (95% CI: 0.3, 21 mL; p = 0.04) and FEV<sub>0.4</sub> by 10 mL (2, 19 mL; p = 0.02) in infants whose mothers smoked. While V'<sub>maxFRC</sub> was 21 mL.s<sup>−1</sup> (0.3, 42 mL.s<sup>−1</sup>; p = 0.04) significantly lower among infants whose mothers smoked, MEF<sub>25</sub> was not diminished (p = 0.09). A summary of airway function between SGA and AGA infants according to both birthweight and maternal smoking status is presented in Table 4.17 and Table 4.23.

---

1 Data shown as mean (SD) for continuous and n (%) for categorical variables.

† n = 104; ‡ n = 77

* p < 0.05
<table>
<thead>
<tr>
<th></th>
<th>SGA Smoking (n = 39)</th>
<th>SGA Non-smoking (n = 40)</th>
<th>95% CI of Difference: Smoking - Non-smoking</th>
<th>AGA Smoking (n = 39)</th>
<th>AGA Non-smoking (n = 65)</th>
<th>95% CI of Difference: Smoking - Non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>15 (38%)</td>
<td>28 (70%)</td>
<td>-52%, -11% ***</td>
<td>25 (64%)</td>
<td>30 (46%)</td>
<td>-1%, 37%</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>5.7 (2.3)</td>
<td>6.7 (2.6)</td>
<td>-2.1, 0.1</td>
<td>6.3 (1.8)</td>
<td>5.9 (2.3)</td>
<td>-0.5, 1.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.9 (0.8)</td>
<td>4.3 (0.8)</td>
<td>-0.8, -0.1 *</td>
<td>4.8 (0.7)</td>
<td>4.7 (0.8)</td>
<td>-0.2, 0.4</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>53.4 (3.2)</td>
<td>54.6 (2.4)</td>
<td>-2.51, 0.02</td>
<td>56.7 (2.5)</td>
<td>56.4 (2.9)</td>
<td>-0.9, 1.3</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>108 (32)</td>
<td>127 (28)</td>
<td>-32, -6 **</td>
<td>144 (32)</td>
<td>142 (35)</td>
<td>-12, 15</td>
</tr>
<tr>
<td>FEV_{0.4} (mL)</td>
<td>96 (29)</td>
<td>112 (21)^†</td>
<td>-27, -5 **</td>
<td>123 (26)</td>
<td>125 (28)</td>
<td>-12, 10</td>
</tr>
<tr>
<td>FEV_{0.5} (mL)</td>
<td>101 (31)</td>
<td>120 (22)^†</td>
<td>-31, -6 **</td>
<td>131 (28)</td>
<td>134 (30)^‡</td>
<td>-15, 9</td>
</tr>
<tr>
<td>MEF_{25} (mL.s⁻¹)</td>
<td>164 (69)</td>
<td>172 (59)</td>
<td>-37, 20</td>
<td>185 (65)</td>
<td>204 (72)</td>
<td>-46, 9</td>
</tr>
<tr>
<td>MEF_{15} (mL.s⁻¹)</td>
<td>100 (44)</td>
<td>103 (34)</td>
<td>-21, 14</td>
<td>111 (46)</td>
<td>125 (44)</td>
<td>-31, 4</td>
</tr>
<tr>
<td>FEV_{0.4}/FVC</td>
<td>0.89 (0.07)</td>
<td>0.87 (0.06)^†</td>
<td>-0.02, 0.04</td>
<td>0.86 (0.06)</td>
<td>0.88 (0.06)</td>
<td>-0.04, 0.01</td>
</tr>
<tr>
<td>V′_maxFRC (mL.s⁻¹)</td>
<td>124 (68)</td>
<td>134 (67)</td>
<td>-41, 20</td>
<td>120 (69)^†</td>
<td>148 (74)^‡</td>
<td>-58, 0.8</td>
</tr>
<tr>
<td>V′_maxFRC SD score</td>
<td>-0.2 (1.0)</td>
<td>0.1 (1.0)</td>
<td>-0.7, 0.2</td>
<td>-0.4 (1.1)</td>
<td>0.0 (1.1)</td>
<td>-0.79, 0.09</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>48 (10)</td>
<td>46 (9)</td>
<td>-2, 6</td>
<td>46 (10)</td>
<td>46 (10)</td>
<td>-4, 4</td>
</tr>
<tr>
<td>VT (mL)</td>
<td>33 (8)</td>
<td>38 (8)</td>
<td>-8, -1 **</td>
<td>42 (8)</td>
<td>41 (7)</td>
<td>-2, 4</td>
</tr>
<tr>
<td>VT/kg (mL)</td>
<td>8.5 (1.4)</td>
<td>8.8 (1.5)</td>
<td>-0.9, 0.4</td>
<td>8.7 (1.2)</td>
<td>8.8 (1.7)</td>
<td>-0.7, 0.5</td>
</tr>
<tr>
<td>t_{PTE}/t_{E}</td>
<td>0.34 (0.09)</td>
<td>0.35 (0.10)</td>
<td>-0.06, 0.03</td>
<td>0.32 (0.09)</td>
<td>0.36 (0.10)</td>
<td>-0.073, 0.003</td>
</tr>
</tbody>
</table>

* Data shown as mean (SD) for continuous and n (%) for categorical variables.
† n = 39; † n = 37; ‡ n = 60; § n = 64; † n = 38
When comparing the effect of smoking on airway function between SGA infants who were and were not exposed to maternal smoking (Table 4.17), FVC and FEV\(_{0.4}\) were significantly reduced among SGA infants whose mothers smoked. This reduction in central airway function may be due to differences in body size and age at time of test. Body size was similar between AGA infants who were and were not exposed to maternal smoking, and measures of central airway function (FVC and FEV\(_{0.4}\)) were virtually identical. However, among AGA infants exposed to maternal smoking, \(V'_{\text{maxFRC}}\) (120 vs. 148 mL.s\(^{-1}\)) and \(t_{\text{PTEF:I}}\) (0.32 vs. 0.36) were lower than among the infants who were not exposed, and this difference was of borderline significance ([95% CI]: [-58, 0.8], p = 0.05; [-0.073, 0.003], p = 0.06 respectively). These differences were not observed among the SGA infants, as sex distribution and body size in this group probably confounded the results and this may reflect a male excess among non-smokers.

In this study, smoking status was based on pregnancy smoking history as well as smoking habit at test. Thus, infants whose mother smoked beyond eight weeks gestation but who stopped smoking later in pregnancy and post delivery, would still have been classified as smokers. However the mothers of seven of these infants had salivary cotinine values < 2 ng.mL\(^{-1}\), well below levels reported for active smokers (McNeill et al. 1987). As inclusion of these light or infrequent smokers may lead to an underestimation of the influence of maternal smoking on infant airway function, analyses were repeated omitting these seven infants from the smoking group. When airway function from the remaining 71 infants whose mothers smoked was compared with 105 infants of non-smoking mothers, the mean reduction in FVC, FEV\(_{0.4}\) and \(V'_{\text{maxFRC}}\) was of a magnitude similar to that observed in the whole ‘smoking’ group (n = 78; Table 4.16). By contrast, in an the analysis omitting light or infrequent smokers, MEF\(_{25}\) was significantly lower (171 vs. 192 mL.s\(^{-1}\) [95% CI: 0.3, 41 mL.s\(^{-1}\)]; p = 0.05) in infants whose mothers smoked, suggesting that among heavier smokers, there is an association between maternal smoking and peripheral airway function.
4.6.3 Sex differences in airway function

Although boys were heavier and longer than girls at time of test (Table 4.13), in univariate analyses, values for FVC and FEV$_{0.4}$ were similar in boys and girls. However, peripheral airway function, as assessed by MEF$_{25}$ and $V'_{\text{maxFR}}$, was significantly lower in boys than girls (Table 4.18; Figure 4.9 and Figure 4.10).

Table 4.18 Respiratory function results according to infant sex$^1$

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 98)</th>
<th>Girls (n = 85)</th>
<th>95% CI of Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>43 (44%)</td>
<td>36 (42%)</td>
<td>-13%, 16%</td>
</tr>
<tr>
<td>Any maternal smoking after 8 w gestation</td>
<td>40 (41%)</td>
<td>38 (45%)</td>
<td>-18%, 10%</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>134 (36)</td>
<td>130 (34)</td>
<td>-6, 14</td>
</tr>
<tr>
<td>FEV$_{0.4}$ (mL)</td>
<td>115 (29)$^\dagger$</td>
<td>116 (29)</td>
<td>-8, 8</td>
</tr>
<tr>
<td>FEV$_{0.5}$ (mL)</td>
<td>123 (31)$^\ddagger$</td>
<td>124 (31)$^\ddagger$</td>
<td>-10, 9</td>
</tr>
<tr>
<td>MEF$_{25}$ (mL.s$^{-1}$)</td>
<td>174 (66)</td>
<td>197 (70)</td>
<td>-42, -3 $^*$</td>
</tr>
<tr>
<td>MEF$_{15}$ (mL.s$^{-1}$)</td>
<td>106 (41)</td>
<td>119 (46)</td>
<td>-26, -0.8 $^*$</td>
</tr>
<tr>
<td>FEV$_{0.4}$/FVC</td>
<td>0.86 (0.07)$^\dagger$</td>
<td>0.89 (0.06)</td>
<td>-0.05, -0.01 $^*$</td>
</tr>
<tr>
<td>$V'_{\text{maxFR}}$ (mL.s$^{-1}$)</td>
<td>115 (65)$^\dagger$</td>
<td>156 (71)$^*$</td>
<td>-61, -21 $^*$</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>47 (10)</td>
<td>47 (9)</td>
<td>-3, 3</td>
</tr>
<tr>
<td>VT (mL)</td>
<td>40 (10)</td>
<td>37 (7)</td>
<td>0.6, 5.4 $^*$</td>
</tr>
<tr>
<td>VT/kg (mL)</td>
<td>8.6 (1.4)</td>
<td>8.9 (1.6)</td>
<td>-0.7, 0.2</td>
</tr>
<tr>
<td>$t_{\text{PTFE}}: t_{E}$</td>
<td>0.329 (0.093)</td>
<td>0.358 (0.097)</td>
<td>-0.057, -0.001 $^*$</td>
</tr>
</tbody>
</table>

$^1$ Data shown as mean (SD) for continuous and n (%) for categorical variables.
$^{\dagger}$ n = 97; $^\ddagger$ n = 94; $^\ast$ n = 84
$^*$ p < 0.05; $^{**}$ p < 0.01; $^{***}$ p < 0.001

In univariate analyses, respiratory rate and tidal volume adjusted for body weight were similar in both boys and girls. However, the tidal expiratory flow ratio, $t_{\text{PTFE}}: t_{E}$, was significantly lower in boys than girls. These results will be discussed further in Section 4.6.7.
Figure 4.9 Maximal expiratory flow at 25% of FVC plotted against body length according to sex

Figure 4.10 Maximal expiratory flow at FRC plotted against body length according to sex
4.6.4 Factors influencing central airway function

In univariate analyses, FVC and FEV\textsubscript{0.4} were significantly diminished in SGA compared with AGA infants (Table 4.14). These parameters were also significantly associated with infant’s body length (Figure 4.11 and Figure 4.12), postnatal age, maternal smoking and social class (Table 4.19 and Table 4.20). In multivariate analyses, FVC remained significantly lower in SGA infants, being on average 9 mL (95% CI: 0.6, 17) lower in SGA than AGA infants, after adjusting for body length at test, postnatal age, maternal smoking and social class. Similarly, FEV\textsubscript{0.4} was on average 8 mL (0.6, 16) lower in SGA than AGA infants after adjusting for the same variables (Table 4.20).

**Figure 4.11** FVC plotted against body length according to birthweight status
Figure 4.12  \( \text{FEV}_{0.4} \) against body length according to birthweight status

[Graph showing \( \text{FEV}_{0.4} \) against body length with data points for SGA and AGA groups.]
Table 4.19  FVC: associations with birthweight status and other factors

<table>
<thead>
<tr>
<th></th>
<th>Difference in FVC (mL)</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-25</td>
<td>-35, -16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-26</td>
<td>-36, -16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length at test</td>
<td>8</td>
<td>7, 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per cm)</td>
<td>8</td>
<td>7, 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal age</td>
<td>9</td>
<td>7, 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per week)</td>
<td>9</td>
<td>7, 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-11</td>
<td>-21, -0.3</td>
<td>0.043</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-12</td>
<td>-23, -2</td>
<td>0.025</td>
</tr>
<tr>
<td>Maternal social class</td>
<td>-19</td>
<td>-31, -8</td>
<td>0.001</td>
</tr>
<tr>
<td>(baseline: non-manual occupation)</td>
<td>-20</td>
<td>-32, -8</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>4</td>
<td>-6, 14</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-7, 14</td>
<td>0.526</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>-4</td>
<td>-16, 7</td>
<td>0.452</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-4</td>
<td>-16, 7</td>
<td>0.455</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-6</td>
<td>-22, 9</td>
<td>0.429</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-6</td>
<td>-21, 10</td>
<td>0.464</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-9</td>
<td>-17, -0.6</td>
<td>0.036</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-10</td>
<td>-18, -1</td>
<td>0.028</td>
</tr>
<tr>
<td>Length at test</td>
<td>6</td>
<td>4, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per cm)</td>
<td>6</td>
<td>4, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal age</td>
<td>3</td>
<td>0.7, 5.3</td>
<td>0.012</td>
</tr>
<tr>
<td>(per week)</td>
<td>3</td>
<td>0.6, 5.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-5</td>
<td>-12, 3</td>
<td>0.230</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-4</td>
<td>-12, 4</td>
<td>0.299</td>
</tr>
<tr>
<td>Maternal social class</td>
<td>-3</td>
<td>-12, 6</td>
<td>0.485</td>
</tr>
<tr>
<td>(baseline: non-manual occupation)</td>
<td>-3</td>
<td>-13, 6</td>
<td>0.456</td>
</tr>
</tbody>
</table>

Data presented in BLACK relate to data from ALL infants while data presented in RED relate to analyses when infants of light or infrequent smokers were excluded.

* data adjusted for those variables found to be significant in univariate analyses, i.e. birthweight status, length at test, postnatal age, maternal smoking and maternal social class.

Constant (95% CI) for multivariate analysis: -209 mL (-304, -114); -204 (-302, -106).
Table 4.20 FEV\(_{0.4}\): associations with birthweight status and other factors

<table>
<thead>
<tr>
<th></th>
<th>Difference in FEV(_{0.4}) (mL)</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-20</td>
<td>-28, -12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-21</td>
<td>-29, -13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length at test</td>
<td>6</td>
<td>5, 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per cm)</td>
<td>6</td>
<td>5, 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal age</td>
<td>6</td>
<td>5, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per week)</td>
<td>6</td>
<td>5, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-10</td>
<td>-19, -2</td>
<td>0.016</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-12</td>
<td>-20, -3</td>
<td>0.007</td>
</tr>
<tr>
<td>Maternal social class</td>
<td>-14</td>
<td>-24, -5</td>
<td>0.004</td>
</tr>
<tr>
<td>(baseline: non-manual occupation)</td>
<td>-15</td>
<td>-25, -5</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>-0.03</td>
<td>-8, 8</td>
<td>0.994</td>
</tr>
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<td></td>
<td>-0.8</td>
<td>-9, 8</td>
<td>0.854</td>
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<tr>
<td>Family history of asthma</td>
<td>-8</td>
<td>-17, 2</td>
<td>0.107</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-8</td>
<td>-17, 2</td>
<td>0.103</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-10</td>
<td>-22, 2</td>
<td>0.110</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-10</td>
<td>-22, 3</td>
<td>0.126</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-8</td>
<td>-16, -0.6</td>
<td>0.034</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-9</td>
<td>-17, -1</td>
<td>0.023</td>
</tr>
<tr>
<td>Length at test</td>
<td>4</td>
<td>3, 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(per cm)</td>
<td>4</td>
<td>2, 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal age</td>
<td>2</td>
<td>-0.1, 4.1</td>
<td>0.064</td>
</tr>
<tr>
<td>(per week)</td>
<td>2</td>
<td>-0.1, 4</td>
<td>0.060</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-6</td>
<td>-13, 1</td>
<td>0.112</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-6</td>
<td>-13, 1</td>
<td>0.114</td>
</tr>
<tr>
<td>Maternal social class</td>
<td>-2</td>
<td>-10, 6</td>
<td>0.599</td>
</tr>
<tr>
<td>(baseline: non-manual occupation)</td>
<td>-2</td>
<td>-11, 6</td>
<td>0.606</td>
</tr>
</tbody>
</table>

Data presented in BLACK relate to data from ALL infants while data presented in RED relate to analyses when infants of light or infrequent smokers were excluded.

* data adjusted for those variables found to be significant in univariate analyses, i.e. birthweight status, length at test, postnatal age, maternal smoking and maternal social class.

Constant (95% CI) for multivariate analysis: -138 mL (-225, -51); -125 mL (-214, -35)
4.6.5 Factors influencing peripheral airway function

In univariate analyses, MEF$_{25}$, which is thought to reflect primarily peripheral airway function, was significantly lower in SGA than AGA infants (mean [95% CI of the difference], SGA:AGA: 168 vs. 197 mL.s$^{-1}$ [9, 49]; p = 0.004) (Table 4.14). In addition, MEF$_{25}$ was positively associated with body length and was significantly lower in boys but not in infants of mothers who smoked (Table 4.21). However, when infants of mothers who smoked infrequently and had salivary cotinine ≤ 2 ng.mL$^{-1}$ were excluded from analysis, MEF$_{25}$ among infants whose mother smoked was on average 21 mL.s$^{-1}$ [95% CI: 0.3, 41] lower than among infants whose mother did not smoke (p = 0.05) (Table 4.21). A maternal history of asthma was also significantly associated with MEF$_{25}$, being on average [95% CI]: 48 mL.s$^{-1}$ [19, 76] (p < 0.01) lower in infants whose mothers had a history of asthma. In multivariate analyses, birthweight status was of borderline significance (95%CI: -45, 1; p = 0.07) after allowing for length, sex, postnatal age and maternal history of asthma (Table 4.21). This association remained unchanged even when light or infrequent smokers were excluded from the analysis (95% CI: -39, 2; p = 0.08). Nevertheless, sex remained significant in this model, with MEF$_{25}$ being on average [95% CI] 29 mL.s$^{-1}$ [10, 48] lower in boys compared with girls after allowing for differences in body size (Table 4.21 and Figure 4.9).

In univariate analyses, $V'_{\text{maxFRC}}$ was significantly associated with infant sex, maternal smoking, maternal history of asthma but not birthweight status (Table 4.22 and Figure 4.10). These three factors remained significantly associated with $V'_{\text{maxFRC}}$ on multivariate analysis (Table 4.22).
Table 4.21  MEF$_{25}$: associations with birthweight status and other factors

<table>
<thead>
<tr>
<th></th>
<th>Difference in MEF$_{25}$ (mL·s$^{-1}$)</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-29</td>
<td>-49, -9</td>
<td>0.004</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-29</td>
<td>-49, -10</td>
<td>0.004</td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>-23</td>
<td>-42, -3</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>-27</td>
<td>-46, -7</td>
<td>0.009</td>
</tr>
<tr>
<td>Length at test (per cm)</td>
<td>5</td>
<td>2, 8</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1, 8</td>
<td>0.008</td>
</tr>
<tr>
<td>Postnatal age (per week)</td>
<td>5</td>
<td>0.2, 9</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-0.3, 9</td>
<td>0.066</td>
</tr>
<tr>
<td>Maternal smoking (baseline: no maternal smoking)</td>
<td>-17</td>
<td>-37, 3</td>
<td>0.091</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>-30</td>
<td>-52, -9</td>
<td>0.007</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-31</td>
<td>-53, -9</td>
<td>0.005</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-48</td>
<td>-76, -19</td>
<td>0.001</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-47</td>
<td>-75, -18</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-22</td>
<td>-45, 1</td>
<td>0.066</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-18</td>
<td>-39, 2</td>
<td>0.084</td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>-29</td>
<td>-48, -10</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>-33</td>
<td>-52, -13</td>
<td>0.001</td>
</tr>
<tr>
<td>Length at test (per cm)</td>
<td>3</td>
<td>-3, 8</td>
<td>0.320</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.3, 7</td>
<td>0.072</td>
</tr>
<tr>
<td>Postnatal age (per week)</td>
<td>3</td>
<td>-4, 9</td>
<td>0.426</td>
</tr>
<tr>
<td>Maternal smoking (baseline: no maternal smoking)</td>
<td>-15</td>
<td>-34, 4</td>
<td>0.121</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-45</td>
<td>-73, -17</td>
<td>0.002</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-45</td>
<td>-72, -17</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data presented in BLACK relate to data from ALL infants while data presented in RED relate to analyses when infants of light or infrequent smokers were excluded.

* data adjusted for those variables found to be significant in univariate analyses, i.e. birthweight status, infant sex, length at test, postnatal age and maternal history of asthma.

Note: As mothers with history of asthma were also included in infants with ‘family history of asthma’, only ‘maternal history of asthma’ variable was included in the multivariate analysis. Constant (95% CI) for multivariate analysis: 54 mL·s$^{-1}$ (-210, 319); 46 mL·s$^{-1}$ (-149, 240).
### Table 4.22  $V'_{\text{maxFRC}}$: associations with birthweight status and other factors

<table>
<thead>
<tr>
<th></th>
<th>Difference in $V'_{\text{maxFRC}}$ (mL/s')</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-8</td>
<td>-29, 13</td>
<td>0.437</td>
</tr>
<tr>
<td>(baseline: AGA infant)</td>
<td>-9</td>
<td>-30, 13</td>
<td>0.430</td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>-41</td>
<td>-61, -21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-42</td>
<td>-63, -22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-21</td>
<td>-42, -0.3</td>
<td>0.047</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-22</td>
<td>-44, -1</td>
<td>0.041</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>-21</td>
<td>-44, 2</td>
<td>0.070</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-23</td>
<td>-46, 0.2</td>
<td>0.052</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-33</td>
<td>-63, -2</td>
<td>0.034</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-33</td>
<td>-63, -2</td>
<td>0.034</td>
</tr>
<tr>
<td>*<em>Multivariate analysis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (baseline: female)</td>
<td>-43</td>
<td>-62, -23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-45</td>
<td>-64, -25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-22</td>
<td>-41, -2</td>
<td>0.031</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
<td>-23</td>
<td>-43, -3</td>
<td>0.027</td>
</tr>
<tr>
<td>Maternal history of asthma</td>
<td>-36</td>
<td>-64, -7</td>
<td>0.015</td>
</tr>
<tr>
<td>(baseline: no history of asthma)</td>
<td>-35</td>
<td>-64, -7</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Data presented in BLACK relate to data from ALL infants while data presented in RED relate to analyses when infants of light or infrequent smokers were excluded.

* data adjusted for those variables found to be significant in univariate analyses, i.e. infant sex, maternal smoking and maternal history of asthma.

Constant (95% CI) for multivariate analysis: 153 mL.s$^{-1}$ (137, 168); 172 mL.s$^{-1}$ (155, 189)
<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th>Smoking</th>
<th>95% CI of Difference: SGA-AGA</th>
<th>95% CI of Difference: (Smoking) SGA-AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA (n = 40) AGA (n = 65)</td>
<td>SGA (n = 39) AGA (n = 39)</td>
<td>95% CI of Difference: SGA-AGA</td>
<td>95% CI of Difference: (Smoking) SGA-AGA</td>
</tr>
<tr>
<td>Boys</td>
<td>28 (70%)</td>
<td>30 (46%)</td>
<td>5%, 43% *</td>
<td>15 (38%)</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>6.7 (2.6)</td>
<td>5.9 (2.3)</td>
<td>-0.2, 1.7</td>
<td>5.7 (2.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>4.3 (0.8)</td>
<td>4.7 (0.8)</td>
<td>-0.7, -0.1 *</td>
<td>3.9 (0.8)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>54.6 (2.4)</td>
<td>56.4 (2.9)</td>
<td>-2.9, -0.7 **</td>
<td>53.4 (3.2)</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>127 (28)</td>
<td>142 (35)</td>
<td>-28, -3 **</td>
<td>108 (32)</td>
</tr>
<tr>
<td>FEV_{0.5} (mL)</td>
<td>108 (20)^†</td>
<td>121 (28)</td>
<td>-23, -3 *</td>
<td>96 (29)</td>
</tr>
<tr>
<td>FEV_{0.3} (mL)</td>
<td>116 (21)^‡</td>
<td>131 (30)^‡</td>
<td>-25, -4 **</td>
<td>101 (31)</td>
</tr>
<tr>
<td>MEF_{25} (mL.s^{-1})</td>
<td>172 (59)</td>
<td>204 (72)</td>
<td>-58, -5 *</td>
<td>164 (69)</td>
</tr>
<tr>
<td>MEF_{50} (mL.s^{-1})</td>
<td>103 (34)</td>
<td>125 (44)</td>
<td>-37, -6 *</td>
<td>100 (44)</td>
</tr>
<tr>
<td>FEV_{0.5}/FVC</td>
<td>0.87 (0.06)^†</td>
<td>0.88 (0.06)</td>
<td>-0.03, 0.02</td>
<td>0.89 (0.07)</td>
</tr>
<tr>
<td>V_{maxFRC} (mL.s^{-1})</td>
<td>134 (67)</td>
<td>148 (74)^‡</td>
<td>-42, 15</td>
<td>124 (68)</td>
</tr>
<tr>
<td>V_{maxFRC} SD score</td>
<td>0.1 (1.0)</td>
<td>0.0 (1.1)</td>
<td>-0.3, 0.5</td>
<td>-0.2 (1.0)</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>46 (9)</td>
<td>46 (10)</td>
<td>-4, 4</td>
<td>48 (10)</td>
</tr>
<tr>
<td>VT (mL)</td>
<td>38 (8)</td>
<td>41 (7.)</td>
<td>-6, -0.5</td>
<td>33 (8)</td>
</tr>
<tr>
<td>VT/kg (mL)</td>
<td>8.8 (1.5)</td>
<td>8.8 (1.7)</td>
<td>-0.7, 0.6</td>
<td>8.5 (1.4)</td>
</tr>
<tr>
<td>t PREF: E&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0.348 (0.099)</td>
<td>0.357 (0.097)</td>
<td>-0.048, 0.030</td>
<td>0.335 (0.094)</td>
</tr>
</tbody>
</table>

¹ Data shown as mean (SD) for continuous and n (%) for categorical variables.
* P < 0.05;  ** P < 0.01;  *** P < 0.001
† n = 39;  ‡ n = 37;  § n = 60;  ‡‡ n = 64;  ‡‡‡ n = 38

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4.6.6 Association of birthweight with airway function

In order to assess the association of birthweight and airway function in infants not exposed to maternal smoking, data from such infants (40 SGA and 65 AGA) were analysed separately (Table 4.24) (Lum et al. 2001).

Table 4.24 Influence of birthweight status on airway function parameters

<table>
<thead>
<tr>
<th></th>
<th>SGA (n = 40)</th>
<th>AGA (n = 65)</th>
<th>95% CI SGA – AGA (unadjusted)</th>
<th>95% CI SGA – AGA (adjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures of central airway function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>127 (28)</td>
<td>142 (35)</td>
<td>-28, -3</td>
<td>-11, 9†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.019</td>
<td>p = 0.873</td>
</tr>
<tr>
<td>FEV_{0.4} (mL)</td>
<td>112 (21)</td>
<td>125 (28)</td>
<td>-23, -3</td>
<td>-12, 5†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.015</td>
<td>p = 0.429</td>
</tr>
<tr>
<td>Measures of peripheral airway function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEF_{25} (mL.s^{-1})</td>
<td>172 (59)</td>
<td>204 (72)</td>
<td>-58, -5</td>
<td>-53, 1‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.021</td>
<td>p = 0.063</td>
</tr>
<tr>
<td>\nu_{maxFRC} (mL.s^{-1})</td>
<td>134 (67)</td>
<td>148 (74)</td>
<td>-42, 15</td>
<td>-34, 24‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.339</td>
<td>p = 0.731</td>
</tr>
</tbody>
</table>

† Data shown as mean (SD) and 95% Confidence Interval of the difference.
‡ adjusted for length
§ adjusted for sex

FVC and FEV_{0.4} were significantly lower in SGA compared to AGA infants not exposed to maternal smoking (Table 4.23 and Table 4.24) and were also significantly associated with body length at test but not sex. However, after allowing for body length, birthweight status was no longer significantly associated with FVC and FEV_{0.4} (Table 4.24). Thus, among infants not exposed to maternal smoking, the apparent association with birthweight status appears to be mediated primarily through the reduction in body size rather than a specific effect on lung and airway size.

MEF_{25} was also significantly lower in SGA than AGA infants not exposed to maternal smoking. However, after allowing for sex (Table 4.24) birthweight status
was of borderline significance, reflecting in part the relative excess of boys in the SGA group. In univariate analyses, $V_{\text{maxFRC}}$ was significantly associated with sex but not birthweight status.

### 4.6.7 Tidal breathing parameters

At approximately six weeks of age, respiratory rate was similar between SGA and AGA infants (Table 4.14), infants who were and were not exposed to maternal smoking (Table 4.16) and also between boys and girls (Table 4.18). Tidal volume ($V_T$) was significantly lower in SGA infants, in those infants whose mothers smoked and in girls when compared to their appropriate counterparts. However, when $V_T$ was adjusted for body weight, there was no longer any significant difference between the groups.

$t_{\text{PTEF}}:t_E$ was similar in SGA and AGA infants (Table 4.14) and in infants whose mothers did and did not smoke (Table 4.16) but was significantly diminished in boys compared to girls (Table 4.18). These associations with birthweight status were similar when infants were grouped by maternal smoking status (Table 4.23). Inspiratory ($t_i$) and expiratory time ($t_E$) were also similar between SGA and AGA infants (mean [95% CI of the difference]: $t_i$, 0.57 vs. 0.59 [-0.05, 0.006]; $t_E$, 0.76 vs. 0.78 [-0.07, 0.04]). Similarly, no differences were observed in $t_i$ and $t_E$ between infants who were and were not exposed to maternal smoking, nor between boys and girls.

### 4.6.8 Association of obstetric complications with airway function

Although not a hypothesis of this study, previous publications have suggested that obstetric complications and interventions such as amniocentesis (Working Party on Amniocentesis, 1978; Vyas et al. 1982), chorionic villus sampling (Yüksel et al. 1997) or administration of antenatal glucocorticoids (Wong et al. 1982; Vyas and Kotecha, 1997) may influence growth and development of the fetal lung. Thus this section will explore the potential associations of antenatal interventions on subsequent airway function in this dataset. The following scatter graphs of FVC,
FEV$_{0.4}$ and MEF$_{25}$, were plotted against length according to the antenatal procedures undertaken.

**Figure 4.13  Association between antenatal procedures and FVC**

Definition of abbreviation and number of cases (n):
CVS: Chorionic villus sampling (n = 5)
Amniocentesis (n = 6)
Antenatal steroid (n = 5)
There was no apparent diminution of lung volume (FVC; Figure 4.13) or airway function (FEV\textsubscript{0.4}, Figure 4.14 and MEF\textsubscript{25}, Figure 4.15), but numbers are too small to reach meaningful conclusions about the association of antenatal procedures or antenatal steroids with subsequent infant lung function.

**Figure 4.14** Association between antenatal procedures and FEV\textsubscript{0.4}

Definition of abbreviation and (n) as for Figure 4.13.
Figure 4.15  Association between antenatal procedures and MEF$_{25}$

Definition of abbreviation and (n) as for Figure 4.13.
Figure 4.16 Association between obstetric complications and FVC

Definition of abbreviations and number of cases (n):
PIH: pregnancy induced hypertension (n = 6)
APH: antepartum haemorrhage (n = 8)
PROM: prolonged rupture of membranes (n = 11)

No associations were observed between lung volume (FVC) and various obstetric complications (Figure 4.16). Similarly, no associations were observed between airway function (FEV$_{0.4}$ and MEF$_{25}$) and the various obstetric complications (data not shown).
4.7 Influence of methodological issues on lung function parameters

Methodological studies were conducted to assess potential factors which could influence results from the RVRTC technique, such as any variations in inflation pressures within and between infants, the tightness of jacket fit and the efficiency with which pressure is transmitted from the jacket to the intra-thoracic airways, and the method of assessing flow limitation in infants (Section 3.10). As there is currently no consensus or standardised approach to this technique between centres, the potential influence of these methodological issues on lung function parameters were examined and findings are presented below.

4.7.1 Influence of inflation pressure on RVRTC parameters

Paired measurements of airway function using inflation pressures of 2.7 kPa and 3.0 kPa were obtained in 32 infants, details of whom are summarised in Table 4.25. Measurements from 11 infants were obtained using an initial inflation pressure \( P_{inf} \) of 2.7 kPa while measurements from the remaining 21 infants were obtained using an initial \( P_{inf} \) of 3.0 kPa. There were no significant differences in any of the background characteristics in those in whom initial measurements were made at 2.7 or 3.0 kPa.

<table>
<thead>
<tr>
<th>N</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) boys</td>
<td>11  (34%)</td>
</tr>
<tr>
<td>Age at test (wk)</td>
<td>8.3 (3.9 – 39.3)</td>
</tr>
<tr>
<td>Weight at test (kg)</td>
<td>5.2 (3.8 - 9.9)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
<td>57.5 (51.4 – 74.8)</td>
</tr>
</tbody>
</table>

\(^1\) Data shown as median (range) for continuous variables and n (%) for categorical variables.

Respiratory function results are summarised in Table 4.26. Although the pressure relief valve on the Neopuff Infant Resuscitair was set to deliver a \( P_{inf} \) of 3 kPa, due to the slight flow dependence of the Neopuff system, the average \( P_{inf} \) of the breath immediately preceding forced expiration was in fact 2.9 kPa. Thus there was on average an 8% increase in \( P_{inf} \) between the two sets of measurements.
Table 4.26  Comparison of different inflation pressure on respiratory function results¹

<table>
<thead>
<tr>
<th>$P_{\text{inf}}$ (kPa)</th>
<th>2.9 (0.1)</th>
<th>2.7 (0.1)</th>
<th>Mean difference (2.9 - 2.7 kPa)</th>
<th>95% CI of difference: (2.9 - 2.7 kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacket pressure (kPa)</td>
<td>5.2 (1.4)</td>
<td>5.3 (1.4)</td>
<td>-0.03 (0.2)</td>
<td>-0.1, 0.03</td>
</tr>
<tr>
<td>$t_{\text{FE}}$ (s)</td>
<td>1.2 (0.4)</td>
<td>1.1 (0.4)</td>
<td>0.03 (0.2)</td>
<td>-0.05, 0.1</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>208 (96)</td>
<td>195 (89)</td>
<td>13 (14)</td>
<td>8, 18 **</td>
</tr>
<tr>
<td>FEV$_{0.4}$ (mL)</td>
<td>180 (73)</td>
<td>169 (71)</td>
<td>11 (12)</td>
<td>6, 15 ***</td>
</tr>
<tr>
<td>MEF$_{25}$ (mL.s$^{-1}$)</td>
<td>238 (110)</td>
<td>221 (116)</td>
<td>17 (32)</td>
<td>5, 28 **</td>
</tr>
</tbody>
</table>

¹ Data shown as group mean (SD) and 95% confidence interval (CI) of the difference using paired-sample t-test;  ** $p < 0.01$;  *** $p < 0.001$

The effect of a small change in $P_{\text{inf}}$ on forced expiratory manoeuvres is illustrated in Figure 4.17 which shows an overlay of the best forced expiratory flow-volume curves generated from raised lung volume using $P_{\text{inf}}$ 2.7 and 3.0 kPa in the same infant. By overlaying the curves along the final descending portion of the expiratory loops, it can be seen that the infant appears to breathe out to the same end expiratory level, but that a larger inspiratory volume and hence a bigger FVC was observed at the higher $P_{\text{inf}}$ (Figure 4.17).

The rise in $P_{\text{inf}}$ was accompanied by a small but significant group mean increase in FVC of 13 mL, equivalent to an increase of 6 % in volume. A similar increase was observed in FEV$_{0.4}$ and hence there was no change in FEV$_{0.4}$/FVC. MEF$_{25}$ also increased by an average of 8%, which was also significant ($p < 0.01$; Table 4.26). The duration of forced expiration was almost identical (95% CI of the difference - 0.05, 0.11 s), as was the jacket pressure applied (95% CI, -0.1, 0.03 kPa), under both measurement conditions.
Figure 4.17 Comparison of RVRTC curves using different inflation pressures

![Diagram showing RVRTC curves with different inflation pressures](image)

- $P_{inf}$ 3.0 kPa
- $P_{inf}$ 2.7 kPa

a: inspiratory volume; b: forced vital capacity (FVC) using $P_{inf}$ 2.7 kPa
Start of inspiration/augmented breath is defined as zero on the volume axis. Overlay of the loops generally occurred along the final 40-50% of the expired volume.

4.7.1.1 Influence of variation on inflation pressure on airway function in infants

At commencement of this study, the inflation pressure used to raise lung volume for forced expiratory manoeuvres was pre-set to 2.7 kPa. Due to the flow dependence of the neopuff system and the relative inexperience of the team in using this new technique, the actual inflation pressure delivered to the breath immediately prior to forced expiration was in fact often less than the pressure set. Later, as collaboration between centres was established to build a reference dataset of raised volume parameters, the decision was made to standardise the pre-set inflation pressure to 3 kPa. In order to assess the extent to which variations in inflation pressures could influence any conclusions regarding the association of birthweight on airway function in infants, all earlier work where inflation pressure delivered was $< 2.7$ kPa was excluded and the remaining dataset was re-examined.
Raised volume RTC parameters from 40 SGA and 61 AGA infants whose lung function data were collected with an inflation pressure of ≥ 2.7 kPa on the breath immediately prior to forced expiration were examined and results are presented in Table 4.27.

As expected, there was a systematic bias towards obtaining higher absolute values of FVC, FEV₀.₄ and MEF₂₅ in infants whose lung volume was raised to a mean inflation pressure of 2.9 kPa in the breath immediately prior to the forced expiration manoeuvre compared to the whole study population where mean inflation pressure of that breath was only 2.7 kPa (Table 4.27). However, despite the smaller number of observations remaining (40 SGA and 61 AGA), after exclusion of those with lower inflation pressure (39 SGA and 43 AGA), FVC, FEV₀.₄ and MEF₂₅ remained significantly lower among SGA than AGA infants. Hence, the variation in inflation pressure used in this study did not compromise the significance of the findings in this thesis. This is mainly because data from both SGA and AGA infants were collected concurrently and any variations in methodology would have occurred in similar proportion in each group.
Table 4.27  Comparison of RVRTC parameters according to mean inflation pressures delivered and the association with birthweight status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (SD) $P_{in}$: 2.7 (0.3) kPa</th>
<th>Mean (SD) $P_{in}$: 2.9 (0.1) kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA (n = 79)</td>
<td>AGA (n = 104)</td>
</tr>
<tr>
<td>$P_{in}$ (kPa)</td>
<td>2.65 (0.27)</td>
<td>2.72 (0.27)</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>118 (31)</td>
<td>143 (34)</td>
</tr>
<tr>
<td>FEV₀.₄ (mL)</td>
<td>104 (26)</td>
<td>124 (28)</td>
</tr>
<tr>
<td>FEV₀.₅ (mL)</td>
<td>110 (28)</td>
<td>133 (30)</td>
</tr>
<tr>
<td>MEF₂₅ (mL.s⁻¹)</td>
<td>168 (64)</td>
<td>197 (69)</td>
</tr>
<tr>
<td>MEF₁₅ (mL.s⁻¹)</td>
<td>102 (39)</td>
<td>119 (45)</td>
</tr>
<tr>
<td>FEV₀.₄/FVC</td>
<td>0.88 (0.06)</td>
<td>0.87 (0.06)</td>
</tr>
</tbody>
</table>

95% CI of Difference: SGA - AGA

1 Data shown as mean (SD)
Definition of abbreviation: $P_{in}$ - Inflation pressure

**p < 0.05
***p < 0.01
****p < 0.001
4.7.2 Influence of jacket placement on airway function results

During augmented breathing it has been shown that inflation volume and respiratory system compliance are significantly lower when measurements are obtained with a fastened but uninflated jacket in situ compared to those obtained without (Hoo et al. 2001). Thus, another potential factor that could influence results from the RVRTC technique is the tightness of jacket fit and the efficiency with which pressure is transmitted from the jacket to the intra-thoracic airways.

Paired measurements of airway function using standard jacket placement (snug fit, allowing space for two adult fingers width between jacket and thorax, according to recent recommendations (Sly et al. 2000)) and loosened jacket placement (loose fit, allowing 4 adult fingers width) (Section 3.10.2) were obtained in 20 infants, details of whom are summarised in Table 4.28. All measurements were obtained using the standard jacket placement initially. Respiratory function results are summarised in Table 4.29.

Table 4.28 Infant characteristics from jacket placement comparison¹

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Age at test (wk)</td>
<td>8.4 (4.7 – 44.7)</td>
</tr>
<tr>
<td>Weight at test (kg)</td>
<td>5.4 (4.1 – 8.5)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
<td>56.8 (51.9 – 71.4)</td>
</tr>
</tbody>
</table>

¹ Data shown as median (range).
Table 4.29 Comparison of jacket placement on respiratory function results

<table>
<thead>
<tr>
<th>Jacket placement</th>
<th>Loosened (SD)</th>
<th>Standard (SD)</th>
<th>Mean difference (Loosened-Standard)</th>
<th>95% CI of difference: (Loosened-Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflation pressure (kPa)</td>
<td>3.0 (0.1)</td>
<td>3.0 (0.1)</td>
<td>0.01 (0.06)</td>
<td>-0.03, 0.02</td>
</tr>
<tr>
<td>Jacket pressure [Pj] (kPa)</td>
<td>5.1 (1.2)</td>
<td>5.1 (1.3)</td>
<td>0.01 (0.2)</td>
<td>-0.1, 0.1</td>
</tr>
<tr>
<td>Pj transmission (kPa)</td>
<td>2.8 (0.6)</td>
<td>2.5 (0.6)</td>
<td>0.4 (0.4)</td>
<td>0.2, 0.6 **</td>
</tr>
<tr>
<td>Pj efficiency (%)</td>
<td>58 (12)</td>
<td>54 (13)</td>
<td>4 (8)</td>
<td>-0.02, 7.8</td>
</tr>
<tr>
<td>tFE (s)</td>
<td>1.15 (0.34)</td>
<td>1.16 (0.45)</td>
<td>-0.01 (0.25)</td>
<td>-0.12, 0.11</td>
</tr>
<tr>
<td>PEF (mL.s⁻¹)</td>
<td>961 (231)</td>
<td>847 (142)</td>
<td>114 (178)</td>
<td>23, 206 *</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>208 (74)</td>
<td>203 (71)</td>
<td>5 (15)</td>
<td>-1, 12</td>
</tr>
<tr>
<td>FEV₀.₄ (mL)</td>
<td>165 (52)</td>
<td>163 (51)</td>
<td>1 (8)</td>
<td>-3, 5</td>
</tr>
<tr>
<td>MEF₂₅ (mL.s⁻¹)</td>
<td>232 (85)</td>
<td>253 (107)</td>
<td>-21 (38)</td>
<td>-38, -3 *</td>
</tr>
<tr>
<td>Cᵣ (mL.kPa⁻¹)</td>
<td>65.6 (26.8)</td>
<td>62.9 (24.9)</td>
<td>2.7 (4.7)</td>
<td>0.5, 4.9 *</td>
</tr>
</tbody>
</table>

¹ Data shown as group mean (SD) and 95% CI of the difference using paired-samples t-tests: * p < 0.05; ** p < 0.01. Abbreviations: tFE = duration of forced expiration; PEF = peak expiratory flow; Cᵣ = respiratory system compliance.

Jacket pressure and inflation pressure applied during both measurement conditions were almost identical (Table 4.29). The effect of a loosened jacket placement on forced expiratory manoeuvres is illustrated in Figure 4.18, which shows an overlay of the best forced expiratory flow-volume curves generated from raised lung volume using both the standard and the loosened jacket placement in the same infant.

There was a group mean increase by 0.4 kPa (18%) in jacket pressure transmission during RVRTC manoeuvres using loosened jacket placement. This increase in efficiency of the jacket to transmit pressure to the intra-thoracic airways was accompanied by a significant increase in peak expiratory flow (PEF). However, there were only minimal changes in FVC and FEV₀.₄ which were not significant, while group mean values for MEF₂₅ showed a decrement of 21 mL.s⁻¹, equivalent to a reduction of 6% in flow when a loosened jacket placement was applied (Table 4.29). In addition, a significant reduction in Cᵣ by an average of 4% was noted when the standard jacket placement was applied, suggesting that the standard placement of
the jacket may be associated with a more marked effect of splinting of the chest wall during raised volume manoeuvres. Nevertheless, loosened jacket placement had little effect on lung function parameters.

**Figure 4.18 Comparison of RVRTC curves using different jacket placements**

Note: Start of inspiration/augmented breath is defined as zero on the volume axis. Forced expiratory flow volume curves are overlaid at end inspiration/inflation.
4.7.3 Influence of jacket pressure on lung function parameters

In this study, the optimal jacket pressure for raised volume manoeuvres was determined as the jacket pressure applied above which no further increase in $V'_{\text{maxFRC}}$ was achieved. However, there is some debate as to whether this 'optimal' jacket pressure is sufficient to achieve flow limitation at raised lung volume.

Thus, paired measurements of airway function at raised lung volume, using 'optimal' and higher jacket pressure (1-2 kPa above 'optimal') were obtained in 14 infants, details of whom are summarised in Table 4.30. All measurements were obtained using standard jacket placement and 'optimal' jacket pressure initially.

Table 4.30 Infant characteristics from jacket pressure comparison

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
</tr>
<tr>
<td>Age at test (wk)</td>
<td>7.4 (4.4, 43.0)</td>
</tr>
<tr>
<td>Weight at test (kg)</td>
<td>4.8 (3.0 – 8.5)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
<td>57.3 (50.5 – 71.4)</td>
</tr>
</tbody>
</table>

1 Data shown as median (range)

The effect of 1-2 kPa change in jacket pressure on raised volume forced expiratory manoeuvres is illustrated in Figure 4.19, which shows an overlay of the best forced expiratory flow-volume curves generated using 'optimal' and higher jacket pressures in the same infant. By overlaying the curves at end inspiration (inflation), it can be seen that the infant appeared to breathe out to the same expiratory level, and the higher jacket pressure did not appear to influence the volume parameters (FVC and FEV$_{0.4}$).
Figure 4.19  Comparison of RVRTC curves using different jacket pressures

Note: Start of inspiration is defined as zero on the volume axis. Loops are overlaid at end inflation.

Table 4.31 Comparison of jacket pressure on respiratory function results¹

<table>
<thead>
<tr>
<th>Jacket pressure (kPa)</th>
<th>‘Optimal’ 5.2 (1.1)</th>
<th>Higher 6.4 (1.1)</th>
<th>Mean difference (Optimal – Higher)</th>
<th>95% CI of difference: (Optimal – Higher)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{inf}}$ (kPa)</td>
<td>3.0 (0.04)</td>
<td>3.0 (0.04)</td>
<td>0.01 (0.03)</td>
<td>-0.01, 0.03</td>
</tr>
<tr>
<td>PEF (mLs$^{-1}$)</td>
<td>897 (230)</td>
<td>920 (243)</td>
<td>-23 (199)</td>
<td>-143, 97</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>177 (59)</td>
<td>177 (61)</td>
<td>-1 (6)</td>
<td>-4, 3</td>
</tr>
<tr>
<td>$FEV_{0.4}$ (mL)</td>
<td>145 (44)</td>
<td>146 (46)</td>
<td>-1 (6)</td>
<td>-4, 3</td>
</tr>
<tr>
<td>MEF$_{25}$ (mLs$^{-1}$)</td>
<td>208 (88)</td>
<td>204 (88)</td>
<td>5 (20)</td>
<td>-7, 16</td>
</tr>
<tr>
<td>MEF$_{50}$ (mLs$^{-1}$)</td>
<td>458 (141)</td>
<td>457 (137)</td>
<td>1 (56)</td>
<td>-30, 33</td>
</tr>
<tr>
<td>MEF$_{75-25}$ (mLs$^{-1}$)</td>
<td>403 (123)</td>
<td>408 (127)</td>
<td>-5 (39)</td>
<td>-27, 17</td>
</tr>
<tr>
<td>MEF$_{50-25}$ (mLs$^{-1}$)</td>
<td>458 (141)</td>
<td>457 (137)</td>
<td>1 (56)</td>
<td>-31, 33</td>
</tr>
</tbody>
</table>

¹ Data shown as group mean (SD) and 95% CI of the difference using paired-samples t-tests:
Definition of abbreviations: $P_{\text{inf}}$ = Inflation pressure; PEF = Peak expiratory flow; MEF$_{75-25}$ = Mean expiratory flow from 75-25% of FVC; MEF$_{50-25}$ = Mean expiratory flow from 50-25% of FVC.
Respiratory function results are summarised in the Table 4.31. While peak expiratory flow (PEF) was on average 23 mL.s\(^{-1}\) higher when using the higher jacket pressure, this difference was not significant (95% CI: -143, 97). All other parameters including mean expiratory flows (from 75-25% FVC and 50-25% FVC) were virtually identical (Table 4.31 and Figure 4.19). Hence, the ‘optimal’ jacket pressure that was ascertained during tidal RTC manoeuvres as the jacket pressure above which no further increase in \(V'_{\text{maxFRC}}\) was achieved, was adequate for RVRTC manoeuvres. Thus, any further increase in jacket pressure would not elicit higher flows during RVRTC manoeuvres.

4.7.4 Which parameters?

While there has been increasing interest in and use of the RVRTC technique in infants, there is as yet no consensus regarding equipment, methodology or analysis when performing this technique. In addition, it is unclear as to what the parameters measured by the RVRTC technique actually represent in infancy, or which are the most appropriate parameters to report for specific research or clinical applications.

4.7.4.1 Timed volume parameters (FEV\(_1\))

In infants, due to their rapid respiratory rate and short expiratory time, some of the values conventionally reported in older children or adults such as FEV\(_1\) cannot be calculated. Within this population (n = 183), duration of forced expiration (\(t_F\)) ranged from 0.36 – 1.77s (mean [SD]: 0.83s [0.25]) and 13 infants had a \(t_F\) shorter than 0.5s. Thus, FEV\(_{0.3}\) could be calculated in all infants while FEV\(_{0.4}\) and FEV\(_{0.5}\) could be calculated in 181 (99%) and 170 (93%) respectively. The relationship of FEV\(_{0.4}\) to FEV\(_{0.5}\) is shown in Figure 4.20.
Figure 4.20  The relationship between FEV$_{0.4}$ and FEV$_{0.5}$

$r^2 = 0.995$
Typical flow-volume curves obtained using the RVRTC technique in a younger and older infant with the position of relevant timed intervals for FEVi are shown in Figure 4.21.

**Figure 4.21  RVRTC flow-volume curves and FEVi**

a) Test age: 6 weeks

b) Test age: 61 weeks
4.7.4.2 \textit{MEF}_ \% parameters

The relationship of MEF$_{25}$ to MEF$_{10}$ and MEF$_{15}$ is shown in the figures below.

Figure 4.22  Relationship between MEF$_{25}$ and MEF$_{10}$

![Graph showing the relationship between MEF$_{25}$ and MEF$_{10}$.](image)

$r^2 = 0.7$

Figure 4.23  Relationship between MEF$_{25}$ and MEF$_{15}$

![Graph showing the relationship between MEF$_{25}$ and MEF$_{15}$.](image)

$r^2 = 0.82$
The relationship between MEF$_{25}$, MEF$_{15}$ and MEF$_{10}$ can be represented by the regression equations:

$$\text{MEF}_{25} = 1.8 \times \text{MEF}_{10} + 39.9 \ (r^2 = 0.70)$$

$$\text{MEF}_{25} = 1.4 \times \text{MEF}_{15} + 25.6 \ (r^2 = 0.82).$$

Within the population studied, MEF$_{15}$ was closest in magnitude to $V'_{\text{maxFRC}}$. However, there was considerable inter-subject variability in this relationship, with wide limits of agreement (Figure 4.24). $V'_{\text{maxFRC}}$ was greater than MEF$_{15}$ in 114/181 (63%) infants while MEF$_{25}$ was greater than $V'_{\text{maxFRC}}$ in 156/181 (86%).

**Figure 4.24** Measuring agreement between MEF$_{15}$ and $V'_{\text{maxFRC}}$
4.7.4.3 *Within-subject variability*

For all the respiratory function parameters, the within-subject SD increased proportionately to the mean, thereby justifying expression of variability as the coefficient of variability (CV) (Hutchison et al. 1981). Table 4.32 shows the CV for selected parameters for the 167 infants for whom results for all parameters were available.

As expected, measures of MEF were more variable than the volume parameters. However, $V'_{\text{max FRC}}$ measured by the RTC was less variable than any of the MEF% parameters measured using the RVRTC.

**Table 4.32** Within-subject coefficient of variation for forced expired volume and flow parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Coefficient of variation Mean (SD)</th>
<th>Coefficient of variation 95% CI of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (mL)</td>
<td>3.1 (2.1)</td>
<td>2.8, 3.4</td>
</tr>
<tr>
<td>FEV$_{0.3}$ (mL)</td>
<td>4.5 (3.0)</td>
<td>4.1, 5.0</td>
</tr>
<tr>
<td>FEV$_{0.4}$ (mL)</td>
<td>3.7 (2.3)</td>
<td>3.4, 4.1</td>
</tr>
<tr>
<td>FEV$_{0.5}$ (mL)</td>
<td>3.3 (2.1)</td>
<td>3.0, 3.6</td>
</tr>
<tr>
<td>MEF$_{10}$ (mL.s$^{-1}$)</td>
<td>10.9 (7.3)</td>
<td>9.8, 12.0</td>
</tr>
<tr>
<td>MEF$_{15}$ (mL.s$^{-1}$)</td>
<td>10.6 (7.3)</td>
<td>9.5, 11.7</td>
</tr>
<tr>
<td>MEF$_{25}$ (mL.s$^{-1}$)</td>
<td>8.8 (5.6)</td>
<td>7.9, 9.6</td>
</tr>
<tr>
<td>$V'_{\text{max FRC}}$ (mL.s$^{-1}$)</td>
<td>6.0 (4.8)</td>
<td>5.2, 6.7</td>
</tr>
</tbody>
</table>

$^{1}n = 167$
4.8 Summary of results

In summary, the main findings of this study are:

- At six weeks of age, SGA infants remained significantly shorter and lighter than AGA infants.

- In univariate analyses, FVC, FEV$_{0.4}$, MEF$_{25}$ were significantly reduced in SGA infants when compared with AGA infants. After adjustment for body size, maternal smoking and social class, FVC and FEV$_{0.4}$ remained significantly lower in SGA infants.

- In infants not exposed to maternal smoking after eight weeks gestation, the reduction in FVC and FEV$_{0.4}$ appears to be mediated primarily by body size of SGA infants in the first six weeks of life.

- In univariate analyses, peripheral airway function, as reflected by MEF$_{25}$ and $V'_{\text{maxFRC}}$ was significantly reduced in infants whose mothers smoked and in boys compared to girls. In multivariate analysis, MEF$_{25}$ and $V'_{\text{maxFRC}}$ remained significantly reduced in boys compared to girls. However, $V'_{\text{maxFRC}}$, but not MEF$_{25}$ remained significantly lower in infants whose mothers smoked.

- In multivariate analyses, MEF$_{25}$ and $V'_{\text{maxFRC}}$ were significantly diminished in infants of mothers with asthma.

- Whilst absolute inflation pressure applied has a significant effect on airway function parameters, initial use of slightly lower inflation pressures did not bias comparison of SGA and AGA infants.

- The method used to fit the jacket and to assess optimal jacket pressure when using the RVRTC technique appears to be valid and robust.
5 Discussion
5.1 Overall summary of results

To our knowledge, this is the only prospective study to be large enough to examine the association of airway function in SGA infants. Successful measurements of airway function using both tidal RTC and the raised volume RTC technique were obtained in 79 SGA and 104 AGA infants at a mean age of six weeks of age. Results from this study have shown that central airway function (FVC and FEV<sub>0.4</sub>) appears to be diminished in early postnatal life in infants born SGA compared to those born AGA. However, in infants not exposed to maternal smoking, this reduction in central airway function appears to be mediated primarily by diminished body size of SGA infants at the time of test. In addition, in multivariate analyses, peripheral airway function (V<sub>maxFRC</sub> and MEF<sub>25</sub>) was also significantly reduced in boys compared to girls and V<sub>maxFRC</sub> but not MEF<sub>25</sub> was also significantly lower in infants whose mothers smoked. Peripheral airway function was not significantly reduced in SGA infants compared to AGA infants. V<sub>maxFRC</sub> and MEF<sub>25</sub> were also diminished in infants of mothers with asthma.

5.2 Strengths of the study design

These findings are unlikely to be due to biases arising from the study design. First, in order to reduce errors due to misclassification, estimation of gestational age for the classification of birthweight status was based on sonographic assessments before 20 weeks gestation, which are currently considered to be the most accurate means of estimating gestation and hence birthweight centiles. In addition, maternal reports of post-natal smoking were confirmed by cotinine assay of maternal saliva and infant urine samples collected at time of test. Thus, random errors arising from misclassification of birthweight or smoking status were minimised, as these errors might lead to an underestimation of the strength of the association between birthweight status and airway function. Although the possibility of residual confounding (discussed in section 5.10.1) may still be present, reasonably accurate adjustments for maternal smoking were made in the model.
Secondly, lung function tests were performed prior to any lower respiratory illness (LRI), thus enabling respiratory function in SGA and AGA infants to be compared without confounding due to LRI.

Thirdly, the use of the raised volume technique to measure airway function allowed measures of forced expiration to be compared between infants over an extended volume range (Le Souëf et al. 1996).

Finally, this study had sufficient power to detect clinically important differences in airway function between SGA and AGA infants. The calculations for power of study suggested that a sample size of approximately 40 per group (SGA non-smoking, SGA smoking, AGA non-smoking and AGA smoking) would provide 80% power to detect a 10% difference in adjusted estimates of forced expiratory flows and volumes between birthweight groups, significant at the 5% level. Although, as with any statistical testing, for a given total sample size, power is maximised if group sizes are similar, it is not essential that they are absolutely equal, and any power calculation can only ever be an approximation. Details on the power of study and sample size calculations are given in Appendix I. This estimate was based on knowledge from previous studies conducted in our laboratory, which has shown that age, length, sex, maternal smoking status and family history of asthma explain a high proportion of variability in airway function at this age (Dezateux et al. 1999; Dezateux et al. 2001). In this study, retrospective analyses have shown that 47% of the variability observed in FEV$_{0.4}$ was accounted for by variables such as body size, age, maternal smoking and maternal social class.

According to power calculations, this should provide 80% power to detect a (0.05) 5% of the total variability in FEV$_{0.4}$ that can be explained by group differences due to birthweight status and/or smoking status (Figure 5.1). A difference of this magnitude is equivalent to 9% (0.05/0.53) of the variability remaining after accounting for known confounding factors, i.e. this would account for the power to detect true difference between SGA and AGA infants, with and without smoking mothers.
In reality this equates to an average (95% CI) reduction in FEV$_{0.4}$ of 8 (1, 16) mL in SGA compared to AGA infants after allowing not only for maternal smoking, but for differences in length, age and maternal social class (Table 4.20). Thus, as total variability of FEV$_{0.4}$ in this study population was 159 mL (ranged 44 – 203 mL) and being SGA was associated with on average a reduction in FEV$_{0.4}$ of 8 mL, this is equivalent to 5% of total variability observed (as predicted). After adjustment for other confounding factors, for an infant of average length (54 cm) and age (6 wk), FEV$_{0.4}$ = - 138 - 8 (SGA) + 4 (length, cm) + 2 (age, wk) (Table 4.20). Thus, for such an SGA infant, FEV$_{0.4}$ predicted is 82 mL while that for an AGA infant with similar characteristics is 90 mL. This actually equates to a 9% reduction in FEV$_{0.4}$ when comparing between an SGA and AGA infant of similar body size and age. A similar reduction was observed in FVC. Therefore this study proved to be adequately powered to detect variability in airway function attributable to low birthweight of a magnitude that is clinically and aetiologically relevant (see Section 5.7).
In this study population, the total between subject variability for MEF$_{25}$ was 320 mL·s$^{-1}$ (ranged 56 – 377 mL·s$^{-1}$), the variability of which was virtually twice that for FEV$_{0.4}$. The increased between subject ‘unexplained’ variation in MEF$_{25}$ will to some extent reflect the increased ‘noise’ associated with flow parameters, as reflected by a much higher within subject coefficient of variation for MEF$_{25}$ compared to that for FEV$_{0.4}$ (MEF$_{25}$ vs. FEV$_{0.4}$, mean [95% CI]: 8.8 [7.9, 9.6] vs. 3.7 [3.4, 4.1]) as shown in Table 4.32. In this study, although being born SGA was also associated with an average reduction in MEF$_{25}$ of 9% (22 mL·s$^{-1}$) compared to an AGA infant of similar characteristics after adjustment for sex and family history of asthma, this difference just missed statistical significance (p = 0.07; Table 4.21). This adjusted difference in MEF$_{25}$ according to birthweight status did in fact become significant when an additional 40 infants were recruited after completion of this thesis (bringing the final total to 91 SGA and 132 AGA infants) in order to attain the numbers required for subsequent follow-up of respiratory function in this cohort (Dezateux et al. Lancet; submitted). This illustrates the practical difficulties in estimating power of study prospectively when using several outcome measures, with differing degrees of total and unexplained variability, especially when new techniques are being used, with little prior evidence on which to base such estimates. The results from this study indicate that significant differences in airway function according to birthweight status were detectable with fewer subjects when FEV$_{0.4}$ rather than MEF$_{25}$ was selected as a primary outcome measure, due to the lower between subject variability of the former. Nevertheless, additional important information regarding other determinants of airway function (e.g. influence of sex and maternal history of asthma, Table 4.21) were obtained by including assessments of MEF$_{25}$.

5.3 Modifications to original study design

The original study design was proposed to test the ‘Barker’ hypothesis that airway function is impaired in infants of low birthweight compared to those who achieved an optimal weight. Modifications to the original study design were implemented within the first year of study. These were to ensure that adequate power of study was achieved within the study period to allow meaningful interpretation of the results and to test the proposed hypothesis. Factors relating to study design which could
potentially influence the interpretation of these data will be briefly discussed, together with methods used in the identification of SGA infants, accuracy of anthropometric data, assessment of maternal smoking status, recruitment bias and the methods used in the assessment of infant lung function.

5.3.1 Early modification to study protocol

Modifications to original study protocol were made soon after commencement with respect to inclusion criteria, methods of recruitment and neonatal anthropometry. These are discussed in this section.

5.3.1.1 Definition of SGA

An SGA infant was initially defined as one whose birthweight was \( \leq 3^{rd} \) centile according to the Child Growth Foundation (CGF) algorithm. This criterion was originally proposed to limit recruitment to the mainly growth restricted infants and to minimise recruitment of small but appropriately grown infants. However, it was recognised that with this definition we would not recruit sufficient full-term infants to achieve the necessary power of study required during the study period. Definition of an SGA infant was therefore amended to include those infants whose birthweight was \( \leq 10^{th} \) centile according to the CGF program.

It was acknowledged that more infants who were small but appropriately grown might be recruited by this amendment. However, the use of the GROW program, which calculates an individualised birthweight ratio (Wilcox et al. 1993), may assist in identifying growth restricted infants. Thus we proposed, once data collection was completed, to conduct final analyses for all SGA infants as a group, and also separately for those under the \( 3^{rd} \) birthweight centiles to examine the extent to which potential misclassification of IUGR might influence study findings.

5.3.1.2 Gestational age

Recruitment of all infants was originally limited to infants who were \( \geq 37 \) weeks gestation. This might also limit recruitment of SGA infants, as infants in whom
growth restriction is suspected are often delivered electively before 37 weeks gestation. In order to ensure that such infants, who were otherwise eligible, were not excluded, gestational age for eligible infants was extended to $\geq 35$ weeks gestation.

5.3.1.3 Antenatal steroids

Similarly, infants whose mothers received antenatal steroids were excluded in the original protocol. However, if fetal growth restriction is identified during the second trimester and growth faltering continues to be of concern to both obstetrician and paediatrician, antenatal steroids are often given to these mothers before a planned early delivery (often before 37 weeks gestation). While the effect of antenatal steroids on fetal lung development is unclear, it was decided that such infants would be recruited if they were $\geq 35$ weeks gestation. However, pending the final number of such infants recruited to the study, final analyses would be performed for this group separately or, if the number was low, after their exclusion from the final analysis.

5.3.1.4 Recruitment

An audit of the recruitment process (Section 3.1.5) following the first six months showed no difference in the success rate between face to face and postal recruitment (Appendix J). In view of the time consuming nature of trying to contact mothers on the wards, all subsequent recruitment was initiated by postal contact.

Accrual of eligible subjects was limited due to the large number of women from ethnic minority groups delivering at Homerton Hospital. To ensure adequate numbers, recruitment was extended to infants delivered at University College Hospital, London. Ethical approval for this was granted and recruitment at the second site commenced September 1998.

5.3.1.5 Prenatal recruitment

Prenatal diagnosis of suspected fetal growth restriction may be made following confirmation of reduced growth velocity by ultrasound scan. Hence, SGA infants
who were diagnosed antenatally were originally proposed as the 5\textsuperscript{th} group of infants in the study population. However, despite various attempts to recruit these infants over the first 12 months of the study period, referrals for this group of infants were not forthcoming from the Fetal Medicine Unit. In view of this, it was decided to omit this group from the study protocol.

5.3.1.6 Anthropometric measurement

Accurate birth length measurement is essential for the calculation of ponderal indices [birthweight (g) / length$^3$ (cm) $\times$ 100] and for further classification of SGA infants, as symmetric or asymmetric in terms of body proportions. However, length is a difficult measurement in routine practice, and problems include equipment, training and availability of personnel, as two people are needed to perform this measurement (Section 3.1.6). Our research team offered training on the use of a stadiometer, for the accurate measurement of infant length to ward staff. In order to validate the accuracy of infant body length measurement following the change in practice, two researchers performed length measurements on at least 10 infants prior to their discharge home in random order on the postnatal wards at three to six monthly intervals. The measurements obtained by the researchers were then compared with those obtained by staff on the Delivery Suite. Regrettably, the validation of length measurements showed that ward staff were inconsistent with the method used (Appendix K), as mothers reported that tape measures were frequently used instead of the stadiometer. Hence the decision was taken to accept that birth length, while still recorded, was unlikely to be as accurate as measurements taken in our laboratory due to unreliable technique.

With the extended recruitment to University College Hospital, there were slight policy differences between the hospitals with respect to neonatal anthropometric measurements. At University College Hospital, birth length was measured using a neonatal Rolameter (CMS). Length measurements using the stadiometer were found to be in close agreement with measurements made using the Rolameter. Chest circumference and mid-arm circumference were not measured at University College Hospital. Thus, although birth anthropometry was not routinely available for all the
infants studied, anthropometric measurements were performed on all infants at time of test by the researchers.

5.3.2 Accuracy of methods used to ascertain SGA/AGA infants

5.3.2.1 Antenatal detection of restricted fetal growth

In this study population, only 28% (22/79) of SGA infants were identified as having poor fetal growth antenatally. Unfortunately, no data are available regarding the specificity and sensitivity of antenatal ultrasound screening to detect SGA fetuses at the two recruitment centres. Nevertheless, the proportion of SGA infants identified antenatally in this study was similar to that reported in an earlier perinatal review (de Courcey-Wheeler et al. 1995). Despite increasing technological advancement in ultrasonography and its use for the assessment of fetal growth, antenatal detection of SGA fetuses has been reported to be poor (Neilson and Alfirevic, 1998; Neilson and Alfirevic, 1998). Hence most of the SGA infants were identified from anthropometric measurements made after delivery.

5.3.2.2 Postnatal identification

Since the optimal method of identifying SGA infants postnatally remains unclear, two methods to classify infants' size at birth were used, namely the CGF (Freeman et al. 1995) and GROW (Wilcox et al. 1993) algorithms. There was generally reasonable agreement between these two methods (Lum et al. 1999), with any discrepancies falling between the 11 - 15th centiles on one or other program. By including infants who were identified as SGA by either method, it was hoped that misclassification would have been avoided. Furthermore, in order to maintain a clear dichotomy between the SGA and AGA groups, infants whose birthweight centile fell between 15th – 20th centile according to CGF charts were not recruited into either group. However, it is recognised that the relationship between morbidity and birthweight is not a dichotomy but a continuous distribution, as it is dependent on the exposure to risk factors such as smoking, nutrition and poor socio-economic status (Kramer, 1987).
While it may be argued that birthweight centiles should be considered as a continuous variable rather than dichotomised into SGA and AGA groups, the latter option was chosen as the most cost-effective study design available. As we were interested in examining the implications of more severe growth restriction after allowing for some potentially confounding factors, we considered it advisable to actively recruit larger numbers of infants of low birthweight for gestation than would have been possible had we simply recruited a general population sample (Section 5.3.1.1). As these tests are time consuming and require sedation and furthermore as SGA infants tend to be disproportionately exposed to maternal smoking, we actively recruited SGA infants to ensure that the study had adequate power to detect clinically important differences and to adjust for recognised confounding factors. As this was the recruitment strategy, we therefore analysed all data accordingly using a dichotomous variable to express birthweight status. Using this option allowed comparison to be made between the two extremes of population with a clear gap (CGF 15-20\textsuperscript{th} centile) between the two groups.

However, we have also undertaken analyses using 'Birthweight SD score' as a continuous variable and found that the conclusions of the study are similar irrespective of whether birthweight status is expressed as continuous or dichotomous (Figures 5.2 and 5.3). Thus, as for the dichotomous analysis presented in sections 4.6.4 and 4.6.5, univariate analysis revealed a significant relationship between both FEV\textsubscript{0.4} (Figure 5.2) and MEF\textsubscript{25} (Figure 5.3) and birthweight SD score, whereas this relationship only remained significant for FEV\textsubscript{0.4} after adjusting for known confounders (see Table 4.20 and Table 4.21).
Figure 5.2  \( FEV_{0.4} \) plotted against birthweight SD score according to maternal smoking status

Figure 5.3  \( MEF_{25} \) plotted against birthweight SD score according to maternal smoking status
5.3.2.3 *CGF or GROW?*

There was no 'reference' birthweight standard for the local population with which to compare birthweight status of infants recruited to this study. Thus the widely used population based CGF reference standard for the UK (Cole et al. 1995) was used in conjunction with the assumption that an SGA infant is one whose birthweight lies below the 3rd or the 10th centile. However, some infants may be poorly grown, but on the basis of their birthweight, may appear to be of appropriate size for their gestational age. The GROW program, which calculates an individualised birthweight ratio from the relative contributions of gestation, maternal weight, infant sex, maternal height, parity and ethnic origin, was therefore used to provide a measure of the difference between the actual birthweight and the predicted birthweight of the infant (Wilcox et al. 1993).

Although there was reasonable agreement between these two methods of birthweight classification (Figure 4.4 and Figure 4.5), these methods are not interchangeable as shown by the wide limits of agreement according to the method by Bland and Altman (Bland and Altman, 1986). Centiles derived from the CGF were on average 3 centiles (95% CI: 2.2, 3.7) higher than GROW among SGA infants and 5.3 centiles (3.1, 7.4) higher than GROW among AGA infants.

Nevertheless, recent studies examining perinatal outcome in SGA births as defined by customised (GROW) versus population based birthweight standards have reported that the former method has an improved capacity to identify adverse effects related to fetal growth restriction such as stillbirth, neonatal death and Apgar score less than four at five minutes (de Jong et al. 1998; Claussen B et al. 2001).

5.3.2.4 *Practical difficulties in calculating birthweight centiles*

There are additional practical difficulties in attempting to use either of these methods. The calculation of birthweight centiles using the CGF algorithm is based on infant's sex, gestational age and birthweight. As these factors are routinely recorded for each birth, and centile charts published by CGF are widely available,
this method of birthweight centile estimation is readily accessible for use in postnatal wards. By contrast, for birthweight centiles calculated using the GROW algorithm, information on maternal height, booking weight, ethnic group and parity are needed together with the GROW software to calculate an individualised birthweight centile. This information was not routinely recorded in the obstetric records, specifically details of maternal height and booking weight were often missing. When maternal booking weight was available, the gestational age at which this was recorded varied from nine to 20 weeks. Thus, classification of infants using the GROW program was not feasible as a routine procedure to identify and recruit eligible SGA infants from postnatal wards. Hence some of the SGA infants who might have been identified as such according to GROW algorithm but who were above the 10th centile according to CGF algorithm might have been missed and hence not approached for recruitment to this study.

During the first year of this study, the identification of eligible infants for recruitment was based on using the CGF paper charts rather than software on the postnatal ward. While the resolution of the CGF chart was adequate for the estimation of birthweight centile up to 40 weeks gestation, it was much harder to use reliably for infants delivered after this gestation, due to the smaller scale of the chart (Appendix L). As a result, five infants who attended for respiratory function tests were later calculated to have birthweight between 15th – 20th centile according to the CGF algorithm and were therefore excluded from the study. In order to minimise this potential source of error, software produced by the Child Growth Foundation was subsequently used to identify eligible infants for recruitment. This meant that access to a computer and appropriate software was required for both methods.

As race and ethnic group have significant effects on birthweight (Zhang and Harville, 1998; Pang et al. 2000), birthweight standards such as the CGF method, derived from predominantly ‘Caucasian’ populations cannot be used for all racial/ethnic groups. Nevertheless, Gardosi et al. suggests that differences between the ethnic groups disappear when maternal size is also taken into consideration as proposed in the GROW program (Gardosi et al. 1992). However, this information was lacking in more than half of the eligible study population. Furthermore, the time of the
woman's first antenatal visit may vary greatly and the measurement of maternal weight and height is no longer routinely performed in many maternity units.

5.3.3 Issue of introducing change in practice for research

While it would have been interesting to compare birth length as well as birthweight between SGA and AGA infants, unfortunately the accuracy of such data could not be assured despite various attempts by the research team to introduce routine infant length measurement using a neonatal stadiometer (Section 5.3.1.6). Similarly, only limited anthropometric measurements on head, chest and mid arm circumferences were performed prior to discharge despite support from neonatal consultants and midwifery managers. Thus the opportunity to classify and assess somatic growth and airway function of SGA infants according to potential symmetrical or asymmetrical growth retardation as proposed by Barker (Barker et al. 1993) was lost.

In addition, prenatal recruitment of SGA infants had to be abandoned due to lack of referrals from the Fetal Medicine Unit. This highlights the difficulty in implementing change in practice especially when time is limited and when changes are perceived to be for research purposes only. While these limitations have not curtailed interpretation of the main outcome measures in the current study, for large epidemiological studies to be successful, support from the NHS may not be feasible. This has implications and consideration must be given for the resources required to mount such a study.

5.3.4 Time of recruitment

Another limitation of the study design was the time of recruitment. As mothers and infants were recruited after birth, collection of prospective records of tobacco smoke exposure and nutritional intake of the mother during pregnancies, which have been reported to be strongly associated with fetal growth restriction, was not possible (Bassi et al. 1984; Chen et al. 1987; Sebire NJ et al. 2001; England et al. 2001). For such data to be included, the focus of recruitment would have had to be at the time of booking, which would have had significant cost implications.
5.3.5 Difficulties of recruitment

Initially infants were only recruited from the Homerton Hospital NHS Trust. However, within the first six months of commencement of this study, it was evident that recruitment, particularly of SGA infants, was too low. Thus a second site, University College London Hospital NHS Trust, was established to supplement recruitment to this study.

In order to assess the reason for the shortfall at the original recruitment centre, an audit of births at this centre was performed. As there has been no fluctuation in the birth rate at Homerton Hospital over the past few years (~4,000 births per annum), the audit was performed for all births delivered in 2000. While information on ethnic status of the mother is normally recorded in the obstetric records, this information is not routinely entered onto the maternity database or into the main patient administration system (PAS) of the hospital. Thus individual obstetric records were accessed for this information. Of the 3,996 infants delivered at Homerton Hospital, 36.6% (1463) were born to Caucasian mothers, 32.6% (1303) mothers were African/Caribbean origin, 10% (399) were of Mediterranean origin; 12% (481) mothers were Asian/oriental, 1.5% (60) were of mixed origin while the ethnic origin of 7.3% (291) mothers of infants was unavailable. Of those infants born to Caucasian mothers, 16.8% (210) were below the 10th birthweight centile. Thus, while recognising the rich ethnic mix in this population, the percentage of Caucasian population eligible for recruitment to this study was overestimated. Furthermore, approximately 25% of the Caucasian population delivered at Homerton Hospital were Orthodox Jews, most of whom were unwilling to participate in research for various reasons (see Figure 4.1). Despite the difficulties encountered, 23% of the eligible population contacted were successfully recruited and studied, similar to that reported in previous epidemiological studies of infant lung function tests (Martinez et al. 1988; Tager et al. 1993; Dezateux et al. 1999; Young et al. 2000).

5.3.6 Assessment of smoking status

Maternal smoking during pregnancy is a known risk factor for low birthweight, sudden infant death syndrome, and increased respiratory morbidity through infancy.
However, there are well-recognised difficulties in objectively documenting tobacco smoke exposure during early life, and even in deciding who should be classified as 'a smoker'.

There are several biochemical methods for the assessment of tobacco smoke exposure in the mother and her young infant such as, nicotine or cotinine assay of serum, salivary, urinary or hair samples. However, nicotine is lipid soluble and readily absorbed and has an elimination half-life of 1-3 hours, hence monitoring of this chemical is not likely to reflect the extent of smoking (Eliopoulos et al. 1994; Perez-Stable et al. 1998). Cotinine, which is the major metabolite of nicotine, has a much longer elimination half-life than nicotine (20-24 hours) (Benowitz et al. 1983) and thus provides a better index of tobacco exposure than nicotine (Haddow et al. 1987; McNeill et al. 1987). Assay of serum, salivary and urinary cotinine provides an assessment of tobacco exposure at time of test. It has been suggested that assay of hair concentration of cotinine may reflect long-term systemic exposure to these toxins but this requires confirmation (Feher et al. 1996).

Self-reports of smoking status have been widely used as the only measure to assess detrimental effects of smoking on fetal growth and to orient counselling (Sexton and Hebel, 1984; Ahlsten et al. 1993; Lieberman et al. 1994; Horta et al. 1997; Cooke, 1998; Wisborg et al. 2000). This method of assessment can be unreliable if the subject is under pressure because of social or medical disapproval. While population surveys of adults generally find a high level of agreement between self-report and cotinine levels, previous studies of pregnant women have reported misclassification rates as high as 38% and 14% among those who claim to be non-smokers and smokers respectively (Bardy et al. 1993; Boyd et al. 1998; Klebanoff et al. 2001). In this study, 4.5% (5/110) of mothers who were self-reported non-smokers were reclassified as smokers on the basis of cotinine results, while 9% (7/78) who were classified as smokers because they reported minimal smoking or had given up smoking later than eight weeks gestation had salivary cotinine < 2ng.mL⁻¹. Previous studies (England et al. 2001; Owen and McNeill, 2001) have concurred with our observation that there was considerable overlap in the cotinine levels of those who reported smoking between 5 - 20 cigarettes per day (Figure 4.7). These findings
highlight the difficulties in assessing the 'dose' of passive smoke exposure received by the fetus.

5.3.6.1 Assessment of tobacco smoke exposure in the fetus and young infant

In this study, as mothers and their infants were recruited after delivery, it was not possible to assess tobacco smoke exposure prospectively. Thus assessment of in-utero exposure in the fetus was dependent on maternal recall which may be unreliable (Section 5.3.4). While, cotinine assay of the infant's first urine following birth may offer a more precise method of assessing in-utero exposure this will only reflect exposure during the last few days prior to delivery (Etzel et al. 1985; Jordanov, 1990; Hoo et al. 1998). In this study, where infants were recruited postnatally, the first objective measure of tobacco smoke exposure in young infants was from cotinine assay of the infant's urine at time of test. As in previous studies (Greenberg et al. 1984; Hoo et al. 1998; Dezateux et al. 1999), we found that infant urinary cotinine levels were significantly higher in those whose mothers smoked compared to those infants not exposed to maternal smoking (Table 4.5). Among those infants exposed to maternal smoking, there was a wide distribution of cotinine levels (range: 0.01 – 191.7 ng.mL⁻¹).

A number of factors may be responsible for the variation observed in passive exposure, including proximity to the source and amount of smoke and ventilation in the environment (Henschen et al. 1997). For young infants such as in this study group, the main source of tobacco smoke exposure is often the mother as she is the chief carer for her infant at this young age. Thus maternal salivary cotinine levels could be used as a proxy of passive tobacco smoke exposure in the infant. In older children (5 – 7 years old), cotinine levels were related to the number of sources of exposure or smokers within the household, though it has been suggested that maternal smoking remains the most important influence (Cook et al. 1994). In addition, observations from earlier studies have shown that nicotine and cotinine are present in breast milk of mothers who smoked, and therefore contribute to urinary cotinine levels in breast-fed infants (Luck and Nau, 1985; Schulte-Hobein et al. 1992; Mascola et al. 1998). However, the amount of cotinine and nicotine ingested via the mother’s milk is dependent on the individual smoking patterns of the mother.
(e.g. depth of inhalation, number of puffs per cigarette) (Armitage et al. 1975; Forbes et al. 1976) and on the time difference between the last cigarette smoked and breast feeding (Hamada et al. 1994).

It is unclear whether ingestion of cotinine and other tobacco products through breast milk contributes to adverse health consequences of environmental tobacco smoke exposure by inhalation. As cotinine is only a quantitative biomarker for smoking, the identity of the compounds in tobacco smoke that are actually responsible for the adverse health impact on infants and young children and the degree to which their concentrations in breast milk are correlated are largely unknown.

5.3.6.2 **Misclassification of smoking status**

Earlier studies have shown that misclassification of smokers as non-smokers is a source of bias in epidemiological studies estimating the risk associated with tobacco smoking (Riboli et al. 1995; Suadicani et al. 1997). While self-report of smoking can be validated by an objective biochemical method as described earlier, and smokers re-classified on the basis of cotinine levels, the question remains as to how smokers with low levels of exposure should be categorised. In this study, in an attempt to identify those infants whose exposure to maternal smoking was significant during the period of airway growth and development in-utero, a smoker was defined as one who continued to smoke beyond eight weeks gestation (Section 3.1.4.3). When infants of light smokers, as assessed by salivary cotinine were included in the smoking exposed group, we found that the effect of maternal smoking on peripheral airway function, specifically MEF_{25}, was attenuated (Section 4.6.2).

5.4 **Study population - how representative?**

5.4.1 **Exclusion criteria**

Since prematurity, respiratory disease and ventilatory assistance during the neonatal period are all likely to have a negative impact on airway function, such infants were excluded from this study. As this potentially excludes those infants with more severe fetal growth retardation born by elective premature delivery or with severe
respiratory disease, the SGA population studied may be potentially biased towards those with less severe growth restriction.

In order to maintain a clear dichotomy between SGA and AGA infants, those infants with birthweight between 15th - 19th centile according to CGF algorithm were excluded.

5.4.2 Social and demographic characteristics of mother

The social and demographic characteristics of the mothers of both SGA and AGA infants were similar (Table 4.2), but mothers in this study were older, of higher social class and better educated than women in a similar study of term infants carried out within the inner London area (Dezateux et al. 1999) or when compared to the national average for age at first delivery (Office for National Statistics, 1998). This suggests that SGA infants recruited to this study may have come from more affluent or better-educated families, as maternal age is associated with social class. Also as SGA mothers are likely to be from more socially disadvantaged groups, this bias is likely to be greater than predicted. Unfortunately such details are not available for those who were eligible but not recruited. However any such potential biases in recruitment would tend to attenuate the associations observed and lead to conservative estimates of the association between airway function and low birthweight for gestational age.

When categorised according to smoking status, mothers who smoked were significantly shorter, but not lighter, at time of booking. Significantly more mothers who smoked were in manual occupations (54% vs. 10%) and were less well educated (18.5 vs. 21.6 years) than those who did not, as observed in many reports (Kramer, 1987). Thus, smoking is clearly associated with social disadvantage.

5.4.3 Sex imbalance in SGA non-smoking group

A further issue when interpreting these results arises from the relatively low proportion of SGA girls in this study population who were born to non-smoking mothers. The reason for this is unclear. An audit of births confirmed that equal
numbers of SGA boys and girls were born during the period of recruitment (Appendix M), and this is reflected by the fact that a similar proportion of SGA boys and girls were recruited (Table 4.2). However, a higher proportion of SGA girls recruited to this study were born to mothers who smoked, thus SGA girls unexposed to maternal smoking are relatively under-represented in this analysis. This imbalance was taken into account when interpreting results by adjusting for sex in the multivariate regression analyses. Interestingly, in a study incorporating all live births during one week in Finland, the authors reported that girls tended to outnumber boys in the group exposed to maternal smoking, but this difference just failed to reach statistical significance (Bardy et al. 1993). In addition, a recent study of 11,815 liveborn singleton infants, has shown that periconceptional smoking is associated with a lower male to female sex ratio of offspring (Fukuda et al. 2002).

5.4.4 Other factors

While maternal smoking remains one of the strongest associated factors for fetal growth restriction (Kramer, 1987), maternal nutrition in pregnancy has received increasing attention during recent years (Godfrey et al. 1996; Mathews et al. 1999; Sebire NJ et al. 2001). There has also been considerable interest in the intergenerational influences on birthweight (Kramer, 1987; Emanuel et al. 1992; Coutinho et al. 1997; Klebanoff et al. 1997; Hennessy and Alberman, 1998). These published studies suggest that there is a significant positive association between the infant’s birthweight and the mother’s birthweight, as was shown in the current study, since the mean self-reported maternal birthweight for mothers of SGA infants was significantly lower than that reported by mothers of AGA infants. It has been suggested that this may be a multigenerational effect and that the most critical factor for determining birthweight of an infant is the growth and development of the mother during her intrauterine life as an embryo/fetus (Emanuel et al. 1992).

These observations were similar to those reported in a meta-analysis of 895 publications on the determinants of low-birth weight (Kramer, 1987), which suggested that maternal genetic potential for growth could impose physical limitations on the growth of the uterus, placenta and fetus. However, other
contributory factors such as smoking, socio-economic status, parity, pre-pregnancy weight and maternal age may reflect an accumulation of risk (Kramer, 1987).

While recognising that birthweight is influenced by birth order, notably being lower in a first pregnancy, a similar number of SGA and AGA infants in this study were firstborn. Furthermore, the incidence of antenatal intervention and obstetric complications, which may predispose to a growth restricted infant (such as pregnancy induced hypertension), was low within the study population and was similarly distributed between mothers of SGA and AGA infants (Table 4.3). The low recruitment of infants whose mothers had pregnancy induced hypertension, a common cause of low birthweight, may have been due to the fact that infants of mothers with severe pregnancy induced hypertension or pre-eclampsia were invariably electively delivered before 35 weeks gestation and were therefore excluded from the study. Thus, infants recruited to this study only comprise the healthiest of SGA infants and the changes observed may have been more marked had we studied those more severely affected. Although it would have been interesting to assess the influence of antenatal interventions such as amniocentesis or chorionic villus sampling on infant airway function, the number of cases with these interventions was too small for meaningful analysis.

5.4.5 Optimal age for respiratory function test

A number of studies have shown that impaired airway function in term infants precedes and predicts wheezing in early childhood (Martinez et al. 1991; Dezateux and Stocks, 1997; Dezateux et al. 2001). In addition, the incidence and age of onset of lower respiratory illnesses (LRI) in infancy appears to be determined in part by diminished airway function which is detectable shortly after birth (Dezateux and Stocks, 1997; Dezateux et al. 2000; Dezateux et al. 2001). While assessment of premorbid respiratory function necessitates early measurements before any LRI, within and between subject variation in tidal breathing and other parameters may be very high during early infancy (Stocks et al. 1994b; Le Souèf et al. 1996; Henschen and Stocks, 1999). In addition, the feasibility of conducting a large population based study will be determined by parental acceptance of these tests, which is likely to be higher beyond the neonatal period.
The decision to perform respiratory function measurements within four to ten weeks of postnatal age was based on the factors mentioned above. While attempting to obtain measurements before the onset of any LRI, these measurements were undertaken after initial physiological instability had resolved. This was particularly important with respect to the highly variable end expiratory level during the neonatal period and the fact that the breathing strategy of very young infants is strongly influenced by the effect of sleep state on expiratory timing and diaphragmatic braking (Stark and Frantz, 1979; Stocks et al. 1994b). This in turn may have marked effects on the magnitude and variability of measures such as $t_{\text{PTEF}}^{\#E}$ (Stocks et al. 1994b) and $V'_{\text{maxFRC}}$ (Henschen and Stocks, 1999).

However, measurements at this age, as opposed to the first week of life (Stocks et al. 1997), require sedation. In order to minimise intra and inter-subject variability of respiratory function parameters, lung function data were collected during quiet sleep, aided by use of sedation. Sedation such as chloral hydrate or trichlofos sodium, could be administered with minimal risk after 44 weeks post-conceptional age as recommended (Gaultier et al. 1996). The small increase in respiratory rate observed after administration of trichlofos (the active breakdown product of chloral hydrate) though statistically significant was not clinically important and is thought not to influence the interpretation of lung function tests (Jackson et al. 1991).

The recent development of the raised volume technique for measuring forced expiratory flows from full flow-volume curves in infants allowed flows to be related to a more stable lung volume, hence providing a more stable volume landmark.

### 5.5 Choice of lung function technique

In older children and adults, forced expiratory manoeuvres are routinely used to determine pulmonary function in both clinical and research settings. Partial expiratory flow volume manoeuvres obtained by the rapid thoraco-abdominal compression (RTC) technique have been widely used to assess airway function in infants, as this technique provides the first practical, non-invasive method for assessing airway physiology in both healthy and diseased infants (Martinez et al. 1990; Hanrahan et al. 1992; Hoo et al. 1998; Young et al. 2000; Hoo et al. 2002).
These studies have shown that $V'_{\text{maxFRC}}$ is significantly lower in infants exposed to maternal smoking and also lower in boys than in girls. However, a major disadvantage of this method is the lack of a reliable volume landmark. The measurement of $V'_{\text{maxFRC}}$ is dependent on a stable functional residual capacity (FRC) between manoeuvres, and recent studies have shown that in young infants, FRC is not stable, is usually dynamically elevated and may be influenced by changes in airway calibre, sleep state and addition of dead space (Le Souef et al. 1996; Henschen and Stocks, 1999; Modl et al. 1999). In addition, whether or not flow limitation can be reached in all healthy infants using this technique remains controversial (Le Souef et al. 1996).

During the last few years adaptations of this technique have been applied, wherein the infant’s lungs are passively inflated towards total lung capacity before applying the compressive pressure (Turner et al. 1995; Feher et al. 1996). This enables full forced expiratory manoeuvres to be obtained in infants as in older children and adults. It has been suggested that this raised lung volume rapid thoraco-abdominal compression (RVRTC) technique may be more reproducible and sensitive than the partial flow volume curves and may provide data comparable with FEV$_1$ in older subjects (Le Souef et al. 1996; Modl et al. 2000).

Recent applications of the RVRTC have shown that MEF$_{\%}$ was significantly lower in infants whose mothers smoked during pregnancy than in infants whose mothers did not smoke. MEF$_{25}$ was also significantly lower in boys than girls (Jones et al. 2000). Studies on school age children have also shown that FVC and FEV$_1$ were significantly reduced in children with low birthweight for gestational age (Rona et al. 1993). Thus it would be of great interest to ascertain if deficits in airway function are present soon after birth in SGA infants.

However, there is currently no standardised approach to either data collection or analysis for this promising new technique. Potential factors which may influence results from the RVRTC include: the number and rate of augmented breaths prior to forcing expiration; the tightness of jacket fit and efficiency with which pressure is transmitted from the jacket to the intra-thoracic airways; the methods used to assess
flow limitation and most importantly, the pre-set pressure used to inflate the lungs (Gappa, 1999; Allen and Gappa, 2000; Gappa, 1999).

5.5.1 Influence of inflation pressure on RVRTC parameters

It has been recognised that one of the advantages of the RVRTC method is that lung volume can be standardised by using a pre-set airway pressure (Le Souèf et al. 1996). However, the results from a study of 32 infants (section 4.7.1) indicate that when using the RVRTC technique, relatively minor variations in $P_{\text{inf}}$ (± 8%) will be accompanied by highly significant changes in the major outcome variables (FVC, FEV$_{0.4}$ and MEF$_{25}$) derived from this technique.

The very high degree of within-subject repeatability of parameters derived from the RVRTC technique, and their strong dependence on the inflation pressure delivered means that even minor differences in equipment or technique may result in a significant bias between data collected in different laboratories. A difference of 5-10% in FEV$_{1}$ or MEF$_{25}$ has been considered clinically significant in recent epidemiological studies (Gilliland et al. 2000; Li et al. 2000), a magnitude of differences that could easily occur due to slight variations in the application of the RVRTC technique (Li et al. 2000). Meticulous attention therefore needs to be paid to all aspects of data collection during the raised volume technique if meaningful comparisons are to be made within and between infants. This is crucial if interpretation of results is to be based on reference data collected elsewhere.

There is as yet no consensus as to which is the optimal inflation pressure to use for raising lung volume. Currently, most centres are applying 3 kPa (Modl et al. 1999; Jones et al. 2000) but others have used 2 kPa equally successfully and with similar reproducibility (Hayden et al. 1998) and this may be more applicable in very small or immature infants (Henschen et al. 1998). Since virtually all parameters derived from the RVRTC are strongly dependent on $P_{\text{inf}}$, separate reference data will naturally have to be established according to the selected $P_{\text{inf}}$, which can be a very time consuming and complex undertaking (Stocks and Quanjer, 1995; Stocks et al. 2000). Equally important, as discussed above, despite selecting a specific $P_{\text{inf}}$, there may be subtle variations in the actual $P_{\text{inf}}$ delivered, which could bias the results.
When first introducing any new lung function technique there is usually a steep learning curve, before any degree of standardisation can be introduced. This is particularly true for the RVRTC technique and even when fully automated systems are used, there are few if any laboratories around the world that can claim to inflate the lungs to exactly 3 (or 2) kPa in the breath immediately prior to expiratory forcing. In reality, despite attempts to provide a standardised pressure, this is quite likely to vary by ± 0.2 kPa between infants or centres. Such differences may arise as a result of slight flow dependence of pressure valves, variations in the duration of the inflated breath, minor calibration errors, or simply the algorithms used to calculate mean $P_{inf}$.

Furthermore, the ability to check the pressure actually delivered, rather than that preset by the equipment, is not routinely possible in all currently available systems. Consequently, an assessment of the potential effect of subtle changes in $P_{inf}$ was undertaken to validate the RVRTC technique (Section 3.10.1).

### 5.5.1.1 Influence of variations in inflation pressures on airway function results

While some variation in inflation pressure was inevitable, especially when using a new technique during the initial study period, its effect on lung function parameters did not appear to compromise the findings in this study or alter their significance (section 4.7.1.1). This was because data from both SGA and AGA infants were collected concurrently and thus any variation in methodology occurred in a similar proportion in each group.

### 5.5.2 Influence of jacket placement on airway function

As mentioned previously, there is currently no standardised approach to the raised volume technique between centres (Gappa, 1999; Allen and Gappa, 2000) and considerable debate had arisen as to whether the jacket should be applied more loosely when undertaking measurements at raised lung volume than recommended for the tidal RTC manoeuvre. A small study was undertaken to compare the influence of a loosened versus standard jacket placement on RVRTC parameters. Results indicated that loosened jacket placement had minimal effect on lung function parameters (Section 4.7.2). A loosened jacket placement was associated with an improved jacket pressure transmission by an average of 18% during RVRTC.
manoeuvres. This increase in transmitted pressure to the intra-thoracic airways was
accompanied by a significant increase (13%) in peak expiratory flow and significant
decrement (8%) in MEF25, but no change in FVC, FEV0.4 or tFE.

5.5.2.1 Physiological interpretation

In the main study, the protocol used for adjusting and fastening the jacket during the
raised volume manoeuvres was according to recent recommendations for
standardised measurements of tidal RTC in infants (Sly et al. 2000). During
augmented breathing, it has been shown that inflation volume and respiratory system
compliance (CrS) are significantly lower (8% respectively) when measurements are
obtained with a fastened jacket in situ compared to those obtained without (Hoo et al.
2001). This suggests that fastening the jacket restricts chest wall movement which in
turn reduces the chest wall, and hence total respiratory, compliance in infants. By
contrast, in this small study, investigating the influence of jacket placement on
RVRTC parameters, the increase in FVC and CrS when measured with a loosened
versus a ‘standard’ jacket was on average only 2% and 4% respectively. Thus, the
effect was less than half that observed when jacket was removed altogether,
suggesting that even with a looser fit, there were other ongoing influences resulting
from jacket placement.

The reasons for this are unclear but may be related to stimulation of chest wall
reflexes by the presence of a jacket which therefore still splints or restrict chest
expansion even when jacket is loosely applied, compared to lung inflation without
jacket in-situ. Earlier studies have noted that both compliance and lung volume were
reduced during tidal breathing following jacket placement (Steinbrugger et al. 1988)
or with respiratory inductance bands (Dundas et al. 1995).

The significant change observed when a loosened jacket was applied was a marked
reduction (8%) in MEF25. The reason for this may be twofold. First, in the presence
of a slight increase in FVC, due to the increase in CrS, MEF25 will be measured at a
slightly lower lung volume, which will have a more marked effect on flows than
volume parameters due to the sharp slope of the flow volume loop in young infants
(Figure 5.4).
Figure 5.4  Measurement of MEF$_{25}$ in relation to different FVCs

- Standard jacket placement: MEF$_{25} = 211$ mL s\(^{-1}\)
- Loosened jacket placement: MEF$_{25} = 188$ mL s\(^{-1}\)
Alternatively, in infants where there is already some degree of tidal flow limitation present and flow limitation had already been reached at raised lung volume, any increase in jacket pressure transmitted to the intra-thoracic airways such as when a loosened jacket is applied, may cause negative flow dependency to occur (Figure 5.5).

Figure 5.5 Negative flow dependency resulting from higher jacket pressure transmission

Note: This infant was very flow limited during tidal RTC (Best $V'_{\text{maxFRC}} = 32 \text{ mL.s}^{-1}$). Jacket pressure used to achieve $V'_{\text{maxFRC}}$ and for RVRTC manoeuvres was 3.4 kPa. Mean jacket pressure transmission was 2.3 and 2.5 kPa for standard and loosened jacket application respectively.

Thus if results of RVRTC are to be compared between laboratories, while there is no systematic bias with respect to volume parameters resulting from different jacket applications, a significant reduction in MEF$_{25}$ will be observed with looser jacket applications.
5.5.3 Influence of jacket pressure on lung volume parameters

In the main study, the jacket pressure ($P_j$) used for raised volume manoeuvres was determined as the ‘optimal’ $P_j$ applied above which no further increase in $V'_{\text{maxFRC}}$ was achieved. In order to clarify the issue of whether this ‘optimal’ $P_j$ was sufficient to achieve flow limitation at raised lung volume, a methodological study comparing paired data from 14 infants was undertaken. This showed that further increase in $P_j$ did not elicit higher flows during RVRTC manoeuvres (section 4.7.3).

5.5.3.1 Physiological interpretation of observed results

While no significant group change was observed measured between ‘optimal’ and higher $P_j$ manoeuvres for any parameter, application of the higher $P_j$ appear to cause peripheral airway closure and negative flow dependence in some infants.

Figure 5.6 RVRTC curves – showing influence of high jacket pressure on airway mechanics

Figure 5.6 shows an overlay of RVRTC curves from the same infant, generated using similar $P_j$, which was approximately 1 kPa above the ‘optimal’ $P_j$ (2.9 kPa) derived from the standard RTC manoeuvres. Results of parameters from the initial manoeuvre at higher $P_j$ were similar to those obtained at ‘optimal’ $P_j$. However, when a higher $P_j$ was applied, despite identical inflation pressure and measurement
conditions used for both manoeuvres, the second manoeuvre produced marked reduction in FVC. This probably reflects peripheral airway closure due to external forces (jacket pressure) being greater than transmural pressure within the peripheral airways. Mean jacket pressure transmission at ‘optimal’ $P_j$ was 1.4 kPa while jacket pressure transmission at the higher $P_j$ was 2.2 kPa.

However, in some infants, it may not be possible to achieve flow limitation at high lung volume without inducing marked chest wall and upper airway reflexes as shown in Figure 5.7.

**Figure 5.7** Higher jacket pressure induced marked glottic activity at high lung volume

![Figure 5.7](image)

5.5.4 Relevance of current findings

Results from these studies have clearly demonstrated that a looser jacket application or the use of a jacket pressure that is higher than ‘optimal’ not only fails to elicit higher flows but could stimulate chest wall and upper airway reflexes such as glottic closure (Figure 5.7), and cause peripheral airway closure (Figure 5.6) and negative flow dependence (Figure 5.5). These unfavourable outcomes appear to be very much related to the pressure transmitted from the jacket to the intra-thoracic airways at a given lung volume. However, due to time constraints, it was not possible to re-assess
optimal $P_j$ in the presence of a looser jacket placement during the RVRTC manoeuvres in this study, in order to make a direct comparison of results under these conditions. It may well be that, had a slightly lower $P_j$ been used in the presence of a looser jacket, identical flows at low lung volumes would have been achieved as when using a 'standard' fit of jacket and 'optimal' $P_j$. As jacket pressure transmission was improved when a looser jacket placement was applied, perhaps a looser jacket i.e. allowing four adult fingers breadth between jacket and sternum should be recommended for both partial and full forced expiratory manoeuvres in infants.

By using the 'optimal' $P_j$ from the tidal RTC (above which no further increase in $V'_{mFRC}$ was achieved) manoeuvres for the RVRTC technique, we have shown that this method of ascertaining optimal jacket pressure for the latter to be robust. In centres where only the RVRTC technique is used for assessing airway function in infants, forced expiratory manoeuvres have been repeated with increasing jacket compression pressures until the highest expired volumes and flows are obtained (Tepper et al. 1999; Jones et al. 2000; Castile et al. 2000). However, this method of estimating optimal jacket pressure for RVRTC technique requires many more manoeuvres and augmented breaths, thus increasing the risk of gastric distension from repeated manoeuvres.

Jacket pressure transmission is not routinely assessed in all centres using the RVRTC technique. In this study and in centres that have routinely undertaken such assessments, jacket pressure transmission has been generally assessed at end tidal inspiration and not at raised lung volume (Hayden et al. 1997; Modi et al. 2000; Lum et al. 2000; Ranganathan et al. 2001). While it has been suggested that assessment of pressure transmission at raised lung volume (i.e. end inflation) may be more informative (Turner et al. 1995; Le Souëf et al. 1996), this approach has not been attempted in manoeuvres where $P_{inf}$ is pre-set to 3 kPa, because the infant would have to be exposed to very high intra-thoracic pressures (> 5-6 kPa). Data collection may also be difficult due to increased risk of leaks between the mask and face from the high pressure applied.

The pressure transmitted from the jacket to the intra-thoracic airways is dependent on the tightness of jacket application and may differ between application in different
infants. However, the question remains as to whether the assessment of transmission pressure is useful or indeed necessary. Hayden et al. suggested that a constant transmission pressure of 2 - 2.5 kPa would be most suitable for all RVRTC manoeuvres in infants (Hayden et al. 1997). We would disagree with this recommendation as we have shown that this level of transmission pressure will fail to achieve flow limitation in some infants while causing negative flow dependence and peripheral airway closure in others. As the 'optimal' $P_j$ differs between individuals, assessment of transmission pressure may not always provide useful information on any given occasion. Its assessment does, however, allow comparisons of technique to be made between different centres studying similar population of infants and may be particularly useful as a quality control measure in multi-centre trials.

5.6 Lung function parameters

In this study, airway function in infants was measured using both partial and full forced expiratory manoeuvres in order to address some of the analytical issues surrounding these techniques. Within this population, $\text{FEV}_{0.4}$ could be calculated in 99% of infants studied. As expected, since volume is integrated from flow, volume parameters ($\text{FEV}_1$) were less variable than any of the flow parameters ($\text{MEF}_{\%}$ and $V'_{\text{maxFRC}}$). $V'_{\text{maxFRC}}$ was most closely related to $\text{MEF}_{15}$ and $V'_{\text{maxFRC}}$ was less variable than any of the $\text{MEF}_{\%}$ parameters measured using the RVRTC technique (section 4.7.4).

5.6.1 Timed volume parameters ($\text{FEV}_1$)

$\text{FEV}_1$ is used in older children and adults to assess intrathoracic airways obstruction, monitor disease progression and individual response to therapy (Pride, 1999). However, the underlying physiology of this parameter is complex. $\text{FEV}_1$ is relatively independent of the resistance of the upper airways and of the effort applied. However, it is dependent on factors which determine the maximum expiratory flow at a particular lung volume (lung recoil pressure and the resistance of the intrapulmonary airways) and on the change in maximal expiratory flow with lung volume (which reflects change in recoil pressure with change in lung volume, i.e. pulmonary compliance and the change in intrapulmonary resistance with change in
lung volume) (Pride, 1971). In older subjects, FEV₁ is thought to reflect primarily large and central airway function (Hogg et al., 1968) although there is limited evidence to support this. It is likely that FEV₁ in infants reflects similar mechanical properties of the airways and alveoli but there may not be anatomical concordance with FEV₁ in older subjects. Measuring FEV at different intervals (0.3s, 0.4s or 0.5s) after the start of forced expiration on any one occasion, or even measuring the same FEV₁ in the same infant longitudinally, is unlikely to provide physiological information from the same airway generations on each occasion.

FEV₁ is not an appropriate parameter to report in young infants as only 146 (80%) infants from this study population completed their forced expiration before 1s. Furthermore, as tests such as the RVRTC technique are labour intensive and require specialist equipment and sedation of the infant, it is essential that parameters selected for use as major outcome variables should be feasible in most infants. In this study, only FEV₀.₃ could be calculated in all infants. Although it could be argued that FEV₀.₃ would therefore be the most appropriate parameter in young infants, theoretically the first 0.3s of forced expiration is more subject to artefact, and volumes measured at this time point may occur too early in the forced expired breath (especially those with forced expiratory time > 1s) to encompass the flow-limited portion of the forced expiratory flow-volume curve. In addition, the variability of this parameter was greater than that of FEV₀.₄ or FEV₀.₅ (Table 4.32). Therefore, reporting FEV₀.₄ appears to be the best compromise between a measurement made at a short enough time period to be feasible in most infants during the first three months of life and minimal variability.

5.6.2 MEF₉₄ parameters

Flow limitation occurs when the maximum flow obtained is independent of driving pressure (Pride, 1999). The transmission of jacket pressure to the intra-thoracic airways via the chest wall, in addition to the elastic recoil pressure, forms the pressure that drives flow during forced expiratory manoeuvres in infants. Once flow limitation has been achieved, expiratory flow reflects the mechanical properties of the lung (Dawson and Elliot, 1977; Mead, 1980b). Forced expiratory flows measured at the airway opening, which represent the integrated output of the whole
respiratory system, may not provide accurate information about the mechanical properties of the lung if flow limitation cannot be achieved.

As lung volume decreases towards late expiration, smaller airways are more likely to be flow limited (Mead, 1980b) so that the integrated output of MEF at lower lung volume (e.g. MEF25), while not directly indicative of smaller airway function per se, may contain more clinically useful information regarding small airway function. The usefulness of flows measured at even lower lung volumes (e.g. MEF15 or MEF10) is not known. The absolute magnitude of the MEF% parameters within an infant depends not only on the inflation pressures used but also whether full forced expiration to residual volume has been achieved. In infants, particularly those with airway disease, it may not be possible to achieve this as inspiration may occur prematurely. When this occurs FVC is apparently reduced, leading to overestimation of MEF% as these parameters will be measured at relatively higher lung volumes under such conditions. Consequently, some authors have chosen not to report FVC or MEF% when applying the RVRTC technique to infants with bronchiolitis (Modi et al. 2000), while others have reported that, despite the higher within subject variability, MEF% may be more discriminative than FEV1 (Castile et al. 1999). In order to overcome the problem of early inspiration, increasing the number of inflation breaths prior to forcing expiration may override the infant’s own respiratory reflexes. However, the operator needs to examine the time-based records and shape of the flow volume curves to ascertain completeness of expiration. As expected and previously reported in older children (Hutchison et al. 1981), within-subject variability for MEF% was higher than that for volume parameters (FVC, FEV1). In this study, MEF15 and MEF10 were more variable than MEF25, which may in part be explained by a low signal to noise ratio at such low measurements of flow.

5.6.3 MEF% vs. \( V'_{\text{maxFRC}} \)

The measurement of \( V'_{\text{maxFRC}} \) using the standard RTC technique, relies on the functional residual capacity (FRC) as a volume landmark. However, it is recognised that FRC may be relatively unstable especially in young infants and may vary with sleep state and disease (Stark et al. 1987; Henschen and Stocks, 1999). In addition, variability in \( V'_{\text{maxFRC}} \) may be related to a failure to achieve flow limitation
especially in healthy infants (American Thoracic Society/European Respiratory Society, 1993). Nevertheless, results from this study have shown that if strict quality control criteria are applied, forced expired flows measured with the RTC are no more variable than those measured using the RVRTC technique. The potential variability caused by an unstable FRC and lack of flow limitation can be minimised by ensuring that the RTC is performed during quiet sleep, with stable end expiratory levels, checking that selected curves overlay on the descending portion of the flow-volume curve and ensuring an adequate driving pressure. Indeed, while the within-subject variability for all parameters derived from the RVRTC technique in this study was similar to that reported by others (Turner et al. 1995), the coefficient of variability for $V'_{\text{maxFRC}}$ was considerably less than that documented in many previous studies (Taussig et al. 1982; Wall et al. 1984; Tepper et al. 1986; Turner et al. 1995). The latter, were however performed prior to the introduction of standardised methods (Sly et al. 2000).

Over the past 20 years, the RTC technique for measuring $V'_{\text{maxFRC}}$ from partial expiratory flow-volume curves has been the most popular method of assessing peripheral airway function in infants (American Thoracic Society/European Respiratory Society, 1993; Le Souëf et al. 1996). However, the recent development of the RVRTC technique has allowed investigators to assess forced expiration from raised lung volume in infants, similar to that used in adults and older children. While the latter technique has several advantages (section 3.8.5) there is currently no consensus or standardised approach to this technique between centres, nor is it clear as to what the parameters measured by the RVRTC technique actually represent in infancy. Thus respiratory function data were collected using both techniques.

Interestingly, while MEF$_{25}$, MEF$_{15}$ and $V'_{\text{maxFRC}}$ are all considered to be measures of peripheral airway function, MEF$_{25}$ and MEF$_{15}$ but not $V'_{\text{maxFRC}}$ were significantly lower in SGA infants during early infancy on univariate analysis. One possible reason for this discrepancy is that these parameters may reflect mechanical properties of different generations of peripheral airways. Alternatively, the relatively higher inter-subject variability of $V'_{\text{maxFRC}}$ (Table 4.14), which in part reflects the variable extent to which dynamic elevation of FRC occurs during early infancy (Henschen and Stocks, 1999), may mean that it identifies SGA infants less well than MEF$_{25}$ and
MEF\textsubscript{15}. However, sex differences in expiratory airflow were detected by both techniques, suggesting that the distributions of \( V'_{\text{maxFRC}} \) values are distinct for sex but not for birthweight status at the level of power of this study (Table 4.18). The fact that observed sex differences in peripheral airway function were larger both in absolute terms and in relation to the inter-subject variability for \( V'_{\text{maxFRC}} \) than either MEF\textsubscript{25} or MEF\textsubscript{15} (Table 4.18) again suggests that these parameters may be reflecting different aspects of airway function. It also suggests that any decrements of airway function associated with low birthweight may be operating at a slightly different site to those associated with being male.

5.6.3.1 Effects of deep inhalations

A vital aspect of the RVRTC technique that has yet to be elucidated concerns the effect if any, of administering a series of augmented breaths prior to forcing expiration. It is particularly important to ascertain whether the effects of inflating the lungs passively by application of a positive pressure at the airway opening are comparable to the effects on airway mechanics that have been demonstrated in older subjects of taking a deep breath.

Intrathoracic airway calibre is physiologically determined by a balance between forces that tend to constrict the airways (airway smooth muscle) and those that prevent narrowing (lung elastic recoil). While lung elastic recoil is determined by lung volume and volume history, the response of airways to stimuli such as deep inhalation (DI) is the result of interdependence between airways and lung parenchyma (Pellegrino et al. 1998).

In healthy subjects, flows are usually higher at any given lung volume following deep inhalation than during tidal breathing, due to the bronchodilator effect of a deep breath which relaxes airway smooth muscle (Pellegrino et al. 1998; Kapsali et al. 2000; Jensen et al. 2001). This effect is not seen in asthmatics. It has been proposed that airway inflammation, remodelling and peripheral bronchoconstriction could prevent airways from stretching (Pellegrino et al. 1998) in these subjects. It has also been postulated that the primary defect in asthma is in the airway smooth muscle and
that it exists in a distinct ‘latchlike’ manner because of long standing inflammation and remodelling (Jensen et al. 2001).

A ratio of maximal to partial flow (M/P) at a given lung volume is the most popular method of assessing the effect of DI on airway calibre in adults (Pellegrino et al. 1998). If DI results in bronchodilation M/P will be > 1, whereas if bronchoconstriction occurs, M/P < 1. However, a change in M/P will also occur if there is a change in residual volume (RV), either as a result of bronchial challenge or the effect of DI per se. These assessments are critically dependent on knowledge of absolute lung volume and therefore are very difficult to emulate in infants.

In this study, it was sometimes possible to overlay partial and full F-V loops along the descending portion of the expiratory curve, suggesting absence of any effects of DI (Figure 5.6). By contrast, in other infants, much higher flows were observed during the RVRTC than were expected from the partial manoeuvres (Figure 5.7). When this occurred, the most usual pattern appeared to be a parallel shift of the F-V loop which is suggestive of a change in lung volume rather than a change in slope, which might have been observed had there been a direct bronchodilator effect (Pellegrino et al. 1998). This in turn suggests that there may be recruitment of atelectactic areas during lung inflations in some infants (Figure 5.7).

A bronchoconstrictor effect could potentially have been elicited in this study due to the fact that cold dry air direct from a wall supply was delivered via the Neopuff to augment the breaths for raised lung volume. While the possibility cannot be excluded, it remained highly unlikely in that when any discrepancy existed between the full and partial manoeuvres, flows were generally higher at similar lung volumes during raised volume technique, not lower as would be expected in the presence of bronchoconstriction. Furthermore, induction of bronchoconstriction by cold and/or dry air challenge requires a much more prolonged and intensive period of hyperventilation (Gustafsson and Kjellman, 2000; Nielsen and Bisgaard, 2001) than infants were exposed to during the 4-5 augmented breaths prior to each forcing manoeuvre in this study. Nevertheless, for future studies it would seem prudent to deliver gas that had been pre-conditioned to at least ambient conditions.
In animal studies, morphometric analysis on lung development has shown that fetal growth restricted lambs had a smaller number of alveoli per respiratory unit, thicker interalveolar septa and a greater volume density of lung tissue when compared to controls (Maritz et al. 2001). Similarly, exposure to nicotine during pregnancy and lactation in rats appears to interfere with neonatal alveolar development and lung cell growth (Maritz, 1988; Maritz et al. 1993). A recent study also demonstrated a gender difference and reported that girls have a greater increase in flows after maximal inhalation than boys (Marotti et al. 2001). Thus differences in airway wall compliance, parenchymal tethering and the extent to which airway wall closure occurs during tidal breathing and the association with the high chest wall compliance in infants may contribute to the variety of responses observed (Figure 5.6 and Figure 5.7).
Figure 5.8  Overlay of Tidal and RVRTC curves showing no apparent ‘Big Breath’ effect

No effect from lung inflations prior to forced expiration and hence infant was observed to breathe down to the same residual volume in both tidal and RVRTC manoeuvres.

Figure 5.9  Overlay of Tidal and RVRTC curves showing ‘Big Breath’ effect

Had the partial and full F-V curves been overlaid on the descending portion as in Fig 5.6, the end expiratory level observed during tidal breathing would have been below the elastic equilibrium volume (EEV) extrapolated from the expiratory portion of the passive inflation prior to forcing expiration.
There was no clear pattern observed as to which group of infants did or did not have the ‘Big breath’ effect. Nevertheless, the mechanical relationship between airways, lung volume and its response to deep inhalation may be associated with cellular changes resulting from adverse intra-uterine events. However, the mechanism underlying these observations will remain highly speculative until improved methods of simultaneously measuring forced expiratory manoeuvres and absolute lung volumes can be developed for use in infants. The reason why so many healthy babies do not apparently demonstrate any ‘Big breath’ effect also needs to be elucidated.

5.7 Association between low birthweight and airway function

The results of this study suggest that both lung volume and airway function as reflected by FVC, FEV₀.₄ and MEF₂₅, are diminished during the first few months of life in infants who are born small for gestational age. FVC and FEV₀.₄ remained significantly lower in SGA than AGA infants after adjustment for body size, maternal smoking and social class but MEF₂₅ was no longer significantly reduced in SGA infants after adjustment for sex.

To illustrate the influence of birthweight status and other contributary factors on FVC and FEV₀.₄ during early infancy, prediction equations derived from multivariate analyses (Table 4.19 and Table 4.20) were applied to a best and worst case scenario for AGA/SGA infants of average age and length for the current study population i.e. 6 weeks of age with a crown-heel length of 55 cm.

\[
\text{FVC} = -209 - 9(\text{SGA}) + (6 \times \text{length}) + (3 \times \text{age}) - 5(\text{smoking}) - 3(\text{manual occupation});
\]

\[
\text{FEV₀.₄} = -138 - 8(\text{SGA}) + (4 \times \text{length}) + (2 \times \text{age}) - 6(\text{smoking}) - 2(\text{manual occupation});
\]

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Table 5.1 Comparison of FVC and FEV$_{0.4}$ between BEST and WORSE case scenarios

<table>
<thead>
<tr>
<th></th>
<th>BEST case scenario</th>
<th>WORST case scenario</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(girls of non-smoking mother with a non-manual occupation and no history of maternal asthma)</td>
<td>(boys of smoking mother with a manual occupation and a history of maternal asthma)</td>
</tr>
<tr>
<td>AGA</td>
<td>SGA</td>
<td>% difference (95% CI)</td>
</tr>
<tr>
<td>girl</td>
<td>girl</td>
<td>6% (7%, 12%)</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>139</td>
<td>130</td>
</tr>
<tr>
<td>FEV$_{0.4}$ (mL)</td>
<td>94</td>
<td>86</td>
</tr>
<tr>
<td>% difference: (AGA - SGA)/AGA</td>
<td>9% (1%, 17%)</td>
<td>7% (7%, 12%)</td>
</tr>
</tbody>
</table>

From these prediction equations, it can be seen that after adjusting for confounding factors, in the ‘BEST’ case scenario (i.e. girl of non-smoking mother with a non-manual occupation and no history of maternal asthma), being SGA was associated with an average of 6% (9/139) reduction in FVC and an average 9% (8/94) reduction in FEV$_{0.4}$. When applied to the ‘WORSE’ case scenario (i.e. boy with mother who smokes, had a manual occupation and with a history of maternal asthma), a similar reduction in FVC and FEV$_{0.4}$ (7% and 9% respectively) was observed. Such reductions in respiratory function might contribute to the increased incidence of respiratory morbidity observed among SGA infants in the first year of life (Vik et al. 1996) since infants with diminished premorbid airway function are known to be at increased risk of subsequent wheezing illnesses (Martinez et al. 1991; Dezateux et al. 1999; Dezateux et al. 2001).

5.7.1 Relation to previous studies

In a study based on nine SGA infants, whose gestational age ranged from 33 - 41 weeks, Dahms et al. reported elevated compliance and crying vital capacity but normal functional residual capacity when compared with appropriately grown infants of similar birthweight and concluded that intrauterine stress leads to increased pulmonary maturity (Dahms et al. 1974). However, this study was small and gestational age was determined from physical appearance and neurologic...
characteristics, which are less accurate than sonographic assessments. We are not aware of any other attempt to assess lung function in SGA infants.

A number of studies have been published reporting an association of low birthweight with diminished airway function in older children (Rona et al. 1993) and adults (Barker et al. 1991; Stein et al. 1997; Shaheen et al. 1998; Barker et al. 1991; Barker et al. 1991). With the exception of one study of British school children by Rona et al., which included maternal report of birthweight and gestational age, other published studies (Barker et al. 1991; Stein et al. 1997) have not taken account of gestational age when assessing associations between airway function and birthweight in their study population. Thus it is unclear whether the associations reported between low birthweight and diminished airway function reflect prematurity or low birthweight for gestational age.

Fetal and early postnatal life are periods of rapid growth and development of the respiratory system. Bronchial development and airway branching are mainly complete by the 16th week of gestation (Hislop, 1995). Risk factors for fetal growth restriction such as exposure to maternal smoking or malnutrition occurring during critical periods of growth and development may have detrimental effects on growth and maturation of organs and tissues. Thus any insult occurring in the first few months of pregnancy may cause developmental alterations, resulting in permanent changes to the airway branching system (Hislop, 1995) and it has been suggested that this 'programming' is to some extent irreversible (Barker and Fall, 1993). Furthermore, minor alterations in lung structural development during fetal life may have marked postnatal consequences, leading to critical disturbances in airway calibre in response to subsequent respiratory infections and resulting in severe, and potentially fatal, respiratory compromise (Martinez et al. 1988).

However, this programming hypothesis has been challenged, and it has been proposed that health in later life may have been due to the cumulative effect of life events along developmental trajectories (Power and Hertzman, 1997). Recent evidence from the 1958 British Birth Cohort suggests that health in later life is strongly associated with social class at birth for factors such as birthweight, childhood material circumstances, height, educational attainment and smoking
behaviour (Power, 1992). Thus, while Rona and colleagues reported a significant association between birthweight (adjusted for gestational age) and lung function (FVC and FEV₁) in children aged 5 to 11 years, it is possible that this association reflects the impact of other intervening exposures, related to low birthweight and impaired airway function but occurring prior to the test occasion. In our study we have looked at the association between airway function and birthweight before any insult or intervening factor occurs to confound results.

A number of mechanisms whereby diminished fetal growth may affect airway growth and development were discussed in Chapter 2. Of these, maternal smoking is most important and may confound the association between low birthweight and airway function. Hence the confounding effects of maternal smoking and low birthweight for gestational age will be discussed in section 5.10.

5.8 Association between sex and the airways

Our findings suggest that peripheral airway function is reduced in boys shortly after birth. This is consistent with most previously published observations during infancy and childhood (Hanrahan et al. 1990; Rona et al. 1993; Hibbert et al. 1995; Stocks et al. 1997; Hoo et al. 1998; Jones et al. 2000). In a recent multi-centre collaborative study for reference standards for $V'_\text{maxFRC}$, Hoo et al. reported that the rate of increase in $V'_\text{maxFRC}$, which is thought to reflect airway growth, proceeds more slowly in boys than girls from birth to 6-9 months of age, then accelerates faster than girls, so that when predicted on age, flows are similar by 15 months and 10% greater in boys by 18 months. However, when based on length, $V'_\text{maxFRC}$ remained higher in girls until at least 75 cm (Hoo et al. 2002) These differences appear to persist in childhood and adolescence (Taussig, 1977; Pagtakhan et al. 1984).

As airway function appears to be reduced in boys when compared with girls, further decrements in airway function associated with low birthweight for gestational age might increase the risk of respiratory symptoms and the need for assisted ventilation. Given the exclusion criteria used in this study, this could have resulted in only the fittest of SGA boys being eligible for inclusion in this study. There was however no
evidence from our audit of births that SGA boys were more likely than girls to be admitted to neonatal special or intensive care units during the study period.

5.8.1 Differences in airway structure

There is another reason for the clear sex differences noted in all measures of peripheral airway function in this study. As discussed in Section 2.9.1, airway structure has been shown to differ in male and female infants, and it has been suggested that the greater amount of smooth muscle as well as the thicker inner airway wall in boys may provide part of the explanation for sex differences in airway function in early life (McKay, 2000). The observed sex differences in flow may also be attributed to differences in airway tone (Landau et al. 1993) and/or lung mechanical properties between boys and girls (Taussig et al. 1981).

A recent study of 475 children at age 11 years has also suggested that after maximal inhalation, girls demonstrated a greater increase in flows than boys (Marotti et al. 2001), suggesting that a sex specific bronchodilator effect of deep inhalation occurs during childhood. These authors also reported that tobacco smoke exposure had a sex specific effect, which was dependent on the timing of the exposure (Marotti et al. 2001). Exposure to smoking during pregnancy (independent of current smoking) was associated with minimal bronchodilatory effect of deep inhalation in girls compared to the non-exposed group, but this difference was not apparent in boys. Conversely, current maternal smoking was associated with a bronchodilatory effect of deep inhalation in boys compared to the non-exposed group, but not in girls.

5.9 Association between maternal smoking and low birthweight

In common with other studies, we have shown that the birthweight of infants whose mothers smoked in pregnancy and/or postnatally was on average 200 g less than that of infants of non-smoking mothers (Ahlsten et al. 1993; Oyen et al. 1997; Zaren et al. 2000; England et al. 2001). In addition, more SGA infants were born to mothers who smoked (Kramer et al. 1990; Schellscheidt et al. 1998; Allen et al. 1998) and a significantly higher proportion of these mothers were multiparous and less likely to breast feed their infants (Schulte-Hobein et al. 1992; Ahlsten et al. 1993).
Furthermore, significantly more mothers who smoked were in manual occupations prior to delivery (Kramer, 1987).

Published data reporting the association of maternal smoking and intra-uterine growth are consistent. While many reports have demonstrated a dose-response relationship, with birthweight reduction being inversely related to the number of cigarettes smoked per day (Kramer, 1987; Eskenazi et al. 1995; Horta et al. 1997; Peacock et al. 1998) and the risk of sudden infant deaths (Wisborg et al. 2000), others have reported that the effect appears to be dependent on the period in pregnancy when the mother smoked (Ahlsten et al. 1993; Eskenazi et al. 1995; Lindley et al. 2000). However, a recent study has reported that while mean adjusted birthweight decreased as the number of cigarettes smoked per day increased, this relationship was not linear, with the sharpest decline in birthweight occurring at low levels of cigarette smoking (England et al. 2001). Thus it may appear that the strict criteria set for this study in the classification of a smoker were justified.

Smoking is also increasingly associated with lower socio-economic class and educational status (Brooke et al. 1989; Tuthill et al. 1999). While demographic details of this study population suggest that mothers recruited to our study may have come from more affluent or better-educated background, nevertheless a similar trend was observed among those who smoked. Interestingly, in this study population, the percentage of non-smoking mothers who breast-fed their infants is similar to that of the Maternity Unit’s overall rate. Among mothers who smoked, the proportion who breast-fed their infants was halved. An earlier study has reported that 39% of smoking mothers, but 23% of non-smoking mothers changed to bottle-feeding within four weeks of delivery, the apparent reason being insufficient milk supply (Schulte-Hobein et al. 1992). It has been proposed that a nicotine-induced lack of prolactin is responsible for the insufficient milk supply (Baron et al. 1986). However, to date this hypothesis has not been tested comparing prolactin levels of lactating women who do or do not smoke.
5.10 Association between maternal smoking and airway function

Infants of both smokers and non-smokers were recruited to this study, as maternal smoking during pregnancy is known to be a major risk factor for both reduced airway function and being SGA. While there is considerable evidence to suggest an adverse effect of maternal smoking on forced flows derived from partial flow-volume curves (Hanrahan et al. 1992; Tager et al. 1995; Hoo et al. 1998; Young et al. 2000) there are minimal data to quantify this effect when using the raised volume technique. Although Jones et al examined the effects of smoking on parameters derived from the raised volume technique, infants were studied at an older age (mean [SD] age: 48 wk [34]), after considerable postnatal exposure (Jones et al. 2000). It was therefore important to include infants of smokers in this study, in order to determine the effects of maternal smoking shortly after birth, to examine any interactions between maternal smoking and birth status on airway function, and to establish the relative effect of such exposure on parameters derived from the partial and raised volume technique within the same infant.

This study has shown that peripheral airway function as reflected by $V'_{\text{maxFRC}}$ was significantly reduced in infants whose mothers smoked. However, the association between maternal smoking and MEF$_{25}$ was not significant. This may reflect the attenuation of the effects of smoking on MEF$_{25}$ by the deep inhalation bronchodilator response as well as a sex specific effect of smoking exposure (Section 5.6.3.1) (Marotti et al. 2001). Nevertheless, within an individual the effect of maternal smoking was associated on average with a 10% (95% CI: -3%, 23%; $p = 0.1$) reduction in MEF$_{25}$ and on average a 14% (95% CI: 1%, 27%; $p = 0.03$) reduction in $V'_{\text{maxFRC}}$ among ‘worse’ case scenarios (Table 4.21 and Table 4.22).

Numerous studies and reviews have demonstrated that maternal smoking is strongly associated with increased health problems in infants and children (Dezateux and Stocks, 1997; Strachan and Cook, 1997). In common with other studies of term infants and our own study of preterm infants, a significant reduction in $V'_{\text{maxFRC}}$ was observed among those exposed to maternal smoking (Hanrahan et al. 1992; Brown et al. 1995; Hoo et al. 1998; Young et al. 2000). This association remained significant even after adjusting for sex and maternal history of asthma.
Although both $V'_{\text{maxFRC}}$ and MEF$_{25}$ are thought primarily to reflect peripheral airway function, it may be that both these parameters are reflecting different generations of airway branching or are influenced by different mechanical properties (Section 5.6.3.1). Thus the measurement of $V'_{\text{maxFRC}}$ may reflect a particular generation of peripheral airways most affected by effects of maternal smoking, rather than the observed discrepancy being attributed to varying sensitivities of the two parameters or due to misclassification of smokers (Section 5.10.1). While adverse effects of prenatal exposure to tobacco on airway function in preterm and term infants have been clearly demonstrated (Stick et al. 1996; Hoo et al. 1998), we were not able in this study, to separate the effects of prenatal from postnatal tobacco smoke exposure. Nevertheless, others have reported that exposure to maternal smoking was associated with reduced MEF$_{25}$ in school age children (Gilliland et al. 2000) and FEV$_1$ in adults (Upton et al. 1998). In addition, maternal smoking during pregnancy has also been linked to an increase in the prevalence of asthma (Gilliland et al. 2001) and wheezing during infancy and childhood (Dezateux et al. 1999; Young et al. 2000). However, the association between maternal smoking and the growth and development of airway structure and function remains unclear.

A recent study of children who died from sudden infant death syndrome, in which the structure of the infant airway wall was compared between those who were and were not exposed to maternal smoking, reported that inner airway wall thickness in small respiratory and terminal bronchioles was increased in infants whose mothers smoked $> 20$ cigarettes per day (Elliot et al. 1998). It is unclear whether the structural changes reported were a result of in-utero exposure or passive exposure via inhalation postnatally, causing tissue oedema or excessive structural fibres being laid down in these areas. Nevertheless, alterations to the airway wall structure, particularly increased inner airway wall thickness in peripheral airways would have had major effects on airway physiology and could explain the observed reduction in peripheral airway flow as discussed above and increased airway reactivity observed in other studies (Young et al. 1991).

There is also compelling evidence from animal studies which may explain the possible mechanism by which tobacco smoke exposure influences lung development and airway function. The recent identification of nicotinic acetylcholine receptors
(nAChR) in airway epithelial cells, air space parenchymal cells and fibroblast layers surrounding blood vessels and airways, suggests that the direct interaction between nicotine and nAChR in fetal lung may underlie many of the postnatal airway function abnormalities seen in human infants and as observed in this study (Sekhon et al. 1999; Sekhon et al. 2001). With prenatal nicotine exposure, levels of α7-nAChR increase in the fibroblast layers. Airway wall thickness and collagen expression both increase in parallel with the increase in α7 expression. Collagen, which accounts for the bulk of the extracellular matrix protein in the lung, is important for tensile strength and rigidity and together with elastin is important for lung recoil. Increased wall thickness and accumulation of collagen in the airway wall would reduce the calibre of airways and make them less pliable. Such alterations in the morphometric dimensions or airway compliance would be expected to produce significant changes in pulmonary resistance and expiratory flow, as indeed were observed by Sekhon et al (Sekhon et al. 2001). In addition, these authors have shown that in utero nicotine exposure adversely affected fetal lung development as reflected by decreased lung weight and lung volume in the nicotine-exposed newborn monkey (Sekhon et al. 1999). This study demonstrates that prenatal nicotine exposure alters pulmonary function at birth independently of any socio-economic or nutritional confounders and suggests that nicotine, transported across the placenta may be the key constituent of cigarette smoke to impair fetal lung development, alter lung function and increase respiratory illness in the offspring (Sekhon et al. 2001). These findings also have implications for the safety of nicotine replacement therapy during pregnancy, although the dose of nicotine delivered by gum or patch is typically less than that from cigarettes. Such therapies is currently contraindicated and extensive animal studies would be required prior to amending such recommendations. The ultimate cost benefit would obviously depend to a large extent on how heavily the mother smoked prior to intervention.

5.10.1 Residual confounding and effect of smoking classification

To examine the main effect of birthweight on airway function in infants, adjustments for potentially relevant factors have been made in the analyses, as confounding is the most important threat to the validity of results. However, residual confounding remains a potential serious problem in research. Residual confounding arises
whenever a confounding factor cannot be measured with sufficient precision, a situation that often occurs in epidemiological studies (Phillips and Smith, 1991).

By including those who were light smokers or those who stopped smoking after 8 weeks gestation, we may have masked the effects of smoking in our study population. This is suggested by the fact that when mothers who were 'light smokers' were excluded from the univariate analysis, MEF$_{25}$ was significantly diminished in infants whose mothers were 'definite smokers' (Table 4.21), but not significantly diminished when 'light smokers' were included.

Although infant exposure to tobacco smoke exposure was confirmed by an objective biochemical assay of cotinine in infant urine at time of test, this reflects not only exposure to maternal smoking but also to smoking from other household members or caregivers as well as to ingestion of cotinine via breast milk. However, cotinine is only a quantitative bio-marker for smoking and it is likely that there are other chemicals or compounds in tobacco smoke that are responsible for the adverse effects of smoking. Furthermore the degree to which these compounds are correlated with cotinine in breast milk is still unknown. However, at present assessment of cotinine levels is widely used to confirm smoking exposure (Jarvis et al. 2000).

5.10.2 $t_{PTEF:TE}$ - association with smoking exposure

Previous studies on premorbid airway function in infants have reported that $t_{PTEF:TE}$ is reduced in infants exposed to maternal smoking and that this precedes and predicts wheeze in infancy (Stick et al. 1996; Hoo et al. 1998; Dezateux et al. 1999). However, this has not been confirmed by others who have reported no difference in premorbid measures of $t_{PTEF:TE}$ among those with subsequent wheezing (Clarke et al. 1994; Adler et al. 1995). In the current study, $t_{PTEF:TE}$ did not differ between infants whose mothers smoked and those whose mothers did not. This lack of association may reflect the inclusion of light smokers. In fact, when mothers who smoked > 10 cigarettes per day were compared with non smokers, $t_{PTEF:TE}$ was significantly diminished in those infants heavily exposed to maternal smoking (mean [SD]: 0.31 [0.1] vs. 0.35 [0.1] s; 95% CI of the difference: 0.004, 0.08; p = 0.03). These results are similar to those reported by Stick and colleagues when a comparison between
non-smokers and the same classification of smokers (> 10 cigarettes per day) was performed (Stick et al. 1996).

5.10.3 Sex imbalance within the smoking subgroups

In this study, two thirds of SGA girls who were recruited and tested were born to mothers who smoked, compared with one third among the boys. Among the AGA infants born to mothers who smoked, one third were girls and two thirds were boys. As various studies have consistently shown that girls have better respiratory function than boys during infancy (section 5.8) and that maternal smoking is strongly associated with poorer airway function from infancy to adulthood (section 5.10), this imbalance complicates interpretation of results. For example, it is likely in our study that on univariate analysis, some of the negative impact of smoking may be negated by the positive effect of being female. Results from a recent study investigating the association between maternal smoking and fetal growth, which compared pregnant women who smoked heavily (> 10 cigarettes per day) with those who did not smoke, suggested that the fetal growth restricting effect from maternal smoking may affect boys more than girls (Zaren et al. 2000). It has been postulated that factors limiting fetal growth could have a greater impact in fetuses with greater intra-uterine growth velocity. As boys have a higher rate of growth than girls, but mature more slowly than girls, it could be that boys are more vulnerable as they may be more dependent on a ‘perfect’ ambience in the hormonal milieu for organ maturity.

5.11 Association between maternal asthma and airway function

A surprising finding of this study was the significant association between diminished peripheral airway function ($V'_{\text{maxFRC}}$ and MEF$_{25}$) and maternal asthma. While similar associations between family history of atopy and airway responsiveness in a group of young infants have been reported (Young et al. 1991), our findings suggest that maternal asthma may be the most important aspect. Young et al. reported that airway responsiveness was increased in infants with a family history of atopy compared to infants without this history, suggesting the heritability of airway responsiveness. Furthermore, it has also been reported that specific airway conductance was significantly lower among infants with a positive family history of
asthma than those without and that infants with wheezing were more likely to have a first degree relative with asthma (Dezateux et al. 1999; Dezateux et al. 2001). While not suggesting that these infants are likely to be asthmatic in later childhood, our findings appear to be consistent with evidence from epidemiological studies that there is an important genetic contribution to the aetiology of asthma (Dold et al. 1992; Le Souëf, 1995; Litonjua et al. 1998; Los et al. 1999). From multivariate analyses for MEF$_{25}$ and $V'_{\text{maxFRC}}$ (Table 4.21 and Table 4.22), it can be seen that a history of maternal asthma could be associated with up to a 30% (95% CI: 11%, 49%; $p = 0.002$) reduction in MEF$_{25}$ and a 33% (95%CI: 6%, 58%; $p = 0.02$) reduction in $V'_{\text{maxFRC}}$ in boys whose mothers smoked. However, these data had not been able to clarify the pattern or mechanism of the inheritance of asthma.

It has also been suggested that the environment has a greater influence than genetics in the development of asthma, though the interaction between heredity and environment is a major problem, as separating the environmental and genetic effects has proved very difficult (Le Souëf, 1995).

Nevertheless, maternal environmental characteristics such as smoking (Weitzman et al. 1990) and infections (Xu et al. 1999) during pregnancy, have also been strongly associated with subsequent asthma development. Within the developing fetus, a weak Th2 response normally develops as a result of fetal priming to help maintain pregnancy. In utero exposure to allergens may significantly enhance the Th2 response (Piccinni et al. 1993; Jones et al. 1996). The fetal response to allergens may also vary according to genetic disposition. Thus, Tantisira and Weiss proposed that the result of interactions between genetics and the in utero environment is a Th2 skewed immunophenotype in the neonate, and that subsequent interactions with external environmental exposures (including infections), in conjunction with genetic predisposition, lead to development of asthma (Tantisira and Weiss, 2001).
5.12 Implications and future directions

This study has focussed on the influence of being low birthweight for gestation on respiratory development and function during early infancy. As discussed, the findings presented in this thesis were from the healthiest of SGA infants and therefore the differences observed in airway function between SGA and AGA infants were an underestimation of this association, such that our findings may only reflect the tip of the iceberg. Furthermore, associations of airway function in the infant with maternal height and social class have not been reported previously, and indicate the complexity of the causal chain linking socio-economic disadvantage to low birthweight (Dezateux et al. 2002, submitted).

In addition to compromises in respiratory health as discussed, IUGR is a risk factor for impaired somatic growth (Section 1.3.2) and neurological and behavioural deficits. SGA infants appear to be at increased risk for neurodevelopmental abnormalities and decreased cognitive performance, although data are difficult to interpret due to small sample size and inclusion of infants with underlying conditions and neonatal complications that affect outcome. Affected children with neonatal complications had significantly lower IQ scores and poorer neurodevelopmental outcome at three years than did those without complications (Fattal-Valevski et al. 1999). When complications such as birth asphyxia were excluded, term SGA infants had a good prognosis for cognitive and neurological development at 13 – 19 years
(Westwood et al. 1983). However, being born SGA at term is associated with poorer school performance at 12 and 18 years (Larroque et al. 2001). While the precise biological mechanism underlying these associations is unclear, animal studies have shown that IUGR induced during the second half of pregnancy is associated with reduced numbers of neurons in the hippocampus and the cerebellum in conjunction with retarded dendritic and axonal growth within these structures (Mallard et al. 2000). Thus alterations in neurodevelopment in these regions may therefore lead to a broad range of functional deficits in the postnatal animal, including the human infant.

Similarly, a broad range of epidemiological evidence supports the hypothesis that risk of essential hypertension, coronary heart disease and non-insulin dependent diabetes is, in part, determined before birth (Barker and Fall, 1993; Barker, 1995; Barker, 1995). An increasing number of human studies indicate that the developing kidney is particularly vulnerable to the adverse effects of fetal growth retarding influences. In animals, growth retarding diets or other insults which have an impact upon the development of cardiovascular function, also appear to impact upon nephron number (Marchand and Langley-Evans, 2001). Glomeruli number was significantly reduced in IUGR rabbit fetuses (Bassan et al. 2000), which may contribute to impaired renal function, predisposing to neonatal renal dysfunction and late sequelae, such as adult hypertension (Barker, 1990). The various organ adaptations that result from growth restriction in-utero and, which may in turn result in subsequent adult diseases, emphasises the clinical importance of early IUGR diagnosis and prevention.

5.12.1 Implications for policy and practice

We have shown the importance of gestational age when classifying birthweight status in infants. Yet the Office of National Statistics (ONS) does not collect this information as part of routine birth data. We would propose that gestational age is included as routine ONS birth data to allow the percentage of SGA births to be reported and its relevance to later health outcomes assessed.
It is generally recognised that low birthweight is the end of a causal chain and that modifiable factors such as nutrition, smoking and socio-economic conditions, which have large effects on intrauterine growth should be targeted for public health intervention. While there is little evidence of benefit in nutritional supplementation during pregnancy (Mathews et al. 2000) and that changes in the socio-economic status may require long term planning, the intervention that is likely to have the largest impact on intrauterine growth is to reduce maternal smoking. A recent report from the Department of Health has recommended that the timing and nature of advice provided by doctors and midwives to pregnant smokers should be standardised and the effectiveness of such measures should be evaluated (Hijazi et al. 2000). Furthermore, the committee also recommended that a randomised trial is needed on the efficacy and safety of nicotine replacement therapy for pregnant women who smoke heavily and are unable to give up smoking with current advice and support. In the Cochrane review based on 34 trials, Lumley et al. concluded that smoking cessation programs in pregnancy appear to reduce smoking, low birthweight and preterm births but no effect was detected for perinatal mortality (Devereux et al. 2002). Nevertheless, smoking cessation programs should be actively incorporated as part of routine maternity care and general health promotion.

Early delivery of growth restricted fetuses to prevent further compromise may not improve subsequent airway growth, since recent work from our own laboratory has shown that for otherwise healthy infants born at a mean age of 33 weeks gestation, $V'_{\text{maxFRC}}$ was significantly lower (-2 SD) when compared to healthy term equivalents at approximately one year of age (Hoo et al. 2001). Furthermore, it has also been suggested that premature delivery is likely to affect subsequent airway and alveolar development since airway size increases together with multiplication and maturation of the alveoli during the last trimester (Zeltner et al. 1986; Hislop et al. 1987; Hislop, 1997).

5.12.2 Implications for research

The subject numbers reported in this thesis were frozen at an earlier date (February 2001) to allow for the writing of this thesis. Despite the complexity of the techniques used, the need to sedate infants and the time consuming nature of the
tests, which limits the number of infants that can be studied per day, we have been successful in achieving measurements on the first occasion in 224 infants (92 SGA; 132 AGA) to date. In order to clarify whether this reduction in respiratory function in SGA infants persists through infancy, further follow-up of this cohort is required to establish the pattern of growth and development of the airways in relation to sex, birthweight status and subsequent somatic growth. We are currently funded for follow-up measurements at 6-8 months of age and to collect morbidity data from GP records at 1 year of age. Thus, the association between initial airway function and morbidity at one year of age could then be further examined as adverse influences during this period may diminish airway or alveolar growth and hence maximal lung and airway size attained. For those in whom maximal fetal and early childhood growth potential has not been achieved, a steeper age-related decline in respiratory function (which normally commences in mid adult life) may occur, such that the critical threshold at which respiratory symptoms become manifest may occur at an earlier age (Brown and Weiss, 1991). Thus it would be interesting if follow-up of this cohort could be achieved up to at least school age, such that airway function could be repeated at 1 year, 3-4 years and 7 years of age, using multiple breath washout by mass spectrometry (Gustafsson et al. 1994) and forced expiratory manoeuvres or spirometry. The former technique has recently been adapted for use in infants and pre-school children in our department and may be more sensitive to early changes in small airway function than the RVRTC.

The significant association between diminished peripheral airway function in infants and maternal asthma was a surprising finding in our study. Since this was not an aim in our study, this finding needs confirming with a larger population. It may also be possible to distinguish whether the basis of the association is genetic or environmental, by comparing airway function of infants whose mothers smoked but had no physiological or clinical evidence of asthma to those infants with mothers who did not smoke but with a history of asthma.

Maternal smoking during pregnancy is a known risk factor for low birthweight, sudden infant death syndrome and increased respiratory morbidity through infancy. Thus, to document fetal and infant exposure to maternal smoking accurately, data should be collected prospectively. In addition, it would be helpful to be able to
distinguish tobacco smoke exposure from the difference sources, such as the mother and father separately and the extent of these influences on the infant's airway function. However, a larger sample size would be required.

To assess the best method for classifying birthweight centiles in infants properly, further analysis of CGF and GROW methods in larger populations is needed.

The RVRTC technique is widely used for measuring airway function in infants. However, the effect of deep inhalation from augmented breaths during RVRTC in infants is still unclear. We have observed that there was no clear pattern as to which group of infants did or did not have the 'Big breath' effect and the mechanism underlying these observations remains speculative. Therefore, improved methods, which allow simultaneously measurement of forced expiratory flows and volumes and absolute lung volume needs to be developed for use in infants. Better understanding of what the parameters are actually reflecting is also essential.

In addition to evidence provided regarding the effects of SGA on airway function in infancy, data collected from this study could potentially make a substantial contribution towards development of reference values for the various parameters derived from the RVRTC technique. To construct such reference data, it would be essential to: a) exclude data from infants in whom inflation pressure less than 2.7 kPa had been used; b) randomly exclude all but 10% of SGA infants in order to create a sample that would be representative of the normal population, i.e. one in which only 10% of infants would have birthweight ≤ 10th centile for gestational age; c) obtain more measurements in older infants during the first 2 years of life.

5.13 Summary

The findings observed in this study suggest that there is an independent effect of being small for gestational age on airway function during early infancy. This effect is small in relation to other factors. It suggests limited support for the programming hypothesis at this age. It is however possible that the fetal environment may also 'programme' later postnatal lung and airway growth and development. Therefore
further follow-up of this cohort of infants is being undertaken and will be specifically examined in infants of non-smoking mothers.
References


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WHO (1999) International consultation on environmental tobacco smoke (ETS) and child health. Consultation report.


Appendices

Appendix A: Parents information leaflet

Appendix B: Project questionnaire

Appendix C: Ethical approval from East London and The City Health Authority Research Ethics Committee

Appendix D: Ethical approval from the Institute of Child Health, London

Appendix E: Parents consent form

Appendix F: Post sedation advice

Appendix G: Equipment used and manufacturer's details

Appendix H: Group characteristics at test according to smoking status and birthweight

Appendix I: Sample size calculations

Appendix J: Comparison of recruitment process

Appendix K: Validation of neonatal anthropometric measurement

Appendix L: Child Growth Foundation paper chart

Appendix M: Audit of infants born during 1999-2000 at Homerton Hospital
The influence of low birthweight on lung development in infancy

Information for Parents

While not usually causing any problems, babies with narrower breathing tubes are more likely to wheeze when they catch a cold during the first year of life. In order to find out more about the factors which affect breathing in babies, we are measuring the size of the lungs and airways in healthy babies who were smaller than expected at birth, as well as those with normal birth weight.

We should like to invite you and your baby to take part in this research study.

- Breathing tests with your baby will be carried out when s/he is about a month old, at the infant lung function room, in Special Care Baby Unit, at the Homerton, on a day convenient to you.
- Travel costs will be provided to allow you to come by taxi if that is helpful.
- The tests will be explained to you in detail, and a routine questionnaire completed.
- Your baby will breathe through a small mask which sits around the nose and mouth and therefore it is important that s/he is soundly asleep. We usually give a mild sleeping syrup called chloral, by mouth, to ensure the baby has a good nap while we are doing the tests. This syrup has been used in thousands of babies without any problems.
- At this visit, we will try to collect a small specimen of urine from the baby, and saliva from mother (this simply requires placing a cotton wool bud in the mouth for a few minutes).
- Babies usually stay asleep throughout the measurements but will begin to wake up towards the end of the test or immediately afterwards. They usually take a feed before going home. Although the measurements themselves do not take very long, we have to wait for the baby to fall asleep so the whole visit usually lasts for a morning or an afternoon. Parents are very welcome to remain for the whole time if they like or are free to come and go as they wish.

Where can I get more information?

You can speak to our Research Midwives Ah-fong Hoo and Sooky Lum about this work, either

- on the Special care Baby Unit at the Homerton Hospital, telephone no. 020 8510 7868, or
- at the Respiratory Lab, Great Ormond Street Hospital, on 020 7405 9200 ext.5454

Thank you for your time.
Appendix B  Project questionnaire

SGA PROJECT

Baby's first name

Baby's surname

Baby's hospital number

Baby's NHS number

Baby's date of birth

Parent's names

Home address

Telephone numbers:  

or

GP

Practice address:

Telephone number:
### Maternal Details

**Previous Pregnancies (from notes)**

- **Mother’s Name:**
- **Mother’s hospital number:**
- **Mother’s NHS number:**
- **Mother’s date of birth:**
- **Booking weight (kg):**

### Details of previous pregnancies:

List outcome of previous pregnancies including miscarriages, terminations and stillbirths.

<table>
<thead>
<tr>
<th>Date of Delivery (month/year)</th>
<th>Gest. age (weeks)</th>
<th>Birth weight (g)</th>
<th>Sex</th>
<th>Centile</th>
<th>Complications/comments</th>
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</tr>
</tbody>
</table>

### Relevant medical history:

- Essential hypertension □ No □ Yes
- Renal problems □ No □ Yes
- Diabetes □ No □ Yes
- Hyperthyroidism □ No □ Yes
- Haemoglobinopathy □ No □ Yes

**Other:**
give details
Infant Background Details
(for completion at discharge from maternity wards)

Date of birth

Place of birth

Birthweight (g)

Birth crown-heel length (cm)

Gestational Age

GA determined from:

Sex

Birth centile (CGF program)

Birth centile (GROW)

Admission to SCBU?

Discharge date

OFC (cm)*

Midarm circ. (cm)*

Chest circ. (cm)*

*to be determined by SHO at post-natal check

Placental weight (g)
Current Maternal Details
(to be completed at time of respiratory function tests)

Date of interview

Are details of home address and GP correct? (amend if necessary) Yes □

Mother’s measured height (cm):
If known - Mother’s birthweight:
Today’s weight (kg):

Did you smoke at any time while you were pregnant with [name] □ No □ Yes
If yes, approximately how many cigarettes per day?

Did you give up at any time? □ No □ Yes – when? (e.g. completed weeks):

Did you resume smoking during this pregnancy? ?
□ No □ Yes – when? (e.g. completed weeks):

Are you still smoking, or have you smoked since [name] was born? □ No □ Yes
If yes, approximately how many a day?

When did you have your last cigarette? (state time)

Maternal saliva collected? □ No □ Yes - time of collection

Does your partner smoke? □ No □ Yes - how many a day?

Was anyone else in your household a smoker during your pregnancy?
□ No □ Yes - how many people?

Were you exposed to any (other) cigarette smoke at work during your pregnancy?
□ No □ Yes - give details

Have you spent anytime with anyone who smokes in the past 24 hrs?
□ No □ Yes - give details

Does your baby spend anytime with anyone who smokes?
□ No □ Yes - If so, who?.................................

Has the baby spent any time with anyone who smokes during the past 24 hours?
□ No □ Yes

Urine/saliva sample □ Yes (Give time of collection: )
(delete where appropriate) □ No
I should now like to ask you some questions about your baby's (1st degree) family's health (i.e. parents, siblings and/or half siblings)

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has a doctor ever said that any member of your family has asthma?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>If any, specify who:</td>
<td></td>
</tr>
<tr>
<td>Has any member of your family, (not diagnosed with asthma) ever had</td>
<td>No  Yes</td>
</tr>
<tr>
<td>wheezing or whistling in the chest?</td>
<td></td>
</tr>
<tr>
<td>If any, specify who:</td>
<td></td>
</tr>
<tr>
<td>Has any member of your family ever had eczema?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>If any, specify who:</td>
<td></td>
</tr>
<tr>
<td>Apart from the illnesses I have mentioned above, have you or [baby’s]</td>
<td>No  Yes</td>
</tr>
<tr>
<td>father ever suffered from chest trouble?</td>
<td></td>
</tr>
<tr>
<td>If any, specify who &amp; what type of chest trouble?</td>
<td></td>
</tr>
<tr>
<td>Does anyone in your family (1st and 2nd degree) have cystic fibrosis?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>(incl. parents, siblings, grandparents, aunts, uncles and 1st cousins)</td>
<td></td>
</tr>
</tbody>
</table>

I should now like to ask you some questions about the work that you and [baby’s name] father do. It may seem surprising but babies’ breathing can be affected by the work their parents do. These questions won’t take long to answer and will be treated in strictest confidence.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>How old were you when you left full time education? (years)</td>
<td></td>
</tr>
<tr>
<td>Adjusted age (allowing for time out/part-time) (years)</td>
<td></td>
</tr>
<tr>
<td>Have you been in regular employment since leaving school/college?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>What is/was your most recent job before you had [baby]?</td>
<td></td>
</tr>
<tr>
<td>Did this involve supervising others?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>Are you self-employed? ?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>What is/was [baby’s] father’s most recent job?</td>
<td></td>
</tr>
<tr>
<td>Does/did this involve supervising others?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>Is he self-employed?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>Has he been in regular employment since leaving school?</td>
<td>No  Yes</td>
</tr>
<tr>
<td>If not, is this because of further education?</td>
<td>No  Yes</td>
</tr>
</tbody>
</table>
Infant Details
Lung Function Test

Date of birth
Date of test
Test weight (kg)
Crown-heel length (cm)
Crown-rump length (cm)
OFC (cm)
Chest circumference (cm)
Mid-arm circumference (cm)
Method of feeding: ☐ Breast fed ☐ Bottle fed ☐ Breast / bottle
Any solids? ☐ No ☐ Yes
(discuss)

Respiratory health of infant (only to be coded ** and used if no GP data available)
Since birth, has [baby’s name] had any breathing problems such as cough or runny nose? ☐ No ☐ Yes – details:
If ‘Yes’, has this been in the last 3 weeks? ☐ No ☐ Yes but asymptomatic for ☐ days ☐ Yes and still symptomatic.

(For coding later **)

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>URTI</td>
<td></td>
</tr>
<tr>
<td>probable LRI</td>
<td></td>
</tr>
<tr>
<td>definite LRI – no wheeze</td>
<td></td>
</tr>
<tr>
<td>definite LRI – with wheeze</td>
<td></td>
</tr>
</tbody>
</table>
Pre-sedation oxygen saturation (%):  

Sedation:  □ No  □ Yes ( = mg or mg/kg)  
Preparation used (delete as appropriate): Chloral hydrate : oral/suppositories Triclofos sodium

Post sedation/pre LFT respiratory rate (bpm):  

Post-sedation oxygen saturation (%):  

Posture?  □  (1 = supine  2 = prone  3 = lateral (R/L)*  4 = semi-recumbent)  

Barometric pressure (mbar)  

Size of Hans Rudolph PNT used*:  

Size of Jaeger PNT used*  

*circle as appropriate

Mask size:

Team members:  

Follow up (Month / year):
Appendix C  Ethical approval from East London and The City Health Authority Research Ethics Committee

Dr K L Cosicloe
Head of Academic Dept.
Child Health
Room 416 Alexandra House
The Royal London Hospital
Whitechapel
LONDON
E1 1BB

ref:meje/P/97/250  8 January 1998

Dear Dr Cosicloe

Re: Fr97/250 - The influence of low birthweight on respiratory function in infancy

Thank you for your letter, dated 19 December 1997, enclosing suggested amendments to the above mentioned study.

With specific reference to your revised recruitment of babies to below the 5th centile and below the gestational age of 34 completed weeks, I confirm that I am able to take Chairman's Action and approve these amendments as ethically satisfactory.

Yours sincerely

PROFESSOR M SWASH MD FRCP FRCPath
Chairman
ELCHA Research Ethics Committee

cc Dr J Stocks
Paediatric Anaesthesia
Intensive Care and Respiratory Medicine Unit
Institute of Child Health
30, Guildford Street
London WC1N 1EH
25 November 1998

Dr J Stocks
Reader in Respiratory Physiology
ICH

Dear Dr Stocks,

96EB23 The influence of suboptimal intrauterine growth on airway development and function in infancy.

Thank you for your recent correspondence. The Chairman of the Research Ethics Committee, Dr Duncan Macrae, has on behalf of the Committee approved the extension of measurements to take place at the Institute of Child Health.

The decision will be ratified at the full Committee meeting that will take place on Wednesday 9 December 1998.

Yours sincerely

Orlagh Sheils
Secretary to the Research Ethics Committee
Appendix E    Parents consent form

WRITTEN CONSENT FORM:         REC Number:

Title of research proposal: The influence of low birthweight on respiratory function in infancy.

Name of Parent (Block Capitals):

Address:

- The study organisers have invited me to take part in this research. □
- I understand what is in the leaflet about the research. I have a copy of the leaflet to keep. □
- I have had the chance to talk and ask questions about the study. □
- I know what my baby’s part will be in the study and I know how long it will take. □
- I have been told that my baby will be given some medicine to help to sleep through the tests. I understand about the breathing tests and the follow-up measurements. □
- I know how the study may affect my baby. I have been told if there are possible risks.  □
- I understand that my baby should not take part in more than one study at a time. □
- I know that the local East London and The City Health Authority Research Ethics Committee has seen and agreed to this study. □
- I understand that personal information is strictly confidential: I know the only people who may see information about my baby’s part in the study are the research team or an official representative of the organisation which funded the research. □
- I know that the researchers will/might tell my general practitioner (GP) about my baby’s part in the study. □
- I freely consent to my baby taking part in this study. No one has put pressure on me. □
- I know that my baby can stop taking part in the study at any time. □
- I know if my baby does not take part, he/she will still be able to have normal treatment. □

- I know that if there are any problems, I can contact:

Dr/Mr/Ms.......................................................... Tel. No .................................................

Parent’s Signature: .................................. Witness’s Name: .................................

Date: ................................................. Witness’s Signature: .................................

The following should be signed by the Clinician/Investigator responsible for obtaining consent

As the Investigator responsible for this research or a designated deputy, I confirm that I have explained to the parent named above the nature and purpose of the research to be undertaken.

Investigators Name: .............................Investigator’s Signature: ..............................

Date: ..............................................
Appendix F  Post sedation advice

Breathing Measurements in Babies and Toddlers

Information for parents

_______________________ has had a medicine (chloral syrup) to help him/her to sleep during lung function measurements. Although awake before being taken home, you will find ________________________ may remain rather sleepy for a few hours afterwards as the medicine wears off and, unless asleep, should not be left alone during this time in case he/she falls over. Although there has never been any problems with chloral syrup in the past, parents bringing healthy babies for measurements are given the telephone number of a contact with the hospital should they have any reason for concern after arriving home, however unlikely this may be.

Thank you for bringing ________________________ and allowing us to measure his/her lung function.

Contact name ____________________________

Telephone number ____________________________

Date_________________ Length ________________ Weight __________________
## Appendix G  Equipment used and manufacturer’s details

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂SMO/SpO₂ monitor</td>
<td>Novametrix Medical System INC. Connecticut USA</td>
</tr>
<tr>
<td>Digitron P200 Manometer</td>
<td>Digitron Instrumentation Ltd Herts. England</td>
</tr>
<tr>
<td>Face Masks (Rendell Baker Soucek)</td>
<td>Southern Syringe Service Ltd New Universl House 303 Chase Road Southgate London N14 6JB</td>
</tr>
<tr>
<td>Furness Transducers FCO44</td>
<td>Furness Control Ltd Redhill England</td>
</tr>
<tr>
<td>Hans Rudolph pneumotachometer, heater control and calibration syringe</td>
<td>Hans Rudolph INC. 7200 Wyandolte Kansas City MO 64114 USA</td>
</tr>
<tr>
<td>Harpenden Infantometer</td>
<td>CMS Ltd, Harpenden, England</td>
</tr>
<tr>
<td>Pressure regulator</td>
<td>Therapy Equipment Ltd England</td>
</tr>
<tr>
<td>Rotameter</td>
<td>KDG Instruments Ltd Taylors and Rotameter Works 59-61 Victoria Road Burgess Hill West Sussex RH15 9LY England</td>
</tr>
<tr>
<td>Seca electronic scales</td>
<td>Seca Ltd Birmingham, England</td>
</tr>
<tr>
<td>Therapeutic putty</td>
<td>Promedics Ltd Moorgate Street Blackburn Lancashire BB2 4PB England</td>
</tr>
<tr>
<td>Tubing – Vinyl (translucent) 800/012/200 ID 3.0mm</td>
<td>Southern Syringe Services Ltd New Universal House 303 Chase Road Southgate London N14 6JB</td>
</tr>
</tbody>
</table>
### Appendix H  Group characteristics at test according to smoking status and birthweight

<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th>Smoking</th>
<th>95% CI of Difference:</th>
<th>95% CI of Difference:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA (n = 40)</td>
<td>AGA (n = 65)</td>
<td>(Non-smoking) SGA-AGA</td>
<td>(Smoking) SGA-AGA</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td>28 (70%)</td>
<td>30 (46%)</td>
<td>5%, 43% *</td>
<td>15 (38%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 (64%)</td>
</tr>
<tr>
<td><strong>Age (wk)</strong></td>
<td>6.7 (2.6)</td>
<td>5.9 (2.3)</td>
<td>-1.7, 0.2</td>
<td>5.7 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.3 (1.8)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>4.3 (0.8)</td>
<td>4.7 (0.8)</td>
<td>-0.7, -0.1 *</td>
<td>3.9 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8 (0.7)</td>
</tr>
<tr>
<td><strong>Weight SD score</strong></td>
<td>-1.0 (0.8)</td>
<td>0.1 (0.9)</td>
<td>-1.4, -0.7 ***</td>
<td>-1.3 (0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0 (0.9)</td>
</tr>
<tr>
<td><strong>Length (cm)</strong></td>
<td>54.6 (2.4)</td>
<td>56.4 (2.9)</td>
<td>-2.9, -0.7 **</td>
<td>53.4 (3.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56.7 (2.5)</td>
</tr>
<tr>
<td><strong>Length SD score</strong></td>
<td>-0.7 (0.6)</td>
<td>0.6 (1.0)</td>
<td>-1.6, -1.0 ***</td>
<td>-0.8 (1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.4 (0.9)</td>
</tr>
<tr>
<td><strong>Head circumference (cm)</strong></td>
<td>38.2 (1.6)</td>
<td>39.0 (1.7)</td>
<td>-1.5, -0.2 *</td>
<td>37.5 (1.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.1 (1.4)</td>
</tr>
<tr>
<td><strong>Head circumference SD score</strong></td>
<td>-0.2 (0.8)</td>
<td>0.9 (1.0)</td>
<td>-1.5, -0.7 ***</td>
<td>-0.2 (1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7 (0.9)</td>
</tr>
<tr>
<td><strong>Chest circumference (cm)</strong></td>
<td>37.8 (2.5)</td>
<td>39.0 (2.2)</td>
<td>-2.2, -0.3 *</td>
<td>36.2 (2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39.2 (1.9)</td>
</tr>
<tr>
<td><strong>Mid arm circumference (cm)</strong></td>
<td>12.1 (1.4)</td>
<td>12.4 (1.2)</td>
<td>-0.8, 0.3</td>
<td>11.4 (1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5 (1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.7, -0.4 **</td>
</tr>
</tbody>
</table>

1 Data shown as mean (SD) for continuous and n (%) for categorical variables.

2 age after expected date of delivery

* p < 0.05; ** p < 0.01; *** p < 0.001
Appendix I  Sample size calculation

Variability in airway function due to known confounding factors

From previous studies conducted at this laboratory, it was known that a proportion of variability in airway function in infants can be explained during univariate analyses, by known confounders such as sex (5%), length (40%), age (20%), family history of asthma (5%) and smoking (5%) (Stocks et al. 1994; Stocks et al. 1997; Hoo et al. 1998; Dezateux et al. 1999; Dezateux et al. 2001). However, these figures can only provide a broad estimate as proportion variability ($R^2$) since growth and development will vary not only for each parameter but also for the age range studied. For example, while the $R^2$ of FVC for length may account for 0.9 of the total variability when examining a population from birth to 2 years of age (Jones et al. 2000), the relative contribution of length to overall variability will be much less when studying infants over a smaller age/size range as in this study. Similarly, while sex and smoking have marked effects on $V'_{maxFRC}$, these factors explain far less of the variability for FVC or FEV$_{0.4}$. Despite these caveats, for the purposes of determining the sample size necessary to attain a desired power to detect differences between groups, it is necessary to use a broad estimate of the likely variability accounted for by the independent variables in the calculation.

Calculation of sample size

The F test used in fixed multiple regression and correlation analysis is a test of the null hypothesis that the proportion of the variance in a parameter accounted for by some source (proportion variation, $R^2$) is zero in the population. All power analyses and calculation of sample sizes are based on 'guesstimates'. Such calculations also require the investigator to specify the size of the difference that would be clinically or aetiologically important to detect. The decision regarding what is a clinically or physiologically significant effect in turn will be determined at least in part by the within and between subject variability for any given parameter. In terms of sample size, the smaller the difference that must not be missed in relation to the between subject variability of the parameter after accounting for all other known confounders, the greater the number of subjects that will need to be measured. Similarly, the greater the confidence (power) that is required to ensure that such a difference is not
missed, the larger the sample size is required. For this study, it was decided that a difference in airway function due to being SGA of 10% after accounting for all other known variables would be aetiollogically important. Consequently, the calculations shown below (Cohen 1988) were used during the conception of this study.

Hence, in this study where the proportion of total variance of parameter $Y$ (e.g. $FEV_{0.4}$) which was accounted for by a set $B$ (i.e. study groups) and consisting of $u$ variables ($R^2_{Y,B}$, e.g. birthweight status, then $u = 1$) over and above the variance accounted for by a set $A$ consisting of $w$ variables ($R^2_{Y,A}$, e.g. sex, age, length and family history of asthma, and $w = 4$) is determined. This quantity is given by:

$$R^2_{Y,B} = R^2_{Y,A,B} - R^2_{Y,A} \quad \text{[equation 1]}$$

The error variance proportion (i.e. variance left after effects of both $A$ and $B$ are removed) is given by:

$$1 - R^2_{Y,A,B} \quad \text{[equation 2]}$$

$f^2$ or effect size index, is the ratio of these two (i.e. variability accounted for by $B$ over and above $A$ divided by the error variance).

Example: If $A$ accounts for 0.3 of the $Y$ variance ($R^2_{Y,A} = 0.3$) and $A$ and $B$ together account for 0.45 of the $Y$ variance ($R^2_{Y,A,B} = 0.45$) then $B$ uniquely accounts for 0.15, the error variance is 0.55 (= 1 - 0.45) and $f^2 = 0.15/0.55$ i.e. 0.27. [equation 3]

To calculate sample size, Cohen has provided a table whereby power values for the F test on the proportion of $Y$ variance accounted for by a set of $u$ variables $B$ are given (Table I.1).

<table>
<thead>
<tr>
<th>$u$</th>
<th>$v$</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>27</td>
<td>48</td>
<td>64</td>
<td>77</td>
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<td>80</td>
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<td>93</td>
<td>96</td>
<td>98</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>$\infty$</td>
<td>29</td>
<td>52</td>
<td>69</td>
<td>81</td>
<td>89</td>
<td>93</td>
<td>96</td>
<td>98</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

Note: Only 1 value of $u$ is shown here. In the original Cohen table, power values are given for the following 23 values of $u$: 1 to 15, 18, 20, 30, 40, 48, 60, 120.
v reflects the relative number of variables (u, w) to be adjusted in relation to total number of subjects (N) to be studied,

i.e. \[ v = N - u - w - 1 \] [equation 4]

\( \lambda \) is the noncentrality parameter of the noncentral F distribution and is a simple function of the effect size index \((f^2)\) and the number of variables \(u\) within the study group (e.g. birthweight status),

i.e. \[ \lambda = f^2 (u + v + 1) \] [equation 5]

Therefore, substituting equation 4 into equation 5,

\[ \lambda = f^2 (u + (N - u - w - 1) + 1) \]

\[ \lambda = f^2 (N - w) \]

Hence, \[ N = (\lambda / f^2) + w \] [equation 6]

At the inception of this study, the aim was to investigate the effects of birthweight status on airway function. As maternal smoking is potentially an important determinant of both airway function and birthweight, we proposed to consider the effects of maternal smoking exposure separately. Thus, the sample size was initially calculated to examine the effects of birthweight status on airway function in infants of non-smoking mothers. As discussed earlier and in Section 5.2, the proportion of variability for any given parameter is in part determined by the within and between subject variability for that parameter after accounting for known confounders. Thus, we estimated that known confounders would account for 60% of the between subject variability for volume parameters such as FVC and FEV\(_{0.4}\), but only 20% of the variability for flow parameters such as MEF\(_{25}\). Therefore in order to estimate a suitable sample size for measurements of both volume and flow parameters, an average of proportion of variation of 40% \((R_{Y,A} = 40\%)\) was used.

As discussed earlier, we have found that sex, age, length and maternal history of asthma are independent variables \((w = 4)\) of airway function in infants. In addition,
we postulated that a 10% difference in adjusted estimates of forced expired flows and volumes between birthweight groups \((R_{Y,B})\) would be clinically and aetiologically significant.

Therefore, \(R_{Y,B} = 10\% \times (100\% - 40\%) = 6\%\) of total proportion of variation and total proportion of variability \(R_{Y,A,B} = 46\%\).

The error variance proportion \((1 - R_{Y,A,B})\) is 54\% \[from equation 2\]

and the effect size index, \(r^2 = 6/54 = 0.11\) \[from equation 3\].

Thus, to detect this difference between birthweight groups with 80\% power, the sample size required is \(N = (\lambda / r^2) + w\) \[from equation 6\].

From Table I.1, for 80\% power at the 5\% significance level, the approximate value of \(\lambda\) is 8. Therefore total sample size required, \(N = (8 / 0.11) + 4 = 77\). As the value of \(\lambda\) was obtained by interpolation from the table given and in order to ensure that the study was adequately powered at 80\%, the sample size agreed for this study was 40 infants per group (SGA/AGA of non-smoking mothers; \(N = 80\)).

As maternal smoking during pregnancy is known to be a major risk factor for both reduced airway function and being SGA, it was also agreed that two groups of infants of smoking mothers (SGA/AGA; 40 per group) will also be recruited in order to assess how maternal smoking exposure affects airway function in these groups of infants.
### Appendix J  Comparison of recruitment process

<table>
<thead>
<tr>
<th></th>
<th>Face to face approach</th>
<th>Postal approach</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Approached</td>
<td>Agreed</td>
</tr>
<tr>
<td>SGA</td>
<td>39</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>AGA</td>
<td>21</td>
<td>7 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>13 (22%)</td>
</tr>
</tbody>
</table>

### Appendix K  Validation of neonatal anthropometric measurement

<table>
<thead>
<tr>
<th>Date of validation</th>
<th>Hospital no.</th>
<th>Crown-heel length (cm) Measured by Delivery Suite staff</th>
<th>Crown-heel length (cm) Validated by SL/IG</th>
<th>Absolute difference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/06/99</td>
<td>792760</td>
<td>58.0</td>
<td>51.8</td>
<td>6.2</td>
</tr>
<tr>
<td>23/06/99</td>
<td>794196</td>
<td>52.0</td>
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</tr>
<tr>
<td>23/06/99</td>
<td>794210</td>
<td>49.0</td>
<td>48.2</td>
<td>0.8</td>
</tr>
<tr>
<td>23/06/99</td>
<td>858044</td>
<td>49.0</td>
<td>48.7</td>
<td>0.3</td>
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<tr>
<td>23/06/99</td>
<td>794201</td>
<td>48.0</td>
<td>48.1</td>
<td>0.1</td>
</tr>
<tr>
<td>23/06/99</td>
<td>794201</td>
<td>55.5</td>
<td>50.0</td>
<td>5.5</td>
</tr>
<tr>
<td>5/07/99</td>
<td>857900</td>
<td>50.0</td>
<td>48.1</td>
<td>1.9</td>
</tr>
<tr>
<td>5/07/99</td>
<td>794132</td>
<td>53.0</td>
<td>48.3</td>
<td>4.7</td>
</tr>
<tr>
<td>5/07/99</td>
<td>857919</td>
<td>46.5</td>
<td>45.2</td>
<td>1.3</td>
</tr>
<tr>
<td>5/07/99</td>
<td>794175</td>
<td>51.0</td>
<td>49.3</td>
<td>1.7</td>
</tr>
<tr>
<td>5/07/99</td>
<td>857901</td>
<td>50.0</td>
<td>51.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

N = 11
Group mean difference in crown-heel length measurements between Delivery Suite staff and validated by SL/IG was on average 2.1 cm (95% Confidence Interval of the mean difference: 0.5, 3.7); This difference is significant (p = 0.02).
The portion of the CGF chart for classifying birthweight centiles for girls is shown above. The chart above is equivalent to 3/4 of the actual size of the CGF chart.
Appendix M  Audit of SGA infants born during 1999 – 2000 at Homerton Hospital

<table>
<thead>
<tr>
<th>Year</th>
<th>Total SGA</th>
<th>SGA girls</th>
<th>SGA boys</th>
<th>Eligible SGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>631</td>
<td>308</td>
<td>323</td>
<td>105</td>
</tr>
<tr>
<td>2000</td>
<td>648</td>
<td>340</td>
<td>308</td>
<td>90</td>
</tr>
</tbody>
</table>
Publications
The Association between Birthweight, Sex, and Airway Function in Infants of Nonsmoking Mothers

SOOKY LUM, AH-FONG HOO, CAROL DEZATEUX, IRIS GOETZ, ANGIE WADE, LAURA DROOY, KATE COSTELOE, and JANET STOCKS

Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit and Center for Paediatric Epidemiology and Biostatistics, Institute of Child Health and Great Ormond Street Hospital NHS Trust; Neonatal Unit, University College Hospital, and Department of Child Health, Barts and the Royal London School of Medicine and Dentistry, Homerton Hospital, London, United Kingdom

The risk of respiratory illness and death is increased in infants of low birthweight for gestational age, but the underlying physiologic mechanisms remain unclear. We examined the hypothesis that airway function is diminished in infants of low birthweight for gestational age, independent of exposure to maternal smoking. Respiratory function was measured using partial and raised volume forced expiratory maneuvers in 103 infants (> 35 wk gestation; 56 boys) not exposed pre- or postnatally to maternal smoking who, according to birthweight, were either small (SGA; n = 38) or appropriate (AGA; n = 65) for gestational age. At testing, SGA infants were of similar postnatal age (mean [SD]: SGA 6.8 [2.4] wk, AGA 5.9 [2.3] wk), but remained shorter and lighter than AGA infants. In univariate analyses, FVC, forced expired volume in 0.4 s (FEV), and FE F were significantly diminished in SGA compared with AGA infants (mean [95% CI] of difference: FVC: 127 versus 143 ml [-29, -2]: FEV: 112 versus 125 ml [-24, -2]; and FE F: 173 versus 203 ml s [-57, -3], respectively), but these differences were no longer significant after allowing for sex and body size. Furthermore, FE F was on average 35 ml s lower in boys than girls (95% CI: -61, -8). We conclude that diminished airway function in SGA infants shortly after birth appears to be primarily mediated through impaired somatic growth.

Keywords: infant; small for gestational age; fetal growth retardation; birthweight; function test; respiratory; sex

The clinical importance of fetal growth restriction was first recognized by Lutchenco and coworkers in 1963, who reported that perinatal mortality and morbidity were increased among infants whose birthweight fell at or below the 10th percentile for gestational age (1). More recently, it has been shown that infants who are small for gestational age (SGA) are at increased risk of sudden death in infancy (2) and of wheezing and respiratory infection in early childhood (3, 4). Furthermore, diminished airway function has been reported in adults who were of low birthweight (5, 6), leading to the speculation that this association is mediated by "fetal programming" (5). The physiologic mechanisms underlying these associations remain unclear. If the "fetal programming" hypothesis is correct, this might suggest an association between birthweight and airway function in infancy and early childhood. However, there have been few published studies specifically examining this association (7, 8) and this aspect has received little attention in previous epidemiologic studies of infant respiratory function (9). The associations between maternal smoking in pregnancy and low birthweight (10, 11) and between impaired airway function in infancy (12,14) and an increased risk of lower respiratory illness in early childhood are well recognized (15), but it is unclear whether there is an association between low birthweight and impaired airway function in infancy that is independent of exposure to maternal smoking. This study was therefore established to examine the hypothesis that airway function is impaired in infants who are of low birthweight for gestational age but who have not been exposed to maternal smoking pre- or postnatally.

METHODS

Study Population

Infants were recruited from the maternity units at the Homerton and University College Hospitals, London. Healthy, singleton infants (> 35 wk gestation) were eligible for inclusion if born to a white northern European mother who did not smoke in pregnancy or postnatally. Infants with congenital abnormalities or with neuromuscular or cardiovascular disorders were ineligible, as were those who required any ventilatory assistance during the neonatal period or who had experienced any lower respiratory illness (LRI) prior to testing.

Infants were classified according to birthweight and gestational age using the sex-specific Child Growth Foundation (CGF) algorithms (16) as well as the Gestation Related Optimal Weight or "GROW" program (17). The latter takes maternal characteristics such as height, body mass index, ethnic group, parity into account as well as infant birthweight, gestation, and sex. Gestational age was based on ultrasound assessment before 20 wk. Infants with a birthweight < 10th percentile according to either the CGF or GROW programs were classified as SGA, whereas those between the 20th and 95th percentile were classified as appropriate for gestational age (AGA). Local Research Ethics Committees approved this study and informed written consent was obtained from parents.

Family history of respiratory illnesses, first-degree family history of asthma, maternal age on leaving full time education, and parental occupational status were obtained from the mother at the time of the lung function test. Additional details were obtained from the obstetric notes. Exposure to maternal smoking pre- and postnatally was assessed from parental report. Current smoking exposure was validated by cotinine assay of infant urine and maternal saliva obtained at the time of lung function testing (18).

Five infants whose mother's salivary cotinine concentrations ranged from 20.8 to 434.6 ng ml were excluded from the study as these are consistent with values reported from active smokers (> 15 ng ml) (19, 20). Maternal salivary cotinine for the remaining study population was negligible (geometric mean [range]: 0.125 [0.0001–4.096] ng ml), with no significant difference between mothers of SGA and AGA infants.

Respiratory function was measured between 4 and 12 wk postnatally, when infants had been well and free from upper respiratory tract infections for at least 3 wk. Body weight and crown-heel length were measured as described previously (21) and expressed as sex-specific z

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Correspondence and requests for reprints should be addressed to Ms. Sooky Lum, Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom. E-mail: s.lum@ich.ucl.ac.uk

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scores (16). All infants were studied supine following sedation with 60 mg kg⁻¹ chloral hydrate, administered orally. Heart rate and oxygen saturation were monitored continuously during the test (CO2SMO, Model 171/00, Novametrics Medical Systems Inc., Wallingford, CT).

Respiratory Function Tests
Airway function was assessed from both partial (22) and raised lung volume (32, 24) forced expiratory manoeuvres, using the rapid thoraco-abdominal compression (RTC) technique as described previously (13, 25, 26). Measurements were performed in accordance with recent recommendations (22), ensuring that flow limitation, as indicated by reproducible flow-volume (F-V) curves with no further increase in maximal flow at FRC (VmaxFRC), was achieved despite increasing jacket pressures. VmaxFRC was reported as the mean (SD) of the three highest flows at FRC (22, 27).

Measurements of airway function at raised lung volume were performed using an adaptation of the technique described by Feher and coworkers (23). Briefly, the respiratory muscles were relaxed by administering four or five lung inflations to a pressure of 3 kPa before inflating the jacket to force expiration from raised lung volume. This maneuver was repeated until a minimum of three acceptable and reproducible F-V curves was obtained. Parameters calculated from the raised volume technique, including forced vital capacity from an inflation pressure of 3 kPa (FVC), forced expiratory volume at 0.1 s (FEV₀.₁), and forced expired flow at 75% of expired FVC (FEF₇₅) were reported from the "best" raised volume curve. This was defined as the technically acceptable forced expiratory F-V curve with the highest sum of FVC and FEF₇₅.

Sample Size and Statistical Analysis
It was estimated that 40 infants per group would provide 90% power at the 5% significance level to detect a difference of one standard deviation (SD) in estimates of forced expiratory flows and volumes between SGA and AGA infants after adjustment for potential confounding factors. Comparisons of group characteristics and respiratory function between the groups were performed using t tests, chi-square, or exact tests as appropriate (StatXact v 4.01). The extent to which low birthweight for gestational age is associated with residual variance in forced expiratory flows and volumes was examined using multiple linear regression (SPSS for Windows, Release 8.0.2) after adjustment for body size and sex and after examining for the effects of other potential confounding factors.

RESULTS
Lung function measurements were attempted in 123 infants but were unsuccessful in 15, due to poor quality data or inability to complete the study protocol. Of the 108 infants successfully measured, five were excluded subsequently because maternal salivary cotinine concentrations were consistent with nonmanual occupations. The proportion of SGA and AGA pregnancies complicated by prolonged ruptured membranes (more than 24 h but less than 1 wk), antepartum hemorrhage, and pregnancy-induced hypertension was similar (data not shown), but more SGA (21%) than AGA (8%) infants exhibited meconium staining of liquor during labor (95% CI, SGA - AGA: 29%, 0.13%; p = 0.001).

Five SGA (13%) and six AGA (9%) infants experienced an upper respiratory illness prior to testing but all infants had been free from respiratory symptoms for at least 3 wk at the time of the test. There were no significant differences in any airway function between those who did or did not have upper respiratory illness prior to testing.

Characteristics at time of test are summarized in Table 2 according to birthweight status. At time of testing, SGA infants weighed less, were shorter, and of smaller head and chest circumference than AGA infants.

AgA infants, reflecting the selection criteria used. A positive family history of asthma was reported in a similar proportion of SGA and AGA infants, and in four (11%) SGA and eight (12%) AGA infants, the mother was one of the affected family members. There were no significant differences between the groups with respect to maternal age, maternal age at completion of full time education, or the percentage of mothers in nonmanual occupations. The proportion of SGA and AGA pregnancies complicated by prolonged ruptured membranes (more than 24 h but less than 1 wk), antepartum hemorrhage, and pregnancy-induced hypertension was similar (data not shown), but more SGA (21%) than AGA (8%) infants exhibited meconium staining of liquor during labor (95% CI, SGA - AGA: 29%, 0.13%; p = 0.001).

Five SGA (13%) and six AGA (9%) infants experienced an upper respiratory illness prior to testing but all infants had been free from respiratory symptoms for at least 3 wk at the time of the test. There were no significant differences in any airway function between those who did or did not have upper respiratory illness prior to testing.

Characteristics at time of test are summarized in Table 2 according to birthweight status. At time of testing, SGA infants weighed less, were shorter, and of smaller head and chest circumference than AGA infants.

Table 1. Group Characteristics* at Birth According to Birthweight Classification

<table>
<thead>
<tr>
<th></th>
<th>SGA (n = 38)</th>
<th>AGA (n = 65)</th>
<th>95% CI of Difference: SGA - AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>40.2 (1.3)</td>
<td>39.8 (1.5)</td>
<td>-0.2, 0.9</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>2.8 (0.3)</td>
<td>3.5 (0.4)</td>
<td>-0.8, 0.6</td>
</tr>
<tr>
<td>CGF birthweight z score</td>
<td>-1.6 (0.4)</td>
<td>0.1 (0.6)</td>
<td>-1.9, -1.6</td>
</tr>
<tr>
<td>CGF birthweight percentile</td>
<td>6.4 (3.7)</td>
<td>54.6 (19.3)</td>
<td>-53.1, -43.2</td>
</tr>
<tr>
<td>GROW percentile</td>
<td>3.4 (3.9)</td>
<td>50.7 (23.8)</td>
<td>-53.4, -41.2</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>33.0 (3.3)</td>
<td>34.5 (1.4)</td>
<td>-2.1, -0.9</td>
</tr>
<tr>
<td>Head circumference z score</td>
<td>-1.6 (0.9)</td>
<td>0.2 (1.0)</td>
<td>-1.8, -1.0</td>
</tr>
<tr>
<td>Boys</td>
<td>27 (71%)</td>
<td>29 (45%)</td>
<td>8%, 45%</td>
</tr>
<tr>
<td>Firstborn</td>
<td>28 (74%)</td>
<td>43 (66%)</td>
<td>-11%, 24%</td>
</tr>
<tr>
<td>Maternal age at delivery, yr</td>
<td>33.2 (6.8)</td>
<td>33.5 (4.4)</td>
<td>-2.2, 1.5</td>
</tr>
<tr>
<td>Maternal age at completion of full-time education, yr</td>
<td>21.7 (3.1)</td>
<td>21.5 (3.2)</td>
<td>-1.1, 3.5</td>
</tr>
<tr>
<td>Maternal weight at booking, kg</td>
<td>66.0 (13.3)</td>
<td>66.1 (12.2)</td>
<td>-0.7, 4.5</td>
</tr>
<tr>
<td>Mothers in nonmanual occupation</td>
<td>33 (87%)</td>
<td>58 (91%)</td>
<td>-19%, 8%</td>
</tr>
<tr>
<td>First-degree family history of asthma</td>
<td>9 (24%)</td>
<td>17 (26%)</td>
<td>-18%, 16%</td>
</tr>
</tbody>
</table>

Data shown as mean (SD) for continuous and n (%) for categorical variables.

Definition of abbreviations: AGA = appropriate for gestational age; CGF = Child Growth Foundation algorithm (16); CI = confidence interval; GROW = Gestation Related Optimal Weight algorithm (17); SGA = small for gestational age.

Footnotes:
1. p < 0.001.
2. p < 0.01.
3. According to CGF algorithms (16).
### TABLE 2. GROUP CHARACTERISTICS AT TEST ACCORDING TO BIRTHWEIGHT CLASSIFICATION

<table>
<thead>
<tr>
<th></th>
<th>SGA (n = 38)</th>
<th>AGA (n = 65)</th>
<th>95% CI of Difference: SGA - AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>6.8 (2.4)</td>
<td>5.9 (2.3)</td>
<td>-0.1, 1.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>4.3 (0.8)</td>
<td>4.7 (0.8)</td>
<td>-0.7, -0.1</td>
</tr>
<tr>
<td>Weight z score</td>
<td>-1.0 (0.8)</td>
<td>0.1 (0.9)</td>
<td>-1.5, -0.8</td>
</tr>
<tr>
<td>Length, cm</td>
<td>54.6 (2.4)</td>
<td>56.5 (2.9)</td>
<td>-2.9, 0.7</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>38.2 (1.6)</td>
<td>39.0 (1.7)</td>
<td>-1.5, -0.2</td>
</tr>
<tr>
<td>Head circumference z score</td>
<td>-0.3 (0.8)</td>
<td>0.9 (1.0)</td>
<td>-1.6, -0.8</td>
</tr>
<tr>
<td>Chest circumference, cm</td>
<td>37.8 (2.6)</td>
<td>39.0 (2.2)</td>
<td>-2.2, -0.3</td>
</tr>
</tbody>
</table>

Definition of abbreviations: AGA = appropriate for gestational age; CI = confidence interval; SGA = small for gestational age.

1 Age after expected date of delivery.
2 p ≤ 0.05.
3 According to CGF algorithms (16).
4 p = 0.001.
5 p ≤ 0.01.
6 n = 64.

### DISCUSSION

The results of this study suggest that both lung capacity and airway function, as reflected by FVC, FEV_{0.4}, and FEF_{75}, are diminished during the first few months of life in infants who are born small for gestational age, but this association appears to be primarily mediated through impaired somatic growth rather than through a specific effect on lung and airway growth. In addition, we found that measures that reflect peripheral but not central airway function were significantly diminished in boys compared with girls.

This is the first study to explicitly test the hypothesis that low birthweight for gestational age is associated with impaired airway function in infancy. By limiting analysis to infants who had not been exposed to maternal smoking, the influence of low birthweight relative to duration of gestation (and by inference impaired fetal growth) on airway function in infancy can be examined.

There are several strengths in the current study design. First, lung function tests were performed prior to any lower respiratory infections (LRI), thus enabling respiratory function in SGA and AGA infants to be compared without potential confounding by the effects of LRI on airway function. Second, gestational age was determined from sonographic assessments before 20 wk gestation, which are currently considered to be the most accurate means of estimating gestation and hence

### TABLE 3. INFLUENCE OF BIRTHWEIGHT STATUS ON AIRWAY FUNCTION PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>SGA (n = 38)</th>
<th>AGA (n = 65)</th>
<th>95% CI of Difference: SGA - AGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures of large airway function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC, ml</td>
<td>127 (28)</td>
<td>143 (35)</td>
<td>-29, -2</td>
</tr>
<tr>
<td>p = 0.02</td>
<td>p = 0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV_{0.4}, ml</td>
<td>112 (21)</td>
<td>125 (28)</td>
<td>-24, -2</td>
</tr>
<tr>
<td>p = 0.02</td>
<td>p = 0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measures of peripheral airway function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF_{75}, ml s^{-1}</td>
<td>173 (60)</td>
<td>203 (71)</td>
<td>-57, -3</td>
</tr>
<tr>
<td>p = 0.03</td>
<td>p = 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V'_{maxFRC}, ml s^{-1}</td>
<td>135 (68)</td>
<td>148 (74)</td>
<td>-42, 16</td>
</tr>
<tr>
<td>p = 0.38</td>
<td>p = 0.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Definition of abbreviations: AGA = appropriate for gestational age; CI = confidence interval; SGA = small for gestational age; V'_{maxFRC} = maximal flow at functional residual capacity.

1 Data shown as mean (SD) and 95% confidence interval of the difference.
2 Adjusted for length.
3 Adjusted for sex.
birthweight percentiles. Third, we were able to validate maternal reports of postnatal smoking by measuring cotinine, a breakdown product of nicotine, in maternal saliva and infant urine. This allowed the association of low birthweight and airway function to be examined independently of the known associations between maternal smoking, low birthweight, and impaired airway function. Finally, the use of the raised volume technique to measure airway function allowed measures of forced expiration to be compared between infants over an extended volume range (24).

How Representative Is This Population?

Because prematurity, respiratory disease, and ventilatory assistance during the neonatal period are all likely to have a negative impact on airway function, such infants were excluded from this study. As this potentially excludes those infants with more severe fetal growth retardation born by elective premature delivery or with severe respiratory disease, the AGA population studied may be potentially biased toward those with less severe growth restriction. A similar bias could result from excluding those exposed to maternal smoking during pregnancy.

### Table 4. Group Characteristics at Test and Respiratory Function Results According to Sex

<table>
<thead>
<tr>
<th>Boys (n = 56)</th>
<th>Girls (n = 47)</th>
<th>95% CI of Difference: Boys – Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>27 (48%)</td>
<td>11 (23%)</td>
</tr>
<tr>
<td>Age, wk*</td>
<td>6.5 (2.4)</td>
<td>5.9 (2.2)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>4.7 (0.9)</td>
<td>4.4 (0.7)</td>
</tr>
<tr>
<td>Length, cm</td>
<td>56.1 (2.8)</td>
<td>55.4 (2.9)</td>
</tr>
<tr>
<td>Length z score*</td>
<td>-0.1 (1.0)</td>
<td>0.5 (1.2)</td>
</tr>
<tr>
<td>FVC, ml</td>
<td>136 (34)</td>
<td>138 (33)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;, ml</td>
<td>118 (27)</td>
<td>122 (27)</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25&lt;/sub&gt;-&lt;sub&gt;75&lt;/sub&gt;, ml s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>177 (67)</td>
<td>209 (67)</td>
</tr>
<tr>
<td>V' maxFRC, ml s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>126 (66)</td>
<td>164 (73)</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** CI = confidence interval; FEF<sub>25</sub>-<sub>75</sub> = forced expiratory flow at 75% of FVC; FEV<sub>1</sub> = forced expired volume at 0.4 s; FVC = forced vital capacity; SGA = small for gestational age; V' maxFRC = maximal flow at functional residual capacity.

* Data shown as mean (SD) for continuous and n (%) for categorical variables.

* p < 0.01

* Age after expected date of delivery.

* p < 0.05

* According to CGF algorithms (16).

The social and demographic characteristics of the mothers of both SGA and AGA infants were similar (Table 1), but mothers in our study were older, of higher social class, and better educated than women in a similar study of preterm infants carried out in this maternity unit (13) or when compared to the national average for age at first delivery (28), suggesting that the SGA infants recruited to this study may have come from more affluent or better educated families. Unfortunately, such details are not available for those who were eligible but not recruited. However, any such potential biases in recruitment would tend to attenuate the associations observed and lead to conservative estimates of the association between airway function and low birthweight for gestational age. A further issue when interpreting our results arises from the relatively low proportion of SGA girls in our study population who were born to nonsmoking mothers. The reason for this unexpected discrepancy is unclear. We were able to confirm, through an audit, that equal numbers of SGA boys and girls were born in the study hospitals during the period of recruitment (data not shown). However, a higher proportion of SGA girls recruited to this study were born to mothers who smoked, thus SGA girls are relatively underrepresented in this analysis based on infants of nonsmoking mothers. Hence, adjustment...
was made in the regression analyses to investigate differences in outcomes among SGA infants after accounting for the resulting sex imbalance.

Because the optimal method of identifying SGA infants remains unclear, two methods to classify infants’ size at birth were used, namely the CGF (16) and GROW (17) algorithms. There was generally good concordance between these two methods (29), with any discrepancies falling between the 11th and 15th percentiles on one or the other chart. By including infants who were identified as SGA by either method, we hoped to avoid misclassification. Furthermore, to maintain a clear dichotomy between the SGA and AGA groups, infants between the 15th and 20th percentile according to CGF charts were not recruited into either group. However, it is recognized that the relationship between morbidity and birthweight is not a dichotomy but a graded risk, as it is dependent on the exposure to risk factors such as smoking, nutrition, and poor socioeconomic status (30).

Although maternal smoking remains one of the strongest associated factors for fetal growth restriction in developed countries (30), maternal nutrition in pregnancy has received increasing attention during recent years (31, 32). In this study, maternal characteristics such as weight at booking and other socioeconomic factors were similar in the SGA and AGA groups (Table 1). In addition, although recognizing that birthweight is influenced by birth order, notably being lower in a first pregnancy, a similar number of SGA and AGA infants studied were firstborn. Furthermore, the incidence of obstetric complications that may predispose to a growth-restricted fetus (such as pregnancy-induced hypertension) was low within the study population and was similarly distributed between mothers of SGA and AGA infants (data not shown). Although it was interesting to note that the incidence of meconium liquor was higher during SGA labors, none of the infants studied had any clinical evidence of meconium aspiration or required any ventilatory assistance during the neonatal period.

Lung Function Parameters

Although FEV₁ is the most frequently used measure of airway function in adults and older children, young infants have a rapid respiratory rate and short expiratory time, which usually preclude its measurement at this age (33). Within the current population, the time of forced expiration (Treff) ranged from 0.36 to 1.77 s (mean 0.82 s). Treff was less than 0.5 s in nine infants, but greater than 0.4 s in all but two infants who had to be excluded when calculating FEV₁. In those infants in whom both parameters of timed FEV could be calculated (n = 94) the difference observed between the groups was similar (data available from authors on request).

Interestingly, although both FEF75 and V’maxFRC are considered to be measures of peripheral airway caliber, there was no significant difference in V’maxFRC during early infancy between the SGA and AGA infants. One possible reason for this discrepancy is that these two parameters may reflect the mechanical properties of different generations of peripheral airways. Alternatively, the relatively higher intersubject variability of V’maxFRC (Table 3), which in part reflects the variable extent to which dynamic elevation of FRC occurs during early infancy (34), may mean that it discriminates less well between groups than FEF75. However, as sex differences in expiratory airflow were detected equally well by either technique, this does not appear to be the case (Table 4). The fact that observed sex differences in peripheral airway function were larger both in absolute terms and in relation to the intersubject variability for V’maxFRC than for FEF75 (Table 4) again suggests that these two parameters may be reflecting different aspects of airway function. It also suggests that any decrements of airway function associated with low birthweight may be operating at a site slightly different from those associated with being male.

Association between Low Birthweight and Airway Function

In a study based on nine SGA infants, whose gestational age ranged from 33 to 41 wk, Dahms and coworkers (7) reported elevated compliance and crying vital capacity but normal functional residual capacity when compared with appropriately grown infants of similar birthweight and concluded that intrauterine stress leads to increased pulmonary maturity. However, this study was small and gestational age was determined from physical appearance and neurologic characteristics, which are less accurate than sonographic assessments. We are unaware of other attempts to assess lung function in SGA infants. A number of studies have been published reporting an association of low birthweight with diminished airway function in older children (8) and adults (5, 6, 35). With the exception of one study of British school children by Rona and coworkers (8), which included maternal report of birthweight and gestational age, other published studies (5, 6) have not taken account of information on gestational age when assessing associations between airway function and birthweight in their study population. Thus, it is unclear whether the associations reported between low birthweight and diminished airway function reflect prematurity or low birthweight for gestational age.

Although the “fetal programming” hypothesis has been suggested as a possible explanation for an association between birthweight and adult airway function, other factors such as social class at birth and various intervening social and biologic events merit consideration (36). Recent evidence from the 1958 British Birth Cohort suggests that health in later life is strongly associated with social class at birth for factors such as birthweight, childhood material circumstances, height, educational attainment, and smoking behavior (36). Thus, although Rona and colleagues (8) reported a significant association between birthweight (adjusted for gestational age) and lung function (FVC and FEV₁) in children aged 5 to 11 yr, it is possible that this association reflects the impact of other intervening exposures, related to low birthweight and impaired airway function but occurring prior to the test occasion. Although the current report focuses on a cross-sectional comparison of airway function between SGA and AGA infants shortly after birth, follow-up studies of this cohort are currently being undertaken to determine whether there is impairment of airway function with subsequent growth and development.

Sex and the Airways

Our findings suggest that peripheral airway function is reduced in boys shortly after birth. This is consistent with most previously published observations during infancy and childhood (13, 25, 37–41). Preliminary anatomic evidence is consistent with the finding of diminished airway function in boys. Airway structure has been shown to differ in male and female infants, and it has been suggested that the greater amount of smooth muscle and thicker airway wall in boys may provide part of the explanation for sex differences in airway function and susceptibility to respiratory disease in early life (42).

As airway function appears to be reduced in boys when compared with girls, any further decrements in airway function associated with low birthweight for gestational age might increase the risk of respiratory symptoms and the need for
assisted ventilation. Given the exclusion criteria used in this study, this could have resulted in only the fittest of SGA boys being eligible for inclusion in this study. There was, however, no evidence from our audit of births that SGA boys were more likely than girls to be admitted to neonatal special or intensive care units during the study period (data not shown).

**Interpretation**

Fetal and early postnatal life are periods of rapid growth and development of the respiratory system. Bronchial development and airway branching are mainly complete by Week 16 of gestation (43). Thus, any insult occurring in the first few months of pregnancy may cause developmental alterations, resulting in changes to the airway branching system (44). As hypothesized by Martinez (45), minor alterations in lung structural development during fetal life may have marked postnatal consequences, leading to critical disturbances in airway caliber in response to subsequent respiratory infections and resulting in severe and potentially fatal respiratory compromise. In this study, we have found that SGA infants have diminished airway function. Although this appears to be mediated primarily through the reduction in body size, it could contribute to the increased incidence of respiratory morbidity observed among SGA infants in the first year of life (3) as infants with diminished premorbid lung function are known to be at increased risk of subsequent wheezy illnesses (14, 46–48). Bearing in mind the early formation of airways during prenatal development, it will be particularly important to examine the pattern of subsequent growth and development in these SGA infants to ascertain whether somatic growth is associated with a “catch up” of airway function or continuing impairment.

**Conclusions**

The findings of the present study suggest that airway function is diminished in infants who are small for gestational age at birth but who have not been exposed to maternal smoking. Although this appears to be mediated primarily through reductions in body size it could contribute to the increased incidence of respiratory morbidity observed in such children during early life. Furthermore, peripheral airway function as reflected by FEF 25–75 and V max FRC is significantly lower in boys when compared with girls. Further follow-up of this cohort is required to establish the pattern of growth and development of the airways in relation to sex, birthweight status, and subsequent somatic growth.

**Acknowledgement:** We thank the parents of infants studied for their participation and commitment to the project. We also thank Dr. Jane Hawdon, Consultant Neonatologist, for her support and help with recruitment at University College Hospital, and Sarah Davies and Rosie Castle for their help in data collection and analysis.

**References**


Influence of Jacket Placement on Respiratory Compliance During Raised Lung Volume Measurements in Infants

Ah-Fong Hoo, MPhil,1* S.Y. Lum, RM,1 I. Goetz, MD,1 C. Dezateux, FRCP,2 and J. Stocks, PhD1

Summary. Recent introduction of the raised lung volume rapid thoraco-abdominal compression (RVRTC) technique for measuring forced expiratory maneuvers in infants provides the potential opportunity to assess respiratory mechanics simultaneously by using multiple linear regression (MLR) of the relaxed breaths preceding jacket inflation to force expiration. This study was undertaken to investigate whether data obtained from raised lung volume are influenced by placement of the rapid thoraco-abdominal compression (RTC) squeeze jacket. Paired measurements of tidal volume (Vt) and respiratory rate (RR) during tidal breathing, and of inflation volume (VInf), respiratory system compliance (Crs), and resistance (Rrs) during passive lung inflations were made in 60 (30 male) healthy term infants with and without a fastened, but uninflated RTC jacket in place.

Jacket placement was associated with a significant reduction (P < 0.0001) in weight-corrected Vint [−1.86 (95% confidence interval, −2.46, −1.27) mL kg−1] and Crs [−0.77 (−1.04, −0.49) mL kPa−1 kg−1]. This represented a reduction in weight-corrected Crs from 9.00 to 8.24 mL kPa−1 kg−1, with the fall being >10% in 42% of infants studied. There was no significant change in Rrs or weight-corrected Vt.

If passive respiratory mechanics are to be measured during raised lung volume maneuvers, they should be performed prior to the jacket being fastened, unless considerable care is taken with each infant to ensure that the jacket does not restrict chest wall movement during maximum inflation. Pediatr Pulmonol. 2001; 31:51-58. © 2001 Wiley-Liss, Inc.

Key words: methods; infant; respiratory mechanics; Inflation; pulmonary function tests.

INTRODUCTION

Recent introduction of the raised lung volume rapid thoraco-abdominal compression (RVRTC) technique to measure forced expiratory maneuvers in infants1-3 provides the potential opportunity to assess total passive respiratory mechanics simultaneously by using multiple linear regression (MLR) analysis4-6 of the relaxed breaths preceding jacket inflation to force expiration. MLR is commonly used to assess passive respiratory mechanics in ventilated subjects in the intensive care unit,5,6 but is dependent on complete absence of respiratory activity. During RVRTC measurements, passive inflations prior to jacket inflation evoke the Hering-Breuer inflation reflex (HBIR),7,8 thereby inducing respiratory muscle relaxation and passive expiration, hence fulfilling one of the major criteria for applying MLR. The validity of this approach would, however, depend on measurements being unaffected by jacket placement.

Currently, there is relatively little guidance as to how tightly the squeeze jacket should be wrapped during forced expiratory maneuvers and the extent to which this should vary according to whether full or partial maneuvers are being performed. The aim of this study was to assess the effect of a fastened but uninflated squeeze jacket, wrapped as recommended for production of a tidal forced expiratory maneuver, on both tidal venti-
lation and the assessment of respiratory mechanics from raised lung volume.

MATERIALS AND METHODS

Subjects

Healthy full-term infants were recruited from the Maternity Unit at the Homerton Hospital, East London, to an epidemiological project. They were studied at a mean corrected postnatal age of 6.2 weeks (range, 2.7–11.0 weeks). Respiratory function tests were scheduled to coincide with the infant’s feeding regime and were carried out when the infants were free from respiratory symptoms for at least 3 weeks prior to testing. Infants were sedated with chloral hydrate syrup (60 mg/kg) and then offered a feed. Once asleep, infants were settled in the supine position. Heart rate and oxygen saturation were monitored continuously during the test period, using a CO$_2$SMO monitor (Model 7100, Novametrics Medical Systems, Inc., Wallingford, CT). Respiratory data were collected during consecutive epochs of behaviorally determined quiet sleep, with room temperature maintained between 21–23°C. The study was approved by the East London and City Research Ethics Committee. Informed written consent was obtained from the infants’ parents, who were usually present during the measurements. Characteristics of the infants are summarized in Table 1.

Equipment and Data Collection

Flow was measured by a heated Hans Rudolph pneumotachometer (PNT; Model 3500, Hans Rudolph, Inc., Kansas City, MO; linearity, 0–35 L·min$^{-1}$) connected to a ± 0.2 kPa (± 2 cm H$_2$O) differential pressure transducer (Furness Controls Ltd., Bexhill, East Sussex, UK). The PNT was calibrated before respiratory tests were performed, and the calibration was rechecked with known signals at the end of the tests. A transparent Rendell-Baker face mask (size 1 or 2, Rusch UK Ltd., High Wycombe, Bucks, UK) was connected to the PNT and placed over the infant’s nose and mouth. An airtight seal was created with a thin rim of silicone therapeutic putty (Carters, Bridgend, Mid Glamorgan, UK) around the edge of the mask. Volume was derived by digital integration of the flow signal. Pressure at the airway opening ($P_{a o}$) was measured with a ± 5 kPa (± 50 cm H$_2$O) differential pressure transducer (Furness Controls Ltd.).

The equipment used for passive inflation and RVRTC was reported previously. The PNT was attached to a three-way Y-piece connector (total resistance, 0.57 kPa·L$^{-1}$·s at a flow of 100 mL·s$^{-1}$). A constant airflow of 12 L·min$^{-1}$ passed through the inspiratory limb of the Y-piece connector via a pressure relief valve (Neopuff, RD1000; Fisher & Paykel Healthcare, Auckland, New Zealand), which was set to approximately 3 kPa (∼30 cm H$_2$O) (Fig. 1).

During forced expiratory maneuvers a jacket, which extended from under the infant’s axillae to the iliac crest, was wrapped snugly around the infant’s torso with the arms outside the jacket, according to recent recommendations, i.e., with space to insert 2–3 adult fingers between the jacket and sternum (Fig. 1b). The jacket consisted of a 17 x 16 cm polythene inflatable plate (Hannover, Germany) surrounded by a stiff outer fabric covering (Columbus, OH) which could be rapidly inflated from a 100-L pressurized reservoir connected to the inflatable plate via a rigid, large-bore (28-mm ID) tubing (Fig. 1b). Jacket pressure ($P_j$) was measured with a ± 10 kPa (± 100 cm H$_2$O) differential pressure transducer (Furness Controls Ltd.). Flow and pressure signals were amplified and filtered above 10 Hz. Analog signals were digitized at 200 Hz (RASP, Physiologic Ltd., Newbury, Berks, UK).

Respiratory Test Protocol

Recordings of tidal and augmented breathing, with and without the RTC jacket being fastened, were made in each infant. Measurements with the jacket in situ preceded those without the jacket in the first 25 infants studied, since initial measurements were performed immediately before forced expiratory maneuvers from raised lung

**TABLE 1**—Infant Characteristics (n = 60)$^1$

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>39.9 (1.2)</td>
</tr>
<tr>
<td>Corrected postnatal age at test (weeks)</td>
<td>6.2 (1.9)</td>
</tr>
<tr>
<td>Weight at test (kg)</td>
<td>4.5 (0.8)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
<td>55.4 (3.0)</td>
</tr>
</tbody>
</table>

$^1$Mean (SD).
Jacket Placement Reduces Infant Respiratory Compliance

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Both tidal and relaxed augmented breaths had been recorded with or without the jacket in situ, measurements were repeated under the alternative measurement conditions. In infants in whom initial measurements were obtained with the jacket in situ, the outer fabric jacket was unfastened from the front and folded to the side of the torso, and the polythene inflatable plate was removed, hence completely freeing the infant’s chest.

Jacket pressure transmission was assessed by calculating the change in pressure at the airway opening when the jacket was inflated during an end-inspiratory airway occlusion (ΔPaoj). By relating ΔPaoj to jacket inflation pressure (ΔPj), the relative efficiency of jacket pressure transmission can be calculated.

Fig. 1. Equipment for measuring passive respiratory mechanics at raised lung volume. a: Before jacket placement. b: With jacket fastened prior to forced expiratory maneuvers.

Data Analysis

The analysis software package ("Squeeze," software for the analysis of lung function test recordings, version 1.44, P. Dixon and J. Stocks, Imperial College, London, 1997) was developed and validated in collaboration with the Imperial College of Science, Technology and Medicine. The time-based signals and flow-volume curves from separate epochs of tidal and augmented breaths were inspected for data quality prior to data analyses.

A minimum of 30 regular tidal breaths and 8 relaxed augmented breaths, with and without the jacket fastened, were analyzed for each infant to obtain paired measurements of tidal breathing parameters, inflation volume (V_{inj}) and passive mechanics during both measurement conditions. C_{rs} and R_{rs} were calculated by MLR using the algorithm

$P = E_L \cdot V + R_L \cdot V' + k$,\(^\text{16}\)

where P is the transpulmonary pressure, E_L is lung elastance, V is the volume above the elastic equilibrium volume, R_L is lung resistance, V' is flow, and k is a constant.

The following criteria governed the acceptability of the data for analysis:

- Stable pattern of ventilation with each inflation being held long enough to achieve a relaxed P_{a0} plateau at approximately 3 kPa (Fig. 2);
- Relaxed ventilated breath with no distortion of the V'-V loop due to inspiratory or expiratory effort;
- $r^2$ for MLR ≥ 0.95.

The “Squeeze” analysis software applies MLR to the pressure, flow, and volume data over the entire portion of each augmented breath, and allows several ventilated V'-V loops per epoch to be analyzed simultaneously. The quality of these V'-V loops was inspected in an ensembled format before the calculated parameters were accepted for reporting.
Statistical Analysis

Statistical analysis of the data was performed using SPSS version 8.0 for Windows. Within-subject paired difference and 95% confidence intervals (CI) of this difference were calculated for $C_{rs}$ and $R_{rs}$ with and without the jacket in situ, using the method of Bland and Altman.\(^{17}\)

RESULTS

Paired respiratory function measurements, with and without a jacket in situ, were obtained in 60 infants. The anthropometric characteristics of these infants are summarized in Table 1. There was no significant difference in any of the characteristics between those in whom measurements were initially made with or without the jacket in situ. The effect of jacket placement is illustrated in Figure 3; it shows passive $V$-$V$ curves collected at an identical $P_{inf}$ in an infant with and without the jacket fastened. By using the relaxed elastic equilibrium level as the volume landmark to overlay the $V$-$V$ curves, it can be seen that the inflation volume ($V_{inf}$), which was taken as the mean from between 8 to 12 relaxed augmented breaths, was reduced when the jacket was fastened. In addition, fastening the jacket was
TABLE 2—Effects of a Fastened Jacket on Respiratory Mechanics

<table>
<thead>
<tr>
<th></th>
<th>“Jacket on”</th>
<th>“Jacket off”</th>
<th>Mean within-pair difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal breathing (n = 55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR (bpm)</td>
<td>48.0 (8.6)</td>
<td>45.1 (8.7)</td>
<td>2.8 (1.2, 4.4)*</td>
</tr>
<tr>
<td>(V_r) (mL·kg(^{-1}))</td>
<td>8.5 (1.2)</td>
<td>8.6 (1.1)</td>
<td>-0.19 (−4.1, 0.03)</td>
</tr>
<tr>
<td>(t_e) (S)</td>
<td>0.56 (0.08)</td>
<td>0.58 (0.09)</td>
<td>-0.02 (−0.03, −0.04)*</td>
</tr>
<tr>
<td>(t_t) (S)</td>
<td>0.74 (0.16)</td>
<td>0.80 (0.18)</td>
<td>-0.07 (−0.03, −0.10)**</td>
</tr>
<tr>
<td>Augmented breathing (n = 60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P_{inf}) (kPa)</td>
<td>2.71 (0.18)</td>
<td>2.71 (0.18)</td>
<td>0.02 (−0.01, 0.02)</td>
</tr>
<tr>
<td>(R_{rs}) (kPa·L(^{-1})·s(^{-1}))</td>
<td>3.93 (1.21)</td>
<td>3.98 (1.14)</td>
<td>-0.05 (−0.29, 0.18)</td>
</tr>
<tr>
<td>(C_{rs}) (mL·kPa(^{-1})·kg(^{-1}))</td>
<td>8.24 (1.28)</td>
<td>9.00 (1.56)</td>
<td>-0.77 (−1.04, −0.49)**</td>
</tr>
<tr>
<td>(V_{inf}) (mL·kg(^{-1}))</td>
<td>21.5 (3.4)</td>
<td>23.4 (3.9)</td>
<td>-1.9 (−2.5, −1.3)**</td>
</tr>
<tr>
<td>Inflation rate (min(^{-1}))</td>
<td>36.7 (7.4)</td>
<td>33.4 (7.1)</td>
<td>3.3 (2.2, 4.4)**</td>
</tr>
<tr>
<td>(t_e) (S)</td>
<td>0.93 (0.20)</td>
<td>1.0 (0.21)</td>
<td>-0.07 (−0.03, −0.11)**</td>
</tr>
<tr>
<td>(t_t) (S)</td>
<td>0.79 (0.18)</td>
<td>0.89 (0.20)</td>
<td>-0.10 (−0.06, −0.14)**</td>
</tr>
<tr>
<td>(t_{rs})</td>
<td>0.33 (0.10)</td>
<td>0.39 (0.11)</td>
<td>-0.05 (−0.03, −0.07)**</td>
</tr>
</tbody>
</table>

Cl, confidence interval; RR, respiratory rate; bpm, breaths per minute; \(V_r\), tidal volume; \(t_e\), expiratory time; \(t_t\), respiratory time; s, second; \(P_{inf}\), inflation pressure; \(V_{inf}\), inflation volume; \(C_{rs}\) and \(R_{rs}\), total compliance and resistance of the inspiratory system, respectively, calculated using multiple linear regression; \(t_{rs}\), time constant of respiratory system.

1Mean (SD).

*P < 0.05.

**P < 0.0001.

accompanied by a change in the expiratory time constant (seen as the descending linear expiratory portion of the curves) which became significantly shorter, i.e., had a steeper V'/V slope (Fig. 3, Table 2).

Results from all the infants are summarized in Table 2. During tidal breathing, a small but statistically significant rise in respiratory rate (2.8 bpm) was observed with the jacket fastened, but there was no change in tidal volume. The rise in respiratory rate was primarily due to a shortening of expiratory time (\(t_e\)) when the jacket was in place.

During augmented breathing, \(R_{rs}\) was similar with and without the jacket in situ (P = 0.64). However, despite using an identical \(P_{inf}\) during passive inflations, fastening the jacket was associated with a highly significant (P < 0.0001) reduction in \(C_{rs}\) \((-0.77 \text{ mL} \cdot \text{kPa}^{-1} \cdot \text{kg}^{-1}\) and \(V_{inf}\) \((-1.86 \text{ mL} \cdot \text{kg}^{-1}\). This represented a mean (95% CI) fall in \(C_{rs}\) by \(-7.6% \) (−10.4, −4.9%). These changes were accompanied by a small but significant increase in inflation rate (3 min\(^{-1}\)), which was associated with a significant reduction in the expiratory time constant from 0.39 to 0.33 s when the jacket was in place.

The relationship of \(C_{rs}\) with and without the jacket fastened is shown as a Bland and Altman plot, in which percent change in \(C_{rs}\) (jacket on-off) is plotted against mean \(C_{rs}\) (jacket on-off) (Fig. 4). \(C_{rs}\) was lower in all but 12 of the infants when the jacket was fastened, this difference being greater than 10% in 21 (41.7%) of the infants studied. By contrast, repeat measurements of \(C_{rs}\) within the same subject without the jacket in situ were always within 10% of each other (mean difference, 0.23%). The within subject, within epoch coefficient of variation for \(C_{rs}\) range from 2 to 6%. Crs with and without the jacket in place was 8.2 and 9.1 mL·kPa\(^{-1}\)·kg\(^{-1}\) respectively in the 25 infants in whom initial measurements were made with the jacket in situ, and 8.2 and 9.0 mL·kPa\(^{-1}\)·kg\(^{-1}\) in the 35 in whom initial measurements were made without the jacket (P = 0.70).

Mean (SD) jacket pressure transmission after inflation was 52 (8%), similar to that reported by other centers.16

![Mean Crs (mL·kPa\(^{-1}\)·kg\(^{-1}\)); jacket on-off](image)

Fig. 4. Percent change in respiratory system compliance plotted against mean compliance with and without the jacket in situ, according to Bland and Altman.17 Solid line represents mean difference in respiratory compliance (jacket on-off) of −7.6% (95% CI, −10.4, −4.9%). Dashed lines represent 95% limits of agreement.
DISCUSSION

The results from this study indicate that jacket placement is associated with a small but significant reduction in both weight-corrected \( V_{\text{inf}} \) and \( C_{\text{rs}} \) during passive lung inflations. This suggests that fastening the jacket restricts chest wall movement, which in turn reduces chest wall compliance, and hence total respiratory compliance in a significant proportion of infants. Thus, although investigators may wish to calculate passive mechanics from relaxed breaths immediately prior to forced expirations from raised lung volumes, to maximize the amount of data that can be collected in the limited time that infants remain asleep during respiratory function tests, we have shown that this may result in an underestimation of \( C_{\text{rs}} \).

Administration of inflations was performed manually, with inflation rate being adapted according to the age and spontaneous respiratory rate of the infant, rather than attempting to use a standard rate for all infants. Despite the achievement of a virtually identical \( P_{\text{inf}} \) under both sets of measurement conditions (Table 2), and holding inflations to ensure that a clear pressure and volume plateau occurred at end inspiration (Fig. 2), inflation rate was found to be slightly higher (3 min\(^{-1}\)) with the jacket fastened. This probably reflects the shorter time constant and hence filling time of the lung, that would have accompanied the observed reduction in respiratory compliance, when the jacket was fastened (Fig. 3, Table 2). Interestingly, a similar increase in respiratory rate also occurred during tidal breathing when the jacket was fastened. The reasons for this are unclear but may be related to stimulation of chest wall reflexes by the presence of the jacket, or a slight decrease in \( C_{\text{rs}} \) even during tidal breathing. The latter was not measured in this study due to time constraints. A reduction of both compliance and lung volume during tidal breathing following jacket fastening was reported previously, and it has been noted that even the placement of respiratory inductance bands may decrease \( C_{\text{rs}} \).

A few years ago, preliminary recommendations were made that the jacket should be applied loosely enough during the raised volume technique to allow full expansion of the chest without restriction, and that this could potentially be checked by measuring inflation volumes immediately prior to and after jacket placement. However, there has been no indication in any of the publications reporting the use of the raised volume technique that this practice has in fact been routinely implemented. If such comparisons of inflation volume were to be made with and without the jacket in situ prior to performing forced expirations, automatic online analysis would be required to ensure immediate feedback to the operator. In addition, it would be essential to ensure that identical conditions occur with respect to all other variables, particularly the presence of either intrinsic or extrinsic PEEP during the inflations, both of which will influence the \( V_{\text{inf}} \) achieved for any given \( P_{\text{inf}} \).

If forced expiratory maneuvers are only performed from raised lung volume, there may be no problem in simply applying the jacket more loosely to avoid any chest wall restriction. However, there are still many unresolved issues regarding the relative sensitivity and specificity of the tidal versus raised volume technique, which several centers are currently trying to address by using both techniques in healthy infants and those with disease. One is then faced with a potential dilemma: if the jacket is wrapped loosely enough for the raised volume technique, insufficient pressure might be transmitted to ensure flow limitation during the tidal RTC or even when measuring forced flows at low lung volumes. By contrast, attempts to alter the way in which the jacket is fitted in the middle of the study carries the risk of waking the child. As a first step, this study aimed to investigate the effect of the jacket when adjusted as recommended for the tidal RTC. The evidence provided indicates that this will have a small, but significant effect on \( V_{\text{inf}} \) and \( C_{\text{rs}} \) and hence presumably on parameters derived from forced expiration from raised lung volume. This emphasizes the need to use a looser jacket when using the raised volume technique. The extent to which this might influence respiratory mechanics or achievement of flow limitation at low lung volumes during forced expirations remains to be ascertained. In this study, since the jacket was vented to atmosphere prior to jacket inflation, only minimal changes in jacket pressure (<0.02 kPa) were observable during maximal lung inflation and none during tidal breathing. Nevertheless, fastening of the jacket was associated with a small rise in respiratory rate during tidal breathing, suggesting that monitoring of jacket pressure per se, even when displayed on an expanded axis, will not be sufficient to detect its potential influence on underlying respiratory parameters. It would therefore be advisable to remove the jacket prior to performing any lung function tests in infants that do not require chest wall compression.

There may be additional limitations to calculating passive mechanics during augmented breaths, including the fact that the pressure-volume curve of the respiratory system is not linear. Measurements made at inflation pressures above 2 kPa are likely to encompass the stiffer, flatter upper portion of the curve, such that the applica-
tion of simple MLR analysis may become unreliable unless a volume-dependent term is included in the equation. Furthermore, the pattern of recruitment of the respiratory musculature during spontaneous breathing may be more efficient at inflating the lungs than that resulting from imposition of positive airway pressure at the airway opening, especially in young infants in whom volume dependency of the HBIR is so strong. It has been shown that infants occasionally take a deep sigh at the end of inflation, thereby almost doubling the inflation volume achieved at 2–3 kPa. Both these factors may have contributed to the relatively low values of weight-corrected baseline Crs observed in many of the infants in this study. When measured over the tidal range, values for Crs in healthy infants generally range from 9–16 mL·kPa⁻¹·kg⁻¹. This has potential implications when interpreting results of respiratory mechanics from intubated, ventilated children using MLR analysis over the whole breath, and emphasizes the fact that normative data collected during spontaneous breathing over the tidal range cannot be extrapolated for use under different conditions of measurement. Indeed, considerable further validation work to elucidate the numerous factors which can influence the assessment of passive respiratory mechanics over an extended volume range are required before such measurements can be used routinely.

In conclusion, the results from this study indicate that the presence of a jacket fastened according to recommendations for obtaining partial expiratory maneuvers will result in a significant reduction in Crs. The extent to which such changes can be mitigated if the jacket were to be applied more loosely and exactly how loose a jacket can be without influencing measurements of forced flow and volume from raised lung volume has yet to be ascertained. Irrespective of whether or not a jacket is in situ, routine assessment of respiratory mechanics from passive inflations cannot yet be recommended due to several unresolved problems. Continued investigation in this field is essential to elucidate the influence of various methodological factors such as the pattern and magnitude of inflations, as well as the linearity of respiratory compliance at augmented lung volumes, and potential developmental changes. In the meantime, it is recommended that passive respiratory mechanics and any other parameter of lung function that does not require chest wall compression are measured without a jacket or any other restraints around the chest wall.

ACKNOWLEDGMENTS

J.S. was funded by SIMS Portex PLC. We thank the parents of infants studied for their participation and commitment to the project.

REFERENCES


Effect of Airway Inflation Pressure on Forced Expiratory Maneuvers From Raised Lung Volume in Infants

Sooky Lum, RM, * Ah-Fong Hoo, MPHI, and Janet Stocks, PhD

Summary. The raised lung volume technique is increasingly used to measure forced expiratory maneuvers in infants. However, there is no consensus regarding the optimal airway inflation pressure (P_{in}) required for such maneuvers, or the influence of small changes in P_{in} within and between infants. The aim of this study was to assess the effect of small differences (0.2–0.3 kPa) in P_{in} on forced vital capacity (FVC), forced expired volume in 0.5 sec (FEV_{0.5}), and forced expired flow at 75% of vital capacity (FEF_{75}), all derived from the raised volume rapid thoraco-abdominal compression (RVRTC) technique. Randomized paired forced expiratory maneuvers were obtained in 32 healthy infants (3.9–39.3 weeks old, 3.8–9.9 kg) with the safety pressure relief valve for P_{in} set to 2.7 kPa or 3.0 kPa (27 or 30 cm H{sub}2O).

When mean (SD) P_{in} was increased by 8.4 (2.8)%, there was a significant (P < 0.01) increase in mean (SD) FVC, FEV_{0.5}, and FEF_{75} by 5.8 (5.7)%, 6.1 (6)% and 8.3 (16.2)%, respectively.

In conclusion, relatively small differences in P_{in} will result in significant differences in FVC, FEV_{0.5}, and FEF_{75} by RVRTC technique. Precision in setting and reporting the applied P_{in} is therefore essential, particularly if data are to be compared between centers. Pediatr Pulmonol. 2002; 33:130–134. © 2002 Wiley-Liss, Inc.

Key words: maximal expiratory flow-volume curves; pulmonary function test; infants; inflation pressures; methods, standardization; spirometry.

INTRODUCTION

The raised lung volume rapid thoraco-abdominal compression (RVRTC) technique is increasingly used to measure forced expiratory maneuvers in infants over an extended volume range.1 2 However, there is currently no standardized approach to either data collection or analysis.3 4 One of the major methodological differences has been the different airway inflation pressure (P_{in}) used in various centers. Some investigators obtained measurements after raising lung volume to a P_{in} of 2 kPa (20 cm H{sub}2O),5 6 while others used a P_{in} of 3 kPa (30 cm H{sub}2O).7 8 While results will obviously not be comparable if inflation pressures vary to this extent between centers, the effect of subtle variations in P_{in}, such as may occur within and between infants studied at any one center, is as yet unknown.

The aim of this study was to assess the effect of small differences in P_{in} on measurements derived by the RVRTC technique.

MATERIALS AND METHODS

Healthy full-term infants were recruited from the maternity unit at Homerton Hospital, London, to an epidemiological project,9 and were studied at a median corrected postnatal age of 8.3 (range, 3.9–39.3) weeks. Respiratory function tests were carried out when infants were free from any respiratory tract infections, and at least 3 weeks had elapsed since any respiratory symptoms. Measurements were made during behaviourally determined quiet sleep10 11 following sedation with chloral hydrate syrup (60–80 mg kg^{-1}). The study was approved by the East London and City Research Ethics Committee, and informed consent was obtained from the infants’ parents.

Airway function was assessed from forced expiratory flow-volume (FEFV) curves obtained at raised lung volume (RVRTC), using a technique adapted from Feher...
et al., and Hensch et al. Raised lung volume was achieved by manually inflating the infant’s lungs, using a fresh gas flow at a rate of 12 L min⁻¹, via a Neopuff Infant Resuscitaire (Fisher & Paykel Healthcare, Auckland, New Zealand), which has a safety pressure relief valve set to a given airway pressure. Four to five augmented breaths were delivered before inflating the jacket to force expiration from the raised lung volume. Lung inflations were maintained until a plateau was observed on both the pressure and volume recordings (Fig. 1). The study was designed to assess the influence of a 10% change in inflation pressure (0.3 kPa) on forced vital capacity (FVC), forced expired volume in 0.5 sec (FEV₀.₅), and forced expired flow at 75% of vital capacity (FEF₇₅) as such differences in P-inf had been observed both during interlaboratory visits and within our own department due to the slight flow dependence of the Neopuff system. Consequently, the inflation pressure valve was preset to either 2.7 or 3.0 kPa prior to forcing expiration. In each infant an entire set of measurements was performed, which comprised 4–6 forced expiratory manoeuvers to yield at least three reproducible (within 10%), technically satisfactory (see data and statistical analysis) FEFV curves at the preset (2.7 or 3.0 kPa) airway inflation pressure. The entire procedure was then repeated at the other inflation pressure. The order of application of these inflation pressures was randomized.

Within each infant, an identical jacket pressure was used during both sets of measurements. This was selected as the jacket pressure above which no further increase in maximal expiratory flow at functional residual capacity (V-maxFRRC) was achieved for that infant during partial forced expiratory manoeuvers. The latter had been performed immediately prior to the raised volume manoeuvres as part of the epidemiological study (data not shown). The extent to which pressure was transmitted from the jacket to the intrathoracic airways was assessed by performing a brief airway occlusion at end-tidal inspiration immediately prior to jacket inflation, and then measuring the subsequent change in pressure at the airway opening, which in this study was on average 2.3 kPa (SD, 0.8).

Recent application of the RVRTC technique to over 100 healthy infants in this department established a mean (SD) within-subject coefficient of variation of 3.2 (2.1)% for FVC, 3.3 (2.0)% for FEV₀.₅, and 8.9 (6.4)% for FEF₇₅. Thus, despite the use of a manual inflation system in this study, the intrasubject variability for all RVRTC parameters was similar to that reported by others using more automated systems.

Data and Statistical Analysis

Technically acceptable manoeuvres, defined as a rapid rise to peak expiratory flow following jacket inflation without evidence of early inspiration, and a smooth flow-volume curve without significant glottis closure or flow transients, especially during the last half of expiration, were analyzed using previously validated software (“Squeeze” version 2.04, P. Dixon and J. Stocks, Imperial College, London, 1999) to calculate FVC, FEV₀.₅, and FEF₇₅. Within-subject comparisons were made, using the best curve obtained at each inflation pressure. The “best” curve was defined as the technically acceptable curve with the highest sum of FVC and FEV₀.₅. Criteria for acceptance of the data included the observation that both FVC and FEV₀.₅ from the “best” curve were within 10% of those from the next best manoeuvre, recorded under the same measurement conditions. All results were cross-checked by an independent observer (J.S.). The P-inf delivered to the infant was taken as the mean pressure at the airway opening during the plateau immediately prior to jacket inflation to force expiration (Fig. 1). Statistical analysis of data was performed using a paired t-test with 95% confidence intervals (CI) of the difference (SPSS version 8.0 for Windows).

Pilot studies suggested that the SD of within-pair differences (P-inf 3.0–2.7 kPa) in FEF₇₅, the most variable

**ABBREVIATIONS**

CI  | Confidence interval
FEFV | Forced expiratory flow-volume
FEF₇₅ | Forced expiratory flow when 75% of vital capacity has been expired
FEV₀.₅ | Forced expiratory volume in 0.5 sec
FVC | Forced vital capacity
P-inf | Pressure at airway opening
P-air | Inflation pressure
RVRTC | Raised volume rapid thoraco-abdominal compression
V-maxFRRC | Maximal expiratory flow at functional residual capacity
of the three outcome measures, would be approximately 30 mL s$^{-1}$. Using this estimate, paired measurements from 29 subjects would provide 95% power at the 5% significance level to detect a 20 mL s$^{-1}$ change in FEF$_{75}$ (i.e., ~10%) in response to a 10% change in P$_{inf}$.

RESULTS

Paired measurements of airway function using inflation airway opening pressures of 2.7 and 3.0 kPa were obtained in 32 infants, details of whom are summarized in Table 1. There were no significant differences in any of the background characteristics in the infants in whom initial measurements were made at 2.7 or 3.0 kPa. Respiratory function results are summarised in Table 2. Although the pressure valve on the Neopuff Infant Resuscitaire was set to deliver a P$_{inf}$ of 3 kPa, due to the slight flow dependence of the Neopuff system, the average P$_{inf}$ of the breath immediately preceding forced expiration was in fact 2.9 kPa. Thus there was on average an 8% increase in P$_{inf}$ between the two sets of measurement conditions.

The effect of a small change in P$_{inf}$ on forced expiratory maneuvers is illustrated in Figure 2, which shows an overlay of the best forced expiratory flow-volume curves generated from raised lung volume using P$_{inf}$ 2.7 and 3.0 kPa in the same infant. By overlaying the curves along the final descending portion of the expiratory loops, it can be seen that the infant appeared to breathe out to the same end-expiratory level, but that a larger inspiratory volume and hence a bigger FVC were observed at the higher P$_{inf}$ (Fig. 2 and Table 2).

This rise in P$_{inf}$ was accompanied by a small but highly significant (P < 0.001) group mean increase in FVC of 13 mL, equivalent to an increase of 6% in expired volume. A similar increase was observed in FEV$_{0.5}$, and hence there was no change in FEV$_{0.5}$/FVC. FEF$_{75}$ also increased by an average of 17 mL s$^{-1}$, equivalent to an increase of 8% in flow, which was also significant (P < 0.01; Table 2). The duration of forced expiration (t$_{exp}$) was almost identical (95% CI of the difference, -0.05 to 0.1 sec; P = 0.48), as was the jacket pressure applied (95% CI, -0.10 to 0.03 kPa; P = 0.31; Table 2), under both measurement conditions.

<table>
<thead>
<tr>
<th>TABLE 1—Infant Characteristics$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) boys</td>
</tr>
<tr>
<td>Corrected postnatal age at test (weeks)</td>
</tr>
<tr>
<td>Weight at time of test (kg)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
</tr>
</tbody>
</table>

$^1$Data shown are medians (range) for continuous variables and n (%) for categorical variables.

DISCUSSION

It has been recognized that one of the advantages of the RVRTC method is that lung volume can be standardised by using a preset airway opening pressure.$^{14}$ However, the results from this study indicate that when using the RVRTC technique, relatively minor variations in P$_{inf}$ (± 8%) will be accompanied by highly significant changes in the major outcome variables (FVC, FEV$_{0.5}$, and FEF$_{75}$) derived by this technique.

Potential factors which may influence results from the RVRTC include: 1) number and rate of augmented breaths prior to forcing expiration; 2) tightness of jacket fit and efficiency with which pressure is transmitted from the jacket to the intrathoracic airways; 3) methods used to assess flow limitation; and 4) most importantly, the preset airway inflation pressure used to inflate the lungs.$^{3-4}$ There is as yet no consensus as to which is the optimal inflation pressure to use for raising lung volume. Currently, most centers are applying 3 kPa,$^{3-6}$ but others have used 2 kPa equally successfully and with similar reproducibility,$^3$ and this pressure may be more appropriate in very small or immature infants.$^6$ Since virtually all parameters derived from the RVRTC are strongly dependent on P$_{inf}$, separate reference data will have to be established according to the selected P$_{inf}$ which can be a very time-consuming and complex undertaking.$^{15,16}$ Equally important, despite selecting a specific P$_{inf}$, there may be subtle variations in the actual P$_{inf}$ delivered, which could bias the results. When first introducing any new lung function technique, there is usually a steep learning curve before any degree of standardization can be introduced. This is particularly true for the RVRTC technique. Even when fully automated systems are used, there are few if any laboratories around the world that can claim to inflate the lungs to exactly 3 (or 2) kPa in the breath immediately prior to expiratory forcing. In reality, despite attempts to provide a standardized pressure, this is quite likely to vary by ±0.2 kPa between infants or centers. Such differences may arise as a result of slight flow dependence of pressure valves, variations in duration of the inflated breath, minor calibration errors, or simply the algorithms used to calculate mean P$_{inf}$. Furthermore, the ability to check the pressure actually delivered rather than that preset by the equipment is not routinely possible in all currently available systems. The aim of this study was to investigate the effect of such subtle changes.

The high degree of within-subject repeatability of parameters derived by the RVRTC technique, and their strong dependence on the inflation pressure delivered at the airway opening, mean that even minor differences in equipment or technique may result in a significant bias between data collected in different laboratories. A difference of 5–10% in FEV$_{0.5}$ or FEF$_{75}$ was considered
TABLE 2—Respiratory Function Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2.9 (0.1)</th>
<th>2.7 (0.1)</th>
<th>Mean difference (2.9–2.7 kPa)</th>
<th>95% CI of difference (2.9–2.7 kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacket pressure (kPa)</td>
<td>5.2 (1.4)</td>
<td>5.3 (1.4)</td>
<td>−0.03 (0.2)</td>
<td>−0.1, 0.03</td>
</tr>
<tr>
<td>$t_{FE}$ (s)</td>
<td>1.2 (0.4)</td>
<td>1.1 (0.4)</td>
<td>0.03 (0.2)</td>
<td>−0.05, 0.1</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>208.0 (96)</td>
<td>195.0 (89)</td>
<td>13.0 (14)</td>
<td>8.18**</td>
</tr>
<tr>
<td>FEV$_{0.5}$ (mL)</td>
<td>180.0 (73)</td>
<td>169.0 (71)</td>
<td>11.0 (12)</td>
<td>6.15**</td>
</tr>
<tr>
<td>FEF$_{75}$ (mL s$^{-1}$)</td>
<td>238.0 (110)</td>
<td>221.0 (116)</td>
<td>17.0 (32)</td>
<td>5.28*</td>
</tr>
</tbody>
</table>

1Data shown as group mean (SD) and 95% confidence interval (CI) of the difference, using paired-samples $t$-test; $t_{FE}$, duration of forced expiration; FVC, forced vital capacity; FEV$_{0.5}$, forced expiratory volume in 0.5 sec; FEF$_{75}$, forced expiratory flow when 75% of FVC has been expelled; CI, confidence interval.

$^*$P < 0.01.

$^{**}$P < 0.001.

significant in recent epidemiological studies. Meti­culous attention, therefore, needs to be paid to all aspects of data collection during the RVRTC technique if meaningful comparisons are to be made within and between infants. This is particularly crucial if the interpretation of results is to be based on reference data collected elsewhere.

CONCLUSIONS

This study has shown that it is important to ensure that precise airway inflation pressures are delivered in the breath immediately prior to the forced expiratory maneuver in order to minimize variations in results derived from FEFV curves and caused by subtle changes in such inflation pressure. Most currently available reference data derived from the RVRTC technique have been obtained using an inflation pressure of 3 kPa. In order to facilitate collaboration and comparison of results between centers, it may be advisable to standardize RVRTC measurements to this pressure, unless there are specific contraindications. In addition, the precise inflation pressure delivered should be measured and reported for comparison. The potential influence of other factors that may influence results obtained from the RVRTC technique remain to be elucidated.

ACKNOWLEDGMENTS

We thank the parents of infants studied for their participation and commitment to the project, and Professor Kate Costeloe for her continued support and permission to recruit and perform respiratory function tests at Homerton Hospital.

REFERENCES


Influence of Jacket Tightness and Pressure on Raised Lung Volume Forced Expiratory Maneuvers in Infants

Sooky Lum, RM,* Ah-Fong Hoo, MPhil, and Janet Stocks, PhD

Summary. While the use of the raised volume rapid thoraco-abdominal compression (RVRTC) technique has been shown to provide new insights into airway and pulmonary pathophysiology in infants, and appears to resemble the spirometric techniques used in older subjects, there is as yet no consensus regarding measurement procedures, which are known to vary considerably between laboratories (Gappa [1999] Pediatr Pulmonol 28:391–393). The aims of this study were to assess the effects of tightness of jacket fit, the efficiency with which pressure is transmitted from the jacket to the intrathoracic airways, and the effect of jacket pressure on parameters derived from the RVRTC technique. Paired forced expiratory maneuvers were performed in 20 infants with the jacket snugly or loosely wrapped around the infant’s torso, and in a further 21 infants using “optimal” or a higher jacket pressure (Pj) (1–2 kPa above “optimal” Pj).

When either a loosened jacket or a higher than “optimal” Pj was used, forced expired flow at low lung volumes (FEF75) was significantly reduced by, on average, 8% and 7%, respectively. There were, however, minimal changes in forced vital capacity (FVC) or forced expired volume in 0.4 sec (FEV0.4). The observed changes may have been due to the increased pressure transmitted to the intrathoracic structures under these experimental conditions, and emphasize the need to assess optimal jacket pressure within each infant when using the RVRTC technique. In addition, when using a loosened jacket or a higher than “optimal” Pj, chest wall and upper airway reflexes such as glottic closure, peripheral airway closure, and negative flow dependence were more evident.


Key words: maximal expiratory flow-volume curves; pulmonary function test; infant; methods; standardization; spirometry.

INTRODUCTION

In recent years, adaptations of the tidal rapid thoraco-abdominal compression (RTC) technique have been applied, wherein the infant’s lungs are passively inflated towards total lung capacity before applying compressive pressure.1,2,3 This enables full forced expiratory maneuvers to be obtained in infants as in older children and adults.

However, there is currently no standardized approach to either data collection or analysis for this promising new technique.1,4,5 We recently showed that relatively minor (8%) variations in lung inflation pressure applied during the raised volume RTC maneuver (RVRTC) will be accompanied by significant changes in major outcome variables.6 Thus the precise lung inflation pressure delivered must be reported if meaningful comparisons are to be made between results, within or between different infants, and/or centers. During augmented breathing, we also showed that inflation volume and respiratory system compliance (Crs) are significantly lower when measurements are obtained with a fastened but uninflated jacket in situ, compared to those obtained without.7 In addition, there has been considerable debate whether the “optimal” jacket pressure determined during standard RTC maneuvers is sufficient to achieve flow limitation at raised lung volume, and whether the jacket should be applied more loosely during the raised volume than the tidal RTC technique.

The aims of this study were to assess the effect of tightness of jacket fit, the efficiency with which pressure is transmitted from the jacket to the intrathoracic airways, and the effect of jacket pressure on parameters derived from the RVRTC technique.

Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit, Institute of Child Health and Great Ormond Street Hospital NHS Trust, and Neonatal Unit, Homerton University Hospital, London, United Kingdom.

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*Correspondence to: Ms. Sooky Lum, Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit, Institute of Child Health, 30 Guilford St., London WC1N 1EH, UK. E-mail: s.lum@ich.ucl.ac.uk

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MATERIALS AND METHODS

Healthy full-term infants were recruited from the Maternity Units at Homerton and University College London Hospitals, London, to an epidemiological study, which included parameters derived from both the tidal and raised volume RTC techniques as outcome measures. Respiratory function tests were carried out when infants were free from any respiratory tract infections, and when at least 3 weeks had elapsed since any respiratory symptoms. Measurements were made during behaviorally determined quiet sleep following sedation with chloral hydrate syrup (60–80 mg · kg⁻¹). Tidal RTC (results from which are not reported here) was performed prior to the RVRTC technique. The studies were approved by the local Research Ethics Committees, and written informed consent was obtained from the infants’ parents.

Airway function was assessed from forced expiratory flow-volume (FEFV) curves obtained at raised lung volume, using a technique adapted from Feher et al. and previously described. During forced expiratory maneuvers, a jacket which extended from the infant's axillae to the iliac crest was wrapped around the infant's torso, with the arms outside the jacket as per protocol (see below). The jacket consisted of a polythene inflatable bladder (Hannover, Germany) surrounded by a stiff outer fabric covering (Columbus, OH) which could be rapidly inflated from a 100-L pressurized air reservoir connected to the inflatable bladder via rigid large-bore (28-mm ID) tubing (Fig. 1). Two sizes of jackets were available for infants of different ages. The polythene inflatable bladder for the smaller jacket measured 17 × 16 cm, while that for the larger jacket measured 20 × 20 cm. Raised lung volume was achieved by manually inflating the infant's lungs, using a fresh gas flow at a rate of 12 L · min⁻¹ via a Neopuff Infant Resuscitaire (Fisher & Paykel Healthcare, Auckland, New Zealand) which had a safety relief valve set to an airway pressure of 3 kPa. Lung inflations were maintained until a plateau was observed on both the pressure and volume recordings, and were released once zero flow was observed at end inflation. The mean duration of plateaux on the pressure and volume signals was 150–250 msec and 50–100 msec, respectively. Four to five augmented breaths were delivered before inflating the jacket (using a predetermined "optimal" pressure; see below) to force expiration from raised lung volume (Fig. 2). Jacket inflation was triggered as passive inflation approached zero flow on the flow-volume loop, by manually switching the three-way tap connecting the inflatable plate and the pressurized air reservoir, to force expiration. The mean duration of the rise time of jacket inflation (i.e., from start to full jacket inflation) was 70–100 msec. Jacket inflation was maintained until forced expiration was complete, as seen by zero flow crossing on the FEFV curve. Following the last passive inflation and the RTC maneuver, an expiratory pause was usually

ABBREVIATIONS

ATS  American Thoracic Society  
CI  Confidence interval  
Crs  Respiratory system compliance  
FEF  Forced expiratory flow  
FVC  Forced vital capacity  
FEF₇₅  FEF when 75% of FVC has been expired  
FEF₅₀  FEF when 50% of FVC has been expired  
FEF₂₅–₇₅  Mean FEF between 25–75% of expired FVC  
FEF₅₀–₇₅  Mean FEF between 50–75% of expired FVC  
FEFV  Forced expiratory flow volume  
FEF₉₄  Forced expired volume in 0.4 sec  
PEF  Peak expiratory flow  
P₉₅  Absolute pressure transmitted to intrathoracic airways as a result of jacket inflation  
P₉₅  Inflation pressure  
Pj  Jacket pressure  
RTC  Rapid thoraco-abdominal compression  
RVRTC  Raised volume RTC  
VmaxFRC  Maximal flow at functional residual capacity

Fig. 1. Schematic diagram of equipment for RVRTC technique.

Fig. 2. Time-based trace of RVRTC maneuver, showing relaxed augmented breaths with plateaux on volume and airway opening pressure signals prior to jacket inflation.
observed. Data recording was maintained until regular tidal breaths recommenced. 

Crs was measured from the passive inflations and deflations prior to forcing expiration, using multilinear regression analysis as described previously.

Influence of Jacket Tightness on RVRTC Parameters

The first study was designed to assess the influence of standard vs. loosened jacket placement on RVRTC parameters. The former was a practice within our own department, while the latter had been observed during interlaboratory visits to other centers using the raised volume technique.

The RTC jacket was wrapped snugly according to recent recommendations for the tidal RTC maneuver (i.e., with space to insert 2 adult fingers between the jacket and sternum), or loosely (i.e., allowing the breadth of 4 adult fingers between jacket and sternum) around the infant's torso. An entire set of measurements, which comprised 4–6 forced expiratory maneuvers to yield at least 3 reproducible (within 10%), technically satisfactory FEFV curves (see Data and Statistical Analysis, below) was obtained at each jacket placement in each infant. Within each infant, an identical lung inflation pressure (3 kPa) and jacket pressure (Pj) were used during both sets of measurements. The "optimal" Pj was defined as that above which no further increase in maximal flow at functional residual capacity (VmaxPRC) was achieved using standard jacket placement. The extent to which pressure was transmitted from the jacket to the intrathoracic airways, using different jacket fittings, was assessed by performing a brief airway occlusion at end-tidal inspiration immediately prior to jacket inflation, and then measuring the subsequent change in pressure at the airway opening following jacket inflation.

Influence of Jacket Pressure (Pj) on RVRTC Parameters

The second study compared paired measurements of airway function at raised lung volume, using both "optimal" and higher Pj (1–2 kPa above "optimal" Pj). Within each infant, an identical lung inflation pressure (3 kPa) and standard jacket placement were used during both sets of measurements. Jacket pressure transmission was assessed as described above.

Data and Statistical Analysis

Technically acceptable maneuvers (defined as rapid rise to peak expiratory flow following jacket inflation, no evidence of early inspiration, and a smooth flow-volume curve without significant glottic closure or flow transients, especially during the last half of expiration) were analyzed using previously validated software ("Squeeze" version 2.04, P. Dixon and J. Stocks, Imperial College, London, 1999) to calculate FVC, FEV0.40.5, and FEF75. Within-subject comparisons were made, using the best curve obtained at each inflation pressure. The "best" curve was defined as the technically acceptable curve with the highest sum of FVC and FEV0.4,* as adapted from the ATS spirometry guidelines for adults and older children. Criteria for acceptance of data included the fact that both FVC and FEV0.4 from the "best" curve should be within 10% of those from the next best maneuver, recorded under the same measurement conditions.

The absolute pressure transmitted to the intrathoracic airways as a result of jacket inflation (Paoj) was calculated as the difference between the airway opening pressure during a brief airway occlusion at end-tidal inspiration (P1) and subsequent airway opening pressure achieved following jacket inflation (P2), i.e., Paoj = P2 – P1. The relative efficiency of the jacket pressure transmission was assessed by (Paoj/Pj) × 100.

All results were cross-checked by an independent observer (J.S.). Statistical analysis of data was performed using paired t-tests, with 95% confidence intervals of the difference (SPSS version 10.0 for Windows).

RESULTS

Comparison of Jacket Placement

Paired measurements of airway function using standard and loosened jacket placement were obtained in 20 infants, whose anthropometric details are summarized in Table 1. All measurements were obtained using the standard jacket placement initially. Respiratory function results are summarized in Table 2. Pj and lung inflation pressure (Pml) applied during both measurement conditions were almost

<table>
<thead>
<tr>
<th>TABLE 1—Infant Characteristics</th>
<th>Jacket tightness comparison</th>
<th>Jacket pressure comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% boys)</td>
<td>20.0 (50%)</td>
<td>21.0 (48%)</td>
</tr>
<tr>
<td>Age at test (weeks)</td>
<td>8.4 (4.7–4.7)</td>
<td>7.9 (4.4–4.0)</td>
</tr>
<tr>
<td>Weight at test (kg)</td>
<td>5.4 (4.1–8.5)</td>
<td>4.9 (3.0–8.5)</td>
</tr>
<tr>
<td>Crown-heel length at test (cm)</td>
<td>56.8 (51.9–71.4)</td>
<td>57.0 (50.5–71.4)</td>
</tr>
</tbody>
</table>

1Data shown as median (range).  
2Three infants were common to both studies.
TABLE 2—Effect of Jacket Tightness on Respiratory Function Results

<table>
<thead>
<tr>
<th>Jacket tightness</th>
<th>Loosened</th>
<th>Standard</th>
<th>Mean difference (loosened — standard)</th>
<th>95% CI of difference (loosened — standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{inj}$ (kPa)</td>
<td>3.0 (0.1)</td>
<td>3.0 (0.1)</td>
<td>0.01 (0.06)</td>
<td>-0.03, 0.02</td>
</tr>
<tr>
<td>$P_j$ (kPa)</td>
<td>5.1 (1.2)</td>
<td>5.1 (1.3)</td>
<td>0.01 (0.2)</td>
<td>-0.1, 0.1</td>
</tr>
<tr>
<td>$t_{PE}$ (sec)</td>
<td>1.15 (0.34)</td>
<td>1.16 (0.45)</td>
<td>-0.01 (0.25)</td>
<td>-0.12, 0.11</td>
</tr>
<tr>
<td>PEF (mL/ sec⁻¹)</td>
<td>961.0 (231)</td>
<td>847.0 (142)</td>
<td>114.0 (178)</td>
<td>23, 206*</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>208.0 (74)</td>
<td>203.0 (71)</td>
<td>5.0 (15)</td>
<td>-1, 12</td>
</tr>
<tr>
<td>FEV₀.₄₀ (mL)</td>
<td>165.0 (52)</td>
<td>164.0 (51)</td>
<td>1.0 (8)</td>
<td>-3, 5</td>
</tr>
<tr>
<td>FEV₀.₅₀ (mL)</td>
<td>182.0 (58)</td>
<td>181.0 (57)</td>
<td>1.0 (11)</td>
<td>-4, 7</td>
</tr>
<tr>
<td>FEF₇₅ (mL/ sec⁻¹)</td>
<td>232.0 (85)</td>
<td>253.0 (107)</td>
<td>-21.0 (38)</td>
<td>-38, -3*</td>
</tr>
<tr>
<td>FEF₅₀₋₇₅ (mL/ sec⁻¹)</td>
<td>509.0 (135)</td>
<td>504.0 (138)</td>
<td>5.0 (72)</td>
<td>-28, 39</td>
</tr>
<tr>
<td>FEF₇₅₋₇₅ (mL/ sec⁻¹)</td>
<td>456.0 (128)</td>
<td>461.0 (152)</td>
<td>-5.0 (53)</td>
<td>-30, 19</td>
</tr>
<tr>
<td>FEF₀.₄₀₋₀.₅₀ (mL/ sec⁻¹)</td>
<td>359.0 (110)</td>
<td>368.0 (126)</td>
<td>-9.0 (38)</td>
<td>-27, 9</td>
</tr>
<tr>
<td>$C_{rs}$ (mL/ kPa⁻¹)</td>
<td>63.6 (26.8)</td>
<td>62.9 (24.9)</td>
<td>2.7 (4.7)</td>
<td>0.5, 4.9*</td>
</tr>
</tbody>
</table>

*Data shown as group mean (SD) and 95% CI of the difference, using paired-sample t-tests. $P_{inj}$, inflation pressure; $P_j$, jacket pressure; $t_{PE}$, duration of forced expiration; PEF, peak expiratory flow; FVC, forced vital capacity; FEV₀.₄₀, forced expired volume in 0.4 or 0.5 secs; FEF₀.₄₀₋₀.₅₀, forced expiratory flow at 50-75% of expired FVC; FEF₅₀₋₇₅, mean forced expiratory flow between 25-75% of FVC; FEF₀.₄₀₋₀.₅₀, mean forced expiratory flow between 50-75% of FVC; $C_{rs}$, respiratory system compliance.

^n = 19.*P < 0.05.

The effect of a loosened jacket placement on forced expiratory maneuvers is illustrated in Figure 3, which shows an overlay of the best FEFV curve generated from raised lung volume, using both the standard and loosened jacket placement in the same infant. There was a group mean increase by 0.39 kPa (16%) in $P_j$ transmission (loosened vs. standard, 2.84 vs. 2.45 kPa) using the loosened jacket placement. This increase in efficiency of the jacket to transmit pressure to the intrathoracic airways was accompanied by a significant increase in peak expiratory flow (PEF). There were only minimal changes (<2%) in FVC and FEV₀.₄₀₋₀.₅₀ which were not significant, but the group mean values for FEF₇₅ showed a decrement of 21 mL/sec⁻¹, equivalent to a reduction of 8% in flow, when the jacket was loosened (Table 2). In addition, there was a small but significant increase in respiratory system compliance ($C_{rs}$) by a mean of 4% when the jacket was loosened, suggesting that the standard placement of the jacket may be associated with slightly more restriction of chest expansion during lung inflations.

**Comparison of Jacket Pressure**

Paired measurements of airway function at raised lung volume using "optimal" and higher $P_j$ (1-2 kPa above "optimal" $P_j$) were obtained in 21 infants, whose anthropometric details are summarized in Table 1. Three of these infants had also participated in the study comparing loose and standard jacket placement. All measurements were obtained using standard jacket placement and "optimal" $P_j$ initially. The effect of a 1-2-kPa change in $P_j$ on raised volume forced expiratory maneuvers is illustrated in Figure 4, which shows an overlay of the best FEFV curves generated using "optimal" and higher $P_j$ in the same infant. By overlaying the curves at end inspiration (inflation), it can be seen that the infant appeared to breathe out to a similar end expiratory level, and that using a higher $P_j$ did not appear to influence the volume parameters. Respiratory function results are summarized in Table 3. Volume parameters such as FVC and FEV₀.₄₀₋₀.₅₀ were virtually identical (Table 3 and Fig. 4) under both sets of measurement conditions. Peak expiratory flow (PEF) was approximately 3% higher when using the higher $P_j$, but this difference was

![Fig. 3. Comparison of RVRTC curves using different jacket tightness. Start of inspiration/augmented breath is defined as zero on the volume axis. Forced expiratory flow volume curves using a lung inflation pressure of 3 kPa are overlaid along descending portion of flow volume curves.](image-url)
not statistically significant (95% CI, -125, 66). By contrast, both FEF\textsubscript{75} and FEF\textsubscript{50–75} were significantly lower (by 7% and 6%, respectively) when higher jacket pressures were applied. Application of the higher P\textsubscript{j} was also more likely to be associated with glottic narrowing (Fig. 5) and/or evidence of negative flow dependence (Fig. 6).

**DISCUSSION**

The results from this study suggest that use of the “optimal” jacket pressure as determined during the tidal RTC maneuvers is sufficient to achieve flow limitation at raised lung volume, provided the jacket fit is not adjusted between the two techniques. The protocol used for adjusting and fastening the jacket during the RVRTC maneuver in this ongoing epidemiological study was based on recent recommendations for standardized measurements of tidal RTC in infants,\textsuperscript{11} since we were also collecting V\textsuperscript{maxFRC} data. We previously showed that during augmented breathing, fastening the jacket restricts inflation volume and reduces respiratory system compliance (C\textsubscript{rs}) in infants by an average of 8\% when compared to measurements obtained without the jacket in situ.\textsuperscript{7} In this study, the increase in FVC and C\textsubscript{rs} when measured with a loosened vs. a “standard” jacket was on average only 2% and 4%.

**TABLE 3—Comparison of Jacket Pressure on Respiratory Function Results\textsuperscript{1}**

<table>
<thead>
<tr>
<th>Jacket pressure (P\textsubscript{j})</th>
<th>“Optimal”</th>
<th>Higher</th>
<th>Mean difference (optimal – higher)</th>
<th>95% CI of difference (optimal – higher)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P\textsubscript{j} (kPa)</td>
<td>5.0 (1.1)</td>
<td>6.1 (1.2)</td>
<td>-1.1 (0.2)</td>
<td>-1.2, -1***</td>
</tr>
<tr>
<td>P\textsubscript{ao} (kPa)</td>
<td>3.0 (0.08)</td>
<td>3.0 (0.07)</td>
<td>-0.01 (0.04)</td>
<td>-0.01, 0.03</td>
</tr>
<tr>
<td>t\textsubscript{ao} (sec)</td>
<td>1.01 (0.31)</td>
<td>1.12 (0.28)</td>
<td>-0.06 (0.23)</td>
<td>-0.17, 0.04</td>
</tr>
<tr>
<td>PEF (mL/sec\textsuperscript{-1})</td>
<td>972.0 (252)</td>
<td>1,002.0 (282)</td>
<td>-30.0 (199)</td>
<td>-125, 66</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>181.0 (62)</td>
<td>180.0 (59)</td>
<td>1.0 (10)</td>
<td>-4, 5</td>
</tr>
<tr>
<td>FEV\textsubscript{1.00} (mL)</td>
<td>147.0 (44)</td>
<td>146.0 (43)</td>
<td>1.0 (9)</td>
<td>-3, 5</td>
</tr>
<tr>
<td>FEV\textsubscript{1.00} (mL\textsuperscript{2})</td>
<td>160.0 (50)</td>
<td>158.0 (49)</td>
<td>2.0 (8)</td>
<td>-3, 6</td>
</tr>
<tr>
<td>FEF\textsubscript{75} (mL/sec\textsuperscript{-1})</td>
<td>205.0 (78)</td>
<td>191.0 (79)</td>
<td>14.0 (24)</td>
<td>3, 25*</td>
</tr>
<tr>
<td>FEF\textsubscript{50–75} (mL/sec\textsuperscript{-1})</td>
<td>471.0 (146)</td>
<td>459.0 (131)</td>
<td>12.0 (71)</td>
<td>-20, 45</td>
</tr>
<tr>
<td>FEF\textsubscript{25–75} (mL/sec\textsuperscript{-1})</td>
<td>414.0 (122)</td>
<td>405.0 (121)</td>
<td>9.0 (48)</td>
<td>-13, 30</td>
</tr>
<tr>
<td>FEF\textsubscript{50–75} (mL/sec\textsuperscript{-1})</td>
<td>318.0 (100)</td>
<td>300.0 (101)</td>
<td>18.0 (40)</td>
<td>-1, 36**</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Data shown as group mean (SD) and 95% CI of the difference, using paired-sample t-tests. Definition of abbreviations, same as for Table 2.

\textsuperscript{2}n = 20.

\textsuperscript{*}P < 0.05.

\textsuperscript{**}P = 0.059.

\textsuperscript{***}P < 0.001.
Applying a higher Pj, may cause negative flow dependency airways, as a result of either loosening the jacket or jacket placement continues to exert some effect on any further increase in Pj transmitted to the intrathoracic flow limitation has already been achieved at "optimal" Pj, infants with relatively compliant airways, and in whom flow than volume parameters (Fig. 3). Alternatively, in lung volume, which will have a more marked effect on FVC, secondly, in the presence of a small increase in chest wall reflexes by the presence of a jacket or slight restriction of chest expansion during lung inflations, even when the jacket is loosely applied. Previous studies also noted a reduction in both lung volume and compliance during tidal breathing following jacket placement or with inductance bands.

The most consistent change observed when either a loosened jacket or higher than "optimal" Pj was applied during RVRTC was a marked reduction in FEF75 by an average of 8% and 7%, respectively. While the magnitude of this reduction may be of minimal clinical significance in individual infants, these findings are of considerable relevance to the current international debate regarding standardization of data collection and analysis for the RVRTC technique. The reasons for the reduction in FEF75 may be threefold. First, due to the steepness of the slope of the FEFV curve, a small increase in FVC could explain an 8% decrease in flow. Secondly, in the presence of a small increase in FVC, due to the slight increase in Cns, when the jacket is loosened, FEF75 might be measured at a slightly lower lung volume, which will have a more marked effect on flow than volume parameters (Fig. 3). Alternatively, in infants with relatively compliant airways, and in whom flow limitation has already been achieved at "optimal" Pj, any further increase in Pj transmitted to the intrathoracic airways, as a result of either loosening the jacket or applying a higher Pj, may cause negative flow dependency to occur (Fig. 6). Application of a higher Pj may also induce marked upper airway reflexes, such as glotic narrowing or closure at high lung volume (Fig. 5).

The results from this study suggest that the approach used in our previous clinical and epidemiological studies, wherein "optimal" Pj was assessed during the tidal RTC technique and then applied during RVRTC in order to minimize both the time required for data collection and the number of lung inflations and forced expirations to which the infant is exposed, is valid provided the jacket fit is not adjusted. Since the amount of pressure transmitted is critically dependent on how tightly the jacket is placed, optimal pressure must be assessed individually for each infant under the precise measurement conditions being used during testing. Although loosening the jacket did result in an increase in FVC, this effect was very small (on average, only 2%). The observation that a looser jacket was associated with an increase in pressure transmission was surprising, but may reflect a more even distribution of applied pressure around the infant's chest and abdomen under these circumstances. Nevertheless, it would appear that careful estimation of "optimal" jacket pressure to ensure that this is high enough to achieve maximal expiratory flows, while not resulting in negative flow dependence at low lung volumes, may be more important than trying to assess exactly how tightly a jacket is wrapped.

In centers where only the RVRTC technique is used for assessing airway function in infants, forced expiratory maneuvers have been repeated with increasing jacket compression pressures until the highest volumes and flows are obtained. This method of estimating "optimal" Pj for the RVRTC technique is obviously equally valid, but requires many more lung inflations, which could potentially increase the risk of gastric distension. In an attempt to standardize the technique and minimize the number of maneuvers required, it has been suggested that a constant transmission pressure of 2–2.5 kPa would be suitable for all infants during RVRTC maneuvers. However, we have shown that this level of transmission pressure will fail to achieve flow limitation in some healthy infants while causing negative flow dependence and glotic closure in others, and we therefore do not recommend this approach.

Jacket pressure transmission is not routinely assessed in all centers that use the RVRTC technique, but in those that do, it is most commonly assessed at end tidal inspiration. While it has been suggested that assessment of pressure transmission at raised lung volume (i.e., end inflation) may be more informative, this approach has not generally been attempted where the inflation pressures are preset to 3 kPa, because the infant would be exposed, albeit very briefly, to very high intrathoracic pressures (>5–6 kPa). In addition, accurate data collection may be compromised due to increased risk of leaks between the mask and face from the high pressure applied under such circumstances.
The question still remains as to whether the assessment of transmission pressure is indeed useful or necessary. As the “optimal” $P_j$ differs between individuals, assessment of transmission pressure may not always provide useful information on any given occasion. Its assessment does, however, allow comparisons of technique between different centers studying similar populations of infants, and it may therefore be particularly useful as a quality-control measure in multicenter trials.

Due to time constraints, it was not possible to reassess optimal $P_j$ in the presence of a looser jacket during the RVRTC maneuvers in this study, in order to make a direct comparison of results under these conditions. It may well be that, had a slightly lower $P_j$ been used in the presence of a looser jacket, identical flows at low lung volumes would have been achieved as when using a “standard” fit of jacket and “optimal” $P_j$. The achievement of higher peak flows but lower flows at low lung volumes when using a higher $P_j$ presents a potential problem. Increasing the $P_j$ will obviously help to achieve flow limitation at high lung volume, which is desirable. However, since this may be accompanied by an increasing number of failures due to glottic closure and reflex stiffening of the chest wall, together with negative flow dependence at low lung volumes, the use of excessive $P_j$ must obviously be avoided, especially since the most important information regarding respiratory mechanics in infants is probably obtained from flows at low lung volumes.

Measurements for these two studies were achieved in lightly sedated infants who remained in quiet sleep after completion of the standard protocol for an ongoing epidemiological study. Although the study design for these studies could potentially be improved by randomization of the study protocol, to do so would have required infants to be recruited specifically for this validation, which was not felt to be ethically justifiable. The issue of sedating healthy infants is contentious in some countries. However, over the past 20 years we have never experienced any adverse effects when sedating healthy infants ($n > 1,000$). Given the numerous determinants of lung and airway function during early life (e.g., age, body size, low birth weight, maternal smoking), it is impossible to interpret lung function results from infants with lung disease (for whom risks of sedation may be considerably greater) unless appropriate control/reference data are available. We therefore believe the benefits of properly conducted epidemiological studies (such as the one from which infants participating in this methodological study were recruited) far outweigh any risks, a view that is shared by our Research Ethics Committee.

Results from the current study do not provide clear evidence regarding the ‘best’ approach to estimating optimal jacket pressure, nor whether this should be done during tidal or raised volume maneuvers. They do, however, show that whereas raised volume indices such as $FV_{50}$ and $FEV_{T}$ are very sensitive to changes in inflation pressure, they are remarkably robust to changes in jacket tightness or pressure. Over the past 4 years, the mean (range) of “optimal” jacket pressure during forced expiratory maneuvers achieved in 363 healthy infants (age range, 0–90 weeks) measured in our laboratory was 5.2 (2.2–8.4) kPa, and the mean (range) for transmitted jacket pressure was 2.3 (1–3.9) kPa. In addition, within any given infant, we found that jacket pressure can vary by up to 3 kPa from which similar $V_{maxFRC}$ (within 10%) can be obtained once flow limitation is reached. These findings suggest that “optimal” $P_j$ cannot be predetermined, but needs to be ascertained for each infant on each occasion, as it will vary according to how tightly the jacket is wrapped and the efficiency of pressure transmission. When assessing optimal $P_j$ during the RVRTC technique, it will obviously be essential to base the selection of “best curves” on a combination of $FVC$ and $FEF_{T5-75}$. Results since, provided expiration proceeds to residual volume, the former is primarily dependent on inflation pressure used and may be achieved at “suboptimal” forcing pressures, whereas the latter will be falsely elevated if there is early inspiration. Tepper et al. and Jones et al. selected the best curve as that with the highest product of $FVC$ and $FEF_{T5-75}$. However, in very young infants, we found that flow limitation may not always be achieved at high lung volume, and hence $FEF_{T50-75}$ may be a more appropriate parameter on which to base such decisions.

Further studies are required to ascertain whether flow and volume parameters derived from the “best curve,” defined as the maneuver with the highest sum of $FVC$ and $FEF_{T}$ as adapted from the ATS recommendations for adults and older children, having previously determined “optimal” $P_j$ are comparable to those derived when the “best curve” is defined as that with the highest product of $FVC$ and $FEF_{T5-75}$. Until such comparisons are available, caution will be necessary when collating or using normative data from different centers.

CONCLUSIONS

This study demonstrated that there is no diminution of any of the key parameters derived from the RVRTC technique, if the “optimal” $P_j$ and “standard” jacket fitting ascertained during the tidal RTC maneuvers are used. In order to minimize any restriction of chest wall movement, and the accompanying small reduction in FVC, use of a slightly looser jacket than has been recommended in the past would seem advisable. However, it is essential to estimate the optimal $P_j$ for each infant, with the jacket fitted exactly as it is to be used, and any repositioning of the jacket during the testing session should be accompanied by a reassessment of this “optimal” jacket pressure in order to ensure that maximal forced expirations really are achieved. This study also raised the important issue of
selection criteria for the “best” flow volume curve from RVRTC maneuvers, which will need further investigation in order to minimize any systematic bias when collating or comparing results from different centers.

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REFERENCES


Q1: Spell out insp and exp in legend.
Q2: Add city of publisher.
Q3: Give volume and page numbers.
Low birthweight for gestation and airway function in infancy: exploring the fetal origins hypothesis

Carol Dezateux FRCP
Sooky Lum PhD
Ah-Fong Hoo MPhil
Jane Hawdon FRCP
Kate Costeloe FRCP
Janet Stocks PhD

1Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, London, UK
2Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit Institute of Child Health and Great Ormond Street NHS Trust, London, UK
3University College London Hospital, London, UK
4Barts and the London, Queen Mary School of Medicine and Dentistry, Homerton University Hospital, London, UK.

Correspondence to:
Carol Dezateux
Centre for Paediatric Epidemiology and Biostatistics
Institute of Child Health
30 Guilford Street
London WC1N 1EH, UK
Email: c.dezateux@ich.ucl.ac.uk
Tel: 0207 905 2605
Fax: 0207 242 2723

Keywords: [MESH headings] Birthweight; fetal growth retardation; respiratory function tests; forced expiratory volume; infant; maternal smoking; social class;
Background  Poor fetal growth has been associated with impaired airway function in adult life but evidence linking birthweight and airway function in early childhood is sparse. We examined the hypothesis that low birthweight for gestation is associated with impaired airway function shortly after birth.

Methods  Airway function was measured using the raised volume technique in healthy infants of low (<10th centile) or appropriate (≥20th centile) birthweight for gestation and expressed as forced expiratory volume at 0.4s (FEV0.4) and the maximal expired flow at 25% of forced vital capacity (MEF25). Infant length, maternal height and weight, maternal report of smoking pre- and post-natally and parental occupation were recorded.

Findings  Mothers of low birthweight for gestation infants (n=91) were lighter, shorter and more likely to smoke and have partners in manual occupations. At 6 weeks their infants remained lighter and shorter than those of appropriate birthweight (n=132). Both FEV0.4 and MEF25 were diminished in infants of low birthweight for gestation, in those whose mothers smoked in pregnancy or who were in manual occupations. After adjusting for relevant maternal and infant characteristics, FEV0.4 was an average 9mL lower (95% CI: 2, 16; p=0.01), and MEF25 an average 22 mL.s⁻¹ lower (95% CI: 1, 42; p=0.04) in the low birthweight for gestation group.

Interpretation  Airway function is diminished in early postnatal life as a consequence of a complex causal pathway, which includes social disadvantage as expressed by maternal social class, smoking and height, birthweight as a proximal and related consequence of these factors, and genetic predisposition to asthma. Further work is needed to establish the relevance of these findings to subsequent airway growth and development in later infancy and early childhood.
Introduction
Low rates of fetal growth have been associated with impaired airway function in adult life\(^1\). While this hypothesis has been investigated in a number of studies exploring the relation of low birthweight to adult airway function\(^2,3\), evidence linking birthweight and airway function in early childhood is sparse\(^4,5\). The association between fetal development and airway function is likely to be complex, involving causal pathways that include both genetic and pre- and postnatal environmental factors\(^6\). The potential for confounding, particularly by socio-economic status, in studies examining the fetal origins of adult disease has been discussed by Kramer\(^7\), who, with others, has highlighted the need to develop study designs which provide a more robust and explicit test of the fetal origins hypothesis\(^8,9\).

We report here the findings of a prospective epidemiological study comparing airway function in early infancy in full term infants considered to be of low and appropriate birthweight for gestational age. We aimed to test the hypothesis that low birthweight for gestation was associated with impaired airway function shortly after birth, and that this association was independent of maternal socio-economic status and fetal exposure to maternal smoking.
Methods

Parents of infants delivered in the maternity units at the Homerton University Hospital and University College London Hospital, London were contacted by post. Healthy infants (>35 wk gestation) of white mothers and with no congenital abnormalities, neuromuscular or cardiorespiratory disorders were eligible for inclusion. Infants were ineligible if they needed ventilatory assistance during the neonatal period, had experienced any lower respiratory illness prior to testing or were more than 12 weeks postnatal age at test. Infants were classified according to birthweight and gestational age using the sex-specific Child Growth Foundation (CGF) algorithms as well as the Gestation Related Optimal Weight or 'GROW' program. The latter takes maternal characteristics such as height, booking weight, ethnic group and parity into account as well as infant birthweight, gestation and sex. Gestational age was based on ultrasound assessment before 20 weeks. Infants whose birthweight fell at or below the tenth centile according to either algorithm were assigned to the low birthweight for gestation group, with those between the 20th and 95th centile on either algorithm assigned to the appropriate birthweight for gestation group. Infants of intermediate birthweight (>10th and <20th centile) were excluded. Local Research Ethics Committees approved this study and informed written consent was obtained from parents.

Respiratory function was measured between 4 and 12 wk postnatally, when infants had been well and free from upper respiratory tract infections for at least 3 weeks. Measurements were made following sedation with chloral hydrate syrup (60mg.kg⁻¹), during behaviourally determined quiet sleep using previously reported procedures. Body weight, crown-heel length, chest and mid-arm circumferences were measured and weight and length expressed as sex-specific SD scores.

Airway function was assessed from the forced expiratory volume at 0.4s (FEV₀₄) and the maximal expired flow at 25% of forced vital capacity (MEF₂₅) during the raised volume technique as described previously. These parameters were calculated according to previously described quality control criteria from the best of at least three acceptable and reproducible flow-volume curves obtained from raised volume...
techniques, where best is defined as the technically acceptable loop with the highest sum of FVC and FEV$_{0.4}^{13,16}$.

At the time of lung function testing, mothers were asked about their own smoking pre- and postnatally, their age at leaving full time education, parental occupational status, and family history of asthma in infant’s first degree relatives. Maternal height and weight were measured and infant urine and maternal saliva obtained for cotinine assay$^{17}$. Maternal salivary cotinine concentrations ranged from 20.8 to 434.6 ng.mL$^{-1}$ in five infants whose mothers reported themselves as non-smokers. As these values are consistent with values obtained from active smokers ($>15$ ng.mL$^{-1}$) these mothers were considered as smokers in subsequent analyses$^{18,19}$.

**Sample Size and Statistical Analysis**

The study was designed to provide 90% power at the 5% significance level to detect a difference of one standard deviation (SD) in estimates of forced expiratory flows and volumes between the two groups after adjustment for potential confounding factors. Comparisons of group characteristics and respiratory function between the groups were performed using t tests, chi-square, or exact tests as appropriate (StatXact v4.01). The extent to which low birthweight for gestation is associated with forced expiratory flow and volumes was examined using multiple linear regression (SPSS for Windows, Release 10.1.3) after adjustment for sex and current body size and after examining for the effects of other potential confounding factors.
Results

We traced 1061 of 1634 potentially eligible infants born over a four year period (1998-2001) at Homerton University and the University College London hospitals. Parental consent was given for 359 infants (34%) to take part, 53 of whom became subsequently ineligible either because they developed a lower respiratory illness (n=12) or because cancellations due to upper respiratory infections meant they no longer met the age eligibility criterion (n=41). A further 63 infants did not attend because their parents withdrew from the study (n=30) or did not have time to attend the laboratory (n=33). Thus 243 infants attended for respiratory function testing, and measurements were successfully obtained in 223 infants, 91 from the low birthweight for gestation and 132 from the appropriate birthweight for gestation groups.

Infants in the low birthweight for gestation group were of similar gestation but shorter and of smaller head circumference at birth than those of appropriate birthweight for gestation (Table 1). Although the groups did not differ with respect to maternal age at delivery or maternal social class, maternal age and the percentage of fathers in non-manual occupations were higher in the study population overall than predicted from national data. Mothers of low birthweight infants were significantly lighter and shorter and were more likely to smoke. Maternal social class, stature and smoking status were interrelated, with more mothers from manual occupations being below average height (163.7 cm) for the group ($\chi^2 = 11.54; p = 0.001$) or smokers ($\chi^2 = 25.31; p < 0.001$). Mothers who smoked were more likely to be below average height than those who did not ($\chi^2 = 4.86; p = 0.027$).

At about 6 weeks of age, infants of low birthweight remained significantly lighter and shorter, with smaller head, chest and mid arm circumferences than those of appropriate birthweight (Table 1). At this age, urinary cotinine was significantly higher in infants whose mothers reported smoking (geometric mean [interquartile range]: 12.2 ng.mL$^{-1}$ [5.6 – 31.9]) than in those whose mothers did not (1.3 ng.mL$^{-1}$ [0.7 – 2.7]; 95% CI of the ratio smokers: non-smokers: 6.7 to 14.2; $p <0.001$).

In univariate analyses, flow and volume parameters were diminished in infants of low birthweight for gestational age, in those with mothers who smoked in pregnancy or
who were in manual occupations (Table 2). There was a marked difference in the pattern of associations with maternal, biological and environmental factors for the flow and volume parameters, which were both related to the infant's age and length at test. FEV<sub>0.4</sub> was diminished in infants whose mothers were shorter, smoked and were in a manual occupation (Table 3). By contrast, MEF<sub>25</sub> was diminished in boys and those with a family history of asthma and, to a lesser extent, in infants whose mothers smoked (Table 4).

In multivariate analyses, FEV<sub>0.4</sub> was an average 9mL (95% CI: 2, 16; p=0.01) lower in the low birthweight for gestation group after adjusting for variables found to be significant in univariate analyses, namely age, body length at test, maternal smoking, social class and height (Table 3). Similarly, MEF<sub>25</sub> was an average 22 mL.s<sup>-1</sup> (95% CI: 1, 42; p=0.04) lower in the low birthweight group after adjusting for age, sex, length at test and family history of asthma (Table 4). This compares with an adjusted average (95% CI) reduction in MEF<sub>25</sub> of 26 mL.s<sup>-1</sup> (9,43) in boys relative to girls and 25 mL.s<sup>-1</sup> (6,43) in infants with a family history of asthma.

A model incorporating birthweight status, age and body length accounted for 47% of the total variance in FEV<sub>0.4</sub>, with birthweight status accounting for 1.7%. By contrast, the model for MEF<sub>25</sub> explained only 14% of the total variance in this parameter, with birthweight status, sex and a family history of asthma accounting for 1.7%, 3.6% and 2.7% respectively.
Discussion

In this population-based study, low birthweight for gestation was associated with diminished airway function when measured in early infancy and prior to the onset of any lower respiratory illness. This was evident whether assessed from forced flows or volumes from the raised volume technique. In older children and adults, FEV$_1$ and MEF$_{25}$ are traditionally considered to reflect primarily large and peripheral airway function respectively, but such relationships are less clear when these measures are obtained during infancy. During early childhood, measurement of FEV$_1$ is rarely feasible due to the rapidity of lung emptying during a forced expiration and FEV$_{0.4}$ or FEV$_{0.5}$ are usually reported$^{16}$. As these timed expired volumes still encompass the majority of the forced expiration, they probably reflect the integrated output from both central and peripheral airways. Despite this, different patterns of associations with maternal, biological and environmental factors were evident for the various flow and volume parameters. Thus FEV$_{0.4}$ was associated with maternal height, smoking and social class, which were inter-related as well as being associated with low birthweight for gestation. By contrast, MEF$_{25}$ was inversely related to infant characteristics such as male sex and a family history of asthma with a weaker association with maternal smoking. Although statistically significant, low birthweight for gestation accounted for less than 2% of the total variation in airway function, as assessed from forced flow or volume parameters. These novel observations help to shed some light on the biological pathways linking fetal growth and airway development.

These findings are generalisable to healthy white infants of low and appropriate birthweight for gestation. The study population excluded infants with factors associated with alterations in airway function, for example, neonatal ventilation and prematurity$^{21}$. The proportion of parents consenting to take part in the study among those contacted was comparable to that reported from other population based studies$^{22-24}$. The study population was however biased towards the more educated and older mother, and, overall, the prevalence of maternal smoking was higher than the national average$^{25}$, but comparable to other studies of antenatal populations in this part of London$^{22}$. This might attenuate the strength of any observed association.
Our study population was biased towards those with milder impairment of growth in utero as we excluded infants delivered electively before 35 weeks gestation and those with respiratory problems at birth. The parameters used to assess airway function are sensitive to impaired airway function and all results were checked to ensure adherence to quality control criteria by an independent observer masked to the birthweight status and smoking exposure of the infants. Thus we consider that these observations are unlikely to be biased or due to chance.

These findings were independent of maternal smoking, which is known to confound the association between low birthweight and impaired airway function in infancy. Associations of infant airway function with maternal height and social class have not been reported previously, and indicate the complexity of the causal chain linking socio-economic disadvantage to low birthweight for gestation. Both forced expiratory volume and flow were positively associated with age and body length and negatively with birthweight status. However forced expiratory flows were also significantly diminished in boys and in infants with a family history of asthma. Our findings are in accord with the findings from studies of airway function and birthweight in school aged children. Rona et al reported a significant association between birthweight and lung function in primary school aged children which was independent of parental smoking and social factors. Similarly Chan et al reported that low birth weight (<2000g) was closely associated with poor airway function at 7 years of age and noted that male sex and exposure to maternal smoking were also important factors. Earlier studies of adult airway function have taken birthweight as a measure of intrauterine growth but should more correctly be adjusted for gestational age to ensure that the effects of prematurity can be separated from those of poor fetal growth.

During fetal development, all airway branches are formed by the 16th week of gestation, with subsequent pre and postnatal growth of the airways resulting from an increase in size rather than number. By contrast, there is a rapid increase in alveolar number during the first two years of life, resulting in a greater increase in lung volume than airway size during this period, a phenomenon known as dysanaptic growth. Age, sex and body length are important determinants of infant airway function during this critical period of growth and development. We chose to measure infants as soon as
possible after birth at an age when the pattern of breathing had stabilised and infants were able to tolerate sedation, but before they had experienced a lower respiratory illness.

Impaired airway function in adult life is an important and independent indicator of mortality risk. Evidence to suggest that reduced size at birth is associated with impaired airway function in adult life is accumulating but the biological and social pathways that mediate these associations remain unclear. Our study suggests that, in early postnatal life, airway function is diminished as a consequence of a complex causal pathway which includes social disadvantage as expressed by maternal social class, smoking and height, birthweight as a proximal and related consequence of these factors, and genetic predisposition to asthma. Further follow up will be needed to establish the relevance of these early findings to subsequent airway growth and development in later infancy and early childhood.
Sources of support

This work was carried out with grants from the Dunhill Medical Trust and the Foundation for the Study of Infant Death. A-F Hoo and Janet Stocks are supported by Portex PLC. Research at the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from R&D funding received from the NHS Executive.

Statement of Authorship

CD and JS conceived the study and, with KC, were responsible for the study design; JH and KC assisted with recruitment; SL and AFH recruited and measured infants, and, together with JS, calculated airway function parameters; CD and SL were responsible for statistical analyses and drafted the manuscript. All authors contributed to interpretation and commented on the manuscript.

Acknowledgements

We are grateful to Sarah Davies and Anne Cantarella for help with recruitment and to Angela Wade for statistical advice.
<table>
<thead>
<tr>
<th>Birthweight group</th>
<th>Low for gestation</th>
<th>Appropriate for gestation</th>
<th>95% CI of Difference: Low-Appropriate</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>91</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>47 (52%)</td>
<td>69 (52%)</td>
<td>-14%, 13%</td>
</tr>
</tbody>
</table>

**Infant characteristics at birth**

- **Gestational age (wk)**: 39.9 (1.5) vs. 39.8 (1.4), -0.3, 0.5
- **Birthweight (kg)**: 2.7 (0.3) vs. 3.5 (0.4), -0.9, -0.7
- **Birthweight SD score**: -1.7 (0.4) vs. 0.05 (0.5), -1.8, -1.6
- **Crown-heel length (cm)**: 49.0 (3.0) vs. 52.0 (2.7), -3.8, -2.2
- **Crown-heel length SD score**: -0.8 (1.3) vs. 0.8 (1.3), -2.0, -1.3
- **Head circumference (cm)**: 33.0 (1.5) vs. 34.5 (1.3), -2.0, -1.2

**Maternal and family characteristics**

- **Maternal age at delivery (yr)**: 32.0 (5.3) vs. 33.0 (5.6), -2.5, 0.5
- **Primipara**: 59 (65%) vs. 84 (64%), -12%, 14%
- **Maternal smoking in pregnancy**: 43 (47%) vs. 51 (39%), -5%, 22%
- **Maternal weight at booking (kg)**: 59.4 (10.0) vs. 63.6 (10.8), -6.9, -1.3 **
- **Maternal height (cm)**: 162.1 (6.6) vs. 164.8 (6.7), -4.4, -0.9 **
- **Mother in non-manual occupation**: 66 (73%) vs. 105 (80%), -18%, 4%
- **Father in non-manual occupation**: 50 (57%) vs. 95 (73%), -29%, -3% *

**Infant characteristics at test**

- **Age (wk)**: 6.4 (2.4) vs. 6.2 (2.0), -0.4, 0.8
- **Weight (kg)**: 4.2 (0.8) vs. 4.8 (0.7), -0.8, -0.4 ***
- **Weight SD score**: -1.1 (0.9) vs. 0.02 (0.9), -1.4, -0.9 ***
- **Length (cm)**: 54.1 (2.8) vs. 56.4 (2.6), -3.0, -1.5 ***
- **Length SD score**: -0.8 (0.8) vs. 0.4 (0.9), -1.4, -1.0 ***
- **Head circumference (cm)**: 37.9 (1.7) vs. 38.9 (1.5), -1.4, -0.6 ***
- **Chest circumference (cm)**: 37.2 (2.8) vs. 39.1 (2.2), -2.6, -1.2 ***
- **Mid arm circumference (cm)**: 11.8 (1.4) vs. 12.4 (1.2), -0.9, -0.2 **
Footnotes to Table 1

Data shown as mean (SD) for continuous and n (%) for categorical variables. SD scores were calculated using CGF algorithms.\textsuperscript{10}

\textsuperscript{§} age after expected date of delivery

* p < 0.05; ** p < 0.01; *** p < 0.001

Definition of symbols

<table>
<thead>
<tr>
<th>Low birthweight for gestation (n)</th>
<th>Appropriate birthweight for gestation (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¥ 78</td>
<td>123</td>
</tr>
<tr>
<td>‡ 81</td>
<td>123</td>
</tr>
<tr>
<td>¥ 88</td>
<td>130</td>
</tr>
<tr>
<td># 90</td>
<td>130</td>
</tr>
<tr>
<td>Birthweight status</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------------------------</td>
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<tr>
<td></td>
<td>Low birthweight for gestation</td>
</tr>
<tr>
<td>n</td>
<td>91</td>
</tr>
<tr>
<td>FEV_{0.4} (mL)</td>
<td>105 (26)</td>
</tr>
<tr>
<td>MEF_{25} (mL.s^{-1})</td>
<td>168 (62)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maternal smoking in pregnancy</th>
<th>Yes</th>
<th>No</th>
<th>Smoking – non-smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>94</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>FEV_{0.4} (mL)</td>
<td>112 (31)</td>
<td>121 (26)</td>
<td>-9</td>
</tr>
<tr>
<td>MEF_{25} (mL.s^{-1})</td>
<td>175 (68)</td>
<td>191 (64)</td>
<td>-16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>45</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>FEV_{0.4} (mL)</td>
<td>107 (30)</td>
<td>120 (28)</td>
<td>-13</td>
</tr>
<tr>
<td>MEF_{25} (mL.s^{-1})^{†}</td>
<td>174 (60)</td>
<td>188 (68)</td>
<td>-14</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001

^{†} n = 46 and n = 170 respectively.

Abbreviations: FEV_{0.4} = forced expired volume in 0.4s; MEF_{25} = maximal expired flow at 25% of forced vital capacity; CI = confidence interval.
### Table 3  Association of FEV$_{0.4}$ with birthweight status and other factors

<table>
<thead>
<tr>
<th></th>
<th>Difference in FEV$_{0.4}$ (mL)</th>
<th>95% Confidence Interval of difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analyses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-20</td>
<td>-27, -12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(baseline: appropriate birthweight for gestation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length at test (per cm)</td>
<td>7</td>
<td>6, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal age (per week)</td>
<td>7</td>
<td>5, 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>-9</td>
<td>-17, -1</td>
<td>0.020</td>
</tr>
<tr>
<td>(baseline: no maternal smoking)</td>
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<tr>
<td>Maternal social class</td>
<td>-13</td>
<td>-22, -4</td>
<td>0.007</td>
</tr>
<tr>
<td>(baseline: non-manual occupation)</td>
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<tr>
<td>Maternal height (per cm)</td>
<td>0.8</td>
<td>0.3, 1.4</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Multivariate analysis$^*$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight status</td>
<td>-9</td>
<td>-16, -2</td>
<td>0.010</td>
</tr>
<tr>
<td>(baseline: appropriate birthweight for gestation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length at test (per cm)</td>
<td>4</td>
<td>3, 6</td>
<td>&lt;0.001</td>
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<tr>
<td>Postnatal age (per week)</td>
<td>3</td>
<td>0.8, 5</td>
<td>0.006</td>
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<tr>
<td>Maternal smoking</td>
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<td>-10, 2</td>
<td>0.172</td>
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<tr>
<td>(baseline: no maternal smoking)</td>
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<tr>
<td>Maternal social class</td>
<td>-1.3</td>
<td>-7, 5</td>
<td>0.664</td>
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<tr>
<td>(baseline: non-manual occupation)</td>
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</tr>
<tr>
<td>Maternal height (per cm)</td>
<td>0.2</td>
<td>-0.2, 0.7</td>
<td>0.275</td>
</tr>
</tbody>
</table>

$^*$ Data adjusted for those variables found to be significant in univariate analyses, i.e. birthweight status, length at test, postnatal age, maternal smoking, maternal social class and maternal height.
| Table 4 Association of MEF\textsubscript{25} with birthweight status and other factors |
|----------------------------------|------------------------------|-----------------|--------|
|                                  | Difference in MEF\textsubscript{25} (mL.s\textsuperscript{-1}) | 95% Confidence Interval of difference | p-value |
| **Univariate analyses**          |                                             |                              |        |
| Birthweight status               | -28                                        | -46, -11                  | 0.002  |
| (baseline: appropriate birthweight for gestation) |
| Sex (baseline: female)           | -20                                        | -38, -3                   | 0.023  |
| Length at test (per cm)          | 5                                          | 2, 8                      | <0.001 |
| Postnatal age (per week)         | 5                                          | 1, 9                      | 0.016  |
| Maternal smoking (baseline: no maternal smoking) | -17                                        | -34, 1                    | 0.067  |
| Family history of asthma (baseline: no history of asthma) | -25                                        | -44, -6                   | 0.011  |
| Maternal height (per cm)         | 0.9                                        | -0.4, 2.0                 | 0.185  |
| **Multivariate analysis\textsuperscript{a}** |                                             |                              |        |
| Birthweight status               | -22                                        | -42, -1                   | 0.039  |
| (baseline: appropriate birthweight for gestation) |
| Sex (baseline: female)           | -26                                        | -43, -9                   | 0.003  |
| Length at test (per cm)          | 4                                          | -1, 9                     | 0.142  |
| Postnatal age (per week)         | 2                                          | -4, 8                     | 0.544  |
| Family history of asthma (baseline: no history of asthma) | -25                                        | -43, -6                   | 0.010  |

\textsuperscript{a} Data adjusted for those variables found to be significant in univariate analyses, i.e. birthweight status, infant sex, length at test, postnatal age and family history of asthma.
Reference List


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