

**LONG TERM CARDIORESPIRATORY  
OUTCOME IN CHILDREN WITH CHRONIC  
LUNG DISEASE OF PREMATURITY**

**A thesis submitted in candidature for the degree of  
Doctor of Philosophy**

**by**

**Suchita Joshi**

**Department of Child Health**

**Cardiff University**

**Cardiff, Wales**

**UK**

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**To Sneha and Neeraj,**  
**For your unconditional love and unwavering belief**



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## **Publications arising from this research**

**Joshi S, Kotecha S.** Lung growth and development. *Early Hum Dev.* 2007 Dec; 83(12):789-94.

**Joshi S, Edwards J M, Wilson D G, Wong J K, Kotecha S, Fraser A G.** Reproducibility of myocardial velocity and deformation imaging in term and preterm infants. *Eur J Echocardiogr.* 2010 Jan;11(1):44-50.

**Poon CY, Edwards JM, Joshi S, Kotecha S, Fraser AG.** Optimisation of myocardial deformation imaging in term and preterm infants. *Eur J Echocardiogr.* 2011,12:247-254

**Joshi S, Powell T, Drayton M, Williams EM, Kotecha S.** Lung function and exercise capacity in school-aged children who had chronic lung disease in infancy. (Submitted for publication to *European Respiratory Journal*)

### **Abstracts presented**

**Joshi S, Edwards JM, Wilson DG, Uzun O, Fraser AG, Kotecha S.** Preterm infants have subclinically reduced myocardial function compared with healthy term controls. Poster presentation: European Respiratory Society meeting (Berlin, 4-8 October 2008).

**Joshi S, Edwards JM, Wilson DG, Uzun O, Drayton M, Fraser AG, Kotecha S.** Subclinical left and right ventricular dysfunction in preterm infants, identified by tissue Doppler imaging. Oral presentation: Welsh Paediatric Society meeting (Abergavenny, 14 November 2008).

**Joshi S, Edwards JM, Wilson DW, Drayton M, Fraser AG, Kotecha S.** Cardiovascular response to acute hypoxia in 8-12 year old children with chronic lung disease of prematurity. Poster presentation: European Respiratory Society meeting (Vienna, 12-16 September 2009).

**Joshi S, Edwards JM, Wilson DG, Wong JK, Kotecha S, Fraser AG.** Can echocardiography be used to measure right ventricular dysfunction and pulmonary hypertension in newborn infants? Poster presentation: European Respiratory Society meeting (Vienna, 12-16 September 2009)

**Joshi S, J M. Edwards, D W. Wilson, S. Kotecha, A G. Fraser** Subclinical myocardial dysfunction in premature infants. Poster presentation: EUROECHO meeting (Madrid, 9-12 December 2009)

**Joshi S, Edwards JM, Wilson DW, Drayton M, Fraser AG, Kotecha S.** Cardiovascular response to acute hypoxia in 8-12 year old children with chronic lung disease of prematurity. Oral presentation: Welsh Paediatric Society meeting (Saundersfoot, 13 November 2009)

## **Prize**

**Best oral presentation, Welsh Paediatric Society Autumn Meeting, 14th November 2008**

## **List of contributors**

**Cardiopulmonary exercise test**

**Assisted by Dr Thomas Powell**

**ELISA test for urinary cotinine level**

**Dr Malinath Chakravarty**

**Measurement of body composition**

**Mrs Sarah Kotecha**

**Acquisition of images for**

**‘Tissue Doppler reproducibility study’**

**Assisted by Mrs Julie Edwards**

**Power calculation and regression analysis**

**Professor Sailesh Kotecha and**

**Dr William John Watkins**

### List of commonly used abbreviations

AT	Acceleration time
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CI	Confidence interval
CLD	Chronic lung disease
CO	Carbon monoxide
CPET	Cardiopulmonary exercise testing
CV	Coefficient of variation
DL <sub>CO</sub>	Alveolar diffusion capacity for carbon monoxide
Ds'	Annular displacement
DXA	Dual-x-ray-absorptiometry
ET	Ejection time
FFMI	Fat free mass index
FiO <sub>2</sub>	Concentration of inhaled oxygen
FMI	Fat mass index
FRC <sub>He</sub>	Functional residual capacity measured by He dilution
FRC	Functional residual capacity
FRC <sub>pleth</sub>	Functional residual capacity measured by plethysmography
Hb	Haemoglobin
HCT	Hypoxic challenge test
He	Helium
ITGV	Intra thoracic gas volume
IVA	Isovolumic acceleration
IVRT	Isovolumic relaxation time

KCO	Carbon monoxide transfer coefficient (Krogh factor)
LV	Left ventricle
MVI	Myocardial velocity imaging
MVV	Maximal voluntary ventilation
O <sub>2</sub>	Oxygen
PAH	Pulmonary arterial hypertension
PaO <sub>2</sub>	Partial pressure of oxygen
PAP	Pulmonary arterial pressure
PR	Pulmonary regurgitation
Q <sub>Peff</sub>	Effective pulmonary blood flow
RDS	Respiratory distress syndrome
R <sub>eff</sub>	Effective airway resistance
RV	Residual volume
SaO <sub>2</sub>	Oxygen saturation measured by pulse oxymetry
SD	Standard deviation
S <sub>Reff</sub>	Specific airway resistance
Ss (al)	End-systolic strain at the apical segment of the lateral wall
Ss (bl)	End-systolic strain at the basal segment of the lateral wall
TDI	Tissue Doppler imaging
T <sub>Q-vs</sub>	Time interval between onset of Q wave and the peak systolic velocity
TLC	Total lung capacity
TR	Tricuspid regurgitation
Va (bl)	Late diastolic velocity at the basal segment of the lateral wall
VA	Alveolar volume

$V_e$ (bl)	Early diastolic velocity at the basal segment of the lateral wall
VR	Ventilatory reserve
$V_s$ (bl)	Systolic velocity at the basal segment of the lateral wall
VT	Vital capacity
VTI	Velocity time integral
$\dot{V}CO_2$	Peak carbon dioxide consumption
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Peak oxygen consumption

## **Symbols used to indicate significant differences between groups**

p <0.05 †(CLD vs. Preterm control), ††(CLD vs. Term control), †††(Preterm control vs. Term control)

p <0.01 ‡(CLD vs. Preterm control), ‡‡(CLD vs. Term control), ‡‡‡(Preterm control vs. Term control)

p <0.001 §(CLD vs. Preterm control), §§(CLD vs. Term control), §§§(Preterm control vs. Term control)

## **Symbols used to indicate significant differences in echocardiographic parameters with hypoxia**

p <0.05 †(21% O<sub>2</sub> vs. 15% O<sub>2</sub>), ††(21% O<sub>2</sub> vs. 12% O<sub>2</sub>), †††(15% O<sub>2</sub> vs. 12% O<sub>2</sub>)

p <0.01 ‡(21% O<sub>2</sub> vs. 15% O<sub>2</sub>), ‡‡(21% O<sub>2</sub> vs. 12% O<sub>2</sub>), ‡‡‡(15% O<sub>2</sub> vs. 12% O<sub>2</sub>)

p <0.001 §(21% O<sub>2</sub> vs. 15% O<sub>2</sub>), §§(21% O<sub>2</sub> vs. 12% O<sub>2</sub>), §§§ (15% O<sub>2</sub> vs. 12% O<sub>2</sub>)



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## Abstract

The aim of this cross sectional study was to compare cardiopulmonary function in children with chronic lung disease of prematurity (CLD) with preterm and term-born controls. Whole-body plethysmography, alveolar diffusion capacity ( $DL_{CO}$ ) and spirometry before and after cycle ergometry were assessed in 92 children: 29 with CLD, 33 preterm ( $\leq 32$  weeks gestation) and 30 term-born controls. They also underwent echocardiographic assessment of myocardial function and pulmonary arterial pressure while breathing room air and after breathing 15% and 12% oxygen for 20 minutes each.

Children with CLD had lower  $FEV_1$  and  $FEF_{25-75}$  compared with preterm control ( $p < 0.001$ ) and term ( $p < 0.001$ ) groups and raised residual volume ( $p < 0.01$ ).  $DL_{CO}$  was significantly lower in the CLD ( $p < 0.05$ ) group. There were no differences in oxygen uptake and carbon dioxide output at maximum exercise between the groups but maximal voluntary ventilation and ventilatory reserve were significantly lower in the CLD group compared with the preterm ( $p < 0.05$ ) and term control groups ( $p < 0.001$ ).  $FEV_1$  was reduced by 10.9% after exercise but increased by 15.1% after bronchodilator in the CLD group. Although those with CLD desaturated more than the control groups after the hypoxic challenge, surrogate markers of the pulmonary arterial pressure altered similarly in all three groups in response to hypoxia. Echocardiographic markers of the right ventricular function did not vary between groups at baseline or after the hypoxic challenge.

The findings of this thesis suggest that children with CLD continue to have airway abnormalities including *reversible* airway obstruction, air trapping and impaired gas transfer compared to term and preterm controls at 8-12 years of age. Although they had similar

exercise capacity to preterm and term control groups, this was at the expense of using greater proportion of their ventilatory reserve. At school age, they do not have evidence of myocardial dysfunction or subclinical pulmonary arterial hypertension.

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## **Chapter One: General Introduction**

## **1.1 Development of the cardiopulmonary system**

The association between fetal factors and the origin of complex adult diseases has been proposed by the epidemiologist David Barker (Barker 1989). Most of the current evidence from epidemiological studies that support Barker's 'fetal origin hypothesis' points towards the relationship between low birth weight and environmental factors, with adult cardiovascular disease (Mi 2000; Eriksson 2001; Barker 2002). When infants are born prematurely, their cardiovascular and pulmonary systems are still in the developmental stages, which may have important implications to the development of adult cardiopulmonary disease; yet our knowledge on association between premature birth and cardiopulmonary disease in later life is limited.

### **1.1.1 Lung growth and development**

Human lung growth starts as early as 3 weeks of embryonic life and undergoes several morphological stages that continue into postnatal life up to early adulthood. The stages of human lung growth are summarised in Table 1.1.

The pseudoglandular stage marks an important landmark in human lung growth. By 24 weeks of intrauterine life, surfactant protein is detectable, and thus, a possible platform for gas exchange is established. Further development of pneumocytes and division and enlargement of alveoli continue in the saccular and alveolar stages. Alveolar multiplication continues in the postnatal period at least up to 2-3 years and alveolar size and surface area increases until after adolescence (Kotecha 2000; Hislop 2002). In prematurely born infants, lung growth is interrupted in the pseudoglandular or saccular stages, which has implications for infant respiratory and cardiovascular functions, in the short and long terms.

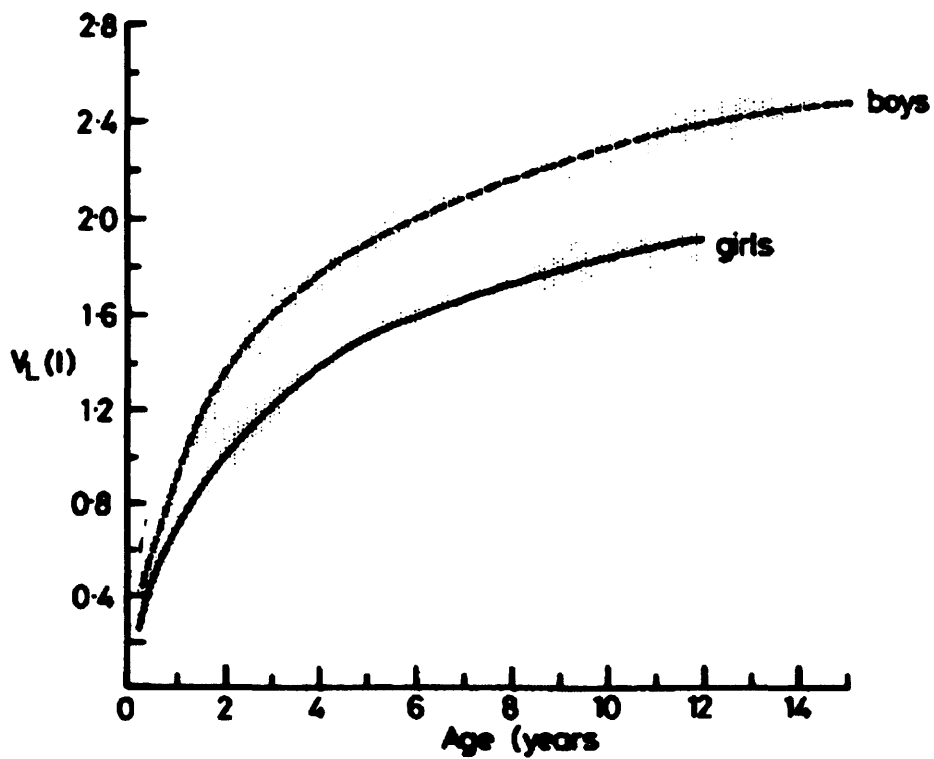
Table 1.1 Stages of lung growth (Joshi and Kotecha 2007)

Stage	Time	Events
Embryonic	0-7 wks	Formation of trachea, right and left main bronchi, and vasculogenesis around airway buds
Canalicular	7-17 wks	Differentiation of epithelial cells, formation of pulmonary arteries and veins
Pseudoglandular	7-27 wks	Formation of respiratory bronchioles, alveolar ducts and primitive alveoli, differentiation of type I and type II pneumocytes and formation of alveolar capillary barrier
Saccular	8-36 wks	Increment in gas exchange areas, further differentiation of type I and type II cells
Alveolar	36 wks- 2 yrs	Septation and multiplication of alveoli
	Until 18-22 yrs	Enlargement of terminal bronchioles and alveoli
Microvascular maturation	Birth to 2-3 yrs	Fusion of double alveolar capillary network into a single layer

There are limited data on postnatal lung growth. Almost 40 years ago, Dunhill published data from morphometric studies on 10 children showing that these children had 20 million alveoli at birth which multiplied rapidly in the first few years of life but slowed down after 4 years of age and stopped at 8 years (Dunhill 1972). This was supported by other studies (Davies and Reid 1970, Thurlbeck and Angus 1975) albeit in small numbers of children. In 1982, Thurlbeck studied post-mortem lungs of 36 boys and 20 girls (aged 6 weeks to 14 years) dying as a result of trauma or after a short illness. The morphometric studies showed that there was rapid alveolar multiplication during the first 2 years of life. Alveolar dimensions and number of alveoli per unit area and volume did not change much during this period. There was minimal growth in the alveolar number after 2 years of age (Thurlbeck 1982) (Figure 1.1).

With recent advances on MRI imaging of lungs using hyperpolarized  $^3\text{He}$  (Ebert 1996, Peces-Barba 2003), it may be possible to quantify linear alveolar growth more precisely. Until longitudinal studies are carried out from birth onwards using these new technologies, our knowledge on postnatal human lung growth is limited. Based on current literature, it is assumed that alveolar hyperplasia is minimal after 8 years of age.

Figure 1.1 Postnatal lung growth



Regression equations for lung volume against age plotted for boys and girls: Mean  $\pm$  1 standard error shown.  $V_L$  = lung volume at a transpulmonary pressure of 25 cm formalin (Thurlbeck 1982)

### 1.1.2 Development of heart and pulmonary vessels

The heart and corresponding major blood vessels develop between 3-8 weeks of gestational age. Unlike most other human organs, the fetal heart does not develop as a miniature adult heart. In fact, it develops as a tubular structure, which undergoes a series of morphological changes, to form the four chambers as well as the inflow and outflow tracts (Table 1.2).

Table 1.2 Development of cardiac structures (Koff 1993)

Neonatal structures	Corresponding embryonic structures
Superior and inferior venae cavae	Sinus venosus
Atria	Dilation of heart tube
Septum	Endocardial cushion
Left ventricle	Dilation of heart tube
Right ventricle	Bulbus cordis
Pulmonary and aortic arteries	Truncus arteriosus
Pulmonary veins	Outgrowth of posterior wall of left atrium

During early stages of lung development, the developing airways act as a template for pulmonary vascular development, and the pulmonary blood vessels form around the branching airway by vasculogenesis, the process by which blood vessels are formed by de novo production of endothelial cells (Hislop 2005). In later development, however, new pulmonary blood vessels are formed only from pre-existing blood vessels, the process known as angiogenesis. This physiological process takes place only as long as alveoli increase in size and number (Hislop 2005). During the postnatal period, the existing capillaries split to form new capillary meshes so that there is a double capillary network. Burri's group has also described that, besides the multiplication and enlargement of airspaces, some important

microvascular changes occur in postnatal lung growth, which they termed as the stage of microvascular maturation (Burri 2006). Essentially, during this period, the double capillary network gradually merges as a single capillary layer. This is an extremely important event in postnatal lung growth, as angiogenesis and alveolization go hand in hand, and once the capillary network fuses into a single layer, new alveolar septa cannot be formed. This has now been postulated as the major cause of premature arrest of lung growth following postnatal steroid treatment in premature infants with chronic lung disease (Roth-Kleiner 2005).

## **1.2 Cardiorespiratory adaptation at birth**

There are some fundamental differences between fetal and postnatal pulmonary physiology and circulation. It is important to understand the mechanisms of fetal circulation and cardiorespiratory adaptation at birth, in order to understand the cardiorespiratory consequences of premature birth.

Fetal lungs are filled with fluid. The fluid secretion is maximal at mid-gestation and plays a vital role in lung growth and development. These fluid filled lungs do not contribute to oxygenation of blood in the foetus. Instead, the placenta acts as an organ of oxygenation. In intrauterine life, only 12-20% of total right ventricular output flows through the pulmonary circulation due to high pulmonary vascular resistance and high right atrial pressure. Besides, there are some key structures in the fetal circulation, which undergo acute changes after birth. In the fetal circulation, the ductus venosus and foramen ovale allow oxygenated blood to flow from the placenta to the left atrium and subsequently to the ascending aorta, to be distributed to the coronary and cerebral circulations. During labour, increasing levels of catecholamine decrease the production of lung liquid. After birth, the inflation of the lungs displaces the lung liquid from air sacs into perivascular space (Bland 1980). The majority of fluid is absorbed into pulmonary lymphatics and capillaries. Along with lung liquid absorption, the distension of lungs and increase in oxygen tension reduces the pulmonary vascular resistance, which in turn is associated with structural reorganization and thinning of the pulmonary vascular wall (Abman 1990; Lakshminrusimha and Steinhorn 1999). This allows pulmonary vascular flow to increase by almost eightfold. At the same time, as the umbilical cord is clamped, venous return to the right atrium is reduced which leads to a drop in right atrial pressure and closure of the foramen ovale. In intrauterine life, pulmonary arterial pressure is equal to or more than the systemic arterial pressure. After birth, it rapidly falls to about 50% of that in the fetus,



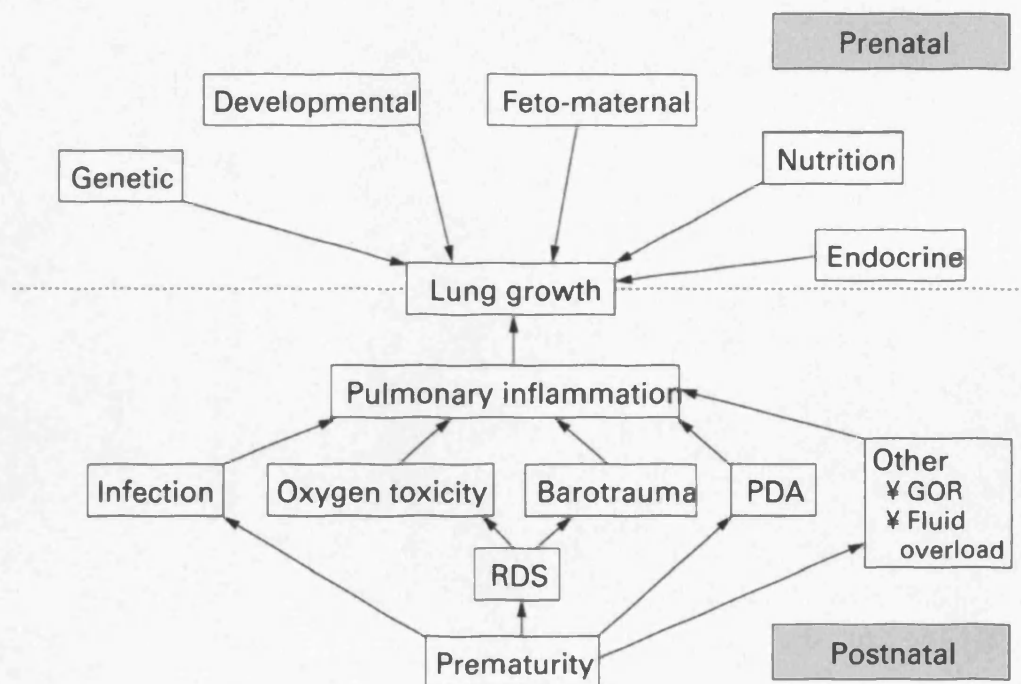
mediated by prostacyclin and endogenous nitric oxide (Leffler 1984; Fineman 1991). This drop in pulmonary arterial pressure initiates the closure of the ductus arteriosus. In the majority of healthy term infants, these steps of cardiorespiratory adaptation are complete by about 24 hours after birth.

In prematurely born infants, postnatal cardiorespiratory adaptation does not occur as smoothly as in the majority of term born infants. Pulmonary hypertension is a recognised complication of neonatal lung diseases, but the mechanisms for persistently high pulmonary vascular resistance in premature infants are not yet well understood (Goodman 1988; Mourani 2004; Danhaive 2005).

### 1.3 Respiratory consequences of premature birth

As evident from the stages of human lung growth, prematurely born infants have interrupted lung development. Various factors such as mechanical ventilation, oxygen, infection, inflammation, nutrition etc. affect their postnatal lung growth (Figure 1.2).

Figure: 1.2 Model of factors affecting lung growth



Top half shows prenatal factors that may be important in normal lung growth; each may cause inflammation, so affecting lung growth. Bottom half shows postnatal factors that may affect lung growth (Kotecha 2000)

Thus, preterm birth is associated with both antenatal and postnatal risk factors that affect lung growth and development which may lead to short term and long term respiratory morbidity.

In sections 1.3.1 and 1.3.2 I shall discuss respiratory distress syndrome and chronic lung disease, the two most important respiratory pathologies associated with premature birth.

### **1.3.1 Respiratory distress syndrome**

#### **1.3.1.1 Introduction**

Respiratory distress syndrome (RDS) is an acute respiratory illness caused by surfactant deficiency, primarily in prematurely born infants. In the majority of affected infants, RDS is characterised by increased respiratory rate (>60 breaths/ min), subcostal, intercostal or sternal recession, expiratory grunting, and cyanosis. Extremely premature infants (<26 weeks), however, may present with apnoea without any other associated respiratory signs. Premature birth is by far the most important factor contributing to development of RDS in infants. Male gender (M:F 1.7:1) and Caucasian race have also been strongly associated with this condition. Other predisposing factors related to development of RDS are, Caesarean section, birth hypoxia, maternal diabetes, hypothyroidism, genetic predisposition, twin pregnancy, hypothermia, malnutrition, intrauterine growth retardation, haemolytic disease of newborn and timing of cord clamping (Greenough 2005).

The incidence of RDS is known to be inversely proportional to the gestational age at birth. Data from previous studies have shown that the risk of RDS reduces from approximately 50% in infants born less than 30 weeks of gestational age to only 2% in those born at 35-36 weeks of gestational age (Rubaltelli 1998). Exposure to antenatal steroids (Ventolini 2008) and postnatal surfactant (Stoelhorst 2005) have been shown to significantly reduce the incidence of RDS. Despite these positive interventions, RDS is still a major cause of morbidity in preterm infants. As the incidence of preterm birth is ever increasing, and comprises about 5-

7% of all live births in the developed world (Tucker 2005), further research into RDS and its consequences still remains an area that needs to be understood better.

#### 1.3.1.2 Pathology

The basis of RDS is surfactant deficiency. Surfactant is a combination of phospholipids and proteins produced by type II pneumocytes, which spread as a layer of thin film at the air liquid interface of the alveolar surface and lowers its surface tension, thereby preventing alveolar collapse. Dipalmitoyl phosphatidyl choline or DPPC is the principal component of surfactant, which is responsible for reducing alveolar surface tension. As discussed in section 1.1.1, differentiation of types I and II pneumocytes occur during 17-27 weeks of gestational age. Type II cells are detectable by 24 weeks of gestation. However, surfactant produced by type II cells at this stage is insufficient to adequately lower alveolar surface tension. Therefore, alveoli tend to collapse, particularly at end expiration, leading to poorly compliant lungs with atelectasis, and sometimes fluid filled lungs, which give rise to the 'white out' appearance in chest x-ray. Other radiological signs are small lungs and presence of air bronchograms.

Histologically, in lungs affected by RDS, the distended terminal and respiratory bronchioles are lined by hyaline membranes; giving its histological name 'hyaline membrane disease'. The hyaline membranes are formed by coagulation of plasma proteins that leak onto the lung surface through damaged capillaries and epithelial cells, and fibrin exudates (Greenough 2005). After about 24 hours, the healing process begins, with appearance of inflammatory cells and macrophages. In uncomplicated RDS, the healing process is complete in the first few days after birth, and hyaline membranes disappear by the end of the first week. However,

in extremely premature infants on mechanical ventilation, this process is markedly altered and delayed (Greenough 2005).

#### 1.3.1.3 Effects of RDS on pulmonary function

The pulmonary function of infants with RDS depends upon the gestational age, type of ventilatory support and severity of RDS among other factors. By definition, the hallmark of RDS is non-compliant, stiff lungs. Pulmonary resistance in infants with RDS has been estimated to be 3-6 folds greater, compared with newborn infants without RDS. Studies have also shown that functional residual capacity is decreased in RDS. Although infants with RDS tend to achieve similar tidal volume as healthy term infants, almost 60-80% of this consists of dead space ventilation (Ainsworth 2005). To compensate for this, spontaneously breathing infants with RDS increase their respiratory rate. There is also evidence that prematurely born infants with RDS tend to have poor pulmonary compliance and reduced functional residual capacity in the first month of life, which is not fully corrected by treatment with surfactant (Kavvadia 1999). In some prematurely born infants, this early structural and functional damage in the lungs may lead to chronic lung disease of prematurity, requiring prolonged oxygen dependency and/or ventilatory support.

### **1.3.2 Neonatal chronic lung disease**

#### 1.3.2.1 Introduction

In 1967 Northway introduced the term bronchopulmonary dysplasia (BPD), describing the pulmonary changes in the surviving population of prematurely born infants with severe respiratory failure (Northway 1967). In current literature, the terms chronic lung disease and

bronchopulmonary dysplasia are used interchangeably to describe chronic respiratory problems following premature birth. The National Institutes of Health workshop in 2001 suggested that the term 'Bronchopulmonary Dysplasia' be used for prematurely born infants who develop persistent respiratory problem, as this is a more specific terminology (Jobe and Bancalari 2001). However, in 2003 the American Thoracic Society (ATS) document on the 'Statement on the care of the child with chronic lung disease of infancy and childhood' emphasized that the term bronchopulmonary dysplasia has certain histological and pathological implications that may not be present in all prematurely born infants. More importantly, the long term management of children and adolescents with different forms of chronic lung disease of infancy are similar in principle. Therefore, the ATS document argued that it may be more appropriate to use the generic term chronic lung disease to describe the residual neonatal pulmonary problems in children and adolescents (ATS 2003).

Throughout this thesis, I have used the term chronic lung disease (CLD) to describe children who were born  $\leq 32$  weeks of gestational age who were dependent on supplemental oxygen at 36 weeks of corrected gestational age or discharge to home, whichever comes first.

The incidence of CLD has been reported to be as varied as 10-40% (Fanaroff 2007). This variation is probably contributed by lack of agreed diagnostic criteria; some using oxygen dependency at 28 days of life and others using oxygen dependency at 36 weeks of corrected gestational age for diagnosis of CLD (Bancalari 1979; Avery 1987; Shennan 1988). Shennan et al (Shennan 1988) made a case that the cut-off mark of oxygen dependency at 36 weeks of corrected gestational age predicts a more accurate incidence of CLD irrespective of the gestational age at birth. In 2001, the National Institute of Child Health and Human Development (NICHD)/ National Heart Lung and Blood Institute (NHLB)/ Office of rare

diseases workshop on CLD agreed on diagnostic criteria for CLD (Jobe and Bancalari 2001) (Table 1.3). The NICHD also reported an incidence of CLD in 23% of prematurely born infants, using the definition of oxygen dependency at 36 weeks of corrected gestational age in prematurely born infants (Lemons 2001).

Table 1.3 Diagnostic criteria for severity of CLD

Gestational age	<32 weeks	>32 weeks
TPA	<b>36 wk PMA or discharge to home, whichever comes first</b>	>28 days but <56 days PNA or discharge home, whichever is first
Mild CLD	Breathing room air at TPA	Breathing room air at TPA
Moderate CLD	Need for <30% oxygen at TPA	Need for <30% oxygen at TPA
Severe CLD	Need for $\geq$ 30% oxygen and/or positive pressure, (PPV or CPAP)	Need for $\geq$ 30% oxygen and/or positive pressure, (PPV or CPAP)

(Jobe and Bancalari 2001)

TPA= Time point of assessment, PMA= Post menstrual age, PNA= Post natal age, PPV= Positive pressure ventilation, CPAP= Continuous positive airway pressure



### 1.3.2.2 Risk Factors for the Development of CLD

Several antenatal and postnatal factors have been associated with the development of CLD. Antenatal inflammation and infections have been explored as potential causes in both animal models and in infants born prematurely. In a sheep model, when chorioamnionitis was induced by injecting endotoxin, there were alveolar and microvascular changes in the fetal lung similar to CLD (Kramer 2008). Maternal preeclampsia (Hansen 2010) and chorioamnionitis (Watterberg 1996; Paul 2009) have been shown to increase the incidence of CLD in very low birth weight infants who are born prematurely. Others have suggested that the exposure to antenatal inflammation is a risk for development of CLD only if the infant is exposed to postnatal infection (Van Marter 2002). Cytomegalovirus (Sawyer 1987) and *Ureaplasma urealyticum* (Cassell 1988) have also been suggested to be risk factors for CLD.

Oxygen toxicity and barotrauma are considered to be important postnatal causes of CLD. As premature infants have a poorly developed antioxidant system, they are at higher risk of oxygen free radical damage (Frank 1992). It has been clearly demonstrated in a piglet model that hyperoxia causes more significant physiological, inflammatory, and histological changes in the lungs than barotrauma alone (Davis 1991). The inverse relationship between hypocarbia and development of CLD has also been demonstrated in prematurely born infants who were hypocarbic prior to receiving surfactant replacement therapy (Garland 1995). Barotrauma caused by high pressure ventilation is an important cause of pulmonary interstitial emphysema (Greenough 1984) which in turn may lead to neonatal CLD (Cochran 1994).

When Cunha et al studied a cohort of 86 infants with birth weight less than 1500g, they reported that apart from birth weight, gestational age and oxygen, the most important factors

for development of CLD were prematurity, patent ductus arteriosus, high peak inspiratory pressure and fluid overload (Cunha 2005).

Although CLD is a disease of prematurely born infants, lack of RDS does not prevent CLD. In fact, in one study, up to 5% of infants who developed CLD did not have any initial lung disease (Charafeddine 1999). Although antenatal corticosteroids have shown to be hugely beneficial in decreasing the incidence of RDS, this has not influenced the incidence of CLD, possibly as a result of increasing survival after extremely premature birth (Crowley 1995).

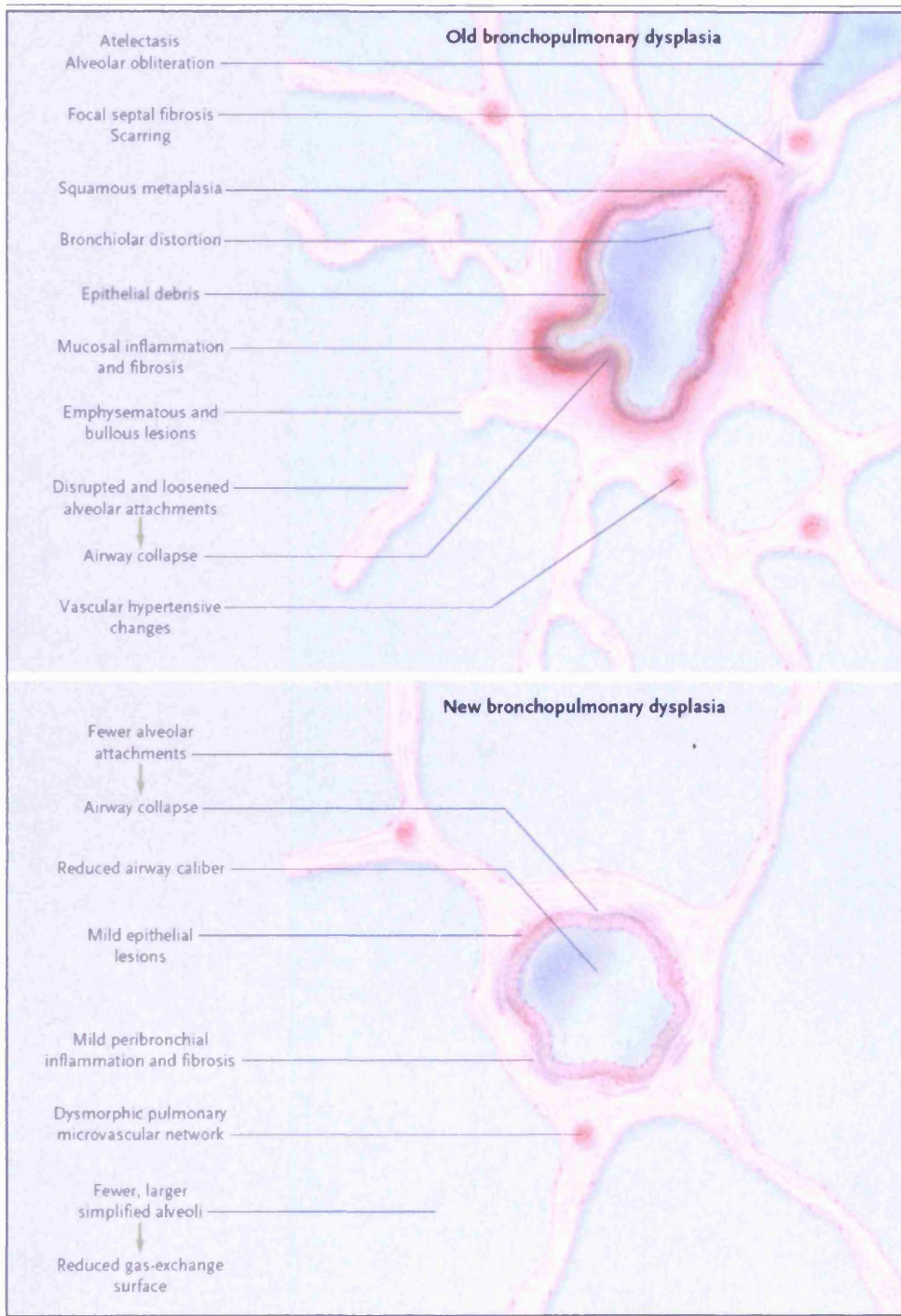
#### 1.3.2.3 Pathogenesis and Pathophysiology of CLD

The population of infants, on whom Northway and his colleagues described CLD, were born moderately premature and were exposed to high concentration of oxygen and aggressive mechanical ventilation, and none of them had received surfactant (Northway 1967). These infants had severe respiratory failure with prolonged healing process. The morphological changes in the radiograph were severe, and included pulmonary hyperinflation, emphysema, atelectasis and fibrosis. Histological characteristics of this classic CLD were epithelial squamous metaplasia and smooth muscle hypertrophy in the airways and in the pulmonary vasculature (Bancalari and Claure 2006). The post-mortem examinations of lung tissue in infants who died of CLD demonstrated normal or increased lung volumes, thin alveolar walls, and most importantly, less than normal total numbers of alveoli for post-natal and postconceptional age (Hislop and Haworth 1990).

With the introduction of antenatal steroids in the 1970s and surfactant replacement therapy in the early 1990s, along with improved mechanical ventilation, careful monitoring of oxygen

therapy and improved nutritional care, the survival of extremely premature infants has improved, and the pathology of CLD has changed over the years. Unlike the 'classic CLD' or the 'old CLD' seen in the pre-surfactant era, the 'new CLD' of the post-surfactant era is seen in extremely premature infants, who may or may not have suffered from RDS. 'New CLD' seems to occur due to arrest of alveolar growth leading to enlarged airspaces with minimal alveolization (Jobe 1999; Coalson 2003), rather than the classic appearance of alternating sites of hyperinflation and fibrosis caused by oxidant injury or barotraumas seen in infants in the pre-surfactant years (Figure 1.3). The radiographic changes in the 'new CLD' are also different to what was observed classically. These infants seem to have small lungs with minimal alveolization. It has been demonstrated that antenatal exposure to steroids caused thinning of the alveolar wall, an increase in alveolar volume by 20% and a decrease in alveolar number by 30%, in fetal lambs (Willet 2000). This gives us the picture of pulmonary pathology in the prematurely born children who have been exposed to antenatal steroids.

Figure 1.3 Histological appearance of 'old' and 'new' CLD



(Baraldi 2007)

### **1.3.3 Long term respiratory outcome of prematurity**

Respiratory consequences of premature birth have been investigated in infants, pre-school and school-aged children, and in young adults. The studies in infants have concentrated mostly on the incidence of respiratory symptoms, lower respiratory tract infection such as respiratory syncytial virus infection, and hospital admissions. Research on school-aged children and adolescents has given us some insight on the pulmonary function in these children in greater detail. However, most of these studies are based on children born in the pre-surfactant era.

#### **Re-hospitalization:**

Various studies have consistently shown that there is an increased incidence of hospital admissions due to respiratory illness in premature infants with CLD. In an American cohort of 133 surviving infants of gestational age  $\leq 32$  weeks and 121 healthy term controls (born 1985-1986), 36% of preterm infants were re-hospitalized in the first 2 years of life compared to only 2.5% of term infants. Among the preterm infants who were hospitalized, 45% had CLD compared to 25% who did not have CLD (Cunningham 1991). In another American study, out of 125 surviving children born at 24-31 weeks of gestational age, 53% with CLD were re-hospitalized within the first 2 years as opposed to 26% of preterm children without CLD (Gross 1998). Similar results have also been shown by an Australian group who followed up 78 infants who were discharged home on home oxygen and 78 birth weight matched infants without CLD. In this study, the overall rates of re-hospitalization (58% vs 35%) and hospitalization for respiratory illness (39% vs 20%) were higher in the CLD group in the first year of life (Chye and Gray 1995).

In a large case control study carried out in the USA, increased risk of hospitalization for respiratory illness has been reported in 18-27 year old adults who had a history of low birth weight. In this study involving 4,674 cases and 18,445 controls, odds ratio for hospitalization for respiratory illness was 1.83 (95% CI 1.28 to 2.62,  $p=0.001$ ) for very low birth weight (birth weight <1,500 grams) and 1.34 (95% CI 1.17 to 1.52,  $p<0.005$ ) for moderate low birth weight (birth weight 1,500 grams to 2,499 grams) subjects. Premature birth and presence or absence of CLD was not considered in this study (Walter 2009).

#### Respiratory symptoms:

The fact that the incidence for re-hospitalization due to respiratory illness is higher in preterm children with CLD implies that ongoing respiratory symptoms must be higher in this group of infants. A four year prospective follow-up of 81 prematurely born children (median gestational age 29 weeks) revealed that 47% of children were symptomatic with chronic wheeze and cough in the first year of life, 36% in the second year and 33% in the third and fourth years (Greenough 1996). Gestational age  $\leq 28$  weeks, ventilation  $\geq 5$  days, supplemental oxygen  $\geq 10$  days, family history of atopy, and parental smoking were noted to be important predictors of respiratory symptoms. In a larger study with 492 infants born before 29 weeks of gestational age, up to 27% were symptomatic with cough and wheeze in the first year. Male sex, oxygen dependency at 36 weeks of corrected gestational age, having older siblings under the age of five and living in rental accommodation were reported to be risk factors for respiratory symptoms at 6 and 12 months of age (Greenough 2005). Premature infants who wheeze in the first year have also been associated with abnormal pulmonary function tests (Broughton 2007). None of these studies have compared respiratory symptoms between prematurely born children with and without CLD.

Whether or not the respiratory symptoms in preterm children persist as they grow older is not well understood, but there is evidence that prematurely born children continue to have respiratory symptoms at school age. When respiratory symptoms over a period of 12 months were studied in 384, 8-year-old children who were born with very low birth weight ( $\leq 1500$  grams), the investigators noted that respiratory symptoms were twice as common in the study group compared to 154 term-born controls (Palta 2001). The investigators in this study also compared the respiratory symptoms in this group of preterm children (born between 1988-1991), prior to the introduction of surfactant therapy, to 2 other groups of preterm children; those born between 1989-1990 when surfactant was used only as a rescue therapy and 1990-1991 when surfactant became generally available. The investigators used a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher 1995). They concluded that with the advent of availability of surfactant, the prevalence of wheezing at 8 years of age decreased from 50% to 16% among children with CLD (defined as use of supplemental oxygen at 25-35 days), but increase in wheezing at 8 years of age in those very low birth weight children who did not meet the criteria for CLD (Palta 2001). Another published data on the follow-up of the extremely premature children ( $\leq 25$  weeks, born in 1985) from the EPICure study cohort (Costeloe 2000) have shown that respiratory symptoms and medication use were more prevalent at 30 months and at 6 years, in the CLD population (n=180) compared to the non-CLD (n=56). They also concluded that need for hospitalization and medication use declined between 30 months and 6 years (Hennessy 2008). The authors found that discharge gestational age, exposure to passive smoking at home, maternal smoking in pregnancy, and CLD were risk factors for respiratory symptoms at 6 years of age.

### Pulmonary function tests:

Respiratory compliance and resistance have been reported to be abnormal (50% predicted) in infants during the acute phase of CLD, but these seemed to have improved during the first year of life with values reaching normal ranges by 2 years of age (Baraldi 1997). However, forced expiratory flow measured at the age of 2 years was abnormal in 70% of the children in this cohort. If prematurely born infants wheeze, they tend to have evidence of gas trapping as opposed to those who are asymptomatic at 1 year of age (Broughton 2007). In the EPICure cohort which consisted of 236 infants born at  $\leq 25$  weeks of gestational age (74%, CLD), peak expiratory flow was lower than in term born classroom controls (mean adjusted difference 39 l/min) at 6 years of age (Hennessy 2008). CLD defined as need for oxygen supplement at 36 weeks of corrected gestational age was found to be the only independent predictor of peak flow.

Very low birth weight (<1000grams) and extremely preterm (<28 weeks) children have been shown to have compromised pulmonary function at school age (8-9 years) with lower forced expiratory volume at 1 second, FEV<sub>1</sub> (<75% predicted) in 20% of the index population compared to just 2% of term-born controls. They also had higher residual volume (RV) and RV:TLC ratio (residual volume: total lung capacity) indicating air trapping (Doyle 2006). 37% (out of 298) of the very low birth weight children in this study had CLD. FEV<sub>1</sub> was lower (<75% predicted in 27% with CLD v 15% without CLD), and RV as well as RV: TLC ratio were higher in the CLD population. The results from the Australian cohort of 52 preterm children with CLD and 62 preterm controls at 8 years of age are slightly different (Kulasekaran 2007). In this cohort, forced expiratory flow at 25-75% of vital capacity (FEF<sub>25-75</sub>) was the only respiratory parameter that was significantly lower in the CLD group compared to the preterm controls, which responded to bronchodilator therapy. There was no



evidence of increased air trapping or bronchial hyper-reactivity in the children with CLD compared with the preterm controls. None of the children in this study had received surfactant therapy. Data from 18 studies on children and young adults with CLD of prematurity (6-19 years) have been extensively reviewed by Baraldi (Baraldi 2007). As shown in Figure 1.4, it is clear that even those children with CLD who received surfactant after birth tend to have lower FEV<sub>1</sub> later in life compared to the control population.

The evidence on the effect of surfactant therapy at birth on respiratory outcome at school age is varied. 38% (out of 298) of the preterm children in the study by Doyle et al had received surfactant therapy, and thus did not influence the respiratory outcome at 8 years of age (Doyle 2006). Similarly, 8 year follow-up on children who were recruited for a randomised control study of surfactant therapy revealed that there were no differences in FRC, forced expiratory flow (FEV<sub>1</sub>, FVC, FEF<sub>25</sub>, FEF<sub>50</sub>), PEF or airway resistance between those who received surfactant therapy and those who received placebo (Gappa 1999). 2/22 in the surfactant group, compared with 9/18 in the placebo group had developed CLD. Another follow-up study at school age (7-12 years), on children who were recruited in a randomised control trial for surfactant therapy gives a different perspective to the influence of surfactant therapy on long-term respiratory outcome. In this Finnish study which included 17 children who had received prophylactic surfactant at birth, 14 children who received surfactant as a rescue therapy and 9 children who received placebo, the recipients of the prophylactic surfactant therapy had better FVC and PEF than the placebo group ( $p < .05$ ) but did not differ to those who received rescue surfactant. All the spirometric parameters were significantly lower in the 11 preterm children who developed CLD (28%) than in those who did not develop CLD. None of the children in this study were exposed to antenatal steroids (Pelkonen 1998). Another Finnish study which reported lung function at 8 years of age in preterm children

received surfactant therapy at birth, demonstrated lower FEV<sub>1</sub>, higher RV: TLC and higher airway resistance in the CLD group compared with the term controls (Korhonen 2004). The limitation of this study is that the investigators defined CLD as oxygen dependency at 28 days of life, and only 14 out of 34 children in the CLD group were oxygen dependent at 36 weeks of corrected gestational age. FEV<sub>1</sub> was lower in the 14 children who were oxygen dependent at 36 weeks compared to both the control groups.

The older population from the pre-surfactant era have had their lung function reported, in their early adulthood. At 18 years, 68% of 26 subjects (born between 1964-1973) who had classic CLD, had evidence of airway obstruction; this was irreversible in 24% (Northway Jr 1990). Compared to the control population, the CLD group also had reductions in vital capacity and higher RV:TLC ratio. Similar results were also reported by another study on 42, 19-year old young adults who were born at <32 weeks of gestational age and <1500 grams of birth weight (born in 1983). Compared to 48 healthy term control subjects, the ex-preterm subjects had lower FEV<sub>1</sub> and lower diffusion capacities. This study also showed lower exercise capacity (lower maximum load), but normal peak oxygen consumption, ( $\dot{V}O_2$ ) in ex-preterm subjects (Vrijlandt 2006). In this study, the authors reported that none of these differences were statistically significant between preterm infants with and without CLD. This conclusion should be carefully interpreted, as only 9/42 preterm subjects in this study had CLD. Halvorsen et al (Halvorsen 2004) and Doyle et al (Doyle 2006) have also demonstrated lower FEV<sub>1</sub> in the ex- CLD population compared to term controls at 18-19 years of age. More recently published data on 60 ex- preterm subjects (7 with CLD) and 50 term-born controls showed that at 21 years of age, the prematurely born young adults still had excess respiratory symptoms but there was no significant difference in measured spirometry (Narang 2008).

There are only limited studies with longitudinal data on preterm cohorts that give prospective evidence about the natural history of lung function in the survivors of CLD. In a study on 17 survivors of CLD, 17 preterm controls and 34 healthy controls, the FEV<sub>1</sub> z- score was consistent in the CLD group at 2 years, 9 years and 15 years. Values for FEV<sub>1</sub> were significantly lower in the CLD group compared with the control groups at 9 and 15 years (Filippone 2009). In the study by Doyle et al (Doyle 2006), there was a larger fall in FEV<sub>1</sub>: FVC ratio in the subjects with CLD (n=29) than in preterm controls (n=129) between 8 and 18 years of age (mean difference in FEV<sub>1</sub>: FVC -3.4%). On the other hand, Blayney et al reported improvement in FEV<sub>1</sub> by 10 years of age in 19 children (out of 32 who had CLD) who had FEV<sub>1</sub><80% predicted at 7 years of age (Blayney 1991). In another study, Koumbourlis et al reported improvement in TLC and RV: TLC ratio over time (from 8 years to 15 years) suggesting resolution in gas trapping. FEV<sub>1</sub> and FEV<sub>1</sub>: FVC remained normal at both the phases but FEF at 25-75% of VC and at FEF at 75% of vital capacity remained low at both the phases (Koumbourlis 1996).

There are also limited data on diffusion capacity and exercise tests on school-aged children and young adults with history of CLD. Currently available literature on alveolar diffusion capacity and cardiopulmonary exercise on preterm subjects is summarized in Table 1.4 (a) and 1.4 (b) respectively.

Measurement of alveolar diffusion capacity (DL<sub>CO</sub>) is a method for indirectly assessing alveolar-capillary gas transfer. There is lack of consistency in the literature regarding information about the alveolar-capillary carbon monoxide diffusion in preterm infants. The only available data from school-aged children have shown lower DL<sub>CO</sub> in children with CLD compared to the controls (Mitchell and Teague 1998). The investigators used the intrabreath

technique rather than the more commonly used single breath method in this study. The EPICure study group recently reported lower diffusion capacity and lower peak oxygen consumption during exercise ( $\dot{V}O_2$ ) in preterm children born at <26 weeks gestation in the surfactant era (Welsh 2010). Balinotti and colleagues have reported significantly lower  $DL_{CO}$  and the ratio of  $DL_{CO}$ :alveolar volume in infancy in preterm infants with CLD compared to term controls suggesting impaired alveolar development (Balinotti 2010). Other studies for  $DL_{CO}$  including those conducted in preterm born children and young adults report variable results (Mitchell and Teague 1998; Vrijlandt 2006; Smith 2008; Narang 2009). Some studies in this older population showed lower  $DL_{CO}$  values in preterm compared to term controls (Vrijlandt 2006; Narang 2009) but they suggested that the alveolar transfer factor was similar in preterm subjects with and without CLD. In the adult CLD population, Northway and colleagues demonstrated that although  $DL_{CO}$  was statistically lower in the CLD group compared to the term controls, their alveolar diffusion capacity was >80% of the predicted value i.e. within normal limits (Northway Jr 1990).

Currently available data on cardio-pulmonary exercise tests in school-aged children (Jacob 1997; Pianosi and Fisk 2000; Kriemler 2005; Welsh 2010) and young adults who were born preterm are also inconsistent (Vrijlandt 2006; Narang 2009). Some studies have shown no differences in exercise capacity or peak  $\dot{V}O_2$  between preterm and term groups (Jacob 1997; Kriemler 2005; Vrijlandt 2006; Narang 2009) whereas other studies have demonstrated reduced peak  $\dot{V}O_2$  in the preterm subjects (Pianosi and Fisk 2000; Welsh 2010). These differences in the results may be due to variation in the study groups and also in the methods of cardiopulmonary exercise testing and, indeed, often do not differentiate between preterm infants with and without CLD.

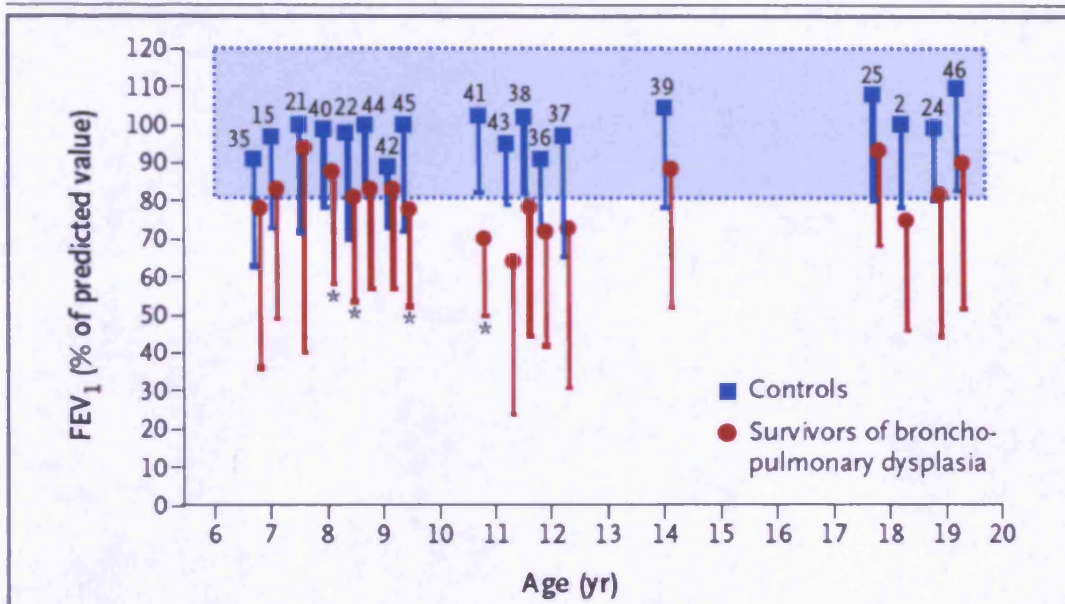
Thus, there is a lack of data on comparing lung function and exercise capacity between age and gender matched preterm children with *and* without CLD with those born at term. Furthermore, the objective effect of exercise on airway function has not been reported nor has the effect of bronchodilator drugs after exercise been assessed in children with or without CLD. Finally, it is unclear which perinatal factors including gestational age, length of oxygen dependency or mechanical ventilation during infancy, influence important parameters such as DL<sub>CO</sub>, exercise tolerance and ventilatory capacity during exercise.

The pathogenesis and pathology of lung disease in prematurely born children in recent years is different to those born before the introduction of surfactant. Currently available literature is based on small cohorts of subjects and there is a lack of evidence based on comprehensive pulmonary function tests including exercise testing in a single cohort. Almost all pulmonary function studies on preterm children mix up preterm children with and without CLD. Therefore, it is unclear if pulmonary function abnormalities in ex-preterm children are due to prematurity *per se* or because of the chronic lung disease.

There is evidence from the older cohort that CLD of prematurity may be associated with early 'ageing' of lungs. CT scans on 21, 17-33 year old (median age 19) adults with a history of CLD revealed abnormal pulmonary CT in all the subjects with evidence of emphysema in 84%. (Wong 2008). Although this is a small study, other studies have also shown similar results. Brostron et al studied 60 (28 with RDS, 32 with CLD) very low birth weight children at 1 month and at 6-8 years of age. 25/32 infants with CLD had abnormal chest x-ray at 1 month of age. Only 26 of these children had high resolution computed tomography (HRCT) scan at 6-8 years of age. 19/26 had evidence of lung fibrosis, emphysema, bronchial wall thickening or linear opacities (Brostron 2010). Similarly, when HRCT was done on preterm

(gestational age  $\leq$  28 weeks) low birth weight (birth weight  $\leq$  1000 grams) subjects who were 10 years old (n=42) and a separate cohort of subjects who were 18 years old (n=32), 64 subjects (86%) had minor abnormalities such as opacities and hypoattenuated areas. The authors have reported that neither age at examination nor birth cohort predicted the HRCT score. However, those who were oxygen dependent for  $>100$  days after birth were noted to be at higher risk for CT abnormality (Auckland 2009). These results suggest that children who are born prematurely and develop CLD are potentially at risk of developing chronic obstructive pulmonary disease (COPD) later in life.

Figure 1.4 FEV<sub>1</sub> in prematurely born children and young adults who had CLD



**Figure 3.** FEV<sub>1</sub> Values in Children, Adolescents, and Young Adults Who Were Born Prematurely and Had Bronchopulmonary Dysplasia, as Compared with Controls Born at Term.

Data (presented as means  $\pm$  2 SD) are from 18 studies, reported since 1990, in which survivors of bronchopulmonary dysplasia who were 6 to 19 years of age were compared with a reference population born at term. In all but two studies, the mean forced expiratory volume in 1 second (FEV<sub>1</sub>) was significantly lower in the patients with bronchopulmonary dysplasia than in the healthy controls. Eighty percent of the predicted FEV<sub>1</sub> value is the accepted lower limit of the normal range. An asterisk indicates that the study was performed after the introduction of surfactant therapy. The studies were conducted in the United States,<sup>2,15,35-37</sup> Australia,<sup>22,24,38,39</sup> Finland,<sup>21,40,41</sup> Canada,<sup>42,43</sup> Italy,<sup>44,45</sup> Norway,<sup>25</sup> and the Netherlands.<sup>46</sup> The numbers within the graph refer to the reference numbers of the studies.

(Baraldi 2007)

Table 1.4 (a): Summary of the literature review on alveolar diffusion capacity in prematurely born children

Reference	Participants (N)	Age	Method	Results
(Balinotti 2010)	CLD (39) Term (61)	12-14 months	Single breath	- DL <sub>CO</sub> adjusted for haemoglobin was lower in the CLD group (p<0.01)
(Welsh 2010)	Extremely preterm (38) Term (38)	11 years	Single breath	-DL <sub>CO</sub> was lower in the extremely preterm group compared to the term controls (p<0.001) -CLD vs. non-CLD was not tested
(Mitchell and Teague 1998)	CLD (10) Preterm control (10) Term (10)	7 years	Intrabreath technique	-Baseline DL <sub>CO</sub> was lower in CLD group.
(Vrijlandt 2006)	CLD (9) Preterm control (42) Term (48)	19 years	Single breath	-Preterm subjects had lower DL <sub>CO</sub> compared to term controls but there were no differences between those with and without CLD
(Narang 2009)	CLD (57) Term (50)	21 years	He dilution mixed expired gas analysis	-Reduced DL <sub>CO</sub> and Q <sub>peff</sub> at baseline in ex-preterm subjects which normalised during exercise and reduced again after recovery.
(Northway Jr 1990)	CLD (26) Preterm control (26) Term (53)	Adult	Single breath	- DL <sub>CO</sub> >80% predicted for patients and controls. -CLD vs. Term p <0.05 -CLD vs. preterm control (not significant)



Table 1.4 (b): Summary of the literature review on cardiopulmonary exercise test in prematurely born children

Reference	Participants (N)	Age	Method	Results
(Welsh 2010)	Extremely preterm (38) Term (38)	11 years	Cycle ergometry	-Peak VO <sub>2</sub> was lower in the extreme preterm compared to term -CLD vs. non-CLD not tested
(Bader 1987)	CLD (10) Term (8)	7-14 years	Treadmill	- No difference in peak VO <sub>2</sub> between CLD and term groups.
(Baraldi 1991)	Preterm (15) [None had CLD] Term (26)	7-12 years	Treadmill	-No difference in peak VO <sub>2</sub> between preterm and term groups
(Jacob 1997)	CLD (15) Preterm control (15) Term (13)	9-12 years	Cycle ergometry	- No difference in peak VO <sub>2</sub> between groups
(Pianosi and Fisk 2000)	CLD (17) Preterm control (15) Term (15)	8-9 years	Cycle ergometry	- Preterms with and without CLD had lower VO <sub>2</sub> compared to the terms (p<0.05)
(Kriemler 2005)	CLD (17) Preterm control (14) Term (24)	5-7 years	Cycle ergometry	- No difference in peak VO <sub>2</sub> between groups
(Vrijlandt 2005)	CLD (9) Preterm control (32) Term (48)	19 years (mean age)	Cycle ergometry	- No difference in peak VO <sub>2</sub> between preterm and term groups -Work load was 15% lower in preterm group (p<0.0001)
(Smith 2008)	CLD (37) Preterm control (89) Term (34)	10 years (mean age)	-6 minute walk - 20 meters shuttle run	- Peak VO <sub>2</sub> was lower in the preterm group compared to the term - Difference between CLD and preterm controls was not tested - No difference in 6 minute walk distance - Mean completed shuttle stage in the preterm control group was 50% lower than that of the Term group
(Narang 2009)	CLD (7) Preterm control (53) Term (50)	21 years (mean age)	Cycle ergometry	- No difference in peak VO <sub>2</sub> between term and preterm groups

Whether or not the short term respiratory morbidity and the long term abnormalities in pulmonary function in prematurely born children with CLD are due to prematurity per se or due to the pathology of CLD is difficult to disentangle. As shown in Figure 1.2, there are various antenatal factors that affect lung growth, and if a baby is born prematurely, lung growth is interrupted. This alone may be a major risk factor for long term respiratory morbidity. In a large American population consisting of 233,844 deliveries (born in 2002 to 2008), when late preterms (34 to 38 weeks) were compared to term born infants (>38 weeks), incidence of RDS was 10.5% in the late preterms vs 0.3% in the term population (Hibbard 2010). This suggests that even in late preterms who may or may not have developed CLD, respiratory morbidity in infancy is higher than in the term-born population. However, postnatal interventions such as mechanical ventilation and oxygen inhalation also contribute to lung inflammation. Factors such as low birth weight and postnatal nutrition may also have a role in long term respiratory outcome. When spirometry was performed on 8-9 year old children who were appropriate or gestational age at birth (n=3,462) and those who had intrauterine growth retardation (n=576), FEV<sub>1</sub> was significantly lower in those with intrauterine growth retardation (Kotecha 2010). This study did not include prematurely born children. Current literature on respiratory outcome of CLD do not differentiate if the respiratory consequences are due to prematurity alone or due to the pathology of CLD. Prematurity, CLD and IUGR are intermingled in most of the literature. The question still remains that if preterm infants do not develop CLD, would they still have abnormal lung function in later life?

## **1.4 Cardiac consequences of premature birth**

Although primary airway disease following premature birth is more obvious, premature infants with RDS and CLD are also at high risk of cardiovascular sequelae, particularly pulmonary arterial hypertension (PAH).

### **1.4.1 Pulmonary vascular changes in RDS and CLD**

Premature arrest of lung development and subsequent exposure to hypoxia has important effects on infant haemodynamics during the transitional period. These infants are more likely to have persistent fetal circulation leading to increased pulmonary arterial pressure in the early postnatal period. Hislop and Haworth have also demonstrated that those infants who died of RDS or subsequent CLD had significant structural abnormalities in their pulmonary vasculature irrespective of whether or not they had clinical or pathological features of cor pulmonale (Hislop and Haworth 1990). In infants who died within the first 3 months, while still being supported by mechanical ventilation, the muscularity of the pulmonary vascular wall was similar to that seen in the normal fetus. This was less evident in those who had recovered from initial RDS and did not have clinical cor pulmonale. However, in those who died of cor pulmonale, the muscularity of the pulmonary vessels was considerably increased. All infants who died of CLD, with or without cor pulmonale, had increased adventitial thickness. These structural changes in the pulmonary vasculature cause narrowing of the vessels and also compromise their compliance, thereby contributing to high pulmonary vascular resistance. These findings clearly demonstrate the importance of the assessment of pulmonary arterial pressure and right ventricular function in premature infants with CLD.

### **1.5 Body composition in prematurely born children**

Prematurely born infants are known to have impaired growth in early infancy (Huysman 2003; Cooke and Griffin 2009). Although the mechanisms underlying growth failure in infants with CLD are poorly understood, this has been speculated to be due to increased work of breathing (Kurzner 1988), infection and a different body composition (Huysman 2003).

Cooke et al studied 149 preterm infants ( $\leq 34$  weeks of gestational age,  $\leq 1750$  grams at birth) at discharge from hospital (Cooke and Griffin 2009). The researchers reported that fat free mass measured by dual-x-ray-absorptiometry (DXA) was significantly less in preterm infants than in the reference infants at the same weight or gestational age. Global fat mass was greater in the same group of preterm infants compared to the control population. The authors concluded that the impaired linear growth and fat free mass in preterm infant may be because dietary protein needs were not met before discharge.

In a study by Fewtrell et al (Fewtrell 2004) when 497 prematurely born children ( $< 37$  weeks of gestational age) and 95 term-born children were studied at 8-12 years of age, the researchers noted that those born prematurely had significantly lower fat mass index (FMI) measured by DXA compared with the term-born controls but fat free mass index (FFMI) was not different between the two groups. There was no association between birth weight, gestational age, or neonatal diet and FMI or FFMI at 8-12 years of age.

Body composition has also been measured in prematurely born children with CLD. Bott et al studied 71 children, aged 4-8 years, with CLD of prematurity (Bott 2006). The researchers did not compare the results with preterm or term-born controls. Instead, this study was designed to compare FMI and FFMI measured using anthropometry, dual x-ray-absorptiometry (DXA)

and bioelectrical impedance analysis (BIA). The authors concluded that both BIA and anthropometry gave good agreement with DXA to evaluate fat mass and fat free mass but BIA overestimated fat mass (mean difference: 0.34 kg±2.06) and underestimated fat free mass (mean difference: -1.24 kg±3.32).

In prematurely born young adults aged 19 years (n=403, <32 weeks of gestational age), weight gain before 32 weeks of corrected gestational age was positively associated with adult body size but not with body composition and fat distribution (Euser 2005). Whether body composition of prematurely born children with CLD varies from those who do not develop CLD is not clear.

### **1.5.1 Validation of bioelectrical impedance for measurement of body composition in children**

Dual-x-ray-absorptiometry (DXA) is a reference criterion for measurement of body composition. The drawbacks of this method are that this is a time-consuming and expensive method that requires an expert technician and sophisticated equipment. DXA also involves radiation, albeit a negligible dose of 0.001 milli Sievert (Radiological Society of North America. <http://www.radiologyinfo.org/en/safety/index>) Bioelectrical impedance (BIA) is a more user-friendly and an easily accessible method for measuring nutritional status. BIA is based on the principle that the electrical conductivity through body fluid is much greater in fat free mass than in fat mass because fat free mass contains all body fluids and electrolytes (Sung 2001).

BIA has been validated in a large study on 1139 girls and 1243 boys, aged 7-16 years (Sung 2001). The 95% limits of agreement between BIA and DXA methods were considered to be acceptable (-3.3 kg to -0.5 for fat mass in kg and -3.9 to 0.6 for % body fat). Bott et al studied 71 children, aged 4-8 years, with CLD of prematurity (Bott 2006). The investigators compared fat mass and fat free mass measured using anthropometry, BIA and DXA. The results showed that BIA overestimated fat mass (mean difference: 0.34 kg, SD: 2.06) and underestimated fat free mass (mean difference: -1.24 kg, SD: 3.32) compared to that measured by DXA in children with CLD. The researchers did not, however, adjust fat mass or fat free mass to body size. As a result, it is also not clear from this study whether the reproducibility for fat mass and fat free mass measured by BIA compared with DXA is different in children with CLD compared with the term-born population. A previous study has suggested that in an undernourished population, body composition data correlate less with a reference method like DXA (Wells 1999). In a more recent study on 69, 8 year old children, the same group of researchers have suggested that body composition data should be normalised to height rather than weight (Wells and Cole 2002).

BIA is simple and quick to use in clinical settings. Although BIA may not be as accurate as DXA in measuring body composition in undernourished children, this is a useful method for cross-sectional and longitudinal studies because of its simplicity and acceptable validity. It has been shown to have a relatively good agreement with DXA.

### **1.5.2 Relationship between body composition and respiratory function**

In adult studies, abnormality in body composition, especially depletion of fat free mass has been associated with increased mortality and lower maximal exercise performance in patients

with chronic obstructive pulmonary disease (COPD) (Andreassen and Vestbo 2003). Low fat free mass and low body mass index have been found to predict a higher mortality even in those with a predominantly early stage COPD (Vestbo 2006). It has been demonstrated that patients with COPD have less fat free mass index and that this may be associated with normal body mass index (17/33 COPD patients had low FFMI but normal BMI) (Bolton 2004). In another study, higher lean-to-fat mass ratio was shown to be associated with a greater distance walked in 6 minutes in adults with COPD (Eisner 2007). FFMI has also been shown to be significantly associated with percentage of predicted FEV<sub>1</sub> and FEV<sub>1</sub>:FVC ratio in adults with clinically stable COPD (Ischaki 2007).

The influence of body composition on respiratory muscle and lung function has been established in adults (Ionescu 1998; Enright 2007) with cystic fibrosis. Loss of fat free mass has particularly been associated with loss of diaphragmatic muscle mass and loss of inspiratory muscle work capacity (Enright 2007). In children with cystic fibrosis appropriate weight gain has been associated with better FEV<sub>1</sub> (Zemel 2000; Peterson 2003). These studies have not explored the individual roles of fat mass and fat free mass on lung function.

Bott et al have reported that in children with CLD undernutrition does not correlate with pulmonary status at childhood (4-8 years), but is associated with nutritional status before the age of 2 years (Bott 2006). The authors also noted that although children with CLD had lower fat mass, resting energy expenditure adjusted for weight and fat free mass was similar in those with CLD and the control population (Bott 2006). There is lack of information on how different components of body composition vary between prematurely born children with CLD and the control population and whether this has any correlation with different aspects of lung function.

### **1.5.3 Relationship between body composition and cardiovascular function**

The relationship between body weight and cardiovascular disease has been explored in numerous studies in the past. Low birth weight has been related to increased cardiovascular morbidity and mortality later in life (Barker 1994; Lurbe 2001; Lackland 2003). The association between low birth weight and elevated blood pressure later on life has been extensively studied on the population in the Bogalusa Heart Study (Mzayek 2007). On the other hand, a high body mass index in children has been associated with various cardiovascular risk factors, atherosclerosis and mortality in adulthood (Berenson 1998; Reilly 2003; Bjorge 2008).

There is a gap of knowledge in the current literature on the relationship of different components of the body mass index and cardiovascular risk factors in children. As high body mass index can reflect increase in either fat mass or fat free mass, it would be useful to understand the relationship between specific components of body composition with physiological and biochemical indices of cardiovascular disease. Furthermore, it is not clear if altered body composition is related to subclinical myocardial dysfunction. Understanding the basis of these relationships can help in the holistic management of prematurely born children, particularly those with CLD, who may be at high risk of developing cardiopulmonary disease later in life.



## **1.6 Assessment of pulmonary arterial pressure by echocardiography**

The technical challenges in the assessment of pulmonary arterial pressure (PAP) and RV function in infants and children cannot be overstated. Although the reference criterion for diagnosis of pulmonary arterial hypertension (PAH) is right heart catheterization, this is an invasive procedure, which is highly impractical and unethical, particularly for diagnosis of subclinical disease in small infants and children. Therefore, over the last two decades, non-invasive echocardiographic features have been explored extensively for diagnosis of PAH.

### **1.6.1 Pulsed Doppler methods**

Pulmonary arterial hypertension (PAH) is defined as mean PAP >20 mm Hg at rest, or >30 mm Hg during exercise. Commonly used equations for assessment of PAP by Doppler echocardiography are,

**1. Systolic PAP** =  $4 \times (\text{Tricuspid regurgitation peak velocity})^2 + \text{right atrial pressure}$  (Hatle 1981)

**2. Mean PAP** =  $79 - 0.45 (\text{Pulmonary arterial acceleration time, AT})$  (Dabestani 1987)

**3. Diastolic PAP** =  $4 \times (\text{Pulmonary regurgitation end diastolic velocity})^2 + \text{right atrial pressure}$  (Masuyama 1986)

#### **1.6.1.1 Tricuspid regurgitation peak velocity**

The assessment of peak systolic pressure across the tricuspid valve using tricuspid regurgitation (TR) peak velocity is perhaps the most widely used echocardiographic

parameter for direct assessment of systolic PAP. In this method, maximum peak velocity of blood flow through tricuspid valve is measured by continuous wave Doppler ultrasound (Figure 4.7) and right ventricular to right atrial pressure gradient is calculated by applying the modified Bernoulli equation ( $\Delta P=4V^2$ ). RV pressure is calculated by adding estimated right atrial pressure to the calculated pressure. The right atrial pressure can be estimated by the degree of collapse of the inferior vena cava on inspiration (Kircher 1990). In the absence of RV outflow tract obstruction RV systolic pressure should be equal to the PAP.

This method has been validated both in adults, and in children. When a measurable TR jet is visible, this has been demonstrated to be highly accurate in diagnosis of PAH in adults and correlated well with catheterization measurements ( $r=0.97$ ) (Skjaerpe and Hatle 1986). It has also been shown to be accurate and reproducible in infants with congenital heart disease ( $r=0.95$ ) (Skinner 1993). The problem however is, that a TR jet was detectable only in 47% of patients with chronic obstructive airway disease (COPD) with only 30% patients having good quality TR jet in one study (Tramarin 1991). Similarly, in young children <2 years with chronic lung disease of various aetiology, TR jet could be detected in only 63% of patients and TR velocity could diagnose presence of PAH in 79% of patients, but could correctly estimate PAP in only 47%. 58% of children (7/12) who did not have measurable TR jet were diagnosed to have PAH by subsequent catheterization (Mourani 2008). Thus, although in the presence of a measurable TR jet velocity, the presence of PAH can be diagnosed with fairly good accuracy, its absence does not exclude PAH.

#### 1.6.1.2 Pulmonary regurgitation end diastolic velocity

Continuous-wave or pulse-wave Doppler echocardiography can be used to measure pulmonary regurgitation flow velocity (Figure 4.8), to estimate PAP by modified Bernoulli equation. This method has also been validated in a small study of 21 patients with PAH and 24 controls without PAH (Masuyama 1986). The Doppler determined pressure gradient at end diastole correlated well with catheter measurements ( $r=0.94$ ) in this study. However, measurable PR velocity was visible in only half of the controls and 78% of patients with PAH.

#### 1.6.1.3 RV outflow systolic time intervals

RV systolic time intervals are probably the most extensively studied surrogate markers of PAH. In this method, the forward flow through the pulmonary artery is visualized by pulse-wave Doppler ultrasound. Acceleration time (AT) is measured from the onset of ejection to the time of peak flow velocity, and ejection time is measured from the onset to the end of systolic flow velocity (Figure 4.9) The advantage of this method is that unlike the TR and PR jets, pulmonary flow can invariably be measured in all patients, irrespective of age and presence or absence of PAH.

The negative linear correlation with mean PAP in adult population has been demonstrated in various studies (Matsuda 1986; Dabestani 1987).

Table 1.5 Sensitivity and specificity of acceleration time (AT) for detecting pulmonary arterial hypertension (PAP  $\geq$ 20 mm Hg)

Doppler index	Sensitivity	Specificity
AT $\leq$ 120 ms	91%	63%
AT $\leq$ 110 ms	87%	88%
AT $\leq$ 100 ms	78%	100%

(Dabestani 1987)

In the study by Dabestani et al (Dabestani 1987), all patients with pulmonary systolic flow acceleration time (PA AT)  $\leq$ 75 ms had severe PAH (PAP of at least 40 mm Hg). There was significant correlation between PA AT and mean PAP ( $r=0.84$ ,  $p<0.001$ ). In this study, the use of acceleration time to ejection time ratio (AT: ET) did not improve the correlation with mean pulmonary arterial pressure. All patients in this study had heart rate (HR)  $<110$  beats per minute. The high specificity of PA AT for diagnosis of PAH does not hold true unless the heart rate is 60-100 beats per minute. This caveat needs to be taken into account when using PA AT as a surrogate marker of PAH in infants and children. Reassuringly, when AT was corrected for heart rate by dividing AT by square root of R-R interval, significant inverse correlation with ATc ( $r = -0.78$ ) and AT/ETc ( $r=0.87$ ) was noted in children aged 4 days to 16 years (Akiba 1988).

AT:ET ratio has been used to study changes in PAP in preterm infants during the acute phase of RDS (Evans and Archer 1991), during recovery (Evans and Archer 1991), and in infants with CLD of prematurity (Fitzgerald 1994; Subhedar and Shaw 2000). In one of these studies, using AT:ET ratio of  $\geq 0.35$  as normal and  $<0.31$  as definitely low, 24% of infants with CLD were suggested to have raised PAP (Fitzgerald 1994). The evidence of lower AT:ET ratio in

preterm infants with RDS compared to controls is evident in all these studies. However, this measurement has been reported to have considerable intraobserver variability in preterm infants (Subhedar and Shaw 1996). The coefficient of repeatability for measurement of AT:ET ratio was only 25.4% in this study. The PA AT measurement also depends upon the sampling site, possibly due to varying angle of incidence to pulmonary arterial flow and also sample volume of pulsed Doppler (Panidis 1986).

In summary, pulmonary systolic flow acceleration time (PA AT) is a specific, but not a sensitive index of PAP.

## **1.6.2 Tissue Doppler methods/ Myocardial velocity imaging**

### **1.6.2.1 Right ventricular relaxation time 'IVRT'**

Myocardial pulsed Doppler imaging of the tricuspid annulus can be used to measure RV relaxation time 'IVRT (Figure 4.11)'. In adults, IVRT correlates well ( $r=0.74$  to  $0.87$ ) with simultaneously measured PAP by RV catheterization (Dambrauskaite 2005; Brechot 2008). RV relaxation time was longer in patients with PAH, compared to those who did not have PAH. RV relaxation time 'IVRT'  $\leq 40$  ms has been shown to exclude PAH with 100% negative predictive value (Brechot 2008). Although prolonged IVRT is suggestive of elevated PAP, this cannot confirm the diagnosis of PAH by itself. At present, there is a lack of normal values and validation of this technique in infants and children.

### 1.6.2.2 RV lateral wall peak systolic strain

Strain is the percent magnitude of myocardial deformation during cardiac cycle. Systolic strain is percent shortening of myocardium in a defined region of interest, during systole (Figure 4.14). RV systolic strain has been explored as a promising new technique for diagnosis of PAH. When used as a surrogate marker of PAH, RV systolic strain had significant inverse correlation with PAP ( $r = -0.67$ ;  $p < 0.001$ ) and pulmonary vascular resistance ( $r = 0.6$ ;  $p < 0.001$ ) (Rajagopalan 2008). In another large study on adults, when 111 patients with PAH and 35 patients with normal PAP had tissue Doppler myocardial imaging and cardiac catheterization, RV systolic strain  $< -20\%$  was shown to predict PAP  $> 40$  mm Hg with a sensitivity of 60% and specificity of 87% (Lopez-Candales 2008). In this study, RV dyssynchrony measured by delayed time to peak strain between the ventricular septum and RV lateral wall of  $> 25$  ms was demonstrated as a good predictor of systolic PAP  $> 40$  mm Hg with sensitivity of 78% and a specificity of 80%. In view of these promising results in adult studies, it is possible that RV systolic strain may be a useful surrogate marker for diagnosis of PAH in infants and children as well. Strain and strain rate imaging has been proved to be feasible and reliable in infants (Nestaas 2007). This has not yet been validated for diagnosis of PAH in infants or children. Nevertheless, normal values for RV strain have been described in children, albeit in a wide age range of 1.1-18 years (Kutty 2008). The mean RV basal strain in this study for 30 normal children was,  $-18.9 \pm 4.7\%$ . Thus, the cut-off value of strain  $< -20\%$  cannot be blindly applied in children for diagnosis of PAH.

### **1.6.3 Echocardiographic evidence of pulmonary arterial hypertension in respiratory distress syndrome and chronic lung disease of prematurity**

Evans and Archer have used pulmonary acceleration time to ejection time ratio (AT: ET) for indirect assessment of PAP in 57 preterm infants (38 infants with RDS) at different time periods during the acute phase of RDS (Evans and Archer 1991). In this study, AT:ET ratio did not differ in preterm infants with and without RDS during the first 6 hours after birth, but the ratio was significantly different by 73 to 96 hours. The mean AT: ET ratio did not show significant correlation with gestation, birth weight, maximum supplemental oxygen, or the length of acute phase of illness. Importantly, the investigators noted that the AT: ET varied considerably among infants with RDS, and also depending upon the sampling site. The same group of investigators followed up 37 infants with RDS, during recovery phase of the illness. 12/37 premature infants in this study continued to have low AT: ET ratio at discharge, despite having no clinical signs of respiratory illness. The infants in this group had lower gestational age at birth, lower birth weight, and had required longer duration of additional oxygen compared to the groups in which the ratio returned to normal at discharge (Evans and Archer 1991). Following this, a study involving preterm infants who developed CLD (oxygen dependency at 28 days) has also demonstrated that 24% of infants with CLD, who had no clinical evidence of PAH, had low AT: ET ratio ( $<0.31$ ), suggesting raised PAP. In a more recent study involving 248 examinations on 54 infants with CLD and 44 healthy preterm controls who were born between 1995 and 1997, the AT: ET ratio was significantly lower in the preterm CLD group compared to preterm controls (0.31 vs 0.37) (Subhedar and Shaw 2000). All infants in this study, who had required mechanical ventilation, had been treated with 2 doses of surfactant, 12 hours apart. The infants had longitudinal echocardiographic studies from 36 weeks of corrected gestational age till 40-52 weeks of corrected age. This longitudinal study showed that in infants with CLD, the AT: ET ratio was significantly

independently associated with age and inversely correlated with duration of supplemental oxygen treatment. Thus, although PAP falls with increasing age in the premature infants, there is evidence that some infants with CLD may have persistent subclinical PAH, even in the post-surfactant era. Whether this subclinical PAH seen in infants with RDS and CLD persist later in life, is yet to be seen.

In adults with chronic obstructive airway disease, left ventricular function has also been found to be compromised (Acikel 2010; Sabit 2010) and left ventricular diastolic function has been found to be closely related to the level of pulmonary hypertension (Acikel 2010). This has not yet been tested in children with CLD of prematurity.

In most of the studies assessing PAP in infants with RDS or CLD, the investigators have used blood Doppler echocardiography for indirect assessment of PAP. Tissue Doppler or myocardial velocity imaging methods have not yet been applied in prematurely born infants and children for diagnosis of PAH.

## ***1.7 Cardio-Respiratory Effects of Hypoxia***

### **1.7.1 Hypoxic effects of altitude**

The atmosphere consists primarily of nitrogen (78%) and oxygen (21%). This percentage of inspired oxygen remains constant at different altitudes. What varies is the atmospheric pressure. As the altitude increases, the atmospheric pressure decreases, which results in the reduction of the driving pressure for gas exchange in the lungs i.e. reduction in the inspired alveolar and arterial partial pressure of oxygen (Dalton's Law).



Atmospheric pressure is the pressure at any point of the earth's atmosphere and is determined by hydrostatic pressure caused by the weight of air above the point of measurement. At sea level, the weight of air above us is normally 100kPa. Atmospheric pressure is calculated as the sum of the partial pressure of the constituent gases (oxygen and nitrogen) and the partial pressure of water vapour (6.3kPa at 37°C). As oxygen is 21% dry air, the inspired oxygen pressure at sea level is  $0.21 \times (100 - 6.3) = 19.6 \text{ kPa}$  (Peacock 1998). As we ascend, the air becomes less dense resulting in hypobaric hypoxia and is effectively equivalent to breathing oxygen at lower concentration.

The accessibility of commercial flights means that more and more people are travelling by long distance air flights. Similarly, visits to high altitude ski resorts or hiking in the mountains are becoming increasingly popular. Some of the ski resorts in Europe and North America are less than 2,438m (8,000ft) high. Others are higher than 8,000ft. The highest ski resort in North America is 3,962m (13,000ft) in Colorado, and in Europe, it is 3,794m (12,450ft). Destinations such as the Everest base camp (5,500m) and Tibet (3,658m), which were once considered remote, are now relatively easily accessible. The impact of this fast pace, adventurous life means that increasingly more people are exposed to altitude related hypoxia and its consequences.

#### 1.7.1.1 Air flight and altitude

Commercial aircrafts generally cruise at altitudes of 9,000 to 12,000m (30,000 to 40,000ft) above sea level, where atmospheric partial pressure of oxygen,  $\text{PO}_2$  is  $\leq 5 \text{ kPa}$ . This level of oxygen would normally cause lethal alveolar hypoxia. To reduce the hypoxic effect of

altitude, aircraft cabins are pressurised to an altitude of 1,500-2,400m (5,000-8,000ft) above sea level, giving atmospheric pressure of about 75kPa and an atmospheric PO<sub>2</sub> 15-17kPa. This is equivalent to breathing 15-17% oxygen at sea level (Samuels 2004; Bossley and Balfour-Lynn 2007). In emergency, if the aircraft has to cruise above this altitude, oxygen masks are automatically deployed to all the passengers. For practical and commercial reasons, it is not possible to pressurise aircrafts to sea level. Maintaining aircraft at a sea level pressure would reduce the energy available for other aircraft systems, increase fuel consumption and reduce the working life of the aluminium airframes (Samuels 2004).

The current code of US Federal Aviation Regulations specifies that the air flight cabins and compartments should be pressurised to not more than 2,438m (8,000ft) at the maximum operating altitude of the aeroplanes under normal operating conditions (Anonymous 1986). One important fact to be noted with this regulation is that, it is practically not possible to always adhere to this limit and also, that the cabin altitude does not remain constant during the flight. This has been demonstrated when cabin pressures were directly measured in commercial aircraft flights (Cottrell 1988; Kelly 2007).

#### 1.7.1.2 Flight hypoxia in adults and children

With the growing number of passengers flying with pre existing cardiorespiratory conditions, insight into the hypoxic effects of flight and its possible consequence has become an area of intense scrutiny. When oxygen saturation (SaO<sub>2</sub>) was monitored in 84 passengers (aged 1-78 years) during short and long haul flights, the average change in oxygen saturation was noted to be from 97% (93-100%) at ground level to 93% (85-98%) at cruising altitude (Humphreys 2005). In this study, the authors presented the results from long and short haul flights together

and did not present the data from adults and children separately. Lee and his colleagues have also carried out a similar study on 80 healthy, 0-15 year old children, on a long distance flight from Honolulu to Taipei, lasting 8-10 hours. In this study, the mean oxygen saturation declined from a mean sea level SaO<sub>2</sub> of 98.5% (± 1.2) to 95.7% (± 1.7) after 3 hours and 94.4% (± 1.8) after 7 hours of flight (Lee 2002). It is now well established that most children and adults experience some degree of oxygen desaturation in long haul flights. This degree of hypoxia may not be clinically significant in a well, asymptomatic passenger. On the other hand, passengers with pre-existing cardiopulmonary conditions with chronic mild hypoxemia may develop exaggerated hypoxemia during commercial airline flights or in any other high altitude conditions. There is no strong evidence at present to argue for or against this hypothesis.

#### 1.7.1.3 British Thoracic Society guidelines

With the growing concerns regarding patients with respiratory conditions embarking on commercial flights and lack of clear guidance for recommendation of oxygen for these passengers, there was clearly a need for consistent, practical, and comprehensive advice. To meet this demand, in 2002, British Thoracic Society Standards of Care Committee provided recommendations for respiratory physicians, for those passengers who travel by commercial aircraft and have predisposing lung disease (Coker 2002). In summary, the 2002 BTS guidelines for children recommended that,

- It is prudent to wait for 1 week after birth before allowing infants to fly to ensure the infant is healthy
- If the infant has had any neonatal respiratory problems, the proposed journey should be discussed with a paediatrician and a hypoxic challenge test considered

- For oxygen-dependent children including ex-premature infants with chronic lung disease where flying is imperative, oxygen requirements should be titrated in a body box as follows:

The infant, receiving oxygen via nasal cannula is placed in the body box in the company of a parent or a carer, and SaO<sub>2</sub> monitored. The air in the body box is then diluted to 15% oxygen with nitrogen for 20 minutes. Any fall in SaO<sub>2</sub> can be restored to the original value by titration of the flow of oxygen through the nasal cannula. This flow of oxygen should then be supplied during the flight.

For adults with respiratory disease, the BTS guidelines suggest that those with sea level SaO<sub>2</sub> of 92-95% should undergo hypoxic challenge with 15% oxygen for 20 minutes, while those with SaO<sub>2</sub> <92% at sea level should receive in-flight oxygen. For those undergoing hypoxic challenge, the guidelines recommend that if either the SaO<sub>2</sub> during the challenge falls <85% or post-test PaO<sub>2</sub> is <6.6, in-flight oxygen (2 l/min) should be advised. However, the 2004 update of these guidelines suggested that in-flight oxygen should be recommended if SaO<sub>2</sub> falls <90% during the hypoxic challenge (instead of <85%). Thus, the cut off value of SaO<sub>2</sub><90% during hypoxic challenge for in-flight oxygen requirement is an arbitrary consensus view rather than evidence based.

### **1.7.2 Pre-flight hypoxia test**

Since the publication of the BTS guidelines for passengers with lung disease, there have been prospective as well as retrospective studies on pre-flight hypoxia challenge tests. In the paediatric age group, the pre-flight hypoxia tests have been mainly concentrated on preterm

infants (Buchdahl 2004; Udomittipong 2006; Hall 2007; Martin 2008) and children with cystic fibrosis (Oades 1994).

Buchdahl and colleagues used 15% oxygen delivered in a body box to test 20 infants (8, CLD). 6/8 infants with CLD had baseline SaO<sub>2</sub> of >95% but overall 8/20 children dropped their SaO<sub>2</sub> to <90% during the hypoxic challenge (Buchdahl 2004). In a retrospective review of infants who had pre-flight hypoxic challenge test by Udomittipong et al (Udomittipong 2006), 14% oxygen had been delivered using face masks. 32/47 infants in the study had CLD. All had baseline SaO<sub>2</sub> >95% but 23/32 infants with CLD dropped their SaO<sub>2</sub> to <85% during the hypoxic challenge. In a larger study by Martin et al (Martin 2008), 69 children who had pre-flight hypoxic challenge test using 14% oxygen were reviewed. In this study, 14/23 children with CLD (<2 year old) desaturated to <90% whereas 6/23 children with CLD (>2 year old) dropped their oxygen saturation to <85%. Interestingly, 12/24 healthy children (<2 year old) also desaturated to <90% and 1/24 healthy child (> 2 year old) desaturated to <85%.

It is interesting that SaO<sub>2</sub> in room air was not predictable of the response to hypoxic challenge in any of the above studies. More importantly, in the study by Martin et al (Martin 2008), when SaO<sub>2</sub> < 90% was considered as a failure to pass a hypoxic challenge as suggested by 2004 BTS guidelines, 50% of healthy infants failed hypoxic challenge. SaO<sub>2</sub> <85% seems a more reasonable cut-off according to this study.

#### 1.7.2.1 Hypobaric hypoxia V normobaric hypoxia

Hypobaric hypoxic chambers can simulate aircraft cabin pressure and partial pressure of oxygen (PaO<sub>2</sub>) and would be an ideal method of carrying out pre-flight hypoxic challenge.

However, hypobaric hypoxic chambers are not readily available. Alternatively, hypoxic challenge can be carried out by diluting the oxygen concentration in a body box as suggested in the 2002 BTS guidelines or by letting the child breathe reduced concentration of oxygen via a face mask or nasal cannula. Both these methods would create normobaric hypoxia as opposed to hypobaric hypoxia encountered in an aircraft or at high altitude.

To assess the validity of a normobaric hypoxia inhalation test compared with actual in-flight responses, Kelly et al (Kelly 2006) studied 15 healthy adults, who underwent pre-flight normobaric hypoxia inhalation testing by inhaling 15% oxygen, and compared the SaO<sub>2</sub> results with actual in-flight SaO<sub>2</sub> results. They noted that although there was no difference between the final normobaric hypoxia inhalation test SaO<sub>2</sub> (92±2) and mean in-flight SaO<sub>2</sub> (92±2), there was a significant difference between the lowest normobaric hypoxia inhalation test SaO<sub>2</sub> (90±2%) and the lowest in-flight SaO<sub>2</sub> (88±2%). Reassuringly, a study on adult patients with Chronic Obstructive Airway Disease (COPD), comparing PaO<sub>2</sub> at 15% hypobaric hypoxia inhalation test and 2,438m (8,000ft) hypobaric chamber has shown that the PaO<sub>2</sub> relationship was comparable (Dillard 1995). Another validation of normobaric hypoxia testing has been carried out on 7 children aged 2-51 months in Perth, Australia (Hall 2007). These children were exposed to 14% oxygen at high flow rate of 15 l/min via a mask for 20 minutes. 2 separate periods of 30 seconds of quiet breathing were recorded and inhaled oxygen concentration for each inspiration was calculated. It was noted that the median concentration of inhaled oxygen was 13.5% (SD 0.2%). Therefore, use of normobaric hypoxia inhalation test seems to be a good method to simulate hypobaric hypoxia.

### **1.7.3 Hypoxic pulmonary vasoconstriction**

Hypoxic pulmonary vasoconstriction is an adaptive mechanism whereby blood is shunted from segments of poorly perfused segments of the lung to the well perfused segments, thereby optimizing the ventilation-perfusion match and thus reducing shunt function. This also results in optimal systemic delivery of oxygen in patients with focal lung disease such as pneumonia and atelectasis (Thomas and Garrett 1982; Brimiouille 1996). However, with global hypoxic conditions such as in high altitude hypobaric hypoxia or sleep apnoea, hypoxic pulmonary vasoconstriction results in constriction of pulmonary arteries throughout the pulmonary circulation resulting in increased pulmonary vascular resistance (Moudgil 2005). Bindsler and his colleagues observed that during anaesthesia, hypoxic pulmonary vasoconstriction increased pulmonary vascular resistance by 50% to 300%, and that the response to hypoxia starts within minutes of exposure and peaks within 15 minutes (Bindslev 1985). Similarly, inhalation of 12.5% oxygen at sea level has been shown to decrease systemic PaO<sub>2</sub> to below 50 Torr and increase pulmonary vascular resistance by 100% -150% in normal adult volunteers (Naeije 1987). Interestingly, it is the alveolar oxygen tension that determines the hypoxic pulmonary vasoconstriction and not the blood PaO<sub>2</sub>. As long as the alveolar oxygen tension is maintained above 60 Torr, there seems to be little hypoxic pulmonary vasoconstriction even when blood PaO<sub>2</sub> is reduced to 10 Torr (Marshall and Marshall 1983). To combat the hypoxic pulmonary vasoconstriction, hypoxia also results in hyperventilation and respiratory alkalosis (Bindslev 1985). All these effects of hypoxia are modulated by pulmonary vascular endothelium and the mechanisms surrounding this complex process, including the Redox Theory and the role of K<sup>+</sup> and Ca<sup>++</sup> channels have been extensively reviewed by Moudgil et al (Moudgil 2005).

#### **1.7.4 Effects of acute hypoxia on children with neonatal hypoxic pulmonary arterial hypertension**

Epidemiological studies have clearly suggested that adverse events in utero may lead to cardiovascular and metabolic diseases in adulthood (Barker 1994; Barker 2001). With regards to hypoxic pulmonary arterial hypertension, although there is enough evidence that localized or generalized pulmonary hypoxia leads to hypoxic pulmonary vasoconstriction, there is little evidence to prove that hypoxia in perinatal period leads to susceptibility for hypoxic pulmonary arterial hypertension (PAH) in adulthood. Animal studies on rats have demonstrated that exposure to hypoxia in the first few days of life induces transient pulmonary arterial hypertension and predisposes to augmented pulmonary arterial hypertension in response to hypoxia in adulthood (Hakim and Mortola 1990; Hampl and Herget 1990).

In the only relevant human study in this field, Claudio Sartori and his team tested this hypothesis on 15 adults (mean age 21 years), who had transient hypoxic pulmonary hypertension in the first week of life (Sartori 1999). These volunteers had their systolic pulmonary arterial pressure (PAP) measured by echocardiography at sea level with hypoxic breathing (12% oxygen for 20 minutes), and then at a high altitude research laboratory at 4,559m (4,600m ~ inhalation of 12% oxygen at sea level). They also had their PAP measured after inhalation of nitric oxide at high altitude. The results were compared with that of 10 healthy volunteers who were born at full term and had normal perinatal period. The results showed that at sea level, PAP between the two groups was similar but 24-36 hours after arrival at 4559m, the mean increase in PAP in the group who had neonatal pulmonary hypertension was significantly higher compared to the control group ( $p < 0.01$ ). In this experiment, the decrease in PAP after inhalation of nitric oxide at high altitude was also



significantly higher in the neonatal pulmonary hypertension group compared to the control group.

We know from post mortem studies that infants who died from neonatal chronic lung disease showed thickening of pulmonary vascular smooth muscle (Bush 1990; Margraf 1991). Similar findings were also noted on those infants who died from pulmonary hypertension (Haworth 1988; Stenmark 1988). Whether this early life pathology persists in children who survive neonatal chronic lung disease is not known. If it does, it could predispose to exaggerated vasoconstrictor response to acute hypoxia. Specifically, in light of the observations made by Sartori et al in the above study, it seems prudent that we look for evidence of pulmonary arterial hypertension in response to acute hypoxia in the surviving children with chronic lung disease of prematurity.

In summary, fetal lung growth and the development of pulmonary vasculature are closely related to each other. Pulmonary function studies on ex-preterm children suggest that they continue to have evidence of airway obstruction and impaired alveolar diffusion as they grow older, but most studies do not differentiate whether this impaired pulmonary function is due to prematurity per se or due to CLD. Besides, current literature on exercise capacity in children with CLD is not consistent. It is also not clear if these children have exercise induced bronchospasm and whether the CLD related airway obstruction is reversible. Premature birth is also associated with abnormal pulmonary vascular development. There is some evidence that prematurely born infants who develop CLD have thickened pulmonary arterial smooth muscle and develop pulmonary arterial hypertension. Whether this early life pathology persists as these children grow older is not clear. It is also not known if children with CLD of prematurity are at risk of developing subclinical right ventricular dysfunction and pulmonary

arterial hypertension secondary to impaired lung function, especially if they are exposed to hypoxic conditions.

As lung function and cardiovascular function have both been generally related to nutritional status, it would also be useful to learn if children with CLD have a different body composition compared to the control population and whether there is any relationship between body composition and cardio-respiratory function in children.

## **1.8 Hypothesis and study aims**

### **1.8.1 Hypothesis**

I hypothesized that prematurely born children who had chronic lung disease in infancy would have:

- 1(a) Impaired pulmonary function at rest and after exercise
- 1(b) Reduced exercise capacity compared to both preterm and term controls
- 1(c) Greater exercise induced bronchoconstriction
- 1(d) Greater response to bronchodilator administered after exercise.

Children who had chronic lung disease in infancy would also have:

- 2(a) Subclinical right ventricular dysfunction
  - 2(b) Evidence of raised pulmonary arterial pressure after exposure to acute hypoxia
  - 2(c) Subclinical left ventricular dysfunction
- compared with preterm and term-born controls.

## **1.8.2 Specific aims**

### **Primary aims:**

1(a) To assess pulmonary function at rest in children with CLD and in term and preterm control subjects, using spirometry, single breath test for alveolar carbon monoxide diffusion capacity and whole body plethysmography for static lung volumes

1(b) To measure spirometry after cardiopulmonary exercise test to assess exercise induced bronchospasm in children with CLD and in control subjects

1(c) To assess exercise duration and exercise load, oxygen consumption, carbon dioxide production, maximum voluntary ventilation and ventilatory reserve at maximum exercise in those with CLD compared with preterm and term-born control subjects

1(d) To test response to inhalation of bronchodilator agent in children with CLD compared with the control subjects, to assess the presence of reversible airway disease

2(a) To assess baseline right and left ventricular function in children with CLD compared with term and preterm control subjects, using physiological, biochemical and echocardiographic markers

2(b) To assess right ventricular function and evidence of subclinical pulmonary arterial hypertension in response to acute hypoxia in children with CLD compared to healthy term and preterm controls, by using pulsed-wave Doppler and tissue-Doppler echocardiography.

**Secondary aims to generate hypothesis for future studies:**

1(a) To assess body composition using bioelectrical impedance in children with CLD and compare it with preterm and term-born control population.

1(b) To assess subclinical relationships between body composition and indices of pulmonary function and cardiovascular function.

## **Chapter Two: Pulmonary Function Study**

## **2.1 Introduction**

Survivors of chronic lung disease of prematurity (CLD) have increased respiratory morbidity (Greenough 1996; Greenough 2005), increased hospitalization (Cunningham 1991; Chye and Gray 1995; Gross 1998) and poor lung function during infancy and early childhood (Baraldi 1997; Hennessy 2008). Increased respiratory morbidity is thought to be related to limited ventilatory capacity in infancy but it is unclear if this improves with age. Children with CLD have evidence of airway obstruction (Fawke 2010; Doyle 2006; Hennessy 2008), bronchial hyper-reactivity (Bader 1987; Korhonen 2004) and air trapping (Doyle 2006) compared with term-born controls at school age and in early adulthood (Northway Jr 1990). Preterm children without CLD have also been demonstrated to have lung function abnormalities in early adulthood (Hakulinen 1996; Vrijlandt 2006). Recent studies have reported lower diffusion capacity ( $DL_{CO}$ ) in preterm infants (Balinotti 2010) and children (Welsh 2010) suggesting impaired alveolar development in preterm children born in the surfactant era. As described in section 1.3.3 currently available data on  $DL_{CO}$  (Mitchell and Teague 1998; Vrijlandt 2006; Smith 2008; Narang 2009) and cardiopulmonary exercise test (Jacob 1997; Pianosi and Fisk 2000; Kriemler 2005; Vrijlandt 2006; Narang 2009; Welsh 2010) in prematurely born children and young adults are inconsistent.

Currently available data on lung function in preterm children do not differentiate those with and without CLD. There is also lack of information on the objective effect of exercise on airway function and the effect of bronchodilator drugs after exercise in children with or without CLD in infancy.

I hypothesized that children who had CLD in infancy would (a) have impaired pulmonary function at rest and after exercise; (b) have reduced exercise capacity compared to both

preterm and term controls; (c) have greater exercise induced bronchoconstriction; and (d) have greater response to bronchodilator administered after exercise. Finally, I modelled the data to assess which perinatal factors may influence  $DL_{CO}$ , exercise tolerance and ventilatory capacity.

## **2.2 Methods**

### **2.2.1 Recruitment of the subjects**

#### **Inclusion criteria:**

1. 8-12 year old children, who were born at  $\leq 32$  weeks of gestational age and who were oxygen dependent at either 36 weeks of corrected gestational age or at discharge to home, were included in the preterm CLD group.
2. 8-12 year old children, who were born at  $\leq 32$  weeks of gestational age and who did not require oxygen at 36 weeks of corrected gestation age, were included in the preterm control group.
3. 8-12 year old children, who were born at  $\geq 37$  weeks of gestational age, were included in the term control group.

#### **Exclusion criteria:**

1. Children with congenital structural cardiac defect
2. Children with congenital structural respiratory tract defect
3. Children with any neuromuscular disease that could compromise cardiac or lung function
4. Children with severe neurodevelopmental impairment who would not be able to comply

with the research protocol

Temporary exclusion criteria:

1. Recent respiratory tract infections, i.e. within last 3 weeks
2. Hospital admission within the last 6 weeks for any reason

These children were invited to attend at a later date.

### **Recruitment of prematurely born children:**

Prematurely born children were recruited from Cardiff and Vale NHS trust and Gwent Healthcare NHS trust.

#### **1. Cardiff and Vale NHS Trust**

Two sources were used to identify children from Cardiff and Vale NHS trusts: Neonatal Unit Database at University Hospital of Wales, and Neonatal Outreach Team Register for all children who were followed up by Neonatal Outreach Team once they are discharged from the Neonatal Unit at University Hospital of Wales and Llandough Hospital.

204 children who were born at  $\leq 32$  weeks gestational age between January 1996 to December 1999, and cared for at the Neonatal Unit at University Hospital of Wales were identified by a Consultant Neonatologist (Dr Drayton) from the patient database which is maintained by the Neonatal Unit. A further 101 children who were born at  $\leq 32$  weeks of gestational age at University Hospital of Wales or Llandough Hospital between January 1996 and December 2001, who were followed up by the Neonatal Outreach Nursing Team were identified from the Neonatal Outreach Team register.



From the computer database, each child's name, date of birth, gestational age at birth, duration of oxygen dependency was identified. The deceased children were identified from the hospital 'Patient Management System- PMS' programme.

Out of the 305 children (91 children with CLD, 214 children without CLD) who were identified, 31 (10%) children were excluded for the following reasons;

- 9 children were deceased (7 CLD, 2 without CLD)
- 7 had been diagnosed with congenital structural cardiac defects: 2 Pulmonary stenosis, 2 atrial septal defect and ventricular septal defect, 1 Pulmonary atresia and 2 cardiomyopathy (1 CLD, 6 without CLD)
- 15 children resided outside South Wales

General practitioners for the remaining 274 children were contacted by telephone to ensure that the children are alive, and to confirm their home addresses prior to sending out the letters to the parents inviting the children to participate in the study.

- The current General Practitioner could not be located for 117/274 (43%) children.  
Letters (Appendix C) were sent out by a Consultant Neonatologist (Dr M Drayton), to the parents of the remaining 157 children inviting the children to participate in the study, along with the information sheets for the parents (Appendix D) and for the children (Appendix E), and reply letter with a self addressed return envelope.
- 53/157 (34%) (25 CLD, 28 without CLD) children and parents agreed to participate in the study
- 16/ 157 (14%) (6 CLD, 10 without CLD) children/ parents declined to participate
- 87/157 (56%) parents did not reply

## **2. Gwent Healthcare NHS Trust**

In order to obtain the desired number of prematurely born children to maintain the power of the study, 8 children (5 CLD, 3 without CLD) were recruited from Royal Gwent Hospital. Children who were born at  $\leq 32$  weeks of gestational age, who were admitted in the Special Care Baby Unit at the Royal Gwent Hospital were identified from the admission register. Letters of invitation was sent out by a Consultant Neonatologist (Dr S Sen) at Royal Gwent Hospital to the parents of 23 children (10 CLD, 13 without CLD) out of which 5 did not agree to participate, 9 agreed to participate and were recruited. The others did not reply.

### **Recruitment of subjects for the term control group:**

Four different sources were used to recruit term-born healthy children for the control population of the study.

#### **1. Siblings/ friends of the participants in the preterm groups**

All the parents of the prematurely born children who agreed for their children to participate in the study were asked if the child had a term-born sibling or friend between the ages of 8-12 who may want to participate in the study as a term control.

- 4 children in the term control group are siblings of children in the preterm groups
- 1 child in the term control group is a friend of a child in the preterm control group

#### **2. Children who attended General Paediatric and Orthoptic Clinics at University Hospital of Wales**

A research nurse (A Russell) attended the General Paediatric Clinics and Orthoptic Clinics at the University Hospital of Wales twice a week to identify children who were suitable for the

study. If the parents of these children were interested in the study, they were given the information pack for the study, which included information sheets for the parents, information sheets for the children and reply letter with a self-addressed envelope.

- 8 children in the term control group were recruited from the clinics.

### **3. Children of Staff working in University Hospital of Wales and Cardiff University**

Flyers were posted on various notice boards in the University Hospital of Wales and Cardiff University buildings at Heath site, inviting children between the ages of 8-12 years to participate in the study.

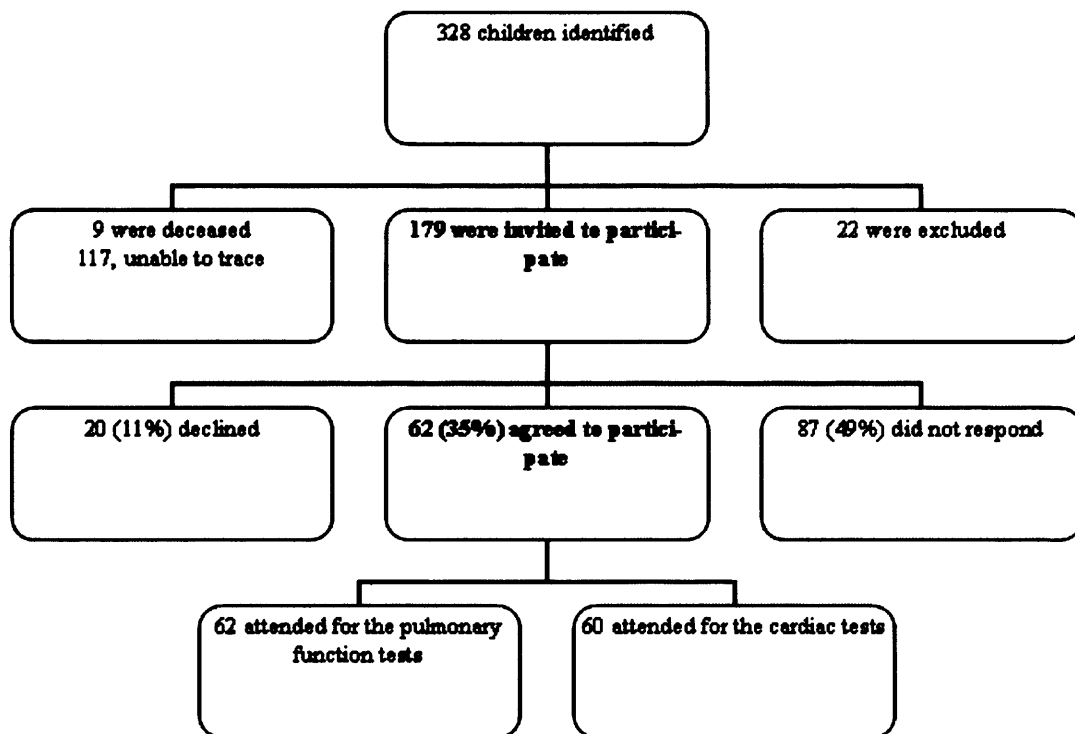
- 10 children in the term control group are the children of the staff from the University Hospital of Wales or Cardiff University, or the children of their friends and colleagues

### **4. Schools in Cardiff**

Electronic flyers were distributed to nine Primary and Secondary schools in Cardiff who agreed to post the flyer in their school websites.

- 7 parents responded to the flyer, out of which 5 participated in the study

Figure 2.1. Flow chart showing recruitment of prematurely born children



### **2.2.2 History and general examination**

For the prematurely born infants, their neonatal history including exposure to antenatal and postnatal steroids, surfactant therapy, gestational age at birth, birth weight, treatment with oxygen and mechanical ventilation, age and weight at discharge were all obtained from the hospital case notes and/or hand-held neonatal records. The birth weight and gestational age at birth for the term-born controls were obtained from the hand-held neonatal records. Case notes for 2 preterm children could not be traced.

The parents of all the participants completed a history proforma that included questions regarding family history of parental smoking, atopy, respiratory and cardiac conditions as well as the child's history of atopy, asthma, respiratory and cardiac conditions and self assessment of physical activity. The proforma for the medical history is in Appendix B.

### **2.2.3 Physiological measurements**

Height and weight were measured using a Stadiometer and a balance scale (Weylux, Model 424, UK). Blood pressure, pulse rate and oxygen saturation were measured using a Dynamap Procare monitor (GE Healthcare, UK). The children had 10-15 minutes rest prior to the measurement of these parameters.

### **2.2.4 Urinary cotinine**

A 10-20 ml urine sample was obtained in a sterile container. The urine sample was immediately divided into 3 individual containers and stored at -80° centigrade.

The samples were analysed in batches of 44. The urine samples were defrosted in the water bath at 36° centigrade before being analysed. The samples were tested for 'Urinary Cotinine', using Urine Cotinine Enzyme Immunoassay (Cozart EIA Cotinine Urine Kit, M155U1). Each sample was tested in duplicate using the following method;

- 10 µL of sample was added to each well
- 100 µL of Cotinine Enzyme was added into each well
- The 96 well plate with sample and enzyme was incubated at room temperature for 30 minutes
- The plate was washed four times with 300 µL of Wash Buffer
- 100 µL of Substrate Solution was added to each well and incubated for 30 minutes at room temperature
- 100 µL of STOP Solution was added to each well
- The absorbance was measured at 450 nm immediately

Urinary cotinine level was measured in 85 (92%) children to assess level of exposure to smoking. 6 children did not provide the urine sample.

### **2.2.5 Pulmonary function tests**

The 'Pulmonary function tests' were carried out in the 'Physiological Assessment Suite' at Glyntaff Campus, University of Glamorgan in Pontypridd. The study comprised of

- Baseline spirometry
- Measurement of alveolar diffusion capacity of carbon monoxide using a single breath helium dilution test
- Measurement of static lung volumes using whole body plethysmography
- Exercise test using bicycle ergometry
- Post exercise spirometry
- Reversibility test with inhaled  $\beta_2$  agonist (Salbutamol)

#### 2.2.5.1 Spirometry

##### Baseline spirometry

Prior to the spirometry tests, I explained and demonstrated the forced expiratory methods to the children. The children had the opportunity to practice the manoeuvre using an animated programme (Flow screen, Viasys Healthcare GmbH, Hoechberg, Germany) until they were comfortable with it and I was satisfied with the child's technique.

Baseline spirometry was carried out in a whole body plethysmograph (Masterscreen, Jaeger, Germany) (Figure 2.2). All the equipments were calibrated using the manufacturer's (Jaeger) instructions each morning after adjustment for ambient conditions. Volume and measures of flow rate were calibrated using a certificated 3 litre syringe, and gas concentrations set against certificated cylinders of known concentration. The equipment was serviced before the study began by the manufacturers.

The test was carried out with the child in sitting position and with nose clip in place and the body box door kept open. The subjects were instructed to place their lips around the

mouthpiece of the pneumotachometer so that the air did not leak around the mouthpiece when breathing. They were then instructed to take the deepest breath possible and blow out as hard and as fast as they could for as long as possible. The absolute values for FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub> and PEF along with % predicted values based on age, gender, and heights were calculated. Each child performed at least three to five spirometric tests and at least three reproducible manoeuvres as per the European Respiratory Society, ERS recommendations (Miller 2005). The reproducibility was assessed by visually observing the flow volume curves and also by ensuring that the differences in the FVC and FEV<sub>1</sub> between the manoeuvres were within 5%.

#### Post-exercise spirometry

Following the exercise test on bicycle ergometry as described in section 2.2.5.4, the children underwent a series of spirometry tests at 5 minutes, 10 minutes, 15 minutes, 30 minutes and 40 minutes after the exercise was stopped at peak  $\dot{V}O_2$ . At each stage, they performed at least three spirometric manoeuvres with at least two reproducible and acceptable tests.

#### Reversibility test

Inhaled bronchodilator (Salbutamol) was delivered as 4 doses of 100 microgram puffs using a paediatric aerochamber with a mask (Figure 2.5). The children were asked to take 10 normal breaths through the aerochamber in between the puffs. Spirometry was repeated soon after inhalation of the last dose of Salbutamol and after 15 minutes, to test for evidence of reversible airway disease. Reversibility was defined as improvement in FEV<sub>1</sub> of  $\geq 10\%$  after bronchodilator therapy (Quanjer 1993).



#### 2.2.5.2 Carbon monoxide diffusion capacity

A single breath method for measuring the diffusion capacity of lungs has been most widely used and validated. Both the ERS and the ATS documents for recommendations for a standard technique for measuring  $DL_{CO}$  (Cramer 1993; ATS 1995) propose the use of single breath method for this purpose. Therefore, I used the single breath method to study the alveolar diffusing capacity of lungs in the subjects in my study.

MS-PFT analyzer unit (Jaeger, Germany) was used to measure  $DL_{CO}$  in this study (Figure 2.3). The participants were seated in a comfortable chair in upright position. A nose clip was used in all participants. After 4-5 tidal breaths, the participants were instructed to expire to residual volume (RV) followed by deep inspiration to total lung capacity (TLC). As soon as they reached the maximal inspiration, they were asked to hold the breath at TLC for 10 seconds. The countdown was visible on the screen for the benefit of both the participant and the investigator. The children then continued expiration and tidal breathing until the analyser signalled the end of test.

All subjects were trained prior to the actual tests using the 'training mode' to avoid inhalation of carbon monoxide during the training period. The tests were started only when the subject and I were both satisfied with the technique. At least three consecutive tests were performed on all subjects with 4 minutes of interval between the manoeuvres to allow carbon monoxide to wash out and to avoid the carbon monoxide back-pressure due to formation of carboxyhaemoglobin. For the same reason, the tests were not repeated more than 5 times. The subjects were seated during the intervals in between the manoeuvres.

### 2.2.5.3 Static lung volumes

Static lung volumes were measured by whole body plethysmography method using a body box (Jaeger Masterscreen Plethysmograph, Jaeger, Hoechberg, Germany). The tests were explained to the child in detail including the fact that the door of the body box would be closed. The child was then seated in the chair inside the body box and the height adjusted so that the child's neck was not hyperextended or flexed while breathing through the mouth piece. The child was then instructed on how to breathe through the mouthpiece, and to breathe against the shutter when the shutter is closed. This manoeuvre was demonstrated by asking the child to breathe through one end of the mouthpiece while briefly closing the other end by hand to replicate shutter closure. Once the child and I were both satisfied that the child had understood the procedure, the door of the body box was closed. After 1-2 minutes of waiting time to maintain the temperature equilibrium inside the body box, the child was then asked to breathe normally at the rate of 15-20 breaths per minute. Once tidal volume breaths were maintained, specific airway resistances (SReff) and effective resistance (Reff) measurements were performed with at least 5 consecutive loops established. Immediately after the SReff measurements, the shutter was closed and the child was instructed to breathe in and out against the shutter to measure FRC. This was repeated at least 3 times to obtain at least 2 satisfactory tests. After the final occlusion, the child was asked to inspire maximally, followed by maximal expiration and then full inspiration to measure vital capacity (VC), total lung capacity (TLC) and residual volume (RV). The door of the body box was closed and the nose clip was used throughout the test.

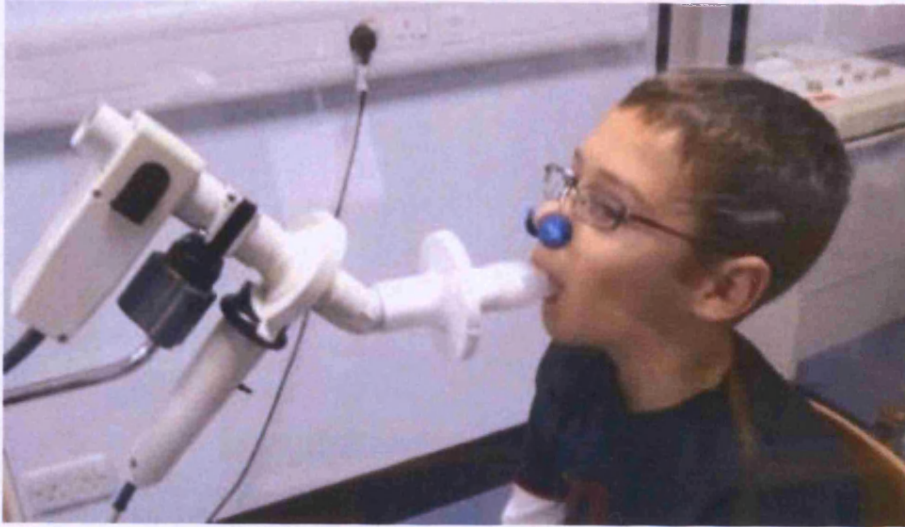
#### 2.2.5.4 Cardiopulmonary exercise testing

Maximal exercise capacity was measured using an incremental symptom limited bicycle ergometer test using electrically braked cycle ergometer (Jaeger, Germany) by a fellow researcher, T Powell and me (Figure 2.4). Pedalling was started on an unloaded cycle, and then every 3 minutes thereafter the load was incremented by 30W until volitional exhaustion or until the heart rate reached 80-90% of the predicted maximum ( $220 - \text{age in years}$ ). Pedalling cadence was held at approximately 60 rpm. Children were verbally encouraged to cycle as long as possible. Heart rate was monitored throughout the exercise test using a telemetric monitor (Polar t31, Polar Electro Inc, UK). The mouth-piece was connected to a computerised  $\text{O}_2/\text{CO}_2$  breath-by-breath analysis system (Jaeger MS-CPX, Germany). The test was considered to be acceptable if at least 2 of the following 4 criteria were met;

- Maximum heart rate  $\geq 80\%$  of predicted maximum
- Respiratory exchange ratio  $> 1$
- $\dot{V}\text{O}_2$  Plateau was reached
- Volitional exhaustion as assessed by Borg's scale

Peak oxygen uptake at peak exercise ( $\dot{V}\text{O}_{2\text{p}}$ ), carbon dioxide output ( $\dot{V}\text{CO}_{2\text{p}}$ ) and minute ventilation ( $\dot{V}_E$ ) were calculated as a mean from the final 15 seconds of exercise. Maximum voluntary ventilation (MVV) was calculated as baseline  $\text{FEV}_1 \times 35$  (Stein 2003). Ventilatory reserve (VR) was calculated as  $(1 - \text{peak } \dot{V}_E / \text{MVV}) \times 100\%$  (Medoff 1998).

Figure 2.2 Photograph of a child performing a spirometry test



A child inside the body-box performing a spirometry test

(A written parental consent and the assent from the child was obtained for the publication of this image)

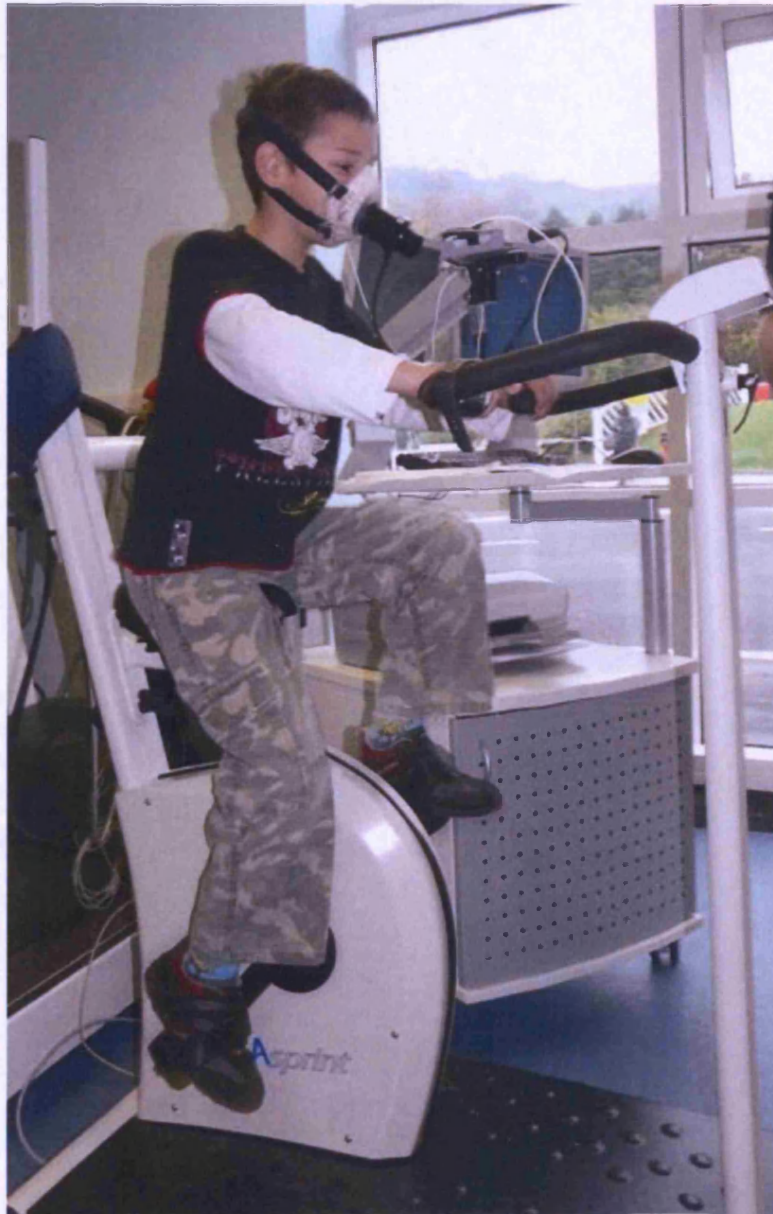
Figure 2.3 Photograph of a child performing a 'single breath test'



A child performing a 'Single-breath test' for measurement of alveolar diffusion capacity  
(A written parental consent and the assent from the child was obtained for the publication of  
this image)



Figure 2.4 Photograph of a child performing a 'cardiopulmonary exercise test'



A child performing 'cardiopulmonary exercise test' using a bicycle ergometer.

(A written parental consent and the assent from the child was obtained for the publication of this image)

Figure 2.5 Photograph of a child having bronchodilator medication using a spacer device



Salbutamol administered using a spacer device

(A written parental consent and the assent from the child was obtained for the publication of this image)

## **2.2.6 Statistical methods**

### **2.2.6.1 Sample size**

I aimed to study 90 subjects (30 CLD, 30 preterm controls and 30 term controls) to give 80% power and  $p < 0.05$  to detect 30% difference in FEV<sub>1</sub> between preterm children with CLD and term-born controls.

### **2.2.6.2 Statistical analysis of data**

Data are given as mean  $\pm$  standard deviation, SD. Differences between groups were tested by ANOVA with Tukey's posthoc analyses. Multiple linear regression modelling used haemoglobin corrected DL<sub>CO</sub>, total exercise time and ventilatory reserve as dependent variables and perinatal factors including gestational age, oxygen duration in the neonatal period and days of mechanical ventilation as explanatory variables and adjustments were made for age, gender and height or body mass index (BMI) as appropriate.  $p < 0.05$  was considered significant. SPSS 16.0 (SPSS Inc., Chicago, IL) was used to perform analyses.

## **2.2.7 Ethical approval**

'South East Wales Regional Ethical Committees' gave ethical approval for this study on 10 October 2007. (Reference: 07/WSE03/77)

Written informed consent from parents (Appendix F) and assent from children (Appendix G) were obtained prior to the study.



## **2.3 Results**

### **2.3.1 Baseline characteristics**

I studied 92 children, 29 CLD, 33 preterm control and 30 term-born controls with one CLD re-classified into the preterm control group after detailed review of the patient's neonatal records. Characteristics of the population studied are given in Table 2.1 and reported symptoms in Table 2.2. As expected, the CLD group was significantly more immature and had a lower mean birth weight than the preterm or term groups. They also received mechanical ventilation and oxygen therapy for longer. All infants in the CLD group had received exogenous surfactant compared to 45% in the preterm group and none in the term group. The weight at the time of lung function was significantly lower in the CLD group compared to the preterm and term groups but height and body mass index (BMI) were not statistically different between the groups.

Doctor diagnosed asthma and exercise-induced wheeze were reported more frequently in the CLD group compared to the preterm and term groups (Table 2.2). Whilst reporting of dry cough at night and current asthma treatment were greater in the CLD group, this difference did not reach statistical significance. Self-reported physical activity was significantly less in the CLD group compared to the other two groups.

Parents also reported the respiratory symptoms of the children by completing a questionnaire. 39% (24/62) of prematurely born children had been diagnosed with asthma at some stage in life. The prevalence was similar in preterm children with or without CLD (45% vs. 33%), but only 4/30 (13%) of the term born children had ever been diagnosed with asthma. Similarly, 42% (26/62) of preterm children had parents and/or siblings with asthma whereas family

history of asthma was reported in only 11% (3/30) term-born children. None of the term-born children were symptomatic within the 12 months prior to the study, whereas 31% (9/29) of children with CLD and 28% (9/33) of preterm children without CLD were symptomatic with some form of chronic respiratory symptom, and 10 ( 6, CLD and 4, preterm control) of them continued to use inhalers for asthma. Significantly more parents of the prematurely born children were reported to be smokers (41% CLD, 47% preterm control) compared to the term-born controls (11%).

Table 2.1. General characteristics of the subjects (Pulmonary function study)

Parameters	CLD (n=29)	Preterm control group (n=33)	Term group (n=30)	p-value
<b>Demographics</b>				
Male gender	17 (59%)	15 (46%)	15 (50%)	
Gestation (weeks)	27.3 (2.1)	30.0 (2.0)	39.8 (1.5)	§, §§
Birth weight (kg)	1.1 (0.4)	1.5 (0.4)	3.4 (0.5)	§, §§
Age (years)	10.2 (1.4)	10.3 (1.1)	10.5 (1.5)	
Height (cm)	137.1 (9.6)	140.9 (9.0)	143.2 (11.0)	
Weight (kg)	32.1 (8.2)	37.3 (10.9)	39.6 (13.7)	††
Basal metabolic index (kg m <sup>-2</sup> )	16.3 (3.2)	17.6 (3.8)	17.9 (4.6)	
<b>Antenatal and postnatal respiratory status</b>				
Antenatal steroids	24 (83%)	20 (61%)	None	
Postnatal steroids	9 (32%)	1 (3%)	None	‡
Surfactant	29 (100%)	15 (45%)	None	§
Supplemental oxygen (days)*	84 (30-410)	4 (0-61)	None	§
Assisted ventilation (days) *	28 (5-80)	3 (0-50)	None	§

Results are expressed in Mean (SD) unless otherwise specified, \*Median (range),

§ p<0.001 (CLD vs. preterm control), §§ p<0.001 (CLD vs. term), †† p <0.05 (CLD vs. term),

‡ p<0.01 (CLD vs. preterm control)

Table 2.2. Prevalence of parent reported respiratory symptoms and measured urine cotinine levels

	<b>CLD (n=29)</b>	<b>Preterm control group (n=33)</b>	<b>Term group (n=30)</b>	<b>p value</b>
Asthma ever (Doctor diagnosed)	13 (45%)	11 (33%)	4 (13%)	†
Current asthma on treatment (Past 12 months)	3 (10%)	4 (12%)	Nil	
Exercised induced wheeze (Past 12 months)	5 (18%)	2 (6%)	Nil	†
Dry cough at night (Past 12 months)	9 (31%)	9 (28%)	1 (4%)	
Eczema ever	7 (24%)	12 (36%)	9 (32%)	
Eczema (Past 12 months)	4 (14%)	6 (19%)	4 (14%)	
Family history of asthma	11 (38%)	12 (36%)	9 (32%)	
Family history of eczema	10 (35%)	12 (36%)	11 (39%)	
Family history of hay fever	9 (31%)	18 (55%)	16 (57%)	
Parental smoking	11 (41%)	15 (47%)	3 (11%)	‡
Self-reported physical activity per week (hours)*	2.0 (0-24)	3.0 (0-10)	3.5 (0-24)	††
Urine cotinine (ng/ml)*	11.3 (7.6-74)	19.6 (0-186.8)	12.3 (0-104.2)	

Results are expressed in Count (%) or Mean (SD) unless stated otherwise. \* Median (Range)

† Pearson's Chi square significance  $p < 0.05$ , ‡ Pearson's Chi square significance  $p < 0.01$ ,

††  $p < 0.05$  (CLD vs. preterm control, CLD vs. term)

### 2.3.2 Urinary cotinine

Mean urinary cotinine levels were 22.6, 38.7 and 19.6 nanograms/ml in the CLD, preterm control and term control groups respectively. Urinary cotinine is considered to be 'not detected' if the level is <10 nanograms/ml. Levels of 10-14 nanograms/ml is considered to signify low exposure, 15-40 nanograms/ml as moderate exposure and >40 nanograms/ml as heavy exposure to tobacco smoking. Taking this into account, the level of exposure to tobacco smoking in different groups are summarised in table 2.3

Table 2.3. Levels of exposure to tobacco smoking

	<b>Cotinine not detected</b>	<b>Low exposure to tobacco smoke</b>	<b>Moderate exposure to tobacco smoke</b>	<b>High exposure to tobacco smoke</b>
<b>CLD</b>	8 (28%)	14 (48%)	2 (7%)	5 (17%)
<b>Preterm control</b>	7 (22%)	4 (13%)	14 (44%)	7 (22%)
<b>Term control</b>	8 (30%)	11 (41%)	4 (15%)	4 (15%)

The mean difference of urinary cotinine levels were not statistically different between the groups although when split into different categories, more children in the preterm control group seem to have been exposed to moderate level of exposure to tobacco smoking than the other two groups.

### **2.3.3 Pulmonary function tests**

#### **2.3.3.1 Spirometry**

All reported results from the forced spirometry manoeuvre are the best out of 3 reproducible measurements. The measurements were considered to be reproducible if they were within 5% of each other. The best measurements for FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub> and PEF are reported, although they may be from different tests.

All 92 participants were able to perform the manoeuvre for baseline spirometry with at least 3 reproducible and acceptable measurements. As some of the children were unable to perform the exercise test successfully as mentioned in section 2.3.3.4, there are some missing data for the post-exercise spirometry series.

The results of the baseline spirometry are summarised in Table 2.4. Baseline FEV<sub>1</sub>, FEF<sub>25-75</sub> and PEF were significantly lower in the CLD group when compared to the preterm and term control groups. Although the preterm control group had lower values, they were not statistically different from the term group. 13 (45%), 6 (18%) and 1 (3%) respectively from the CLD, preterm control and term groups had a predicted FEV<sub>1</sub> of <80%.

Following maximal exercise, FEV<sub>1</sub> decreased from baseline by 10.6% (95% CI 6.6 to 14.7%,  $p < 0.012$ ) in the CLD group compared to 7.4% (4.1 – 10.6%,  $p = \text{ns}$ ) and 6.9% (3.5 – 10.4%,  $p = \text{ns}$ ) decrease in the preterm control and term control groups (Figure 2.6) respectively. After bronchodilator was given 45–60 minutes after exercise, post-exercise FEV<sub>1</sub> improved by 15.1% (95% CI 10.9 – 19.3%,  $p < 0.001$ ) in the CLD group and by 8.5% (4.4 – 12.6%,  $p = \text{ns}$ ) and 5.6% (2.4 – 8.7%,  $p = \text{ns}$ ) in the preterm control and term groups (Figure 2.6). After bronchodilator therapy, FEV<sub>1</sub> remained lower in CLD children when compared to preterm

(-6.2%, 95% CI -16.2 to 3.8%, p=ns) and term (-9.9%, -19.6 to -0.2%, p=0.043) groups.

FVC did not alter with bronchodilator therapy in the CLD group whereas it decreased in the control groups but remained >90% predicted in both the preterm and the term control groups.

Table 2.4 Results for the baseline spirometry

	<b>CLD</b> (n=29)	<b>Preterm control</b> (n=33)	<b>Term</b> (n=30)	<b>p value</b> (ANOVA)
<b>FEV<sub>1</sub></b>				
<i>Measured (L)</i>	1.5 (0.37)	1.8 (0.44)	2.1 (0.47)	
<i>% Predicted</i>	81.9 (12.6)	92.0 (14.2)	97.5 (11.6)	<0.001 <sup>‡, §§</sup>
<b>FVC</b>				
<i>Measured (L)</i>	2.2 (0.51)	2.3 (0.47)	2.5 (0.60)	
<i>% Predicted</i>	98.9 (1.1)	100.8 (11.3)	102.0 (2.8)	0.600
<b>FEF<sub>25-75</sub></b>				
<i>Measured (L)</i>	1.2 (0.44)	1.8 (0.85)	2.1 (0.64)	
<i>% Predicted</i>	49.2 (16.1)	69.2 (24.6)	80.0 (17.1)	<0.001 <sup>‡, §§</sup>
<b>PEF</b>				
<i>Measured (Lmin<sup>-1</sup>)</i>	2.9 (0.98)	3.6 (1.1)	4.2 (1.1)	
<i>% Predicted</i>	71.6 (19.5)	82.2 (17.5)	90.8 (16.7)	<0.001 <sup>§§</sup>

Results are expressed in Mean (SD).

<sup>‡</sup> p < 0.01 (CLD vs. preterm control), <sup>§§</sup> p < 0.001 (CLD vs. term control),

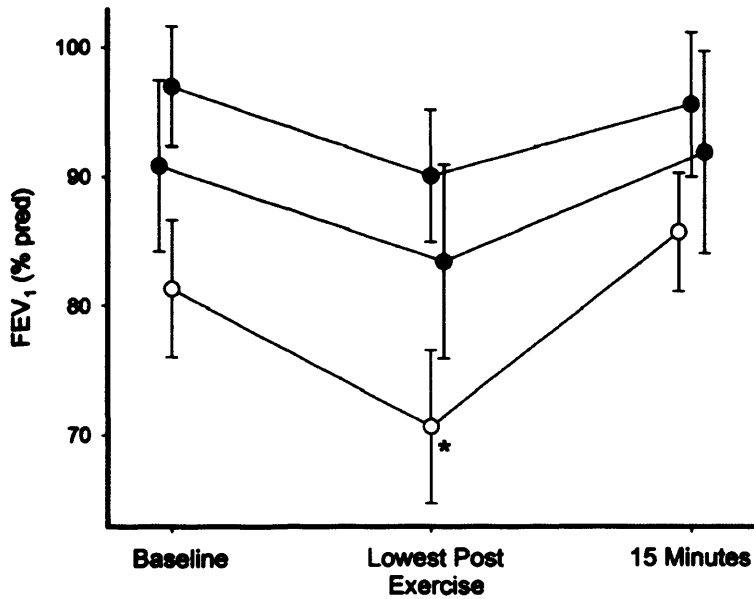


Table 2.5 Spirometry after exercise and bronchodilator therapy

Variables	Groups	Baseline	Post-exercise					Post-bronchodilator	
			5 minutes	10 minutes	15 minutes	30 minutes	40 minutes	0 minutes	15 minutes
FEV1 (%)	CLD	81.9 (12.6)	79.3 (14.1)	77.4 (14.1)	78.8 (14.1)	76.6 (14.6)	78.0 (13.0)	84.4 (11.7)	85.8 (11.5)
	PT	92.0 (14.1)	88.7 (16.4)	87.5 (17.3)	90.4 (14.3)	88.2 (15.7)	89.3 (16.1)	89.7 (17.3)	92.1 (17.1)
	Term	97.5 (11.7)	95.5 (12.9)	95.4 (13.6)	95.6 (12.1)	94.7 (12.9)	94.8 (12.8)	96.7 (12.4)	97.1 (13.8)
FVC (%)	CLD	98.9 (11.1)	94.4 (12.4)	92.6 (11.9)	94.8 (10.9)	92.2 (14.1)	92.7 (11.5)	93.6 (12.4)	94.0 (15.6)
	PT	100.8 (11.3)	98.6 (12.8)	98.3 (12.8)	99.0 (11.9)	97.0 (12.6)	97.4 (14.7)	97.5 (11.8)	97.3 (11.4)
	Term	102.0 (12.8)	100.9 (13.4)	100.0 (13.1)	99.5 (14.3)	99.8 (12.5)	99.3 (13.5)	99.6 (12.6)	99.2 (11.6)
PEF (%)	CLD	71.6 (19.5)	68.7 (17.5)	68.0 (17.6)	71.4 (18.8)	73.3 (20.6)	76.1 (20.2)	80.1 (21.1)	84.2 (20.1)
	PT	82.2 (17.5)	81.5 (19.6)	80.6 (19.8)	80.4 (19.70)	85.6 (22.3)	85.1 (23.7)	85.1 (23.9)	89.7 (24.4)
	Term	90.8 (16.7)	89.1 (19.2)	91.5 (18.0)	91.9 (17.0)	91.5 (18.9)	88.9 (23.6)	92.7 (20.5)	92.0 (23.4)
FEF <sub>25-75</sub> (%)	CLD	49.2 (16.2)	50.4 (22.2)	48.5 (19.7)	48.1 (19.0)	49.2 (21.3)	49.3 (20.1)	61.1 (23.4)	64.5 (22.8)
	PT	69.2 (24.6)	67.0 (26.9)	65.8 (25.1)	68.7 (26.0)	67.7 (28.3)	70.2 (28.2)	72.0 (28.0)	77.9 (28.1)
	Term	80.0 (17.1)	75.6 (21.7)	78.8 (19.3)	76.7 (16.3)	76.5 (18.1)	77.8 (20.7)	83.6 (20.5)	85.9 (24.2)

Results are expressed in Mean (SD) for % predicted values

Figure 2.6 Percent predicted FEV<sub>1</sub> at baseline, after exercise and 15 minutes after bronchodilator therapy



After exercise, FEV<sub>1</sub> decreased by 10.6% in the CLD group compared with 7.4% in the preterm and 6.9% in the term control groups. FEV<sub>1</sub> increased by 15.1% in the CLD group from the post-exercise FEV<sub>1</sub> but the increases of 8.5% and 5.6% were not significant in the preterm and term groups after bronchodilator therapy. \* p<0.12 and p<0.001 in the CLD group between the baseline and post-exercise FEV<sub>1</sub> and between the post-bronchodilator FEV<sub>1</sub> and post-exercise FEV<sub>1</sub> respectively.

Symbols: chronic lung disease, CLD (○), preterm controls (●), term controls (○)

### 2.3.3.2 Carbon monoxide diffusion capacity

Out of the 92 children who were recruited for the study, 'Single breath test' was performed on 87 children. The test could not be performed on 5 subjects due to equipment failure. Out of the 87 children who performed the 'Single breath test', 3 did not meet the acceptability criteria. Therefore, 84 children successfully completed the test.

The test was repeated a minimum of 3 times and a maximum of 5 times to document at least 2 acceptable measurements. Following the ATS guidelines, the average of the two most acceptable tests that meet the reproducibility requirement of  $\pm 10\%$  of the average DLCO are reported.

64 out of 92 subjects (70%) had their haemoglobin concentration measured using a finger prick method and analysed by a blood gas analyser (ABL 735, Radiometer, Copenhagen). The average haemoglobin was 13.8 (12.4 - 15.3) for CLD group, 13.6 (11.4 - 15.2) for the preterm control group and 13.1 (11.9 - 14.3) for the term control group. The average haemoglobin for each group of subjects was used to complete the missing data on 30% of the subjects who did not consent for the blood test.  $DL_{CO}$  was adjusted for haemoglobin using the equation recommended by the American Thoracic Society (ATS 1995); haemoglobin adjusted  $DL_{CO} = \text{observed } DL_{CO} (9.38 + \text{haemoglobin}) / 1.7 \times \text{haemoglobin}$ .

Haemoglobin adjusted  $DL_{CO}$  was significantly different between the groups [ $p < 0.05$  between CLD, 4.8 (95% CI, 4.5 to 5.2) mmol/min/kPa and preterm control group, 5.5 (95% CI, 5.1 to 5.6) mmol/min/kPa, and  $p < 0.05$  between CLD and term group: 5.5 (95% CI, 5.1 to 6.0) mmol/min/kPa]. However, predicted haemoglobin adjusted  $DL_{CO}$  was significantly decreased in the CLD children compared to the preterm group but not the term group. Thus, I used linear regression modelling to assess which factors including perinatal ones may have affected

haemoglobin adjusted  $DL_{CO}$ . Unsurprisingly, height ( $p < 0.001$ ), age ( $p = 0.002$ ) and gender ( $p = 0.001$ ) were significantly associated with a lower  $DL_{CO}$  as was duration of oxygen dependency ( $p < 0.05$ ) after adjustment for age, gender and height but not gestational age.

Table 2.6 Results of the 'Single breath test' for alveolar diffusion capacity

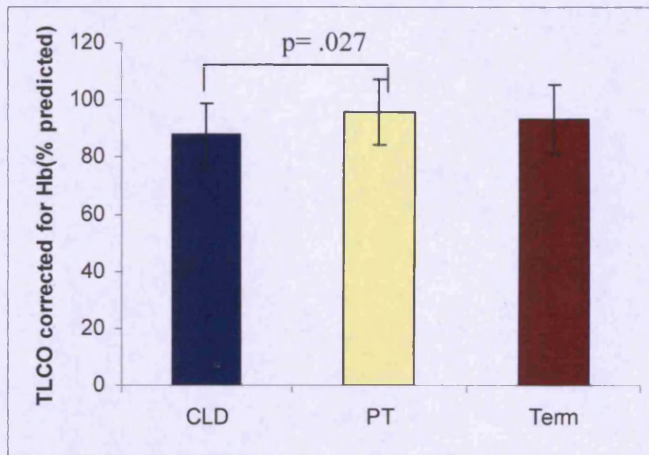
	<b>CLD</b> (n=26)	<b>Preterm control</b> (n=32)	<b>Term</b> (n=26)	<b>p-value</b> (ANOVA)
<b>DL<sub>CO</sub> (mmol<sup>-1</sup>min<sup>-1</sup>kPa)</b>				
<i>Measured</i>	4.9 ± 0.81	5.5 ± 0.97	5.5 ± 1.2	
<i>% predicted</i>	89.1 ± 10.8	96.3 ± 10.6	92.8 ± 12.7	0.059
<b>DL<sub>CO</sub> adjusted for Hb</b> (mmol <sup>-1</sup> min <sup>-1</sup> kPa)				
<i>Measured</i>	4.8 ± 0.81	5.5 ± 1.0	5.5 ± 1.2	
<i>% predicted</i>	88.0 ± 10.7	95.9 ± 11.3	93.5 ± 12.1	0.033 <sup>†</sup>
<b>KCO</b>				
<i>Measured</i>	1.8 ± 0.26	2.1 ± 0.25	2.0 ± 0.36	
<i>% predicted</i>	75.4 ± 11.4	90.1 ± 13.6	89.3 ± 15.8	<0.001 <sup>§, §§</sup>
<b>KCO adjusted for Hb</b>				
<i>Measured</i>	1.8 ± 0.27	2.1 ± 0.26	2.0 ± 0.35	
<i>% predicted</i>	74.6 ± 11.9	89.7 ± 13.9	90.0 ± 15.5	<0.001 <sup>§, §§</sup>
<b>VA (L)</b>				
<i>Measured</i>	2.8 ± 0.51	2.7 ± 0.45	2.8 ± 0.75	
<i>% predicted</i>	115.4 ± 17.3	106.5 ± 16.1	105.3 ± 17.5	0.064

Results are presented as Mean (SD)

<sup>†</sup> p<0.05 (CLD vs. preterm control)

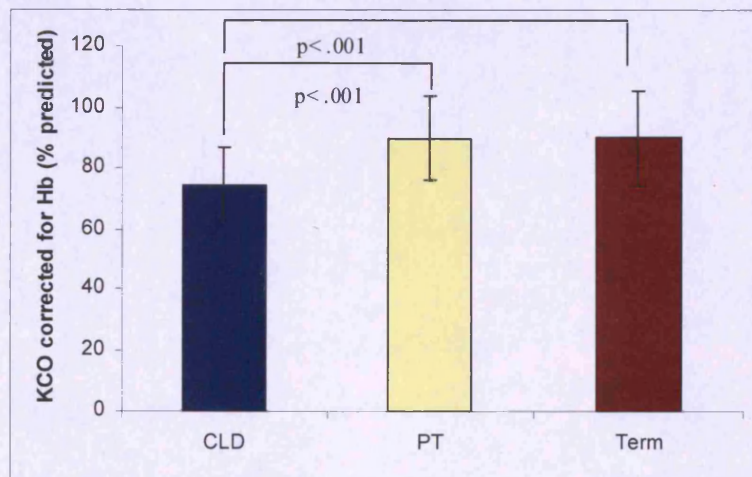
<sup>§</sup> p<0.001 (CLD vs. preterm control), <sup>§§</sup> p<0.001 (CLD vs. term)

Figure 2.7 Bar diagram showing differences in the percent predicted DL<sub>CO</sub> corrected for haemoglobin, between groups.



Data are presented as mean % predicted values and the error bars represent SD

Figure 2.8 Bar diagram showing differences in the percent predicted KCO corrected for haemoglobin, between groups.



Data are presented as mean % predicted values and the error bars represent SD

### 2.3.3.3 Static lung volumes

The resistance measurements are presented as a mean of 3-5 acceptable measurements. The measurements were included only if they were within  $\pm 10\%$  of the mean. Similarly, the reported lung volumes are also the mean of 2-3 acceptable measurements that are within  $\pm 10\%$  of the mean. The vital capacity (VC) manoeuvre was performed only once at the end of the test.

All 92 children who participated in this study underwent the whole body plethysmography test. Out of them, 1 child from the CLD group and 1 child from the term control group were unable to perform the manoeuvre correctly. Therefore, the following results are from the remaining 90 participants.  $FRC_{He}$ , which was measured using 'Single breath He test' while measuring the  $DL_{CO}$ , could be performed on only 87 children. Out of these, only 84 children were able to perform the manoeuvre satisfactorily.

Children with CLD had a significantly higher specific airway resistance ( $S_{Reff}$ ) than the children in the preterm control group (mean difference 61%, 95% CI 10% to 111%,  $p=0.014$ ) and the children in the term control group (mean difference 104%, 95% CI 52% to 156%,  $p<0.001$ ).

Functional residual capacity measured by plethysmography ( $FRC_{Pleth}$ ) and by single breath test ( $FRC_{He}$ ) was higher in the children with CLD compared to both the control groups. The difference in  $FRC_{Pleth}$  between the CLD and preterm control group was 21% (95% CI 5% to 37%,  $p=0.005$ ), and that between the CLD and the term group was 27% (95% CI 11% to 43%,  $p<0.001$ ). Similarly, the difference in the  $FRC_{He}$  between the CLD and the preterm control group was 21% (95% CI 11% to 43%,  $p=0.015$ ), and that between the CLD and the term control group was 24% (95% CI, 6% to 42%,  $p=0.006$ ).

Residual volume (RV) was higher in the children with CLD compared to both the control groups. RV was 28% higher in the children with CLD compared to the preterm control group (95% CI 4% to 53%,  $p = 0.020$ ) and 35% higher than the term control group (95% CI 9% to 60%,  $p=0.005$ ). Total lung capacity (TLC) and vital capacity (VC) were similar in all three groups of children, but RV: TLC ratio was significantly higher in the CLD group compared to the term control group (mean difference 0.08, 95% CI 0.02 to 0.13,  $p=0.002$ ).



Table 2.7 Results of the static lung volumes measured by body plethysmography

	<b>CLD</b> (n=28 )	<b>Preterm control</b> (n=33 )	<b>Term</b> (n=29 )	<b>p value</b> (ANOVA)
<b>SReff (kPa.sec)</b> <i>Measured</i>	1.4 (0.56)	1.0 (0.45)	0.78 (0.28)	
<i>% predicted</i>	253.4 (103.1)	192.6 (85.7)	149.4 (52.6)	0.001 <sup>†, §§</sup>
<b>Reff (kPa.sec.L<sup>-1</sup>)</b> <i>Measured</i>	0.69 (0.27)	0.58 (0.31)	0.45 (0.17)	
<i>% predicted</i>	243.3 (93.2)	210.5 (121.7)	171.0 (63.3)	0.021 <sup>††</sup>
<b>FRC<sub>pleth</sub> (L)</b> <i>Measured</i>	1.8 (0.54)	1.6 (0.31)	1.5 (0.37)	
<i>% predicted</i>	120.6 (38.7)	99.4 (16.0)	93.8 (18.3)	<0.001 <sup>†, §§</sup>
<b>FRC<sub>He</sub> (L)</b> <i>Measured</i>	1.6 (0.40)	1.4 (0.41)	1.4 (0.49)	
<i>% predicted</i>	114.6 (32.9)	93.9 (25.9)	90.5 (21.9)	0.004 <sup>†, ††</sup>
<b>FRC<sub>pleth</sub>: FRC<sub>He</sub></b>				
<i>Measured</i>	1.2 (0.49)	1.3 (0.72)	1.2 (0.49)	0.787
<i>% predicted</i>	1.1 (0.47)	1.2 (0.67)	1.1 (0.48)	0.743
<b>RV (L)</b> <i>Measured</i>	1.1 (0.43)	0.86 (0.31)	0.83 (0.26)	
<i>% predicted</i>	131.6 (55.6)	103.2 (32.4)	96.9 (29.7)	0.004 <sup>†, ††</sup>
<b>TLC (L)</b> <i>Measured</i>	3.1 (0.72)	2.9 (0.56)	3.1 (0.68)	
<i>% predicted</i>	104.9 (20.9)	96.7 (14.9)	99.0 (12.0)	0.144
<b>RV:TLC</b>				
<i>Measured</i>	0.34 (0.09)	0.29 (0.09)	0.27 (0.07)	0.003 <sup>††</sup>
<b>VC (L)</b> <i>Measured</i>	2.0 (0.45)	2.1 (0.41)	2.3 (0.55)	
<i>% predicted</i>	89.9 (12.3)	92.1 (12.7)	94.7 (13.4)	0.362

Results are presented as mean (SD),

<sup>†</sup> p<0.05 (CLD vs. preterm control), <sup>††</sup> p<0.05 (CLD vs. term)

<sup>‡</sup> p<0.01 (CLD vs. preterm control), <sup>‡‡</sup> p<0.01 (CLD vs. term), <sup>§§</sup> p<0.001 (CLD vs. term)

#### 2.3.3.4 Cardiopulmonary exercise tests

Out of the 92 children who attended for the pulmonary function tests, 4 children (2 CLD, 1 preterm controls, 1 term) were too short for cycle ergometry test. 8 children (3 CLD, 3 preterm controls and 2 term controls) did not meet the study criteria and were therefore excluded. Further 2 children (1 preterm control, 1 term) were unable to do the cardiopulmonary exercise test, CPET due to equipment failure and 2 children from the preterm control group missed the cardiopulmonary exercise test for other reasons (1 was temporarily on crutches and 1 missed the test due to time constraint). Thus, there are missing data for 16 children (5 CLD, 7 preterm controls and 4 term).

The results for the cardiopulmonary exercise tests are shown in Table 2.8. Baseline heart rate, respiratory rate and oxygen saturations were similar between the groups. Peak  $\dot{V}O_2$ ,  $\dot{V}CO_2$  and  $\dot{V}_E$  were similar in the three groups. Duration of exercise was decreased in the CLD group but failed to reach statistical significance ( $p=0.085$ ). Since a range of obstructive lung disease was observed in the CLD and preterm groups, I used linear regression modelling to assess if length of exercise was affected by perinatal factors. After adjusting for age, height and gender, only length of oxygen dependency was significantly associated with exercise duration. Maximum voluntary ventilation (MVV) and ventilatory reserve (VR) were both markedly decreased in the CLD group when compared to the preterm and term control groups (Figure 2.9A) with the latter being 25.8% (95% CI: 19.7 to 31.9) compared to 37.5% (32.2 to 42.8) in the preterm control and 43.7% (38.6 to 48.7) in the term control groups. Linear regression modelling was used to assess if the VR was associated with any perinatal factors including gestational age at birth, length of oxygen dependency and length of mechanical ventilation after correction for age, gender and height. VR was significantly associated with haemoglobin adjusted  $DL_{CO}$  and gestational age after correction for age, gender and height. The relationships between VR and gestational age and  $DL_{CO}$  are shown in Figure 2.9B and

2.9C. VR was also closely correlated to maximum respiratory rate after maximal exercise, peak  $\dot{V}O_2$  and with load per Kg at maximum exercise.

Table 2.8 Results of the 'cardiopulmonary exercise test'

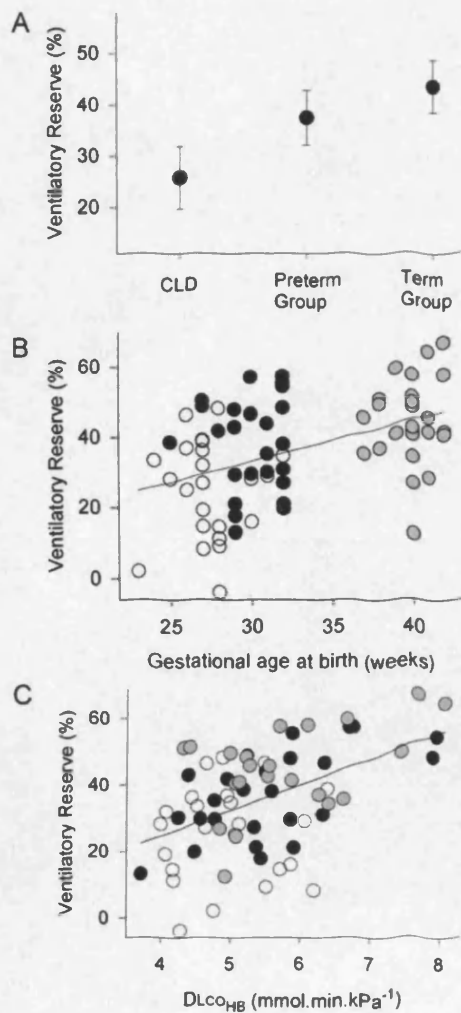
Variable	CLD (n=24)	Preterm control (n=26)	Term (n=26)	p value ANOVA
Baseline oxygen saturation (%)	99.1 (0.98)	99.5 (0.71)	99.2 (0.91)	0.178
Baseline respiratory rate (per minute)	25.3 (7.2)	27.4 (6.5)	25.6 (5.9)	0.412
Maximum respiratory rate (per minute)	53.8 (11.3)	52.5 (11.9)	48.1 (11.6)	0.189
Baseline heart rate (per minute)	80.5 (10.9)	77.5 (13.1)	77.9 (10.5)	0.553
Maximum heart rate (per minute)	175.3 (9.1)	178.7 (8.9)	171.4 (15.9)	0.090
Exercise duration (seconds)	554 (100)	614(133)	625 (120)	0.085
Load (Watts)	78.8 (17.3)	84.4 (23.1)	83.2 (20.9)	0.421
$\dot{V}O_2$ max (ml.kg.min <sup>-1</sup> )	35.4 (5.7)	34.9 (7.2)	31.1 (8.3)	0.073
$\dot{V}CO_2$ max (ml.kg.min <sup>-1</sup> )	37.2 (7.3)	36.9 (9.2)	32.6 (9.9)	0.125
RER	1.04 (0.06)	1.05 (0.09)	1.07 (0.06)	0.232
Peak VE (L min <sup>-1</sup> )	40.7 (7.8)	41.0 (8.6)	40.9 (8.2)	0.991
MVV (L min <sup>-1</sup> )	54.1(12.9)	64.7 (15.5)	72.0 (16.3)	<0.001 <sup>†, §§</sup>
VR (L min <sup>-1</sup> )	25.8 (14.4)	37.5 (13.2)	43.7 (12.6)	<0.001 <sup>†, §§</sup>
Borg's scale	16.2 (3.6)	14.6 (4.0)	15.6 (2.6)	0.311

Results are expressed in mean (SD)

<sup>†</sup> p<0.05 (CLD vs. preterm control), <sup>§§</sup> p<0.001 (CLD vs. term control),

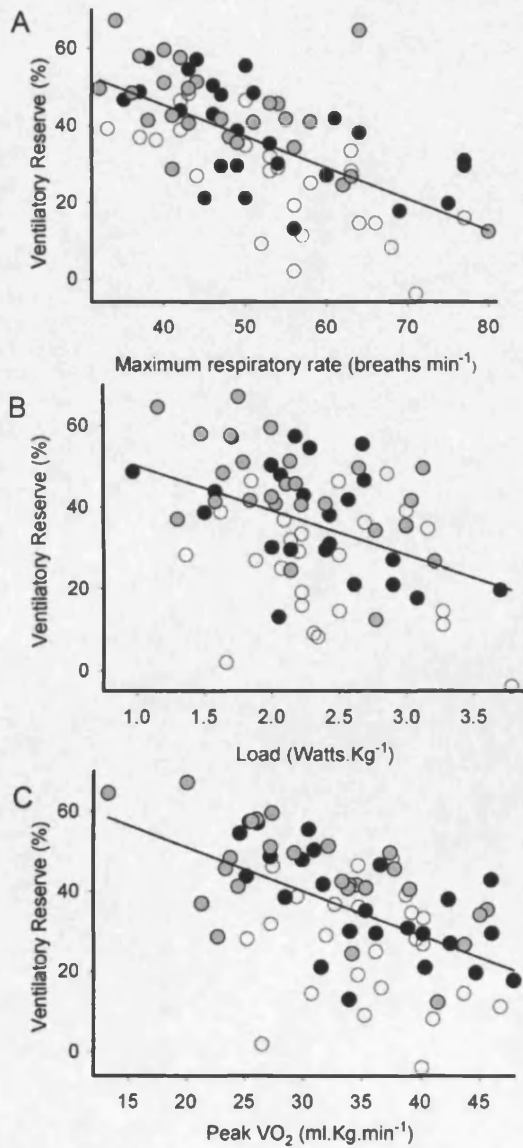
<sup>‡</sup> p< 0.01 (CLD vs. preterm control)

Figure 2.9 Scatter plot showing ventilatory reserve in different groups and its relationship with gestational age and alveolar diffusion capacity



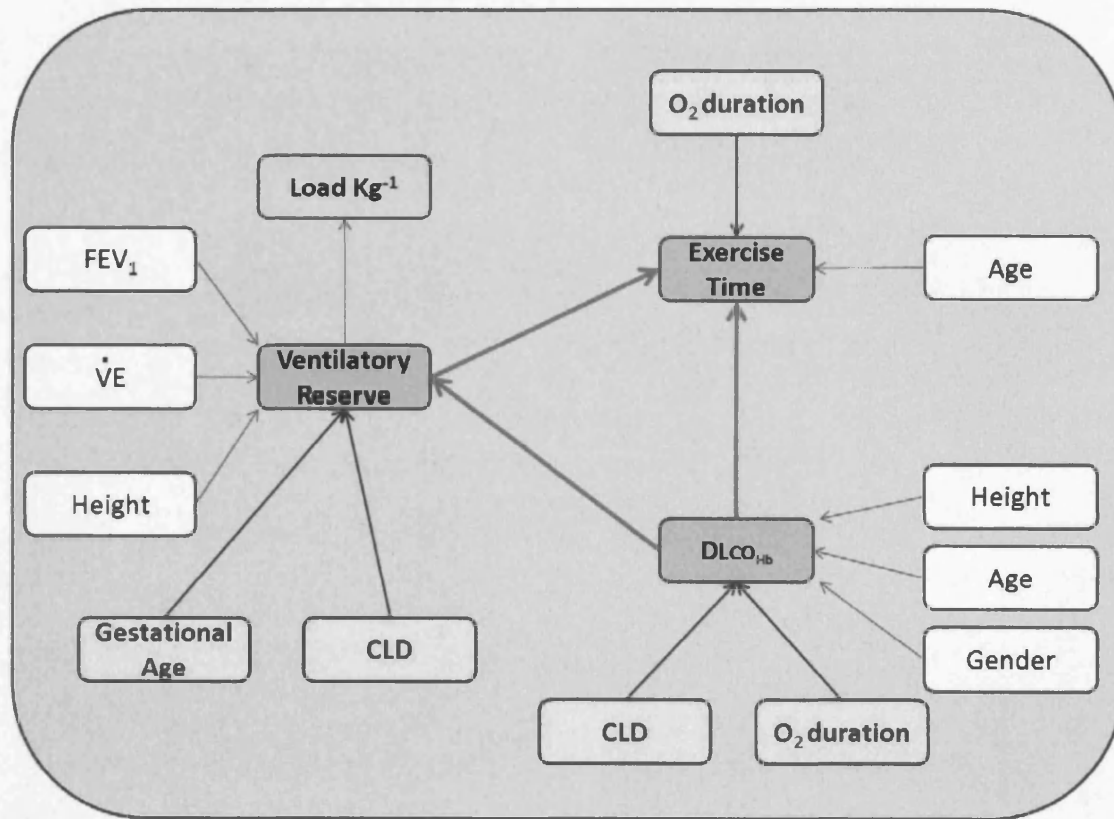
(A) Mean  $\pm$  95% confidence intervals for ventilatory reserve (VR) are shown for subjects with chronic lung disease, preterm group and term controls.  $p < 0.01$  (CLD vs. preterm control) and  $p < 0.001$  (CLD vs. term). Relationships are shown between ventilatory reserve and (B) gestational age ( $r^2 = 0.45$ ,  $p < 0.001$ ) and (C) haemoglobin adjusted  $DL_{CO}$  ( $r^2 = 0.49$ ,  $p < 0.001$ ). Symbols: chronic lung disease, CLD ( $\circ$ ), preterm ( $\bullet$ ), term controls ( $\circ$ )

Figure 2.10 Scatter plot showing relationship between ventilatory reserve and maximum respiratory rate during exercise, maximum load and peak  $\dot{V}O_2$



Relationships are shown between ventilatory reserve and (A) maximum respiratory rate during peak exercise ( $r^2=0.62$ ,  $p<0.001$ ), (B) load at peak exercise ( $r^2=0.42$ ,  $p<0.001$ ), and (C) peak  $\dot{V}O_2$  ( $r^2=0.54$ ,  $p<0.001$ ). Symbols: chronic lung disease, CLD ( $\circ$ ), preterm ( $\bullet$ ), term controls ( $\ominus$ )

Figure 2.11 A proposed model showing relationships between ventilatory reserve, alveolar diffusion capacity and exercise duration with other lung function parameters and possible influences of perinatal factors



Abbreviations: CLD = chronic lung disease, FEV<sub>1</sub>= forced expiratory volume in 1 second,  $\dot{V}_E$  = minute ventilation, O<sub>2</sub> duration = duration of oxygen dependency in infancy.

## **2.4 Discussion**

In this study I have comprehensively studied each child with and without CLD of infancy for their symptoms, lung function, exercise capacity as well as effect of exercise and bronchodilator on airway obstruction. I also assessed the possible effects of perinatal factors on  $DL_{CO}$ , ability to exercise and on ventilatory reserve. I report increased diagnosis of asthma and marked airway obstruction in children with CLD; decreased self-reported physical activity and decreased markers of large and small airway function. Air-trapping was still evident at 8–12 years of age. Furthermore,  $DL_{CO}$  was decreased and although peak  $\dot{V}O_2$  was similar between the groups; this was at the expense of the children who had CLD in infancy using much greater ventilatory reserve (VR). VR was related closely to  $DL_{CO}$ , maximum respiratory rate during exercise and with peak  $\dot{V}O_2$ . Most interestingly, perinatal factors seem to affect several parameters either directly or indirectly: oxygen duration in infancy seem to have an effect on  $DL_{CO}$  and on duration of exercise whilst gestational age appeared to affect ventilatory reserve (Figure 2.11).

Children born preterm are known to have higher incidence of respiratory illness during infancy and early childhood. In my study group, 45% of the CLD group and 33% in the preterm group had been diagnosed with asthma in the past compared to only 4% in the term group. By 8-12 years of age, only 10% of children with CLD and 12% children born preterm but none in the term group were on medication for asthma. However, the prevalence of respiratory symptoms associated with asthma such as exercise-induced wheeze and night cough were still higher in the CLD population compared to the term controls (31% vs. 4%); this is likely to reflect small airway obstruction as shown by spirometry in this group of children.



41% of parents in the CLD group and 47% parents in the preterm group admitted to smoking regularly, in comparison to only 11% of parents in the term group. However, there was no statistically significant difference in the urine cotinine levels in the subjects between groups and no correlation was noted between the urine cotinine level and the percent predicted FEV<sub>1</sub>. Thus, it is unlikely that passive smoking may have influenced differences in the lung function between children in the different groups in this study. Our lung spirometry data for the CLD group, in which children had received surfactant as an infant, was similar to that reported in the pre-surfactant era (Bader 1987; Northway Jr 1990; Gross 1998; Smith 2008) showing FEV<sub>1</sub> and FEF<sub>25-75</sub> at the lower end of normal at 81.9% of predicted values. I am unable to explain why FVC decreased with bronchodilator in the control group but FVC remained within normal range in this group at rest, after exercise and after bronchodilation. Therefore, this is unlikely to be of clinical consequence. RV and RV as a proportion of TLC both were increased in the CLD group suggesting on-going airway trapping even for 8–12 years of age. It is unclear why the term group had low predicted values for FEF<sub>25-75</sub> at 80% but the relative differences with the CLD group were significantly different. The primary aim of this study was not to measure the airway resistance. However, I have reported these results as they were available as a part of the whole body plethysmography. The methodology I used for the whole body plethysmography is not the optimal way of measuring airway resistance and this is reflected in the wide variation in the results including high airway resistance in the control population as well. Therefore, this data should be interpreted with caution.

In this study, haemoglobin corrected DL<sub>CO</sub> was lower in the CLD group compared to both the preterm and term groups but predicted values were lower in the CLD compared to the preterm group but not the term infants. I, therefore, used linear regression modelling to assess which perinatal factors, after adjustment for age, gender and height may be important in its value and noted that duration of oxygen adversely affected its value. This is perhaps not surprising

as oxygen therapy is likely to affect the gas exchange apparatus. My data is in keeping with the results from infants studied by Balinotti's group (Balinotti 2010), the 8-year-old ex-preterm subjects in the EPICure cohort (Welsh 2010) and 19-year-old cohort from the Dutch study (Vrijlandt 2006). Thus, there is evidence of limitation in alveolar-capillary gas exchange in the ex-preterm children with CLD. Narang and colleagues have measured  $DL_{CO}$  combined with effective pulmonary blood flow ( $\dot{Q}_{PEFF}$ ) at rest and during exercise in preterm born young adults from the pre-surfactant era (Narang 2009). The investigators demonstrated that  $DL_{CO}$  was reduced in the preterm group compared to the control population; this normalised during exercise. The investigators suggested that the rise of  $DL_{CO}$  during exercise may be a mechanical consequence of increased  $\dot{Q}_{PEFF}$  and not a true increase.

For the exercise tests, the peak  $\dot{V}O_2$  was lower in the CLD but the differences to the preterm and term groups was not significant ( $p=0.073$ ). Similarly, peak  $\dot{V}CO_2$  and peak  $\dot{V}_E$  did not differ between the groups. However, there were significant differences in the self-reported hours of physical activity per week between the CLD and control groups. I thus, modelled the data to assess which perinatal factors may affect duration of cycle ergometry and noted that duration of neonatal oxygen therapy and  $DL_{CO}$  were important factors after correction for age, gender and height. In view of the explicit airway obstruction of both the large and small airways in the CLD group, and a drop of 11% in  $FEV_1$  after exercise, the variously reported studies' (Mitchell and Teague 1998; Vrijlandt 2006; Smith 2008; Narang 2009) failure to show limitation of exercise capacity in this group is perhaps surprising. Most interestingly, however, I noted that both MVV and ventilatory capacity were markedly decreased in the CLD group with intermediate values noted in the preterm group compared to the term controls. Thus the CLD and preterm groups were able to exercise similarly to the term infants but at the expense of using greater proportion of their ventilatory reserve capacity. Clearly VR

at maximum exercise is dependent on peak  $\dot{V}_E$  and FEV<sub>1</sub> by definition but after correction for age, height and gender, DL<sub>CO</sub> and gestational age were both significantly associated with VR at maximum exercise.

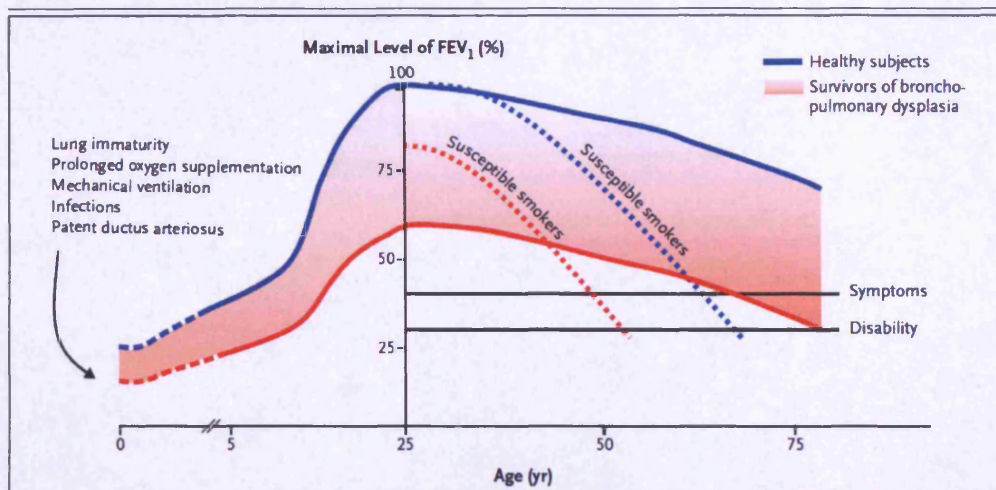
Taken together these data appear to demonstrate that several perinatal factors affect DL<sub>CO</sub>, duration of exercise and VR. In particular, duration of oxygen or CLD affected DL<sub>CO</sub> and duration of exercise; and gestational age and CLD appear to affect VR. Using these data, I have proposed the model shown in Figure 2.11 showing the effects of perinatal factors on important elements that may affect exercise capacity in children who had CLD in infancy.

During this study, we also assessed both the effect of maximum exercise on FEV<sub>1</sub> and the response to bronchodilator after 45 minutes of rest after exercise. I noted a drop in FEV<sub>1</sub> of 11% after exercise in the CLD group but <10% in the two control groups. After exercise, the response to bronchodilator was greater in the CLD group suggesting under-diagnosed exercise induced bronchoconstriction which clearly is an area that needs further study and intervention if confirmed by other studies. If >30% prematurely born children smoke when they are young adults as reported by previous studies (Doyle 2006, Halvorsen 2004), the increased rate of decline of FEV<sub>1</sub> due to smoking may make CLD a major risk factor for chronic obstructive pulmonary disease in these patients (Baraldi 2007, Figure 2.12).

In conclusion, 8-12 year old children with CLD in infancy have on-going pulmonary abnormalities including a greater incidence of doctor diagnosed asthma, exercise induced bronchoconstriction and a greater response to bronchodilation than do preterm or term controls. Furthermore, these children have on-going airflow limitations and also abnormalities of gas exchange. Although they reached similar exercise load to control children, this was at the expense of using greater ventilatory reserve. Finally, several perinatal factors including

gestational age and oxygen duration seem to have a direct effect on several parameters of lung function.

Figure 2.12 Theoretical model of changes in FEV<sub>1</sub> in survivors of CLD and healthy subjects according to age



**Figure 4. Theoretical Model of Changes in FEV<sub>1</sub> in Survivors of Bronchopulmonary Dysplasia and Healthy Subjects According to Age.**

Theoretical curves are shown for the forced expiratory volume in 1 second (FEV<sub>1</sub>) in healthy subjects and survivors of bronchopulmonary dysplasia. Survivors of bronchopulmonary dysplasia may have variable airflow limitation from the first years of life, with little evidence of "catch-up" growth in lung function. In some of these patients, FEV<sub>1</sub> does not reach the normal maximal value in early adulthood, and the phase of declining FEV<sub>1</sub> values starts from a substantially reduced maximal value. Whether the rate of decline with advancing age will parallel that among healthy persons or will be accelerated is not known. The dashed lines represent the potential effect of smoking on the rate of decline of FEV<sub>1</sub> in susceptible subjects. Values for FEV<sub>1</sub> in the first 3 years of life are extrapolated from measurements of maximal flow at functional residual capacity. Adapted from Fletcher and Peto.<sup>62</sup>

(Baraldi 2007)

## **Limitations**

### Recruitment

The prematurely born subjects in this study were traced from the contact details of the children at the time of their birth and their current hospital records. As it would have been insensitive to contact the parents of these children without ensuring that their child is alive and well, I did not contact any child/ parent for whom the current General Practitioner could not be traced. Thus, I was unable to trace 117/328 (36%) of the preterm children who were originally identified. 49% of those contacted (87/179) i.e. 27% (87/328) of the initially identified population did not respond to the 'letter of invitation' (Figure 2.1). Only one letter was sent to the parents/ guardians inviting the children to take part in the study, as advised by the ethics committee. The reasons for non-response for these children are unknown. Both the above caveats may have caused unintentional and unavoidable selection bias.

### Sample size

Power calculations for this study were done to assess evidence of increment in pulmonary arterial pressure in response to acute hypoxia. The power calculation for the pulmonary function study was post-hoc. This is one of the limitations of the study.

### Pulmonary function study

As the pulmonary function studies and the cardiovascular studies were conducted in separate sites, the subjects were invited to attend for the pulmonary function study and the cardiovascular study on separate days. This meant that all aspects of the pulmonary function tests had to be accommodated on a single sitting and therefore, the study protocol had to be compromised in the following aspects:

- Ideally, a multiple breath test to study  $FRC_{He}$  in order to assess  $FRC_{Pleth}$ :  $FRC_{He}$  would have been extremely useful to assess evidence of air trapping in children with CLD.

However, the study was designed to perform a 'single breath test' to assess alveolar diffusion capacity. Due to the time constraint it was not practical to perform both the single breath and the multiple breath tests on the same day.

- Similarly, it would have been ideal to assess exercise induced bronchoconstriction and the reversibility test on separate occasions. As both these tests had to be performed on the same day, I have presented the findings as 'change in FEV<sub>1</sub> from baseline to after exercise' as a measure of exercise induced bronchoconstriction and 'increase in FEV<sub>1</sub> from its lowest point after exercise to 15 minutes after Salbutamol inhalation' as a measure of response to bronchodilator.

## **Chapter Three: Reproducibility of Myocardial Velocity and Deformation Imaging**

### **3.1 Introduction**

Historically, it has not been easy to quantify right ventricular function using non-invasive methods such as 2-D and Doppler echocardiography. In recent years, myocardial velocity imaging (MVI) or tissue Doppler imaging (TDI) has been validated and widely used in the adult population for diagnosis of left ventricular (LV) and right ventricular (RV) dysfunction. MVI is an echocardiographic technique which directly measures the velocities of myocardial motion during systole and diastole. This has been suggested as a potentially useful diagnostic tool for quantitative assessment of myocardial function in infants (Mori 2004) and children as well (Kapusta 2000; Mori 2000). Certain tissue Doppler parameters such as RV relaxation time 'IVRT' (Dambrauskaite 2005; Lindqvist 2006; Brechot 2008) and RV systolic strain (Dambrauskaite 2007; Lopez-Candales 2008; Rajagopalan 2008) have been validated as useful markers for estimation of pulmonary arterial pressure in adults. Regional myocardial strain has also been shown to be feasible in healthy term infants (Nestaas 2007; Pena 2009).

The feasibility and reproducibility for off-line tissue Doppler measurements in adults has been extensively studied by the MYDISE study group (Fraser 2003). This has not yet been tested or validated in prematurely born infants or children. Limited data on children have suggested that reproducibility for measurement of myocardial velocities in children are acceptable (intra and interobserver variability <10%) (Pauliks 2005) but there is lack of data on reproducibility of parameters such as isovolumic acceleration (IVA), deformation index, strain and isovolumic relaxation time 'IVRT' in children. In adult studies, coefficient of variation for parameters such as relaxation time was >10% (Lindqvist 2006) and for IVA >20% (Margulescu 2010). Thus, understanding the feasibility and reproducibility for these echocardiographic parameters was vital prior to using these methods for assessment of myocardial function in children with CLD for this thesis. I had the opportunity to study the



reproducibility of myocardial velocity and deformation imaging in great detail with a separate ongoing research on the neonatal population, which I shall describe in this chapter.

In this study, I assessed the intra and interobserver variability for off-line analysis of longitudinal systolic and diastolic myocardial velocities, myocardial strain, annular displacements, and isovolumic acceleration compared with conventional blood pool Doppler parameters such as mitral inflow velocities and pulmonary arterial acceleration time.

### **3.2 Methods**

Feasibility and reproducibility of myocardial velocity and deformation indices was established using infants who were recruited for a separate study on ‘myocardial velocity imaging in term and preterm infants’. Thirty healthy term infants ( $\geq 38$  weeks of gestational age) and 25 preterm infants ( $\leq 34$  weeks of gestational age) were recruited from the post-natal wards and the Special Care Baby Unit of University Hospital of Wales. Written informed consent was obtained from parents prior to recruitment. The South East Wales Regional Ethics Committee gave ethical approval for this study.

All infants had their first scan within the first 72 h after birth. The infants were screened for congenital cardiac defects and those with structural cardiac defects were excluded from the study, with the exception of patent ductus arteriosus. A second scan was performed a month later. Images were acquired using a commercially available system (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway) with a 7.0 or 5.0 MHz transducer.

We selected a random sample of 16 studies for the reproducibility study, by drawing lots: 8 out of 30 studies from term infants and 8 out of 25 studies from preterm infants. The results presented for the reproducibility of echocardiographic indices are from these 16 scans.

In order to establish the reproducibility of myocardial velocity and strain indices, myocardial velocity loops acquired from 16 infants were analysed by four observers including myself (S Joshi, research fellow, JM Edwards, research sonographer, DG Wilson, consultant paediatric cardiologist and JK Wong, paediatric cardiology staff grade) for interobserver variability, and re-analysed after 6 months by myself for intraobserver variability. Images were analysed using commercially available EchoPAC software. Observers were blinded to the clinical status of the infants.

Intra and interobserver variability were assessed for thirteen parameters including 4 blood Doppler parameters: LV VTI (Figure 4.4 B), mitral E and A velocities (Figure 4.5), and pulmonary arterial AT (Figure 4.9) and 9 tissue Doppler parameters from LV and RV images: annular displacement (Figure 4.13), myocardial systolic, early and late diastolic velocities (Figure 4.12), RV relaxation time (Figure 4.11), QRS to peak systolic time interval (Figure 4.12), isovolumic acceleration (Figure 4.12), myocardial systolic strain at the apical and the basal segments (Figure 4.14). All parameters were measured in 3 beats and averaged, except if the signal from an individual beat was too noisy to be analysed in which case 1 - 2 beats were measured. The details of the methods and results of this study have been published in the paper, 'Reproducibility of myocardial velocity and deformation imaging in term and preterm infants' (Appendix H2). The methodologies for off-line analysis of the individual parameters are described in section 4.2.3.5.

The echocardiographic equipment used for acquiring the images, the equipment used for post-processing of the tissue Doppler images and the methodology used for acquisition and measurement of echocardiographic indices were similar to that for the study carried out on 8-12 year old children studied for this thesis (Chapter 4).

### **Statistical methods**

The sample size of 16 scans observed by four observers for the interobserver reproducibility gave 48 degrees of freedom [number of studies  $\times$  (number of observers-1)], and the power to demonstrate differences with 95% confidence intervals estimated at  $0.4 \times$  standard deviation (SD).

Intra and interobserver reproducibility were reported as coefficients of variation (CVs, in %), calculated using the formula:  $CV = (SD/\text{arithmetic mean of measurements}) \times 100$ , where SD is the standard deviation of residuals (measurement 1 - measurement 2). As there were four observers, there were six pairs of data for comparison (each derived from repeated analysis of the 16 patients); the interobserver reproducibility of each variable is therefore expressed as a pooled CV, which is the mean value of the six comparisons. Intraobserver variability is also presented as CV for each variable.

## **3.3 Results**

### **Feasibility**

Most pulsed and tissue Doppler parameters could be measured in 90% of neonates. A single diastolic velocity at the mitral valve was noted in 5% (1 infant), and using tissue Doppler in

31% (5 infants) at the LV, 20% (3 infants) at the RV, and 12% (2 infants) at the septum. In these cases, a single diastolic velocity was measured and noted as early diastolic velocity (E or Ve'). The fusion of early and late diastolic velocities may be due to rapid heart rates in infants. Missing values for the myocardial velocity occurred because a segment could not be imaged or because the traces were of the poor quality. The feasibility for measuring LV apical strain was only 75%.

### **Reproducibility**

The mean values for all parameters, and the results of coefficients of variation (CV) for both intra- and interobserver variability, are summarized in Table 3.1.

#### Indices for assessment of LV function

Mitral annular early diastolic velocity by pulsed tissue Doppler (Ve') had reproducibility of 7-13% (intraobserver) and 9% (interobserver). Intra- and interobserver variability for LV myocardial systolic and diastolic velocities as well as annular displacements, obtained by processing stored digital loops, were between 10 and 15% except for the diastolic velocity (Va) (20% interobserver). More advanced parameters such as QRS to Vs time interval ( $T_{Q-Vs}$ ) and LV strain had intraobserver CVs of 15%, but interobserver reproducibility for these parameters was sub-optimal at CVs 35%. LV IVA was not reproducible (CVs 30-60%).

In comparison, blood Doppler parameters such as LV velocity time integral and mitral inflow Doppler had CVs of 3-6% for both intra- and interobserver variability.

### Indices for assessment of RV function

Intraobserver CVs for the RV indices were generally higher compared with the corresponding LV indices. Intra and interobserver CVs for the RV myocardial systolic and diastolic velocities, annular displacement, and QRS to V<sub>s</sub> time interval (T<sub>Q-V<sub>s</sub></sub>), were between 15 -19% and 18 -24%, respectively. IVA was poorly reproducible in the RV, with CV of 40%.

### Indices for assessment of pulmonary arterial pressure

Pulmonary arterial acceleration time, which has been traditionally used to estimate pulmonary arterial pressure, had CV of 12- 15%. Tissue Doppler parameters that are relatively new methods of indirect estimation of pulmonary arterial pressure were less reproducible. CV for tricuspid annular IVRT was 16% (intraobserver) and 25% (interobserver), and that for RV basal strain was 19% (intraobserver) and 25-37% (interobserver).

Table 3.1 Reproducibility of echocardiographic indices

	Intraobserver variability CV %	Interobserver variability CV%, pooled mean (SD)
VTI (cm)	3.6	6.0 (1.9)
Mitral E (m s <sup>-1</sup> )	2.5	2.8 (0.4)
Mitral A (m s <sup>-1</sup> )	5.5	4.5 (0.8)
PA AT (ms)	11.9	15.2 (2.9)
Displacement (mm)		
LV Ds'	6.2	12.2 (3.4)
RV Ds'	12.8	18.2 (4.6)
Mitral annular velocity (cm s <sup>-1</sup> )		
Lateral Ve'	12.6	9.4 (4.2)
Medial Ve'	6.9	9.2 (3.9)
Myocardial velocity (cm s <sup>-1</sup> )		
LV		
Vs (bl)	9.8	14.3 (1.8)
Ve (bl)	11.1	13.5 (1.9)
Va (bl)	9.9	20.3 (4.7)
RV		
Vs (bl)	17.7	18.3 (4.5)
Ve (bl)	15.0	23.3 (8.4)
Va (bl)	18.5	24.1 (7.8)
Timing (ms)		
RV relaxation time 'IVRT'	16.1	27.0 (8.8)
T <sub>Q-Vs</sub>		
LV	13.3	34.6 (18.3)
RV	9.4	22.2 (12.3)
Isovolumic acceleration (ms <sup>-2</sup> )		
LV	62.5	46.7 (7.6)
RV	29.3	60.3 (13.3)
Strain (%)		
LV		
Ssbl	14.0	43.0 (13.9)
Ssal	17.1	36.9 (15.7)
RV		
Ssbl	19.0	37.1 (11.5)
Ssal	23.9	25.3 (1.9)

SD, standard deviation; CV, coefficient of variation; VTI, left ventricular outflow tract velocity time integral; PA AT, pulmonary arterial acceleration time; LV, left ventricle; RV, right ventricle; Ve', annular early diastolic velocity; Vs, myocardial systolic velocity; Ve, myocardial early diastolic velocity; Va, myocardial diastolic velocity during atrial contraction; IVRT, right ventricular isovolumic relaxation time; T<sub>Q-Vs</sub>, QRS to Vs time interval; Ss, systolic strain; bl, basal segment of lateral wall; al, apical segment of lateral wall; bs, basal segment of septal wall; ms, mid-septal segment.

### **3.4 Discussion**

#### Feasibility of tissue Doppler in infants

Due to RV dominance in newborn infants, the acquisition of images from the LV, without missing the apex and while retaining a narrow sector of the image and thus a high frame rate, was a challenge. This accounts for the fact that LV apical strain had the most missing values (37%). The other tissue Doppler parameters measured in this study were not similarly affected as they were all measured either at the annulus or in the basal segments.

#### Reproducibility of off-line analysis of tissue Doppler measurements

As anticipated, pulsed Doppler recordings of blood flow were more reproducible than myocardial velocities. The reproducibility for measuring the acceleration time of pulmonary arterial flow in this study was better than previously published data in infants by Subhedar and Shaw (Subhedar and Shaw 1996) (intraobserver CV 12 vs. 25%).

The lateral mitral annular early diastolic velocity in this study had interobserver variability of 9% which is comparable to that reported in an adult study (CV 9- 17%) (Vinereanu 1999). Although there is evidence that the relaxation time 'IVRT' of the tricuspid annulus is a useful parameter for excluding pulmonary hypertension in adults (Brecht 2008), the interobserver reproducibility for this index in infants was sub-optimal (CV 27%), compared with 12-13% in adults (Dambrauskaite 2005; Lindqvist 2006). Intraobserver variability was better at CV 16%. When Pauliks et al. addressed myocardial velocities in children undergoing closure of an atrial septal defect (Pauliks 2005), the CVs for systolic ( $V_s$ ) and diastolic ( $V_e$ ) velocities were, 10% for both intra- and interobserver variability, while CVs for diastolic velocity ( $V_a$ ) were higher (21.5% intra- and 15% interobserver) (Pauliks 2005). In infants, I found that the reproducibility of measuring myocardial velocity was more variable in the RV (15-18% intra-

and 18-24% interobserver variability) than in the septum and the lateral wall of the LV (CV, 15%). In adults with LV dyssynchrony, variability for measuring myocardial systolic velocities (Vs) has been as much as 18-56% (Mandysova 2008). There are limited data on the reproducibility of deformation indices or IVA in infants and children. Weidemann et al.(Weidemann 2002) measured radial and longitudinal strain and strain rate in 33 healthy children aged 4-16 years. They reported intraobserver variability of 11% and interobserver variability of 15%; these data are pooled CVs for LV, RV, and septal strain. In my study, when measuring longitudinal peak systolic strain, variation between lowest and highest CVs varied in the LV (14-17%) and RV (19-23%). Pena et al.(Pena 2009) have reported a reproducibility of longitudinal peak systolic strain in infants of only 1.2%, but this is misleading as the figure given is a Bland-Altman difference presented as percentage, and not the CV between observers. One of the major difficulties faced when measuring myocardial strain in infants is the small size of the heart. I used a computation length of 10 mm, as previously recommended (Nestaas 2007). Using smaller distances increased noise and reduced reproducibility. The strain length of 10 mm was therefore necessary, but in small hearts means that it becomes difficult to position the sample volume accurately to avoid influences from surrounding structures such as the atrium. IVA has been validated as a useful, load-independent index for assessing myocardial contractility in animal models (Vogel 2002). It has also been used to assess myocardial contractility in children after surgical closure of atrial septal defect where the variability reported by Pauliks et al. (Pauliks 2005) (intraobserver 10.8% and interobserver 11.5%) is considerably better than was found in infants in my study (CV, 35%).

In conclusion, I have demonstrated that myocardial velocity imaging and its off-line analysis using the available equipment in our laboratory was feasible in newborn infants, and therefore, would be feasible in older children as well. I have also demonstrated that



intraobserver reproducibility for myocardial velocities, displacement and strain are adequate and these parameters can be used in clinical research. However, interobserver reproducibility for more advanced indices such as LV and RV longitudinal strain is sub-optimal suggesting that these measurements should be used cautiously for clinical diagnosis. Reproducibility for myocardial acceleration (IVA), a marker of contractile function, is currently unsatisfactory.

In this study, the images were acquired by two operators and three different GE vivid machines were used. The intra and interobserver variability of the analysis presented in this study does not take into account the inter-machine and inter-operator variation.

## **Chapter Four: Cardiovascular Study**

## **4.1 Introduction**

Right ventricular dysfunction and pulmonary arterial hypertension (PAH) are recognised causes of significant morbidity and mortality in infants with CLD (Evans and Archer 1991; Subhedar and Shaw 2000). Presence of moderate PAH beyond the first few months of life has been associated with 47% mortality within 2 years of diagnosis (Khemani 2007). In addition, cardiac catheterisation studies have shown that even mild hypoxia can cause marked elevation in pulmonary arterial pressure (PAP) (Mourani 2004). In Chapter 2, I have demonstrated that prematurely born children with CLD continue to have evidence of lung function abnormalities at school age. Given the evidence of abnormal myocardial function in adult patients with chronic obstructive pulmonary disease (COPD) (Sabit 2010), the children with prematurity related CLD may also be at high risk for developing subclinical myocardial dysfunction and pulmonary arterial hypertension, especially when exposed to hypoxic conditions. At present there is lack of data regarding natural progression of PAH in survivors of CLD beyond infancy. As the subjects in this study did not have any ongoing clinical problems regarding their cardiac health, I assumed that hypoxia induced pulmonary vascular stress would be a useful method of unmasking the underlying pulmonary vascular abnormalities and that any abnormality detected would be subclinical.

Left ventricular (LV) and right ventricular (RV) function both predict long-term outcome in adult patients with COPD (Barbera 2003, Wright 2005). As children with neonatal CLD continue to have abnormal pulmonary function as they grow older as described in Chapter 2, early diagnosis of left and right ventricular dysfunction in these children may allow early intervention and appropriate follow-up. Because of the right ventricular anatomy, it is not easy to accurately quantify right ventricular function using conventional 2-D and Doppler echocardiography. Myocardial velocity imaging (MVI) or tissue Doppler imaging (TDI)

allows assessment of regional and global left and right ventricular function and thus helps detection of sub-clinical ventricular dysfunction.

In this study I hypothesized that 8-12 year old children with CLD will have

1. Subclinical right ventricular dysfunction
2. Evidence of raised pulmonary arterial pressure when exposed to acute hypoxia
3. Subclinical left ventricular dysfunction

## **4.2 Methods**

The methods used for recruitment of the subjects have already been described in section 2.2.1. Cardiovascular tests were carried out in the Wales Heart Research Institute at Cardiff University, Cardiff. All cardiovascular tests were done in the morning and the children were asked to attend after an overnight fast. The following tests comprised the cardiovascular function;

- 12-lead ECG
- Measurement of biochemical markers of cardiovascular function
- Baseline echocardiography
- Hypoxic challenge test
- Measurement of the flow-mediated dilatation of brachial artery\*
- Conduit arterial function and wave travel\*

\* Measurements of the flow-mediated dilatation of brachial artery and the conduit arterial function of carotid arteries were carried out by other colleagues of the research team (T Powell, E Ellins and N Pickerd) and the methods and results of these measurements are not included as a part of this thesis.

#### **4.2.1 General assessment**

The parents of the participants were requested to fill in their child's medical history proforma that included history of past and current cardiac conditions. The history proforma is shown in Appendix B. The participants also had physical examination including cardiovascular examination, baseline pulse oximetry, heart rate and blood pressure measurements.

#### **4.2.2 Biochemical tests**

The children who consented to having a capillary blood sample were asked to attend the Wales Heart Research Institute after an overnight fast. The children had a finger prick blood sampling using a lancet (Accu-check Softclix, UK) and blood samples were collected in a 15 $\mu$ L, a 40 $\mu$ L and a 100 $\mu$ L capillary blood collectors. A handheld blood analyser (Cardio Check PA analyser, USA) was used to measure blood glucose, cholesterol, high density lipoprotein and triglycerides. Blood sample from the 15 $\mu$ L collector was introduced in the glucose panel in the analyser to measure fasting blood glucose, followed by the sample from the 40 $\mu$ L collector to the lipid panel to measure total cholesterol, high density lipoprotein and triglycerides. The blood sample collected in the 100 $\mu$ L collector was transported immediately to the Special Care Baby Unit at University Hospital of Wales to measure capillary blood gas using a blood gas analyser (ABL 735, Radiometer, Copenhagen).

## **4.2.3 Hypoxic challenge test**

### 4.2.3.1 Hypoxic challenge test protocol

The detailed study protocol for the hypoxic challenge test is given in Appendix A2. After acquisition of the baseline echocardiographic images, the children were asked to breathe through a non re-breathing mask connected to a cylinder containing 15% oxygen. After 20 minutes of inhalation of 15% oxygen, echocardiography was repeated while the children continued to inhale 15% oxygen. Following this, they inhaled 12% oxygen for 20 minutes followed by repeat echocardiography while continuing to inhale 12% oxygen. The hypoxic challenge test was then terminated.

### 4.2.3.2 Delivery of hypoxic oxygen

British Oxygen Company Limited (BOC, UK) provided the premixed cylinders of 15% and 12% oxygen with nitrogen blend. This hypoxic oxygen blend was delivered to the child via a paediatric non re-breathing mask with a bag (Figure 4.2). Oxygen delivery rate was set at 10L/min using a regulator attached to the gas cylinder. High oxygen delivery rate was selected so as to keep the bag inflated throughout the experiment. Although I did not use sealed mask for the delivery of oxygen, by using high gas flow rate and ensuring that the bag was inflated and that the mask was fitted as tightly as practically possible, I maintained the inspiratory oxygen concentration as close as possible to the desired 15% or 12%.

### 4.2.3.3 Monitoring the child during hypoxic challenge

As induction of hypoxia in a child is a matter of anxiety for the parents as well as for the ethical committee, this part of the study had been scrutinized in detail both by the research ethics committee and the external reviewer. This study was initially set-up at Leicester

University by Professor Kotecha where the study protocol had been given a favourable review by an independent external reviewer as well as the local ethics committee. Due to Professor Kotecha's move to Cardiff, the study could not be undertaken in Leicester. Prior to conducting this study at Cardiff University, the study protocol had again been reviewed by the South East Wales regional ethics committee and was granted a favourable opinion in October 2007 (Reference: 07/WSE03/77).

As advised by the ethics committee, per-cutaneous oxygen saturation was monitored and recorded continuously using Nellcor pulse oximetry (Nellcor, Tyco Healthcare, UK) and the low alarm limit in the pulse oximetry was set at 80% in keeping with the study protocol (Appendix A2); which stated that *'the hypoxic oxygen should be stopped if the oxygen saturation drops below 80% for >30 seconds or below 80% for <30 seconds on more than 3 occasions, and should be discontinued immediately if oxygen saturation drops to <75%'*. The pulse oximetry recording stored during the hypoxic challenge test was downloaded in the computer system and the average as well as the lowest oxygen saturation during inhalation of 15% oxygen and 12% oxygen was noted.

At the end of the hypoxic challenge test, pulse oximetry was continued until the oxygen saturation returned to baseline. Heart rate and blood pressure were measured at baseline, at the end of 15% oxygen inhalation and at the end of 12% oxygen inhalation using the 'Dynamap Procare' monitor. The adult size, small adult size or the paediatric size cuffs were selected as appropriate depending upon the size of the child. Respiratory rate was counted for 1 minute.

#### 4.2.3.4 Echocardiography with blood flow and tissue Doppler methods

Before subjecting the child to hypoxia, baseline echocardiography was performed firstly to rule out structural cardiac defects and secondly to assess baseline left and right ventricular function. Blood flow and tissue Doppler images were also acquired to measure the surrogate markers of pulmonary hypertension. The M-mode image of the inferior vena cava was acquired in order to estimate right atrial pressure at baseline (Figure 4.6). While acquiring the M-mode image of the inferior vena cava (IVC), the child was asked to sniff and the inspiratory collapse of the inferior vena cava during the 'sniff test' was assessed. The right atrial pressure can be estimated by the degree of collapse of the IVC on inspiration (Kircher 1990). If the collapse of IVC is less than 50%, the right atrial pressure is estimated to be  $\geq 10$  mm Hg. If the inferior vena caval collapse is more than 50%, the right atrial pressure is estimated to be  $< 10$  mm Hg.

After each phase of hypoxic challenge, echocardiography was repeated whilst the child continued to breathe the prescribed oxygen. Images were acquired to assess RV function and surrogate markers of pulmonary hypertension. The echocardiography acquisition protocol is given in Appendix A3.

All images were acquired as 3-beat loops. Images were acquired using a commercially available system (Vivid 7.0, GE Vingmed Ultrasound AS, Horten, Norway, Figure 4.1) with a 3S MHz transducer.

Pulsed wave Doppler of the left ventricular outflow tract (LVOT) in the apical 4-chamber view was acquired to measure LVOT velocity time integral (Figure 4.4). 2-D image of the long axis view of the LVOT was obtained to measure the LVOT diameter (Figure 4.3). Conventional pulsed wave Doppler of mitral (Figure 4.5) and pulmonary flow (Figure 4.9)



were acquired from the apical 4-chamber and the parasternal short axis views respectively. Continuous wave Doppler image of the tricuspid flow (Figure 4.7) and pulsed wave Doppler of the pulmonary flow (Figure 4.8) were acquired to measure tricuspid regurgitation systolic velocity and pulmonary regurgitation end-diastolic velocity respectively. For lateral and medial mitral annular velocities (Figure 4.10) and right ventricular relaxation time, 'IVRT' (Figure 4.11) real-time pulsed velocity profiles were acquired from the mitral and tricuspid annulus respectively. Colour tissue Doppler images were acquired separately of the left and right ventricles. The Nyquist limit was optimised to avoid aliasing, and the depth of imaging and the sector angle were adjusted to obtain high frame rates. The Nyquist limit, which is 50% of the sampling frequency, is the highest frequency that can be coded at a given sampling rate in order to be able to fully reconstruct the signal.

All images were stored as 3-beat loops in magneto-optical disks for post- processing.

Figure 4.1 Photograph of a GE Vivid 7 equipment



GE Vivid 7 equipment used for echocardiography

Figure 4.2 Photograph of a child having echocardiography while breathing 12% oxygen via a non-rebreathing face mask.



(A written parental consent and the assent from the child was obtained for the publication of this image)

#### 4.2.3.5 Analysis of the echocardiography images

Images were analysed using commercially available EchoPAC software (GE Vingmed Ultrasound EchoPAC 7-00, Horten, Norway). Images were stored only with coded identity so that I was blinded to the clinical status of the subjects while analysing the images.

25 parameters (Tables 4.1 and 4.2) were measured from each study, including 8 blood flow Doppler parameters, 3 tissue Doppler parameters from real-time tissue Doppler images and 7 post-processed parameters from LV and RV tissue Doppler loops. Each tissue Doppler parameter was measured at the basal segments of the LV and RV. The cursor was positioned within each segment so that it did not encroach upon the annulus during systole.

Left ventricular pre-ejection period (PEP) was measured as the time interval between the onset of the QRS complex and the onset of the left ventricular outflow (Figure 4.4).

The velocity time integral of LV outflow (VTI) (Figure 4.4), the mitral E and A velocities (Figure 4.5), tricuspid regurgitation (Figure 4.7) and pulmonary regurgitation (Figure 4.8) velocities, and the pulmonary artery acceleration time (Figure 4.9) were measured conventionally from blood pool Doppler.

The area of the LV outflow tract was calculated as  $\pi r^2$  where  $r$  is the radius of the LV outflow tract ( $r = \text{diameter of LV outflow tract} / 2$ ). Stroke volume was calculated by multiplying the velocity time integral (VTI) by LV outflow tract area. Multiplying stroke volume by heart rate yields cardiac output (Thomas and Popovic 2006). In order to correct cardiac output for body size, cardiac index (CI) was calculated as cardiac output (L/min) / (height in m)<sup>2</sup>.

Pulmonary artery acceleration time (AT) was calculated as the time interval between the onset of flow to the peak velocity and the ejection time (ET) was calculated as the time interval between the onset of pulmonary flow to the end of flow (Figure 4.9). The ratio of the acceleration time to the ejection time (AT:ET) was also calculated.

The mitral annular early diastolic velocity (Ve') was measured at both the medial and lateral mitral annulus and averaged.

Right ventricular relaxation time 'IVRT' was measured as the time interval between the end of systole and the beginning of diastole during the tricuspid annular motion (Figure 4.11).

Myocardial systolic velocity (Vs), early diastolic velocity (Ve) and diastolic velocity during atrial contraction (Va) were measured at the basal segments (Figure 4.12) of the LV and the RV lateral walls. When myocardial Ve and Va were fused due to rapid heart rate, a single diastolic velocity was recorded and noted as Ve. Time to peak systolic velocity ( $T_{Q-Vs}$ ) was measured as the time interval between the start of the QRS complex and the peak myocardial velocity during ejection (Figure 4.12). The measurement for the myocardial isovolumic acceleration (IVA) was made by dividing the peak positive velocity during the IVC by time to this peak velocity from the onset of the signal at the zero crossing (Figure 4.12.).

Annular displacement (Ds') was measured at the mitral and tricuspid annulus using tissue tracking (Figure 4.13).

Peak systolic strain at end-systole (S) was measured within the basal and the apical portions of the lateral wall (Figure 4.14). For computation, strain length of 12 mm was used. To ensure measurement of end-systolic strain during systole, event timing was superimposed from left



ventricular outflow tract and right ventricular outflow tract blood flow Doppler recordings for the LV and RV respectively.

All of the above parameters were measured in 3 beats and averaged.

#### 4.2.3.6 Statistical methods

I studied 90 subjects with 30 in each of the three groups (Preterm CLD, preterm control and term control). Based on the data from Sartori et al (Sartori 1999), I calculated that I would need to study 30 children in each arm to be 90% certain of a difference of 30% in pulmonary arterial pressure in response to hypoxia at a  $p < 0.05$  between the groups.

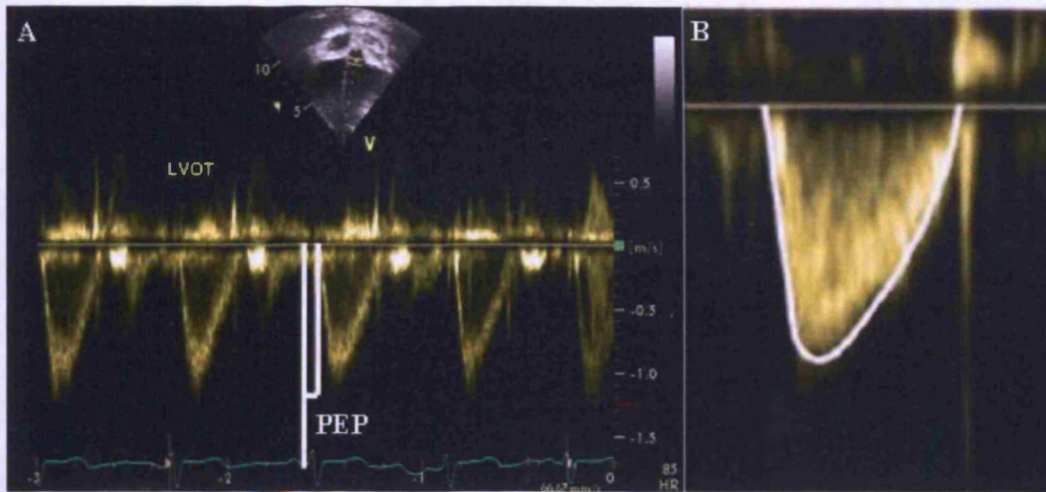
Results are presented as mean and standard deviation (SD). Differences between groups were tested by ANOVA with Tukey's posthoc analyses. SPSS 16.0 (SPSS Inc., Chicago, IL) was used to perform analyses

Figure 4.3 2-D image of the left ventricular outflow tract diameter



2-D parasternal long axis view of the left ventricular outflow tract. The vertical white line shows the measurement of the left ventricular outflow tract diameter when the aortic valve is open.

Figure 4.4 Pulsed wave Doppler of the left ventricular outflow tract, LVOT

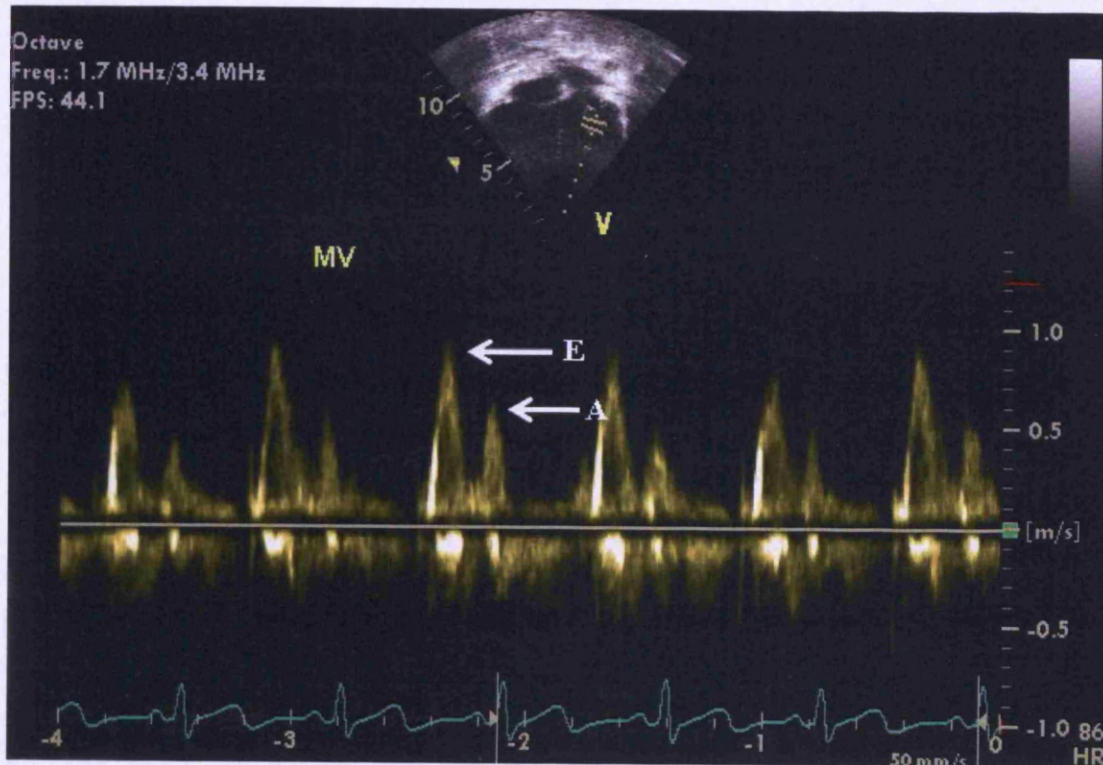


A. Pre-ejection period, PEP is the interval between the beginning of QRS complex and the start of left ventricular outflow.

B. Enhanced view of the left ventricular outflow velocity with thick tracing of the LVOT flow to measure the velocity time integral, VTI.

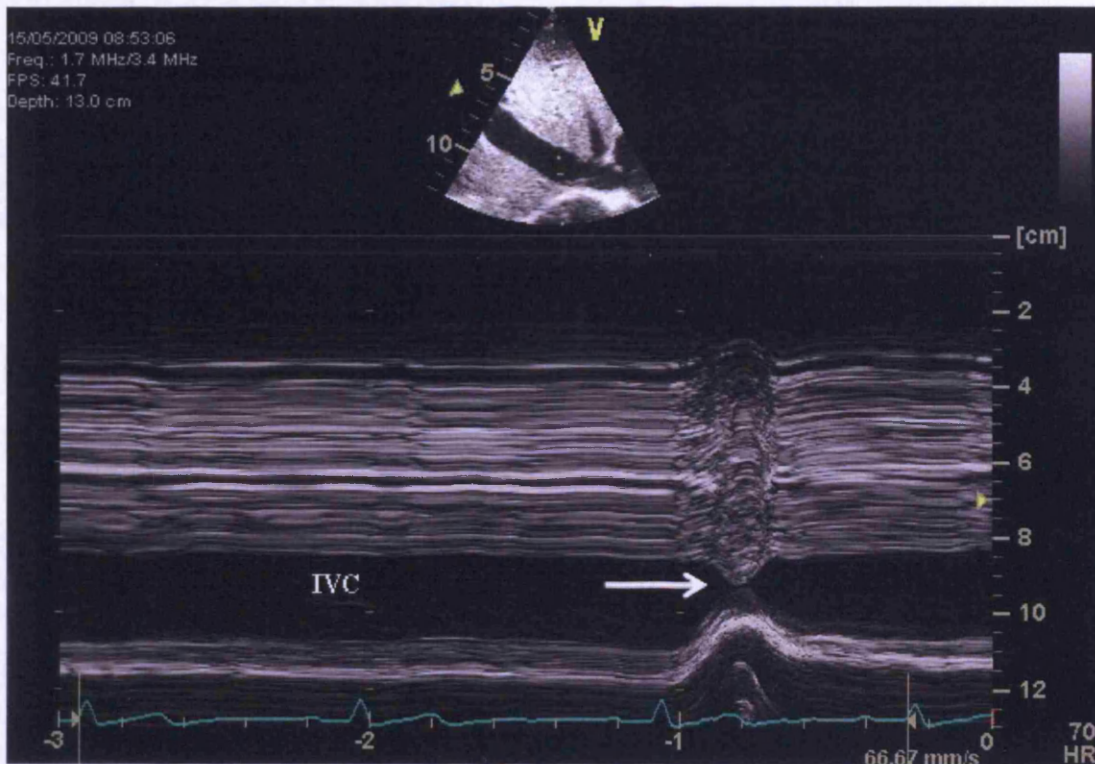


Figure 4.5 Pulsed wave Doppler of the mitral valve showing early (E) and late (A) diastolic velocities



E= Mitral early diastolic velocity, A= mitral late diastolic velocity during atrial contraction

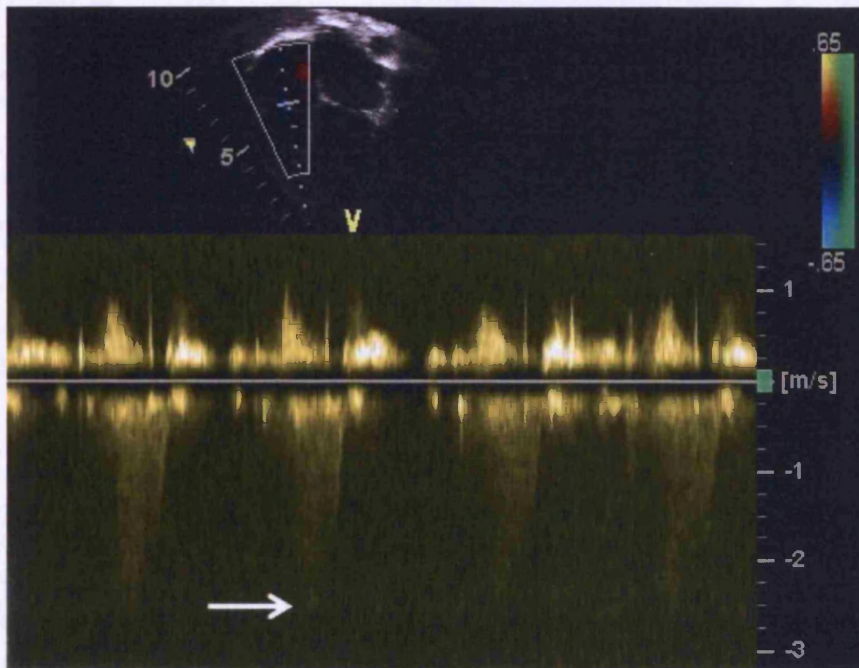
Figure 4.6 2-D image showing collapse of the inferior vena cava during a 'sniff test'



The arrow is pointed to the 'collapse' of the inferior vena cava (IVC) when the child sniffed.

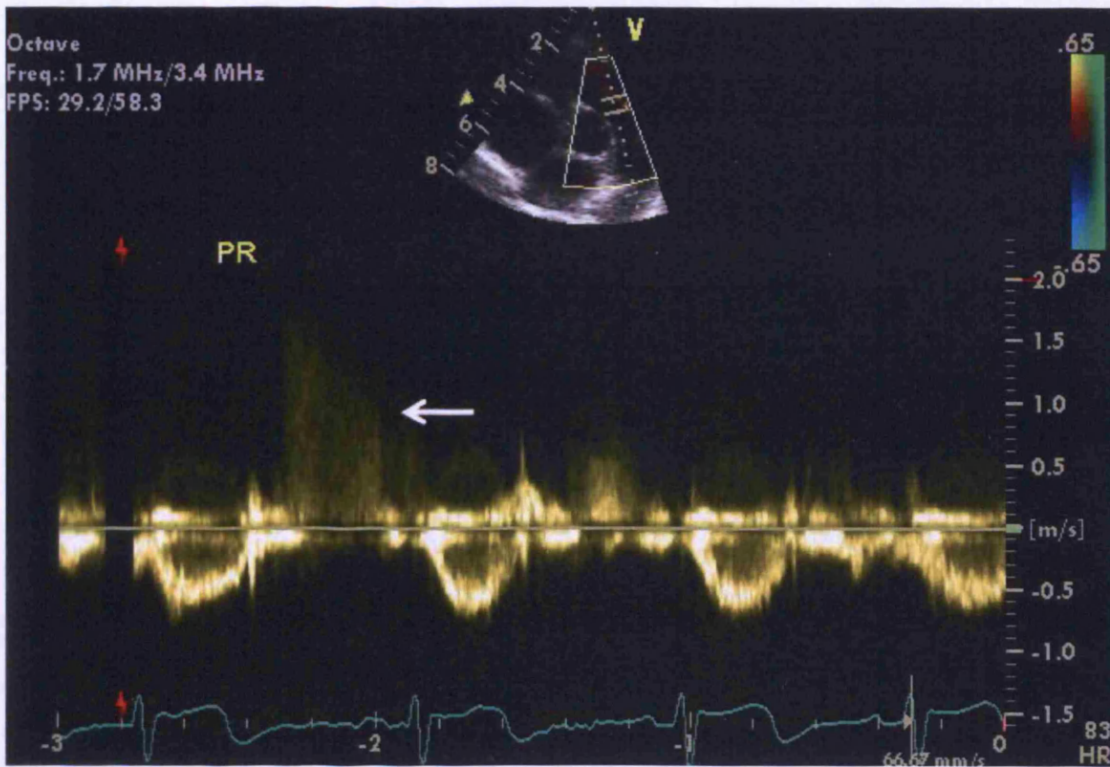


Figure 4.7 Continuous wave Doppler of the tricuspid valve showing tricuspid regurgitation



The arrow is pointed at the peak tricuspid regurgitation velocity

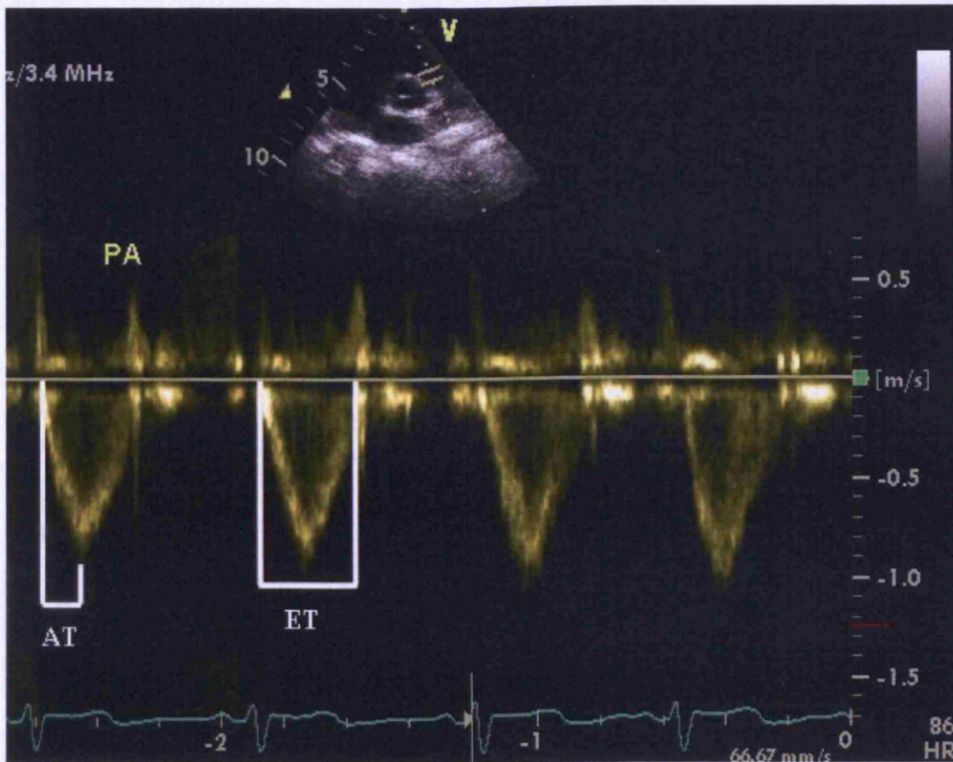
Figure 4.8 Pulsed wave Doppler of the pulmonary valve showing pulmonary regurgitation



The arrow is pointed at the pulmonary regurgitation end-diastolic velocity



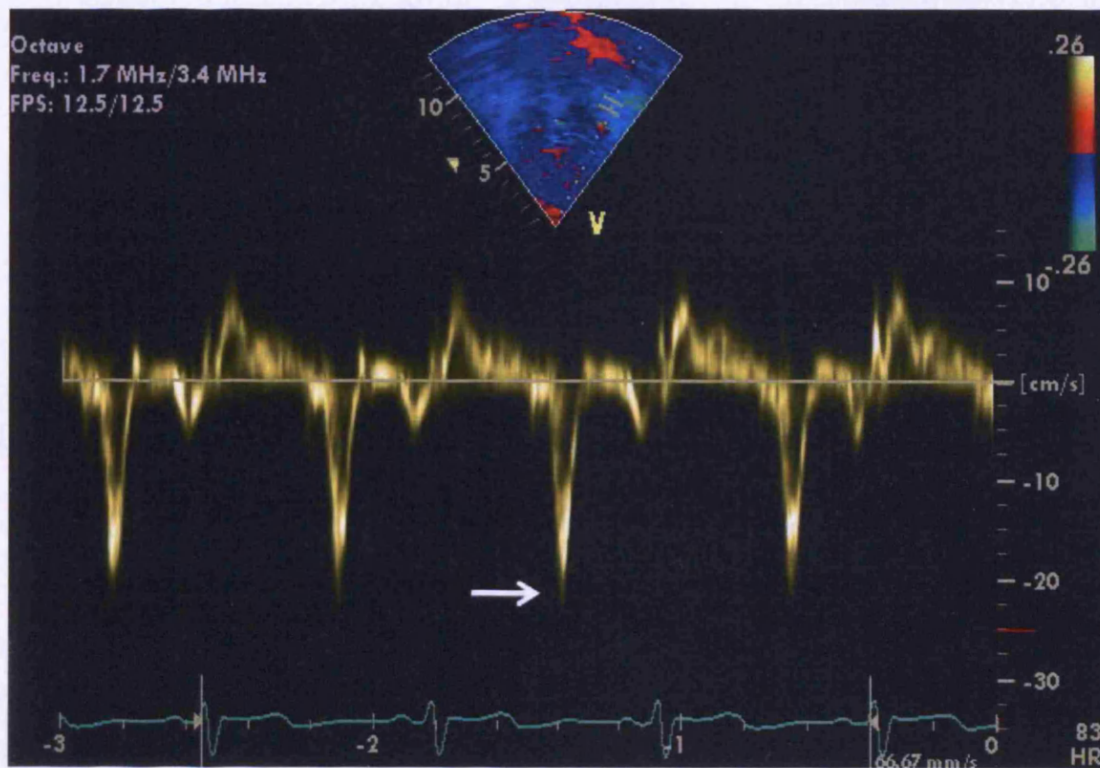
Figure 4.9 Pulsed wave Doppler of the pulmonary valve illustrating measurements of acceleration time and ejection time



Pulmonary arterial acceleration time, AT is measured as the interval between the onset of flow and the peak flow.

Pulmonary arterial ejection time, ET is measured as the interval between the onset of flow and the end of flow.

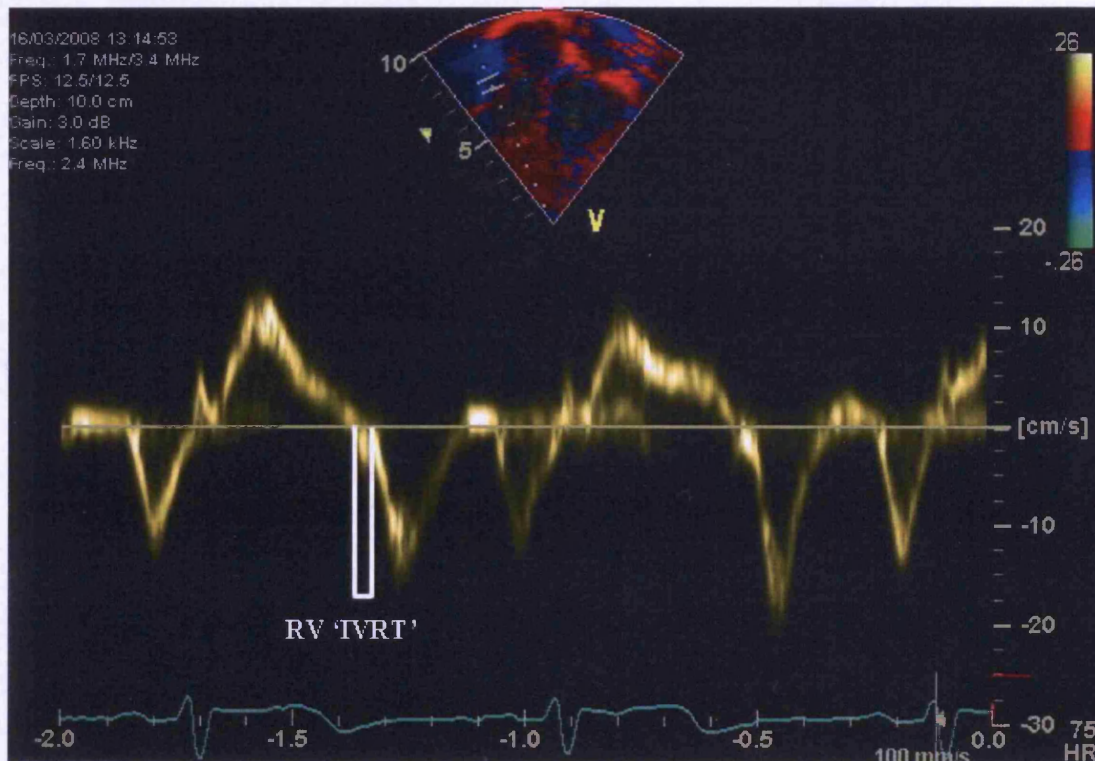
Figure 4.10 Real time pulsed tissue Doppler at the lateral mitral annulus



The arrow is pointed to the lateral mitral annular early diastolic velocity (Ve'). Ve' was measured at lateral and medial mitral annulus and the mean Ve' is reported.



Figure 4.11 Real time pulsed wave Doppler at the lateral tricuspid annulus illustrating right ventricular relaxation time 'IVRT'



Right ventricular 'IVRT' is the time interval between the end of systole and the beginning of diastolic motion of the tricuspid annulus

Figure 4.12 Example of a myocardial velocity trace at the basal segment of the right ventricular free wall

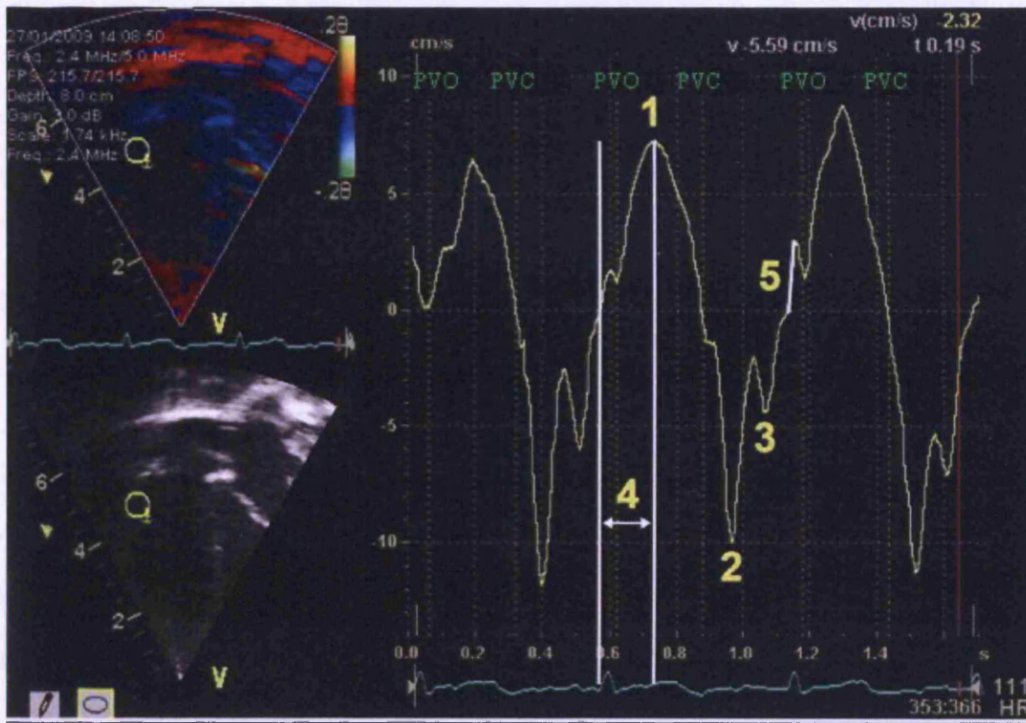


Figure showing the post-processed regional myocardial velocities of the right ventricle. A circular sample volume is placed at the base of the lateral wall.

PVO= Pulmonary valve opening and PVC= Pulmonary valve closure. PVO and PVC were measured from blood-flow Doppler traces of pulmonary flow.

1= Peak systolic velocity ( $V_s$ ), 2= Early diastolic velocity ( $V_e$ ), 3= Late diastolic velocity due to atrial contraction ( $V_a$ ), 4= Time interval between onset of the QRS complex and peak systolic velocity ( $T_{Q-V_s}$ ) and 5= Right ventricular isovolumic acceleration, IVA.



Figure 4.13 Illustration of annular displacement at the lateral tricuspid annulus

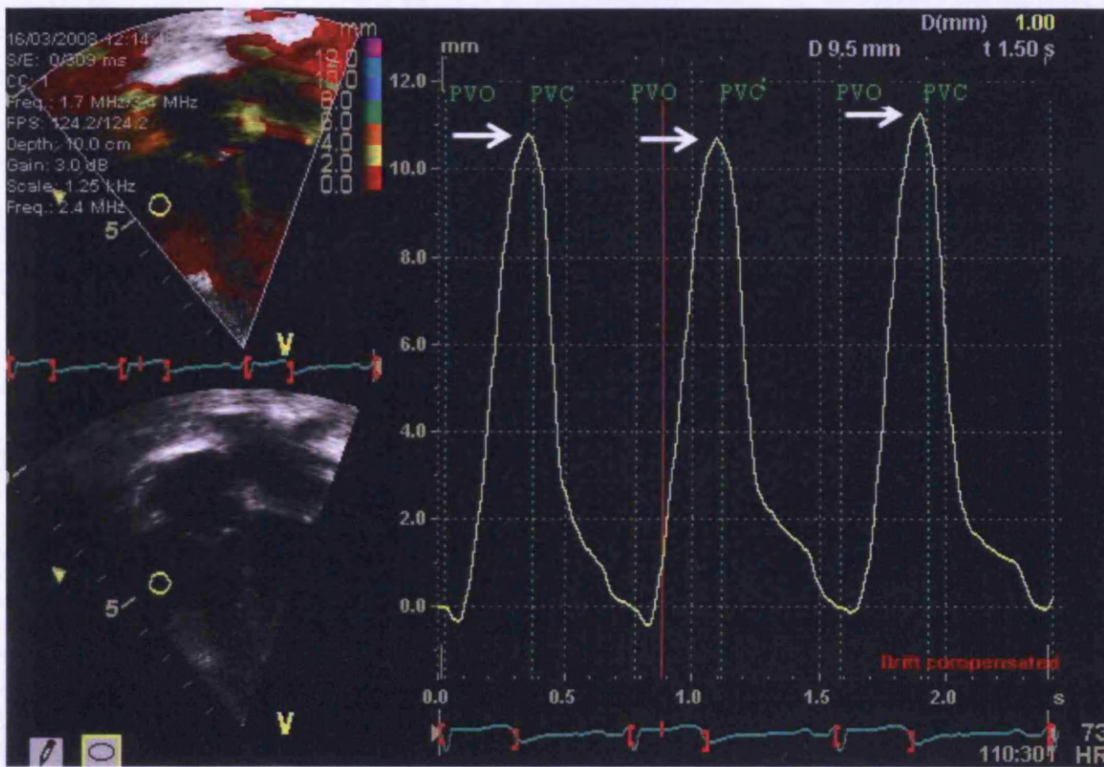


Figure showing the measurement of right ventricular annular displacement using tissue tracking. The arrows point to the annular displacement measured in millimetres.

Figure 4.14 Illustration of strain imaging at the basal segment of the right ventricular free wall

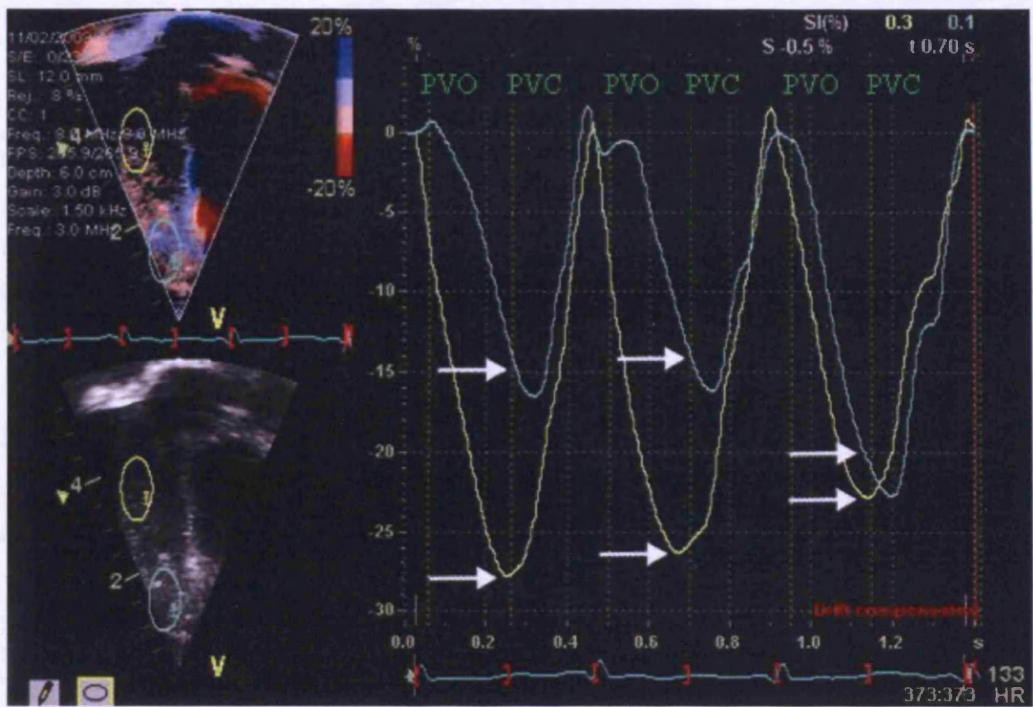


Figure showing right ventricular systolic strain at the basal (yellow) and at the apical (blue) segments of the right ventricle. PVO= Pulmonary valve opening and PVC= Pulmonary valve closure. Pulmonary valve closure marks the end of systole. The arrows point to the end-systolic strain.

#### 4.2.3.7 Echocardiographic indices of left and right ventricular function

The echocardiographic indices that were used to assess left and right ventricular function are summarised in Table 4.1. Both systolic and diastolic function were assessed using 2-D, conventional blood flow Doppler and tissue Doppler methods also known as myocardial velocity imaging.

Cardiac index and LV pre-ejection period (LV PEP) were used as markers of LV systolic function. The ratio of the mitral inflow velocities E:A was measured to assess the LV filling pattern. The mitral annular Ve' velocity measured by myocardial velocity imaging was used to calculate E:Ve' ratio which gives an estimate of mean LV filling pressure.

Myocardial velocity imaging was used to quantify both LV and RV global and regional long-axis function. Mitral annular displacement (Ds') is annular excursion, which in turn correlates with the global myocardial systolic function. Isovolumic acceleration, IVA, is a marker of myocardial contractile function. The myocardial function at the basal segments of the LV and RV lateral walls was quantified by myocardial systolic velocity, Vs, early diastolic velocity, Ve, and the late diastolic velocity, Va. T<sub>Q-Vs</sub>, the time interval from the onset of QRS complex to the peak systolic velocity (Vs), is a measure of electromechanical delay for myocardial systolic function. RV and LV systolic strain are indices of regional myocardial deformation.

Echocardiographic evidence of increased pulmonary arterial pressure (PAP) was also assessed using standard blood-flow Doppler methods and myocardial velocity imaging as summarised in table 4.2. Standard Doppler methods were used to measure tricuspid regurgitant velocity, and RV systolic pressure was calculated by applying the modified Bernoulli equation ( $\Delta P=4V^2$ ). As none of the children had evidence of increased right atrial pressure, right atrial pressure was estimated at 5 mm Hg for all the subjects. Therefore,

pulmonary arterial systolic pressure was estimated as, calculated RV systolic pressure + 5 mm Hg.

Pulmonary arterial acceleration time, AT, and ejection time, ET, and the ratio of ET:AT, were also used to assess pulmonary arterial systolic pressure.

The relaxation time of the myocardium in the basal lateral segment of the free wall of the RV 'IVRT', measured by using tissue Doppler imaging was also used as a surrogate for pulmonary arterial systolic pressure. In patients with raised PA pressure, due to increased RV pressure, there is delay in the onset of diastole. This time lag is the isovolumic relaxation time 'IVRT', which is used as a surrogate of PA pressure.

RV basal strain was also measured before and after hypoxic stimulus as a surrogate of PA hypertension (Dambrauskaite 2007).

Table 4.1 Echocardiographic markers of left and right ventricular function

Markers of LV function	Markers of RV function
<u>Pulsed-Doppler parameter</u>	
LV velocity time integral (VTI)	
LV pre ejection period (LV PEP)	
Mitral early diastolic velocity (E)	
Mitral late diastolic velocity (A)	
<u>Real-time annular myocardial velocity imaging</u>	
Medial mitral annular velocity (MMA Ve')	
Lateral mitral annular velocity (LMA Ve')	
<u>Colour processed myocardial velocity imaging</u>	
LV annular displacement (LV Ds')	RV annular displacement (RV Ds')
LV basal systolic velocity (LV Vsbl)	RV basal systolic velocity (RV Vsbl)
LV basal e velocity (LV Vubl)	RV basal e velocity (RV Vubl)
LV basal a velocity (LV Vabl)	RV basal a velocity (RV Vabl)
LV isovolumic acceleration (LV IVA)	RV isovolumic acceleration (RV IVA)
LV end systolic apical and basal strain	RV end-systolic apical and basal strain

Table 4.2 Surrogate markers of pulmonary arterial pressure

Blood pool Doppler markers	Tissue Doppler markers
Tricuspid regurgitation velocity (TR)	RV relaxation time (IVRT)
Pulmonary regurgitation end-diastolic velocity (PR)	RV basal systolic strain (RV Ssbl)
Pulmonary artery acceleration time (AT)	
Pulmonary artery ejection time (ET)	

### **4.3 Results**

Out of 92 children who were recruited for the study, a total of 90 children (28 with CLD, 32 PT controls and 30 Term controls) participated in the cardiovascular studies. One child from the CLD group and one child from the preterm control group attended the pulmonary function tests but did not keep the appointment for the cardiovascular studies.

#### **4.3.1 Demographics and general characteristics**

General characteristics of the participants including baseline heart rate, blood pressure and oxygen saturation at rest are summarised in Table 4.3. The gender and the age of the children were similar between the three groups. Children who had CLD were lighter than the term-born controls ( $p < 0.05$ ) but there was no significant difference in the mean height. Baseline heart rate, respiratory rate, oxygen saturation and blood pressure were also similar between the 3 groups.

#### **4.3.2 Biochemical markers of cardiovascular function**

75/90 (84%) subjects consented to the finger prick blood test. The fasting blood glucose, total cholesterol, triglycerides and high density lipoprotein were within normal range in all the subjects and were similar between the 3 groups. 68 children (76%) also had the capillary blood gas analysis. Mean  $\text{PaO}_2$  was 17% lower in the children with CLD compared with the term-born controls ( $p < 0.05$ ). There were no significant differences in the other blood gas parameters between the term and the preterm groups (Table 4.4)



Table 4.3 General characteristics of the subjects (Cardiovascular study)

Parameters	CLD (n=28)	Preterm control (n=32)	Term control (n=30)	p-value
<b>Demographics</b>				
Male: Female	17: 11	15: 17	15: 15	0.539
Gestation (wks)	27.3 (2.2)	30.0 (2.0)	39.7 (1.5)	<0.001 <sup>§, §§, §§§</sup>
Age (years)	10.3 (1.5)	10.4 (1.2)	10.6 (1.5)	0.668
Height (cm)	138.0 (9.8)	141.5 (9.3)	144.3 (10.6)	0.061
Weight (kg)	30.7 (8.9)	35.6 (10.9)	38.0 (13.7)	0.053 <sup>††</sup>
<b>Physiological measurements</b>				
Heart rate (per minute)	77.5 (12.0)	77.0 (11.9)	73.3 (9.5)	0.289
Respiratory rate (per minute)	21.3 (4.6)	20.6 (3.7)	20.5 (3.4)	0.757
SBP (mm Hg)	105.7 (6.9)	103.6 (9.1)	101.7 (7.5)	0.158
DBP (mm Hg)	63.9 (6.7)	61.8 (8.2)	63.5 (5.9)	0.187
PP (mm Hg)	41.8 (7.6)	41.8 (9.1)	38.2 (6.4)	0.123
Oxygen saturation (%)	99.3 (0.84)	98.6 (1.0)	98.8 (0.91)	0.036 <sup>†</sup>

<sup>§</sup> p<0.001 (CLD vs. preterm control), <sup>§§</sup> p<0.001 (CLD vs. term), <sup>§§§</sup> p<0.001 (preterm vs.

term control), <sup>††</sup> p<0.05 (CLD vs. term), <sup>†</sup> p<0.05 (CLD vs. preterm control)

Table 4.4 Biochemical markers of cardiovascular function

Parameters	CLD (n=22)	Preterm control (n=29)	Term control (n=24)	p-value
<b>Biochemical markers</b>				
Glucose	4.3 (0.94)	4.0 (0.58)	4.3 (0.78)	0.411
Cholesterol	3.8 (0.66)	3.6 (0.68)	3.6 (0.53)	0.362
Triglycerides	0.72 (0.31)	0.72 (0.20)	0.74 (0.39)	0.985
HDL	1.2 (0.37)	1.1 (0.4)	1.1 (0.27)	0.176
<b>Capillary blood gas analysis</b>				
pH	7.4 (0.02)	7.4 (0.02)	7.4 (0.01)	0.951
PCO <sub>2</sub>	5.2 (0.33)	5.2 (0.29)	5.1 (0.32)	0.584
PO <sub>2</sub>	9.6 (1.5)	10.4 (1.0)	11.5 (2.0)	0.001 <sup>§§</sup>
HCO <sub>3</sub>	24.5 (0.97)	24.6 (1.0)	24.4 (0.98)	0.811
Base excess	0.11 (1.3)	0.17 (1.2)	0.02 (1.1)	0.905

Results are presented as mean (SD)

<sup>§§</sup> p <0.001 (CLD vs. term control)

### 4.3.3 Electrocardiography

12-lead electrocardiography (ECG) was performed on all participants. All the children were in sinus rhythm. None of the children had ECG evidence of right ventricular hypertrophy. R wave amplitude in lead V1, QRS axis, and duration of the QRS complex, were all within normal limits in the control groups as well as those with CLD (Table 4.5). QRS duration was 7% lower in the preterm children without CLD compared to the term-born children but the values were within normal limits in all children. 4 (15%) children in the CLD group, 4 (13%) children in the preterm control group and 6 (20%) children in the term control group had evidence of incomplete right bundle branch block.

Table 4.5 Electrocardiographic indices of right ventricular hypertrophy

<b>ECG index</b>	<b>CLD (n=28)</b>	<b>Preterm Control (n=32)</b>	<b>Term control (n=30)</b>	<b>p-value</b>
<b>R wave amplitude in V1 (mm)</b>	5.4 (2.2)	5.3 (2.9)	4.5 (1.9)	0.324
<b>QRS axis (degrees)</b>	61.1 (57.6)	57.6 (22.1)	53.5 (28.2)	0.520
<b>QRS duration (milliseconds)</b>	80.6 (9.1)	78.5 (9.1)	84.5 (7.3)	0.024 <sup>†††</sup>

Results are presented as mean (SD).

<sup>†††</sup> p <0.05 (preterm control V Term control)



#### **4.3.4 Echocardiographic markers of left and right ventricular function**

##### Left and right ventricular function

The results of the echocardiography of left and right ventricular function at baseline are summarised in Table 4.7.

While breathing in room air, the preterm and term-born children had normal LV and RV function. There were no significant differences in systolic or diastolic function either in the LV or the RV between those with CLD compared with the preterm and the term control groups. The left ventricular outflow diameter was smaller in the preterm children compared to the term groups but cardiac index was similar between the three groups.

Tricuspid regurgitation was present in 20/28 (71.4%) children with CLD, 20/32 (62.5%) preterm controls and 18/30 (60%) term-born controls. PR end-diastolic velocity was measurable in 25 (89.3%) in the CLD, 25 (78.1%) in the preterm control and 25 (83.3%) in the term control groups.

During the sniff test, all the children had  $\geq 50\%$  collapse of the inferior vena cava. Thus, there was no evidence of increased right atrial pressure in any of the children and RA pressure was estimated to be  $< 10$  mm Hg (Kircher 1990).

The estimated pulmonary arterial systolic pressure at baseline was similar in all three groups: 22 mmHg in the children with CLD, 24 mmHg in the preterm control group and 22 mmHg in the term-born controls.

### Subclinical changes in cardiac function after hypoxia

#### *Oxygen saturation*

After exposure to hypoxia, oxygen saturation measured by pulse oximetry decreased in all three groups. When *average* oxygen saturation during each period of hypoxic challenge was analysed, after inhalation of 15% oxygen for 20 minutes, mean oxygen saturation decreased by 4.4% (95% CI, 3.7% to 5.1%,  $p<0.001$ ) in the CLD group, 3.0% (95% CI, 2.5% to 3.5%,  $p<0.001$ ) in the preterm control group and by 3.3% (95% CI, 2.9% to 3.7%,  $p<0.001$ ) in the term control group. Similarly, after exposure to 12% oxygen for 20 minutes the differences in oxygen saturation from baseline were 7.8% (95% CI, 6.4% to 9.1%,  $p<0.001$ ), 6.1% (95% CI, 5.4% to 6.8%,  $p<0.001$ ) and 6.3% (95% CI, 5.6% to 7%,  $p<0.001$ ) in the CLD, preterm and the term control groups respectively. Although the levels of average oxygen saturation dropped significantly in all three groups after hypoxic exposure, the levels were not significantly different between the three groups at any stage.

It was noted that the average oxygen saturation did not accurately represent the lowest oxygen saturation that the children reached at the end of the hypoxic challenge. The average and the lowest oxygen saturation reached at the end of each phase of the hypoxic challenge are summarised in Table 4.6. After exposure to 15% oxygen for 20 minutes, the mean drop in oxygen saturation was -13% (95% CI, -10.1% to 15.8%,  $p<0.001$ ) in the CLD group, -9% (95% CI, -7.6% to -10.4%,  $p<0.001$ ) in the preterm control group and -10% (95% CI, -8.3% to -11.6%,  $p<0.001$ ) in the term control group. Taking the *lowest* oxygen saturations reached, the mean drop in oxygen saturation at the end of hypoxic challenge with 12% oxygen was -17.4% (95% CI, -15.3% to -19.4%,  $p<0.001$ ) in the CLD group, -13.6% (95% CI, -12.4% to -14.7%,  $p<0.001$ ) in the preterm control group and -14.1% (95% CI, -12.8% to -15.4%,  $p<0.001$ ) in the term control group. At the end of the hypoxic challenge with 15% oxygen,

oxygen saturation level was significantly lower in the CLD group compared to the preterm control group ( $86.2\% \pm 6.7\%$  vs  $89.7\% \pm 3.5\%$ ,  $p < 0.05$ ). Similarly, children with CLD had significantly lower level of oxygen saturation at the end of the hypoxic challenge with 12% oxygen compared with those in the preterm control group ( $81.9\% \pm 5.4\%$  vs  $85.1\% \pm 3.3\%$ ,  $p < 0.01$ ) and the term control group ( $81.9\% \pm 5.4\%$  vs  $84.7 \pm 3.6\%$ ,  $p < 0.05$ ).

Table 4.6 Oxygen saturation at baseline and after hypoxic challenge

Study groups	Oxygen saturations				
	Room air	15% Oxygen		12% Oxygen	
		Mean	Lowest	Mean	Lowest
CLD	99.3% (0.84)	94.9% (1.5)	86.2% (6.7)	91.5% (3.0)	81.9% (5.4)
Preterm control	98.6% (1.0)	95.6% (1.3)	89.7% (3.5)	92.5% (2.0)	85.1% (3.3)
Term control	98.8% (0.91)	95.5 (1.1)	88.8% (4.1)	92.6% (1.9)	84.7% (3.6)
ANOVA	0.036 <sup>†</sup>	0.052	0.032 <sup>†</sup>	0.152	0.008 <sup>†, ††</sup>

Results are expressed as mean (%) and SD

<sup>†</sup>  $p < 0.05$  (CLD vs. preterm control), <sup>‡</sup>  $p < 0.01$  (CLD vs. preterm control), <sup>††</sup>  $p < 0.05$  (CLD vs. term control)

#### *Respiratory rate*

Change in respiratory rate was  $< 1\%$  after acute hypoxia (after inhalation of 15% oxygen and 12% oxygen compared with baseline) in all three groups of children.

After inhalation of 15% oxygen for 20 minutes, heart rate increased by 3.1% (95% CI, 1.0% to 7.2%) in the CLD group, 1.4% (95% CI, 0.7% to 5.5%) in the preterm controls and by

4.5% (95% CI, 1.6% to 7.3%) in the term control group. After inhalation of 12% oxygen for a further 20 minutes, heart rate increased by 4.7% (95% CI, 0.04% to 9.3%), 3.9% (95% CI, 0.3% to 7.6%) and 6.6% (95% CI, 3.8% to 9.5%) in the children with CLD, preterm and term-born controls respectively.

### *Blood pressure*

Systolic blood pressure was reduced by 4.2% (95% CI, -1.4% to -9.8%) in the prematurely born children with CLD after exposure to 12% oxygen compared with baseline. In the control population, the change in systolic blood pressure was <1% after exposure to acute hypoxia. Compared with the baseline reading, diastolic blood pressure increased by 2.0% (95% CI, 1.0% to 4.9%) in the CLD group after inhalation of 12% oxygen for 20 minutes. Similarly, diastolic blood pressure increased by 3.9% (95% CI, 1.2% to 6.6%) in the preterm control group after exposure to 12% oxygen. Change in diastolic blood pressure was <1% in the term control group after hypoxia. Compared to the baseline, pulse pressure was reduced by -6.2% (95% CI, -12.3% to -0.2%) in the CLD group and by -3.6% (95% CI, -6.7% to -0.4%) in the preterm control group after exposure to 12% oxygen for 20 minutes. The mean change in pulse pressure was <1% in the term control group. None of these changes were statistically significant.

### *Echocardiographic parameters*

The change in the echocardiographic markers of right ventricular function and surrogate markers of pulmonary hypertension after inhalation of 15% and 12% oxygen are summarised in Tables 4.8, 4.9 and 4.10 for the CLD, preterm control and term control groups respectively.

Following the hypoxic challenge, the surrogate markers for PAH changed as expected in all three groups of children. After inhalation of 12% oxygen, the maximum velocity of the tricuspid regurgitation increased by  $0.38 \text{ m s}^{-1}$  (95% CI, 0.24 to  $0.53 \text{ m s}^{-1}$ ,  $p < 0.001$ ) in the children with CLD of prematurity compared to  $0.18 \text{ m s}^{-1}$  (95% CI, 0.09 to  $0.26 \text{ m s}^{-1}$ ,  $p > 0.05$ ) in the preterm controls and  $0.25 \text{ m s}^{-1}$  (95% CI, 0.13 to  $0.36 \text{ m s}^{-1}$ ,  $p < 0.001$ ) in the term-born controls.

Pulmonary arterial acceleration time shortened with acute hypoxia in all three groups but the difference was greater in the children with CLD compared to the control groups. Compared to the baseline, the mean difference in acceleration time was  $-23.8 \text{ ms}$  (95% CI,  $-32.9$  to  $14.7 \text{ ms}$ ,  $p < 0.001$ ),  $-15.6 \text{ ms}$  (95% CI,  $-24.2$  to  $-7.0 \text{ ms}$ ,  $p < 0.001$ ) and  $-19.2$  (95% CI,  $-25.8$  to  $-12.6 \text{ ms}$ ,  $p < 0.001$ ) in the CLD, preterm and term control groups respectively after inhalation of 12% oxygen for 20 minutes. Similarly, the ratio of acceleration time to ejection time, AT:ET was reduced in all 3 groups after acute hypoxia. The mean difference in AT:ET after exposure to 12% oxygen compared to the baseline was,  $-0.07$  (95%CI,  $-0.4$  to  $0.09$ ,  $p < 0.001$ ),  $-0.05$  (95% CI,  $-0.02$  to  $-0.07$ ,  $p < 0.05$ ) and  $-0.05$  (95% CI,  $0.02$  to  $0.08$ ,  $p < 0.05$ ) in the CLD, preterm and term control groups.

RV relaxation time 'IVRT' was another surrogate marker of pulmonary arterial pressure that altered with hypoxia. IVRT increased by  $14.3 \text{ ms}$  (95% CI,  $8.2$  to  $14.3 \text{ ms}$ ,  $p < 0.05$ ) in the CLD group,  $8.6 \text{ ms}$  (95% CI,  $4.0$  to  $12.6 \text{ ms}$ ,  $p < 0.05$ ) in the preterm control group and by  $12.8 \text{ ms}$  (95% CI,  $8.4$  to  $17.2 \text{ ms}$ ,  $p = 0.001$ ) in the term control group after exposure to 12% oxygen.

There were no statistically significant changes in the end-systolic strain after the hypoxic challenge in either of the groups. The end-systolic strain remained within normal limits throughout the hypoxic challenge test in all three groups.

Cardiac index, which is a marker of the left ventricular output, increased by  $0.2 \text{ Lmin}^{-1}\text{m}^{-2}$  (95% CI, 0.04 to  $0.35 \text{ Lmin}^{-1}\text{m}^{-2}$ ,  $p < 0.01$ ) after inhalation of 12% oxygen in those with CLD but did not alter significantly in the control groups. The rest of the echocardiographic markers of left ventricular function did not alter with hypoxia.

Table 4.7 Baseline echocardiographic parameters of left and right ventricular function

Echocardiographic indices	Mean (SD)			Significance
	CLD	Preterm control	Term	
<b>LV indices</b>				
LVOT diameter (cm)	1.5 (0.16)	1.6 (0.15)	1.7 (0.20)	0.001 <sup>§§,†††</sup>
LV VTI (cm)	21.6 (3.9)	22.1 (3.4)	23.3 (3.7)	0.200
HR (beats/min)	73.5 (11.6)	72.0 (11.8)	72.1 (8.7)	0.851
Cardiac index (L//min/m <sup>2</sup> )	1.6 (0.41)	1.6(0.41)	1.7 (0.36)	0.364
LV PEP (ms)	65.2 (16.2)	68.6 (13.0)	62.4(16.4)	0.288
Mitral E velocity (m/s)	1.0 (0.13)	1.0 (0.17)	0.95 (0.11)	0.310
Mitral A velocity (m/s)	0.53 (0.11)	0.51(0.13)	0.49 (0.87)	0.523
Mitral E:A	2.0 (0.47)	2.05 (0.45)	2.0 (0.35)	0.637
MMA Ve' (m/s)	0.16 (0.03)	0.14(0.02)	0.15 (0.02)	0.140
LMA Ve' (m/s)	0.18 (0.04)	0.17 (0.03)	0.18 (0.03)	0.758
Mitral Ve' (m/s)	0.17 (0.03)	0.16 (0.02)	0.16 (0.02)	0.058
E:e'	6.1 (1.0)	6.4 (1.2)	6.0 (0.96)	0.332
LV Ds' (mm)	11.0 (1.95)	11.0 (2.2)	11.6 (2.0)	0.463
LV Vsbl (cm/s)	6.3 (1.4)	6.5 (2.0)	6.7 (1.8)	0.673
LV Vebl (cm/s)	14.0 (2.0)	13.9 (2.2)	14.0 (2.7)	0.991
LV Vabl (cm/s)	2.5 (1.2)	2.7 (0.93)	14.0 (2.7)	0.800
LV T <sub>Q-vs</sub> (ms)	89.6 (14.6)	112.7 (42.4)	94.3 (17.7)	0.006 <sup>†,†††</sup>
LV IVA (cm/s <sup>2</sup> )	1.09 (0.47)	1.06 (0.52)	0.87 (0.32)	0.199
LV Ssbl (%)	-21.4 (5.8)	-22.6 (7.5)	-23.7 (5.7)	0.432
LV Ssal (%)	-13.0 (3.8)	-13.1 (4.9)	-15.1 (4.2)	0.148
<b>RV Indices</b>				
RV Ds' (mm)	18.0 (3.3)	18.4 (2.1)	17.6 (2.4)	0.487
RV Vsbl (cm/s)	8.4 (1.9)	8.9 (1.4)	8.8 (2.0)	0.586
RV Vebl (cm/s)	11.4 (2.8)	10.7 (2.5)	10.3 (2.5)	0.257
RV Vabl (cm/s)	4.3 (2.0)	5.4 (2.2)	4.7 (2.0)	0.107
RV T <sub>Q-vs</sub> (ms)	178.5 (27.9)	175.4 (26.4)	177.9 (26.3)	0.895
RV IVA (cm/s <sup>2</sup> )	1.3 (0.64)	1.4 (0.66)	1.0 (0.37)	0.021 <sup>†††</sup>
RV Ssbl (%)	-34.4 (9.5)	-33.6 (8.4)	-30.9 (9.1)	0.290
RV Ssal (%)	-24.0 (8.2)	-22.3 (9.5)	-21.0 (6.7)	0.390
<b>Surrogate markers for PAH</b>				
TR vel (m/s)	2.1 (0.25)	2.1 (0.36)	2.0 (0.20)	0.394
PR vel (m/s)	0.77 (0.13)	0.78 (0.16)	0.76 (0.15)	0.776
PA AT (ms)	127.4 (19.9)	127.5 (18.2)	122.6 (19.8)	0.535
PA ET (ms)	316.8 (27.4)	310.2 (28.5)	320.9 (23.0)	0.285
AT:ET	0.40 (0.05)	0.41 (0.05)	0.38 (0.05)	0.073
IVRT (ms)	53.2 (9.5)	52.6 (11.9)	52.3 (11.9)	0.961

<sup>§§</sup> p<0.001 (CLD vs. term control), <sup>†††</sup> p<0.05 (preterm control vs. term control), <sup>†</sup> p<0.05 (CLD vs. preterm control)

Table 4.8 Changes in echocardiographic parameters with hypoxia in children with chronic lung disease (CLD group)

<b>Echocardiographic Indices</b>	<b>21% oxygen Mean (SD)</b>	<b>15% oxygen Mean (SD)</b>	<b>12% oxygen Mean (SD)</b>	<b>p value</b>
<b>LV indices</b>				
Cardiac index (L min <sup>-1</sup> m <sup>-2</sup> )	1.6 (0.41)	1.7 (0.45)	1.8 (0.57)	0.355
LV PEP (ms)	65.2 (16.2)	62.2 (14.5)	60.4 (13.9)	0.901
Mitral E:A	2.0 (0.47)	1.9 (0.58)	1.8 (0.48)	0.612
Mitral Ve' (m s <sup>-1</sup> )	0.17 (0.03)	0.16 (0.03)	0.17 (0.04)	0.623
Mitral E: Ve'	6.1 (1.04)	6.1 (1.0)	6.0 (1.2)	0.913
<b>RV indices</b>				
RV Ds' (mm)	18.0 (3.3)	17.9 (3.3)	18.1 (3.5)	0.981
RV Vsbl (cm s <sup>-1</sup> )	8.4 (1.9)	8.8 (1.8)	8.9 (2.0)	0.585
RV Vebl (cm s <sup>-1</sup> )	11.4 (2.8)	11.7 (2.0)	12.3 (2.7)	0.468
RV Vabl (cm s <sup>-1</sup> )	4.3 (2.0)	4.7 (1.9)	5.8 (2.2)	0.222
RV T <sub>q-vs</sub> (ms)	178.5 (27.9)	174.2 (25.8)	166.5 (24.8)	0.227
RV IVA (cm s <sup>-2</sup> )	1.3 (0.64)	1.3 (0.62)	1.4 (0.75)	0.373
RV Ssbl (%)	-34.4 (9.5)	-32.7 (9.0)	-33.5 (10.3)	0.800
RV Ssal (%)	-24.0 (8.2)	-24.0 (6.1)	-24.7 (6.3)	0.915
<b>Surrogate markers for PAH</b>				
TR velocity (m s <sup>-1</sup> )	2.1 (0.25)	2.2 (0.26)	2.4 (0.33)	<0.001 <sup>§§</sup>
PR end-diastolic velocity (m s <sup>-1</sup> )	0.77 (0.13)	0.90 (0.16)	0.98 (0.26)	<0.001 <sup>§§</sup>
PA AT (ms)	127.4 (19.9)	115.9 (17.4)	103.6 (21.0)	<0.001 <sup>§§</sup>
AT:ET	0.40 (0.05)	0.37 (0.04)	0.33 (0.06)	<0.001 <sup>§§</sup>
RV relaxation time 'IVRT' (ms)	53.2 (9.5)	64.4 (17.2)	65.7 (18.9)	0.011 <sup>†,††</sup>

<sup>§§</sup> p<0.001 (21% vs. 12%), <sup>†</sup> p<0.05 (12% vs. 15%), <sup>††</sup> p<0.05 (21% vs. 12%)



Table 4.9 Changes in echocardiographic parameters with hypoxia in preterm children without chronic lung disease (Preterm control group)

<b>Echocardiographic Indices</b>	<b>21% oxygen Mean (SD)</b>	<b>15% oxygen Mean (SD)</b>	<b>12% oxygen Mean (SD)</b>	<b>p value</b>
<b>LV indices</b>				
Cardiac index (L min <sup>-1</sup> m <sup>-2</sup> )	1.6 (0.41)	1.6 (0.51)	1.6 (0.44)	0.890
LV PEP (ms)	68.6 (13.0)	68.5 (9.4)	67.4 (10.4)	0.881
Mitral E:A	2.1 (0.45)	1.9 (0.43)	1.8 (0.36)	0.037 <sup>††</sup>
Mitral Ve'	0.16 (0.02)	0.15 (0.02)	0.15 (0.02)	0.495
Mitral E: Ve'	6.4 (1.2)	6.3 (1.3)	6.4 (1.4)	0.958
<b>RV indices</b>				
RV Ds' (mm)	18.4 (2.1)	18.4 (2.5)	18.4 (1.9)	1.00
RV Vsbl (cm s <sup>-1</sup> )	8.9 (1.4)	8.9 (1.8)	8.8 (1.50)	0.974
RV Vebl (cm s <sup>-1</sup> )	10.7 (2.5)	10.8 (2.6)	11.0 (2.7)	0.883
RV Vabl (cm s <sup>-1</sup> )	5.4 (2.2)	5.2 (1.7)	5.6 (2.3)	0.363
RV T <sub>q-vs</sub> (ms)	175.4 (26.4)	173.9 (25.4)	170.1 (26.0)	0.705
RV IVA (cm s <sup>-2</sup> )	1.4 (0.66)	1.2 (0.49)	1.1 (0.47)	0.298
RV Ssbl (%)	-33.6 (8.4)	-35.3 (10.6)	-34.7 (7.8)	0.739
RV Ssal (%)	-22.3 (9.5)	-23.1 (9.5)	-23.6 (9.6)	0.864
<b>Surrogate markers for PAH</b>				
TR velocity (m s <sup>-1</sup> )	2.1 (0.36)	2.2 (0.35)	2.3 (0.30)	0.183
PR end-diastolic velocity (m s <sup>-1</sup> )	0.78 (0.16)	0.80 (0.15)	0.91 (0.20)	0.014 <sup>††</sup>
PA AT (ms)	127.5 (18.2)	120.8 (15.3)	111.4 (18.3)	0.002 <sup>§§</sup>
AT:ET	0.41 (0.05)	0.39 (0.05)	0.37 (0.07)	0.037 <sup>††</sup>
RV relaxation time 'IVRT' (ms)	52.6 (11.9)	55.2 (13.7)	60.5 (11.3)	0.040 <sup>††</sup>

<sup>††</sup> p<0.05 (21% vs. 12%), <sup>§§</sup> p<0.001 (21% vs. 12%)

Table 4.10 Changes in echocardiographic parameters with hypoxia in term-born children

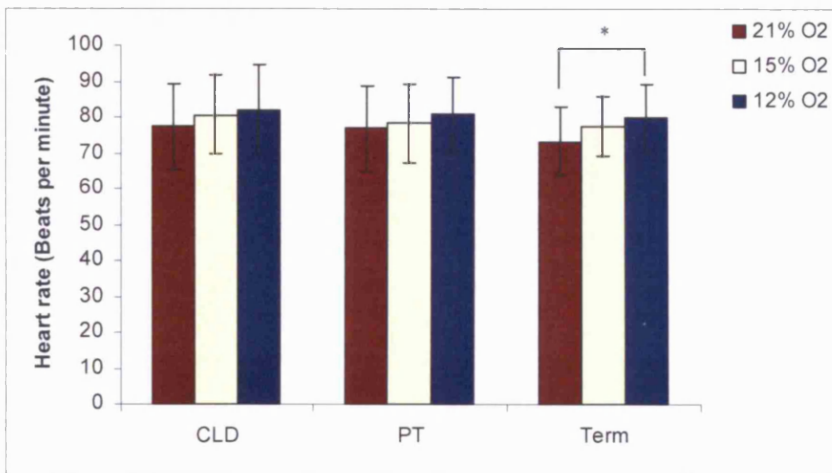
(Term control group)

<b>Echocardiographic Indices</b>	<b>21% oxygen Mean (SD)</b>	<b>15% oxygen Mean (SD)</b>	<b>12% oxygen Mean (SD)</b>	<b>p value</b>
<b>LV indices</b>				
Cardiac index (L min <sup>-1</sup> m <sup>-2</sup> )	1.7 (0.36)	1.7 (0.39)	1.7 (0.48)	0.802
LV PEP (ms)	62.4 (16.4)	65.8 (14.8)	65.0 (16.3)	0.694
Mitral E:A	2.0 (0.35)	1.8 (0.36)	1.7 (0.29)	0.006 <sup>††</sup>
Mitral Ve'	0.16 (0.02)	0.15 (0.02)	0.15 (0.02)	0.124
Mitral E: Ve'	6.0 (1.0)	6.2 (1.1)	6.3 (1.1)	0.546
<b>RV indices</b>				
RV Ds' (mm)	17.6 (2.4)	17.8 (2.7)	18.5 (3.0)	0.418
RV Vsbl (cm s <sup>-1</sup> )	8.8 (2.0)	9.1 (2.2)	9.3 (1.9)	0.615
RV Vebl (cm s <sup>-1</sup> )	10.3 (2.5)	10.4 (2.6)	11.1 (2.6)	0.412
RV Vabl (cm s <sup>-1</sup> )	4.7 (2.0)	4.6 (1.8)	5.6 (2.4)	0.101
RV T <sub>q-vs</sub> (ms)	177.9 (26.3)	180.6 (26.6)	170.7 (28.0)	0.341
RV IVA (cm s <sup>-2</sup> )	1.0 (0.37)	1.1 (0.35)	1.0 (0.36)	0.306
RV Ssbl (%)	-30.9 (9.1)	-32.9 (10.9)	-35.1 (10.9)	0.287
RV Ssal (%)	-21.0 (6.7)	-22.7 (6.0)	-22.5 (8.4)	0.576
<b>Surrogate markers for PAH</b>				
TR velocity (m s <sup>-1</sup> )	2.0 (0.20)	2.1 (0.22)	2.3 (0.16)	<0.001 <sup>§§</sup>
PR end-diastolic velocity (m s <sup>-1</sup> )	0.76 (0.15)	0.84 (0.22)	0.88 (0.20)	0.051
PA AT (ms)	122.6 (19.8)	111.9 (17.6)	103.4 (23.3)	0.002 <sup>††</sup>
AT:ET	0.38 (0.05)	0.36 (0.05)	0.33 (0.08)	0.017 <sup>††</sup>
RV relaxation time 'IVRT' (ms)	52.3 (11.9)	56.2 (14.0)	65.6 (14.4)	0.001 <sup>§</sup>

<sup>††</sup> p<0.01 (21% vs. 12%), <sup>§§</sup> p<0.001 (21% vs. 12%), <sup>††</sup> p<0.05 (21% vs 12%),

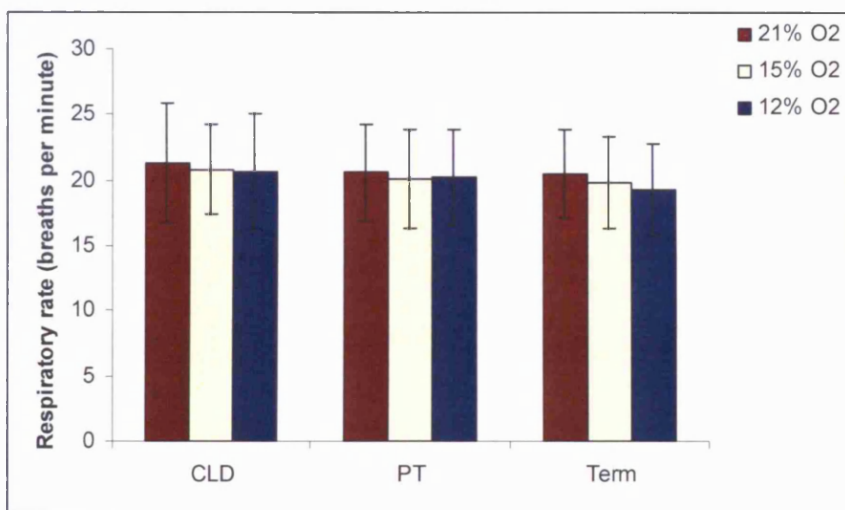
<sup>§</sup> p<0.001 (21% vs. 15%)

Figure 4.15 Bar diagram showing change in heart rate with hypoxia



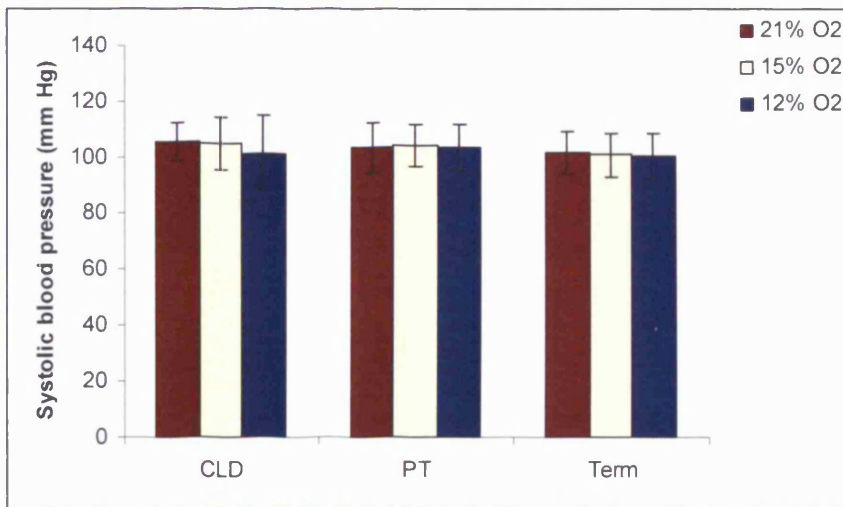
\*  $p < .05$

Figure 4.16 Bar diagram showing change in respiratory rate with hypoxia



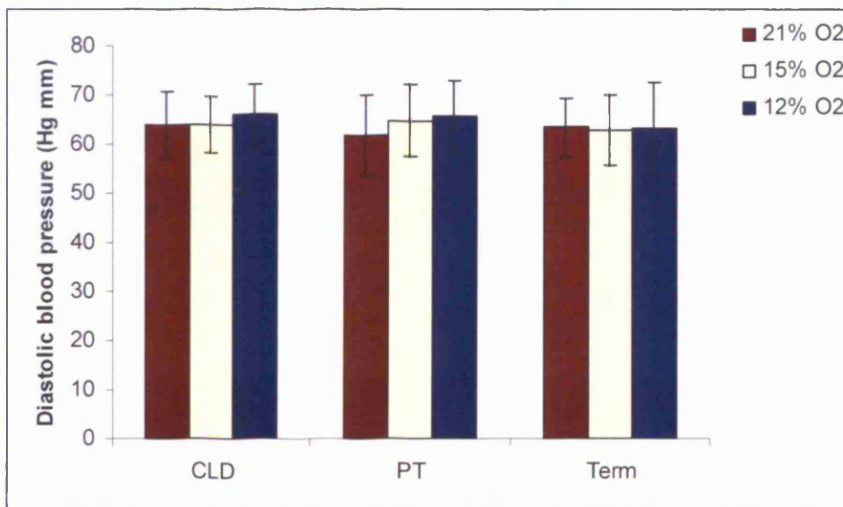
There was no significant difference between groups at baseline or after hypoxia. Respiratory rate did not alter significantly in either of the groups with hypoxia.

Figure 4.17 Bar diagram showing change in systolic blood pressure with hypoxia



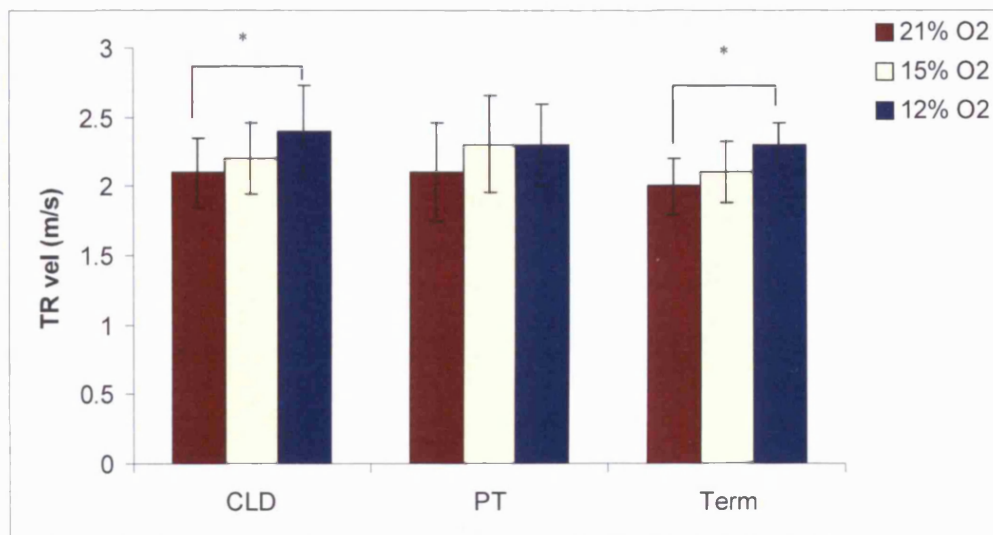
There was no significant difference between groups at baseline or after hypoxia. Systolic blood pressure did not alter significantly in either of the groups with hypoxia.

Figure 4.18 Bar diagram showing change in diastolic blood pressure with hypoxia



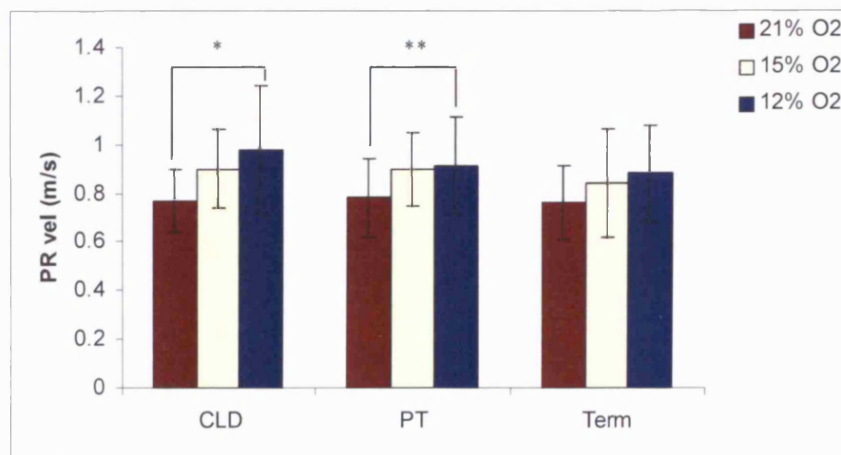
There was no significant difference between groups at baseline or after hypoxia. Diastolic blood pressure did not alter significantly in either of the groups with hypoxia.

Figure 4.19 Bar diagram showing change in tricuspid regurgitation systolic velocity with hypoxia



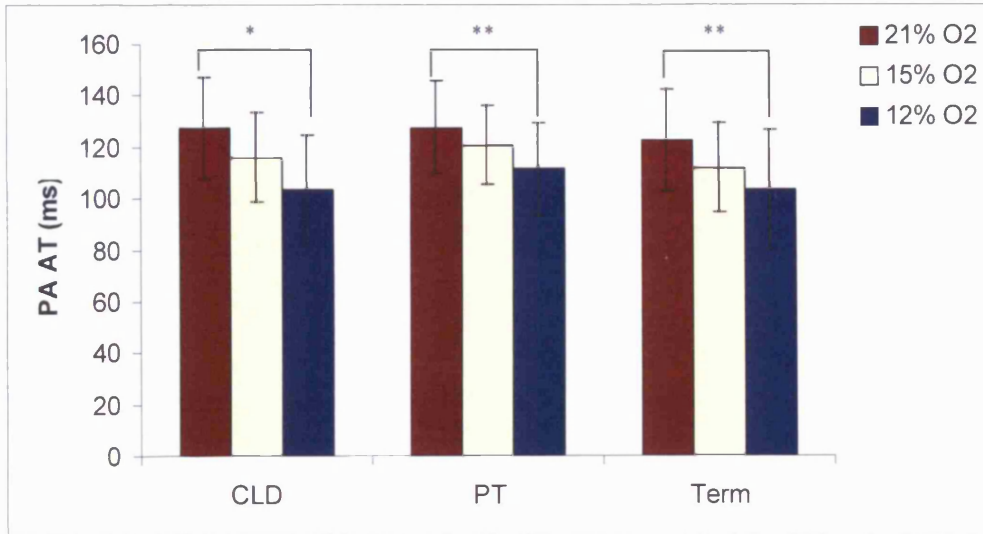
\*  $p < 0.001$

Figure 4.20 Bar diagram showing change in pulmonary regurgitation end-diastolic velocity with hypoxia



\*  $p = 0.001$ , \*\*  $p < 0.05$

Figure 4.21 Bar diagram showing change in pulmonary arterial acceleration time, AT with hypoxia



\*  $p < 0.001$ , \*\*  $p = 0.002$

Figure 4.22 Bar diagram showing change in AT:ET with hypoxia

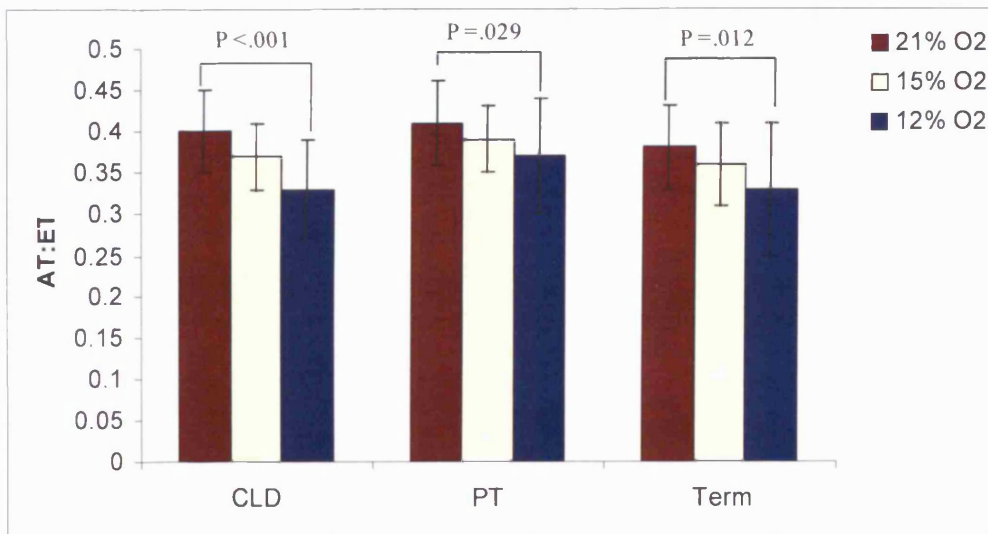
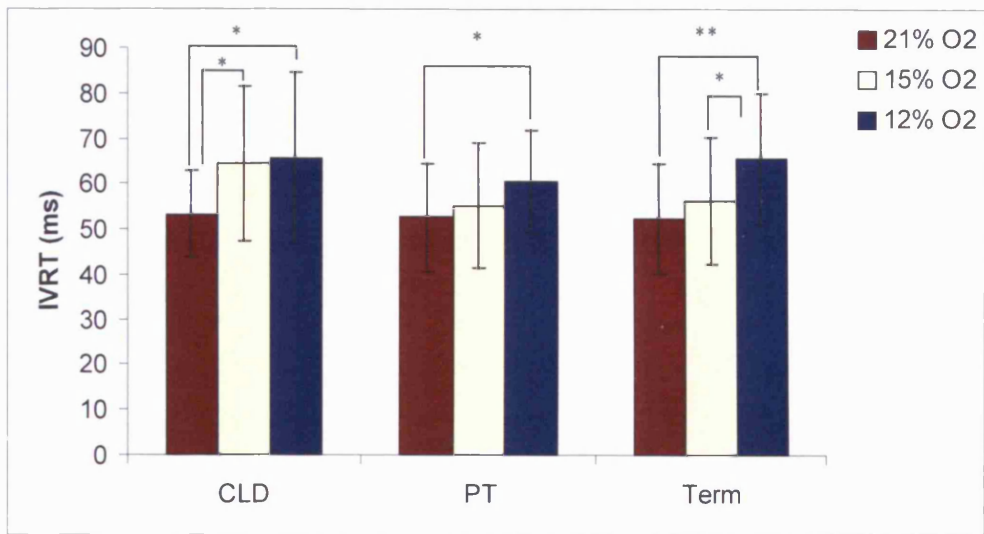
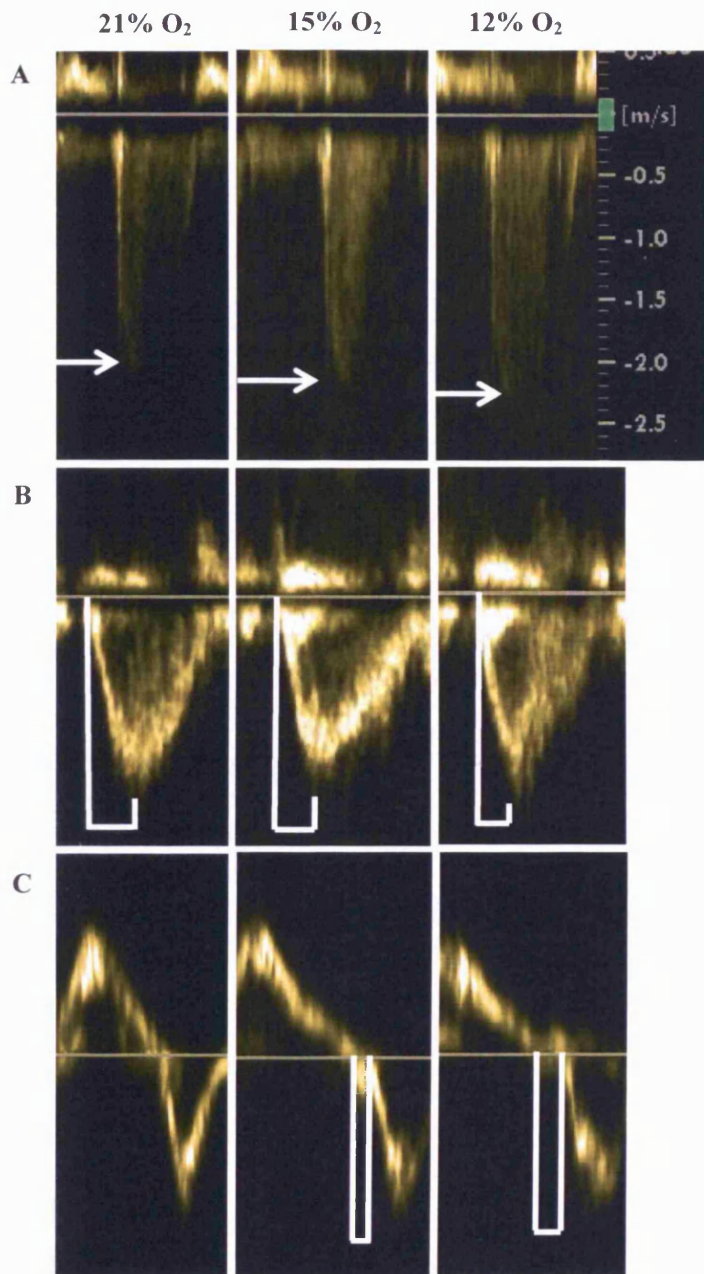


Figure 4.23 Bar chart showing change in RV relaxation time 'IVRT' with hypoxia



\* p<0.05, \*\* p=0.001

Figure 4.24 Figure demonstrating an example of the change in surrogate markers of pulmonary arterial pressure during the 'hypoxic challenge'



A. Velocity of the tricuspid regurgitation increased with increasing hypoxia  
B. Pulmonary arterial acceleration time decreased with increasing hypoxia  
C. Right ventricular relaxation time 'IVRT' increased with increasing hypoxia



#### **4.4 Discussion**

To my knowledge, this is the first study that explores cardiovascular response to acute hypoxia in school aged children who had CLD in infancy. This study gives an insight into the myocardial function in ex-preterm school-aged children who are clinically asymptomatic.

Despite its limitations, at present, echocardiography remains the best available non-invasive diagnostic tool for monitoring ventricular function and for the early diagnosis of PAH in children with CLD. Other non-invasive methods such as CT and MRI can be used to measure RV volume but are more expensive and are not readily available. Claustrophobia can also be an issue with young children undergoing MRI study.

Specific data on echocardiographic parameters of RV function and pulmonary hypertension in children is currently limited.

##### Baseline right ventricular function

Due to the nature of the right ventricular anatomy, it is not convenient to quantify RV function precisely by using conventional 2-D and blood flow Doppler echocardiography. Therefore, I used myocardial velocity imaging to quantify RV long axis systolic and diastolic function. I used the measurements from the term-born control population in our study as a comparison to assess RV myocardial function in those born prematurely with and without the history of CLD in infancy.

RV annular displacement, a marker of global RV systolic function was similar to that of the preterm and term-born controls. Similarly, the peak systolic and diastolic velocities were also similar to the control groups.

The statistical difference in the RV isovolumic acceleration between the preterm and the term control groups is unlikely to have any clinical significance as the indices are within normal limits and there is no 'trend' in the differences between the term and the preterm groups. Besides, the reproducibility of this index is not reliable (Joshi 2010).

RV systolic strain in our study population was normal at baseline. The values of RV systolic strain were comparable to the published data on healthy children (4-16 year old) (Weidemann 2002). In an adult study, non-invasively measured RV peak systolic strain has been shown to correlate well with invasively measured RV haemodynamics in patients with normal LV function (Rajagopalan 2008). In my study, none of the children had LV dysfunction clinically or echocardiographically. Therefore, the normal RV strain in these children should indicate normal RV haemodynamics at rest.

The RV myocardial velocity, displacement indices and strain indices were similar in those with CLD of prematurity and the preterm and term-born control population. This indicates that there was no evidence of RV systolic or diastolic dysfunction in the children with CLD at rest.

### Baseline left ventricular function

The LV outflow tract diameter was smaller in the children with CLD compared to the children in the control groups but cardiac index was within normal limits in all three groups and the difference between the groups was not statistically significant.

Left ventricular filling pressure is an important marker of LV diastolic function. Trans-mitral E and A velocities were measured to assess LV filling pattern. The mean ratio of E:A was  $>1$  in all three groups of children implying normal LV filling pressure.

I also used the myocardial velocity imaging to measure LV annular displacement. The displacement of the mitral annulus during systole provides a surrogate measure of the LV myocardial long axis systolic function. There was no statistically significant difference between the LV annular displacements between the children with CLD and those in the control groups.

The normal cardiac index and normal ratio of the mitral inflow velocities (E:A  $>1$ ) along with normal LV displacement indicate normal LV global systolic function in the children with CLD.

Left ventricular filling pattern (E:A) reflects early diastolic changes and the ratio of early diastolic mitral inflow velocity to early diastolic velocity of the mitral annular motion, E:Ve' has been shown to correlate well with mean ventricular filling pressure. This has been validated in adults as a useful tool to assess LV diastolic function in adults (Sohn 1997; Ommen 2000). In one adult study, E:Ve'  $>10$  was shown to correlate with raised filling pressure when lateral Ve' was used (Nagueh 1997) whereas another study has shown that E:Ve' ratio of  $>15$  correlated with elevated filling pressure when medial Ve' was used

.(Ommen 2000). As it is not yet clear whether the medial or the lateral mitral annular velocity is more useful for diagnosing diastolic dysfunction, I opted to measure both medial and lateral mitral annular velocities and used the average of the medial and lateral mitral annular e' velocity as mean Ve' to calculate the ratio E:Ve'. In this study, all three study groups had mean E:Ve' ratio of <7. Thus there was no evidence of LV global diastolic dysfunction in children with CLD.

In order to assess the regional LV myocardial function, I measured the systolic and diastolic velocities of the basal segment of the LV wall using myocardial velocity imaging. I also measured the LV systolic strain which is a deformation index at the basal and the apical wall of the LV. We used the data from our term-born control population as reference values for healthy subjects. On comparing the LV myocardial systolic and diastolic velocities between different groups, I did not find any difference between the values in the children with CLD to the preterm and term-born controls. Similarly, LV myocardial strain at the apical and the basal segments were also similar between the groups indicating normal deformation indices.

I noted that the mean value for the time interval between QRS complex and peak systolic velocity ( $T_{Q-v_s}$ ) was lower in the preterm group compared to those with CLD and term-born controls.  $T_{Q-v_s}$  is a marker of electromechanical delay in the LV systolic function. However, as there was no trend of this time interval being longer in the children with CLD compared to the control groups, and given the evidence that this echocardiographic index has a wide observer variation (Joshi 2010), I think that this difference may be a result of a type I error due to multiple comparisons and is unlikely to be of clinical significance.

Thus, I noted that at school age, ex-preterm children with CLD had normal global and regional systolic and diastolic LV function at rest.

### Cardiovascular response to hypoxia

Following hypoxic stimulus, I noticed a clear physiological response in all three groups of children. In healthy adult population, Ingram et al had reported that arterial oxygen saturation fell from  $98 \pm 0.2\%$  to  $83 \pm 1.7\%$  following exposure to 12 % oxygen in a hypoxic chamber (Ingram 2010). The drop in oxygen saturation from baseline to the end of hypoxic challenge with 12% oxygen in our study groups ( $99.3 \pm 0.8\%$  to  $81.9 \pm 5.4\%$  in CLD,  $98.6 \pm 1.0\%$  to  $85.1 \pm 3.3\%$  in preterm CLD and  $98.8 \pm 0.9\%$  to  $84.7 \pm 3.6\%$  in term control) were similar to that reported by Ingram et al. The fact that the children had all responded by dropping their oxygen saturation after exposure to 15% oxygen and 12% oxygen, we can be certain that the desired physiological effect had been achieved.

Although those with CLD had a higher mean pulmonary arterial systolic pressure at baseline and after hypoxic stimulus, this difference did not reach statistical significance compared to other groups even after hypoxic stimulus. Similarly, PA AT and AT:ET ratio shortened in all three groups of children following hypoxic stimulus. This reflects the increasing PA pressure with acute hypoxia. However, the difference in PA AT or the AT:ET ratio was not significantly different between those with CLD and the control population.

In addition to the above methods, which have been traditionally used to estimate pulmonary arterial pressure, I also used the more advanced tissue Doppler methods to overcome the drawbacks of the conventional methods as described in section 1.6. In adult patients, RV relaxation time has been shown to have a significant correlation with pulmonary artery systolic pressure ( $r=0.71$ ,  $p<0.01$ ) in adults undergoing cardiac catheterization for different cardiac conditions (Lindqvist 2006) This has not yet been validated in the paediatric

population. In the subjects in this study, I noted that RV relaxation time 'IVRT' increased with hypoxia as expected in all three groups of children. Again the difference in the RV relaxation time remained insignificant between groups even after the hypoxic stimulus. This indicates that although RV early diastolic pressure increased with exposure to acute hypoxia in all groups, the differences between groups were not statistically significant.

Some adult studies have also suggested that RV myocardial systolic strain correlates well with pulmonary vascular resistance (Dambrauskaite 2007; Rajagopalan 2008) Therefore I measured RV myocardial strain at the apical and the basal segments before and after hypoxic challenge test. I noted that the RV systolic strain at the basal segment increased following the hypoxic stimulus but remained stable in the preterm groups with and without CLD. The RV basal strain remained within normal limits in the CLD group as well as the control groups throughout the study, and the differences in the RV basal strain before and after the hypoxic stimulus was not statistically significant.

Thus, I conclude that the 8-12 year old children with CLD had normal cardiovascular function at rest and there was no echocardiographic evidence of subclinical pulmonary arterial hypertension even after exposure to acute hypoxia.

## **Limitations**

### Sample size

As there are no previous studies on pulmonary vascular response to hypoxia in ex-preterm children, I based my sample size on a study by Sartori et al (Sartori 1999). Sartori and colleagues had studied term-born young adults who had persistent pulmonary hypertension

rather than those with CLD of prematurity. Thus, the sample size was based on a study that had a different study population with regards to their disease pathology.

#### Hypoxic challenge test: Challenges and limitations

Induction of hypoxia in children was technically and ethically challenging. I used delivery of high-flow oxygen via a non-rebreathing mask to deliver 15% and 12% oxygen to the subjects. Although this method has been validated for hypoxic challenge tests, I had no means to measure the accuracy of the concentration of inhaled oxygen. Use of an environmental chamber to create hypobaric hypoxic environment would have been an ideal choice to replicate altitude induced hypoxia and would also have ensured continuous gas exchange without alteration in CO<sub>2</sub> level. The problem with using a hypoxic chamber was two-fold. Firstly, the chamber was not available on-site at Cardiff University and secondly, it takes about an hour to change the concentration of oxygen within the environmental chamber (Ingram 2010). To ensure, that the hypoxic challenge was conducted in a safe and controlled manner, the study was designed for step-wise hypoxic induction with initial exposure to 15% oxygen and then to 12% oxygen. This meant that use of environmental chamber would have been impractical.

It can be argued that if the children were allowed to achieve lower levels of oxygen saturation (eg <80%), the hypoxic stimulus may have unmasked subclinical pulmonary hypertension and RV dysfunction more accurately. However, in order to abide by the advice given by the ethics committee, I had to ensure that the children did not desaturate below 80% for >30 seconds.

#### Limitations of tissue Doppler echocardiography

Although myocardial velocity and strain imaging provide accurate measures of regional myocardial function, they are associated with a number of technical problems. Strain images

may be characterised by signal noise compromising image quality. It is also angle dependent and tissue direction should be within 30° of the beam for meaningful measurements to be made. As evident from our reproducibility study (Joshi 2010), the intraobserver variability for most of the myocardial velocity indices are acceptable (CV<15%), but interobserver variability for some of the more complex indices such as strain (CV >30%) and isovolumic acceleration (CV>40%) are sub-optimal.



**Chapter Five: Body Composition and its Subclinical  
Relationship to Lung Function and Cardiovascular  
Function**

## **5.1 Introduction**

Preterm children with CLD can often suffer from malnutrition in infancy (Huysman 2003). In infants with CLD, higher resting metabolic demand has been speculated to be one of the important causes of relative malnutrition (Kurzner 1988). As I discussed in Chapter 2, prematurely born children with CLD continue to have lower body weight compared with age and gender matched control population at school age. My data has also suggested that children who had CLD in infancy use higher ventilatory reserve to reach the same exercise limit as the preterm and term-born control children (Section 2.3.3.4). There is some evidence that malnutrition at a younger age is related to impaired lung function in school aged children with CLD (Bott 2006). Low birth weight has also been shown to be associated with impaired endothelial function in adulthood (Goodfellow 1998). It is not clear if school aged children with CLD continue to have altered body composition and whether this has any effect on their cardiopulmonary health.

Although body mass index (BMI) is widely used as a surrogate measure of adiposity, it is a measure of excess weight relative to height rather than excess body fat relative to height. BMI has in fact been shown to be strongly associated with fat mass index in those with BMI >85<sup>th</sup> centile but associated more with fat free mass index in those with BMI <50<sup>th</sup> centile (Freedman 2005). Bioelectrical impedance analysis (BIA) is a simple and quick method of assessing body composition. This method of measurement of impedance has been validated against DXA in healthy children (Sung 2001). In this study, the 95% limits of agreement between BIA and DXA methods were considered acceptable (-3.34 kg to -0.52 kg for fat mass and -0.39 g to 2.36 kg for fat free mass). BIA has also been validated in children with CLD (Bott 2006). In children with CLD, BIE was shown to overestimate fat mass (mean difference: 0.34 kg ± 2.06) and underestimate fat free mass (mean difference: -1.24 kg ± 3.32)

compared to DXA. The adjustments of fat mass and fat free mass for height as fat mass/height<sup>2</sup> = Fat mass index (FMI) and fat free mass/height<sup>2</sup> = Fat free mass index (FFMI) respectively have also been validated in children (Wells 2002). Using FMI and FFMI as indices for measurement of body composition, Fewtrell et al have demonstrated that prematurely born children had significantly lower FMI compared to term-born children at 8-12 years but there was no difference in FFMI between the two groups (Fewtrell 2004).

I aimed to study the differences in body composition parameters between preterm children with CLD compared with preterm and term-born controls, and also the relationship of body composition with respiratory and cardiovascular functions.

## **5.2 Methods**

### **5.2.1 Measurement of body composition**

All children had their body composition measured by bioimpedance analysis using a leg to leg body composition analyser (Tanita BC-418MA, UK). Prior to the measurement of the body composition, the children were asked to empty their bladder, remove their shoes, socks and heavy clothing, empty their pockets and it was ensured that they did not have any internal medical devices. One child with internal medical device/ implants was excluded from this part of the study. Height was measured using a stadiometer. The child's height and age were entered manually. The children were then asked to stand on the weighing platform of the body composition analyser and to grasp the hand grips with both hands until the analyser calculated the body weight, body mass index, percentage of total body fat, fat free mass and total body water.

Fat mass index (FMI) was calculated as fat mass/ht<sup>2</sup> and fat free mass index (FFMI) was calculated as fat free mass / ht<sup>2</sup>.

### Statistical analysis

Body composition parameters are presented as mean and standard deviation. The difference between the groups was tested using analysis of variance (ANOVA) and p<0.05 was considered to be significant.

Correlations between the body composition parameters and the pulmonary function or the cardiovascular parameters were tested using a Pearson's test. p value of <0.05 was considered to be statistically significant. This was tested across the study population and not for individual groups. Results are presented as Pearson's correlation coefficient (r) and the p value.

## **5.3 Results**

### **5.3.1 Body composition**

One child from the CLD group did not have the body impedance measured because of an internal medical device (cochlear implant).

The results of the body composition are summarised in Table 5.1.

Children with the history of neonatal CLD were 16% lighter than the preterm controls and 24% lighter than the term-born controls (p<0.05) but they had achieved similar height to the

children in the control groups. Body mass index was 10% lower in those with CLD compared with PT controls and 13% less compared with the term-born controls. Those with CLD also had lower fat% (23% less vs preterm controls and 22% less vs term controls), lower fat mass index (38% less vs preterm controls and 45% less vs term controls), lower fat free mass index (4% less vs preterm controls and 5% less vs term controls), lower total body water (11% less vs preterm controls and 16% less vs term controls) and lower basal metabolic rate (4% less vs preterm controls and 7% less vs term controls). Although the mean values for the above parameters were less in the CLD group, apart from the body weight, none of the other differences in the body composition (FMI, FFMI, total body water) were statistically significant.

Table 5.1 Results of the measurement of body composition

Parameters	CLD (n=27)	Preterm control (n=32)	Term control (n=30)	ANOVA
Weight	30.7 (8.9)	35.6 (10.9)	38.0 (13.7)	0.053 <sup>††</sup>
Body mass index (kg m <sup>-2</sup> )	15.8 (2.9)	17.4 (3.7)	17.9 (4.6)	0.122
Fat %	17.6 (4.4)	21.7 (6.2)	21.5 (8.6)	0.044*
Fat mass index (kg m <sup>-2</sup> )	2.9 (1.2)	4.0 (2.2)	4.2 (2.9)	0.068
Fat free mass index (kg m <sup>-2</sup> )	13.0 (1.9)	13.5 (1.7)	13.7 (1.9)	0.341
Total body water (kg)	18.3 (4.8)	20.1 (4.7)	21.2 (5.3)	0.106
Basal metabolic rate (kcal)	1180.9 (180.7)	1225.3 (184.5)	1262.7 (205.6)	0.276

Results are presented as mean (SD)

<sup>††</sup> p<0.05 (CLD vs. Term)

\* Tukey's post hoc test did not show significant difference between the individual groups.

### 5.3.2 Subclinical relationship between body composition and lung function

Across the study population, BMI was positively related to total duration of exercise, maximum load at peak exercise, maximum voluntary ventilation (MVV) and ventilatory reserve (VR). Maximum load at peak exercise was correlated with FFMI, but not with FMI. MVV and VR were correlated to both FMI and FFMI. Oxygen consumption at peak exercise ( $\dot{V}O_2$ ) had negative correlation with BMI. FEV<sub>1</sub> and alveolar diffusion capacity (DL<sub>CO</sub>) did not correlate with BMI (Table 5.2).

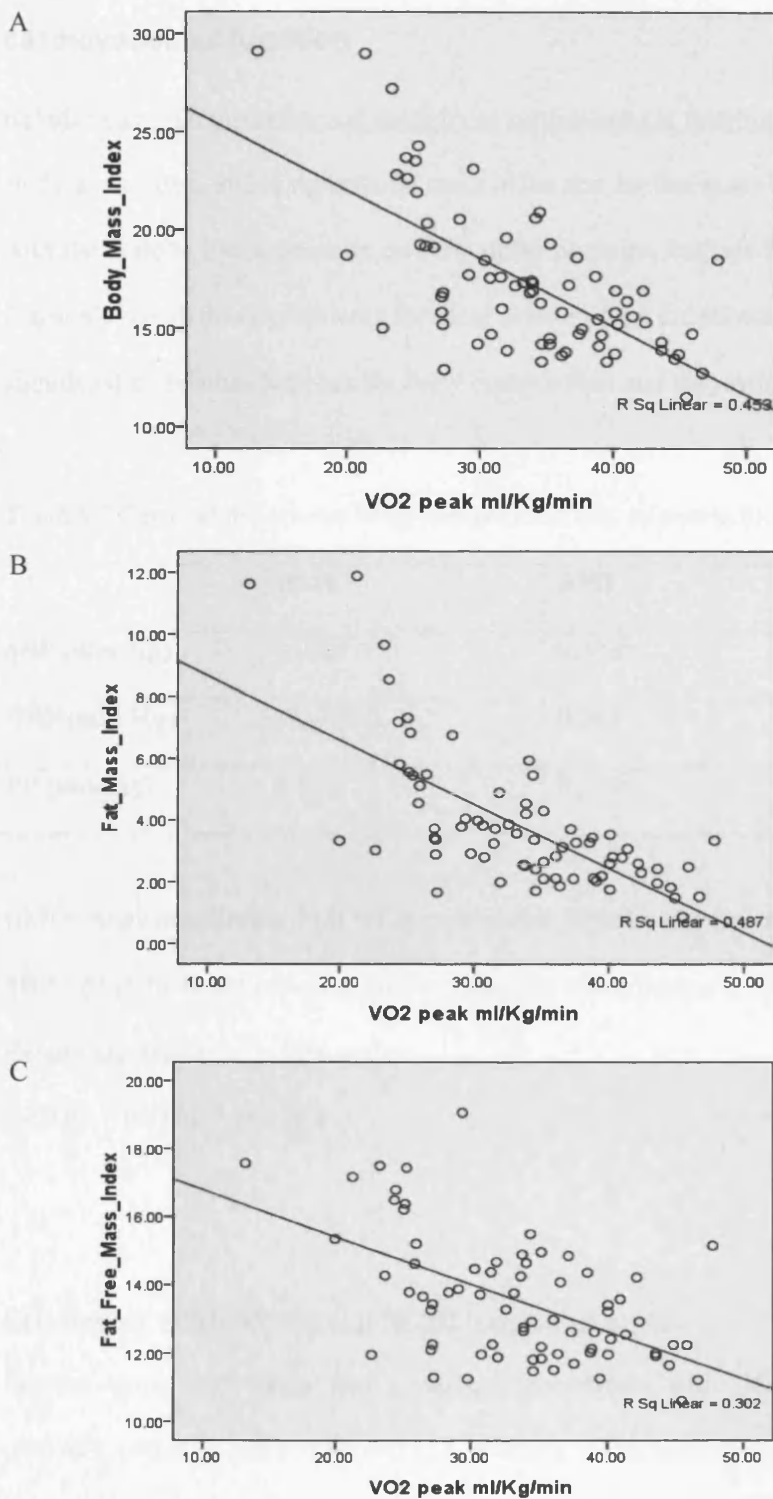
Table 5.2 Correlation between body composition and pulmonary function

	<b>FEV<sub>1</sub></b> (%)	<b>DL<sub>CO</sub></b> (%)	<b>ṠO<sub>2</sub></b> (ml/kg/min)	<b>Exercise</b> <b>Duration</b> (seconds)	<b>Load</b> (Watts)	<b>MVV</b>	<b>VR</b>
<b>BMI</b> (kg m <sup>-2</sup> )	0.093	0.156	-0.673 <sup>§</sup>	0.407 <sup>§</sup>	0.335 <sup>‡</sup>	0.463 <sup>§</sup>	0.305 <sup>‡</sup>
<b>FMI</b> (kg m <sup>-2</sup> )	0.046	0.107	-0.698 <sup>§</sup>	0.234 <sup>†</sup>	0.195	0.349 <sup>‡</sup>	0.287 <sup>†</sup>
<b>FFMI</b> (kg m <sup>-2</sup> )	0.137	0.198	-0.549 <sup>§</sup>	0.549 <sup>§</sup>	0.447 <sup>§</sup>	0.538 <sup>§</sup>	0.282 <sup>†</sup>

Results are expressed in Pearson's r

†p<0.05, ‡ p<0.01, § p<0.001

Figure 5.1 Scatter plot showing relationships between peak  $\dot{V}O_2$  and body composition



Relationships between peak  $\dot{V}O_2$  and A) body mass index, B) fat mass index C) fat free mass index



### 5.3.3 Subclinical relationship between body composition and markers of cardiovascular function

#### Relationship with physiological markers of cardiovascular function

Body mass index, including both fat mass index and fat free mass index moderately correlated with the systolic blood pressure and the pulse pressure, but not the diastolic blood pressure. Pearson's correlation coefficients for these relationships are shown in Table 5.3. There was no significant correlation between the body composition and the resting heart rate.

Table 5.3 Correlation between body composition and systemic blood pressure

	<b>BMI</b>	<b>FMI</b>	<b>FFMI</b>
<b>SBP (mm Hg)</b>	0.323 <sup>‡</sup>	0.338 <sup>‡</sup>	0.225 <sup>†</sup>
<b>DBP (mm Hg)</b>	-0.007	0.063	-0.066
<b>PP (mm Hg)</b>	0.326 <sup>‡</sup>	0.278 <sup>‡</sup>	0.282 <sup>‡</sup>

BMI = Body mass index, FMI = Fat mass index, FFMI = Fat free mass index

SBP = Systolic blood pressure, DBP = Diastolic blood pressure, PP = Pulse pressure

Results are expressed in Pearson's r

<sup>†</sup>p<0.05, <sup>‡</sup> p<0.01, <sup>§</sup> p<0.001

#### Relationship with biochemical tests and body composition

Fasting serum triglyceride had a positive correlation with BMI (r=0.368, p<0.01), FMI (r=0.443, p<0.001) and FFMI (r=0.313, p<0.01). As expected, high density lipoprotein had a negative correlation with BMI (r= -0.406, p<0.001), FMI (r= -0.300, p<0.01) and FFMI (r= -0.387, p<0.001). Fasting blood glucose and cholesterol were not associated with the BMI.

Table 5.4 Correlation between body composition and biochemical markers of cardiovascular function

	<b>BMI</b>	<b>FMI</b>	<b>FFMI</b>
<b>Fasting glucose</b>	0.210	0.148	0.227
<b>Cholesterol</b>	-0.028	0.023	-0.122
<b>Triglycerides</b>	0.368 <sup>‡</sup>	0.443 <sup>§</sup>	0.313 <sup>‡</sup>
<b>High density lipoprotein</b>	-0.406 <sup>§</sup>	-0.386 <sup>‡</sup>	-0.371 <sup>‡</sup>

Results are expressed in Pearson's r

<sup>†</sup>p<0.05, <sup>‡</sup>p<0.01, <sup>§</sup>p<0.001

Relationship with echocardiographic parameters of left ventricular function

BMI was noted to have a negative correlation with the cardiac output (r= -0.475, p<0.001). This relationship was noted both with FMI (r= -0.383, p<0.001) and with FFMI (r= -0.591, p<0.001). Mitral annular e velocity (Ve') at the septal wall also demonstrated a negative correlation with FMI (r= -0.225, p<0.05) but not with the FFMI. The other echocardiographic parameters for LV function did not demonstrate any relationship with the body composition.

Relationship with echocardiographic parameters of right ventricular function

RV annular displacement at rest was noted to be negatively correlated to FMI (r= -0.295, p<0.01). Similar negative relationship with FMI were also demonstrated by RV e velocity (r= -0.236, p<0.05) and RV end-systolic strain at rest (r=0.257, p<0.05) at the basal wall. There were no significant correlations with the FFMI. RV systolic velocity did not demonstrate any relationship with body composition.

#### Relationship with surrogate markers of pulmonary arterial pressure

Positive correlation was demonstrated between the TR maximum velocity and FMI ( $r=0.416$ ,  $p<0.001$ ) and FFMI ( $r=0.415$ ,  $p<0.001$ ). There was a clear negative correlation between FMI and pulmonary arterial acceleration time, AT ( $r=-0.385$ ,  $p<0.001$ ) as well as with the ratio of acceleration time to ejection time, AT: ET ( $r=-0.336$ ,  $p<0.001$ ). RV relaxation time 'IVRT' did not demonstrate any correlation with body composition.

#### **5.4 Discussion**

Previous studies have demonstrated that preterm infants without CLD tend to catch up term-born controls with their height and weight by 2 years of age (Carver 2001; Lucas 2001). The effect of CLD on catch-up growth has not yet been studied. My study has demonstrated that children with CLD continue to have lower body weight compared with the preterm and term-born control subjects at school age. It is also clear from the body composition data that the lower body weight in those with CLD is primarily contributed by their lower FMI rather than the FFMI. BMI in the children with CLD consisted of 18% FMI and 82% FFMI, whereas BMI in both the control groups consisted of 23% FMI and 77% FFMI. This is in keeping with the results from the study by Fewtrell et al. in which the authors reported significantly lower FMI ( $3.34 \text{ kg/m}^2 \pm 1.62$  vs  $3.89 \text{ kg/m}^2 \pm 1.74$ ,  $p < 0.005$ ) in 497 preterm children compared to that of 95 term controls at 8 to 12 years. The authors used DXA to study body composition in this study. FFMI was not significantly different the two groups in this study. The difference in body composition in preterm children with and without CLD was not explored by the authors.

Although there is no current evidence of a clear relationship between nutritional status and airway obstruction in adults with COPD, weight loss and low body weight have been shown

to be associated with decreased diffusing capacity (Engelen 1994; Engelen 1999). Furthermore, lower body weight, which is presumably related to lower fat free mass has been associated with higher mortality in adults with COPD (Landbo 1999).

A previous study on 4-8 year old children with CLD did not show any correlation between body composition at the time of the study with their pulmonary function (Bott 2006). However, Bott et al had not performed cardiopulmonary exercise test in the subjects in their study. The lack of relationship between BMI and FEV<sub>1</sub>/ or DL<sub>CO</sub> in my study may be because of normal FFMI in the subjects, including those with CLD. However, it is clear that those children with high BMI are likely to have lower  $\dot{V}O_2$  at peak exercise despite being able to exercise for a longer period. It is also interesting to note that although the duration of exercise is positively related to BMI (FMI and FFMI), exercise load is positively related only to FFMI and not with FMI.

The adverse effect of childhood obesity on increased cardiovascular morbidity and mortality in adulthood is clear from epidemiological studies (Berenson 1998; Reilly 2003; Bjorge 2008). The relationships between body composition and subclinical myocardial dysfunction in children have not yet been explored. In an adult study, 109 overweight (BMI 25-30 kg/m<sup>2</sup>) or obese (BMI>30 kg/m<sup>2</sup>) subjects without overt heart disease and 33 healthy controls with BMI<25 kg/m<sup>2</sup> underwent transthoracic echocardiography of the left ventricle (Wong 2004). The authors noted that although LV ejection fraction was similar between the subjects and the controls, there were significant differences in regional and global LV strain as well as systolic and early diastolic LV myocardial velocities (p<0.001). Mitral e' velocity and LV early diastolic velocity was lower (p<0.01) and the E/e' ratio was increased in the obese population compared to the healthy controls, signifying LV diastolic dysfunction. In this study, significant correlations were demonstrated between BMI and LV septal strain (r= -0.44,

p<0.001), LV myocardial systolic velocity ( $r = -0.59$ ,  $p < 0.001$ ) and LV myocardial early diastolic velocity ( $r = -0.46$ ,  $p < 0.001$ ). In my study, I have demonstrated that higher BMI was related to increased systolic blood pressure ( $r = 0.32$ ,  $p < 0.01$ ) in children. In the children in my study, cardiac output ( $r = -0.48$ ,  $p < 0.001$ ) and mitral early diastolic velocity ( $r = -0.23$ ,  $p < 0.05$ ) also correlated with FMI. In this study, none of the children had BMI>25. If these relationships are tested in children with wider range of BMI, including obese children, the subclinical correlation of obesity and LV dysfunction in childhood may become clearer.

In summary, as evident from the body composition data from my study, higher FMI seem to be a risk factor for impaired exercise capacity, impaired myocardial function and increased systemic as well as pulmonary hypertension. In view of these results, future studies designed to study the relationships between body composition and myocardial function in preterm children may clarify the role of malnutrition in cardiopulmonary health and thus help in long term management of nutritional and cardiopulmonary health in preterm children with and without CLD.

## **Limitations**

### Measurement of body composition

I used bioelectrical impedance analysis (BIA) to measure body composition in the subjects of this study. Although BIA is not a reference criterion for measurement of body composition, BIA is a non-invasive, quick and easy method which has been validated for good estimation of body fat in general. One potential drawback of BIA is that it may underestimate fat mass and overestimate fat free mass in obese subjects (Coppini 2005). The average BMI of each group of children was below the level of obesity. Therefore, this should not cause inaccuracy in the results of this study. BIA is also hydration dependent. Taking this into account, I

ensured that BIA was measured in the morning after voiding and also ensured that the subjects did not have caffeine prior to the measurement.

#### Subclinical relationships

The present study was powered to detect differences in outcomes other than body composition. Therefore, results presented in this section on body composition in children with CLD and its relationship with cardiorespiratory outcomes should be regarded as descriptive rather than conclusive.

## **Chapter Six: Summary and Conclusions**

## **6.1 Overview**

With improvements in neonatal care over the last two decades, there has been an epidemiological shift and a change in pathology of chronic lung disease of prematurity (CLD). Arrested lung growth and alveolar hypoplasia have replaced severe airway injury and fibrosis seen in children who had 'old CLD' (Coalson 2003). In recent years, the 'vascular hypothesis' of CLD has also been proposed (Abman 2001). In this thesis, I have presented the findings of a detailed study on respiratory, cardiovascular and nutritional status of 8-12 year old children who were born prematurely and had chronic lung disease in infancy, and compared the findings with age and gender matched preterm and term-born control population. I have also studied the effects of acute hypoxia on myocardial function and pulmonary vascular reactivity, and I have explored the subclinical relationships between nutritional, respiratory and cardiovascular functions. All the prematurely born children in this study who required mechanical ventilation after birth had received surfactant. Therefore, this population represents true CLD in the 'surfactant era'.

## **Summary of key findings**

### **Pulmonary function studies (Chapter two):**

- Children with a history of CLD had lower percent predicted values for FEV<sub>1</sub>, FEF<sub>25-75</sub> and PEF than the preterm and term-born control population, thus demonstrating evidence of airway obstruction.
- The drop in FEV<sub>1</sub> after exercise was more profound in those who had CLD in infancy than in the control groups. Children with CLD also demonstrated a greater response to



bronchodilator therapy compared to the two control groups, thus suggesting under-diagnosed exercise induced bronchoconstriction.

- $DL_{CO}$  and  $KCO$  adjusted for haemoglobin were significantly lower in the CLD group compared to preterm control and term control groups. These findings suggest impaired alveolar diffusion capacity in the CLD group.
- Children in the CLD group had significantly higher specific resistance, residual volume, and RV: TLC ratio than the control groups suggesting lung hyperinflation.
- During the cardiopulmonary exercise test, all three groups of children managed to achieve similar exercise load, exercise duration, maximum  $\dot{V}O_2$  and maximum  $\dot{V}CO_2$  at peak exercise. However, those in the CLD group demonstrated lower maximal voluntary ventilation and ventilatory reserve.
- Multiple linear regression demonstrated linear relationship between ventilatory reserve and gestational age at birth, duration of oxygen dependency,  $DL_{CO}$  corrected for haemoglobin, maximum respiratory rate during exercise, maximum load and peak  $\dot{V}O_2$ .
- Finally, prematurely born children who did not develop CLD in infancy i.e. preterm control population did not have any evidence of lung function limitation compared to the term-born population.

#### **Cardiovascular studies (Chapter four):**

- At baseline, indices for right ventricular function, surrogate markers for pulmonary arterial hypertension and left ventricular function were similar in the children who had CLD in infancy and the control groups.
- Arterial oxygen saturation decreased in all three groups of children in response to hypoxic stimulus but the response was greater in those with CLD (17% drop in the CLD V 13%

drop in the preterm and term controls after inhalation of 12% oxygen compared to baseline) (statistically not significant).

- At the end of the hypoxic challenge i.e. after inhalation of 12% oxygen for 20 minutes, lowest oxygen saturation reached was significantly lower in the CLD group compared to both the preterm and the term control groups.
- In response to the hypoxic stimulus, all three groups of children demonstrated indirect evidence of increased pulmonary arterial pressure: Increase in TR systolic velocity, PR end-diastolic velocity, shortening of pulmonary AT, reduction in AT:ET ratio and the increase in RV relaxation time 'IVRT'. The changes in these surrogate markers of pulmonary arterial pressure were statistically significant between the baseline and after inhalation of 12% oxygen. None of the indices were significantly different between the groups.

### **Body composition: (Chapter five)**

- Prematurely born children who had CLD in infancy were significantly lighter than the age and gender matched preterm and term-born controls.
- Although body mass index, including both fat mass index and fat free mass index were lower in the preterm CLD group compared to the control populations, these differences were not statistically significant.

## **6.2 General discussion**

### **6.2.1 Pulmonary function in children with chronic lung disease of prematurity**

It is already known from previous studies that CLD is associated with increased respiratory morbidity (Greenough 1996; Greenough 2005) and increased hospitalization (Cunningham 1991; Chye and Gray 1995) during infancy and early childhood. CLD is also associated with persistent small airway obstruction (Doyle 2006; Hennessy 2008; Fawke 2010) at school age, and impaired alveolar diffusion capacity in infancy (Balinotti 2010), childhood (Welsh 2010) and young adulthood (Vrijlandt 2006). There is limited evidence on reversibility of airway obstruction, exercise induced bronchospasm and exercise capacity in these children. Data on exercise capacity in children with CLD are inconsistent. Current scientific knowledge on association between perinatal factors and different aspects of pulmonary function is also limited.

In keeping with the previous studies (Balinotti 2010; Fawke 2010; Welsh 2010), children with CLD in this study had persistent airway obstruction and impaired diffusion capacity at 8-12 years of age, compared to the age and gender matched preterm and term-born controls. This study also demonstrated exercise induced bronchoconstriction in children with CLD, who had a greater response to inhaled bronchodilator than the preterm and term control groups. Despite significantly higher response to bronchodilator, those with CLD had lower post-bronchodilator FEV<sub>1</sub> than the children in the control groups. Oxygen uptake and carbon dioxide production at peak exercise in children with CLD were similar to the preterm and term-born controls. Although children with CLD were capable of reaching similar exercise load as the control populations, this was at the expense of using greater ventilatory reserve. This study has also demonstrated some evidence that neonatal factors such as gestational age

at birth and duration of oxygen dependency may affect respiratory outcome later in life. This needs to be explored further in future studies.

With increasing number of preterm survivors, there has been concern that these children may have impaired lung function and may develop COPD like disease as they grow older, especially if they smoke (Baraldi 2007). A recently published study on asymptomatic adult smokers has revealed that spirometric, plethysmographic and CO diffusion tests are useful in detecting signs of COPD in these clinically asymptomatic adult smokers. Decreased FEV<sub>1</sub>/FVC and increased RV/TLC were noted in heavy smokers although they did not have any clinical signs of COPD. Lung emphysema was seen in 17/50 chronic smokers and 10 of them had decreased DL<sub>CO</sub> (Nagelmann 2011). Given that the children with CLD in my study had significantly lower FEV<sub>1</sub>, CO diffusion capacity and increased RV/TLC, this study has added to the current evidence that prematurely born children with CLD may be at higher risk of developing COPD as adults.

### **6.2.2 Cardiovascular function in children with chronic lung disease of prematurity**

Premature birth is known to be associated with abnormal pulmonary vascular development (Hislop and Haworth 1990). Preterm infants with CLD have evidence of increased pulmonary arterial pressure (Evans and Archer 2001). Persistence of abnormal lung function in these children may be a potential risk factor for development of pulmonary hypertension and subclinical right ventricular dysfunction but there are limited data on cardiovascular follow-up of children with CLD. In view of the 'vascular hypothesis' suggesting that 'angiogenesis is necessary for alveolization during normal lung development, and that injury to the developing pulmonary circulation during a critical period of growth can also contribute to lung

hypoplasia' (Abman 2001), it is reasonable to assume that impaired lung function in prematurely born children would correlate with subclinical pulmonary hypertension and right ventricular dysfunction.

Historically, it has been assumed that precise assessment of right ventricular function using non-invasive methods is technically difficult. With recent technological advances, it is now possible to assess right ventricular function and pulmonary arterial pressure using 'myocardial velocity imaging'. Although myocardial velocity imaging has previously been used to study subclinical right ventricular dysfunction in adult patient with cystic fibrosis (Ionescu 2001) and chronic obstructive pulmonary disease (Caso 2001; Sabit 2010), this method has not been used to study subclinical myocardial dysfunction in children with CLD of prematurity. I used conventional blood-flow Doppler methods as well as new methods of tissue Doppler imaging or myocardial velocity imaging to assess right ventricular function and surrogate markers of pulmonary arterial hypertension at rest and after exposure to acute hypoxia.

Although 8-12 year old children with CLD had persistently limited lung function, their right and left ventricular function at rest was normal. The echocardiographic parameters for their myocardial function were similar to those of the preterm and term-born control population. The haemodynamic response to acute hypoxia in children with CLD was in general similar to that of children in the control groups but the children with CLD achieved lower oxygen saturation compared to the control population after hypoxic stimulation. Whether or not this has any clinical significance is not yet clear. There was no evidence of myocardial dysfunction or raised RV systolic pressure after exposure to acute hypoxia in either the CLD group or the control groups.

The physiological and biochemical markers of cardiovascular disease were normal in the children with CLD in this study. The results of the body composition suggest that lower fat mass index may have helped the children with CLD to maintain normal systemic blood pressure, blood lipids and normal myocardial function.

### **6.3 Clinical implications of this study**

- Prematurely born children with CLD continue to have impaired lung function at school age, and may benefit with clinical follow-up, including ‘pulmonary function tests’ even if they are clinically asymptomatic.
- If future studies confirm that children with CLD have subclinical reversible airway disease, the benefit of bronchodilator treatment in these children should be considered.
- In view of the evidence that children with CLD have impaired lung function and that they maintain their exercise level at the expense of their ventilatory reserve, these children should be actively discouraged to smoke tobacco as they grow into young adults.
- As children with CLD are at risk of developing right ventricular dysfunction secondary to pulmonary disease, these children may benefit with dietary advice to maintain relatively low fat mass without compromising their general weight.
- In view of the normal cardiovascular function in children with CLD in this study, there is no evidence for routine cardiovascular follow-up in these children if they remain asymptomatic. However, deteriorating pulmonary function may warrant cardiovascular assessment even in asymptomatic children.

#### **6.4 Future and ongoing research**

- Prospective longitudinal studies of pulmonary function and pulmonary vascular reactivity in prematurely born infants will be useful in determining the natural history of airway disease and pulmonary vascular resistance in patients with CLD of prematurity. Longitudinal studies would be the only way to determine whether or not pulmonary dysfunction in these children may lead to early aging of the lungs in young adulthood and whether or not this leads subclinical pulmonary hypertension.
- Although echocardiography is an extremely useful diagnostic tool for assessment of RV function and pulmonary arterial pressure, reproducibility of some of the more complex parameters such as deformation indices and isovolumic acceleration are sub-optimal at present. Magnetic resonance imaging (MRI) is now being explored to study RV function and pulmonary blood flow (Kondo 1992; Champion 2009). Further research on RV function and pulmonary haemodynamics in ex-preterm children using MRI is currently being undertaken within our department as well. Reproducibility of acquisition and analysis of images with these methods are not yet clear and need to be studied.
- Any future research on the pulmonary outcome of prematurity related lung disease should be focused on the need for clinical follow-up of apparently 'normal' preterm children with CLD and the role of bronchodilator therapy in subclinical airway obstruction and exercise induced bronchospasm in these children.
- The results of this thesis suggests that in children there may be subclinical relationships between body composition and cardiopulmonary exercise capacity, which is a measure of

pulmonary function, as well as left and right ventricular function. The current literature on body composition and its role on cardiopulmonary function in children with CLD are limited. Given the clear evidence of the relationship between fat mass index and triglycerides and the negative relationship with HDL, future studies on simultaneous measurement of body composition, preferably with a DXA scan, and vascular function may be useful to further clarify the relationship between nutritional status and cardiovascular function.

- Dietary habits of individuals play a major role on nutritional status and body composition. Assuming that children with CLD may have higher metabolic rates, their dietary intake may be crucial in maintaining their weight without increasing body fat to ensure optimal cardiorespiratory function. Ongoing research in our department includes a detailed study on dietary intake of children with CLD and its relationship with their body composition compared to the preterm and term-born controls.

## **6.5 Conclusion**

This thesis includes a detailed study on respiratory, cardiovascular and nutritional status of prematurely born children who had chronic lung disease in infancy (CLD), at 8-12 years of age. The study population represents children born in the surfactant era and therefore presents outcome of 'new CLD'. Every parameter measured in this study was compared between the prematurely born children with CLD and age and gender-matched preterm and term-born control population. None of the children in this study had clinically evident cardiovascular symptoms. Thus, any relationships between cardiopulmonary and nutritional statuses are sub-clinical.



In this thesis, I have demonstrated that prematurely born children who had CLD in infancy continue to have impaired lung function including airway obstruction, impaired alveolar diffusion capacity, lung hyperinflation, exercise-induced bronchoconstriction and increased response to bronchodilator compared to term and preterm controls. Although their exercise capacity was similar to children in the control groups, this was at the expense of their ventilatory reserve. I have also suggested that there may be relationships between neonatal factors such as gestational age at birth and duration of oxygen dependency, and exercise capacity and alveolar diffusion capacity later in life. Further research is needed in this field.

Using both conventional and new methods of echocardiography, I have clearly demonstrated that school-aged children with CLD, who are asymptomatic, do not have evidence of cardiovascular impairment at rest. Their right ventricular function and pulmonary arterial pressure estimated using surrogate methods were similar to those of the control groups after exposure to acute hypoxia although children with CLD desaturated more after the hypoxic challenge.

In view of persistent lung function abnormalities in children with CLD, right ventricular function and pulmonary artery systolic pressure remain important determinants for long term outcome of these children. Appropriate follow-up and active management of pulmonary disease may help ameliorate early aging of lungs and pulmonary hypertension related to CLD.

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## **Appendices**

## ***Appendix A: Study Protocols***

## A1. Study Protocol for Pulmonary Function Tests

ID:                      Gender:                      DOB:                      Date of study:

Consent  Assent  General + Respiratory examination   
 Baseline HR..... PHR (220-age in years) .....90% PHR.....  
 Blood pressure .....Oxygen saturation.....  
 Height.....m                      Weight.....kg

Calibrate all equipment and check resuscitation equipment

ECG

Checklist   
 - Nose clip  
 - Mouthpiece between teeth

**1. Spirometry- Demonstration and Practice**   
 →Exhale to RV  
 →Inhale to TLC

**2. Carbon monoxide transfer factor**   
 Start in training mode  
 →Tidal breathing (at least 3 breaths, RR>10<25)  
 → Exhale to RV ('Enter' during expiration)  
 → Inhale to TLC  
 → Hold breath until display reads 0.01  
 →Exhale to RV  
 → Tidal breathing

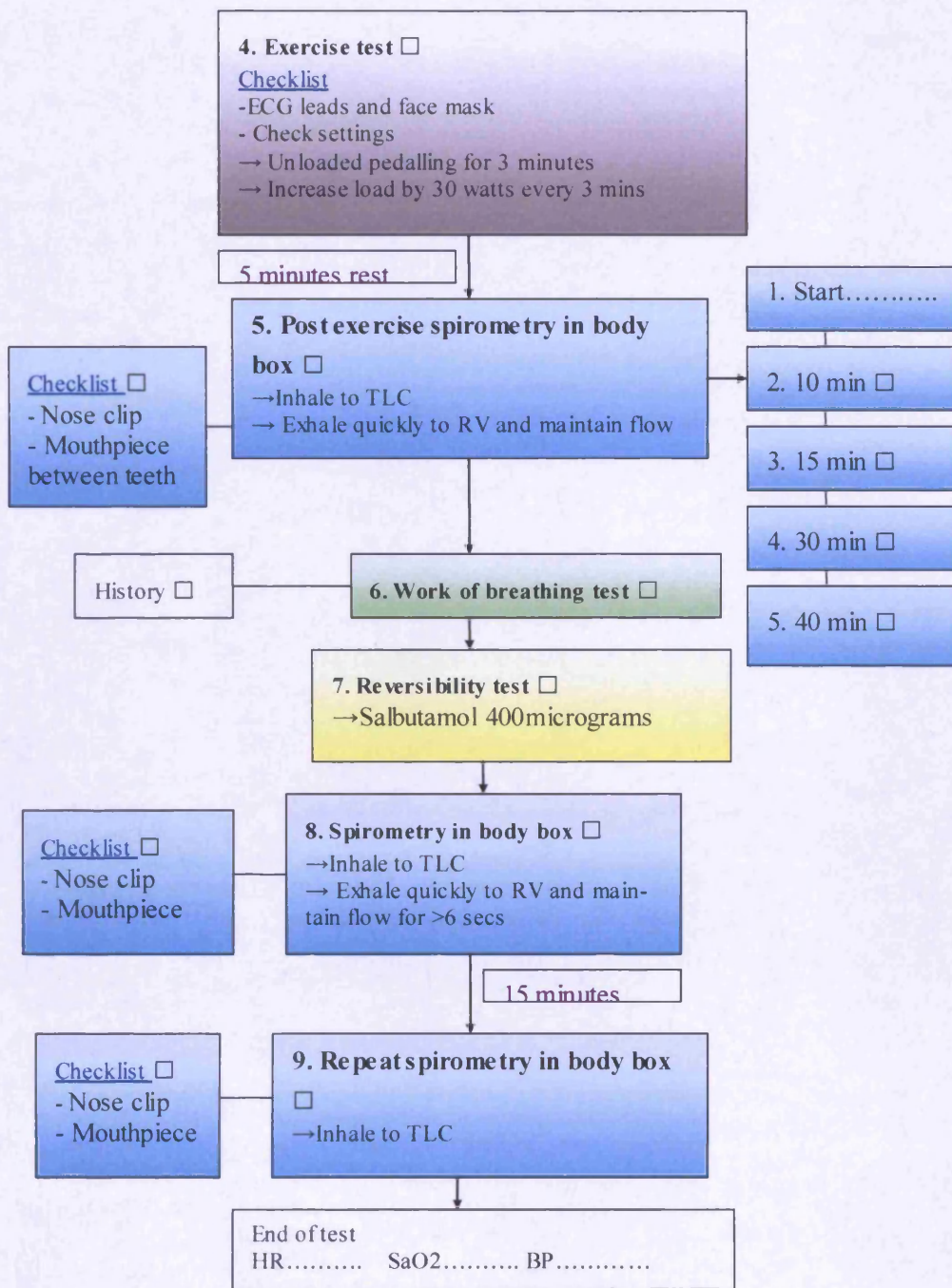
Checklist   
 - Enter ID, height and weight  
 - 'Sniffing' position  
 - Nose clip and mouth piece  
 - Select 'Body plethysmography' from menu  
 -Wait for 1 minute for temperature stabilization

**3. Plethysmography (body box)**   
 →Tidal breathing (RR >10<25continue until satisfactory)  
 → Close the loop (F4 'ASC settings')  
 → Warn the child that the shutter will be closed and instruct to breathe against shutter  
 → Activate ITGV (F3 'ITGV')\*Repeat at least 3 times\*  
 - Exhale→ Inhale to TLC →Exhale to RV

Checklist   
 - Nose clip  
 - Mouthpiece between

**Spirometry**   
 →Inhale to TLC  
 → Exhale quickly to RV and maintain flow for >6 secs  
 \*\* Repeat at least 3 times \*\*





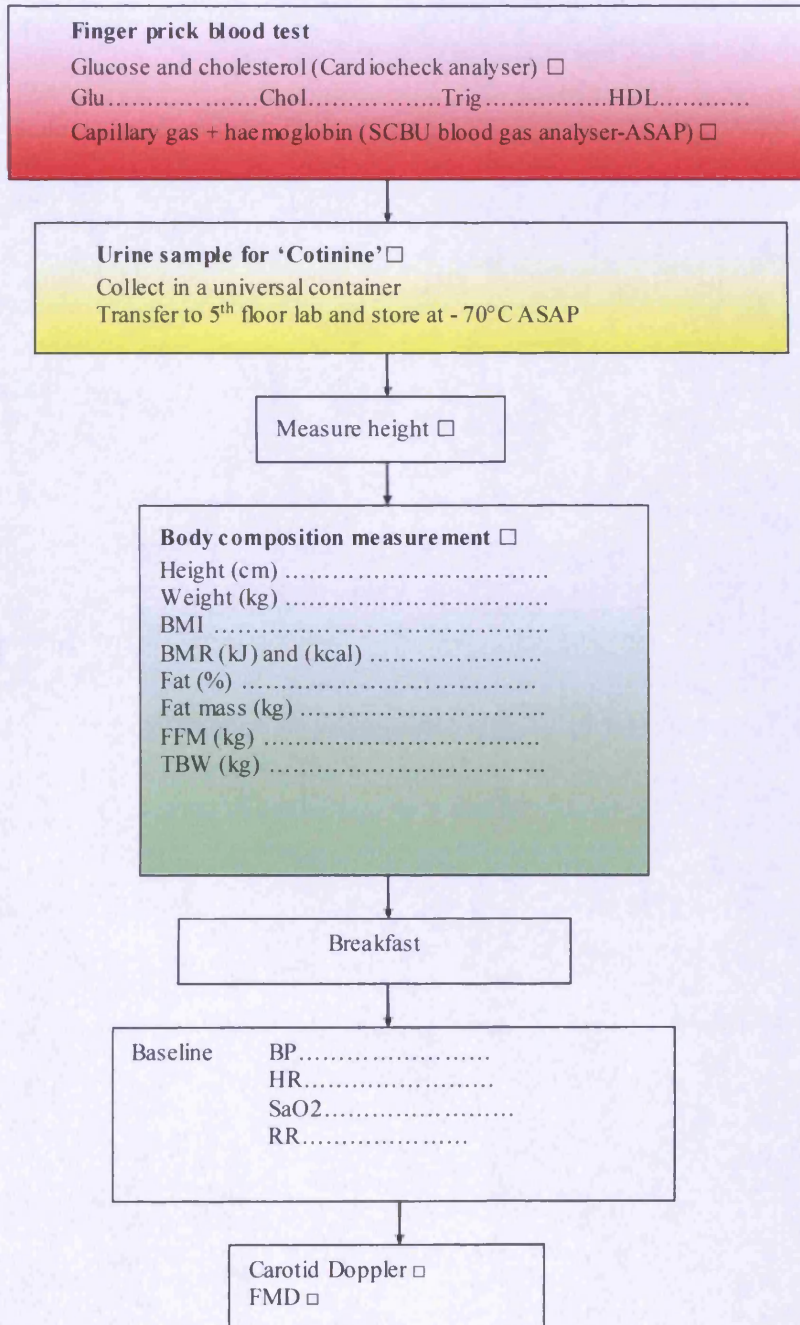
## A2. Study protocol for cardiovascular tests

ID:

Gender:

DOB:

Date of study:



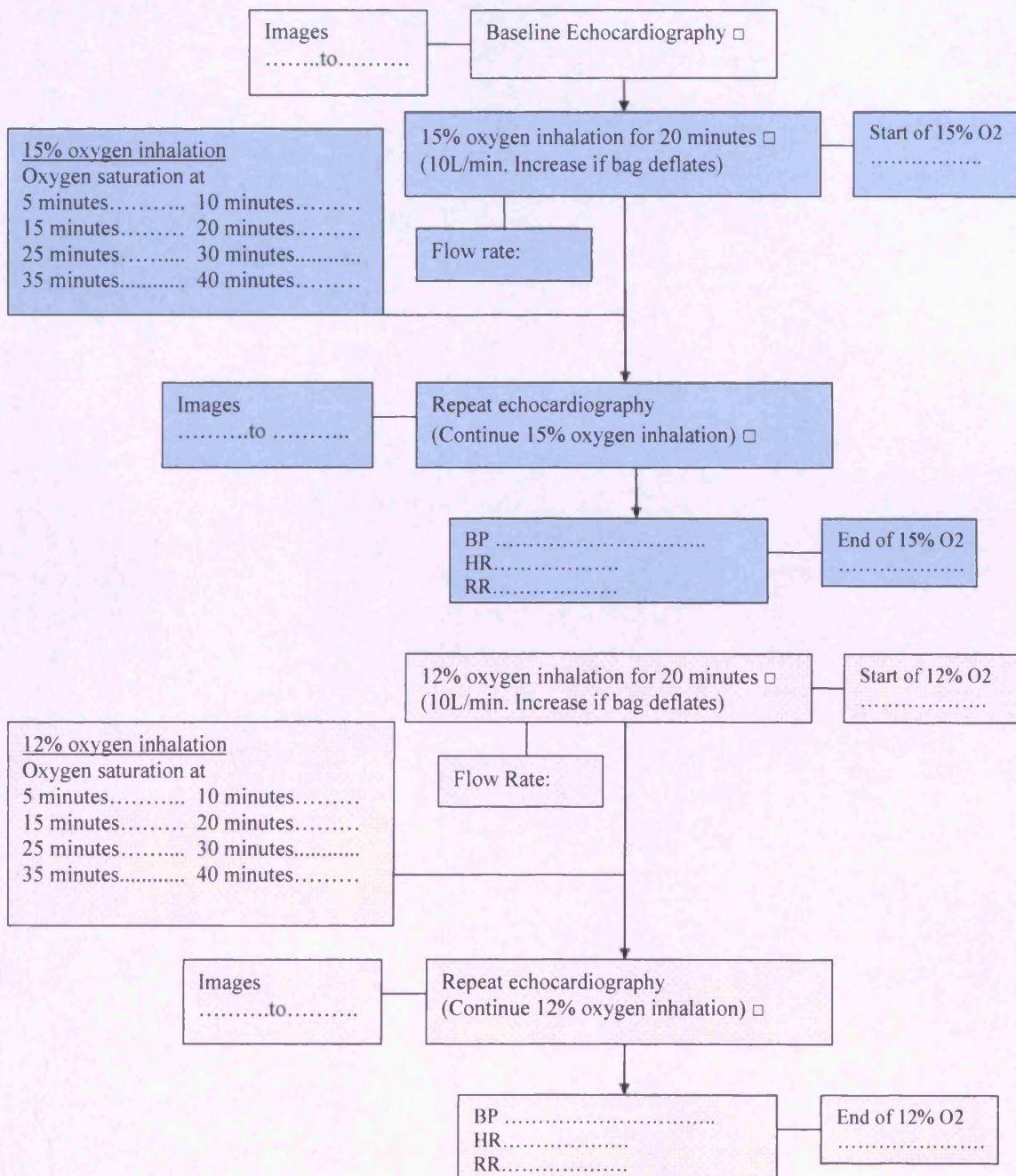


## Hypoxic challenge test

### Checklist

- Image storage (set at 3- beat loops)
- Child in left lateral position
- Keep bag inflated at all times
- Pulse oxymetry recording

Stop-if oxygen saturation drops < 80% for more than 30seconds  
 Stop immediately- If oxygen saturation drops <70%  
 Stop- if oxygen saturation drops to 70% for less than 30 seconds on 3 occasions.





### **A3. Echocardiography acquisition protocol**

#### **Initial Phase Echo: (Baseline)**

##### **A. Parasternal long axis window**

1. Conventional 2 D grey scale

##### **B. Parasternal short axis window**

1. Pulsed wave Doppler (PWD) - Pulmonary artery flow
2. PWD - Pulmonary regurgitation (if present)
3. Continuous wave Doppler (CWD) - T regurgitation (if present)

##### **C. Apical window**

1. 2D - Apical four chamber
2. PWD – Left ventricular outflow tract
3. PWD - mitral flow
4. PWD - tricuspid flow
5. CWD - tricuspid regurgitation (if present)
6. PWD (tissue Doppler, TD) - medial mitral annulus
7. PWD (TD) - lateral mitral annulus
8. PWD (TD) - lateral tricuspid annulus
9. TD- Apical four chamber
10. 2D- Right ventricle only
11. TD- Right ventricle only

##### **D. Subcostal window**

1. PWD- Hepatic venous flow
2. 2D- Inferior vena cava (sniff test)

**\*\* Check AS, VS and all valves to rule out congenital structural defects \*\***

(Echocardiography acquisition protocol continued)

**Repeat Phase Echo after Hypoxic challenge:**

**A. Parasternal short axis window**

1. PWD - Pulmonary artery flow
2. PWD - Pulmonary regurgitation (if present)
3. CWD - Tricuspid regurgitation (if present)

**B. Apical window**

1. PWD- Left ventricular outflow
2. PWD- Mitral flow
3. PWD- Tricuspid flow
4. PWD- Tricuspid regurgitation (if present)
5. PWD (TD) – Medial mitral annulus
6. PWD (TD) - Lateral mitral annulus
7. PWD (TD) – Lateral tricuspid annulus
8. 2D- Right ventricle only
9. TD- Right ventricle only

#### A4. Echocardiography analysis protocol

ID:                      Gender:                      Date of birth:                      Date of scan:

Image	Measurement	Result 1 21% O <sub>2</sub>	Ref	Result 2 15% O <sub>2</sub>	Ref	Result 3 12% O <sub>2</sub>	Ref
Hepatic vein-PWD	Estimated RA pressure			-	-	-	-
IVC- MM	Estimated RA pressure			-	-	-	-
PLAX	LVOT (d) cm			-	-	-	-
	CSA (a) = $\pi r^2$ cm <sup>2</sup>						
<b>Blood Pool data</b>							
LVOT- PWD	Stroke distance (VTI) cm						
	<i>Without angle correction</i>						
	<i>With angle correction.....°</i>						
	Stroke volume (SV) = a × VTI ml						
	Heart rate (HR) /min						
	Cardiac output (CO) = (SV × HR)/wt ml/kg/min						
LVOT - PWD	QRS to onset of flow (LV PEP) ms						
MV inflow-PWD	E m/s						
	A m/s						
	E:A						
TV inflow-CWD	TR vel max m/s						
	TR pg mm Hg						
PA- PWD	PA AT ms						
	PA ET ms						
	HR bpm						
PR-PWD	PR end diastolic vel m/s						
	PR pg mm Hg						

(Echocardiography analysis protocol continued)

Image	Measurement	Result 1 21% O <sub>2</sub>	Ref	Result 2 15% O <sub>2</sub>	Ref	Result 3 12% O <sub>2</sub>	Ref
<b>Pulsed TD Data</b>							
Medial mitral annulus	Early diastolic velocity (Ve') m/s						
Lateral mitral annulus	Early diastolic velocity (Ve') m/s						
	Average Ve' m/s						
Lateral tricuspid annulus	Isovolumic relaxation time (IVRT) ms						
	Heart rate bpm						
<b>Processed TD Data</b>							
	<b>**Event timing from LVOT PWD**</b>	<b>FR/ HR</b>		-		-	
LV	Lateral mitral annular systolic excursion/ Displacement (Ds') mm			-		-	
	Peak systolic velocity (Vsbl) cm/s			-		-	
	Early diastolic velocity (Vebl) cm/s			-		-	
	Late diastolic velocity (Vabl) cm/s			-		-	
	QRS to Peak Vs time interval (Q-Vs) s			-		-	
	Isovolumic acceleration (Asbl) m/s <sup>2</sup>			-		-	
<b>(SL= 10mm)</b>	Strain- LV lateral wall- Base (Ssbl)%			-		-	
	Strain- LV lateral wall- Apical (Ssal) %			-		-	
	<b>Delete LV Event Timing</b>			-		-	

**(Echocardiography analysis protocol)**

<b>Image</b>	<b>Measurement</b>	<b>Result 1</b> 21% O <sub>2</sub>	<b>Ref</b>	<b>Result 2</b> 15% O <sub>2</sub>	<b>Ref</b>	<b>Result 3</b> 12% O <sub>2</sub>	<b>Ref</b>
	<b>**Event timing from RVOT PWD**</b>	FR/ HR		FR/ HR		FR/ HR	
<b>RV</b>	Lateral tricuspid annular systolic excursion/ Displacement (Ds') mm						
	Peak systolic (S) velocity (Vsbl) cm/s						
	Early diastolic (E) velocity (Vebl) cm/s						
	Late diastolic (A) velocity (Vabl) cm/s						
	QRS to Peak Vs time interval (Q-Vs) s						
	Isovolumic acceleration (Asbl) m/s <sup>2</sup>						
<b>(SL= 10 mm)</b>	Strain- RV lateral wall- Base (Ssbl) %						
	Strain- RV lateral wall- Apical (Ssal) %						
	<b>Delete RV Event Timing</b>						

**Appendix B: History proforma**

Serial Number:                      Participant ID:                      Gender: M/F                      Age:

Birth and Neonatal History: (Case notes)

Date of birth:                      Gestational age at birth (in weeks):                      APGAR:

Multiple birth: Yes/ No                      Twin    1                      2                      Triplet 1                      2                      3

Antenatal steroids: Y/ N                      Surfactant: Y/ N                      Postnatal steroids: Y/ N

Assisted ventilation: Y/ N

    Total duration of assisted ventilation .....days

    SIMV/ SIPPV.....days

    HFOV.....days

    CPAP.....days

Duration of oxygen dependency:

    Corrected gestational age ..... weeks

    Days of life .....days

    Maximum FiO2 at any stage after birth.....

Neonatal sepsis:                      Y/ N

    Positive culture 1..... Source.....

    Positive culture 2..... Source.....

    Positive culture 2..... Source.....

Persistent pulmonary hypertension of newborn (PPHN):                      Y/ N

    Echo diagnosis

    Treatment 1.....                      2.....                      3.....

Intraventricular haemorrhage:                      Y/ N

    Worst grade.....

Weight at birth (kg):                      Length at birth (cm):                      OFC at birth (cm):

Feeding at birth      Breast\* / formula/ mixed fed    \* Duration of breast feeding.....weeks

Date of discharge:                      Age at discharge: ..... (days).....(CGA)

Weight at discharge .....Kg

Length at discharge.....cm

OFC at discharge .....cm

Family History in first-degree relatives: (To be filled in by parents)

Cigarette smoking    Y/ N    Not sure

Asthma                      Y/ N    Not sure

Eczema                      Y/ N    Not sure

Hay fever                Y/ N    Not sure

Diabetes                    Y/ N    Not sure

High cholesterol      Y/ N    Not sure

High blood pressure   Y/ N    Not sure

Sudden death at under the age of 40 years    Y\*/ N            \* .....age in years

Medical history: (To be filled in by parents)

Asthma:      Has the child ever been diagnosed with Asthma?    Y\*/ N    Not sure

    \*Age at diagnosis.....

    \*Last episode of asthmatic attack requiring treatment.....years of age

    \*Last episode of asthmatic attack requiring hospital admission.....years of age

Wheeze:      H/O wheeze at any time since birth:    Y\*/ N    Not sure

    \*First episode of wheeze at .....years of age

    \*Last episode of wheeze at.....years of age

    \*Number of attacks of wheezing in the past 1 year .....

    \*Number of attacks requiring 'rescue inhaler' in the past 1 year.....

    \*Number of attacks requiring hospital admission in the past 1 year .....

\*Exercise induced wheeze in the past 1 year Y/ N Not sure

\*Dry cough at night (not cold/flu) in the past 1 year: Y/ N Not sure

Rhinitis: H/O hay fever at any time since birth: Y/ N Not sure

H/O a problem with sneezing, or a runny, or a blocked nose without having cold or the flu: At any time since birth Y/ N Not sure

In the past 1 year Y/ N Not sure

Eczema: H/O eczema at any time since birth: Y/ N Not sure

H/O itchy rash at any time in the past 1 year: Y\*/ N Not sure

\* Itchy rash affecting the folds of the elbow, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes : Y/ N Not sure

Exercise: Periods of activity at school (~30 min=1 period) i.e. walking to school, break time, lunchtime, PE ..... (Periods per day)

After school, in the evenings or at weekends does the child play sport/or participate in any activities (e.g. dancing, swimming, cycling)? Y/N

If yes, estimate of how many hours a week does he/she do this sport/activity for? .....hours

How does the child consider his/ her daily activity compared to friends or siblings?

More active  Less active  Same as friends/ siblings

Cardiac: H/O structural congenital heart disease Y\*/ N Not sure

Diagnosis .....

Surgical correction Y/ N Not sure

Medical treatment Y/ N Not sure

H/O cardiac disease any time since birth (not structural defect) Y\*/ N Not sure

\*Diagnosis .....

Medical treatment Y/ N Not sure



Surgical treatment Y/ N Not sure

Cardiology follow-up in the last 1 year Y/ N Not sure

Symptoms in the past 1 year

Chest pain requiring medical attention Y\*/N \* At rest/ exercise

Breathlessness requiring medical attention Y\*/N \* At rest/ exercise

Palpitation requiring medical attention Y\*/N \* At rest/ exercise

Snoring Y/ N

Undue tiredness Y/ N

Fainting episodes Y/ N

H/O treatment with oxygen in the past 1 year for any reason Y\*/N Not sure

\*Diagnosis.....

Regular medication Y/ N (details if yes).....

Any ongoing medical problem Y/ N (details if yes).....

Number of hospital admissions since birth .....

Nutritional History: (To be filled in by parents)

Mother's height ..... Father's height .....

Has the child ever been a fussy eater? Y/ N

Has the child ever struggled with lumpy food? Y/ N

## **Appendix C: Invitation letter to the parents**

Parent/ Guardian of [Name]  
Address

[Date]

Dear Parent/Guardian

**Re: Long term Cardio- Respiratory Outcome in Children with Chronic Lung disease of Prematurity**

I am writing because [Name] was on the baby unit after he was born. I hope he is keeping well. We are keen to learn more about how our ex-neonatal unit babies do as they grow up so that we can improve our care in the future. I would therefore like to invite [Name] to join the above study at Cardiff University. We have enclosed information leaflets which give more details of the study.

The study will involve spending one morning at the 'Wales Heart Research Institute', Cardiff and another morning or afternoon at the 'University of Glamorgan', Pontypridd. We would be most grateful if you could fill in the enclosed slip and send it in the enclosed envelope to let us know if you are or are not able to join our study. If there are any questions that we may answer before you can make a decision, please contact Dr Suchita Joshi on 029 2074 3438 or email us on [Joshis3@cardiff.ac.uk](mailto:Joshis3@cardiff.ac.uk).

We would very much like to thank you for taking time to consider our study.

Yours sincerely,

Dr Mark Drayton  
Consultant Neonatologist  
University Hospital of Wales  
Heath Park  
Cardiff, CF14 4XN  
(On behalf of the research team)

## **Appendix D: Information sheet for parents**

### **Information Sheet for Parents (1)**

**(For parents of preterm children with lung disease of infancy)**

#### **'Long Term Cardio- Respiratory Outcome in Children with Chronic lung Disease of Prematurity'**

Principal investigator:       Professor Sailesh Kotecha  
  Department of Child Health  
  Cardiff University  
  Cardiff, CF14 4XN

#### **Part 1**

Dear Parent/Guardian

We would like to invite .....to take part in our research study at Cardiff University. This study will be done in collaboration with the Wales Heart Research Institute and University of Glamorgan. Before you and your child decide, whether or not to participate in the study, it is important for you to understand why the research is being done and what it involves. Please take time to read the following information carefully and please feel free to ask any questions that you may have.

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

Many babies who are born prematurely may develop breathing problems at birth. Some of these babies may develop lung disease called chronic lung disease of prematurity or CLD. The majority of children who had CLD as a baby go on to participate in play, exercise and other activities without having any visible problems. It is unknown if there is any difference in the way the lungs work in these children later on in life.

a) We are trying to find out how the blood vessel that connects the heart and the lungs (pulmonary artery) in these children react, when they breathe air containing less oxygen than natural air, as happens when travelling long distance in an aeroplane or when climbing a mountain or a skiing in high altitude ski resorts

b) We are also trying to find out how well their lungs can cope with exercise.

We will compare the results from children A) who were born early and had CLD, with results from B) those who were born early but did not have CLD and C) those who were born at full term. A total of 90 children will take part in this study.

## **2. Why has my child been invited?**

We have invited your child to participate in this study because he/she was born early and had lung disease of infancy as a baby (group A).

## **3. Does my child have to take part?**

No. It is up to you and your child to decide. Your child does not have to take part in this research if either you or your child does not wish to do so. We will describe the study and go through this information sheet, which we will then give to you. Your child will also be given his/her own information sheet. You and your child will have the opportunity to discuss any questions that you may have with the researcher after you have read the information sheets.

We will then ask you to sign a consent form if you agree your child to take part in this research. Your child will also be asked to sign an assent form to show that he/she is happy to take part.

## **4. What will happen to my child if he/she takes part?**

We would like to invite your child to the “**Physiological Assessment Suite**” at the **University of Glamorgan, Pontypridd** for half a day to test how fit his/her lungs are after exercise. This will involve ‘breathing and blowing’ tests (spirometry) and pedalling on an exercise bike. On this visit, we will also ask you some questions regarding your child’s birth details, relevant medical history and family history. We will demonstrate the tests to your child and let him/her practice it until he/she understands how to do the tests. For one of the tests, your child will be asked to sit

inside a cubicle with glass doors and the cubicle door will be closed for a few seconds. Your child will be able to see and hear us. For the exercise test, your child will be asked to pedal an exercise bike for as long as possible. We expect the exercise test to last about 15 minutes. We will be closely monitoring your child during the bicycle exercise test and will stop the test when your child is too tired to continue or if his/her heart rate goes too fast. Your child will have 15 minutes rest after the exercise test after which we will ask him/her to inhale a medicine called 'Salbutamol' (that children with Asthma use) which relaxes the small airways. We do not expect this inhaler medicine to cause any side effects to your child. We will repeat the spirometry test after this. This will allow us to compare the test results before and after relaxing the airways.

We will ensure that your child is comfortable before we allow him/her home. You will be able to stay with your child throughout the test and we expect that you will be spending 2-3 hours with us at the University of Glamorgan.

We would also like to invite you and your child to the **'Wales Heart Research Institute' at Heath Hospital site in Cardiff** for another half day session. This time, we would like to see your child in the morning before he/she has his/her breakfast. This will allow us to check his/her blood sugar, cholesterol and haemoglobin levels, which are thought to be important in the development of heart disease in adulthood. This will involve a finger prick test (similar to the one that diabetic children use for blood sugar tests). We will provide breakfast for your child after the blood test. **If you or your child does not wish for a blood test, we will not do one. If you do not wish your child to have blood test, he/she may come after breakfast but please ensure that he/she does not drink tea, coffee, cola or any drink containing caffeine in the morning for at least 3 hours before the study.** We would also like to do a urine test to check your child's exposure to other people's cigarette smoking.

On this visit, we will record your child's details including height, weight, blood pressure and oxygen levels with a probe on a finger. Then, we will do a heart scan (similar to ultrasound scan done in pregnancy). This will take about 15-20 minutes. We will then ask your child to breathe decreasing levels of oxygen (15% and 12%) for 20 minutes each. The air we breathe is about 21% oxygen, the cabin in a long distance aeroplane is about 15% and skiing in high mountains in Europe is about 12%. We will repeat the heart scans after each interval to see if any changes in heart function are detected with decrease in oxygen. These scans will take about 10-15 minutes each.

On the same visit, we will also do an ultrasound scan of the blood vessel of the right arm before and after applying an inflated blood pressure cuff for 5 minutes around the right forearm of your child. This is similar to the method doctors and nurses use to measure blood pressure but the cuff will be inflated for longer. The pressure of the cuff may cause some discomfort for the child but we will use adult-sized cuff to minimise this discomfort. This test will assess the stiffness of the blood vessel in the arm.

We will provide lunch for you and your child and make sure that your child is comfortable before letting him/her home. We expect that you and your child may have to spend about 3 hours at the WHRI to complete all the above tests.

**5. Can I stay with my child during the tests?**

You are encouraged to stay with your child throughout all the tests.

**6. How long will the different tests take?**

We expect the lung function tests at the University of Glamorgan, Pontypridd to last approximately 2-3 hours including, 30 minutes for recording history and measuring height, weight, heart rate and breathing rate, 15-30 minutes for demonstration of the breathing and blowing tests and practice sessions, 30 minutes to complete these tests, 15-20 minutes for the exercise test and 20-30 minutes of rest periods between different tests.

We expect that you may have to spend about 3 hours at the WHRI, Cardiff. This includes, 5-10 minutes for blood tests, 5-10 minutes for urine test, 2 to 2.5 hours for the oxygen tests and the heart scans. After 20 minutes of oxygen challenge, each heart scan takes about 15 minutes.

**7. Have similar studies been done in the past?**

Often a similar low oxygen (15%) test is performed for 20 minutes in babies who have recently come off their oxygen if they are likely to travel in an aeroplane to determine if the baby requires oxygen during the flight. One study showed that 8 out of 20 babies who had pre-flight hypoxia test required additional oxygen for the flight although they did not normally need additional oxygen. However, we are not aware of any similar

oxygen challenging studies 8 – 12 year-old children (as opposed to babies) who have had chronic lung disease of prematurity when they were babies.

**8. What are the possible benefits of taking part?**

This study may help identify whether children who are born early and had lung disease of infancy are more likely to have lung and heart disease earlier in adulthood and therefore benefit with early monitoring and treatment.

Although we do not anticipate any major difference in children who were born early compared to control population, if we find large differences, then we will firstly explain the findings to you and secondly, your child will be followed up in a regular basis by the research team (Dr DG Wilson, Paediatric Cardiologist).

**9. Is my child likely to experience any discomfort or distress during this study?**

The concentration of oxygen that will be used in this study is similar to that found in aeroplanes (15% oxygen) and in high altitude ski resorts (12% oxygen) and we do not anticipate any serious side effects. Some children may temporarily breathe faster, similar to rates after exercise in the playground. Oxygen levels using a finger probe will be monitored at all times and the study will be discontinued if your child is unable to tolerate the tests or feels any discomfort or distress by breathing reduced oxygen levels. The study will also be stopped if your child's oxygen level drops below 80% for more than 30 seconds but not below 70% at which point it would be stopped immediately. The study will also be stopped if your child's oxygen level drops to 70% for periods of less than 30 seconds on 3 occasions.

We will have provisions for high oxygen (100%) should your child need it.

**10. What if my child is harmed by the study?**

Any complaint about the way your child has been dealt with during the study or any possible harm that your child may suffer will be addressed. The detailed information on this is given in Part 2.

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

There will be no identifiable details of your child in any study information. All information about your child will be handled in confidence.

**12. Will I or my child receive out of pocket expenses for taking part in the study?**

We will provide any travelling expenses that you may incur and provide breakfast for you and your child after the blood test and lunch for both you and your child. Your child will also receive two cinema vouchers with a 'thank you' note for helping us with the project.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision



## **Part 2**

### **13. What happens if my child or I do not wish to participate in the study or wish to withdraw from the study?**

If your child or you do not wish to participate in this study or if you wish to withdraw from the study you may do so without justifying your decision and your child's future treatment will not be affected.

### **14. What if there is a problem?**

Complains:

If you have concerns about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through NHS Complaints Procedure.

Harm:

In the event that something goes wrong and your child is harmed during the research and this is due to someone's negligence then you may have grounds for legal action for complaints against Cardiff University but you may have to pay your legal costs. Medical research is covered for mishaps in the same way as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs.

### **15. Will my child's taking part in this study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about your child will be handled in confidence. With your permission, your GP will be informed of your child's participation in this study. None of your child's identifiable details will be stored or used in any publication or presentation in future.

### **16. What will happen to the results of the study?**

The results of this study will be prepared for publication in medical journals, and for presentation at medical meetings.

### **17. Who is funding the study?**

This study is being funded by the Department of Child Health, Cardiff University and Cardiff and Vale NHS Trust R&D Small Grants Scheme.

### **18. Who has reviewed the study?**

Before any research goes ahead it has to be reviewed by Research Ethics Committee. They make sure the research is fair. This project has been reviewed by the South East Wales Research Ethics Committee.

Thank you for reading this. Please feel free to ask us if there is anything that is not clear or if you would like more information about this study.

Dr Suchita Joshi  
Academic Clinical Fellow  
University Hospital of Wales  
[Joshis3@cardiff.ac.uk](mailto:Joshis3@cardiff.ac.uk)

Prof Sailesh Kotecha  
Professor of Paediatrics and Child Health  
Cardiff University  
[kotechas@cardiff.ac.uk](mailto:kotechas@cardiff.ac.uk)

- **Information Sheet for Parents (2): For parents of preterm children without lung disease of infancy and**
- **Information Sheet for Parents (3): For parents of the term-born children**  
were also provide separately to the parents of the children in the preterm and term groups respectively. (Similar information sheets as above)

## **Appendix E: Information sheet for children**

### Information Sheet (1) for Children

(For children who were born early and needed oxygen for a long time as babies)

#### **‘Long Term Cardio-Respiratory Outcome for Children with Chronic Lung Disease of Prematurity’**

Dear [child’s name],

We would like to invite you to take part in a research project on children who were born early. Before you decide if you want to join in, it’s important that you understand why the research is being done and what you will be asked to do if you take part.

So please read this leaflet carefully with your mum or dad (or ask them to read it to you) and talk about it with them. If you do not understand anything that has been written here or if you have any questions please let us know and we will try to explain it to you.

1. What is research?

Research is a way we try to find out the answers to questions.

2. Why is this project being done?

Some babies are born earlier than they normally should. Some of these babies need oxygen for a long time to help them breathe until their lungs are strong. In this research, we want to find out

- a) How strong their lungs are now, and
- b) How hard their heart has to work to pump blood to their lungs.

To find out the answers to these questions, we will need to do different tests on children who were born early and compare the test results with healthy children who were born on time.

A total of 90 children will take part in this project.

This research project will help us understand if children who are born early and needed oxygen for a long time as babies, need any special tests before they go on an aeroplane or skiing or if they need to have special tests when they grow older.

### 3. Why have I been asked to take part?

You have been asked to take part in this research because you were born earlier than most babies should and you needed oxygen to help you breathe when you were a baby (Group A).

### 4. Did anyone else check the study is OK to do?

Before any research is allowed to happen, it has to be checked by a group of people called Research Ethics Committee (REC). They make sure that the research is fair. This project has been checked by South East Wales REC.

### 5. Do I have to take part?

No. It is up to you. You don't have to take part if you don't want to. If you do, we will ask you to sign a form to say that you are happy to take part in this project.

**You are allowed to stop taking part at any time during the research if you do not wish to continue.** You will not have to give us any reason.

### 6. What will happen to me if I take part?

If you take part in this research, you will be asked to spend one morning with us at the Wales Heart Research Institute (WHRI) at the Heath for about 3 hours for heart scans and another morning or afternoon at the University of Glamorgan in Pontypridd for about 2-3 hours for

breathing tests. **On these occasions, your mum or dad may stay with you throughout the tests.**

**Visit to WHRI (Heath):**

On this visit, we would like you to come in the morning without any breakfast to allow us to do a blood test. If you do not want us to do the blood test, you may come after breakfast, but **please make sure that you do not drink tea, coffee or cola in the morning.** We will give you breakfast after the blood test. We will do following different tests to check how strong your heart has to work to pump blood to your lungs.

Arrive in the morning with your mum or dad.

↓  
Blood test



(This is a finger prick test similar to what children who have diabetes do to check the sugar level in their blood every day. This may feel similar to a pin-prick and will take only a few seconds to collect a few drops of blood. **(We will not do the blood test if you don't want us to do it.)**)

You will then be asked to use our toilet to collect a urine sample in a small bottle for us to test it.↓

Breakfast

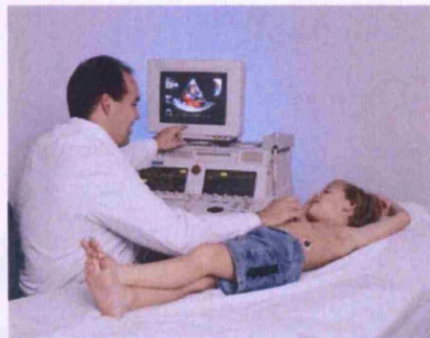
↓



We will then listen to your heart with a stethoscope and record the tracing of your heart beat by using special sticky pads on your chest while you lie down comfortably on a bed.



We will then ask you to lie comfortably on a bed while we do a heart scan. For this, we will put special gel on your chest and take pictures of your heart. You will be able to see how the inside of your heart looks like on a TV screen. This will take about 15-20 minutes. This will not cause you any pain or discomfort.



After this scan, we will help you put on a mask over your nose and mouth and let you breathe two different levels of oxygen for 20 minutes each and then repeat the heart scans. This may make you breathe faster as if you have been running in a playground. We will make sure you are alright during this test but

if you feel unwell or uncomfortable during this test or if you wish to stop the test at any time, you should let us know.



After the heart scans, we will apply a blood pressure cuff around your forearm and inflate it for 5 minutes. The pressure of the cuff may make you feel slightly uncomfortable. You may

let us know if it is too uncomfortable for you.



We will then do a scan of a blood vessel of your arm which is similar to the heart scan but this is much quicker.



Lunch and home



**Visit to the University of Glamorgan (Pontypridd):**

On this visit, we will do different tests to see how strong your lungs are and how well they cope with exercise. You will be spending about 2-3 hours with us. This visit may be in the morning or in the afternoon.

Arrive with mum or dad



Mum or dad will be asked some questions



We will measure your height and weight and listen to your chest



how to do the special breathing and blowing tests, we will do the tests.

We will then show you how to do some special breathing and blowing tests called spirometry. You can then practice the tests to make sure you understand what to do. Once we are sure that you have understood



For one of the tests, you will be asked to sit inside a special box with glass doors. The door will be closed for about 10 seconds. You will be able to hear us clearly and we will be able to see each other.







For the exercise test, we will ask you to pedal an exercise bike. It will be easy to pedal to start with but will get harder and harder. We will stop the test when you let us know that you are too tired to pedal anymore. We expect this test to last for about 15 minutes. We will keep a close eye

on you throughout this test and will stop the test immediately if we need to or if you want us to stop it.



Rest for 15 minutes



After the brief rest, we will ask you to inhale a special medicine that will make the small branches of your breathing tube relax. This will not cause any harm to you and will not be painful or uncomfortable.



Repeat a short breathing and blowing test



Home

You will receive two cinema vouchers as a 'thank you' for taking part in this project.

**7. Might anything about the research upset me?**

Some children get upset about blood tests and don't like having it. If you don't want us to do the blood test, we will not do it.

When you breathe different levels of oxygen, you may breathe faster as if you have been running in the playground. During this test, we will keep a very close eye on you and make sure you are fine. If you feel uncomfortable and want us to stop the test, you have to let us know.

The exercise test will make you breathe faster and make your heart beat faster just as when you run. We will keep a close eye on you throughout this test and ask you to let us know when you are too tired to pedal.

**8. Will joining in help me?**

We cannot promise the study will help you but the information we get might help us look after children who were born too early and who needed oxygen for a long time as babies.

We do not expect to find any problem with you but if we find any abnormal test results then we shall explain them to you and your parents and arrange for a doctor to see you soon.

**9. What happens when the research stops?**

We will write to your parents/ guardians to let them know the results of this research.

**10. What if something goes wrong?**

We do not expect anything to go wrong during this project and we will not ask you to do anything that maybe harmful to you. We will also keep a close eye on throughout the tests and will stop the test immediately if we need to or if you want us to.

11. Will anyone else know I'm doing this?

With your mum or dad's permission, we will let your doctor (GP) know that you are taking part in this project. Apart from your GP and the research team, nobody else will have to know that you are taking part in this project.

12. What will happen to the blood and urine samples that I give?

Once we test the blood and urine samples that you give us, we will throw it away in a special hospital bin.

13. Who is organising and paying for this research?

This research is being organised and paid by the Children's Department at Cardiff University.

14. What if I don't want to do the research anymore?

If at any time you don't want to do the research anymore, just tell us or your mum or dad and you can stop taking part. You do not have to continue if you do not want to do so.

Thank you for reading this leaflet and I hope I can meet you. If you do not understand anything that this leaflet says or if you have any questions for us, please ask your mum or dad to contact me and I will try to answer your questions.

Yours sincerely

Dr Suchita Joshi

Department of Child Health

Cardiff University

02920743438

[Joshis3@cardiff.ac.uk](mailto:Joshis3@cardiff.ac.uk)

- **Information Sheet for Children (2): For preterm children without lung disease of infancy and**
  - **Information Sheet for Children (3): For term-born children**
- were also provided separately to the children in the preterm and term groups respectively.
- (Similar information sheets as above).

## **Appendix F: Consent form for parents**

Patient Identification Number for this study:

### **CONSENT FORM (Version 3.0 17<sup>th</sup> January 2008)**

**Title of Project: 'Long term cardio- respiratory outcome in children with chronic lung disease of prematurity'**

Name of the Researchers: Professor S Kotecha, Dr S Joshi

**Please initial box**

1. I confirm that I have read and understand the information sheet dated 24<sup>th</sup> Sept 2007 (version 4.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my child's participation is voluntary and that my child is free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected.
3. I understand that relevant sections of any of my child's medical notes and data collected during the study, may be looked at by responsible individuals from Cardiff University, from regulatory authorities or from the NHS Trust, where it is relevant to my child's taking part in this research. I give permission for these individuals to have access to my child's records.
4. I agree to my child's GP being informed of my child's participation in the study.
5. I agree to my child having the lung function tests as a part of the above study.
6. I agree to my child having ultrasound scans of the heart and the arm artery as a part of the above study.
7. I agree to my child having a finger prick blood test as a part of the above study.
8. I agree to my child having urine test as a part of the above study.

\_\_\_\_\_  
Name of Parent

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Researcher

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## **Appendix G: Assent form for children**

### **ASSENT FORM FOR CHILDREN**

(To be completed by the child in presence of their parent/ guardian)

Project title: Long Term Cardio- Respiratory Outcome in Children with Chronic Lung Disease of Prematurity

Child (or if unable, parents on their behalf) to circle all they agree with:

1. Have you read (or had read to you) about this project?  
(Child Information Sheet1 Version 2.0 15<sup>th</sup> August 2007 or  
Child Information Sheet 2 Version 2.0 15<sup>th</sup> August 2007 or  
Child Information Sheet 3 Version 3.0 24<sup>th</sup> September) Yes/ No
2. Has somebody else explained this project to you? Yes/ No
3. Do you understand what this project is about? Yes/No
4. Have you asked all the questions you want? Yes/ No
5. Have you had your questions answered in a way you understand? Yes/ No
6. Do you understand it is OK to stop taking part at any time? Yes/ No
7. Are you happy to take part? Yes/ No
8. Are you happy to have heart scans as a part of this project? Yes/ No
9. Are you happy to have breathing and blowing tests and exercise test as a part of this project?  
Yes/ No
10. Are you happy to have finger prick blood test as a part of this project? Yes/ No
11. Are you happy to have urine test as a part of this project? Yes/ No

**If any of the answers 1-9 are 'No' or you don't want to take part, don't sign your name!**

If you do want to take part, you can write your name below:

Your name \_\_\_\_\_ Date \_\_\_\_\_

The doctor who explained this project to you needs to sign too:

Print name \_\_\_\_\_ Date \_\_\_\_\_

Sign \_\_\_\_\_

Thank you for your help.

***Appendix H: Published papers***



# H1. Lung growth and development

Early Human Development (2007) 83, 789–794



available at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

[www.elsevier.com/locate/earhumdev](http://www.elsevier.com/locate/earhumdev)



## Lung growth and development

Suchita Joshi, Sailesh Kotecha\*

Department of Child Health, Cardiff University, Cardiff CF14 4XN, UK

### KEYWORDS

Fetal lung liquid;  
Growth factors;  
Transcriptional factors;  
Prematurity;  
Glucocorticoids;  
Congenital diaphragmatic  
hernia;  
Chronic lung disease of  
prematurity

### Abstract

Human lung growth starts as a primitive lung bud in early embryonic life and undergoes several morphological stages which continue into postnatal life. Each stage of lung growth is a result of complex and tightly regulated events governed by physical, environmental, hormonal and genetic factors. Fetal lung liquid and fetal breathing movements are by far the most important determinants of lung growth. Although timing of the stages of lung growth in animals do not mimic that of human, numerous animal studies, mainly on sheep and rat, have given us a better understanding of the regulators of lung growth. Insight into the genetic basis of lung growth has helped us understand and improve management of complex life threatening congenital abnormalities such as congenital diaphragmatic hernia and pulmonary hypoplasia. Although advances in perinatal medicine have improved survival of preterm infants, premature birth is perhaps still the most important factor for adverse lung growth.  
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### 1. Normal lung development: structural and vascular growth and possible developmental anomalies

In the human embryo, development of lung starts as early as 3 weeks of embryonic life and continues into postnatal life up to early adulthood. The structural and vascular development of the lung is closely related and progresses simultaneously in the human fetus. The events of antenatal growth and development of human lung have traditionally been divided into 5 stages (Table 1). Burri has recently reviewed the role of microvascular maturation during alveolarization [1]. In each of these stages, there are possibilities of congenital malformations associated with abnormal lung development (Table 2).

#### 1.1. Embryonic stage (0–7 weeks in utero)

At around 3–4 weeks of embryonic life, the lung develops as an outgrowth of the ventral wall of the primitive foregut, the laryngotracheal groove. The epithelial cells from the foregut endoderm invade the surrounding mesenchyme to form the trachea. During the embryonic stage, the trachea branches into the right and left main bronchi and subsequently into lobar and segmental bronchi. Lobar and segmental bronchi appear at about the 5th week [2] and by the end of this stage, 18 major lobules are recognisable [3]. Pulmonary arteries and veins develop as a single avascular bud from the 6th aortic arch and continue to grow by vasculogenesis around the airway buds from 4–16 weeks [4].

This earliest stage of lung development may have a principle role in determining the postnatal mortality and morbidity of the fetus. Structural abnormalities such as tracheal or pulmonary agenesis or stenosis may be incompatible with life whereas other forms of anomalies such as tracheomalacia or bronchomalacia, ectopic lobes and congenital lobar cysts may lead to significant respiratory

\* Corresponding author. Tel.: +44 29 20 74 4187; fax: +44 29 20 74 4283.

E-mail address: [kotechas@cardiff.ac.uk](mailto:kotechas@cardiff.ac.uk) (S. Kotecha).



Table 1 Stages of lung growth

Stage	Time	Events
Embryonic	0–7 weeks	Formation of trachea, right and left main bronchi, segmental bronchi, and vasculogenesis around airway buds
Canalicular	7–17 weeks	Differentiation of epithelial cells, formation of conduction airway and terminal bronchioles, formation of pulmonary arteries and veins
Pseudoglandular	17–27 weeks	Formation of respiratory bronchioles, alveolar ducts and primitive alveoli, differentiation of type I and type II pneumocytes and formation of alveolar capillary barrier
Saccular	28–36 weeks	Increment in gas exchange areas, further differentiation of type I and type II cells
Alveolar	36 weeks–2 years	Septation and multiplication of alveoli
	Until 18–22 years	Enlargement of terminal bronchioles and alveoli
Microvascular maturation	Birth to 2–3 years	Fusion of double alveolar capillary network into a single layer

morbidity. Arterio-venous malformations may also form during vasculogenesis.

### 1.2. Pseudoglandular stage (7–17 weeks in utero)

Pseudoglandular stage is marked by further branching of airway and vascular network and progressive differentiation of epithelial cells to form adult structures of cartilage,

Table 2 Congenital malformations associated with abnormal lung growth

<i>Embryonic</i>
Pulmonary agenesis
Tracheal or laryngeal agenesis or stenosis
<i>Tracheo- or broncho-malacia</i>
Bronchial malformation
Ectopic lobes
A-V malformations
Congenital lobar cysts
<i>Pseudoglandular</i>
Cystic adenomatoid malformation
Pulmonary sequestration
Lung hypoplasia
Lung cysts
Congenital pulmonary lymphangiectasia
Congenital diaphragmatic hernia
<i>Canalicular</i>
Lung hypoplasia
Respiratory distress syndrome
Acinar dysplasia
<i>Saccular/alveolar</i>
Pulmonary hypoplasia
Respiratory distress syndrome/ chronic lung disease of prematurity
Acinar dysplasia
Alveolar capillary dysplasia
Reproduced with permission from Kotecha [5].

submucosal gland, bronchial smooth muscle and epithelial cell types. This is the period of fastest division of intra-segmental airways. By 14 weeks, 70% of the total airway generated at birth is formed [2] and by the end of 17 weeks, the formation of conducting airways and terminal bronchioles is complete [5]. Pattern of airway branching and division is determined by epithelial-mesenchymal interactions. Bronchial mesenchyme induces branching in the trachea, whereas tracheal mesenchyme inhibits branching of the bronchial tree [6]. As the pseudoglandular stage progresses, the early pseudo-stratified epithelium is progressively replaced by columnar cells proximally and by cuboidal cells distally. The cuboidal cells, rich in glycogen, represent the immature type II cells. Thus, during pseudoglandular stage, all pre-acinar structures, including, pre-acinar airway, pulmonary arteries and veins are formed.

The failure of normal division of airway structures at this stage may lead to pulmonary hypoplasia or sequestration, cystic adenomatoid malformation and crucially, failure of the pleuro-peritoneal membrane to close at this stage may result in congenital diaphragmatic hernia.

### 1.3. Canalicular stage (17–27 weeks in utero)

The acinar structures comprising the respiratory bronchioles, alveolar ducts and primitive alveoli are formed during the canalicular stage. Canalicular stage is marked by two important steps in the development of the lung: differentiation of type I and type II pneumocytes and formation of the alveolar capillary barrier [3,7]. Surfactant protein is detectable by 24 weeks of intrauterine life. Thus, a possible platform for gas exchange is established. With advances in perinatal medicine and ever increasing survival of extremely preterm infants, this is an important landmark in lung growth and development.

Surfactant deficiency leading to respiratory distress syndrome is inevitable with premature delivery at this stage.

### 1.4. Saccular stage (28–36 weeks in utero)

By this stage, division of the airways is almost complete and further growth and development of lung structures comprise

of enlargement of the peripheral airways with dilatation of acinar tubules forming 'sacculi' and thinning of the airway walls. This ensures increased surface area for gas exchange. There is also further differentiation of type II cells to type I cells and increment in surfactant containing lamellar bodies lining type II pneumocytes.

### 1.5. Alveolar stage (36 weeks in utero—2 years)

Recognition of secondary septa in the terminal airway and formation of definitive cup shaped alveoli marks the alveolar stage. In a newborn rat lung, which is at the saccular stage of lung development, the sacculi are lined by a central sheet of connective tissue supporting a layer of capillary network on either side [1]. Gradually, low ridge like projections, also with double capillary network appear in the airspaces, which eventually divide the airspaces into alveoli. This process of formation of double capillary walled secondary septa and multiplication of alveoli continue rapidly up to the age of at least 2 years in humans.

### 1.6. Postnatal lung growth

It has been estimated that the number of alveoli at birth ranges from 20–50 million [3]. Alveolar multiplication continues in the postnatal period at least up to the age of 2–3 years and alveolar size and surface increases until after adolescence [5,7]. Ochs et al. [8] have reported that the final number of alveoli in a fully developed adult lung reach 300–800 million with approximately 170 alveoli per cubic millimetre. Post-mortem examination of lungs of children who died as a result of trauma or after short illnesses revealed that boys had larger lungs than girls of the same stature which resulted in greater alveolar number and greater alveolar surface area than girls for a given age and stature [9]. In recent years, we have gained further insight into the postnatal growth of distal airways. When air space calibre of small airways and alveoli at their maximal natural distension was measured in 53 children of age 6–22 years and 59 adults of age 23–80 years, it was noted that both the small bronchioles and alveoli expanded significantly in size with increasing age and height up to the age of 22 years but the increase in size thereafter was not statistically significant [10].

### 1.7. Stage of microvascular maturation (several months to 2–3 years)

Burri's group has previously described that, besides the multiplication and enlargement of airspaces, some important microvascular changes occur in postnatal lung development, which he termed as the stage of microvascular maturation [1]. Essentially, during this period, the double capillary network gradually merges as a single capillary layer. This is an extremely important event in the postnatal lung growth as angiogenesis and alveolarization go hand in hand and once the capillary network fuses into a single layer; new alveolar septa cannot be formed. This has now been postulated as the major cause of premature arrest of lung growth following postnatal dexamethasone treatment in premature infants with chronic lung disease.

## 2. Factors that regulate and modify lung growth: prenatal to postnatal stages

Multiple factors that govern lung growth is a topic of growing interest and numerous studies have been performed on animal models, mainly on sheep and rat, to explore the role of physical, hormonal and genetic factors in prenatal lung growth (Table 3).

### 2.1. Fetal lung volume, fetal lung fluid, amniotic fluid and fetal breathing movements

Fetal lung volume maintained by the combined mechanisms of fetal lung fluid secretion and fetal breathing movements is perhaps the most important determinant of fetal lung growth. Experiments on ovine fetus have proved that distension of fetal lung by fetal lung fluid is related to hyperplasia of the lung and drainage of the fluid results in pulmonary hypoplasia [11]. Fetal lung fluid is mainly formed by the epithelial cells of the distal airways. In ovine fetus, lung fluid is secreted at the rate of 2 ml/kg/h at mid gestation and gradually increases to 5 ml/kg/h at near term [5]. The rate and volume of fluid production however decrease as the fetus approaches term in readiness for postnatal adaptation. Arginine vasopressin, catecholamines, cortisol, prostaglandin E<sub>2</sub> and atrial natriuretic hormone are all associated with decrease in production of fetal lung fluid at or around birth. Amniotic fluid volume also contributes to the fetal lung distension and hence lung growth. In the fetal rat, oligohydramnios has been shown to retard lung growth and development of type I cells. However, there was no evidence of its effect on type II cells and surfactant production [12]. Another important factor contributing to the maintenance of adequate lung volume is fetal breathing movements. In the human fetus, breathing movements are noted as early as 11 weeks of gestation and by 30–40 weeks, it occurs 30% of the time. Various animal experiments on abolition of fetal breathing movements including transection of spinal cord at the level of phrenic nerve [13] and ablation of phrenic nerve [14] have demonstrated pulmonary hypoplasia and reduced lung DNA in affected animal fetuses. Increase in carbon dioxide and glucose levels, acidosis and drugs such as caffeine and theophylline are associated with increased fetal breathing movements. Maternal smoking, alcohol and drug use are known to reduce fetal breathing movements. Similarly, hypoxia, hypoglycaemia, intrauterine infection and prostaglandin E<sub>2</sub> may all have adverse effect on these breathing movements [5].

In the last decade, there have been huge advances on research on fetal tracheal occlusion to maintain fetal lung volume and stretching lung tissue to improve lung growth, especially in fetuses with congenital diaphragmatic hernia. A recent review on this topic summarises the pros and cons of this fetal interventional therapy [15]. Numerous studies, on sheep, rabbits, rats and mice, have repeatedly demonstrated that surgical tracheal occlusion applied during canalicular and saccular stages of lung development not only improves dry lung weight but also increases airway branching, alveolarization, alveolar surface area, type II pneumocytes and pulmonary vascular growth [15].

## 2.2. Hormonal, transcriptional and growth factors

The complexity of the role of various hormones as well as growth and transcriptional factors in the growing lung is gaining more and more fascination among researchers in recent years. The list of some of the important hormones, growth factors and transcriptional factors and their possible role in lung growth and development is summarised in Table 3. Thyroid hormone has been well recognised to play an important role in epithelial and mesenchymal cell differentiation and airway branching in early developmental period as well as differentiation of type II cells and surfactant secretion in late development. Pituitary and adrenal hormones have also been implicated in lung maturation. Among the numerous growth hormones and transcriptional factors, fibroblast growth factors (FGF) and Sonic hedgehog (Shh) seem to have influential roles in determining the pattern of airway branching. FGFs are also

important in type II cell differentiation and secretion of Surfactant Protein C later in lung development [16,17]. On the other hand, transforming growth factor (TGF  $\beta$ ) has an opposite but important role of inhibiting cell proliferation and branching morphology, promoting formation of lung matrix and aiding pulmonary repair after injury. Its deficiency can lead to undue lung inflammation and its over-expression may lead to lung fibrosis [16,17]. Vascular endothelial growth factor (VEGF) plays a central role in vasculogenesis and angiogenesis and is therefore critical for normal lung development. Beside its primary role on vascular development, VEGF also has positive effect on differentiation of type II cells and stimulation of surfactant production. Furthermore, VEGF also has an important role on endothelial function and inhibition of VEGF in premature lung has been known to reduce NO bioavailability that may lead to the development of chronic lung disease of prematurity (CLD) [18].

## 2.3. Environmental factors

The influence of environmental factors such as exposure of the developing lung to tobacco smoke both in intrauterine and postnatal life has long been associated with adverse respiratory outcomes such as reduced lung volume and decreased number of alveoli, enlarged alveolar size and abnormal septation leading to reduction of gas exchange area. However, the precise mechanism and timing of events of adverse effects are not clear. Some interesting effects of nicotine on type II cell morphology and metabolism has emerged from a recent study on rats. When rat fetuses were exposed to nicotine in early embryonic life from day 6 to day 20, it was noted that nicotine could permanently alter lung growth of the developing lung by stimulating alveolar type II cells proliferation, differentiation and metabolism and hence stimulating surfactant synthesis [19]. In recent years, there has also been growing interest on the possible influences of other environmental pollutants such as ozone and particulate matters that can result in impaired lung growth [20].

## 2.4. Specific diseases

### 2.4.1. Chronic lung disease of prematurity (CLD)

With advances in perinatal medicine and the resulting improved survival of extremely preterm infants, CLD has inevitably become the most important specific condition that adversely affects postnatal lung development. Unlike term infants, preterm infants are born with lungs still in 'prenatal' stages of development i. e. cannicular or saccular stage. Surfactant deficiency leading to mechanical ventilation, oxygen therapy and pulmonary inflammation, infection and patent ductus arteriosus are some of the determining factors leading to a cascade of events that result in CLD. Hyperoxia and barotrauma both cause lung injury and inflammation that subsequently results in decreased alveolization. Hyperoxia severely disrupts septation, causes increased terminal sac diameter and decreased gas exchanging surface area [3]. Intrauterine infection including with *Ureaplasma Spps.* may also result in disordered lung growth in the fetus and newborn infant [5]. An alternative theory for the dysregulated lung growth seen in infants who develop

Table 3 Possible role of some growth and transcriptional factors in lung growth and development

Transcriptional and growth factors	Possible role in lung growth and development
<i>FOXA1, FOXA2, GATA4 and GATA6</i>	= Formation and maintenance of foregut
<i>Tbx4</i>	= Localisation of bud site
Fibroblast growth factors (FGFs)	= Localisation of organs derived from foregut = Induction of budding and Branching (FGF 10) = Alveolization = Type II cell differentiation and induction of Surfactant Protein C ( FGF2 and FGF7)
Sonic hedgehog (Shh)	= Suppresses <i>Fgf10</i> expression and prevents branching events at sites where branching is stereotypically determined not to take place
Bone Morphogenic Protein 4 (BMP4)	= Formation and control of dorsal and ventral branches
<i>HOX</i> genes	= Defines overall three dimensional orientation
Epidermal growth factor	= Airway proliferation, differentiation and branching
Platelet-derived growth factor (PDGF)	= Alveolization
Retinoic acid (RA)	= Induction of <i>Fgf10</i> and endodermal differentiation
Transforming growth factor (TGF $\beta$ )	= Lung repair after pulmonary insult and matrix production = Inhibits cell proliferation
Insulin-like growth factor (IGF)	= Airway proliferation
Vascular endothelial growth factor (VEGF)	= Vasculogenesis, angiogenesis and lymphangiogenesis
Granulocyte macrophage-colony stimulating factor (GM-CSF)	= Macrophage differentiation

CLD is that their vascular development may be abnormal which may lead to abnormalities of lung growth (reviewed in Ref. [21]).

Postnatal glucocorticoids are widely used in the context of severe respiratory distress syndrome (RDS) and CLD, mostly to wean chronically ventilator-dependent preterm infants. Although postnatal glucocorticoids have proved to be invaluable as a 'rescue' therapy for chronically ventilated preterm infants, there have been concerns over their short-term and long-term adverse effects. From animal studies, it is clear that glucocorticoids directly affect alveolar and microvascular growth of the lungs. Burri et al. have studied the effects of high-dose, short-term as well as low-dose, long-term glucocorticoid administration on alveolarization and pulmonary microvascular development on rats [22]. Low-dose long-term therapy was shown to produce permanent impairment of lung alveolarization, possibly as a result of precocious microvascular maturation. However, when postnatal rats were administered short-term, high-dose glucocorticoids for 4 consecutive days, although there was initial evidence of precocious microvascular maturation by capillary fusion and arrest of parenchymal septation, the effects were temporary. This initial phase of accelerated lung growth was arrested briefly and by day 10, normal alveolar growth resumed [22]. Hence, it is clear that the precise dose, timing and duration of glucocorticoid therapy can have major implications on lung growth and development. Besides the effects of glucocorticoids on lung morphology, their effect on long-term pulmonary function has also been investigated. The results of these studies are difficult to compare or to interpret because of the variations in the use of surfactant, severity of the chronic lung disease, dose, duration and timing of the use of dexamethasone and because of different methods of measuring pulmonary function between different studies. When 145 children, who had participated in a double-blind, randomized controlled trial (1984–1989) of dexamethasone (0.5 mg/kg/day for 1 week) or placebo for treatment of neonatal chronic lung disease were followed up at 13–17 years of age, there was no significant difference in lung function between the two groups, as assessed by spirometry [23]. This study concluded that there is no evidence that use of dexamethasone in neonatal period has long-term consequences for the lung but beneficial effects were not demonstrated either. However, another recent follow-up study by Nixon et al. on 68, 8–11 year old children, who had participated in a randomized controlled trial (1992–1995) of a 42-day tapering course of postnatal dexamethasone (0.5 mg/kg/day) or placebo in the neonatal period to reduce the duration of ventilator dependency, reported that the postnatal dexamethasone exposure was associated with higher expiratory flow but clinically reported asthma scores were similar between the two groups [24].

#### 2.4.2. Congenital diaphragmatic hernia and pulmonary hypoplasia

Congenital diaphragmatic hernia (CDH) affects as many as 1/3000 live births. Despite extensive studies on better understanding of causes of CDH as well as on both prenatal and postnatal interventional therapies, survival still remains poor. A recent review on the genetics of CDH explains in detail the increasing evidence of the contribution of gene mutations to

the development of CDH during early lung development [25]. As summarized in Table 3, transcriptional and growth factors, such as Shh and FGF10, signal and regulate lung morphogenesis and defects at any stage may lead to lung hypoplasia which is an essential component of CDH. In the last decade, radiological advancements in 3D ultrasonography and magnetic resonance imaging (MRI) has made it possible to assess fetal lung volume quantitatively in fetuses suspected to have pulmonary hypoplasia and CDH [26]. As already described above, studies involving intrauterine tracheal obstruction to encourage growth of hypoplastic lung in CDH have been explored but provide many challenges before such therapy becomes routine in clinical practice.

### 3. Conclusion

The close relationship of different stages of morphological and vascular growth of human lung with numerous physical, biological and environmental factors is complex but fascinating. The growing knowledge of molecular biology and human genetics related to lung growth has not only enabled us to have better understanding of this complex and continuous process but also contributed towards antenatal diagnosis and interventional therapy for life threatening congenital conditions such as congenital diaphragmatic hernia and pulmonary hypoplasia. Although there are some important differences in the timing and stages of lung growth in human and animals, numerous animal experiments, mostly on sheep and rats, have greatly enhanced our knowledge of the important roles of various transcriptional and growth factors in lung growth and development. Understanding of the very basic stages of lung growth and recognition of development and maturation of alveoli, formation of functional blood alveolar barrier and production of surfactant has made survival of extremely premature infants possible. The effects of glucocorticoids on postnatal lung maturation need to be investigated further to establish the appropriate dose and duration of the glucocorticoid therapy in severe lung disease of prematurity and to establish possible long-term benefits and adverse effects. Advances in imaging techniques such as 3D ultrasonography and advanced MRI technologies will no doubt play a major role in early and accurate diagnosis of both structural and functional lung pathologies in fetuses with congenital structural defects and children with chronic lung disease of prematurity. However, much work still needs to be done to improve postnatal lung development in premature infants and to prevent chronic lung disease of prematurity.

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## **H2. Reproducibility of myocardial velocity and deformation imaging in term and preterm infants**





## Reproducibility of myocardial velocity and deformation imaging in term and preterm infants

Suchita Joshi<sup>1</sup>, Julie M. Edwards<sup>2</sup>, Dirk G. Wilson<sup>3</sup>, Joon K. Wong<sup>3</sup>, Sailesh Kotecha<sup>1</sup>, and Alan G. Fraser<sup>2\*</sup>

<sup>1</sup>Department of Child Health, Cardiff University, Cardiff, UK; <sup>2</sup>Wales Heart Research Institute, Cardiff University, Cardiff CF14 4XN, UK; and <sup>3</sup>Department of Paediatric Cardiology, University Hospital of Wales, Cardiff, UK

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### Aims

Myocardial velocity imaging has been validated in adults for assessment of ventricular function and indirect indices of pulmonary arterial pressure. To establish whether it could also be used in infants, we investigated the reproducibility of myocardial velocities and deformation indices in term and preterm neonates.

### Methods and results

Myocardial velocity loops acquired from 16 infants were analysed by four observers for inter-observer variability, and re-analysed after 6 months by one observer for intra-observer variability. For myocardial velocities, the coefficients of variation (CVs) for the left ventricle (LV) were 10–11 (intra-observer) and 14–20% (inter-observer) and for the right ventricle (RV) 15–19 and 18–24%, respectively. Reproducibility for annular displacements was <13% (intra-observer) and <18% (inter-observer). CVs for LV strain were 14–17 (intra-observer) and 36–43% (inter-observer) and for RV 19–24 and 25–37%. CVs for isovolumic acceleration were in general >40%. In comparison, the CVs for blood pool indices were 3–15%.

### Conclusion

Intra-observer reproducibility for myocardial velocity and deformation indices in neonates is adequate for these parameters to be used in clinical research. Inter-observer reproducibility is sub-optimal suggesting that these measurements should be used in clinical practice with caution. Myocardial acceleration, a marker of contractile function, was poorly reproducible.

### Keywords

Infants • Preterm • Tissue Doppler • Reproducibility • Myocardial velocity • Strain

## Introduction

Myocardial velocity imaging or tissue Doppler imaging has been validated and widely used in the adult population for the diagnosis of left ventricular (LV) and right ventricular (RV) dysfunction. In recent years, it has also been suggested as a potentially useful diagnostic tool for quantitative assessment of myocardial function in infants<sup>1</sup> and children.<sup>2,3</sup>

Premature infants who develop respiratory distress syndrome are at increased risk of developing structural abnormalities in the pulmonary vasculature and RV hypertrophy.<sup>4</sup> A reliable non-invasive diagnostic tool to monitor associated changes in function would be helpful in this population. Previously, Doppler echocardiography has been used to assess the changes in pulmonary arterial pressure in acute respiratory distress syndrome<sup>5</sup> and during recovery<sup>6</sup> using pulmonary arterial acceleration time. Although

this index can be highly specific for diagnosis of pulmonary arterial hypertension<sup>7</sup> in patients with a heart rate of <100 bpm, this method may not be as accurate in newborn infants with higher heart rates. The velocity of tricuspid regurgitation is another Doppler parameter which correlates highly with pulmonary arterial pressure,<sup>8</sup> but it has been shown to be measurable in only 61% of children aged <2 years who had chronic lung disease.<sup>9</sup> These limitations can be overcome by more advanced tissue Doppler methods. Certain tissue Doppler parameters such as RV isovolumic relaxation time<sup>10,11</sup> (IVRT) and RV systolic strain<sup>12,13</sup> have been validated as useful markers for estimation of pulmonary arterial pressure in adults. Regional myocardial strain has also been shown to be feasible in healthy term infants.<sup>14,15</sup> This has not yet been tested or validated in prematurely born infants.

The feasibility and reproducibility for off-line tissue Doppler measurements in adults has been extensively studied by the

\* Corresponding author. Tel: +44 29 2074 3489; fax: +44 29 2074 3500; E-mail address: fraserag@cardiff.ac.uk

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MYDISE study group.<sup>16</sup> In this present study, we have assessed the intra- and inter-observer variability for off-line analysis of longitudinal systolic and diastolic myocardial velocities, myocardial strain, annular displacements, and isovolumic acceleration (IVA) compared with conventional blood pool Doppler parameters such as mitral inflow velocities and pulmonary arterial acceleration time.

## Methods

### Population

Thirty healthy term infants ( $\geq 38$  weeks of gestational age) and 25 preterm infants ( $\leq 34$  weeks of gestational age) were recruited from the post-natal wards and the Special Care Baby Unit of a university hospital for a study of myocardial function in prematurity. Written informed consent was obtained from parents prior to recruitment. The South East Wales Regional Ethics Committee gave ethical approval for this study.

All infants had their first scan within the first 72 h after birth. The infants were screened for congenital cardiac defects and those with structural cardiac defects were excluded from the study, with the exception of patent ductus arteriosus. A second scan was performed a month later.

To determine the reproducibility of myocardial velocity and deformation indices, we selected a random sample of 16 studies, by drawing lots—8 out of 30 studies from term infants and 8 out of 25 studies from preterm infants. All the data presented in this paper are from these 16 scans.

### Echocardiographic acquisition

Images were acquired using a commercially available system (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway) with a 7.0 or 5.0 MHz transducer, by a cardiac sonographer (J.M.E.) or a clinical research fellow (S.J.). Infants were kept in supine or left lateral position during the scan.

Pulsed wave Doppler of blood flow in the left ventricular outflow tract (LVOT) in the apical four-chamber view was acquired to measure LVOT velocity time integral. Conventional pulsed wave Doppler of mitral and pulmonary flow was also acquired from the apical four-chamber and the parasternal short-axis views, respectively.

For lateral and medial mitral annular velocities and tricuspid annular IVRT, real-time pulsed tissue velocity profiles were acquired from the mitral and tricuspid annulus, respectively.

Colour tissue Doppler images were acquired separately of the LV, RV, and septum. The Nyquist limit was optimized to avoid aliasing, and the depth of imaging and the sector angle were adjusted to obtain high frame rates. All images were stored as 3-beat loops on magneto-optical disks for post-processing.

### Off-line analysis

Images were analysed using commercially available EchoPAC software (GE Vingmed Ultrasound EchoPAC 7.00, Horten, Norway). Images from all 16 studies were analysed independently by four observers (S.J., J.M.E., D.G.W., and J.K.W.) for inter-observer variability. For intra-observer variability, one observer (S.J.) re-analysed the same images after 6 months. Observers were blinded to the clinical status of the infants.

Thirteen parameters were measured from each study, including 4 blood Doppler parameters and 9 tissue Doppler parameters. All parameters were measured in 3 beats and averaged, except if the signal from an individual beat was too noisy to be analysed in which case

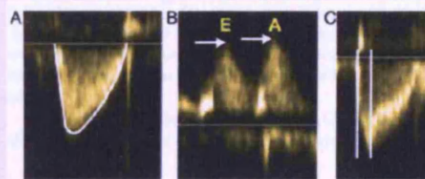
1–2 beats were measured. Each tissue Doppler parameter was measured at the basal segments of the LV and RV, and the base of the septum. The cursor was positioned within each segment, so that it did not encroach upon the annulus during systole.

The velocity time integral of LV outflow, the mitral E and A velocities, and the pulmonary artery acceleration time were measured conventionally from blood pool Doppler (Figure 1A–C).

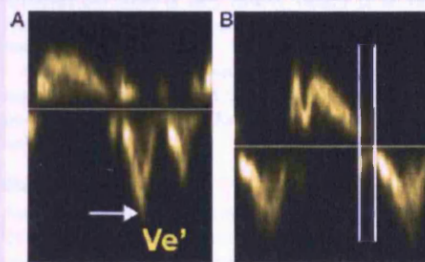
The mitral annular early diastolic velocity ( $Ve'$ ) was measured at both the medial and the lateral mitral annulus (Figure 2A). Tricuspid annular IVRT was measured as the time interval between the end of systolic motion and the beginning of early diastolic motion (Figure 2B).

Myocardial systolic velocity ( $Vs$ ), early diastolic velocity ( $Ve$ ), and diastolic velocity during atrial contraction ( $Va$ ) were measured at the basal segments. When myocardial  $Ve$  and  $Va$  were fused due to rapid heart rate, a single diastolic velocity was recorded and noted as  $Ve$ . Time to peak systolic velocity ( $T_{Q-Vs}$ ) was measured as the time interval between the start of the QRS complex and the peak myocardial velocity during ejection (Figure 3, time interval 4). The measurement for the myocardial IVA was made by dividing the peak positive velocity during the IVC by time to this peak velocity from the onset of the signal at the zero crossing (Figure 3, slope 5).

Annular displacement ( $Ds'$ ) was measured using tissue tracking at the lateral mitral annulus and at the lateral tricuspid annulus (Figure 4A).

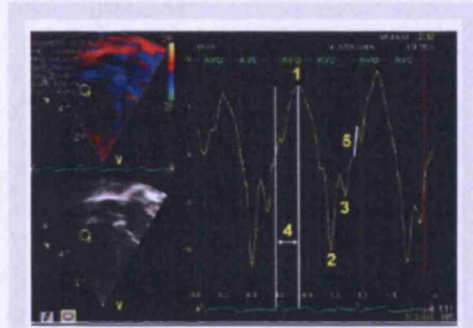


**Figure 1** (A) Tracing of left ventricular outflow tract Doppler to calculate velocity time integral (VTI). (B) Mitral inflow Doppler showing early diastolic velocity (E) and diastolic velocity during atrial contraction (A). (C) Pulmonary artery flow Doppler showing pulmonary arterial acceleration time (PA AT).

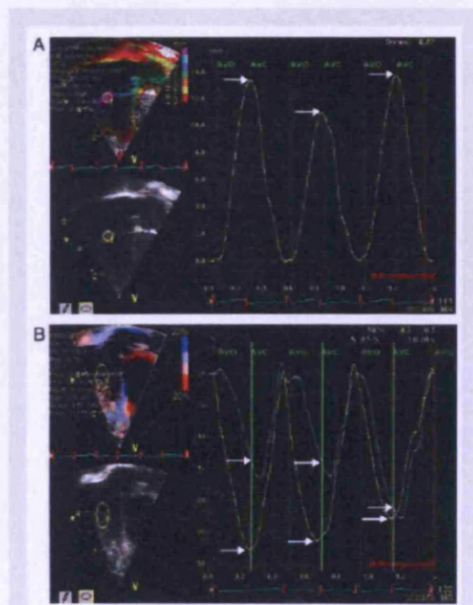


**Figure 2** (A) Lateral mitral annular real-time (pulsed) tissue Doppler showing early diastolic velocity ( $Ve'$ ). (B) Lateral tricuspid annular pulsed tissue Doppler showing measurement of regional relaxation time (IVRT; between the two vertical lines).





**Figure 3** Processed colour tissue Doppler image of RV showing peak systolic velocity (point 1), early diastolic velocity (point 2), diastolic velocity during atrial contraction (point 3), QRS-Vs (time interval 4), and isovolumic acceleration (slope 5).



**Figure 4** (A) Processed colour tissue Doppler image of RV showing tricuspid annular displacement at end systole. (B) Processed colour tissue Doppler image of RV showing basal (lower arrows) and apical (upper arrows) peak strain during systole. The vertical lines represent the timings of pulmonary valve closure. Although labelled by the machine and on these images as 'AVO' and 'AVC', the time intervals refer to pulmonary valve opening and pulmonary valve closure, obtained from the pulsed Doppler trace of pulmonary arterial flow.

Peak systolic strain (S) was measured within the basal and the apical portions of the lateral wall of the LV and the lateral wall of the RV (Figure 4B). In the septum, peak systolic strain was measured only in the mid-septal segment. For computation, strain length of 10 mm was used as recommended by Nestas et al.<sup>15</sup> To ensure measurement of maximal negative strain during systole, event timing was superimposed from LVOT and RV outflow tract blood Doppler recordings for the LV and RV, respectively.

### Statistical methods

The sample size of 16 scans observed by four observers for the inter-observer reproducibility gave 48 degrees of freedom [number of studies  $\times$  (number of observers  $- 1$ )], and the power to demonstrate differences with 95% confidence intervals estimated at  $0.4 \times$  standard deviation (SD).

Mean and SD were calculated for each variable. Intra- and inter-observer reproducibility is reported as coefficients of variation (CVs, in %), calculated using the formula:  $CV = (SD/\text{arithmetic mean of measurements}) \times 100$ , where SD is the standard deviation of residuals (measurement 1  $-$  measurement 2). As there were four observers, there were six pairs of data for comparison (each derived from repeated analysis of the 16 patients); the inter-observer reproducibility of each variable is therefore expressed as a pooled CV, which is the mean value of the six comparisons.

Systematic bias between repeated measurements was assessed using Bland-Altman analysis.<sup>17</sup> The results from these six paired comparisons were also averaged and are presented as the pooled mean difference for each variable (i.e. each summary statistic is the average of the mean difference between two observers repeated six times with different pairs of observers).

Intra-observer variability is also presented as CV and Bland-Altman difference for the paired measurements for each variable.

## Results

### Feasibility

Most pulsed and tissue Doppler parameters could be measured in  $>90\%$  of neonates. A single diastolic velocity at the mitral valve was noted in 5% (1 infant), and using tissue Doppler in 31% (5 infants) at the LV, 20% (3 infants) at the RV, and 12% (2 infants) at the septum. In these cases, a single diastolic velocity was measured and noted as early diastolic velocity (E or  $Ve'$ ). The fusion of early and late diastolic velocities may be due to rapid heart rates in infants.

Missing values for the myocardial velocity occurred because a segment could not be imaged or because the traces were of the poor quality. The feasibility for measuring LV apical strain was only 75%.

### Reproducibility

The average frame rate for the myocardial velocity loops was  $227 \pm 18$  frames per second. The mean values for all parameters, and the results of Bland-Altman analysis and CV for both intra- and inter-observer variability, are summarized in Table 1.

### Indices for assessment of LV function

Mitral annular early diastolic velocity by pulsed tissue Doppler ( $Ve'$ ) had reproducibility of 7–13% (intra-observer) and 9% (inter-observer).



**Table 1** The mean values for all parameters, and the results of Bland–Altman analysis and CV for both intra- and inter-observer variability

	Intra-observer			Inter-observer		
	Mean ± SD (1 observer)	Bland–Altman (95% CI)	CV %	Mean ± SD (4 observers)	Bland–Altman, mean (range)	CV %, pooled mean ± SD
VTI (cm)	11.6 ± 2.4	0.30 (–0.50 to 1.1)	3.6	11.5 ± 2.2	0.19 (0.02 to 0.34)	6.0 ± 1.9
E (m/s)	0.72 ± 0.23	0.02 (–0.01 to 0.05)	2.5	0.73 ± 0.23	0.02 (0.01 to 0.04)	2.8 ± 0.4
A (m/s)	0.66 ± 0.14	0.01 (–0.07 to 0.09)	5.5	0.69 ± 0.15	0.03 (0.00 to 0.05)	4.5 ± 0.8
PA AT (ms)	58.7 ± 13.8	–0.64 (–14.4 to 13.1)	11.9	62.3 ± 5.3	5.03 (0.42 to 8.34)	15.2 ± 2.9
Displacement (mm)						
LV Ds'	3.9 ± 1.7	–0.24 (–0.72 to 0.25)	6.2	4.1 ± 1.6	0.28 (0.12 to 0.32)	12.2 ± 3.4
RV Ds'	7.5 ± 1.9	–0.19 (–2.1 to 1.7)	12.8	4.6 ± 1.2	0.32 (0.03 to 0.49)	18.2 ± 4.6
Septum Ds'	4.7 ± 1.2	–0.15 (–0.68 to 0.38)	5.6	4.8 ± 1.4	0.04 (0.02 to 0.09)	11.8 ± 6.8
Annular velocity (cm/s)						
LV Ve'	8.0 ± 3.0	0.0 (–2.0 to 2.0)	12.6	8.0 ± 2.0	0.3 (0.0 to 0.5)	9.4 ± 4.2
Septum Ve'	7.0 ± 2.5	0.2 (–1.0 to 1.0)	6.9	7.0 ± 3.0	0.3 (0.2 to 0.4)	9.2 ± 3.9
Myocardial velocity (cm/s)						
LV						
Vs (bl)	2.4 ± 0.9	–0.04 (–0.05 to 0.44)	9.8	3.4 ± 1.5	0.18 (0.02 to 0.36)	14.3 ± 1.8
Ve (bl)	4.9 ± 2.4	–0.17 (–1.3 to 0.93)	11.1	4.5 ± 2.6	0.12 (0.04 to 0.22)	13.5 ± 1.9
Va (bl)	2.9 ± 1.0	–0.15 (–0.73 to 0.44)	9.9	3.0 ± 1.1	0.24 (0.06 to 0.43)	20.3 ± 4.7
RV						
Vs (bl)	4.4 ± 1.1	0.18 (–1.3 to 1.7)	17.7	4.6 ± 1.2	0.32 (0.03 to 0.50)	18.3 ± 4.5
Ve (bl)	6.2 ± 2.7	0.48 (–1.3 to 2.3)	15.0	6.3 ± 2.9	0.46 (0.01 to 0.75)	23.3 ± 8.4
Va (bl)	5.8 ± 1.3	0.34 (–1.7 to 2.4)	18.5	5.9 ± 1.2	0.48 (0.07 to 0.85)	24.1 ± 7.8
Septum						
Vs (bs)	2.9 ± 0.9	0.01 (–0.55 to 0.57)	9.9	3.1 ± 1.1	0.35 (0.14 to 0.63)	21.7 ± 5.7
Ve (bs)	4.7 ± 2.3	0.15 (–0.54 to 0.85)	7.7	4.7 ± 2.1	0.14 (0.02 to 0.27)	9.3 ± 0.8
Va (bs)	3.7 ± 1.1	0.21 (–0.36 to 0.78)	8.2	3.9 ± 1.4	0.45 (0.10 to 0.84)	22.7 ± 7.3
Timing (ms)						
RV relaxation time (IVRT)	50.1 ± 8.7	–4.1 (–20.6 to 12.3)	16.1	34.7 ± 15.3	18.5 (10.2 to 33.6)	27.0 ± 8.8
T <sub>Q–V6</sub>						
LV	95.0 ± 25.2	–0.89 (–25.7 to 23.9)	13.3	98.0 ± 29.8	13.6 (1.8 to 26.6)	34.6 ± 18.3
RV	104.5 ± 31.9	0.88 (–21.6 to 23.3)	11.0	104.1 ± 31.1	5.6 (0.9 to 10.8)	11.1 ± 4.2
Septum	106.8 ± 32.1	–4.7 (–24.8 to 15.3)	9.4	112.6 ± 37.2	3.8 (0.8 to 8.7)	22.2 ± 12.3
Isovolumic acceleration (m/s <sup>2</sup> )						
LV	0.80 ± 0.41	0.25 (–0.64 to 1.1)	62.5	0.64 ± 0.33	0.10 (0.02 to 0.20)	46.7 ± 7.6
RV	1.16 ± 0.39	0.16 (–0.78 to 1.1)	43.6	1.0 ± 0.44	0.20 (0.03 to 0.35)	45.0 ± 3.6
Septum	0.96 ± 0.40	0.07 (–0.45 to 0.59)	29.3	0.90 ± 0.57	0.18 (0.00 to 0.28)	60.3 ± 13.3
Strain (%)						
LV						
Ss (bl)	–12.2 ± 4.2	1.5 (–2.1 to 5.1)	14.0	–12.1 ± 5.5	3.2 (1.02 to 5.50)	43.0 ± 13.9
Ss (al)	–12.5 ± 5.4	0.23 (–4.1 to 4.6)	17.1	–12.7 ± 6.4	1.3 (0.83 to 1.82)	36.9 ± 15.7
RV						
Ss (bl)	–23.9 ± 4.2	–0.48 (–9.3 to 8.3)	19.0	–21.6 ± 7.5	2.6 (0.31 to 4.60)	37.1 ± 11.5
Ss (al)	–17.4 ± 6.2	4.0 (–5.2 to 13.3)	23.9	–22.0 ± 7.8	2.4 (0.29 to 5.03)	25.3 ± 1.9
Septum						
Ss (ms)	–17.7 ± 4.1	0.43 (–3.0 to 3.8)	9.9	–19.5 ± 9.4	4.2 (1.21 to 8.70)	52.1 ± 26.4

SD, standard deviation; CI, confidence interval; CV, coefficient of variation; VTI, left ventricular outflow tract velocity time integral; PAAT, pulmonary arterial acceleration time; LV, left ventricle; RV, right ventricle; Ve', annular early diastolic velocity; Vs, myocardial systolic velocity; Ve, myocardial early diastolic velocity; Va, myocardial diastolic velocity during atrial contraction; IVRT, tricuspid annular isovolumic relaxation time; T<sub>Q–V6</sub>, QRS to V6 time interval; Ss, systolic strain; bl, basal segment of lateral wall; al, apical segment of lateral wall; bs, basal segment of septal wall; ms, mid-septal segment.



Intra- and inter-observer variability for LV myocardial systolic and diastolic velocities as well as annular displacements, obtained by processing stored digital loops, were between 10 and 15% except for the diastolic velocity ( $V_a$ ) (20% inter-observer). More advanced parameters such as QRS to  $V_s$  time interval ( $T_{Q-V_s}$ ) and LV strain had intra-observer CVs of <15%, but inter-observer reproducibility for these parameters was sub-optimal at CVs >35%. LV IVA was not reproducible (CVs 30–60%).

In comparison, blood Doppler parameters such as LV velocity time integral and mitral inflow Doppler had CVs of 3–6% for both intra- and inter-observer variability.

#### Indices for assessment of RV function

Intra-observer CVs for the RV indices were generally higher compared with the corresponding LV indices. Intra- and inter-observer CVs for the RV myocardial systolic and diastolic velocities, annular displacement, and ( $T_{Q-V_s}$ ), were between 15–19% and 18–24%, respectively. IVA was poorly reproducible in the RV also, with CV >40%.

#### Indices for assessment of pulmonary arterial pressure

Pulmonary arterial acceleration time, which has been traditionally used to estimate pulmonary arterial pressure, had CV of 12–15%. Tissue Doppler parameters that are relatively new methods of indirect estimation of pulmonary arterial pressure were less reproducible. CV for tricuspid annular IVRT was 16% (intra-observer) and 25% (inter-observer), and that for RV basal strain was 19% (intra-observer) and 25–37% (inter-observer).

#### Comparison between term and preterm infants

At the time of study, preterm infants had lower body weight and slower heart rate (Table 2).

Intra-observer reproducibility was marginally better in the term infants compared with the preterm infants—for the pulmonary arterial acceleration time (CV 9% compared with 14%) and for RV myocardial velocities ( $V_s$  15% compared with 19%;  $V_e$  14% compared with 18%; and  $V_a$  14% compared with 20%). The CV for mid-septal strain was also slightly better in the term infants (7% compared with 13%). On the other hand, intra-observer variability for the tricuspid annular relaxation time had better CV in the preterm infants (7%) compared with the term infants (22%). None of these differences is statistically significant ( $P > 0.05$ ). Analysis of reproducibility by body weight rather than gestational age also did not demonstrate any worse reproducibility in the smallest infants.

## Discussion

Although numerous studies on the use of tissue Doppler have been published in the last decade, there have been surprisingly few detailed studies on the reproducibility of the analysis of myocardial velocity and strain indices especially in children. Our study is the largest and most detailed report of the intra- and inter-observer reproducibility of real-time and processed tissue Doppler parameters in infants.

### Feasibility of tissue Doppler in infants

Due to RV dominance in newborn infants, the acquisition of images from the LV, without missing the apex and while retaining a narrow sector of the image and thus a high frame rate, was a challenge. This accounts for the fact that LV apical strain had the most missing values (37%). The other tissue Doppler parameters measured in this study were not similarly affected as they were all measured either at the annulus or in the basal segments.

### Reproducibility of off-line analysis of tissue Doppler measurements

As anticipated, pulsed Doppler recordings of blood flow were more reproducible than myocardial velocities. The reproducibility for measuring the acceleration time of pulmonary arterial flow in this study was better than previously published data in infants by Subhedar and Shaw<sup>18</sup> (intra-observer CV 12 vs. 25%). This may be because we studied both term and preterm infants, whereas the previous study was restricted to preterm infants with respiratory distress syndrome.

The lateral mitral annular early diastolic velocity in this study had inter-observer variability of 9% which is comparable to that reported in an adult study (CV 9–17%).<sup>19</sup> Although there is evidence that the relaxation time 'IVRT' of the tricuspid annulus is a useful parameter for excluding pulmonary hypertension in adults,<sup>10</sup> the inter-observer reproducibility for this index in infants was sub-optimal (CV 27%), compared with 12–13% in adults.<sup>11,20</sup> Intra-observer variability was better at CV 16%.

When Pauliks et al. addressed myocardial velocities in children undergoing closure of an atrial septal defect,<sup>21</sup> the CVs for systolic ( $V_s$ ) and diastolic ( $V_e$ ) velocities were <10% for both intra- and inter-observer variability, while CVs for diastolic velocity ( $V_a$ ) were higher (21.5% intra- and 15% inter-observer). In infants, we found that the reproducibility of measuring myocardial velocity was more variable in the RV (15–18% intra- and 18–24% inter-observer variability) than in the septum and the lateral wall of the LV (CV < 15%). In adults with LV dyssynchrony, variability for measuring myocardial systolic velocities ( $V_s$ ) has been as much as 18–56%.<sup>22</sup>

There are limited data on the reproducibility of deformation indices or IVA in infants and children. Weidemann et al.<sup>23</sup> measured radial and longitudinal strain and strain rate in 33 healthy children aged 4–16 years. They reported intra-observer variability of 11% and inter-observer variability of 15%; these data are pooled CVs for LV, RV, and septal strain. In our study, when measuring longitudinal peak systolic strain, variation between lowest and highest CVs varied in the LV (14–17%) and RV (19–23%). Pena et al.<sup>14</sup> have recently reported a reproducibility of longitudinal peak systolic strain in infants of only 1.2%, but this is misleading as the figure given is a Bland–Altman difference presented as percentage, and not the CV between observers.

One of the major difficulties faced when measuring myocardial strain in infants is the small size of the heart. We used a computation length of 10 mm, as previously recommended.<sup>15</sup> Using smaller distances increased noise and reduced reproducibility. The strain length of 10 mm was therefore necessary, but in small hearts means



**Table 2** Comparison of term and preterm subjects

	Number	Mean (SD)		
		Gestational age at birth (weeks)	Weight (kg)	Heart rate (bpm)
Term infants	8	39.4 ± 1.6	3.9 ± 0.75	153 ± 26
Preterm infants	8	30.6 ± 2.6	2.0 ± 0.82	140 ± 10
Significance (P-value)		<0.001	<0.001	0.214

SD, standard deviation.

that it becomes difficult to position the sample volume accurately to avoid influences from surrounding structures such as the atrium.

IVA has been validated as a useful, load-independent index for assessing myocardial contractility in animal models.<sup>24,25</sup> It has also been used to assess myocardial contractility in children after surgical closure of atrial septal defect where the variability reported by Pauliks *et al.*<sup>21</sup> (intra-observer 10.8% and inter-observer 11.5%) is considerably better than was found in infants in our study (CV > 35%). One reason for the sub-optimal reproducibility of IVA may be that this index could not always be measured as an average of 3 beats.

### Limitations

In this study, two investigators acquired all the images. Three different GE Vivid seven machines were used. The intra- and inter-observer variability of analysis that we have presented does not take into account possible inter-operator and inter-machine differences during acquisition.

Secondly, despite having experience in echocardiography, the four observers who analysed the images independently had different levels of experience in tissue Doppler. A common protocol was developed jointly between all observers before the start of the study. In our study, the intra-observer variability may represent the best possible reproducibility of myocardial velocity and deformation in a research environment. Inter-observer variability for both acquisition and measurement in a wider clinical context may be much greater.

### Conclusion

We have demonstrated that myocardial velocity imaging and its off-line analysis is feasible in newborn infants. Intra-observer reproducibility for myocardial velocities, displacement, and strain as demonstrated in this study are adequate and these parameters can be used in clinical research. However, inter-observer reproducibility for more advanced indices such as LV and RV longitudinal strain is sub-optimal suggesting that these measurements should be used cautiously for clinical diagnosis. Reproducibility for myocardial acceleration, a marker of contractile function, is currently unsatisfactory in infants.

Thus, myocardial velocity imaging is a promising non-invasive diagnostic tool that may be used in term and preterm infants, but further technical advances are still vital to make this a useful clinical diagnostic tool.

**Conflict of interest:** none declared.

### Funding

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***Appendix I: Posters presented to international meetings***



# Preterm infants have subclinically reduced myocardial function compared with healthy term controls

Suchita Joshi<sup>1</sup>, Julie M Edwards<sup>1</sup>, Dirk G Wilson<sup>2</sup>, Orhan Uzun<sup>2</sup>, Mark Drayton<sup>2</sup>, Alan G Fraser<sup>1</sup>, Sailesh Kotecha<sup>1</sup>

<sup>1</sup>Cardiff University, Cardiff UK, <sup>2</sup>University Hospital of Wales, Cardiff, UK

I1 Preterm infants have sub-clinically reduced myocardial function compared with healthy term controls

## Background:

- There is a need for a reliable, non-invasive diagnostic tool to assess myocardial dysfunction and pulmonary arterial hypertension in preterm infants with Respiratory Distress Syndrome (RDS)
- Tissue Doppler (TD) imaging has been established as a useful imaging modality in adults for assessment of myocardial function and to estimate pulmonary artery pressure by surrogate markers

## Aim:

- To assess if TD imaging can be used to quantify regional myocardial function in infants with RDS

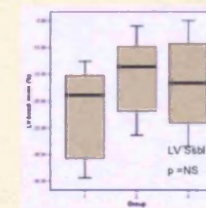
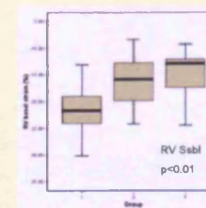
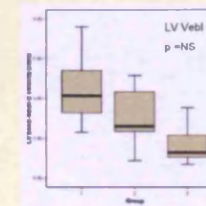
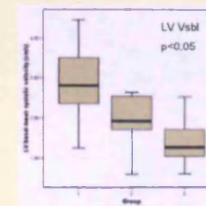
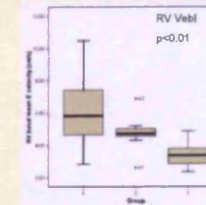
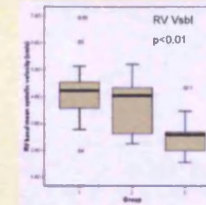
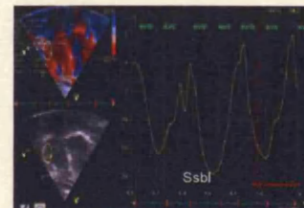
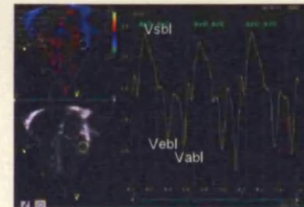
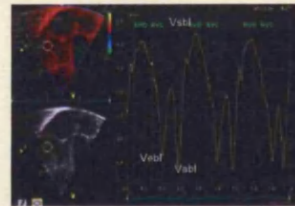
## Methods:

- All Preterm (PT) of  $\leq 34$  weeks gestation and Term infants had 2D, blood flow Doppler and tissue Doppler echocardiograms within 72 hours of birth
- Images were acquired with GE vivid 7, with 7MHz probe
- TD images were post processed using GE Echopac software, to analyse **systolic velocity (Vsbl)**, **early diastolic velocity (Vebl)**, **atrial contraction velocity (Vabl)**, **systolic strain at base (Ssbl)** and **apex (Ssal)** of the lateral walls of the right (RV) and left (LV) ventricles
- Mann-Whitney U Test was used to compare differences between groups for each variable


Group	N (Sex)	Gestation (wks, SD)	Weight (Kg, SD)
Term (1)	23 (F=12)	39.6 (1.2)	3.4 (0.3)
PT Control (2)	9 (F=6)	32 (1.2)	1.8 (0.6)
PT RDS (3)	14 (F=6)	28 (3.2)	1.3 (0.5)

## Conclusion:

- In this pilot study, premature infants with RDS had reduced RV systolic and diastolic velocities, as well as reduced longitudinal strain, compared with healthy term controls. The differences in LV longitudinal function was limited to LV systolic velocity
- Tissue Doppler echocardiography is feasible in the newborn and may be useful to study subclinical relationships between persistent pulmonary hypertension and RV function in preterm infants with RDS








**CARDIFF UNIVERSITY**  
PRIFYSGOL CAERDYDD

## Cardiovascular Response to Acute Hypoxia in 8-12 Year Old Children with Chronic Lung Disease of Prematurity

Suchita Joshi<sup>1</sup>, Julie M Edwards<sup>2</sup>, Dirk G Wilson<sup>1</sup>, Mark Drayton<sup>4</sup>, Alan G Fraser<sup>2</sup>, Sailesh Kotecha<sup>1</sup>

<sup>1</sup> Department of Child Health, <sup>2</sup> Department of Cardiology, School of Medicine, Cardiff University, UK, <sup>3</sup> Department of Paediatric Cardiology, <sup>4</sup> Department of Neonatology, University Hospital of Wales, UK



**Background:**

- Preterm children who die of chronic lung disease of prematurity (CLD) have been shown to have abnormal thickening of pulmonary arterioles.
- It is unknown whether these pathological changes at an early age contribute to diminished right ventricular (RV) function and increased risk of pulmonary arterial hypertension later in life, especially in response to hypoxia.

**Aim:**

- To assess evidence of RV dysfunction and pulmonary hypertension in response to acute hypoxia, in children who had CLD compared to healthy term and preterm controls by using Doppler echocardiography.

**Methods:**

Baseline transthoracic blood and tissue Doppler echocardiography

↓

Inhalation of premixed 15% oxygen for 20 minutes followed by repeat echocardiography (while inhaling 15% oxygen)

↓

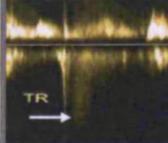
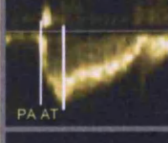
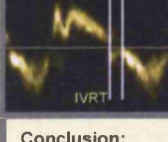
Inhalation of premixed 12% oxygen for 20 minutes followed by repeat echocardiography (while inhaling 12% oxygen)

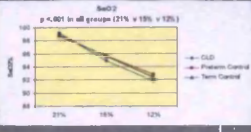
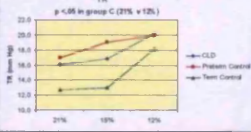
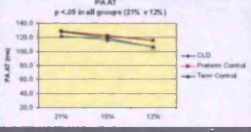
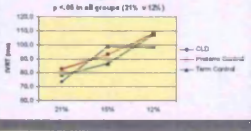
➤ Pulse oximetry was continuously recorded during the procedure

➤ Tricuspid regurgitation maximum velocity (TR), Pulmonary arterial acceleration time (PA AT) and Tricuspid annular isovolumic relaxation time (IVRT) were measured using GE EchoPac system

**Results:**

Doppler indices that varied with hypoxia

	21%	15%	12%
<b>SaO2 (%)</b>	21%	15%	12%
CLD	99.2	94.9	91.8
Preterm Control	98.7	95.7	92.9
Term Control	98.9	95.6	92.4

	21%	15%	12%
<b>TR (mm Hg)</b>	21%	15%	12%
CLD	16.1	16.9	20.2
Preterm Control	17.0	19.1	20.0
Term control	12.7	13.0	18.2

	21%	15%	12%
<b>PA AT (ms)</b>	21%	15%	12%
CLD	128.4	119.0	106.0
Preterm Control	128.9	122.4	116.0
Term Control	122.0	116.4	106.7

	21%	15%	12%
<b>IVRT (ms)</b>	21%	15%	12%
CLD	73.8	98.7	98.0
Preterm Control	82.8	93.3	108.3
Term Control	78.5	86.7	107.5

**Conclusion:**

- The maximum velocity of tricuspid regurgitation (TR) was significantly higher in prematurely born children ( $p < .05$ ), indicating that they may have higher baseline RV systolic pressure compared to children who are born at term.
- Oxygen saturation and Doppler parameters in children with CLD altered in a similar manner to that in the control groups in response to acute hypoxia in this study with statistically insignificant differences between groups, suggesting that these children may not be at increased risk of developing pulmonary hypertension with acute hypoxia.

	No	Gestation (wks)	Age (yrs)	Weight (kg)
Preterm CLD	20	28	10.2	32.1
Preterm control	20	30	10.4	38.6
Term control	16	39	10.1	37.5

12 Cardiovascular response to acute hypoxia in 8-12 year old children with chronic lung disease of prematurity





# Can echocardiography be used to measure right ventricular dysfunction and pulmonary hypertension in newborn infants?



Suchita Joshi<sup>1</sup>, Julie M Edwards<sup>2</sup>, Dirk G Wilson<sup>3</sup>, Joon K Wong<sup>3</sup>, Alan G Fraser<sup>2</sup>, Sailesh Kotecha<sup>1</sup>

<sup>1</sup> Department of Child Health, <sup>2</sup> Department of Cardiology, College of Medicine, Cardiff University, UK. <sup>3</sup> Department of Paediatric Cardiology, University Hospital of Wales, UK

### Background and Aims:

- Myocardial velocity imaging is a useful non-invasive diagnostic tool for assessing pulmonary arterial hypertension in adults. We have shown that this maybe a useful diagnostic tool in preterm infants with respiratory distress syndrome (Joshi et al *ERJ* 2008 *Suppl. 52: 812s*). However, reproducibility and thus clinical utility of off-line analysis of myocardial velocity imaging in newborns has not yet been established.
- Our aim is to establish intra and inter-observer reproducibility for off-line analysis of RV myocardial velocities and strain in newborn infants.

### Methods:

- 3-beat myocardial velocity loops were acquired from newborn infants and were followed up at 1 month. Images from 8 term and 8 preterm infants were randomly selected and analysed by 4 observers for inter-observer variability using commercially available GE EchoPac system. 1 observer re-analysed the images for intra-observer variability.
- Reproducibility is presented as coefficients of variation (CV) %. For inter-observer variability, CV is presented as Pooled CV for the 4 observers.

### Results:

RV indices	Mean (SD)	Intra-observer (CV %)	Inter-observer (Pooled CV %)
PA acceleration time in ms (Fig.1a)	58.7 (13.8)	11.9	15.2
RV relaxation time in ms (Fig.1b)	50.1 (8.7)	16.1	27.0
RV s velocity in cm/s (Fig.2 point 1)	4.4 (1.1)	17.7	18.3
RV e velocity in cm/s (Fig.2 point 2)	6.2 (2.7)	15.0	23.3
RV a velocity in cm/s (Fig.2 point 3)	5.8 (1.3)	18.5	24.1
QRS-Vs time in ms (Fig.2 point 4)	104.5 (31.9)	13.3	34.6
Isovolumic Acceleration in m/s <sup>2</sup> (Fig.2 slope 5, Fig. 3)	1.2 (0.4)	43.6	45.0
Displacement in mm (Fig. 4)	7.5 (1.9)	12.8	18.2
Basal strain % (Fig.5 yellow)	-23.9 (4.2)	19.0	37.1
Apical strain % (Fig.5 blue)	-17.4 (6.2)	23.9	25.3

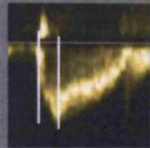


Fig. 1a

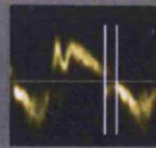


Fig. 1b

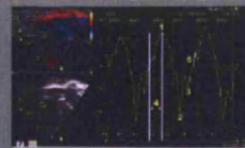


Fig. 2

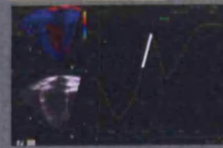


Fig. 3

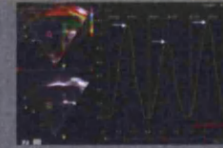


Fig. 4

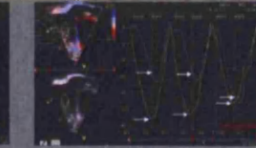


Fig. 5

### Conclusion:

- Intra-observer reproducibility for right ventricular myocardial velocities and displacement are adequate and these parameters can be used in clinical research.
- Inter-observer reproducibility for tissue Doppler myocardial velocity indices are sub-optimal suggesting that that these measures should be used cautiously for clinical diagnosis. Reproducibility for myocardial acceleration, marker of contractile function and deformity indices were unsatisfactory.

Can echocardiography be used to measure right ventricular dysfunction and pulmonary hypertension in newborn infants?