RESPIRATORY HEALTH OUTCOMES IN NEONATES - LUNG FUNCTION, EXERCISE AND AIRWAY MECHANICS

A thesis submitted in consideration for the degree of:

Doctor of Philosophy (PhD)

By

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2021

DEDICATION

For Ann Louise, Samuel, and Emily.

SUMMARY

My thesis uses data collected during the course of the Respiratory Outcomes in Neonates study, which aimed to identify, investigate mechanisms and establish potential treatments for lung disease in a population of preterm-born children in South Wales. As part of this study, 241 children attended our research unit where they underwent in-depth lung function testing, as reported in my thesis.

My results observed preterm-born children with low lung function had increased air trapping on lung volume testing, functional exercise impairment, and greater response to post-exercise bronchodilator. When classifying preterm-born children with low lung function by obstructive versus non-obstructive lung disease, those children with obstructive disease had greater impairment of forced expiratory volume in one second (FEV_1) and greater post-exercise reversibility.

On oscillometry testing, preterm-born children with low lung function, in particular those with obstructive lung disease, had impaired resistance and compliance, with evidence of peripheral airways being most affected. Post-exercise bronchodilator was effective in improving airway mechanical properties in children with low lung function.

Intra-breath oscillometry, unexplored in the preterm population to date, demonstrated impairment throughout the respiratory cycle in children with low lung function, and was not limited to expiration, suggesting a different disease process to that seen in other childhood wheeze disorders.

I demonstrated that my method for classification of obstructive and non-obstructive lung disease was appropriate in my population for identification of children with post-exercise bronchodilator reversibility, and was comparative to other established methods of defining obstructive lung disease.

Finally, I explored differences between two methods of spirometry, and noted systematic bias towards higher results using a pneumotachograph system compared to an infra-red turbine spirometry, with implications for clinical practice.

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ACKNOWLEDGEMENTS

Firstly, I would like to offer my sincere gratitude to Professor Sailesh Kotecha. I thank him for the opportunity to be involved in the RHiNO study and to undertake a PhD, and for his help, support, belief, and guidance along the way. During my many questioning moments getting to this stage, my mantra has been 'In Sailesh I trust'!

I owe a debt of gratitude to Kylie Hart, without whom I know I would not have made it this far. Kylie was such a support and working closely with her was one of the highlights of the whole project. Thank you to Gill Willets and Louise Yendle, the research nurses on RHiNO, who did an incredible job with recruitment, and for their friendship.

I would like to thank my colleagues from the Child Health Department, Drs David Gallacher, Emma Mitchell, Sarah Kotecha and John Watkins, for their help and advice. In particular, Dr John Lowe was a guiding light through the research process, and I am grateful for his friendship. Professors Mark Williams and Iolo Doull have been generous with their knowledge and time. My thanks to Professors Matthias Eberl and Kerry Hood from my appraisal panel – I appreciate the guidance they offered.

The wider RHiNO team, including my co-supervisors, Dr Zöe Hoare and the late Professor John Henderson, have been excellent support. Thank you to the team at NWORTH, especially Dr Nia Goulden and Alison Jenkins, and Louise Richards from NWIS for helping identify participants. We performed the lung function testing in the Children and Young Adults' Research Unit, which was such a wonderful space to have worked in. It has been great to see paediatric research in Wales develop over my time doing this project, and Jenny Muller, Zoe Morrison and Rhian Thomas-Turner were especially helpful and supportive with RHiNO.

A word for Dr Phil Connor, who planted a research seed several years ago, based on his belief I was good enough to do such a thing - I thank/blame him for getting me into this!

Thank you to Dr Bence Radics, Professors Zoltan Hantos and Peter Sly, and the team at University of Szeged, for the supply of the oscillometry equipment, and their exceptional knowledge.

My thanks and love to all my family and friends, in particular my parents Gill and John Cousins, for their kindness and encouragement, and for considerately learning to refrain from asking about how my PhD is going! I am looking forward to having more time to spend in their company in the near future!

Most importantly, the deepest of love and thanks to my wife Ann Louise, and children Samuel and Emily. Their love, patience, support, and sacrifices have been endless. I thank them for putting up with me during this whole process. Words do not do justice to my love and gratitude to them.

Lastly, I am extremely grateful to all the children and their families who participated so generously with their time and enthusiasm, and made this project such a memorable experience, as well as making this thesis possible.

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ABBREVIATIONS

$\%FEF_{25-75\%}$	Percent predicted FEF25-75%
$\%FEV_1$	Percent predicted FEV1
%FVC	Percent predicted FVC
ADHD	Attention deficit - hyperactivity disorder
ANOVA	Analysis of variance
ARV/AXV	Area within resistance/reactance-volume loop
ARV'/AXV'	Area within resistance/reactance-flow loop
ASD	Autistic spectrum disorder
Ax	Area under reactance curve
BMI	Body mass index
BPD	Bronchopulmonary dysplasia
C	Compliance
CI	Confidence interval
CLD	Chronic lung disease of prematurity
CPET	Cardiopulmonary exercise testing
EIB	Exercise-induced bronchoconstriction
ELBW	Extremely low birth weight
F_{br}	Breathing frequency/respiratory rate
Fdep	Frequency dependence of resistance
$FEF_{25-75\%}$	Forced expiratory flow between 25 - 75% of FVC
FEV_1	Forced expiration in 1 second
FRC	Functional residual volume
$FRC_{(He)/(pleth)}$	FRC measured by helium dilution/body plethysmography
*f*Res	Resonant frequency
FVC	Forced vital capacity
ICS	Inhaled corticosteroid
IUGR	Intra-uterine growth restriction
LBW	Low birth weight
LGA	Large for gestational age
MVV	Maximal voluntary ventilation
NEC	Necrotising enterocolitis
NGA	Normal for gestational age
NIPPV	Non-invasive positive pressure ventilation
NNRD	National Neonatal Research Database
Office of National Statistics	ONS
PDA	Patent ductus arteriosus
PEFR	Peak expiratory flow rate
PHVD	Post-haemorrhagic ventricular dilatation
PnOLD	Preterm-associated non-obstructive lung disease
POLD	Preterm-associated obstructive lung disease
PPROM	Preterm premature rupture of membranes

PT_c	Preterm control
PT_{low}	Preterm group with low lung function
PVL	Periventricular leukomalacia
R_{eI}/R_{eE}	End inspiratory/expiratory resistance
X_{eI}/X_{eE}	End inspiratory/expiratory reactance
$R_{meanI/E}$	Mean inspiratory/expiratory resistance
$X_{meanI/E}$	Mean inspiratory/expiratory reactance
$R_{V'maxI/E}$	Resistance at maximal inspiratory/expiratory flow
$X_{V'maxI/E}$	Reactance at maximal inspiratory/expiratory flow
RDS	Respiratory distress syndrome
RER	Respiratory exchange ratio
ROP	Retinopathy of prematurity
Rrs	Respiratory system resistance
RV	Residual volume
$RV_{(He)/(pleth)}$	RV measured by helium dilution/body plethysmography
SD	Standard deviation
SGA	Small for gestational age
T_c	Term control
TLC	Total lung capacity
$TLC_{(He)/(pleth)}$	TLC measured by helium dilution/body plethysmography
TV	Tidal volume
V	Volume
V'	Flow
$\dot{V}CO_2$	Peak carbon dioxide production
$\dot{V}E$	Minute ventilation
VLBW	Very low birth weight
$\dot{V}O_2$	Peak oxygen uptake
Xrs	Respiratory system reactance
ΔR_e	Expiratory to inspiratory difference in end of breath cycle resistance
ΔX_e	Expiratory to inspiratory difference in end of breath cycle reactance
$\Delta R_{V'max}$	Expiratory to inspiratory difference in resistance at maximal flow
$\Delta X_{V'max}$	Expiratory to inspiratory difference in reactance at maximal flow
$\Delta R_{I/E}$	Range of maximum to minimum resistance in inspiration/expiration
$\Delta X_{I/E}$	Range of maximum to minimum reactance in inspiration/expiration
ΔR_{mean}	Expiratory to inspiratory difference in mean resistance
ΔX_{mean}	Expiratory to inspiratory difference in mean reactance

1 INTRODUCTION

In this introduction my aim is to give an overview of preterm birth, respiratory morbidity associated with preterm birth, assess the current knowledge regarding the longer-term respiratory outcomes of preterm birth, and the different modalities of assessing respiratory function in children.

1.1 Preterm birth – short- and long-term respiratory morbidity

1.1.1 Definition and classification of preterm birth

Preterm birth is defined by the World Health Organisation as birth before 37 completed weeks of gestation (World Health Organisation, 2018, February 19). It can be divided into sub-categories depending on the exact gestation (World Health Organisation, 2019), as shown in Table 1.1 below.

Table 1.1 ICD 10 classifications of preterm birth.

Gestation	Definition
< 28 weeks of gestation	Extremely preterm
28 to < 32 weeks of gestation	Very preterm
32 to <37 weeks of gestation	Moderate to late preterm

Infants can also be categorised by birth weight (World Health Organisation, 2019), as per Table 1.2 below.

Table 1.2 ICD classifications of low birth weight.

Birth weight	Definition
< 1000g	Extremely low birth weight (ELBW)
1000 to < 1500g	Very low birth weight (VLBW)
1500 to < 2500g	Low birth weight (LBW)

Additionally all infants can be classified by birthweight in relation to the gestational age, as per Table 1.3 (World Health Organization, 1995). Preterm infants can fall into any category of both the birth weight and relative size definition, i.e. it is possible for an infant born, for example, very low birth weight to be small, normal or large for gestational age. Some of those born small for gestational age are considered to have

intrauterine growth restriction (IUGR), i.e. have an underlying pathological reason for their size (Wollmann, 1998). Unfortunately it is not always possible to separate those small for gestational age infants who have IUGR and so these terms are often used interchangeably (Suhag and Berghella, 2013).

Table 1.3 Case definitions of infant size for gestational age.

Centile for birth weight based on gestation	Definition
<10th centile	Small for gestational age (SGA)
10th to 90th centile	Appropriate for gestational age (AGA)
>90th centile	Large for gestational age (LGA)

All of the above classifications are important to consider as outcomes differ between each (Gill et al., 2013).

Further useful definitions of note include the perinatal period (from 22 weeks completed gestation until 7 completed days following birth) and the neonatal period (from birth up to 28 completed days after birth) (World Health Organisation, 2019). In the event of a death in the neonatal period, this can be classed as early (within the first 7 days after birth) or late (after 7 days and up to 8 completed days after birth) (World Health Organisation, 2019).

1.1.2 Epidemiology of preterm birth

Preterm birth is a common problem, with increasing rates over time, from 9.6% of all live births of all live births in 2005 (Beck et al., 2010) to 11.1% of all live births in 2010 (Blencowe et al., 2012). There are various reasons behind this increase, including increasing maternal age, increased intervention due to impact of chronic diseases on pregnancy, and increase in multiple pregnancies as a result of infertility treatment (Nour, 2012). Additionally better identification of foetuses with growth restriction leads to increased medical intervention.

There are geographical differences in the preterm birth rate, mainly due to the socioeconomic status of a country; rates of preterm birth in 2012 were approximately 12% in low income countries, and 9% in higher-income countries (Blencowe et al.,

2012). Asia and sub-Saharan Africa account for approximately 80% of all preterm births, while Europe has the lowest rates of preterm birth (8.7%) (Chawanpaiboon et al., 2019). The majority of these preterm births, approximately 85%, occur at the moderate – late preterm stage, with ~4% at the extremely preterm stage (Chawanpaiboon et al., 2019).

Rates of preterm birth and survival are affected by ethnicity. Data from the 2013 Office of National Statistics (ONS) birth cohort show preterm birth rates of 10.6% in the Black Caribbean population, 8.1% in the Black African population, and 8% in the Bangladeshi population compared to 7.5-7.6% in the White British, Indian and Pakistani populations (Office for National Statistics, 2013).

1.1.3 Causes of preterm birth

The reasons behind the occurrence of preterm birth are extremely varied. Preterm birth can be broadly categorised as occurring either spontaneously, due to onset of labour or preterm premature rupture of membranes (PPROM, where rupture of membranes occurs before onset of labour at <37 weeks' gestation) which occurs in 3% of pregnancies (Medina and Hill, 2006), or following medical intervention in the form of labour induction or Caesarean section. The former comprises approximately 70% of cases in and the latter, which can result from maternal, foetal or combined reasons, makes up the remaining 30% (Goldenberg et al., 2008). PPROM is defined as the spontaneous rupture of the membranes before 37 weeks' completed gestation and prior to established labour (NICE 2015 Preterm Labour and Birth). While overall rates of preterm birth have been increasing, rates of spontaneous preterm labour have actually been on the decrease, with increasing rates of medically-indicated preterm birth accounting for the overall increase (Ananth et al., 2005).

There are several known pathways that leads to spontaneous preterm birth, and these can occur at differing time points during pregnancy. These pathways, and their mechanistic effects, from Simmons et al (Simmons et al., 2010), are shown in Figure 1.1.

Figure 1.1 Table displaying potential mechanisms underpinning preterm birth (Simmons et al., 2010). Image reproduced with permission of the rights holder.

Pathway	Examples	Mechanistic Effectors	Gestational Age When Predominant
Infection or inflammation	Intrauterine lower genital tract systemic	Proinflammatory cytokine/ prostaglandin cascade matrix metalloproteinases	Early preterm birth (24-32 weeks)
Decidual hemorrhage	Thrombophilias, placental abruption autoantibody syndromes	Thrombin matrix metalloproteinases	Early or late preterm birth
Maternal/fetal hypothalamic-pituitary-adrenal activation	Stress	Maternal/fetal hypothalamic-pituitary-adrenal activation placental corticotropin-releasing hormone estrogens immune modulation	Late preterm birth (32-36 weeks)
Pathologic uterine overdistension	Multifetal gestation polyhydramnios	Expression of gap junctions protein prostaglandins oxytocin receptors	Late preterm birth
Cervical	Cervical insufficiency	Congenital disorders in-utero diethylstilbestrol exposure surgical treatment of cervical dysplasia traumatic damage infection	Very early and early preterm birth

There are many known risk factors that can contribute to one or more of these pathways, which may result in spontaneous labour or a medical intervention to deliver the infant early. Known risk factor include previous spontaneous preterm birth, genetic predisposition, extremes of maternal age, black race, uterine abnormalities, underlying medical problems (i.e. hypertension, pre-existing type I diabetes), multiple pregnancy, bleeding in early pregnancy, infection, shorter times between pregnancies, smoking, and foetal congenital abnormalities or growth restriction (Robinson, 2020).

Interventions to prevent or postpone preterm labour are aimed at reducing the risk factors or managing the mechanistic pathway(s). Some risk factors obviously cannot be adjusted (i.e. race, genetic susceptibility etc). Additionally a review of a number of potential modifiable factors either pre- or during pregnancy only noted two interventions that reduced preterm labour (Barros et al., 2010). These were smoking cessation and progesterone administered to women at higher risk of preterm birth. Others, such as screening and treating asymptomatic bacteria, increasing gap between pregnancies, various supplementations, and cervical cerclage for cervical incompetency, were not observed to have any effect on preterm birth.

Medications are sometimes used in the event of preterm labour, including tocolytic agents such as nifedipine (Songthamwat et al., 2018). The aim of this intervention is

to temporarily prevent the contractions and delay the preterm labour in order to administer antenatal steroids (Medley et al., 2018), a proven benefit to outcomes of preterm children that is covered later in this introduction.

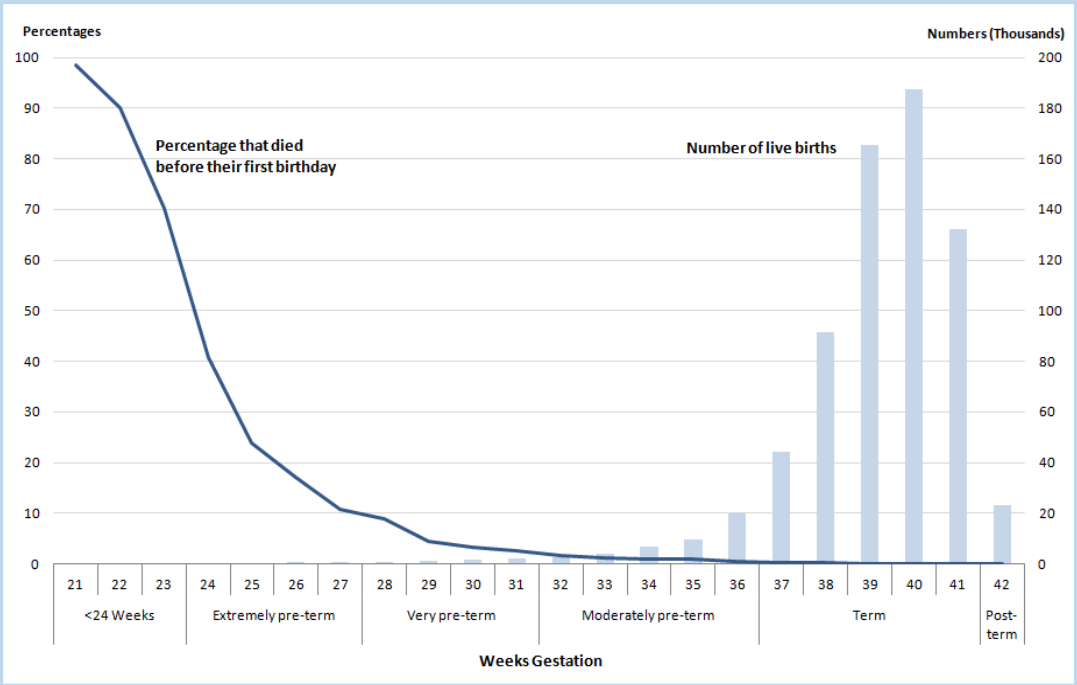
1.1.4 Overview of preterm morbidity and mortality

1.1.4.1 Mortality

Preterm birth is associated with high mortality especially at the extremes of gestation. In 2015 there were 2.7 million deaths worldwide in the neonatal period, with over 944,000 (35%) of these attributed to preterm birth. Additionally, another 111,000 deaths occurring after the age of 1 month were classified with preterm birth as the cause (Liu et al., 2016). As such this makes preterm birth the leading cause of mortality in children under 5 years of age, constituting almost 16% of all reported deaths of under-5s (Liu et al., 2016).

Survival is dependent on gestation. Figure 1.2 from the ONS shows the number of preterm births and the percentage that die in infancy for each gestation from 2013 (Office for National Statistics, 2015). This was collated from all births and infant deaths from England and Wales linked to birth notifications and death registrations. Limitations of this data is a lack of cause of death; however, it clearly demonstrates the effect of gestation on survival.

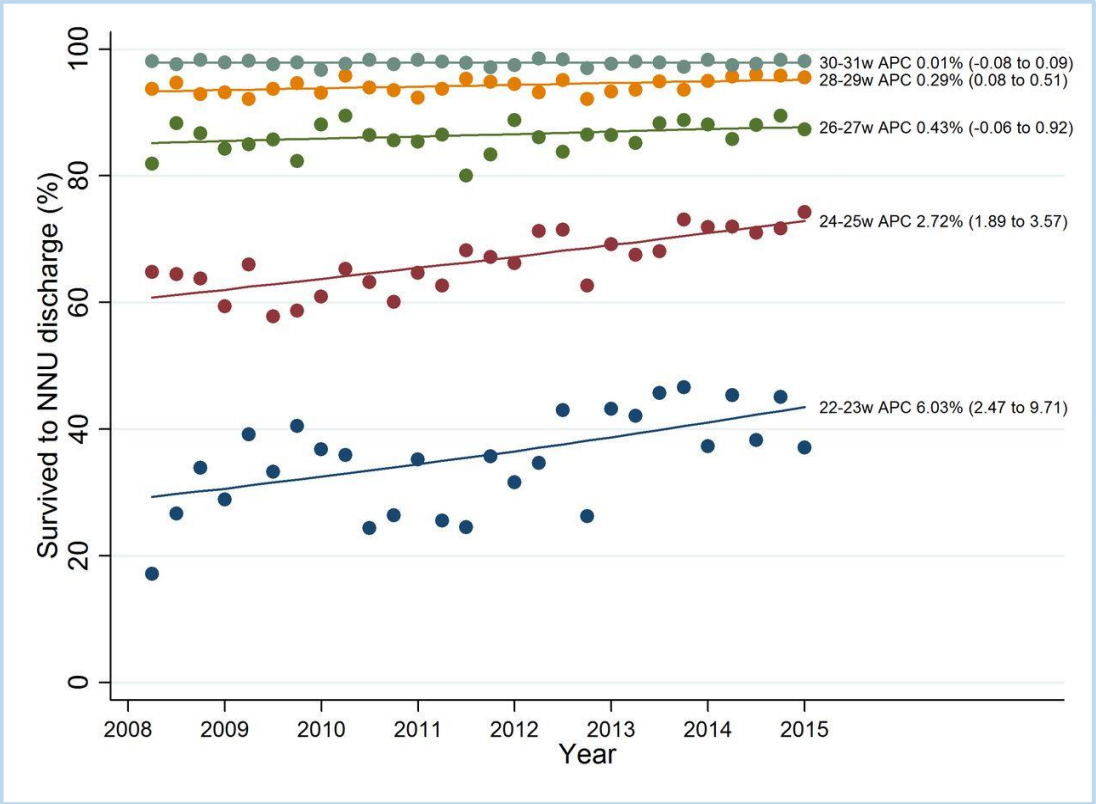
Figure 1.2 Mortality rates in preterm births across gestational ages (Office for National Statistics, 2015). Figure available under Open Government Licence v3.0.



The EPICure group assessed the outcomes of children born at less than 26 weeks' gestation born in 1995 (EPICure) across the UK and Republic of Ireland, and at less than 27 weeks' gestation in 2006 (EPICure 2) across maternity centres in England. Survival rates for infants born at 23 weeks' gestation in the 2006 cohort was 19% and this figure was up to 77% for those infants born at 26 weeks' gestation. There was an improvement in infant survival-to-discharge rates between the 2 time periods from 40% in 1995 to 53% in 2006 for all babies born between 22 and 25 weeks' gestation, suggestive of improved clinical care (Costeloe et al., 2012). Santhakumaran and colleagues used data from the National Neonatal Research Database (NNRD) to assess trends in survival in very preterm infants between 2008 and 2014. The overall survival to discharge rate across this time period for infants born at 23 weeks' gestation was 36% and at 26 weeks gestation was 83%, both higher than reported by EPICure. By 31 weeks of completed gestation, survival to discharge was 98%. Figure 1.3 shows the changes in survival rates across the studied time periods; the greatest improvements in survival were in the lower gestation groups (Santhakumaran et al., 2018). One caveat, acknowledged by the authors, is the data did not include deaths occurring before admission to neonatal units (e.g. on maternity wards), with 36% of

the deaths recorded by the ONS over the same time period not captured by the NNRD.

Figure 1.3 Survival rates to discharge in preterm-born children at different gestation groups, over 2008 – 2015 (Santhakumaran et al., 2018). Abbreviations: NNU – Neonatal Unit; APC – Average Percent Chance. Figure available under Creative Commons CC BY 4.0 license.



Additional analysis compared survival rates of different groups, for example by sex, SGA, multiplicity of pregnancy, administration of antenatal steroids, mode of delivery and maternal age. There were significantly lower rates of survival to discharge for boys, SGA infants, following vaginal delivery, infants of lower maternal age and where antenatal steroids were not given (Santhakumaran et al., 2018).

Ethnicity is also a factor in mortality rate. In the UK, rates of neonatal deaths are highest in the Pakistani population, at 26.5 per 1,000 live births, followed by 20.8 in the Black Caribbean population, 17.7 in the Black African population and 15.3 in the Bangladeshi population, compared to 14.2 and 11.8 per 1,000 live births in the White British and Indian populations respectively (Office for National Statistics, 2013).

1.1.4.2 Morbidity

Preterm birth is also associated with significant morbidity, both short and long term, affecting all systems, however the respiratory system is particularly widely affected. Respiratory Distress Syndrome (RDS) is a disease largely resulting from deficiency of the lipoprotein surfactant, which is produced by type II pneumocytes in the lung. It acts on the air-liquid interface of the alveolar walls, reducing surface tension in the alveoli. Deficiency of this molecule, as occurs in preterm birth, results in atelectasis, and lung collapse or air leak, and ultimately impacts on gas exchange and results in respiratory failure. It manifests clinically with respiratory distress, hypoxia and has a typical appearance on chest radiograph (Gallacher et al., 2016).

Preterm infants who require persistent respiratory support and oxygen therapy are at risk of chronic lung disease of prematurity (CLD), also known as bronchopulmonary dysplasia (BPD). The multiple definitions will be covered in section 1.3.2 but is usually diagnosed by persistent supplemental oxygen requirement at set time points (either 28 days of life or 36 weeks postmenstrual age, depending on gestation at birth) (Jobe, 1999). Chronic lung disease is an important diagnosis as it is associated with increased respiratory symptoms through infancy and childhood.

Respiratory morbidity will be covered in greater detail later in the chapter (section 1.4).

Adverse neurodevelopmental outcomes are also very common. Intraventricular haemorrhage (IVH) is a bleed within the brain, normally originating from the germinal matrix, an area of increased vascularity in the preterm child. It is especially prominent in infants below 31 weeks' gestation, and bleeds often result from cardiovascular instability and fluctuations in blood pressure (another feature of the preterm infant) resulting in altered cerebral perfusion pressures. Intraparenchymal bleeds range from grade I (local haemorrhage at the site of the germinal matrix) to IV (associated with periventricular infarction secondary to bleed). Following IVH, infants are at risk of either post-haemorrhagic ventricular dilatation (PHVD), usually as a result of CSF reabsorption abnormalities following a bleed, and/or periventricular leukomalacia (PVL) where cystic formation occurs in the white matter around the ventricles. The latter in particular is associated with longer term abnormalities such as cerebral palsy.

Cognitive impairment and learning disability, resulting in lower school attainment is also more likely as a result of preterm birth. Neuropsychiatric conditions such as attention deficit - hyperactivity disorder (ADHD) (Franz et al., 2018) or autistic spectrum disorder (ASD) (Johnson et al., 2011) are increased in children born preterm.

Sepsis is another extremely common problem in preterm-born infants; a combination of immature immunity and invasive interventions put the preterm infant at high risk of bacterial and fungal sepsis (Berardi et al., 2019, Stoll et al., 2002).

The immature gut can suffer complications. Spontaneous gastrointestinal perforation is one such problem, but of more severe consequence is the development of necrotising enterocolitis (NEC), a multifactorial gut inflammatory process, causing gut necrosis along with a septic picture. This condition has a high mortality rate (approx. 15-20% of cases) (Christensen et al., 2010) and can require surgical intervention in severe cases. Where surgical intervention is required, further complications can occur resulting in long-term feeding issues, stoma care or short gut syndrome in cases of small bowel resection.

Therapies crucial to survival of the neonate can also cause problems, for instance, oxygen toxicity can result in retinopathy of prematurity (ROP), a phenomenon of abnormal vascularisation of the retina, with resulting visual loss a potential sequelae (Hartnett and Lane, 2013).

Essentially all organs and systems can be affected by preterm birth, its complications and management. All these factors can interact to affect a preterm-born infant's overall short and long-term outcomes. The range of morbidity experienced by an infant is important to consider; those with a greater burden of disease is often a reflection of a sicker infant, and this may be associated with a poorer long-term outcome.

1.2 Respiratory system and preterm birth

As discussed in the previous section, respiratory morbidity is an extremely common outcome of being born preterm. As survival at lower gestations continues to improve, a greater number of infants are potentially at risk of longer-term problems. In order to understand the potential aetiology behind long term respiratory problems, an understanding of the development and adaptation of the respiratory system is important, as well as the pathophysiology of lung disease in the neonatal period and beyond.

1.2.1 Lung development in utero

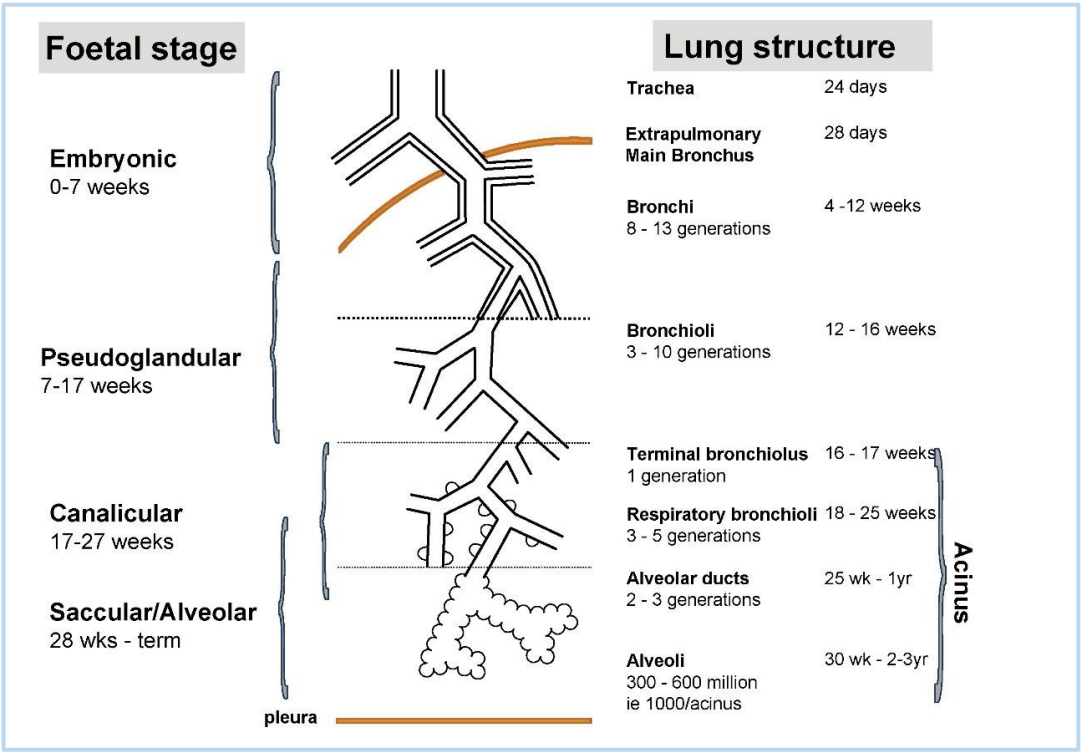
Understanding the development of the lungs in-utero is important for understanding the consequences of preterm birth on the lungs. The gestational timing of various stages of development means that different pathology or severity of pathology occurs after birth at different gestations. There are five well-defined embryological stages in lung development, as demonstrated by Figure 1.4 (Chakraborty et al., 2010).

The *embryonic* stage of lung development starts at approximately 3 weeks of gestation. The lung bud originates from the primitive foregut, with epithelial cells forming the early trachea by invading surrounding mesenchyme. Normal development of the lung requires both epithelial and mesenchymal cells; if the mesenchyme is completely destroyed, the lung will not undergo the necessary divisions (Jeffery, 1998). The embryonic stage continues with the initial divisions into the main bronchi and subsequently lobar and segmental bronchi, with the sixth aortic arches the origin of the pulmonary arteries that follow the airways (Kotecha, 2000).

The *pseudoglandular* stage, so called due to the epithelial-lined, blind-ended air spaces being fully surrounded by mesenchyme and taking on a glandular appearance, starts from approximately 7 weeks of gestation and continues up to 17 weeks. During this stage the bronchi continue rapid segmentation, with each bronchial bud forming 2 new branches with each division. By 14 weeks, approximately 70% of divisions have taken place and by the end of this stage, all the branches will have formed, so

although the lungs continue to develop, the pattern of branching remains unchanged beyond this point (Jeffery, 1998).

Figure 1.4 Diagram showing the stages and evolution of lung development in-utero (Chakraborty et al., 2010). Image reproduced with permission of the rights holder.



The following *canalicular* stage is a crucial time in lung development, starting from 17 weeks and continuing through to 27 weeks gestation. During this stage the primary acinar structures (respiratory bronchioles, alveolar ducts, early alveoli) form. It is during this stage when the alveolar-capillary interface starts to develop with type I pneumocyte formation, which will ultimately allow for gas exchange to take place. Additionally, this is the time when type II pneumocytes start to differentiate, which will subsequently be responsible for surfactant production. It is during this stage of lung development when viability of the foetus is possible; prior to this stage, without the ability to exchange gas, survival is not currently a possibility.

From 27 through to 36 weeks, the lung development is in the *saccular* stage. During this period the lungs are improving their gas exchange capabilities, with an increase in surface area and thinning of airway walls arising from dilatation of the distal airways into saccules (Joshi and Kotecha, 2007). Additionally, there is an increase in

lamellar bodies, the secretory organelles of the type II pneumocyte responsible for surfactant release (Kotecha, 2000).

The final stage of lung development is the *alveolar* stage, commencing at 36 weeks gestation. This is the period when the alveoli mature and vastly increase their numbers. At birth there are approximately 20 to 50 million alveolar (Joshi and Kotecha, 2007); this number increases to 300 to 480 million in a healthy adult (Levitzky, 2018). Originally thought to continue until the age of 2 years, it is now considered to continue until an older age, although the method of septation and forming new alveoli is likely a different process to that which occurs in the initial alveolar stage (Schittny, 2017). This continuation of alveolar development is important, as pathology of the lungs sustained during the perinatal period in preterm-born children may mean that catch-up lung growth will continue for a longer period.

The final macroscopic structure of the normal lung is a tracheobronchial tree with up to 23 generations of branching. The proximal lung contains the conducting airways made up of trachea, bronchi, bronchioles and terminal bronchioles, with the distal lung comprising respiratory bronchioles, alveolar ducts and alveolar sacs. This area is responsible for gas exchange. Each respiratory bronchiole normally separates into 100 alveolar ducts and 2,000 alveoli in healthy adult (Levitzky, 2018).

Microscopically the alveolar surface is comprised of 95% predominantly type I pneumocytes, a type of squamous epithelial cell. Their basement membranes are fused with the basement membranes of the capillaries' endothelial cells, allowing gas exchange across the 0.5 microm barrier. In the healthy adult there is between 50 - 100 square metres of alveolar-capillary interface in the lung (West, 2015). The remaining 5% of the alveolar surface is comprised of the type II pneumocytes, responsible for producing the surfactant.

Another important feature of in-utero lung development is the foetal lung fluid which is produced by the epithelial cells of the distal lungs. It is responsible for distension of the airways, promoting lung growth. The lung fluid is distinct entity from amniotic fluid. The volume of foetal lung fluid is kept in balance by intermittent foetal

breathing movements expelling the lung fluid, against periods of apnoea where a closed glottis produces high resistance in the proximal airways and containment of the fluid, with subsequent distension of the lungs (Hooper and Harding, 1995).

1.3 Respiratory disease in preterm-born children

There are two main, conditions that predominantly affect preterm born neonates, respiratory distress syndrome (RDS) and chronic lung disease of prematurity (CLD). RDS is a condition related to surfactant deficiency as a result of preterm birth, and while there is often an overlap of children who are affected by both and management of RDS may be contributary, CLD is a complex multifactorial disease process that has evolved with changes in how preterm-born infants are managed. CLD is of clinical importance, partly due to its impact later lung health is CLD, as many studies use this diagnosis as a marker potentially worse longer-term outcomes in follow-up studies. Below is a brief summary of both conditions before considering some of the longer-term respiratory outcomes.

1.3.1 Respiratory distress syndrome

The most common respiratory disorder affecting preterm-born neonates is a condition called Respiratory Distress Syndrome (RDS), traditionally known as Hyaline Membrane Disease (HMD) due to the histological appearance of infant lungs following autopsy (Blystad, 1951). This is a disease which affects predominantly preterm neonates as a result of pulmonary surfactant deficiency.

1.3.1.1 Pathophysiology of RDS

Surfactant, a complex molecule comprised of lipids and proteins and produced by type II pneumocytes in the lung epithelium, is responsible for lowering surface tension in the lungs. Type II pneumocytes begin to differentiate from the acinar epithelium during the canalicular phase of lung embryology at 22-24 weeks gestation, which is when surfactant can first be found in the lung surface (Pickerd and Kotecha, 2009). However, it is not until between 32-36 weeks gestation when an accelerated period of maturation of the type II pneumocytes occurs, with subsequent increase in surfactant levels, readying the lungs for delivery at term (Kotecha, 2000). For example, a healthy infant born at full term (≥37 weeks gestational age) is estimated to have approximately 100mg/kg of endogenous surfactant. Conversely a preterm infant may have a surfactant pool of less than 10% of a term baby's (Jobe, 2006).

Without adequate levels of surfactant, and because resultant lung compliance is low, the alveoli are prone to collapse. However due to LaPlace's law, which interpreted clinically means that the pressure needed to overcome the surface tension is greater in alveoli with smaller radii, smaller airspaces are more liable to this collapse, giving a mixture of atelectasis and resulting hyperexpansion of the larger alveoli. This affects oxygenation and ventilation with intrapulmonary shunting leading hypoxaemia and hypercapnia. Other features include acidosis, and extrapulmonary shunt (through the foramen ovale and ductus arteriosus) increasing due to pulmonary vasoconstriction. Endogenous surfactant production decreases and within the airspaces a layer of fibrin and necrotic cells form, known as the hyaline membranes. Again, this further limits gas exchange. In the most severe cases, without treatment, only the more proximal airways remain aerated as a result of ongoing collapse, oedema, bleeds and membrane formation (Locci G, 2014).

1.3.1.2 Clinical features of RDS

The clinical presentation of RDS varies depending on severity. Signs of respiratory distress are present, and blood investigations can reveal hypoxaemia and hypercapnia on blood gas analysis, or dilutional hyponatraemia from fluid retention on electrolyte analysis. Reticulogranular infiltration (also known as 'ground glass appearance') and air bronchograms (from the contrast of air in the bronchioles against consolidated alveoli) are seen on plain chest radiograph.

The typical clinical course in an untreated infant is of worsening disease over the first 48 - 72 hours. Following this the endogenous surfactant pool increases as a result of increased production and the recycling process. The surfactant, once produced, can be recycled with up to 90% re-entering the lamellar bodies and later being re-secreted. This leads to a decrease in alveolar surface tension and better lung aeration resulting in more efficient gas exchange which in turn improves the metabolic environment of the infant.

1.3.1.3 Complications of RDS

Acute complications of RDS include pneumothorax or pneumomediastinum, which can be spontaneous as a result of hyperexpansion of alveoli or secondary to ventilator support. Intraventricular haemorrhage can result from the vascular instability

associated with RDS. Pulmonary interstitial emphysema, which occurs when gas escapes from the alveoli into the perivascular spaces, is a complication normally associated with treatment of RDS (i.e. positive pressure ventilation), and is associated with increased mortality, as well as being an additional risk factor for the above complications (Hart et al., 1983).

1.3.1.4 Management of RDS

The management, or prevention, of RDS begins with antenatal care, aimed at reducing likelihood of preterm birth and/or improving lung maturity in anticipation of preterm birth. Once an infant is born, their care focuses on both treating the respiratory disease and optimising their general care.

1.3.1.4.1 Antenatal care

Prevention of preterm birth, as discussed previously, is obviously the best way to stop the development of diseases associated with preterm birth. However, in those cases where preterm birth is threatened, it is commonplace to give mothers injections of corticosteroids. These act by speeding up type II pneumocyte maturation which in turn increases surfactant production and antioxidant enzyme induction. The introduction of this treatment revolutionised the subsequent postnatal prognosis. The most current Cochrane review by Roberts et al in 2017 showed risk ratios of 0.69 (95% CIs 0.59 to 0.81) for neonatal death and 0.66 (95% CIs 0.56 to 0.77) for RDS in the infants of mothers who received at antenatal steroid versus placebo across 30 trials, however there was no apparent benefit in CLD rates with a risk ratio of 0.86 (95% CIs 0.42 to 1.79) (Roberts et al., 2017). This should be interpretated with some caution as improved survival may result in increase in subsequent CLD cases.

1.3.1.4.2 Postnatal care

Changes in respiratory management have revolutionised neonatal care with improving ventilation strategies to minimise lung damage while supporting the infant with RDS, and surfactant administration markedly improving survival.

1.3.1.4.2.1 Medication

Exogenous surfactant replacement therapy, administered directly into infant lungs, is used for prophylactically in preterm infants, and as a treatment after RDS

symptoms develop. Following its introduction a decrease in infant mortality rate was seen from 51% to 31% (CEMSG 1988), with animal-based surfactants showing better efficacy than synthetic products (Halliday, 2008), and use prophylactically rather than treating once symptoms develop is recommended (Suresh and Soll, 2005). Newer techniques for administering surfactant, such as less invasive surfactant administration (LISA; using a thin catheter inserted into the trachea to deliver the surfactant), show promise in potentially avoiding longer-term complications of prolonged ventilation including CLD (Isayama et al., 2016). Additionally, caffeine, used as a respiratory stimulant for apnoeas of prematurity, has been reported to reduce the rates of BPD, particularly when used early (first 3 days of life). This is possibly due to an anti-inflammatory effect (Abdel-Hady et al., 2015).

1.3.1.4.2.2 Respiratory support

Ventilation of newborn infants has been pivotal for improving outcomes of infants with RDS, however it comes at a potential cost with various types of lung inury due to baro- or volu-trauma that are associated with mechanical ventilation and have long term impact on surviving infants. The aim of ventilation beyond survival is to reduce potential secondary inflammation and in turn reduce CLD rates. Strategies that have shown potential in this regard include using volume rather than pressure based ventilation modes for invasive ventilation (Wheeler et al., 2011), but non-invasive ventilation (Bi-level Positive Airway Pressure or BiPAP, Continuous Positive Airway Pressure or CPAP, and High Flow Nasal Cannula or HFNC), is now an alternative, with systematic reviews showing potential for reduction of CLD using these modalities (Schmölzer et al., 2013).

Another systematic review assessed the use of non-invasive positive pressure ventilation (NIPPV) versus nCPAP in preterm infants with RDS. 3 studies were identified with a total of 180 infants in each group. The primary outcome of the review was treatment failure within 72 hours requiring invasive support; this showed a risk ratio of 0.6 in favour of NIPPV over nCPAP. However, for longer term outcomes the rates of BPD and death were not significantly different (Meneses et al., 2012, Wilkinson et al., 2016). Judicious use of oxygen is also important due to potential

oxidative stress that could contribute to CLD (Perrone et al., 2012), or indeed longer-term long damage (Filippone et al., 2012).

1.3.2 Chronic lung disease

1.3.2.1 Definitions of CLD

Chronic lung disease of prematurity (CLD) is a polymorphous disease state, with the definition, and the underlying pathophysiology, changing over time. The condition was originally defined by paediatric radiologist Dr William Northway, along with colleagues in paediatrics and pathology (Drs Rosan and Porter) in 1967, and named bronchopulmonary dysplasia (BPD). It was identified as a typical pattern seen on chest radiographs, along with corresponding clinical and pathologic features, in preterm infants with hyaline membrane disease (the alternative name for RDS), and treated with oxygen therapy and mechanical ventilation (Northway et al., 1967). The original definition was that of infants requiring ongoing oxygen therapy at 28 days/1 month of life.

Since the original description was published, the disease, both definition and aetiology, has evolved. As a result of advances in perinatal management, a different population of preterm infants (i.e., younger gestations) are affected, with a different underlying disease process causing CLD. As such the definition and diagnosis have adapted to account for the change in population.

The first evolution was following the realisation that in younger gestation infants surviving as a result of improved perinatal care, a persistent oxygen requirement at 28 days did not necessarily reflect chronic disease but just immaturity of the lungs. As such a reclassification was suggested to include an oxygen requirement at 36 weeks' postmenstrual age (Shennan et al., 1988).

A further adaptation of the definition came from a National Heart, Lung and Blood Institute working group in 2000 aimed to grade CLD on the basis of severity, with the view that the gestation of birth affected when a diagnosis of CLD could be made (Jobe and Bancalari, 2001). Table 1.4 summarises this definition.

Table 1.4 Definitions for Chronic Lung Disease of Prematurity (CLD).

Gestational age	< 32 weeks	≥ 32 weeks
Time-point of assessment (ToA)	36 weeks PMA or discharge to home*	> 28 days but < 56 days postnatal age or discharge to home*
Treatment with oxygen > 21% for at least 28 days plus		
Mild CLD	Breathing room air at ToA	Breathing room air at 56 days postnatal age or discharge home*
Moderate CLD	Need for < 30% oxygen at ToA	Need for < 30% oxygen at 56 days postnatal age or discharge home*
Severe CLD	Need for ≥ 30% oxygen and/or positive pressure at ToA	Need for ≥ 30% oxygen and/or positive pressure at 56 days postnatal age or discharge home*

Most recently, another consensus group have suggested a further evolution of the definition which includes use of non-invasive ventilation modalities into the diagnosis, as well as suggesting a definitive test that could be performed to aid the diagnosis, which could ensure cross-centre comparisons are like-for-like.

The importance of having an appropriate definition is in trying to stratify preterm infants for risk of long-term disease, as well as allowing for a measure of outcomes that is consistent across units.

1.3.2.2 Pathophysiology of CLD

The original disease as described by Northway et al, also called 'old BPD', was characterised clinically with several stages of disease progression, from initial respiratory distress syndrome through pulmonary oedema and later progressive chronic lung disease. Associated radiological changes included the initial typical changes associated with RDS developing into areas of cystic lesions and infiltrates along with hyperinflation. Histopathologically these infants showed altered lung structure with interstitial and interalveolar fibrosis, along with bronchiolar smooth muscle hypertrophy, and change in type I epithelial cells into type II pneumocytes (Northway et al., 1967). The cause of these changes was lung injury (barotrauma) from mechanical ventilation and oxygen toxicity on a base of respiratory failure in immature lungs.

With improvements in perinatal care, and greater number of infants surviving at lower gestations with less intensive ventilation and oxygen therapies required, there

has been a change in the aetiology of the disease, termed 'new BPD'. This disease process is manifested pathologically with larger but fewer alveoli, and less fibrosis and inflammatory changes than 'old BPD'. This new pathology is linked to an early interruption of lung development during the saccular period, as a result of infants surviving at younger gestations as a result of interventions such as antenatal steroid administration to mothers at risk of preterm delivery and use of surfactant postnatally (Jobe, 1999).

1.3.2.3 Epidemiology of CLD

The infants at risk of developing CLD has evolved over time with the change in aetiology. The infants who were initially described in Northway's landmark paper were born at higher weights, with over 25% of survivors >1500g developing stage IV BPD (as per original diagnostic criteria) (Northway, 1990).

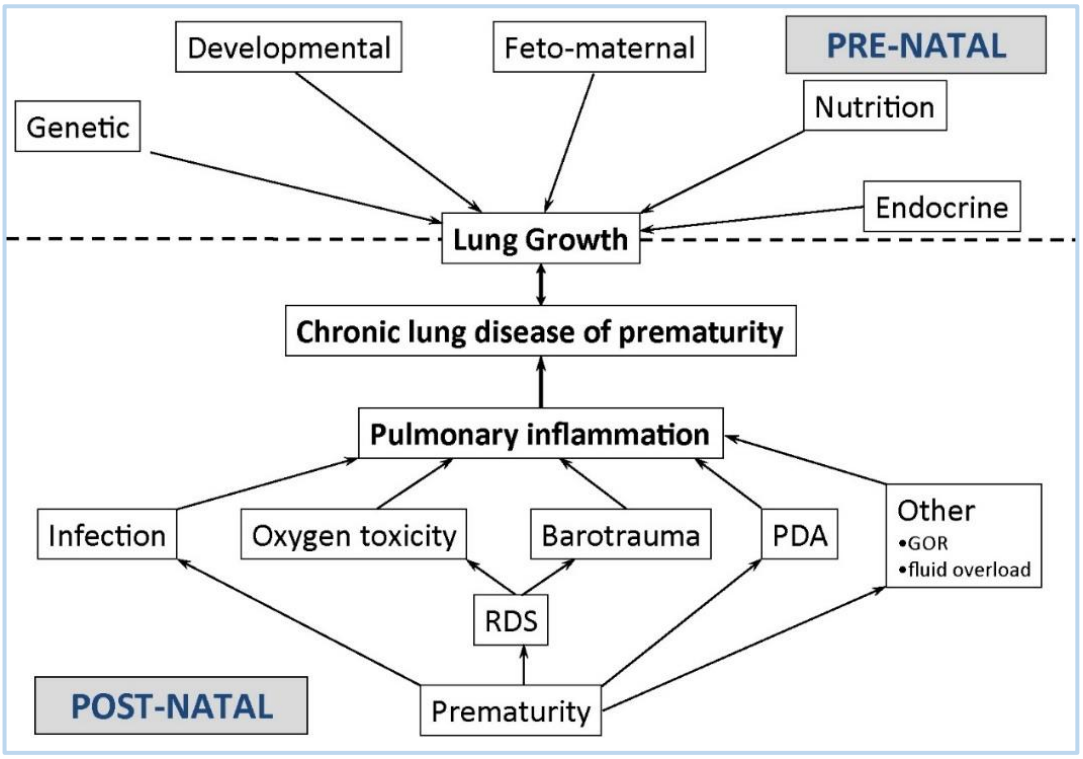
The current demographics of affected infants has changed. Developing CLD is now uncommon in infants born at >1500g (Eber and Zach, 2001). Instead the 'new' disease is primarily a problem of extremely preterm and VLBW infants. The outcomes from 2 cohorts of extremely preterm infants born at 2 time periods showed, out of 312 surviving infants born before 26 weeks' gestation in 1995 and 1041 surviving infants born before 27 weeks' gestation in 2006, CLD rates (supplemental oxygen at 36 weeks' PMA) were 73.6 % and 71.8% respectively (Costeloe et al., 2000, Costeloe et al., 2012).

More broadly, a study across multiple US sites within their Neonatal Research Network between 1993 and 2012 looking at 25,000 surviving infants born at <29 weeks' gestation and <1500g, identified an increasing trend in CLD (at 36 weeks' PMA) from 32% in 1993 to 47% in 2012. During this period overall survival rates had increased from 70 to 79%, with the greatest improvements in survival seen in those born at <26 weeks' gestation. The authors feel this improved survival, along with more active resuscitation of infants at the extremes of gestation along with the intensive care they require, is responsible for this increasing CLD rate (Stoll et al., 2015).

1.3.2.4 Causes of CLD

The aetiology of CLD is complicated due to being multifactorial, with different things contributed in different infants. Figure 1.5 shows the interaction of factors that can lead to the development of CLD (Kotecha, 2000).

Figure 1.5 Demonstration of multi-factorial aetiology of chronic lung disease of prematurity (Kotecha, 2000). Image reproduced with permission of the rights holder.



As is clear from the figure, both pre- and post-natal influences can be responsible for CLD. What is less clear is the direct link between CLD and later lung disease in preterm-born children. As will be described below, preterm-born children have greater respiratory morbidity through childhood and beyond, and while those with a CLD diagnosis are particularly affected, disease is not limited to these individuals.

1.4 Respiratory outcomes

Aside from the respiratory complications that can occur in the neonatal period, those preterm infants who survive the neonatal period are at risk of longer-term respiratory disease. Respiratory health outcomes following preterm birth have been assessed in a manner of ways, including symptomology, health care use/access, medication use, impact on exercise, and lung function testing. As outlined above, at potentially greater risk of respiratory morbidity are infants born towards the extreme of gestation and birthweight, especially in the context of being born small for gestational age, as well as children diagnosed during infancy with CLD. Research into respiratory disease following preterm birth has traditionally focused on those infants diagnosed with CLD; it has been accepted that this diagnosis correlated with a worse respiratory health during infancy, which would project to a negative longer-term outcome. As such there has often been a focus on long-term follow-up of those infants who had a CLD diagnosis from their neonatal period. More recent studies have shifted this focus to either those born extremely preterm or at very/extremely low birth weight (for instance the EPICure or EPIPAGE groups from UK and France respectively). However, as will be highlighted below, long term respiratory morbidity is not confined to these groups, and less is understood about the complex factors that result in a proportion of the ex-preterm population being affected, or why some children with CLD may be spared long-term problems.

1.4.1 Symptoms, medication use and health care utilisation

Respiratory symptoms are common in childhood, irrespective of gestation at birth. Respiratory tract infections account for the majority of these symptoms. For context, looking at frequency of cough in a UK population-based cohort of approximately 6800 1 to 4 year olds (which did include children born preterm and/or with low birth weight), 70% would cough with a cold, and 32% coughed without colds. Nocturnal cough prevalence was 27% in this age group. Wheeze prevalence was 30%. These children were subsequently followed up until maximum age of 17, with symptoms re-assessed at repeated timepoints. Prevalence in the study population of cough with or without cold remained similar to the 1-4 age group. Wheeze prevalence decreased

over time (approximately 15% in children aged 7-9 years). As with any population-based study, there is potential for selection bias, although the initial response rate at the outset was notably high (78%), although authors note that the response rate decreased over time. There is a chance the symptomatic group remained more inclined to continue with the study, resulting in a falsely elevated prevalence of cough at the older age groups. Unfortunately, the study did not consider the impact of preterm children may have on the prevalence, although the overall number of preterms included was 7%, and there would likely be few at the extremes of gestation (Jurca et al., 2017).

1.4.1.1 Pre-school ages

In infancy, preterm-born children are particularly vulnerable to respiratory morbidity. For example, infants <37 weeks' gestation born over a 6-year period in a centre in Switzerland had symptoms assessed at a year of age via questionnaire. Out of 126 respondents, cough and wheeze prevalence was 79% and 48% respectively in the first year of life. In this cohort the median age was ~28 weeks' gestation (Pramana et al., 2011). Unsurprisingly given the greater prevalence of symptoms, preterm infants have a greater impact on health care provision during the first year of life. A large population-based study from the Wales, looking at over 300,000 children born between 1998 and 2008, were retrospectively reviewed for respiratory admissions in infancy and early childhood. Stratifying for gestation and for birth weight (appropriate for gestational age or small for gestational age), ~24% of infants born at <33 weeks gestation required an emergency admission for a respiratory illness in the first year of life compared to 8% in infants born at 40+ weeks. Of note was there was a stepwise increase at every gestation group from 40+ weeks to the very preterm group, including at early term and late preterm. SGA infants were at slightly higher risk (magnitude of 1-3% depending on gestation group) than AGA infants. A similar pattern was seen for hospital admissions in children aged 1-5. Of more clinical significance is the mean number of emergency admissions per 100 child-years (i.e., suggesting recurrent admissions). There were means of 41.5 admissions in AGA infants under 1 year and 48 admissions in SGA infants per 100 child-years – a fourfold increase from the mean admissions at late term (Paranjothy et al., 2013).

Other studies have reported greater prevalence of symptoms in pre-school aged children following preterm birth. For example, Vriljandt et al, in participants born preterm, with and without BPD, noted that symptoms were more prevalent when assessed between age of 3-5 years. Cough within the previous 12 months affected 95% of the 41 children with a history of BPD and 97% of the 36 children without. Of note BPD definition in this cohort was supplementary oxygen therapy at 28 days. Conversely, wheeze appeared less prevalent in this 3-5 year age group, with 32% of BPD and 39% of children without BPD affected (Vrijlandt et al., 2007). Admission rates were much higher in the BPD group (54% requiring admission in the BPD group versus 14% in the no BPD group) before 3 years of age, suggesting that in this group at least, while respiratory symptoms were similarly prevalent, the severity in the BPD group was greater. Medication use in the past 12 months was high with β-agonist use of 39% and 50%, and inhaled corticosteroid use of 37% and 31% in the BPD/no BPD groups respectively. These figures are slightly unexplained. There is greater bronchodilator use in the no BPD group than there are symptoms of wheeze, so the indication for its use is not completely clear. Almost all the BPD group using salbutamol were also using inhaled corticosteroids (ICS). This may be due to the severity of the symptoms, or a lower threshold for using preventer given there was a history of BPD.

Moreno-Galdo et al also focussed on respiratory symptoms in moderate to late preterm children up to the age of 3 years. Almost 1000 children born between 32- and 35-weeks' gestation from multiple centres in Spain were periodically followed up and assessed for episodes of wheeze. Rates of wheeze were 48% in the first year, 44% in the second year and 40% in the third year of life, with infrequent wheeze (<3 episodes within a year) more common than recurrent wheeze (3 or more episodes in 1 year). Hospital admission rates in this moderate to late preterm group were 6.3% in the first year of life, but reduced to 0.75% in the third year of life, however a comparison to term born children was not made in this study (Moreno-Galdó et al., 2020).

A similarly pre-school aged group of children in the UK, taken from the Millennium Cohort Study, had respiratory symptoms assessed at age 3 and 5 years. In particular,

episodes of wheeze within the previous 12 months were obtained from parents during face-to-face interviews with families of children born both preterm and term. In total 1149 children at 3 years and 1165 at 5 years born at <37 weeks' gestation and subsequently stratified into gestation groups (<32 weeks, 33-34 weeks, 35-36 weeks), had odds ratios of recent wheeze calculated in comparison to a term-born children born at 39-41 weeks' gestation. At 3 years, adjusted (for multiple demographic factors) odds ratios for recent wheeze in the 3 preterm groups were 2.6, 1.7 and 1.3 respectively, while at 5 years were 2.9, 1.7 and 1.5 respectively. This clearly shows a gestational effect on later lung morbidity. Of particular interest here is the greater prevalence of symptoms in later preterm children, in whom CLD would be uncommon (although the diagnosis of CLD was not included in the above study). In fact, the same study even looked at early term born children (37-38 weeks gestation) and they too had a slightly greater odds ratio for wheeze at both 3 and 5 (1.1 and 1.2). Also examined were odds ratios for hospital admissions, in infancy (before 9 months) and between 9 months and 5 years of age. Admission risk was greater in the younger age group, again in a stepwise manner from late term to very preterm (OR for <32 weeks 13.7 compared to 5.1 in late preterms). Similarly in pattern but not magnitude, OR for the older age group was 6 compared to 1.9 for late preterms. It should be noted admissions were not limited to respiratory illness but excluded accident-related presentations. Asthma medication use at age 5 (not specified to whether both reliever and preventer medication, so presumably either) was greater towards the lower gestation, again stepwise across gestation groups. In this instance very preterm children conferred an OR of 3.5 compared to late term children (Boyle et al., 2012).

1.4.1.2 School-aged children

As is demonstrated above, respiratory symptoms in preterm-born children are prevalent at all gestations. A cohort of children born both preterm and term were followed up using a questionnaire to assess ongoing respiratory symptoms. Of approximately 7,000 respondents between 1 and 10 years of age, over 4,000 were born preterm. In the school aged children (>5 years of age), those greatest affected by respiratory symptoms were the very preterm (VP; ≤32 weeks' gestation), with an odds ratio (OR) of 3.3 compared to term children reporting wheeze ever. Odds ratios

for the less extreme preterm groups (33 – 34 weeks and 35 – 36 weeks of gestation) were also greater compared to the term controls (1.8 and 1.6 respectively). Hospital admissions for a respiratory problem within 12 months as well as current inhaler use were also greater across the preterm groups, with ORs in the VP group of 2.2 and 2.3 for hospital admissions and inhaler use respectively, as shown in Figure 1.6 (Edwards et al., 2016).

Figure 1.6 Adapted table displaying respiratory characteristics and their unadjusted odds ratios in children >5 years, stratified by gestation group, from the Respiratory and Neurological Outcomes of children born Preterm study (Edwards et al., 2016). Figure available under Creative Commons CC BY 4.0 license.

	Very Preterm	Moderate Preterm	Late Preterm	Full Term
	N = 495	N = 450	N = 1,138	N = 1,456
Wheeze-ever (%)	276 (55.8%)	186 (41.3%)	430 (37.8%)	403 (27.7%)
OR (95% CI)	3.3 (2.7, 4.1)	1.8 (1.5, 2.3)	1.6 (1.3, 1.9)	
p-value	<0.001	<0.001	<0.001	
Recent wheeze (%)	146 (29.5%)	92 (20.4%)	227 (19.9%)	217 (14.9%)
OR (95% CI)	2.4 (1.9, 3.0)	1.5 (1.1, 1.9)	1.4 (1.2, 1.7)	
p-value	<0.001	0.006	0.001	
Doctor diagnosis of asthma (%)	127 (25.7%)	78 (17.3%)	197 (17.3%)	182 (12.5%)
OR (95% CI)	2.4 (1.9, 3.1)	1.5 (1.1, 2.0)	1.5 (1.2, 1.8)	
p-value	<0.001	<0.01	<0.001	
Family history of atopy (%)	91 (18.4%)	98 (21.8%)	275 (24.2%)	330 (22.7%)
OR (95% CI)	0.8 (0.6, 0.99)	1.0 (0.7, 1.2)	1.1 (0.9, 1.3)	
p-value	0.046	0.694	0.370	
Any inhaler medication use (%)	109 (22%)	66 (14.7%)	162 (14.2%)	159 (10.9%)
OR (95% CI)	2.3 (1.8, 3.0)	1.4 (1.0, 1.9)	1.4 (1.1, 1.7)	
p-value	<0.001	0.032	0.011	
Corticosteroid inhaler medication use (%)	66 (13.3%)	36 (8%)	92 (8.1%)	92 (6.3%)
OR (95% CI)	2.3 (1.6, 3.2)	1.3 (0.9, 1.9)	1.3 (0.97, 1.8)	
p-value	<0.001	0.21	0.08	
Hospital admission with breathing related problem (last 12 months) (%)	17 (3.4%)	16 (3.6%)	24 (2.1%)	23 (1.6%)
OR (95% CI)	2.2 (1.2, 4.2)	2.3 (1.2, 4.4)	1.3 (0.7, 2.3)	
p-value	0.014	0.012	0.318	

Focus on early term children (37 – 38 weeks' gestation) has also been carried out by Edwards et al. From the same cohort of children as described above, data was obtained from 272 early term children under the age of 5 years and 273 in children aged 5 years and over, in comparison to a control cohort of over 1,000 full-term (39 – 41 weeks' gestation) in both age groups. Odds ratios were calculated to see whether this group were experiencing greater respiratory morbidity. Indeed, in the younger age group, adjusted ORs (for various factors including sex, maternal smoking and socioeconomic status) were significantly higher for wheeze ever (1.5), recent wheeze (1.7), recent daytime cough (1.8) and inhaler use (2.1). Admissions in the first year of life were also higher (OR 1.6); however, this was not specific to respiratory disease. In the older age group it appeared recent wheeze was less prevalent;

however, exercise-induced symptoms were being seen (OR 1.7), along with continued inhaler use (OR 1.5) (Edwards et al., 2015a).

These outcomes are important to consider in regard to the mechanism underlying respiratory morbidity. Early term children are not affected by CLD, and rarely require ventilation or oxygen therapy, all things that have been linked to lung damage and potentially longer-term sequelae. Their absence in these cases, and indeed a large number of preterm cases, suggest that there are other factors behind any respiratory disease. However, although respiratory morbidity is not confined to those born at extremes of gestation, it is clear that these exceptionally vulnerable children are affected to a greater extent than their later-preterm or early term counterparts, resulting in a research focus towards these lower gestation groups.

1.4.1.3 Extreme prematurity

Extreme prematurity is defined as infants born at <28 weeks' gestation. The 1995 EPICure study in the UK has allowed prospective tracking of respiratory morbidity in childhood following extreme preterm birth. Of 219 children followed-up at 6 years of age, 36% of extremely preterm (EP) children with a history of CLD and 20% of EP children without CLD had experienced wheeze in the past 12 months. This compared to 13% of 148 age and sex matched term-born classmates. Exercise-induced wheeze was also more prevalent, affecting 26% of CLD children, 16% of non-CLD compared to 12% of term controls. Odds ratios were calculated for these differences comparing CLD to non-CLD and non-CLD to term, however not CLD to term. Significant difference was seen in recent wheeze odds ratio for CLD to non-CLD (2.3), but not for the other symptoms mentioned above. Other differences between the CLD and no CLD groups were in recent (past 12 months) bronchodilator or steroid use with OR of 2.2 and 2.4 respectively for the CLD group. This appears to show that while all EP children are more affected by respiratory morbidity, it is children with a history of CLD that are most affected (Hennessy et al., 2008). These children were also followed up at 11 years of age. Of 182 EP children, a significant difference was seen when compared to classroom controls for current asthma diagnosis and asthma medication use, number of wheeze attacks in previous 12 months, exercise induced wheeze and nocturnal cough. There were no significant differences, other than recent wheeze, when

directly comparing children with or without CLD within the EP group (Fawke et al., 2010). Further respiratory evaluation was performed at 19 years of age, where a greater odds ratio for asthma diagnosis ever (3.8) was the only significant outcome for CLD vs no CLD. There were no differences for symptoms within the last year, exertional wheeze, or current inhaler use for CLD vs no CLD, and no differences for CLD vs controls. There had been further attrition from the 11-year follow-up with EP children numbering 123 at this review, which could explain a lack of differences between groups (Hurst et al., 2020).

A comparable prospective follow-up of extremely preterm children born in Norway in years 1999 and 2000 has allowed the tracking of lung disease at older ages. Babies born extremely preterm (<28 weeks' gestation) or with extremely low birth weights (<1000g) were recruited along with a smaller subset of term-born controls. Questionnaires were given to participants at ages 2, 5 and 11 to assess respiratory symptoms. There were 232 respondents to the questionnaire at age 11, with 57 term controls. The proportion of children with wheeze between ages 5 and 11 years was 26% in the 232 extremely preterm children compared to 13% in the 57 term controls, a significant odds ratio of 2.51. Rates of recent wheeze (previous 12 months) at age 11 were 16% and 9%; however, this was not a significant result. Exercise-induced symptoms were, however, greater in the EP group, with 18% of children affected at age 11 compared to 5% of term controls. A history of CLD conveyed a significant odds ratio of having wheeze age 5 to 11 of 1.83. Overall, the rates of symptoms decreased between age 5 and age 11, with significant decreases in the rate of wheeze and cough between these two ages (26 to 16% for wheeze, 23 to 16% for nocturnal cough). An exception was exercise-induced wheeze which remained similar at age 11. 29% of the EP children had required hospital admission between age 5 and 11, whereas a significantly lower proportion of the term cases were admitted (13%). Overall, there were 138 admissions across the 67 EP children who had an admission, compared to 7 separate term children who all had a single admission. Of the total EP admissions, only 16 (12%) were respiratory related, and only 1 of the 7 term admissions (14%). There were no statistically significant differences in inhaler use, either bronchodilator or ICS, despite there being a threefold greater prevalence in their use the EP group.

This suggests that, in this Norwegian population at least, respiratory morbidity is not responsible for causing a substantial health care burden in older childhood (Skromme et al., 2018), although this could represent under-treatment.

Of concern is whether symptoms may persist past childhood. The Skromme study suggested that respiratory morbidity in the EP group may diminish over time; however, this was not the case with Fawke et al's findings. Fortunately, awareness is growing about the potential impact of preterm birth on lung health in adulthood (Bolton et al., 2012).

1.4.1.4 Wheeze

A systematic review of childhood wheezing and its association with preterm birth was performed by Been et al and included some of the studies already mentioned. Across 30 studies from different continents, and over 1.5 million children, preterm children (n=93,616) were noted to be 1.7 times more likely to suffer from a wheeze disorder; this rises to 3 times more likely when looking solely at very preterm children (Been et al., 2014). A conclusion stated by the authors was that preterm birth increases the risk of asthma. This wrongly links wheeze in preterm children solely with a diagnosis of asthma which is incorrect. Wheeze is a heterogenous symptom and can imply different aetiology depending on its course and progression.

Data from the Millennium Cohort Study were used to try and better define what was meant by the term wheeze, and to develop phenotypes for different types of wheeze seen in children, including >1000 preterm children. Data from different ages up to 11 years of children born at term and preterm were used to define wheeze as either no or infrequent wheeze, early (onset before age 3, disappeared by age 7 or 11 years), persistent (present throughout the time points), or late (developing only at age 7 or beyond). 18% of preterm children were classified as having early wheeze compared to 13% of term born, while 13% of the preterm children were classified as having persistent wheeze compared to 9% of term children. Late wheeze had similar proportions in the preterm and term group suggesting that a similar aetiology may be responsible irrelevant of gestation, whereas preterm birth and its associations have a significant link to early and persistent wheeze (Kotecha et al., 2019).

1.4.1.5 Summary of symptoms, medication use and health care utilisation

In summary, preterm-born children suffer from greater respiratory symptoms in comparison to their term-born counterparts. This increase in symptoms is present from infancy, and while may reduce over time continues to be more prevalent. Additionally, children with a background of CLD may be more likely to suffer from respiratory symptoms.

1.4.2 Modalities of testing

In this section a background on some of the different testing modalities which feature through my thesis will be introduced. Their application in children born preterm will be addressed in individual chapters. Additionally, oscillometry will be discussed in detail in the individual chapters.

1.4.2.1 Spirometry

Spirometry is the most widely used and well-established of the lung function tests, used for assessment throughout respiratory conditions, as a screening tool for respiratory disease, and frequently in the research setting.

1.4.2.1.1 Test basics

Spirometry is a technique that was first used as far back as the late 1600s, primarily using displacement of water methods to estimate lung volumes, through Bellows - type kit, with evolution to current high-tech spirometers (Gibson, 2005). It involves predominantly assessment of a forced manoeuvre to empty a person's lungs from full inspiration (i.e., total lung capacity) to full expiration (i.e., residual volume). During this exercise, rather than passive expiration resulting from pressures within the lungs being greater than atmospheric air, resulting in flow from lungs to air, a person will force the air out. This involves active effort to empty the lungs, which is reliant on various factors. These include overcoming any impedance to airflow, most commonly resulting from increased resistance as a result of airway obstruction (relating to calibre of airways); however, also includes restriction from pulmonary disease (e.g. from conditions like interstitial lung disease) or external pathology impacting on the lungs (e.g. chest wall deformities, scoliosis), or from problems related to the muscles of respiration (e.g. from neuromuscular disease) (Liou and

Kanner, 2009). Thus, intra- and extra-pulmonary pathology can impact on spirometry outcomes.

Spirometry can be performed using a variety of equipment, all with the aim to record the expired lung volume, along with additional information. These volumes are usually derived from other parameters which are easier to measure, including pressure or flow. Flow and/or pressure changes can be measured using various types of equipment, including pneumotachographs, turbines and ultrasound methods. Pneumotachographs measure change in pressure across a transducer, with specific pressure changes corresponding directly to specific volumes. Turbines respond directly to airflow, with turbine blades within the spirometry spinning with respiration, which in turn blocks an infrared beam of light. The number and frequency of interruptions to this light equates to specific flows and volumes. Ultrasound methods measure the speed of transit of ultrasound impulses across the flow of gas. If airflow is travelling in the same direction as the impulse, the impulse will travel faster, and vice versa, with flow rates and volume derived from the impulse transit time (García-Río et al., 2013). Consideration needs to be made to the fact that airflow alters at differing temperatures and atmospheric pressures, and as such systems should be reported at body temperature, pressure, saturated with water (BTPS). This is a way of converting from ambient conditions of temperature and pressure that the spirometer is in, to the conditions that the expelled air have been at in the lungs, i.e. at body temperature, and saturated with water vapour, using a correction factor (Graham et al., 2019).

Children are able to perform spirometry although there is a likely age and developmental limit as to in whom successful spirometry is possible. While greater than 85% of 10-year olds and older will yield acceptable and repeatable spirometry results, this decreases to ~55-65% in 6 to 7-year olds and 25% in 4-year olds (Loeb et al., 2008).

1.4.2.1.2 Test procedure

Clear guidance for spirometry testing has been developed by several respiratory organisations to ensure consistency in testing (Miller et al., 2005). The test is performed by a participant while sat wearing a nose clip, who, while connected to

the machine, will breathe to full inspiration before exhaling at full force until they reach residual volume (i.e. cannot breathe out any further). The test is then repeated until they have reproduced a similar test result, within specific specifications for certain volumes. Tests are excluded if expiration is not of sufficient force, if there is artifact during the exhalation (i.e., cough, glottis closure), if there was a slow initiation of breath before reaching peak expiratory flow (back-extrapolated volume), or if there is suspicion of leak. Additionally, if full expiration is not achieved, as determined by no plateau reached on a volume-time curve, then certain results from within the test are not interpretable. A person should not perform greater than 8 attempts at the test due to risk of exhaustion affecting results. Once a minimum of 3 tests have been performed, providing test quality was acceptable, including the two largest FEV_1 and FVC being within 150mls of each other, then testing can stop.

There is also guidance for frequency and method for calibration of volumes and flows, with daily volume calibration using a 3 litre syringe recommended daily, and weekly flow linearity at different flow rates (low-, mid-, and high-flows) (Miller et al., 2005).

1.4.2.1.3 Test interpretation

A lot of information can be obtained from spirometric measurements. Figure 1.7 and Table 1.5 display some of the main parameters seen on flow-volume loops and volume-time curves.

Table 1.5 An overview of frequently used spirometry outcomes.

Outcome measure (Abbreviation)	Explanation
Forced expiratory volume in 1 second (FEV_1)	The volume of air expired in the first second of expiration (the 1 second can be substituted for other units of time, i.e. 0.5 or 0.75 seconds – $FEV_{0.5/0.75}$ – have been suggested as alternatives in children, particularly younger children, due to FEV_1 often contributing a significant proportion to the vital capacity due to relative size of airways compared to lungs (Piccioni et al., 2007)).
Forced vital capacity (FVC)	The total volume expired during forced expiration. In adults, due to prolonged expiration in those with lung obstruction, a suggested substitute is FEV_6 (Bhatt et al., 2014).
FEV_1/FVC ratio	The ratio between volume expired in one second and total vital capacity. Alternative times for measuring set volumes as per above can also be used in the ratio.
Forced expiratory flow at 25/50/75% ($FEF_{25\%}$/$FEF_{50\%}$/$FEF_{75\%}$)	A measurement of the flow rate at the relative volume proportion of the total FVC. These parameters are infrequently used, with the suggestion that $FEF_{75\%}$ potentially does not contribute to identification of pathology besides what FEV_1, FVC and their ratio offers (Quanjer et al., 2014).
Forced expiratory flow between 25-75% of FVC ($FEF_{25-75\%}$)	Also known as maximum mid-expiratory flow (MMEF), this represents the average flow during the mid-part of expiration. Deficits have been associated with airway obstruction, but, as per $FEF_{75\%}$, less utility is placed on this (Lebecque et al., 1993).
Peak expiratory flow rate (PEFR)	Commonly measured independently of spirometry as a monitoring tool in people with a diagnosis of asthma; however, is also measured as part of the spirometry.

Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and their respective ratio (FEV_1/FVC) are the tests most commonly used to identify pathology (Pellegrino et al., 2005); however, visualising the flow-volume loop can also be beneficial in suggesting disease process, as outlined by Figure 1.8 (Patra, 2012).

Importantly, tests need to be normalised to within the population performing them. Age, height, sex, and ethnicity have been shown to impact on spirometry outcomes. Over time there have been reference values derived from many cohorts, predominantly adult populations. However, more recently, reference values have been generated from a large collection of studies under the Global Lung Function Initiative (GLI). This included a larger number of children and is currently the standard for normalising results for the characteristics above (Quanjer et al., 2012).

Figure 1.7 Representations of the A) Flow – volume loop and B) Volume – time curve, annotated to show various outcome parameters.

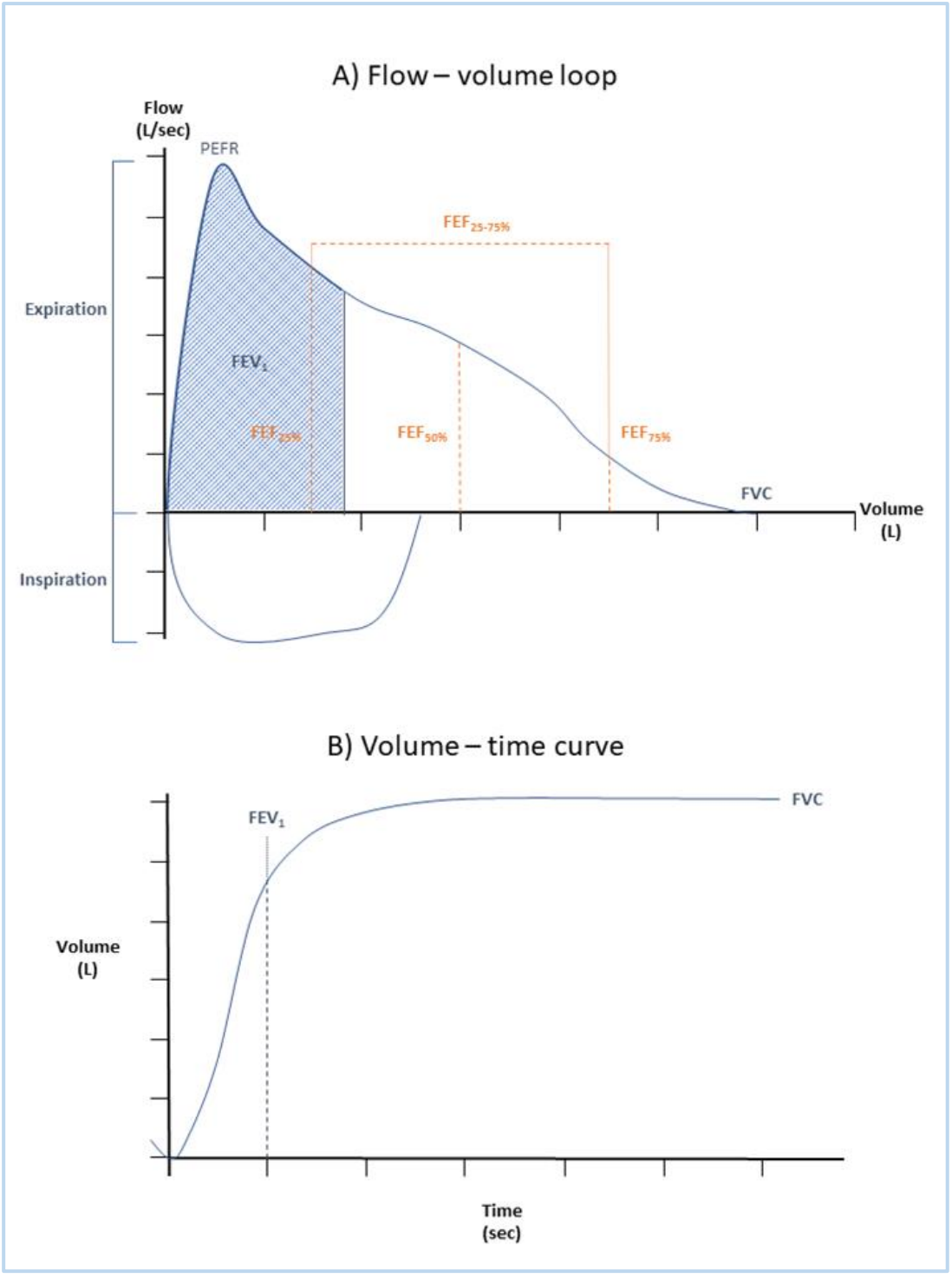
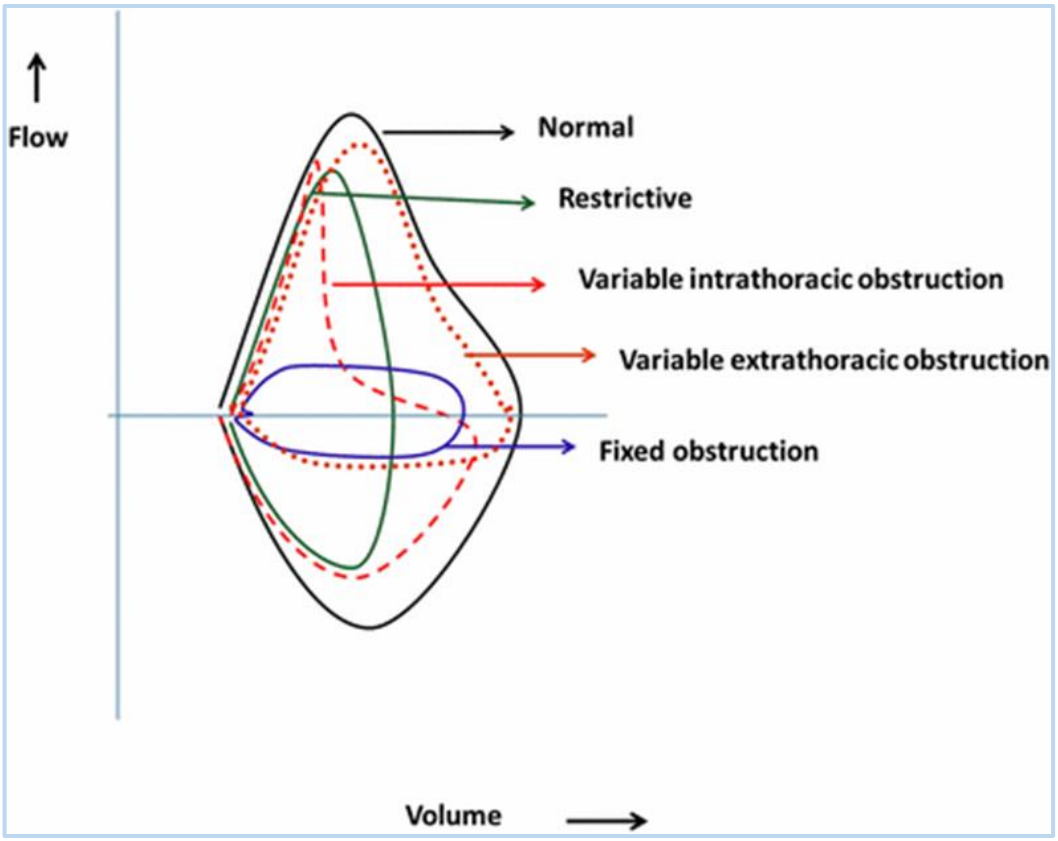


Figure 1.8 Flow – volume loops showing different characteristics representing different disease types (Patra, 2012). Image reproduced with permission of the rights holder.

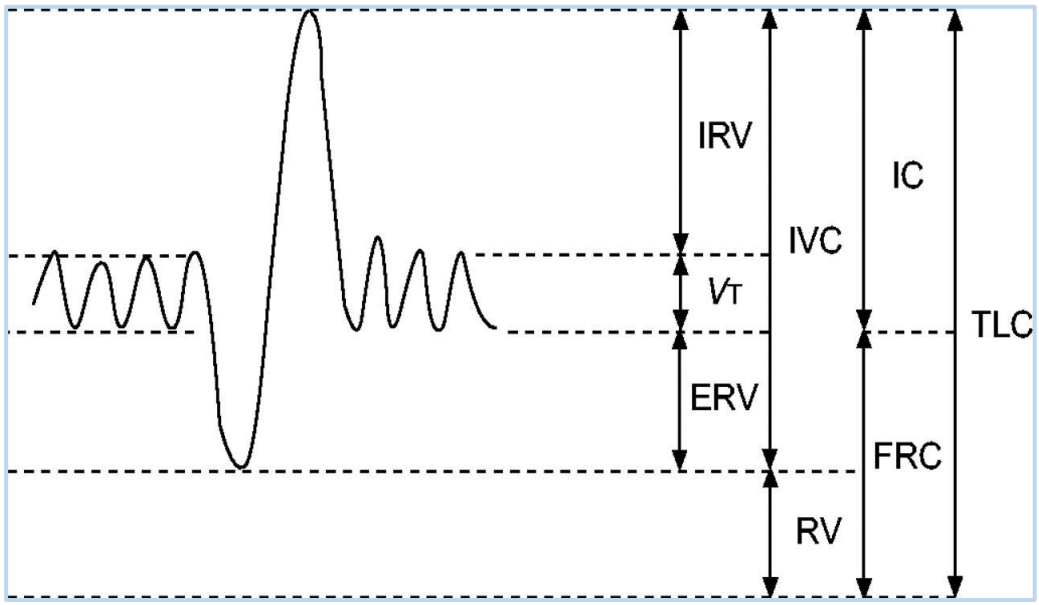


1.4.2.2 Static lung volume testing

Spirometry is a dynamic process that gives information about the how the lung functions, however vital information is missing from this test, in particularly regarding the total lung volume, known as the total lung capacity (TLC). This is comprised of the vital capacity (as can be measured from spirometry), as well as the residual volume (RV) which is the remaining air in the lungs at the end of expiration. Clearly this is more difficult to measure as a person is unable to expel this air to be measured. It is important to be able to clarify the total lung volume, as true restrictive disease (or small volume lungs) is identified by low TLC (Pellegrino et al., 2005). Other parameters include the inspiratory and expiratory reserve volumes (IRV/ERV – the volumes above or below tidal volumes to TLC or RV respectively), and functional residual capacity which is the combined volume of the ERV and RV. If a vital capacity is measured from end expiratory to full inspiration, this is called the inspiratory vital capacity, and if measured following exhaling from full inspiration to full expiration (as per in spirometry), this is called an expiratory vital capacity. There are differing ways

of measuring static lung volumes, the most common being either body plethysmography or gas dilution testing, either in a single or multi-breath format. In this section, I will cover the two types of testing later described in my thesis, body plethysmography and multi-breath helium dilution testing. In the healthy individual, these tests should give equivalent results, however in the presence of disease, especially presence of increased residual volume, differences may be noted (Hall et al., 2021). Figure 1.9 demonstrates the various lung volumes and capacities.

Figure 1.9 Visual representation of different lung volumes and capacities (Wanger et al., 2005). Image reproduced with permission of the rights holder.



IRV: inspiratory reserve volume; VT: tidal volume; ERV: expiratory reserve volume; IVC: inspiratory vital capacity; RV: residual volume; IC: inspiratory capacity, FRC: functional residual capacity; TLC total lung capacity.

1.4.2.2.1 Body Plethysmography

Body plethysmography is performed using the concept of Boyle-Mariotte law that for a fixed volume of gas at constant temperature, pressure and volume are inversely proportional, i.e. a decrease in volume will increase the pressure to the same extent (West, 1999). Additionally, the test is possible due to respiratory physiology, specifically the concept of shift volume. Shift volume refers to the volume change (as generated by increasing thoracic volume) required to generate a pressure change large enough (i.e., enough to overcome airway resistance) for airflow to occur, with changes in volume occurring before any airflow can occur. Because airflow has not

yet occurred at the point in which the (shift) volume is yet to generate a pressure change large enough to overcome airway resistance, Boyle-Mariotte's law applies to the air inside the lung.

The procedure is performed using a volume and pressure constant box which is sealed and allowed to adjust to a stable temperature after a participant sits inside. This box must be able to measure small changes in pressure proportional to that of shift volume, and as such any shift volume occurring within the lung will be reciprocated by a measurable change in the box pressure. A participant will then be connected to the equipment able to measure pressure and volume (as a determinant of flow), i.e., with use of a pneumotachograph. The participant wears a nose clip and holds their cheeks to ensure any air movement or pressure change is a result of respiration rather than artefact. The main part of the test involves respiration against a closed shutter occurring at the end of expiration, with the participant continuing to breathe against the shutter. At the point the shutter closes, the participant has their FRC (denoted as FRC_{pleth}), also called (intra)thoracic gas volume (ITGV or TGV), remaining in their lungs. However, the action of respiration continues, increasing the thoracic cavity but without any airflow, therefore the pressure change, relating to an intrathoracic shift volume, occurring within the lungs can be measured at the airway opening. Simultaneously, the changes in airway opening pressures are measured along with the volume changes within the box (Criée et al., 2011). Therefore the relationship between these can be used to calculate the lung volume, in the form of the equation $ITGV = Pao \times (\Delta V/\Delta P)$ (Clayton, 2007), where ΔV is the change in box volume, ΔP is the change in airway opening pressure, and Pao is the airway opening pressure at end of inspiration, representative of the alveolar pressure. As such, a comparatively large volume change in proportion to the pressure change would give a higher IGTV, with the reverse giving a smaller lung volume.

The test is completed by the participant performing vital capacity manoeuvres to elicit TLC. The shutter test should be repeated, ideally three times with reproducible results, to be able to ensure accuracy of results.

Calibration of the pneumotachograph for volume and flow should be performed similar to that for spirometry. Additionally, regular calibration for ensuring the box pressure and shift volume sensor are working appropriately is required.

1.4.2.2.2 Helium dilution testing

Multiple breath helium dilution testing has a simpler theory behind it. It involves connecting a participant to a closed circuit which includes a reservoir of air of known total volume, breathing at tidal volumes (i.e., at FRC at end of tidal expiration) after connecting to the closed circuit, before introducing helium gas of known concentration into the circuit. The participant continues with tidal breaths until the concentration of helium has equilibrised. If this does not occur, FRC will be underestimated. The FRC (denoted in this instance as FRC_{He}) is calculated as a rearrangement of the formula: *Volume 1 (V_1) x He concentration 1 (He_1) = Volume 2 (V_2) x He concentration 2 (He_2),* where V_1 is the volume of the circuit and He_1 is prior to wash-in. V_2 represents the volume of the circuit plus FRC_{He}, and He_2 is the helium concentration at the end of the wash-in period. FRC_{He} can then be calculated as $V1(He_1 - He_2) / He_2$ (Wanger et al., 2005). Again, the participant must then make a vital capacity manoeuvre in order to calculate TLC and RV. It is important the participant is breathing at FRC when connected to the wash-in period, otherwise a falsely elevated FRC will be measured.

Volume and flow linearity checks are required as per spirometry. Additionally, gas analyser (in particular Helium analyser) and the filling sensor (to ensure accurate volumes within the circuit) calibrations are needed regularly.

1.4.2.2.3 Interpretation of results

1.4.2.2.3.1 Reference values

As with spirometry, interpretation of results requires reference values in order to properly assess for normality. Previously there have only been small cohorts, predominantly Caucasian, used in formulating reference values, with Rosenthal having the greatest number with 772 participants (Rosenthal et al., 1993). The GLI have coalesced results from a number of centres from several countries, from both body plethysmography and helium dilution testing, in order to create more accurate

references (Hall et al., 2021). One finding from this endeavour was that in healthy individuals, the normal values from both tests are similar to each other, enough so that they could be compiled together in generating equations, which are corrected for age, sex, and height. The main limitation of the new reference values is the lack of ethnic diversity across the studies used, meaning the reference values are valid in people of European descent.

1.4.2.2.3.2 Results

The main outcome measures from static lung volume testing are regarding the TLC, RV, FRC and the ratio of RV to TLC. TLC is reduced in restrictive (or mixed) lung disease, generally defined as TLC below the lower limit of normal (Pellegrino et al., 2005), however other interpretation methods sometimes use percent predicted cut-off values. In the case of restrictive lung disease, usually all other parameters are also reduced. A raised RV, and/or RV/TLC ratio may be suggestive of airway obstructive (Sorkness et al., 2008), and indeed this is where differences between measurement modalities are often seen. In airway obstruction, air trapping occurs, and so with helium dilution testing, gas is unable to reach these areas of the lung, whereas body plethysmography is able to detect this part of the lower airways. As such, helium dilution may underestimate lung volumes in the presence of airway obstruction (Dahlqvist and Hedenstierna, 1985).

1.4.2.3 Cardiopulmonary exercise testing

1.4.2.3.1 Background

Cardiopulmonary exercise testing (CPET) is used to assess cardiopulmonary physiology in relation to muscle demands in aerobic and anaerobic states of metabolism, in both disease (cardiac, respiratory disease) and healthy (sports medicine) states. It encompasses a range of methods, designed to stress the cardiopulmonary system and review physiological responses to the exercise, particularly with regards to the relationship between oxygen demand and delivery. Exercise testing, monitoring oxygen content in arterial and mixed blood samples during exercise in patients with heart disease, was being performed in the 1950s (Donald et al., 1954). As with all these tests, this has evolved over time, with non-

invasive methods for assessing oxygen uptake, and carbon dioxide clearance, becoming available (Weber et al., 1982).

Exercise testing is usually performed on either a cycle ergometer or treadmill, however other modalities exist, for instance that involve walking in the form of set time, distance or as a shuttle, useful in patients who are unable to perform more strenuous exercise (Weber et al., 1982). While exercise testing between the modalities of cycle ergometer and treadmill follow similar methods for testing, there are potentially differences in outcomes, particularly related to the peak oxygen uptake, with the treadmill allowing participants to achieve higher peak $\dot{V}O_2$(Radtke et al., 2019). This is likely due to the impact of increasing cycle workload impacted by muscle strength rather than aerobic fitness. Conversely some groups depending on age, development and co-morbidities may have difficulties performing a particular modality of test.

1.4.2.3.2 Test specifics

The aim of most exercise tests is to get the participant to reach a peak of exercise, which theoretically will correspond to a peak cardiopulmonary physiological response. During exercise, participants will use either a face mask or mouthpiece connected to a gas sampler and a flow meter to get breath-by-breath recording of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Minute ventilation ($\dot{V}E$) as a product of tidal volume x respiratory rate is also calculated. The gas sampler and flow meter require regular calibration prior to testing to ensure accuracy of the results.

In general, an exercise test consists of four phases: 1) Rest phase; 2) Unloaded exercise; 3) Incremental exercise; and 4) Recovery phase (Radtke et al., 2019). During the rest phase, baseline parameters are measured. The unloaded (or minimally loaded in the case of cycle ergometer) phase is to warm-up the patient and begin to obtain measurements affected by exercise. The incremental test is generally performed in one of two ways – either as stepwise increments of a determined amount at regular intervals, or as a “ramp” of continuously increasing workload. The recovery phase is partly for safety purposes but also facilitates the start of post-exercise responses to exercise to be monitored.

Using the method of an increasing workload, whether by ramp or incremental steps, a participant is encouraged to continue exercise until they reach exhaustion. At this point it is presumed peak exercise has been reached; however, there are various methods of determining this. Usually a criteria for defining peak exercise is used based on various physiological or subjective parameters reaching certain thresholds (Radtke et al., 2019), including peak heart rate, respiratory exchange ratio or RER ($\dot{V}O_2/\dot{V}CO_2$), and perceived exhaustion score, often based on the Borg (Borg, 1982) or similarly validated scales. Unfortunately achieving peak exercise does not necessarily equate to reaching maximal $\dot{V}O_2$ uptake ($\dot{V}O_{2max}$) (Poole et al., 2008). However, achievement of a $\dot{V}O_2$ plateau (i.e. no further increase in $\dot{V}O_2$ despite increasing workload) is generally accepted as evidence of reaching $\dot{V}O_{2max}$, although children have difficulty achieving this outcome. Alternatively performing a supramaximal verification test has been suggested as a way of confirming that $\dot{V}O_{2peak}$ is indeed synonymous with $\dot{V}O_{2max}$. This involves, following the initial exercise test, recovery phase and a rest period, the participant cycling at high intensity at a workload set greater than the peak achieved during the first exercise test until exhaustion. If the same $\dot{V}O_{2peak}$ (<5% increase from the original test (Sansum et al.)) is obtained on the supramaximal test, this suggests that the $\dot{V}O_{2peak}$ from the initial test was representative of $\dot{V}O_{2max}$ (Barker et al., 2011).

1.4.2.3.3 Explanation of variables

Oxygen uptake per minute ($\dot{V}O2$*)*. Measured throughout testing and at peak exercise ($\dot{V}O_{2peak}$), with the peak theoretically representing the maximal possible uptake of oxygen ($\dot{V}O_{2max}$). $\dot{V}O_{2max}$ is the maximum "integrated capacity of the pulmonary, cardiovascular and muscle systems to uptake, transport, and utilize O_2" (Poole et al., 2008). This means it is reliant on all the systems to be achieved and deficits in one or more areas will impact on exercise capacity.

Carbon dioxide production per minute ($\dot{V}CO_2$*)*. Measured throughout and at peak exercise ($\dot{V}CO_{2peak}$). Typically increases linearly with $\dot{V}E$*,* with an increase in both when CO_2 production increases as a result of an increase in anaerobic respiration. An anaerobic threshold (AT) can be identified at the point in which $\dot{V}CO_2$ increases disproportionally when plotted against $\dot{V}O_2$ due to this change.

Respiratory exchange ratio (RER). RER indirectly reflects how muscles are able to obtain energy, with higher RER suggesting increased use of carbohydrates and lower RER suggesting use of lipids, and may represent physical fitness due to the association of lipid metabolism with oxidative metabolism (Ramos-Jiménez et al., 2008).

Minute ventilation ($\dot{V}E$). Initially increases linearly until anaerobic threshold is reached, at which stage $\dot{V}E$ increases at a greater rate due to increased CO_2 production.

Ventilatory reserve – This is the percentage of the maximum voluntary ventilation (MVV) that is remaining after subtracting the peak $\dot{V}E$. The MVV can be measured by a participant breathing as fast and deeply as possible (to achieve at least 50% of their vital capacity with each breath) for a set time (usually 12 seconds) and the resulting volume extrapolated to a minute (Miller et al., 2005). Alternatively a multiplication of FEV_1 (by a factor of 35 or 40) can be used to estimate MVV (Stein et al., 2003). Ventilatory reserve may be reduced in respiratory disorders (Balady et al., 2010) or in athletes (in the context of high $\dot{V}O_{2peak}$ to distinguish from disease states) (Palange et al., 2007).

1.5 Summary

In summary, preterm births are slowly increasing, including greater survival rates at lower gestations. Respiratory difficulties of preterm birth are among the most common short- and long-term sequelae, including respiratory distress syndrome (RDS) and chronic lung disease of prematurity (CLD) in infancy, with higher rates of respiratory symptoms, respiratory medication use, and health care utilisation compared to those born at term. Children born at the extremes of gestation and birth weight are greatest affected, and a history of CLD also impacts, however disease is not exclusive to these children. Effects are seen throughout childhood and can extend into adulthood, with concern that early life factors may impact on earlier onset of respiratory diseases such as chronic obstructive pulmonary disease, usually seen in later adulthood, particularly if exposed to negative environmental factors. Objective measures of lung function can be used to assess respiratory health, in the form of lung function and cardiopulmonary exercise testing. There are limitations to the age it is possible to perform some of this testing, and so newer techniques including oscillometry are being more widely used, mainly in the research setting, that can conceivably identify pathology at a younger age, potentially opening the door for earlier intervention, if such treatments can be identified. There are also likely different phenotypes within the children with lung disease as a result of preterm birth, with different underlying pathologies and different responses to lung function testing.

As mentioned above, oscillometry is a method of lung function testing which assesses airway mechanics. There are differing modalities within oscillometry, also referred to as forced oscillation technique or FOT, including standard spectral or pseudorandom noise, which involves applying multiple frequencies of soundwaves onto tidal breathing and assessing the resultant changes in pressure and flow, or intra-breath oscillometry which looks at the changes in airway mechanics occurring throughout the breath cycle. Oscillometry is covered in greater detail in individual Chapters 3 and 4.

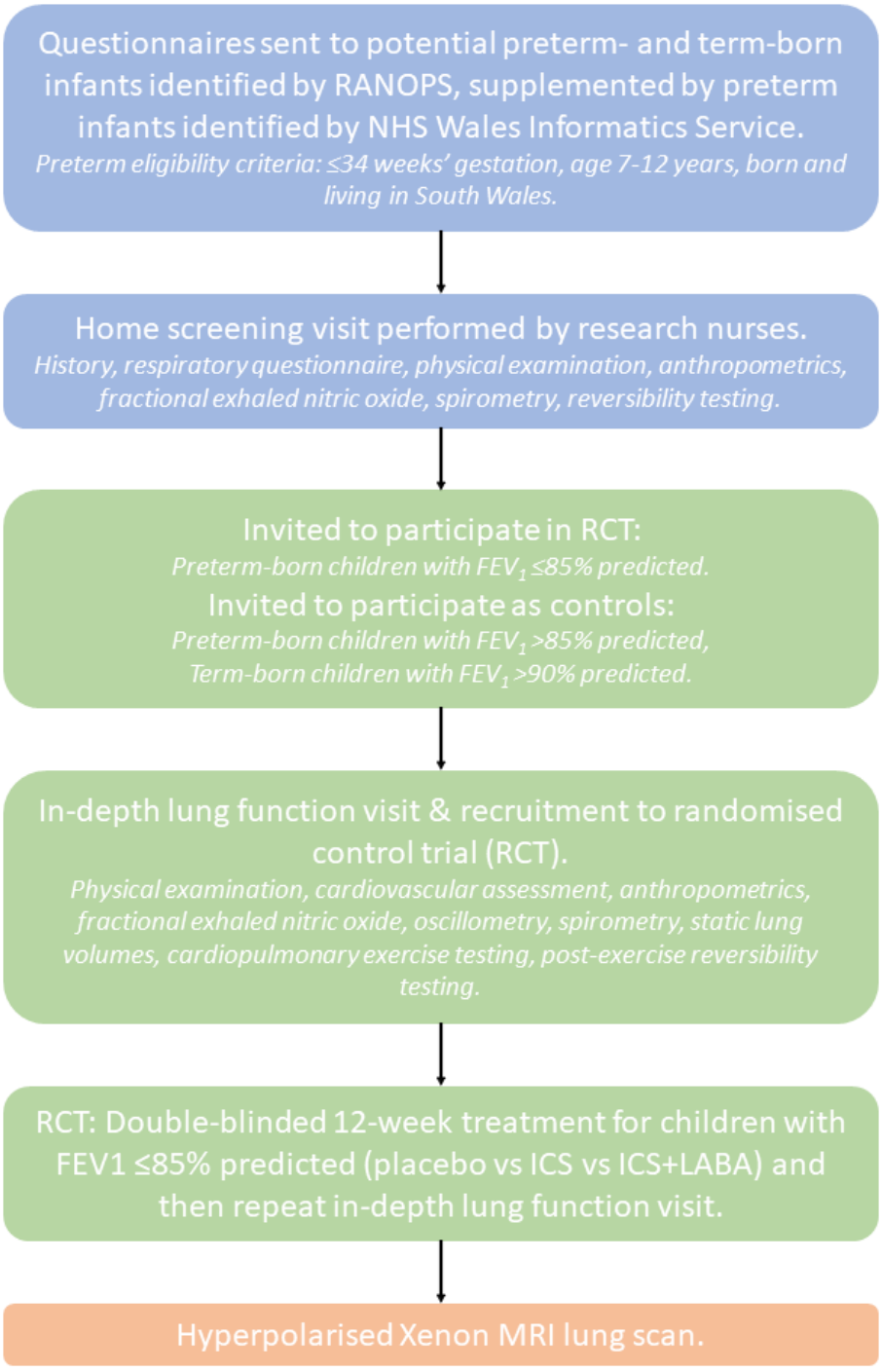
1.6 Respiratory Health Outcomes in Neonates study

1.6.1 Background and relevance to thesis

The Respiratory Health Outcomes in Neonates (RHiNO) study was designed following an original questionnaire-based study, the Respiratory and Neurological Outcomes in children born Preterm Study (RANOPS), which identified greater respiratory morbidity in preterm children across the gestations including those born at early term (Edwards et al., 2015a), and across age groups (Edwards et al., 2016). While this study showed that atopy rates were no higher in affected preterm children, there were still greater numbers classified as having asthma or on asthma medications, without there being any definitive evidence of efficacy in this population, with limited studies assessing the use of medications such as inhaled corticosteroids (Pelkonen et al., 2001).

RHiNO was formed as a follow-up study, designed to objectively determine potential lung dysfunction in the preterm population and to investigate the role of asthma medications in modifying the disease. As such a three-arm, double-blinded, randomised, placebo-controlled trial (RCT) was designed to compare placebo to inhaled corticosteroid and combination of inhaled corticosteroid (ICS) plus long-acting beta-2 agonist (LABA). This was a registered randomised control trial (EudraCT: 2015-003712-20), which received ethical approval from the South-West Bristol Research Ethics Committee (15/SW/0289) and was funded by the Medical Research Council (MR/M022552/1). The RCT was the centre-stage of a broader three-part study exploring potential phenotypes and mechanisms seen in preterm-children with evidence of lung dysfunction. Below I outline the three parts of the wider RHiNO study, with a summary provided in Figure 1.10. The children involved in the RHiNO study are those studied within the course of my thesis. The study allowed generation of my hypotheses and aims which I outline later in section 1.7.

Figure 1.10 Flow diagram outlining RHiNO study as a whole. Part 1 in blue shaded boxes, Part 2 in green shaded boxes, Part 3 in orange shaded boxes. (Abbreviations: RANOPS – Respiratory and Neurological Outcomes in children born Preterm Study; ICS – inhaled corticosteroid; LABA – long-acting beta-2 agonist)



1.6.2 RHiNO Study description

1.6.2.1 Part 1

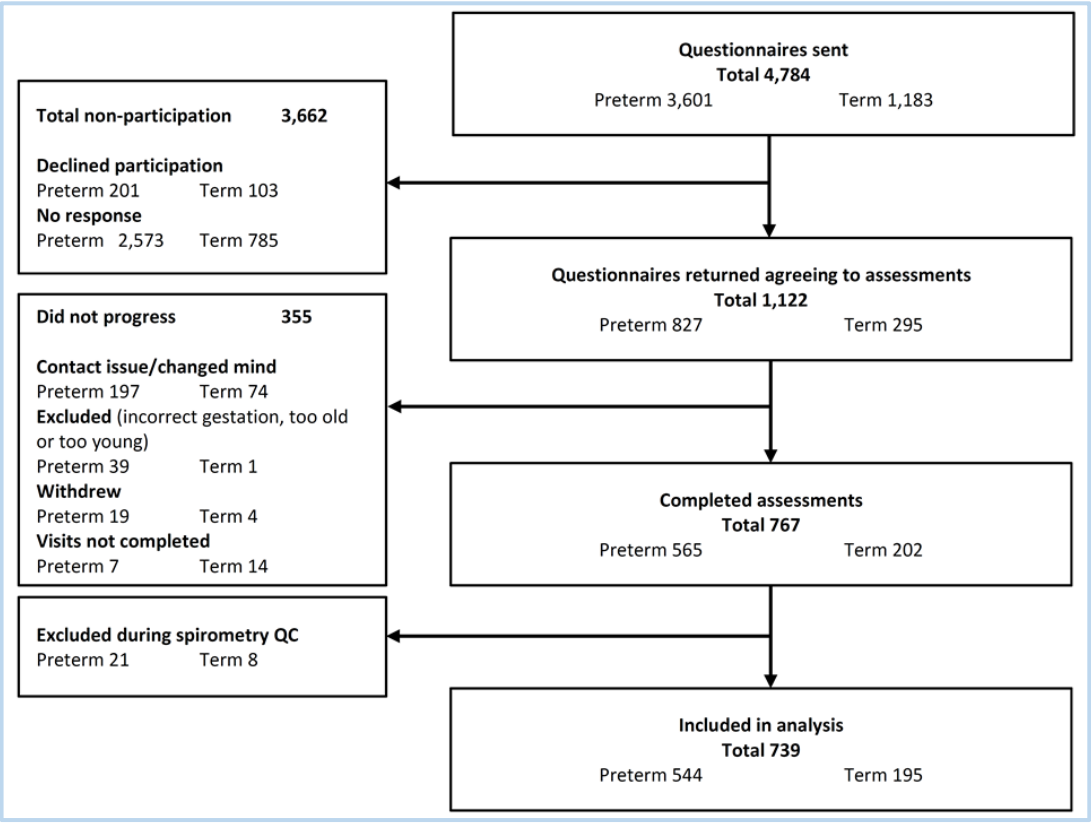
Part 1 of the RHiNO study involved recruitment of participants with an initial questionnaire and a subsequent screening visit to assess lung function performed by research nurses. The children recruited to RANOPS (as above) were firstly invited to take part in RHiNO. For RANOPS, all preterm (<37 weeks' gestation) children born and resident in South Wales, from birth years 2003, 2005, 2007, 2009, 2010 and 2011 were invited to participate in the questionnaire study. The preterm-born children were matched at the time of RANOPS with term (≥37 weeks' gestation) controls based on birth date, sex and location of birth. These children were identified by the NHS Wales Informatics Service (NWIS) database. All preterm-born children of ≤34 weeks' gestation and all term-born children from RANOPS who had consented for involvement in further research were sent a respiratory questionnaire for completion, as well as parent/child information sheet regarding a screening visit and asked whether they were willing to participate. These participants were later supplemented with preterm-born children (≤34 weeks' gestation) from the interval years of RANOPS (i.e. 2004, 2006, 2008), again identified through NWIS database, who were initially contacted by representatives of the health boards in which they were born. These children were again sent a respiratory questionnaire and information leaflets and asked to indicate consent for being contacted by the research team for a screening visit. Inclusion criteria for RHiNO were age 7-12 years (upper threshold chosen to limit children undergoing puberty, and lower threshold due to diminished likelihood of obtaining adequate spirometry), born at ≤34 weeks' (preterm children) or ≥37 weeks' (term children) gestation, and born and resident in South Wales. Children with significant congenital or cardiac abnormalities, or neurodevelopmental impairment were excluded. Recruitment started in December 2016 and continued through until September 2019.

Screening visits were carried out by trained research nurses and performed at either the participant's home or at the Children and Young Adults' Research Unit at the Children's Hospital for Wales. The screening visit involved collecting perinatal and medical history (later supplemented by review of the participant's medical notes),

and performing anthropometric measurements, cardiovascular assessment including blood pressure and augmentation index, fractional exhaled nitric oxide (FE_{NO}), spirometry and reversibility testing. Additionally, urine and saliva samples were taken, the latter for DNA analysis. The aim of the visit was to collect objective lung function data from an undifferentiated preterm population, as well as identifying and stratifying potential patients eligible for Part 2 of the RHiNO study which included the RCT.

Figure 1.11 summarises the numbers recruited during Part 1.

Figure 1.11 Flow diagram showing participant numbers through Part 1 of RHiNO study. (Hart et al., 2021). Image used with permission under author reuse policy.



1.6.2.2 Part 2

Part 2 of the study comprised of in-depth lung function testing for children identified from Part 1, stratified into those with $\%FEV_1$ ≤85% (PT_{low}), along with a number of preterm (PT_c) and term (T_c) controls. Those children in the PT_{low} group (based on repeat lung function at Part 2) were eligible for recruitment into the RCT, the main part of the RHiNO study. All children with $\%FEV_1$ ≤85% at Part 1 were invited to participate, along with all term controls with $\%FEV_1$ >90% at Part 1 and selected preterm controls. Not all preterm children with $\%FEV_1$ >85% were invited; the aim was to maximise the numbers in PT_{low} for RCT recruitment, and a target of 100 preterm controls. As the larger majority of the children screened at Part 1 were anticipated to have $\%FEV_1$ >85%, inviting all these children would have resulted in greater numbers than required. Screening visits at Part 1 started with the children at the higher age limits in order to recruit before they became too old. If all consecutive preterm children with $\%FEV_1$ >85% were invited, this would have resulted in a PT_c

population at Part 2 skewed towards the higher end of the age bracket. As such, preterm children who were within the first 10 screening visits of any calendar month were invited to attend as preterm controls, if $\%FEV_1$ >85%. This was designed to hopefully allow ongoing recruitment throughout the study period (accounting for likely attrition, i.e. not all invited would attend) and would give a range of ages and a balance to recruitment throughout the seasons.

For PT_{low} children potentially eligible for the RCT, decision to recruit to the RCT was based on spirometry ($\%FEV_1$) at the Part 2 visit using a pneumotachograph spirometer. Children with $\%FEV_1$ ≤85% at Part 2 were recruited to the RCT. Due to differences between spirometry methodology (as discussed in detail in Chapter 5) and inherent variability of spirometry, not all children with $\%FEV_1$ ≤85% at Part 1 went on to have $\%FEV_1$ ≤85% at Part 2. In these instances, if that child had been within the first 10 Part 1 visits of a calendar month (i.e., how the PT_c children were invited to Part 2), they were invited to complete the Part 2 visit as PT_c. If they were outside the first 10 visits of a calendar month at Part 1, they did not complete the Part 2 visit. This was to avoid skewing the PT_c group towards the lower end of lung function (with the assumption that children switching groups would likely end up just above the FEV_1 threshold). All children with $\%FEV_1$ ≤85% on definitive spirometry, irrespective of grouping at Part 1, were invited to participate in the RCT, following formal consent/assent.

Part 2 visits involved physical examination, performing anthropometric measurements, cardiovascular assessment including blood pressure and augmentation index, FE_{NO}, oscillometry, static lung volume testing using body plethysmography and helium dilution testing, spirometry using both turbine and pneumotachograph spirometers, cardiopulmonary exercise testing, post-exercise spirometry and oscillometry, and post-exercise reversibility testing. Collection of samples was also performed, including urine, saliva, exhaled breath condensate and induced sputum using nebulised hypertonic saline. Methodology of several of these tests is discussed in greater details within the various chapters. These visits lasted approximately 5-6 hours.

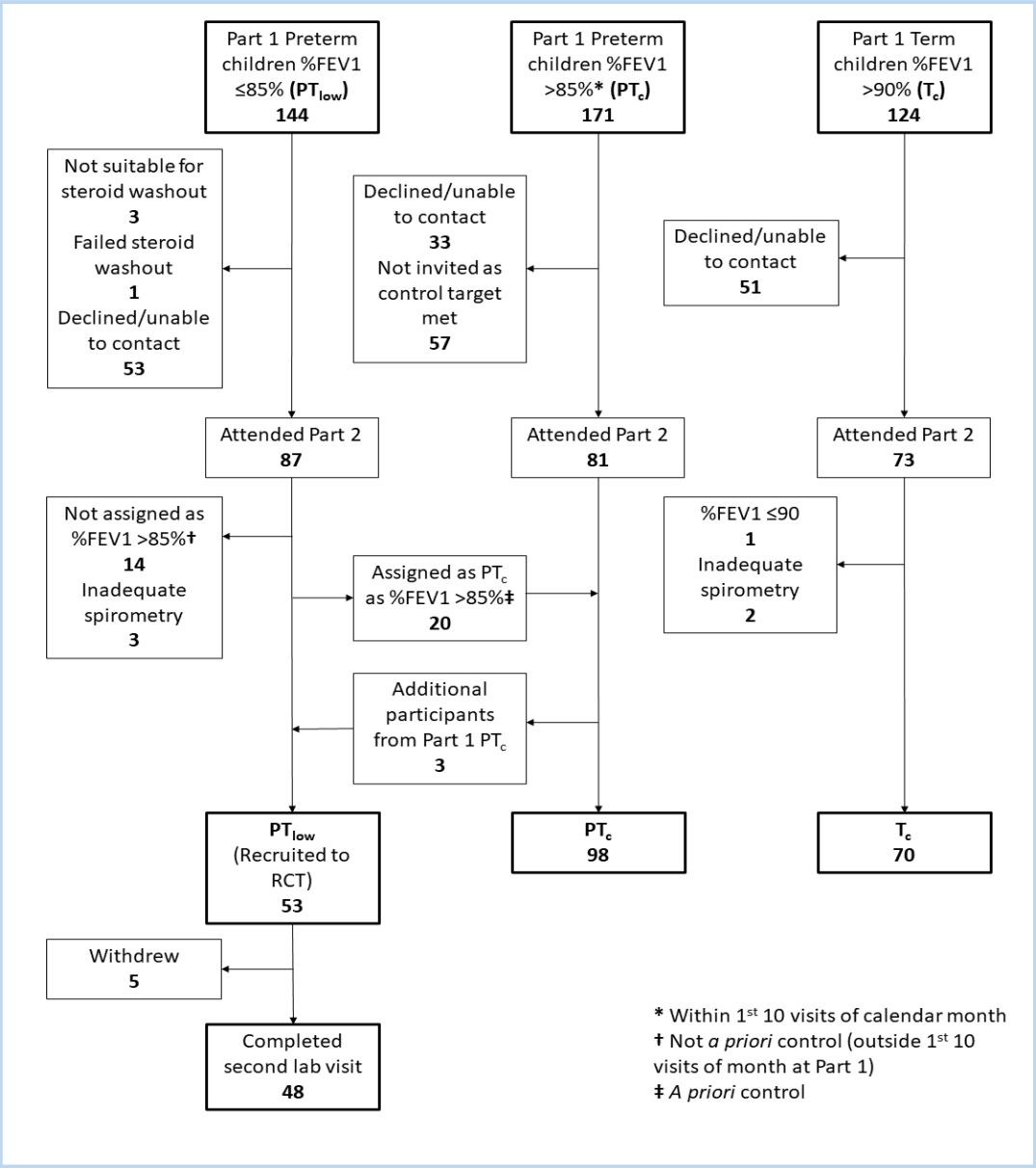
Preterm-born children meeting the above eligibility criteria and who had %FEV_1 ≤85% were recruited to the RCT. This involved randomising to one of three blinded treatment groups: 1) inhaled corticosteroid (fluticasone propionate 50 micrograms); 2) inhaled corticosteroid plus long-acting beta-2 agonist (fluticasone propionate 50 micrograms + salmeterol 25 micrograms); 3) placebo. All participants were instructed to take 2 puffs twice daily of their inhalers via a volumatic spacer for a minimum of 12 weeks, with appropriate instruction given regarding how to take the inhalers. Computer-generated randomisation was performed.

Children potentially eligible for the RCT but who were already on steroid inhalers prior to Part 2 were assessed by a paediatric respiratory consultant to determine whether they were suitable for a 4-week washout period before attending for in-depth lung function testing. Of those who underwent a washout period, regular contact was kept assessing for problems, and in the event of symptoms were advised to restart their preventer medication. Those who remained well, at recruitment, were entered into a two-arm randomisation to ensure they received active treatment.

Following the trial treatment period, participants returned to complete a second in-depth visit, as outlined above. The primary outcome of the trial was looking for a difference in %FEV_1 pre- to post-treatment between the groups, powered to detect a 10% increase in %FEV_1 pre- to post-treatment. The rationale behind the trial was to establish whether there is an optimal treatment in preterm-born children with evidence of lung function impairment. Preterm and term controls did not participate in a second visit.

Figure 1.12 summarises the participant numbers through Part 2.

Figure 1.12 Flow diagram showing participant numbers through Part 1 of RHiNO study.



1.6.2.3 Part 3

The final part of the study involved recruiting children for hyperpolarised xenon MRI scans of the lungs, performed at the University of Sheffield. 20 children from each of the PT_{low}, PT_c and T_C groups who completed a Part 2 visit were invited for participation.

1.6.3 Population

Figures 1 .11 and 1.12 outline the flow and numbers through the RHiNO study, and clearly there was attrition at all stages of the process. The actual response rate to the questionnaire of almost a quarter of the contacted children is impressive. Unfortunately, not all were able to participate in the screening visit as outlined in Figure 1.11. Again, there was a further drop off of children who attended Part 2 visit, with reasons outlined in Figure 1.12. The concern for the attrition is whether this would have an impact on the population that participated in Part 2 of the study, i.e., the data contributing to my thesis.

A comparison was made for preterm and term children who did and did not participate in a screening visit (Hart et al., 2021). Comparison data was limited due to only having specific demographics from NWIS in the non-participators; however, it was possible to compare sex, gestational age and birth weight between responders and non-responders, as shown in the adapted table in Figure 1.13. For the term group there were significantly fewer males and a significantly older gestational age in the term responders. However, the actual numbers (50% vs 52% for male sex, and 39.8 vs 39.6 week's gestational age) clearly show a lack of clinical significance. Similarly for preterm children, the responders were of a younger gestational age and lower birth weight, but again the actual numbers (31 vs 31.6 weeks' gestation, and 1703 vs 1828 grams) suggest there is likely to be little clinical difference between these groups.

Figure 1.13 Adapted table showing comparison between responders and non-responders to the questionnaire of Part 1 of RHiNO study, by preterm or term status.(Hart et al., 2021) Image used with permission under author reuse policy.

	Preterm		Terms	
	Responders	**Non-responders**	**Responders**	**Non-responders**
Subjects (n)	565	3036	202	983
Male	293/565 (52%)	1679/3036 (55%)	100/202 ^{vvv} (50%)	513/983 (52%)
Gestational age (weeks) (mean SD)	31.0 (2.7) ^{vvv}	31.6 (2.6)	39.8 (1.2) ^{v}	39.6 (1.3)
Birthweight (g) (mean SD)	1703 (564) ^{vvv}	1828 (684)	3480 (479)	3443 (507)

Abbreviation: WIMD – Welsh Index of Multiple Deprivation.

Significance ^{v}p<0.05, ^{vv}p,0.01, ^{vvv}p<0.001 comparing the responders and non-responders in the preterm or the term groups.

Number for responders are those who consented for a visit but before any QC.

I made a further comparison of the children who did and did not attend the Part 2 visit after being identified as eligible at screening to see whether there were any differences in the populations, as divided by the group stratification (PT_{low}, PT_c and T_c). Tables 1.6, 1.7 and 1.8 show these comparisons for PT_{low}, PT_c and T_c respectively. There were no differences between Part 2 participators and non-participators for PT_{low} and T_c groups, although there was a non-statistically significant greater number of children with doctor-diagnosed asthma in the PT_{low} children who attended compared to those who did not (30 vs 21%; p=0.223), possibly suggesting a slight selection bias of children with greater respiratory morbidity. The PT_c children who attended were slightly older than those who did not (10.4 vs 9.3 years; p<0.001), likely reflecting the fact that older children were invited first, and that the target for numbers was almost reached before the youngest ages were screened. Other demographic details for PT_c children were similar in both those who did and did not attend. Overall, this suggests that all groups are fairly representative of the population as a whole.

Table 1.6 Table outlining basic demographic details of preterm participants with %FEV$_1$ ≤85% at Part 1 visit who did and did not attend Part 2.

	Participated in Part 2, n=53	Did not participate in Part 2, n=91
Age at Part 1 visit, years	10.0 (9.7 to 10.4)	9.9 (9.6 to 10.2)
Male, n (%)	24 (45%)	46 (51%)
Gestation, decimal weeks	29.7 (28.9 to 30.6)	30.4 (29.8 to 31.0)
Birth weight, grams	1,392 (1,235 to 1,549)	1,546 (1,429 to 1,665)
IUGR, n (%)	12 (23%)	17 (19%)
Doctor-diagnosed asthma, n (%)	16 (30%)	19 (21%)

Results expressed as mean and 95% confidence intervals for continuous data (independent t-test) or number and % proportion (Pearson's χ^2 test). **Abbreviations: IUGR** – Intrauterine Growth Restriction.

Table 1.7 Table outlining basic demographic details of preterm participants with %FEV$_1$ >85% at Part 1 visit who did and did not attend Part 2.

	Participated in Part 2, n=97	Did not participate in Part 2, n=326
Age at Part 1 visit, years	**10.4 *** (10.1 to 10.6)**	9.3 (9.2 to 10.5)
Male, n (%)	49 (51%)	170 (52%)
Gestation, decimal weeks	31.0 (30.5 to 31.6)	31.3 (31.0 to 31.6)
Birth weight, grams	1,730 (1,615 to 1,845)	1,763 (1,701 to 1,825)
IUGR, n (%)	14 (14%)	37 (11%)
Doctor-diagnosed asthma, n (%)	18 (19%)	37 (11%)

Results expressed as mean and 95% confidence intervals for continuous data (independent t-test) or number and % proportion (Pearson's χ^2 test). **Abbreviations: IUGR** – Intrauterine Growth Restriction.
*** $p < 0.001$

Table 1.8 Table outlining basic demographic details of term participants with %FEV_1 >90% at Part 1 visit who did and did not attend Part 2.

	Participated in Part 2, n=53	Did not participate in Part 2, n=91
Age at Part 1 visit, years	9.7 (9.4 to 9.9)	9.7 (9.5 to 9.9)
Male, n (%)	37 (53%)	64 (50%)
Gestation, decimal weeks	40.0 (39.7 to 40.3)	39.7 (39.5 to 39.9)
Birth weight, grams	3,528 (3,404 to 3,651)	3,434 (3,341 to 3,526)
IUGR, n (%)	4 (6%)	6 (5%)
Doctor-diagnosed asthma, n (%)	5 (7%)	7 (6%)

Results expressed as mean and 95% confidence intervals for continuous data (independent t-test) or number and % proportion (Pearson's χ^2 test). **Abbreviations: IUGR** – Intrauterine Growth Restriction.

1.7 Hypothesis and aims

1.7.1 Hypothesis

I hypothesise the following:

1. Preterm-born children with low lung function will have different population characteristics compared to preterm control groups;
2. Preterm-born children with low lung function will have abnormal static lung volumes;
3. Preterm-born children with low lung function will have altered spectral oscillometry at baseline compared to term controls;
4. Preterm-born children with low lung function will have altered single frequency intra-breath oscillometry outcomes at baseline compared to terms, which will show a different pattern to that previously described in children with wheeze phenotype;
5. Preterm-born children with low lung function will have impaired exercise capacity compared to controls;
6. Preterm-born children with low lung function will have greater exercise-induced bronchoconstriction compared to controls;
7. Preterm-born children with low lung function will have greater reversibility to post-exercise bronchodilators compared to controls;
8. Preterm-born children with low lung function are not a single phenotype and within this group there will be children with different clinical and lung function characteristics.

1.7.2 Aims

1. Determine population characteristics relating to birth and respiratory history as per the pre-defined study groups (preterms with low lung function, preterm controls, term controls);
2. Determine baseline lung function results (spirometry, static lung volumes) for these groups;
3. Assess exercise capacity across study groups;
4. Assess for evidence of exercise-induced bronchoconstriction across study groups;

5. Assess for evidence of post-exercise reversibility across study groups;
6. Evaluate airway mechanics using two forms of oscillometry – spectral and single-frequency intra-breath at:
    a. Baseline,
    b. Post-exercise,
    c. Post-exercise bronchodilator;
7. Identify potential phenotypes within the preterm children with low lung function;
8. Re-evaluate aims 1 -6 for any new phenotypes identified;
9. Review any methodological challenges that are identified through the course of my data collection and analysis.

2 LUNG FUNCTION AND EXERCISE TESTING

2.1 Introduction

As outlined in the Introduction, preterm-born children are at greater risk of respiratory health sequelae, both in the short- and long-term, compared to infants born at full term. At potentially greater risk of longer-term morbidity are infants born towards the extreme of gestation and birthweight, especially in the context of being born IUGR, as well as children diagnosed during infancy with CLD. As such there has often been a focus on long-term follow-up of those infants who had a CLD diagnosis from their neonatal period. More recent studies have shifted this focus to either those born extremely preterm or at very/extremely low birth weight (for instance the EPICure or EPIPAGE groups from UK and Franc respectively). However, as will be highlighted below, long term respiratory morbidity is not confined to these groups, and less is understood about the complex factors that result in a proportion of the ex-preterm population being affected, or why some children with CLD may be spared long-term problems. Below I consider lung function and other testing modalities that feature in my thesis, and current evidence of use and outcomes in the preterm population.

2.1.1 Lung function testing

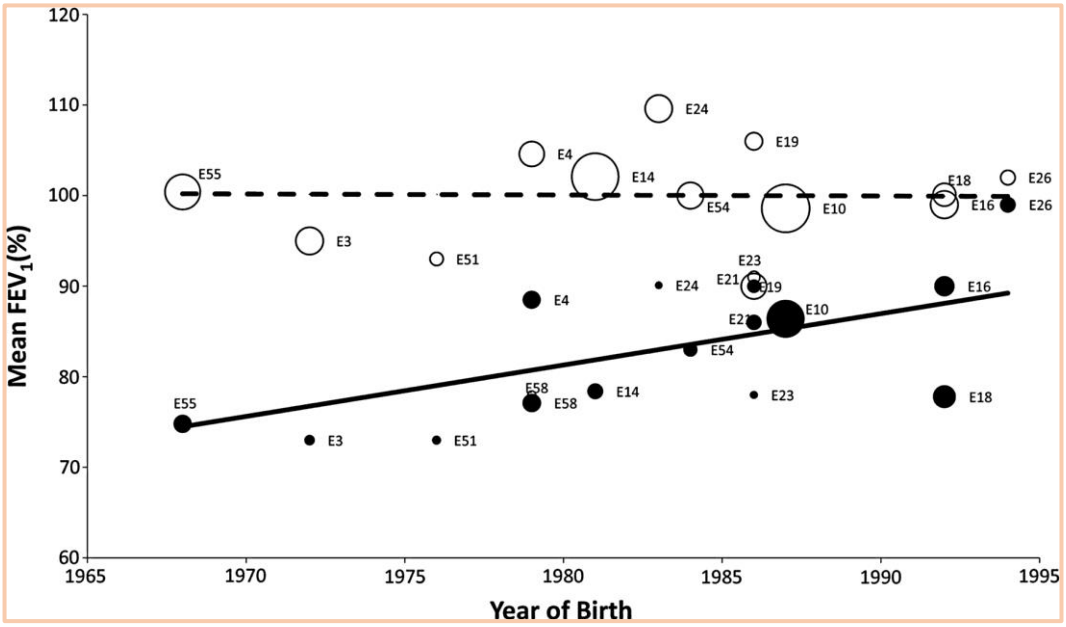
2.1.1.1 Spirometry

Spirometry is the most widely used lung function test in assessment of long-term respiratory follow-up of children born preterm. As such it has been possible to obtain a very clear picture of spirometry outcomes in preterm-born children.

A systematic review of spirometry of over 2,000 preterm-born children (with almost 4,000 term controls) up to the year 2010 demonstrated that FEV_1 was impaired by 8.7% percent predicted ($\%FEV_1$) overall in preterm children (regardless of CLD status) compared to term controls. When focused on preterm children without CLD this deficit reduced to 7.15%. However, when looking at only those children with a CLD diagnosis of ongoing supplemental oxygen requirement at either 28 days (CLD_{28}) or 36 weeks' post-conceptual age (CLD_{36}), this deficit of $\%FEV_1$ increased to 16.2% and

18.9% respectively. Of additional interest from this systematic review was a model looking at the effect of year of birth on the relative deficit of FEV_1 over time (from late-1960s to 1995) in children with CLD_{28}. The earlier-born children had the greatest difference, whereas the children born at more recent dates had less marked lung function deficits compared to their term-born peers, as illustrated in Figure 2.1. This change was likely due to a combination of factors including improved neonatal care (antenatal steroids, surfactant use), as well as an evolution of the pathological processes underlying CLD (Kotecha et al., 2013).

Figure 2.1 Effect of year of birth on %predicted FEV_1 in preterm-born children with chronic lung disease of prematurity (supplemental oxygen at 28 days) – closed circles, compared to term controls – open circles, with weighting of studies contributing to circle size (Kotecha et al., 2013). Image reproduced with permission of the rights holder.



Despite this improvement over time, more recent studies have continued to find that preterm-born children are at greater risk of lung disease. This includes at the extremes of prematurity (Lum et al., 2011, Vollsæter et al., 2015), but also for late preterm children (Yaacoby-Bianu et al., 2019). Of concern is persistent lung function deficits on spirometry into adulthood (Doyle et al., 2019a).

2.1.1.2 Static lung volumes and airway resistance

Body plethysmography has been widely used as an investigation into lung function of children who were born preterm. This includes studies from the pre-surfactant and post-surfactant eras.

A study from the Netherlands investigating children between the age of 8 and 18 years born between 1967 and 1977, all diagnosed with RDS in infancy. 40 children who required ventilation were followed up and compared to a group of gestational and present age plus sex matched (n=38) children who were not ventilated. No differences were found in the TGV, RV or TLC (de Kleine et al., 1990). Similar results with little difference between CLD and controls (Hakulinen et al., 1996) or preterm children with RDS and controls (Cano and Payo, 1997) have been found in other pre-surfactant cohorts

In contrast, Jacob et al. reported differences in FRC and RV, but not TLC, using whole body plethysmography in children with severe CLD (home oxygen) compared to gestational and age matched controls. Additionally, a raised RV:TLC ratio compared to the controls suggested hyperinflation in the CLD group, hypothesised to be secondary to loss of elastic recoil, identified on abnormal pressure-volume using transducers to measure airway opening and oesophageal pressures (Jacob et al., 1997).

Smith et al reported lung volumes data in a study looking at exercise capacity in a group of very preterm infants with extremely low birth weight (ELBW) born between 1992 and 1994. The preterm group (n=94) showed significantly increased %predicted TLC (%TLC), RV (%RV) and FRC (%FRC), and RV/TLC ratio on plethysmography compared to controls (n=30). In particular, the %RV was ~40% higher in the preterm group compared to the control group, again suggesting hyperinflation or air trapping (Smith et al., 2008). Studies evaluating extremely preterm children have similar findings of raised RV and RV/TLC ratio, with CLD appearing to be greatest affected (Welsh et al., 2010). This is consistent with spirometry findings where CLD appears to have worse outcomes.

2.1.1.3 Low birthweight

The link between very low birth weight (VLBW) infants and long-term respiratory sequelae has been investigated with body plethysmography. Korhonen and colleagues explored respiratory outcomes in a group of 34 VLBW children at age 7 with a history of bronchopulmonary dysplasia (BPD) defined as oxygen requirement at 28 days postnatal age, compared to a non-BPD VLBW group and a term group with

the same numbers. A sub-group of 14 BPD infants as defined by oxygen requirement at 36 weeks' corrected gestational age were considered in a separate analysis as well as within the main BPD group analysis. 29 BPD children completed the body plethysmography. Findings were of significantly increased RV and RV/TLC ratio in the BPD group compared to term controls. The authors also used a definition for lung hyperinflation, of RV greater than 30% of the TLC. 52% of the BPD group compared to 27% of the non-BPD VLBW controls and 10% of the term controls met this criterion, with statistically significant differences between the BPD and term groups noted (Korhonen et al., 2004).

Conversely, vom Hove et al reported no significant difference in TLC, RV, or RV:TLC between VLBW school-aged children with and without CLD, despite the CLD group having evidence of lung dysfunction (at least one of FEV_1, FEF_{25-75}, FEV_1:FVC, TLC, or diffusing capacity of the lungs for carbon monoxide (DL_{CO}) < 5^{th} centile) (vom Hove et al., 2014).

Of interest, regarding children outside of those diagnosed with CLD having longer-term lung dysfunction, Lista et al followed-up VLBW infants born at an average gestational age of 27 weeks, enrolled as neonates into a trial comparing high frequency oscillatory ventilation to volume-regulated conventional ventilation in RDS. No patients in either group developed CLD. The study reported increased %RV (194 and 143% in HFOV and conventional modalities respectively). The study also commented on elevated %TLC, however the results displayed showed only 106% and 93% respectively for HFOV and conventional modalities. Repeat testing was performed post-bronchodilator, and while there was a drop in RV in the HFOV group, there are no values displayed to compare statistical significance of pre- and post-treatment. Of note there appeared to be no marked FEV_1 deficit on spirometry. The conclusion from the authors is that these children, despite not having CLD in infancy, still showed significant small airway disease resulting in air trapping (Lista et al., 2014).

2.1.1.4 Summary of lung function testing

In summary, preterm-born children show impairments across lung function tests. Deficits seen on spirometry are clear and consistent, particularly in those with a

history of CLD. Body plethysmography in preterm-born children shows inconsistent results in similar groups of patients. Several of these studies looking at static lung volumes are limited by small numbers, which may explain the variable findings. However, it appears, irrespective of CLD diagnosis, preterm born children, particularly in the VLBW groups, have evidence of increased RV and RV/TLC ratio consistent with air trapping and hyper-expansion, likely to be due to structural abnormalities rather than inflammatory processes. Some of these studies were from the pre-surfactant era when the 'old-BPD' changes were seen in those born preterm, compared to the current form of the disease. Unfortunately, a number of these studies do not reference the use of surfactant during the neonatal management of their patients, presumably due to them being from before surfactant was introduced. Despite this, similar lung volume findings appear to be present in patients from both pre- and post-surfactant eras.

2.1.2 Exercise testing

Probably one of the most important factors for consideration with lung function in preterm born children is not the results of static and dynamic lung volume testing, but the functional aspect of their lung disease. For instance, the ability to perform normal levels of exercise and physical activity, and the causes of any limitations to this ability.

2.1.2.1 Cardiopulmonary exercise testing

Cardiopulmonary exercise testing or CPET has been used to compare outcomes in preterm (with and without CLD) and term groups. There have been inconsistencies in the outcomes when assessing peak $\dot{V}O_2$ in preterm populations. Some studies have found similar peak $\dot{V}O_2$ between preterm and term populations (Bader et al., 1987, Jacob et al., 1997, Kriemler et al., 2005, Joshi et al., 2013) while others have demonstrated lower $\dot{V}O_2$ levels in preterm groups (Santuz et al., 1995, Pianosi and Fisk, 2000, Kilbride et al., 2003, Welsh et al., 2010). Lower body or muscle mass has also been linked to lower $\dot{V}O_2$. Given preterm children are known to have growth issues, this is a potential additional causative factor for the reduced exercise capacity (Pianosi and Fisk, 2000, Welsh et al., 2010).

Minute ventilation ($\dot{V}E$) has also shown inconsistencies across many studies. Higher $\dot{V}E$ has been reported in children with a historical diagnosis of BPD (Bader et al., 1987); however, lower $\dot{V}E$ at peak exercise is the more common outcome (Santuz et al., 1995, Pianosi and Fisk, 2000). Ventilatory reserve (i.e. the proportion of a person's potential respiratory capacity that is remaining at peak exercise, often measured by a comparison between minute ventilation and maximal voluntary ventilation) appears to be impaired in preterm children. While they may be able to perform the same level of exercise, they use up a greater proportion of this respiratory reserve (Jacob et al., 1997, Welsh et al., 2010, Joshi et al., 2013). This is unlikely to have significant impact on a healthy term-born child but may impact on those wishing to perform at a more elite level, as well as impacting those with particularly limited capacity.

Respiratory patterns may be altered in preterm children during exercise. For instance, higher respiratory rates associated with low tidal volumes have been noted in preterm (Pianosi and Fisk, 2000, Welsh et al., 2010). An increase in hyperinflation due to air trapping, resulting in greater volumes of dead space in the lungs, is hypothesised to be responsible.

A research group at the University of Bergen have investigated exercise outcomes in two longitudinal cohorts of extreme preterms. These two cohorts, recruited along with term controls, were treated at different times in the same neonatal unit, with one during the period 1982 – 1985 and the other from 1991 – 1992. Surfactant therapy was only available to the latter cohort with 51% receiving the treatment. Similar rates of BPD were noted between the two cohorts. During the initial follow up in 2001/2, the participants were either approximately 17 or 10 years of age on average. In both age groups, the peak $\dot{V}O_2$ (mL/min) was significantly lower in the EP groups; however, after adjusting for body weight (mL/kg/min) this difference disappeared. The distance completed on the exercise treadmill was significantly shorter for the preterm born in both cohorts. Interestingly, the authors observed that in males, those born at term had significantly higher $\dot{V}O_2$ (mL/kg/min) compared to preterms, a difference not seen in females. There was no association between a diagnosis of BPD and a deficit in exercise capacity (Clemm et al., 2012).

Both the 1980s and 1990s cohorts were followed up again in 2008/9 with the same exercise test, aged 25 and 18 years respectively at time of testing. For the 1980s cohort, there were similar findings to the initial study for the EP group with significantly decreased treadmill distance as well as $\dot{V}O_2$ (mL/min), which again lost statistical significance when corrected for body weight (mL/kg/min). A new statistically significant finding was that of a lower maximum $\dot{V}E$ (mL/kg/min) in the EP group. Between the two study periods, for both EP and term groups, there was a significant decrease in the peak oxygen consumption and distance travelled on the treadmill (Clemm et al., 2014). The follow-up of the 1990s cohort was performed with similar methodology. For this group $\dot{V}O_2$ (mL/min & mL/kg/min) and distance travelled on the treadmill were significantly lower in the EP group compared to term controls. Conversely to the earlier-born cohort, the oxygen consumption (mL/min) and running distance improved over the intervening years between original study and follow-up (Clemm et al., 2015).

A systematic review published in 2015 neatly summarised the deficits seen on exercise testing for preterm children with and without CLD. Considering all preterm-born children irrespective of CLD status, 631 children across 20 studies (and a mixture of methodologies) had a $\dot{V}O_2$ deficit of 2.2mls/kg/min compared to 639 term controls. Excluding children with CLD, there was a $\dot{V}O_2$ deficit of 2.26mls/kg/min in the remaining 246 children compared to 407 term controls. Where there was a historical CLD diagnosis, either CLD_{28} or CLD_{36}, this deficit rose to 3.04 and 3.05mls/kg/min respectively (Edwards et al., 2015b).

2.1.2.2 Exercise-induced bronchoconstriction

Related to exercise capacity and physical activity in the preterm born population is the effect symptoms may have on the ability to exercise. Several studies have gained information on the rates of bronchoconstriction in preterm and CLD groups after performing physical activity, normally following a cycle or treadmill test to exhaustion. Definitions of exercise-induced bronchoconstriction (EIB) have varied; with suggested decreases from baseline of FEV_1 of 10-15% being proposed (Crapo, 2000).

An early study into EIB assessed a group of children followed up from their preterm birth at <32 weeks' gestation. Maximal exercise on a treadmill was performed and spirometry measurements were taken pre- and post-exercise. 96 preterm (43 with history of BPD) and 108 matched term children were compared. Post-exercise spirometry was performed but it is not clear at what interval after exertion this was done. All groups had a drop in FEV_1 post exercise compared to baseline (average drops across the groups were 7% for preterms with and without BPD, and 5% for term controls). The proportion of children in the BPD group with an abnormal FEV_1 (<80% predicted) increased from 42 to 54% with exercise, with the preterm and term groups also seeing an increase in rates from 15 – 30% and 8 – 17% respectively. Overall the BPD group had significantly worse pre- and post-exercise FEV_1 compared to the non-BPD groups (Gross et al., 1998).

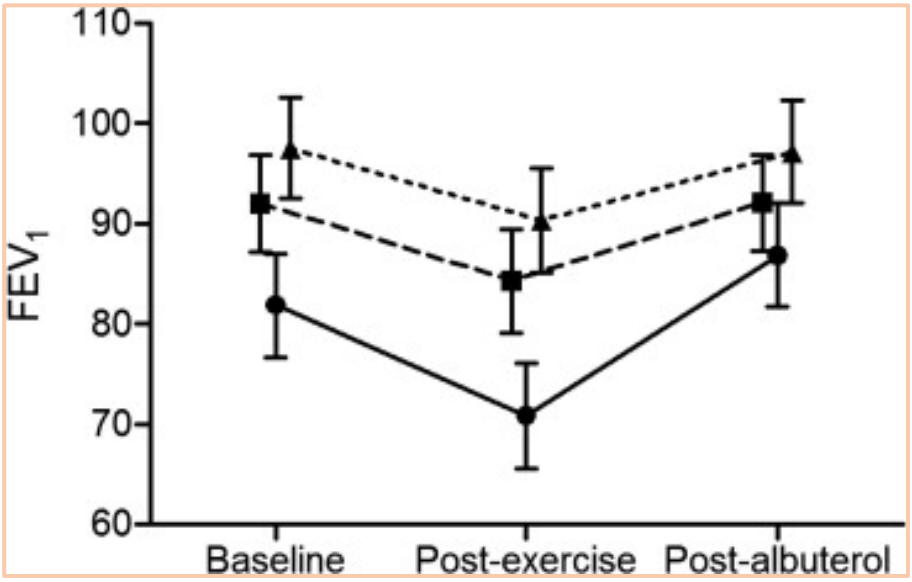
Kriemler performed post-exercise spirometry on their cohort of 5- to 7-year-old children after cycling to exhaustion on a cycle ergometer. In their sample of preterm children with and without CLD, both groups had significantly increased rates of bronchoconstriction, with rates of 44% in the CLD group and 58% in the preterm group compared to only 22% in the control group (≥15% drop in FEV_1 or FEF_{25-75}) up to 10 minutes after completing exercise (Kriemler et al., 2005).

Another study looked at rates of EIB used children who had been enrolled in a trial comparing use of post-natal dexamethasone versus placebo for ventilator dependency during the neonatal period. These two preterm groups were followed up at an average age of 9 years and had spirometry performed post-maximal exercise. This was only done immediately and 5 minutes after exercise ceased. The average drop in FEV_1 was 2% in both groups, with prevalence of EIB (≥15% drop in FEV_1) at 7% in both groups (Nixon et al., 2007).

Hamon also reported increased rates of EIB in preterm children compared to those born at term, although reported that those with CLD were not at any higher risk of EIB. In this instance, 42 children aged 7 years and born between 1999 - 2001 at <32 weeks' gestation underwent submaximal exercise on a treadmill. Post-exercise spirometry was performed, and no term controls had decreases in $\%FEV_1$ of >5% at either 5 or 15 minutes post-exercise compared to 24% of the preterm group who had

decreases ≥7%. The numbers (and their statistical significance) of preterm children who had decreases of at least 12% is not clear from the article and the average drop in FEV_1 for the preterm group was only 4.4% on average (Hamon et al., 2013).

Figure 2.2 %predicted FEV_1 at baseline, post-exercise, and following post-exercise reversibility testing for preterm-born children with chronic lung disease of prematurity (CLD – solid line, circles), without CLD (dashed line, squares) and term controls (dotted line, triangles) (Joshi et al., 2013). Image reproduced with permission of the rights holder.



A clearer description of the impact of exercise on bronchoconstriction was published by Joshi et al from a cohort of 49 preterm children (26 with history of CLD) between the age of 8 -12 years. Post-maximal exercise spirometry was performed at 5, 10, 15, 30 and 40 minutes. They were compared to 26 term controls. The CLD group (who had a lower baseline FEV_1), had an average drop of 11% post-exercise. The preterm and term groups had significantly smaller decreases, with maximum average drops of 7.8% and 7.2% respectively. These peak drops were noted between 17.9 and 20.2 minutes depending on group, later than when spirometry was performed by Nixon, Kriemler and Hamon, perhaps explaining why the results obtained in some of those cases did not show marked EIB (Joshi et al., 2013).

2.1.2.3 Summary of exercise testing

The overall message from studies investigating exercise capacity and exercise-induced bronchoconstriction, is that while individual studies have shown mixed

results, preterm-born children have impaired exercise capacity. A number of these individual studies have had small sample sizes which may explain a lack of clear differences, hence the importance of thorough systematic reviews. Additionally, preterm children are at risk of exercise-induced symptoms.

2.1.3 Summary of introduction

Preterm-born children are at greater risk of long-term respiratory morbidity, as evidenced by objective measures of lung function testing. However, there are still some unknowns within all of this. This cohort of children enrolled in the RHiNO study offered a chance to reframe the thinking behind how we assess preterm children's longer term respiratory health, with a focus on current respiratory health rather than an historical diagnosis. Additionally, a focus on children with low lung function will likely reveal different patterns of results consistent with differing phenotypes.

2.1.4 Aims of study

1. Define population characteristics based on children as stratified by their gestational and FEV_1 status;
2. Assess lung function characteristics for participants within their gestation/lung function as above;
3. Assess exercise capacity, exercise-induced bronchoconstriction, and post-exercise reversibility for participants within their gestation/lung as above;
4. Identify potential differing phenotypes within the preterm low lung function group and repeat steps 1-3 for any potential phenotypes.

2.2 Methods

2.2.1 Recruitment

2.2.1.1 Population

Children aged 7-12 years were recruited as part of the Respiratory Health Outcomes in Neonates (RHiNO) study. All children born preterm in 2003, 2005, 2007 and 2009-2011 were previously contacted for the Respiratory And Neurological Outcomes of Preterm birth Study (RANOPS), a questionnaire-based study looking at prevalence of symptoms in preterm-born children (Edwards et al., 2016). Age-, sex-, and birthplace-matched children born at term were also contacted for RANOPS. These children from RANOPS, along with preterm children born in the intervening years and identified by NHS Wales Informatics Service, were contacted with a further questionnaire study, inviting their parents to complete the questionnaire and indicate their interest in their child taking part in a face-to-face visit for screening tests.

2.2.1.2 Screening visit

Children whose parents agreed to participate were visited by trained research nurses for a screening visit to obtain information regarding perinatal and respiratory history, perform anthropometric measurements, cardiovascular assessment, spirometry (MicroLoop Spirometer, Vyaire, Germany) including reversibility testing with 400 micrograms of salbutamol, exhaled nitric oxide testing (NIOX VERO, Circassia, UK) and urine and saliva collection. Preterm-born children were included if aged 7-12 years, were born before 35 weeks' gestation, living in South Wales, and without congenital abnormalities or significant cardiovascular or neurological disease. Term-born children who participated in RANOPS were invited to participate in the same way as the preterm children. Children were stratified based on their FEV_1 following the screening (Part 1) visit.

2.2.1.3 In-depth lung function testing

Following the screening visit, children were subsequently invited for in-depth lung function testing at the Children and Young Adults' Research Unit at the Noah's Ark Children's Hospital for Wales in Cardiff. All children with $\%FEV_1 \leq 85\%$ (PT_{low}) (using Global Lung Function Initiative reference equations (Quanjer et al., 2012)) were invited for participation, as well as all term children with $\%FEV_1 > 90\%$ (T_c) to create a

normal comparison group. Preterm-born children with %FEV_1 >85% who were within the first 10 visits of a month were eligible to attend as preterm controls (PT_c).

Preterm-born children were designated PT_{low} if their %FEV_1 at Part 2 visit was ≤85% predicted. These children were eligible for the RCT component of the study. If they were invited as PT_{low} but their FEV_1 on the day of testing was >85% they were deemed eligible to continue as preterm controls (PT_c) if they had been within the first 10 Part 1 visits of a given month. This was to ensure the preterm control group was not skewed towards the lower end of normal (with the assumption that if %predicted FEV_1 changed from <85% to >85% that the change would be marginal, and the result would be just over 85 %predicted). Preterm and term controls were defined as per Part 1 (see above). Children were excluded if they were unable to perform adequate spirometry. This is outlined in greater detail in Section 1.6.2.2 in Chapter 1.

The threshold of 85% predicted FEV_1 was used as the eligibility criteria for PT_{low} children being enrolled into the randomised control trial as outlined in Chapter 1. PT_{low} children were also divided by evidence of obstructive lung disease, using FEV_1/FVC ratio of 0.8 as a threshold to classify children into prematurity-associated obstructive (ratio <0.8) or non-obstructive (ratio ≥0.8) lung disease (POLD, PnOLD respectively). Chapter 5 reviews the use of the above thresholds for assigning the relative definitions.

Children were asked to avoid taking certain medications (if applicable) on the day of testing, as below (Graham et al., 2019):

- Short acting beta-2 agonist within past 8 hours
- Inhaled corticosteroids within past 24 hours
- Long-acting beta-2 agonist within past 48 hours
- Leukotriene receptor antagonist within past 48 hours
- Antihistamines within past 48 hours

Children were asked to avoid caffeine products in the 24 hours prior to testing and avoid nitrite-containing foods on the morning of the testing. They also had to be free from respiratory tract infections for 3 weeks and well at time of testing. Written informed assent and consent were obtained from all participants and their

parents/guardians respectively. Ethical approval for the study was granted by the South-West Bristol Research Ethics Committee (15/SW/0289).

2.2.1.4 Terms/Definitions

The following terms and definitions have been used for group designation:

PT_{low}: Preterm with low lung function (born at ≤34 weeks' gestation, FEV_1 ≤85% predicted)

PT_c: Preterm control (born at ≤34 weeks' gestation, FEV_1 >85% predicted)

T_c: Term control (born at ≥37 weeks' gestation, FEV_1 >90% predicted)

The following abbreviations and definitions were used for disease parameters:

IUGR: Birth weight <5th centile;

PROM: Premature rupture of membranes; >24 hours prior to delivery;

CLD: Chronic lung disease of prematurity - combined mild/moderate/severe;

ROP: Retinopathy of prematurity, all stages;

IVH: Intraventricular haemorrhage, all grades;

NEC: Necrotising enterocolitis, requiring medical or surgical treatment;

ROP, IVH or NEC: A diagnosis of any one of these illnesses.

PDA: Patent ductus arteriosus, requiring medical or surgical treatment.

2.2.2 Testing

2.2.2.1 Anthropometry

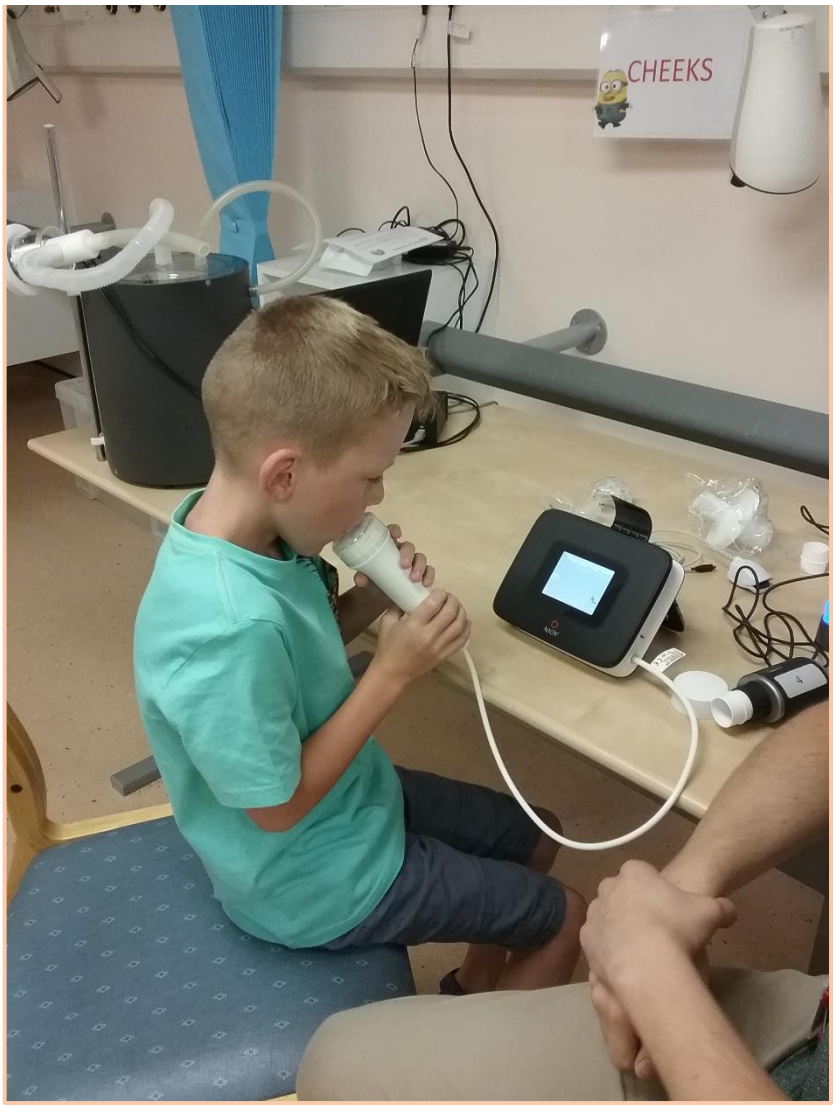
Anthropometric data were collected using a stadiometer for height and the SC-331S Total Body Composition Analyzer (Tanita, USA) for body composition.

2.2.2.2 Fractional exhaled nitric oxide

FE_{NO} was performed using an exhaled nitric oxide analyser (NIOX VERO, Circassia, UK), as shown in Figure 2.3. Children were instructed in its use prior to performing test. The device required a warm-up period after being switched on, following which a

warning would be issued if the sensor or breathing handle were out of date. Providing there were no issues with the above, the child would breathe in deeply through a filter applied to the breathing handle before exhaling at a steady rate and pressure until the test was complete. The child would have to breathe out for 10 seconds in total, although if the child had difficulty exhaling at the required speed for this duration, a shorter test of 6 seconds was performed. An animation was used to help the children achieve the desired flow rate, consisting of blowing a cloud from one side of the screen to the other, without letting it drop off the screen or fly too high. The child performed 2 tests. Both results were documented, and the highest FE_{NO} level was used in analysis.

Figure 2.3 Participant performing FE_{NO} using the NIOX VERO. Consent obtained from parent for image and use in published works.



2.2.2.3 Spirometry

Definitive spirometry was performed using either the MasterScreen Body and PFT systems with SentrySuite measurement software version 2.17 (Vyaire Medical, Germany), as seen in Figure 2.4. ERS/ATS guidelines for obtaining suitable spirometry were used for as a guide for performing the test and test acceptability (Miller et al., 2005). An explanation and a demonstration on how to perform the test were done before the child attempted the spirometry. Spirometry was performed with the child sat upright and wearing a nose clip. They were instructed to take the biggest breath in possible, before blowing out as hard and as fast as they could. Children were vocally encouraged to continue breathing out until they appeared to have reached their residual volume. A minimum of 3 tests were performed, aiming for the intra-test criteria as per Miller et al *(Miller et al., 2005)*. Spirometry was stopped once satisfactory testing was obtained, if the child did not wish to continue or if the child was unable to perform adequate spirometry. Quality control was performed to ensure the correct results from all the measurements were used. Daily volume calibrations and weekly flow calibrations were performed using a three-litre syringe. Results were measured at body temperature, ambient barometric pressure, saturated with water vapour (BTPS) and Global Lung Function Initiative predicted values were used to adjust for height, ethnicity, sex, and age (Quanjer et al., 2012). Spirometry was repeated at 4 separated times following conclusion of the exercise test: at 5-10 minutes; 15-20 minutes; 25-30 minutes; 40-45 minutes. This was performed as outlined above.

Figure 2.4 Participant performing spirometry using the MasterScreen Body. Consent obtained from parent for image and use in published works.

2.2.2.3.1 Reversibility testing

After the final post-exercise spirometry, 400 micrograms of salbutamol (Salamol, TEVA UK Limited) was given via MDI using a Volumatic spacer (GSK, UK). The salbutamol inhaler was shaken before each actuation. Children were instructed to take 10 breaths in and out after each actuation of salbutamol, ensuring the spacer's valve clicked with each breath. Repeat spirometry was performed 15 minutes after administration of the salbutamol, as described as above.

2.2.2.4 Skin prick testing

Skin prick testing was performed using Multi-Test PC lancets (Lincoln Diagnostics, USA). A Dipwell Tray (Lincoln Diagnostics, USA) was pre-prepared with the following

allergens: cat dander; dermatophagoides pterynyssinus; grass mix; dog dander; aspergillus fumigatus; and cladosporium herbarum; as well as a positive histamine control and a negative control (Immunotek, Spain). The procedure was explained to the child and their forearm was cleaned gently with water, after ensuring the skin was free from eczema or any similar skin conditions. The Multi-Test PC lancet was inserted into the Dipwell Tray ensuring all touch-posts were coated with allergen solution. The lancet was slowly removed from the tray, and gently applied to the skin. Following one second of gentle pressure, the lancet was pressed firmly onto the skin with gentle rotation of the lancet device up and down and side to side before removal. Successful application left the imprints of the touch posts on the skin. Any excess allergen fluid on the skin was gently removed with tissue paper ensuring no cross-contamination of sites. A timer was set for 15 minutes. Children were encouraged not to scratch if the arm got itchy. After 15 minutes the arm was inspected for any wheals that developed; the raised aspects of the wheals were drawn around with pen and tape was used to lift the pen mark and stuck to a data sheet. A ruler was then used to measure the widest diameter of any of the wheals. A test was deemed positive if the wheal was greater than 3 mm, along with a positive histamine control test.

2.2.2.5 Body plethysmography

Body plethysmography was performed using the MasterScreen Body system with SentrySuite measurement software version 2.17 (Vyaire Medical, Germany). Testing was performed with reference to the guidelines on static lung volume testing from ERS. In summary, children were seated in the body box and the door closed. A delay of two minutes following door closure allowed for pressure and thermal equalisation. Testing was performed with the child sat upright, wearing a nose clip and holding their cheeks. Following an initial period of tidal breathing to obtain airway resistance measurements, children then breathed against a closed shutter to obtain an intrathoracic gas volume (functional residual capacity) measurement followed by a paired expiratory vital capacity manoeuvre. A minimum of 5 repeatable resistance loops and a minimum of 3 repeatable FRC/VC measurements were obtained. Results were standardised against Global Lung Function Initiative reference values (Hall et

al., 2021). A box calibration (in addition to the previously described volume/flow calibrations) for Tau verification (box seal) and shift volume was performed each day prior to testing, in line with the manufacturer's instructions.

2.2.2.6 Helium dilution testing

Helium dilution testing was performed using the MasterScreen PFT system with SentrySuite measurement software version 2.17 (Vyaire Medical, Germany). Children performed the test sat upright wearing a nose clip. Once connected to the breathing circuit, a period of tidal breathing of 30-60 seconds was performed until a stable FRC was obtained. After starting the helium wash-in, children were instructed to expire to residual volume before continuing tidal breathing. Children were then instructed to return to tidal breathing. Once the helium concentration stabilised children were asked to perform an expiratory vital capacity manoeuvre. A single helium dilution test was performed providing a stable FRC was reached before helium wash-in and appropriate expiratory manoeuvres were performed. Children were watched closely for any leaks that may have given a false FRC. Results were standardised against Global Lung Function Initiative reference values (Hall et al., 2021). Gas analyser and filling sensor calibrations (in addition to the previously described volume/flow calibrations) were performed each day prior to testing, in line with the manufacturer's instructions.

2.2.2.7 Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed on a Paediatric Cycle Ergometer (Lode, Netherlands) linked to a MasterScreen CPX system (Vyaire Medical, Germany), as seen in Figure 2.5. Children wore a fitted facemask and respiratory parameters were measured using a turbine and gas sampling tube. Data were recorded in a breath-by-breath exercise programme on JLab version 5.72 (Vyaire Medical, Germany). Heart rate was recorded using a Polar H10 heart rate sensor (Polar, UK). Oxygen saturations were monitored with a Nellcor oxygen saturation monitor (Medtronic, USA). A ramp protocol was devised to facilitate the exercise testing. This involved 1 minute of baseline measurements at rest, 3 minutes of minimally-loaded cycling (7 Watts), then at an increasing rate of 1 Watt every 6 seconds (10 Watts per minute). The child was vocally encouraged to continue exercise until they could no

longer consistently maintain cadence >60 rpm, with increasing encouragement as the load got higher. Perceived exertion rating was obtained every 3 minutes and at the point the child could no longer continue. Two minutes of minimally loaded pedalling concluded the test. A test was deemed to be 'maximal' if it met ≥2/4 of the following criteria:

- Respiratory exchange ratio (RER) >1.00;
- Heart rate (HR) ≥80% predicted (220 bpm – age);
- ≥9/10 on OMNI scale (pictorial scale for rating of perceived exertion (Barkley and Roemmich, 2008));
- Peak oxygen uptake (V̇O2) plateau based on visual analysis.

Minute ventilation (V̇E), $\dot{V}O_2$ and peak carbon dioxide production ($\dot{V}CO_2$) results were averaged from the last 15 seconds of peak exercise. Maximum load, heart rate and respiratory rate were the highest recorded value at the peak of exercise. Ventilatory reserve was calculated by the following equation: 1-(minute ventilation/maximal voluntary ventilation (MVV))*100, where MVV = FEV_1 x 35 (Joshi et al., 2013). An automated volume calibration and gas analyser calibration were performed on each day of testing, in line with manufacturer's instructions.

Figure 2.5 Participant performing cardiopulmonary exercise testing using the MasterScreen CPX. Consent obtained from parent for image and use in published works.

2.2.3 Ethics

RHiNO was registered with the European Clinical Trials Database (EudraCT: 2015-003712-20). Ethical approval was granted by the South-West Bristol Research Ethics Committee (15/SW/0289). All parents provided written consent and children, where appropriate, provided written assent before participation.

2.2.4 Statistical analysis

Independent t-tests were used for two group comparisons and one-way ANOVA with Bonferroni correction for multiple groups comparisons. Categorical data were assessed using Pearson's χ^2 tests. Within-group (baseline, post-exercise, and post-

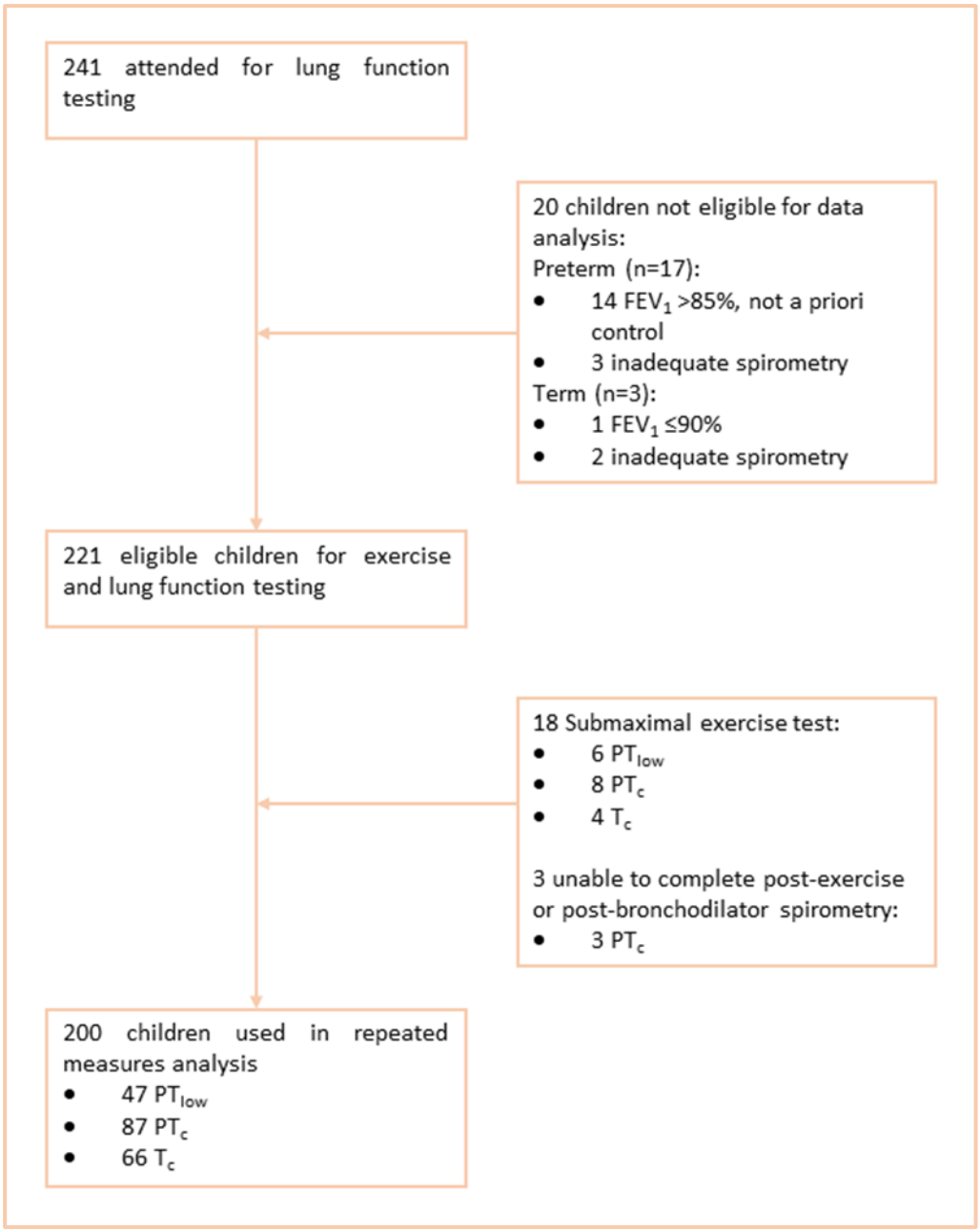
exercise bronchodilator) and between group (lung function groups) comparisons across time points were compared using two-way repeated measures ANOVA. Change scores between groups were compared using one-way ANOVA. All group comparisons were corrected for multiplicity using Bonferroni correction. Statistical analysis was performed using SPSS version 26 (IBM, USA). A p-value of <0.05 was considered significant.

2.3 Results

2.3.1 Participant numbers

241 children attended testing. 20 of these children were excluded from analysis. 5 children were unable to perform adequate spirometry on the day (3 preterm, 2 term); 14 children were invited with low forced expiratory volume in 1 second (FEV_1) following screening visit (potential RCT candidates), however at Part 2 had FEV_1 >85% and were not selected a priori as controls (i.e., they were not within the first 10 screening visits of the month). One term child was excluded due to FEV_1 ≤90%. 18 children did not reach maximal exercise (PT_{low} = 6, PT_c = 8, T_c = 4), and a further 3 PT_c children did not complete spirometry at all 3 time points so were excluded from repeated measures analysis. This meant from a total of 221 eligible children, 47 PT_{low}, 87 PT_c, and 66 T_c were used as part of the repeated measures ANOVA looking at post-exercise and post-exercise bronchodilator spirometry changes. This is summarised in Figure 2.6.

Figure 2.6 Flow diagram representing patient numbers for repeated measures analysis.



2.3.2 Lung function groups

2.3.2.1 Participant characteristics

Demographics (Table 2.1) for the 53 PT_{low}, 98 PT_c and 70 T_c were similar. PT_c children were marginally older than their term counterparts. Otherwise, height, weight, BMI (both actual and z-score) were no different between groups.

PT_{low} children were born earlier (29.7 vs 31.1 vs 40.0 weeks' gestation) and smaller (1392 vs 1729 vs 3528 grams) than PT_c and T_c groups, and both preterm groups had higher rates of IUGR than term counterparts. There were a greater proportion of children with CLD, combined ROP/IVH/NEC, and PDA in the PT_{low} group (38%, 30%, 17% respectively) than in the PT_c group (18%, 13%, 4% respectively). Otherwise, perinatal characteristics were similar in both preterm groups. Perinatal data are summarised in Table 2.2.

Table 2.3 shows children with low lung function were more likely to have ever had bronchiolitis and experienced wheeze compared to PT_c and Tc (32 vs 11 vs 4% for bronchiolitis, and 72 vs 46 vs 27% for wheeze). PT_c's wheeze ever rates were also significantly greater than T_c. Otherwise, the preterm groups were similar in their respiratory history. PT_{low} also had a higher rate of asthma diagnosis than term children (30 vs 7%) and both preterm groups had higher rate of current maternal smoking as a result of there being no maternal smokers in the term group. Rates of recent wheeze were similar across the groups. FE_{NO} showed no statistically significant differences across groups, however PT_c had higher skin prick test positive rate (28%) compared to T_c (11%).

Table 2.1 Anthropometric and perinatal characteristics of participants for preterm children with low lung function (PT_{low}) compared to preterm (PT_c) and term (T_c) controls

Current demographics	**PT_{low}, n=53**	**PT_c, n=98**	**T_c, n=70**
Age, years	10.8 (10.5 to 11.2)	**11.1 (10.9 to 11.4)** ‡‡	10.5 (10.2 to 10.7)
Male, n (%)	24 (45%)	49 (50%)	37 (53%)
Height, cm	143.4 (140.3 to 146.5)	146.7 (144.8 to 148.7)	143.9 (141.6 to 146.1)
Height, Z-score	0.05 (-0.27 to 0.38)	0.33 (0.15 to 0.52)	0.48 (0.25 to 0.72)
Weight, kg	37.2 (34.1 to 40.2)	40.3 (38.2 to 42.4)	37.9 (35.4 to 40.4)
Weight, Z-score	0.03 (-0.36 to 0.41)	0.41 (0.21 to 0.62)	0.46 (0.22 to 0.71)
BMI, kg/m^2	17.7 (16.8 to 18.6)	18.5 (17.8 to 19.2)	18.0 (17.3 to 18.8)
BMI, Z-score	-0.02 (-0.40 to 0.37)	0.26 (0.01 to 0.52)	0.30 (0.05 to 0.56)
Perinatal demographics	**PT_{low}, n=53**	**PT_c, n=98**	**T_c, n=70**
Gestation, decimal weeks	**29.7 (28.9 to 30.6)** ** †††	**31.1 (30.5 to 31.6)** ‡‡‡	40.0 (39.7 to 40.3)
Birth weight, grams	**1,392 (1,235 to 1,549)** ** †††	**1,729 (1,616 to 1,843)** ‡‡‡	3,528 (3,404 to 3,651)
Birth weight, Z-score	-0.22 (-0.60 to 0.16)	0.27 (0.00 to 0.54)	0.08 (-0.15 to 0.31)
IUGR, n (%)	**12 (23%)** †	**15 (15%)** ‡‡‡	4 (6%)
PROM, n (%)	**17 (32%)** †††	**39 (40%)** ‡‡‡	2 (3%)
Caesarean section, n (%)	**29 (55%)** †††	**56 (57%)** ‡‡‡	16 (23%)
Antenatal steroids, n (%)	**48 (91%)** †††	**80 (82%)** ‡‡‡	0 (0%)
Invasive ventilation, n (%)	**25 (47%)** †††	**40 (41%)** ‡‡‡	0 (0%)
CLD, n (%)	**20 (38%)** * †††	**18 (18%)** ‡‡‡	0 (0%)
Home oxygen, n (%)	**5 (9%)** †	2 (2%)	0 (0%)
ROP, IVH or NEC, n (%)	**16 (30%)** * †††	**13 (13%)** ‡‡	0 (0%)
PDA, n (%)	**9 (17%)** * †††	4 (4%)	0 (0%)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified. **Abbreviations: BMI** – Body Mass Index; **IUGR** – Intrauterine Growth Restriction; **PROM** – Premature rupture of membranes; **CLD** – Chronic Lung Disease of prematurity **ROP** – Retinopathy of Prematurity; **IVH** – Intraventricular Haemorrhage; **NEC** – Necrotising Enterocolitis.

PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.

PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Table 2.2 Respiratory and atopy characteristics and fractional exhaled nitric oxide for preterm children with low lung function (PT_{low}) compared to preterm (PT_c) and term (T_c) controls

Respiratory history	**PT_{low}, n=53**	**PT_c, n=98**	**T_c, n=70**
Bronchiolitis, n (%)	**17 (32%)** ** †††	11 (11%)	3 (4%)
Wheeze ever, n (%)	**38 (72%)** ** †††	**45 (46%)** ‡	19 (27%)
Recent wheeze, n (%)	16 (30%)	20 (20%)	9 (13%)
Current salbutamol use, n (%)	**13 (25%)** ††	16 (16%)	4 (6%)
Current preventer use, n (%)	7 (13%)	11 (11%)	3 (4%)
Doctor-diagnosed asthma, n (%)	**16 (30%)** ††	18 (18%)	5 (7%)
Family history of asthma, n (%)	33 (62%)	48 (50%)	35 (50%)
Current maternal smoking, n (%)	**6 (11%)** †	**9 (9%)** ‡	0 (0%)
Skin prick testing	**PT_{low}, n=53**	**PT_c, n=98**	**T_c, n=70**
≥1 positive test(s), n (%)	14 (26%)	**27 (28%)** ‡	8 (11%)
FE_{NO}	**PT_{low}, n=50**	**PT_c, n=97**	**T_c, n=68**
FE_{NO} >35ppb, n (%)	14 (28%)	17 (18%)	8 (12%)
Highest FE_{NO}, ppb	27.3 (19.5 to 35.1)	21.3 (17.4 to 25.3)	19.9 (15.3 to 24.4)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified. **Abbreviations: FE_{NO}** – Fractional Exhaled Nitric Oxide.
PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.
PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.
PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

2.3.2.2 Lung volume & exercise testing

47 PT_{low}, 90 PT_c, and 66 T_c children performed acceptable exercise testing with data summarised in Table 2.3. All three groups reached comparable peak heart rates suggesting a similar degree of exertion was performed. Despite this, both preterm groups achieved lower peak relative workloads (2.29 vs 2.45 vs 2.72 W/kg for PT_{low}, PT_c and T_c respectively) and lower peak relative O_2 uptake (31.9 vs 33.8 vs 38.1 mls/kg/min for PT_{low}, PT_c and T_c respectively) compared to term children. PT_{low} children had lower relative CO_2 production compared to T_c children (37.2 vs 43.7 mls/kg/min) and lower relative peak minute ventilation compared to both control groups (1.33 vs 1.50 vs 1.61 L/kg/min for PT_{low}, PT_c and T_c respectively). Of note, PT_{low} children also used up a greater proportion of their ventilatory reserve while achieving these lower values.

Similar group differences were seen when using helium dilution and plethysmography testing, as summarised in Table 2.4. PT_{low} had lower %TLC, higher absolute and %predicted RV/TLC and proportion with RV/TLC >0.30 compared to both control groups. The main difference seen between testing modalities was a significantly greater %RV on plethysmography for the PT_{low} group compared to both controls (105.9 vs 88.7 vs 89.1 %predicted on plethysmography compared to 99.9 vs 92.5 vs 92.6 %predicted on helium testing, for PT_{low}, PT_c and T_c respectively). Both preterm groups also had lower %predicted TLC than term controls on both plethysmography and helium dilution testing. The proportion of children with TLC <LLN was greatest in PT_{low}, but particularly on helium dilution testing showing almost a quarter had low TLC.

Table 2.3 Cardiopulmonary exercise testing results for preterm children with low lung function (PT_{low}) compared to preterm (PT_c) and term (T_c) controls

Exercise	PT_{low}, n=47	PT_c, n=90	T_c, n=66
Peak heart rate, bpm	187.9 (184.4 to 191.4)	190.2 (187.8 to 192.6)	189.7 (187.2 to 192.3)
Peak respiratory rate, bpm	62.4 (59.5 to 65.3)	62.5 (60.2 to 64.9)	66.3 (64 to 68.6)
Workload, W/kg	**2.29 (2.13 to 2.45)** †††	**2.45 (2.32 to 2.58)** ‡	2.72 (2.58 to 2.86)
Peak O2 uptake, ml/kg/min	**31.9 (30.2 to 33.7)** †††	**33.8 (32.2 to 35.5)** ‡‡	38.1 (36.1 to 40.1)
Peak CO2 production, ml/kg/min	**37.2 (34.8 to 39.5)** †††	40.4 (38.5 to 42.4)	43.7 (41.6 to 45.8)
Peak V̇E, L/min	**49.8 (45.6 to 54.1)** ** ††	59.4 (56.1 to 62.6)	59 (55.1 to 62.8)
Relative peak V̇E, L/kg/min	**1.33 (1.24 to 1.42)** * †††	1.5 (1.41 to 1.58)	1.61 (1.53 to 1.7)
Peak V̇E vs height, L/m/min	**0.34 (0.32 to 0.37)** *** †††	0.4 (0.38 to 0.42)	0.41 (0.39 to 0.43)
Highest RER	1.2 (1.17 to 1.23)	**1.24 (1.22 to 1.26)** ‡	1.2 (1.18 to 1.22)
Breathing reserve max, %	**14.5 (9.4 to 19.6)** *** ††	25.8 (22.4 to 29.2)	24.8 (21.8 to 27.8)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: V̇E** – minute ventilation; **RER** – Respiratory Exchange Ratio.
PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.
PTl_{ow} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.
PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Table 2.4 Body plethysmography and helium dilution results for preterm children with low lung function (PT_{low}) compared to preterm (PT_c) and term (T_c) controls

Static lung volumes (Plethysmography)	**PTl_{ow}, n=49**	**PT_c, n=92**	**T_c, n=68**
FRC, %predicted	98.4 (91.8 to 105)	95.4 (92.3 to 98.5)	97.5 (93.6 to 101.4)
TLC, %predicted	**92.1 (88.4 to 95.7)** * †††	**97.2 (95.2 to 99.2)** ‡	101.6 (99.4 to 103.8)
RV, %predicted	**105.9 (96 to 115.9)** ** ††	88.7 (83.6 to 93.7)	89.1 (83.1 to 95.2)
RV/TLC, ratio	**0.27 (0.25 to 0.28)** *** †††	0.21 (0.20 to 0.22)	0.21 (0.20 to 0.22)
RV/TLC, %predicted	**124.7 (117.1 to 132.3)** *** †††	99.4 (94.2 to 104.6)	96.4 (90.8 to 102.0)
Proportion RV/TLC > 0.30, n (%)	**11 (22%)** * †	6 (7%)	4 (6%)
Proportion TLC < LLN, n (%)	**6 (12%)** *	4 (4%)	0 (0%)
Static lung volumes (Helium dilution)	**PT_{low}, n=47**	**PT_c, n=92**	**T_c, n=65**
FRC, %predicted	94.7 (87.6 to 101.7)	93.2 (90.2 to 96.2)	99.6 (95.3 to 103.8)
TLC, %predicted	**90.7 (86.6 to 94.8)** ** †††	**96.9 (94.8 to 99)** ‡‡	102.2 (99.8 to 104.6)
RV, %predicted	99.9 (88.5 to 111.3)	92.5 (87.2 to 97.9)	92.6 (86.1 to 99.2)
RV/TLC, ratio	**0.25 (0.23 to 0.28)** ** †††	0.22 (0.21 to 0.23)	0.21 (0.2 to 0.23)
RV/TLC, %predicted	**118.8 (109.2 to 128.3)** ** †††	104 (98.7 to 109.4)	99.4 (93.7 to 105.1)
Proportion RV/TLC > 0.30, n (%)	**14 (30%)** * ††	13 (14%)	3 (5%)
Proportion TLC < LLN, n (%)	**11 (23%)** ***	2 (2%)	0 (0%)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified. **Abbreviations: FRC** – Functional Residual Capacity; **TLC** – Total Lung Capacity; **RV** – Residual Volume.
PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.
PTl_{ow} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.
PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

2.3.2.3 Spirometry

As per how the groups were stratified (according to lung function), $\%FEV_1$ was lowest in PT_{low} group (76 vs 99 vs 105 % for PT_{low}, PT_c and T_c respectively). Additionally, they also had lower %FVC (90 vs 103 vs 108 % for PT_{low}, PT_c and T_c respectively) and FEV_1/FVC (74 vs 84 vs 85 % for PT_{low}, PT_c and T_c respectively), as described in Table 2.5 and illustrated in Figure 2.7.

All groups had a significant decrease in $\%FEV_1$ and %FVC following exercise, and a significant increase in $\%FEV_1$, %FVC and FEV_1/FVC following post-exercise bronchodilator, as summarised in Table 2.5. All 3 groups showed similar drop in absolute % FEV_1 following exercise. PT_{low} had a greater decrease in absolute % FVC (5.6%) compared to PT_c (3.7%). Following subsequent bronchodilator therapy, the greatest response was seen in the PT_{low} group compared to PT_c and T_c for FEV_1 (11.9 vs 5.7 vs 6.3 absolute %predicted respectively). 57% of PT_{low} children had a post-exercise bronchodilator response of >10% predicted FEV_1 compared to 17 % in both control groups.

Table 2.5 Spirometry parameters at baseline and response to exercise and post-exercise bronchodilator (BD) therapy, for study group (PT_{low}) compared to preterm (PT_c) and term (T_c) controls

FEV_1	**PT_{low}, n=47**	**PT_c, n=87**	**T_c, n=66**
Baseline, %predicted	**75.8 (73.3 to 78.3)** *** †††	**99.3 (97.4 to 101.1)** ‡‡	104.6 (102.5 to 106.7)
Post-exercise, %predicted	∂∂∂ **72.1 (69.4 to 74.8)** *** †††	∂∂∂ **95.8 (93.9 to 97.8)** ‡‡	∂∂∂ 100.8 (98.6 to 103.1)
Post-exercise BD, %predicted	∂∂∂ **84.0 (81.5 to 86.4)** *** †††	∂∂∂ **101.5 (99.7 to 103.3)** ‡‡‡	∂∂∂ 107.1 (105.1 to 109.2)
Baseline to post-exercise change, %predicted	-3.7 (-5.0 to – 2.4)	-3.4 (-4.3 to -2.6)	-3.7 (-5.0 to -2.5)
Post-exercise to post-exercise BD change, %predicted	**11.9 (9.0 to 14.7)** *** †††	5.7 (4.8 to 6.5)	6.3 (5.0 to 7.6)
Post-exercise BD response >10% FEV_1, n (%)	**27 (57%)** *** †††	15 (17%)	11 (17%)
FVC	**PT_{low}, n=47**	**PT_c, n=87**	**T_c, n=66**
Baseline, %predicted	**90.4 (87.7 to 93.2)** *** †††	**102.5 (100.5 to 104.6)** ‡‡	108.0 (105.6 to 110.3)
Post-exercise, %predicted	∂∂∂ **84.8 (82.1 to 87.6)** *** †††	∂∂∂ **98.8 (96.8 to 100.8)** ‡	∂∂∂ 103.2 (100.9 to 105.5)
Post-exercise BD, %predicted	∂∂∂ **89.7 (86.7 to 92.7)** *** †††	∂∂∂ **101.0 (98.8 to 103.2)** ‡	∂∂∂ 105.5 (102.9 to 108.0)
Baseline to post-exercise change, %predicted	**-5.6 (-7.1 to -4.1)** *	-3.7 (-4.6 to -2.8)	-4.8 (-5.7 to -3.8)
Post-exercise to post-exercise BD change, %predicted	**4.9 (2.9 to 6.8)** * †	2.2 (1.2 to 3.3)	2.3 (1.4 to 3.2)
FEV_1/FVC	**PT_{low}, n=47**	**PT_c, n=87**	**T_c, n=66**
Baseline, ratio	**0.74 (0.72 to 0.76)** *** †††	0.84 (0.83 to 0.86)	0.84 (0.83 to 0.86)
Post-exercise, ratio	**0.75 (0.73 to 0.77)** *** †††	0.85 (0.83 to 0.86)	0.85 (0.83 to 0.87)
Post-exercise BD, ratio	∂∂∂ **0.83 (0.81 to 0.84)** *** †††	∂∂∂ 0.88 (0.87 to 0.89)	∂∂∂ 0.88 (0.87 to 0.90)
Baseline to post-exercise change	0.010 (-0.002 to 0.022)	0.002 (-0.004 to 0.009)	0.008 (-0.001 to 0.017)
Post-exercise to post-exercise BD change	**0.078 (0.060 to 0.095)** *** †††	0.031 (0.023 to 0.038)	0.031 (0.023 to 0.040)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction). **Abbreviations: FEV_1** – Forced Expiratory Volume in 1 second; **FVC** – Forced Vital Capacity; **BD** – bronchodilator.

PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.

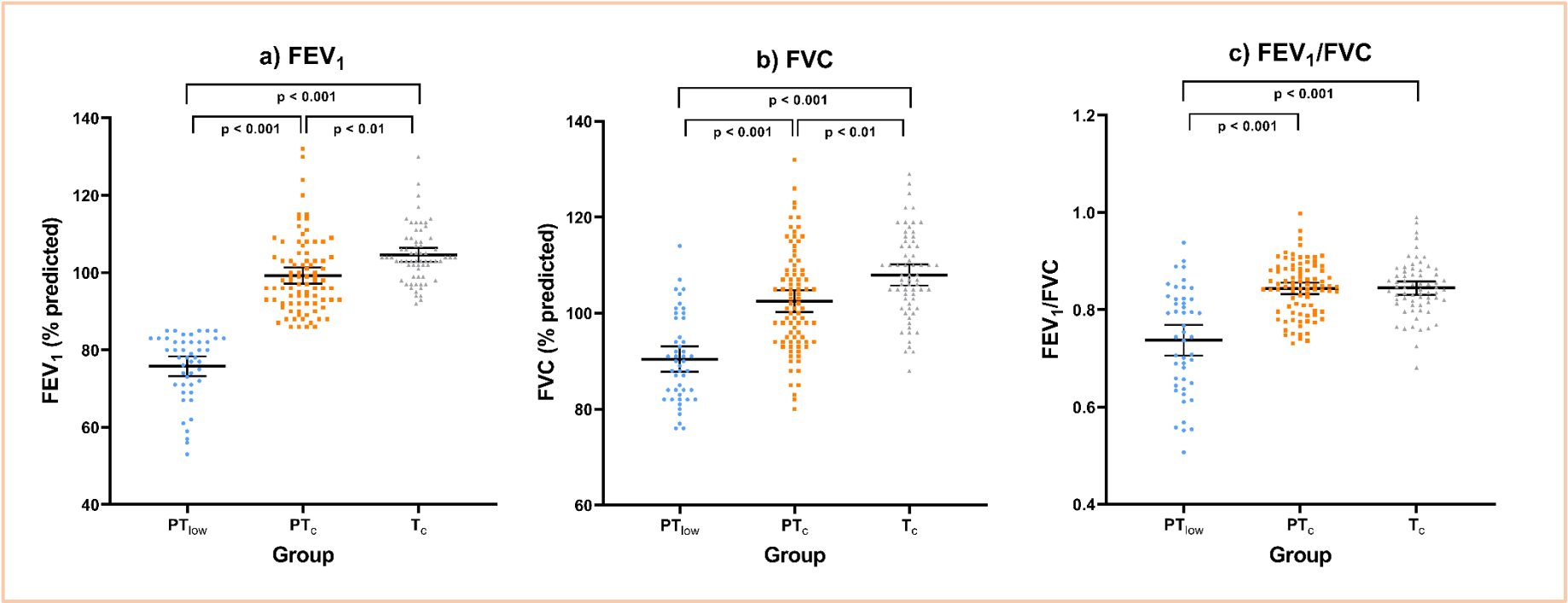
PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise bronchodilator: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Figure 2.7 % predicted FEV_1, % predicted FVC and FEV_1/FVC at baseline for study group (PT_{low}) compared to preterm (PT_c) and term (T_c) controls.



2.3.3 Obstructive & non-obstructive preterm lung disease groups

The spirometry results from the PT_{low} group clearly showed lower FEV_1/FVC compared to controls. Low FEV_1/FVC is associated with evidence of airway obstruction (Pellegrino et al., 2005), so this result was suggestive that within the PT_{low} children were predominantly those with potential airway obstruction. As a result, I classified children with low lung function into two groups base on their FEV_1/FVC ratio, with those children with a ratio of <0.80 defined as having preterm-associated obstructive lung disease (POLD). Chapter 5 outlines rationale and review of using this cut off to determine lung obstruction. Children with $FEV_1/FVC \geq 0.80$ I classified as having preterm-associated non-obstructive lung disease (PnOLD). Potentially these children could have restrictive patterns of lung disease, or mixed patterns, however I felt that this group were harder to define than the obstructive group as a single phenotype.

Patient characteristics were compared directly between POLD and PnOLD, and testing outcomes were compared between POLD, PnOLD and PT_c.

2.3.3.1 Participant characteristics

When dividing the PT_{low} group into those with obstructive lung disease (POLD) and non-obstructive lung disease (PnOLD), few differences were seen for their anthropometrics or their perinatal history. There was a trend towards fewer males in the PnOLD group (only 25% of the 16 were male, compared to just over half in the POLD group). There were higher rates of invasive ventilation in POLD (57% vs 25%), otherwise similar gestation, birth weight and IUGR rates were seen. These characteristics are shown in Table 2.6.

Table 2.7 details respiratory and atopy history of the POLD and PnOLD children. Rates of wheeze ever were higher in POLD (81% vs 50%, $p < 0.05$), however there was no statistical difference seen between the groups for asthma diagnosis, recent wheeze, or salbutamol, although there was a trend towards higher rates in POLD. POLD did have higher FE_{NO} levels than PnOLD (34 vs 13ppb) and a greater proportion of children with FE_{NO} had >35 ppb (38 vs 6%).

Table 2.6 Anthropometric and perinatal characteristics of participants for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups, with preterm controls (PT_c) for comparison.

Current demographics	**POLD, n=37**	**PnOLD, n=16**	**PT_c, n=98**
Age, years	10.7 (10.3 to 11.1)	11.1 (10.5 to 11.7)	11.1 (10.9 to 11.4)
Male, n (%)	20 (54%)	4 (25%)	49 (50%)
Height, cm	142.2 (138.7 to 145.7)	146.1 (139.4 to 152.8)	146.7 (144.8 to 148.7)
Height, Z-score	0 (-0.39 to 0.38)	0.19 (-0.51 to 0.89)	0.33 (0.15 to 0.52)
Weight, kg	36.6 (33.2 to 40.0)	38.5 (31.7 to 45.4)	40.3 (38.2 to 42.4)
Weight, Z-score	0.07 (-0.38 to 0.51)	-0.07 (-0.90 to 0.77)	0.41 (0.21 to 0.62)
BMI, kg/m2	17.8 (16.7 to 18.8)	17.6 (15.6 to 19.6)	18.5 (17.8 to 19.2)
BMI, Z-score	0.08 (-0.37 to 0.54)	-0.25 (-1.04 to 0.54)	0.26 (0.01 to 0.52)
Perinatal demographics	**POLD, n=37**	**PnOLD, n=16**	**PT_c, n=98**
Gestation, decimal weeks	29.8 (28.8 to 30.8)	29.6 (28.0 to 31.3)	31.1 (30.5 to 31.6)
Birth weight, grams	1422 (1236 to 1607)	1324 (1000 to 1648)	1729 (1616 to 1843)
Birth weight, Z-score	-0.14 (-0.57 to 0.28)	-0.39 (-1.24 to 0.47)	0.27 (0.00 to 0.54)
IUGR, n (%)	8 (22%)	4 (25%)	15 (15%)
PROM, n (%)	13 (35%)	4 (25%)	39 (40%)
Caesarean section, n (%)	20 (54%)	9 (56%)	56 (57%)
Antenatal steroids, n (%)	33 (89%)	15 (94%)	80 (82%)
Invasive ventilation, n (%)	**21 (57%) §**	4 (25%)	40 (41%)
CLD, n (%)	15 (41%)	5 (31%)	18 (18%)
Home oxygen, n (%)	4 (11%)	1 (6%)	2 (2%)
ROP, IVH or NEC, n (%)	10 (27%)	6 (38%)	13 (13%)
PDA, n (%)	6 (16%)	3 (19%)	4 (4%)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified.
Abbreviations: BMI – Body Mass Index; **IUGR** – Intrauterine Growth Restriction; **PROM** – Premature rupture of membranes; **CLD** – Chronic Lung Disease of prematurity **ROP** – Retinopathy of Prematurity; **IVH** – Intraventricular Haemorrhage; **NEC** – Necrotising Enterocolitis.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001

Table 2.7 Respiratory and atopy characteristics and fractional exhaled nitric oxide for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups, with preterm controls (PT_c) for comparison.

Respiratory history	**POLD, n=37**	**PnOLD, n=16**	**PT_c, n=98**
Doctor-diagnosed asthma, n (%)	11 (30%)	5 (31%)	18 (18%)
Wheeze ever, n (%)	**30 (81%) §**	8 (50%)	45 (46%)
Recent wheeze, n (%)	14 (38%)	2 (13%)	20 (20%)
Current salbutamol use, n (%)	11 (30%)	2 (13%)	16 (16%)
Current maternal smoking, n (%)	5 (14%)	1 (6%)	9 (9%)
Skin prick testing	**POLD, n=37**	**PnOLD, n=16**	**PT_c, n=98**
≥1 positive test(s), n (%)	11 (30%)	3 (19%)	27 (28%)
FE_{NO}	**POLD, n=34**	**PnOLD, n=16**	**PT_c, n=97**
FE_{NO} >35ppb, n (%)	**13 (38%) §**	1 (6%)	17 (18%)
Highest FE_{NO}, ppb	**34.2 (23.6 to 44.7) §§**	12.8 (7.0 to 18.5)	21.3 (17.4 to 25.3)

Results of independent t-test expressed as mean (95% confidence intervals) or number and % proportion (Pearson's χ^2 test) unless otherwise specified. **Abbreviations: FE_{NO}** – Fractional Exhaled Nitric Oxide.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001

2.3.3.2 *Lung volume & exercise testing*

Few differences were seen between POLD and PnOLD during exercise testing as shown in Table 2.8, although lower peak minute ventilation ($\dot{V}E$) was found in the POLD compared to PT_c. This difference was lost when corrected for weight, however remained when corrected for height. POLD achieved lower respiratory exchange ratio compared to PnOLD and PT_c (1.17 vs 1.26 vs 1.24 for PT_{low}, PT_c and T_c respectively), and had the highest use of their ventilatory reserve.

Similar scope of differences between the groups were seen when using plethysmography and helium dilution. PnOLD had lower %FRC and %TLC than the other preterm groups, consistent with potential restrictive pattern of lung disease. 40% had TLC <LLN on plethysmography and 56% on helium dilution testing. This suggests that the low TLC on a small number (n=3, difference between plethysmography and helium) may have mixed disease. POLD children have greater RV/TLC ratio, again suggestive of hyperinflation, as would be expected of an obstructive lung disease phenotype. These data are summarised in Table 2.9.

Table 2.8 Cardiopulmonary exercise testing results for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c).

Exercise	POLD, n=31	PnOLD, n=16	PT_c, n=90
Peak heart rate, bpm	186.6 (181.9 to 191.2)	190.4 (185.1 to 195.8)	190.2 (187.8 to 192.6)
Peak respiratory rate, bpm	62.9 (59.5 to 66.4)	61.4 (55.8 to 67.1)	62.5 (60.2 to 64.9)
Workload, W/kg	2.28 (2.07 to 2.49)	2.31 (2.04 to 2.58)	2.45 (2.32 to 2.58)
Peak O2 uptake, ml/kg/min	32.6 (30.2 to 34.9)	30.6 (28.0 to 33.3)	33.8 (32.2 to 35.5)
Peak CO2 production, ml/kg/min	37.2 (34.1 to 40.4)	37.1 (33.5 to 40.7)	40.4 (38.5 to 42.4)
Peak V̇E, L/min	**49.3 (44.0 to 54.5)** ¥¥	51.0 (42.8 to 59.2)	59.4 (56.1 to 62.6)
Relative peak V̇E, L/kg/min	1.34 (1.22 to 1.46)	1.31 (1.18 to 1.45)	1.50 (1.41 to 1.58)
Peak V̇E vs height, L/m/min	**0.34 (0.31 to 0.37)** ¥¥	**0.34 (0.30 to 0.38)** ϕ	0.40 (0.38 to 0.42)
Highest RER	**1.17 (1.14 to 1.21)** §§ ¥¥	1.26 (1.22 to 1.30)	1.24 (1.22 to 1.26)
Breathing reserve max, %	**10.69 (4.03 to 17.34)** ¥¥¥	22.41 (15.91 to 28.90)	25.80 (22.40 to 29.20)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: V̇E** – minute ventilation; **RER** – Respiratory Exchange Ratio.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.
PnOLD vs PT_c: ϕ p<0.05; ϕϕ p<0.01; ϕϕϕ p<0.001.

Table 2.9 Body plethysmography and helium dilution testing results for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c).

Static lung volumes (Plethysmography)	**POLD, n=34**	**PnOLD, n=15**	**PT_c, n=92**
FRC, %predicted	**106.1 (98.2 to 113.9)** §§§ ¥¥	**81.1 (74.1 to 88.0)** ϕϕ	95.4 (92.3 to 98.5)
TLC, %predicted	97.2 (93.3 to 101.1)	**80.5 (76.8 to 84.2)** §§§ ϕϕϕ	97.2 (95.2 to 99.2)
RV, %predicted	**114.7 (102.2 to 127.1)** §§ ¥¥¥	86.2 (74.1 to 98.4)	88.7 (83.6 to 93.7)
RV/TLC, ratio	**0.28 (0.26 to 0.30)** ¥¥¥	0.24 (0.22 to 0.27)	0.21 (0.20 to 0.22)
RV/TLC, %predicted	**129.2 (119.6 to 138.8)** ¥¥¥	114.4 (102.6 to 126.3)	99.4 (94.2 to 104.6)
Proportion RV/TLC > 0.30, n (%)	**9 (27%)** ¥¥	2 (13%)	6 (7%)
Proportion TLC < LLN, n (%)	0 (0)	**6 (40%)** §§ ϕϕ	4 (4%)
Static lung volumes (Helium dilution)	**POLD, n=31**	**PnOLD, n=16**	**PT_c, n=92**
FRC, %predicted	**101.9 (92.8 to 111.1)** §§§	**80.6 (72.8 to 88.3)** ϕ	93.2 (90.2 to 96.2)
TLC, %predicted	96.5 (91.8 to 101.3)	**79.3 (75.6 to 83.1)** §§§ ϕϕϕ	96.9 (94.8 to 99.0)
RV, %predicted	**110.1 (95.4 to 124.7)** §§ ¥	80.1 (65 to 95.2)	92.5 (87.2 to 97.9)
RV/TLC, ratio	**0.27 (0.24 to 0.29)** ¥¥	0.23 (0.20 to 0.27)	0.22 (0.21 to 0.23)
RV/TLC, %predicted	**124.1 (112.4 to 135.8)** ¥¥	108.4 (91.2 to 125.5)	104.0 (98.7 to 109.4)
Proportion RV/TLC > 0.30, n (%)	**11 (36%)** ¥	3 (19%)	13 (14%)
Proportion TLC < LLN, n (%)	2 (7%)	**9 (56%)** §§ ϕϕϕ	2 (2%)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test) unless otherwise specified. **Abbreviations: FRC** - Functional Residual Capacity; **TLC** – Total Lung Capacity; **RV** – Residual Volume.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.
PnOLD vs PT_c: ϕ p<0.05; ϕϕ p<0.01; ϕϕϕ p<0.001.

2.3.3.3 Spirometry

Baseline %FEV_1 in POLD vs PnOLD is 74 vs 80% (p=0.073), and PnOLD had a significantly lower %FVC than POLD (83 vs 94 %, p<0.001). This resulted in a lower FEV_1/FVC in POLD than in the PnOLD (68 vs 85 %), as displayed in Table 2.10.

Following exercise, POLD children did not display a greater degree of impairment in repeat spirometry measurements. However, %FEV_1, %FVC and FEV_1/FVC all improved to a significantly greater degree in POLD children compared to PnOLD and PT_c following administration of bronchodilator (%FEV_1: 15.8 vs 4.2 vs 5.7 %; %FVC 6.9 vs 0.9 vs 2.2 %; FEV_1/FVC 9.8 vs 3.8 vs 3.1 for POLD, PnOLD and PT_c respectively). Additionally, 84% of POLD group had a >10 absolute %FEV_1 response to post-exercise bronchodilator therapy, compared to only 6% of PnOLD children. These data are illustrated in Figures 2.8 and 2.9.

Table 2.10 Spirometry parameters at baseline, and response to exercise and post-exercise bronchodilator (BD) therapy for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c).

FEV_1	**POLD, n=31**	**PnOLD, n=16**	**PT_c, n=87**
Baseline, %predicted	**73.6 (70.3 to 76.9)** ¥¥¥	**80.1 (75.5 to 84.6)** ϕϕϕ	99.3 (97.3 to 101.2)
Post-exercise, %predicted	∂∂∂ **69.8 (66.3 to 73.3)** ¥¥¥	∂∂ **76.6 (71.6 to 81.5)** ϕϕϕ	∂∂∂ 95.8 (93.7 to 97.9)
Post-exercise BD, %predicted	ƨƨƨ **85.6 (82.5 to 88.8)** ¥¥¥	ƨ **80.8 (76.3 to 85.2)** ϕϕϕ	ƨƨƨ 101.5 (99.6 to 103.4)
Baseline to post-exercise change, %predicted	-3.8 (-5.6 to -1.9)	-3.5 (-5.4 to -1.6)	-3.4 (-4.3 to -2.6)
Post-exercise to post-exercise BD change, %predicted	**15.8 (12.3 to 19.3)** §§§ ¥¥¥	4.2 (2.5 to 5.9)	5.7 (4.8 to 6.5)
Post-exercise BD response >10% FEV_1, n (%)	**26 (84%)** §§§ ¥¥¥	1 (6%)	15 (17%)
FVC	**POLD, n=31**	**PnOLD, n=16**	**PT_c, n=87**
Baseline, %predicted	**94.4 (91.0 to 97.8)** §§§ ¥¥¥	**82.8 (78.0 to 87.5)** ϕϕϕ	102.5 (100.5 to 104.5)
Post-exercise, %predicted	∂∂∂ **88.5 (85.1 to 92.0)** §§ ¥¥¥	∂∂∂ **77.6 (72.8 to 82.4)** ϕϕϕ	∂∂∂ 98.8 (96.7 to 100.8)
Post-exercise BD, %predicted	ƨƨƨ **95.5 (91.8 to 99.1)** §§§ ¥	**78.6 (73.5 to 83.6)** ϕϕϕ	ƨƨƨ 101.0 (98.9 to 103.2)
Baseline to post-exercise change, %predicted	-5.9 (-8.0 to -3.7)	-5.1 (-7.1 to -3.1)	-3.7 (-4.6 to -2.8)
Post-exercise to post-exercise BD change, %predicted	**6.9 (4.3 to 9.5)** §§ ¥¥¥	0.9 (0.0 to 1.9)	2.2 (1.2 to 3.3)
FEV_1/FVC	**POLD, n=31**	**PnOLD, n=16**	**PT_c, n=87**
Baseline, ratio	**0.68 (0.66 to 0.70)** §§§ ¥¥¥	0.85 (0.82 to 0.88)	0.84 (0.83 to 0.86)
Post-exercise, ratio	**0.69 (0.66 to 0.71)** §§§ ¥¥¥	0.87 (0.83 to 0.90)	0.85 (0.83 to 0.86)
Post-exercise BD, ratio	**0.79 (0.77 to 0.80)** §§§ ¥¥¥	0.90 (0.88 to 0.93)	0.88 (0.87 to 0.89)
Baseline to post-exercise change	0.007 (-0.010 to 0.024)	0.016 (-0.001 to 0.033)	0.002 (-0.004 to 0.009)
Post-exercise to post-exercise BD change	**0.098 (0.076 to 0.121)** §§§ ¥¥¥	0.038 (0.021 to 0.055)	0.031 (0.023 to 0.038)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction) or number and % proportion (Pearson's χ^2 test). **Abbreviations: FEV_1** – Forced Expiratory Volume in 1 second; **FVC** – Forced Vital Capacity; **BD** – bronchodilator.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.
PnOLD vs PT_c: ϕ p<0.05; ϕϕ p<0.01; ϕϕϕ p<0.001.
Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.
Post-exercise vs Post-exercise bronchodilator: ƨ p<0.05; ƨƨ p<0.01; ƨƨƨ p<0.001.

Figure 2.8 % predicted FEV_1, % predicted FVC and FEV_1/FVC at baseline for obstructive (POLD, circles) and non-obstructive (PnOLD, squares) preterm lung disease groups, and preterm controls (PT_C, triangles).

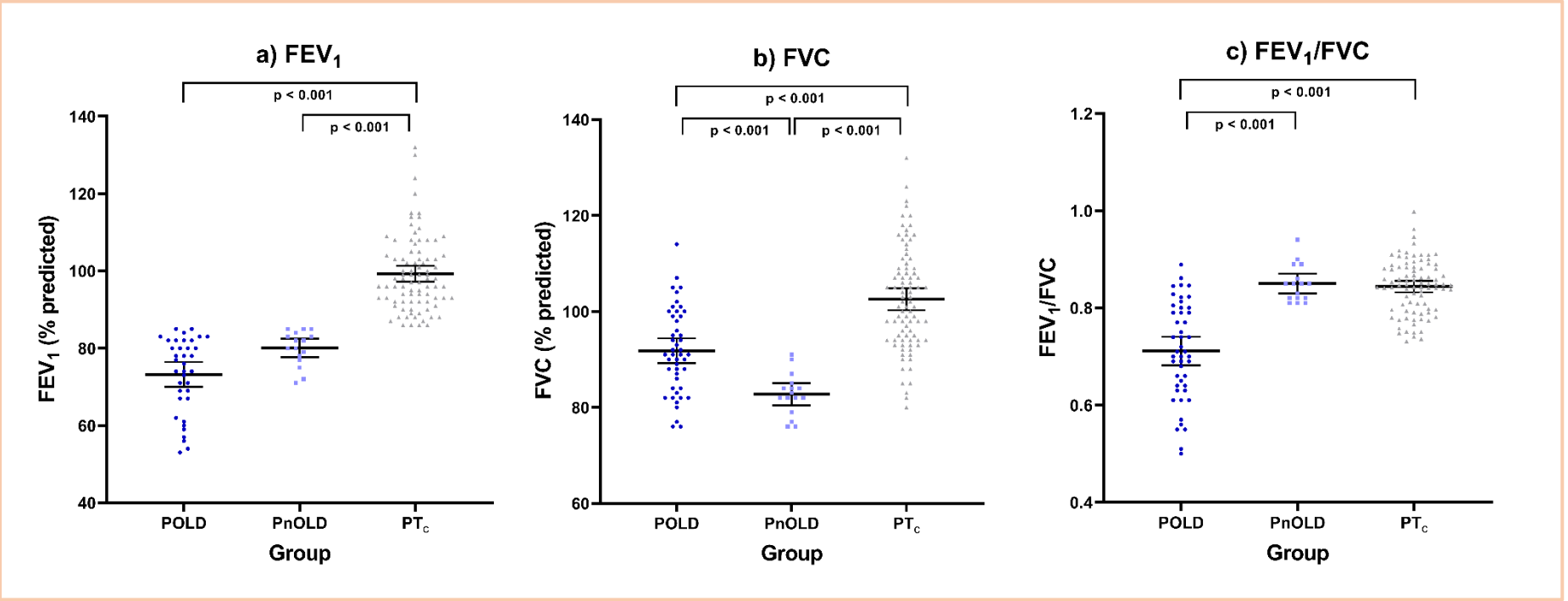
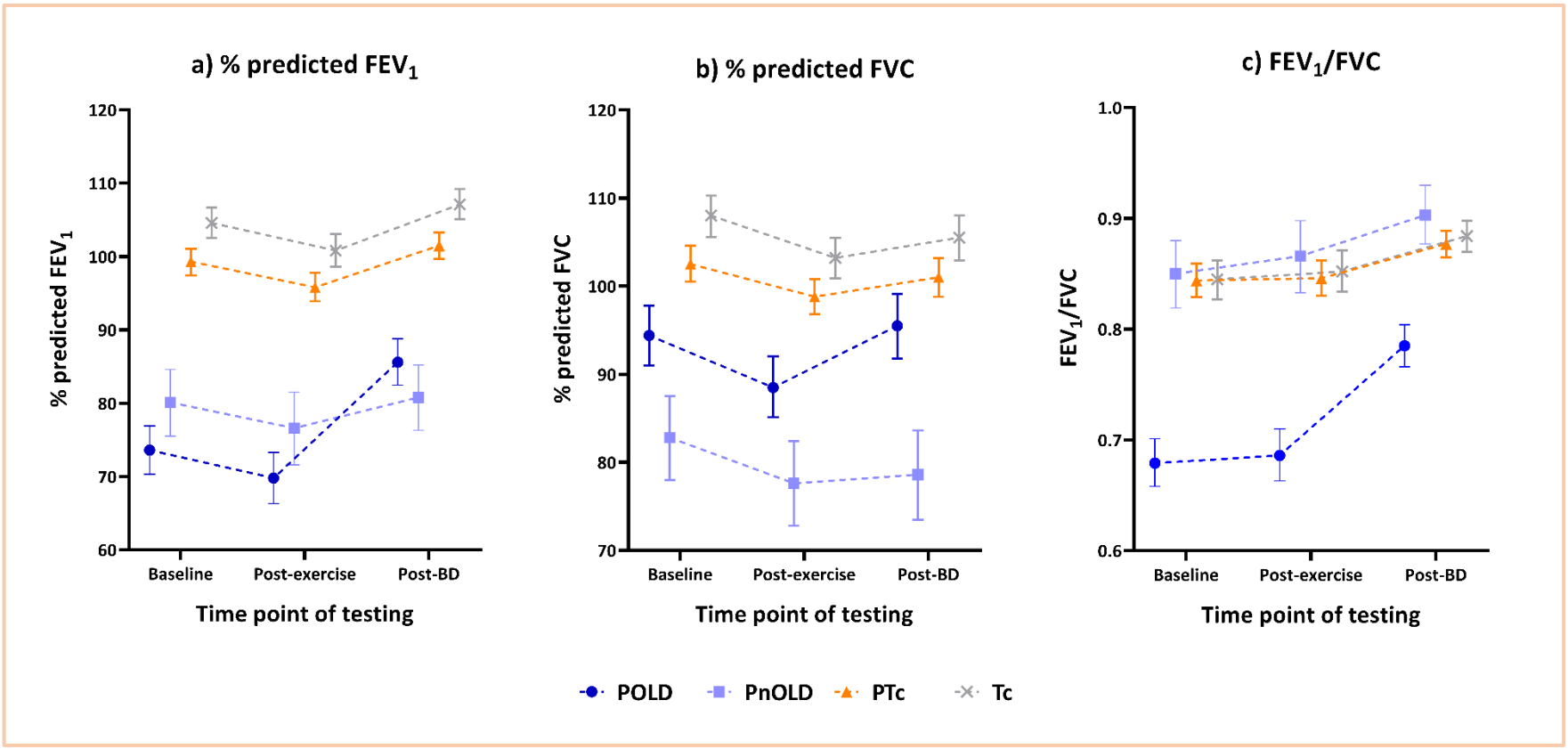


Figure 2.9 % predicted FEV_1, % predicted FVC and FEV_1/FVC at baseline, post-exercise, and following post-exercise reversibility testing for obstructive (POLD, circles) and non-obstructive (PnOLD, squares) preterm lung disease groups, with preterm controls (PT_c, triangles) and term controls (T_c, crosses).



2.4 Discussion

Lung-term respiratory consequences resulting from preterm birth have been well investigated with an emphasis on follow-up for children felt to be at greatest risk, i.e., extremely preterm, lower birth weights, infancy diagnosis of CLD. However, this chapter illustrates that there should be a re-consideration of how we follow-up preterm-born children, giving prominence to identifying those with lung function deficits.

2.4.1 Findings in children with low lung function

This cohort of children were similar in their demographics and anthropometrics across the groups. The PT_{low} children had a greater frequency of respiratory symptoms with almost three-quarters having experienced wheeze, although less than a third have ever received an asthma diagnosis or experienced recent symptoms. This suggests that there is potentially a large proportion of preterm-born children who have impaired lung function which is undetected from either being being asymptomatic or misdiagnosed.

Children with low FEV_1 are more likely to show evidence of air trapping or hyperinflation when evaluating static lung volumes, consistent with some previous findings (Smith et al., 2008) (Welsh et al., 2010). Of interest is evaluation of ongoing lung inflammation using fractional excretion of nitric oxide (FE_{NO}). Meta-analysis has shown that there is no difference between preterm and term born children, although the results show a non-significant trend towards lower values in the preterm groups (Course et al., 2019). Conversely, whilst again there is no statistical difference between groups in this study, the trend seen is of a greater FE_{NO} value in those born preterm, particularly those with lower lung function. This suggests that by actually identifying these preterm children with lung disease we can understand more about the processes behind their pathology, with ongoing inflammation a potential contributory factor. The presence of raised FE_{NO}, a test used predominantly in asthma, clearly raises a question about whether these children have asthma.

However, rates of atopy were similar in the low preterm group compared to the preterm controls, suggesting that this is not the case.

I have shown functional impairment, in the form of reduced exercise capacity, exists in children with low FEV_1, with a 6.2 mls/kg/min lower peak $\dot{V}O_2$ in the PT_{low} group compared to T_c children. This is a greater difference than seen in a systematic review comparing preterm and term children, where peak oxygen consumption differed by between 2.2 to 3.05 mls/kg/min depending on CLD diagnosis (Edwards et al., 2015b). This suggests that functional lung impairment is related to lung function abnormalities, and needs differentiating from children with a CLD diagnosis.

There was little exercise-induced bronchoconstriction, differing from previous findings showing greater decreases of $\%FEV_1$ (Joshi et al., 2013). The reason for this is unclear. What is clear is the positive response to post-exercise bronchodilator seen in the PT_{low} group, with approximately twofold greater increase in FEV_1 compared to controls. This suggests that this group of children need to be actively sought out as they have lung disease responsive to medication.

2.4.2 Obstructive lung disease

I have used this opportunity to look further at the children with low lung function to identify whether there are different phenotypes within the group. The standout result in the PT_{low} children was a reduced FEV_1/FVC ratio below 0.80. Low FEV_1/FVC is seen in obstructive lung disease (Pellegrino et al., 2005), although the use of an absolute ratio could be deemed controversial due to the changing absolute value during growth as a result of dysanapsis (growth of the lungs at a different rate to height) (Quanjer et al., 2010). I have addressed the important methodological concerns surrounding this in Chapter 5. As such defining children with $\%FEV_1 \leq 85\%$ and ratio ≤0.80 was a pragmatic way of distinguishing these children, and classing their lung disease as obstructive is appropriate. More difficult to define are those with FEV_1/FVC ratio >0.80. This would potentially be consistent with restrictive lung disease; however, this would require the TLC to be reduced below the LLN. In my group, 16 children in the low $\%FEV_1$/normal ratio group (40 to 56% depending on

whether plethysmography or helium dilution was used), met this criteria, and as such it is possibly a reasonable surrogate for restrictive disease. To avoid controversy, I felt defining these children as non-obstructive would be more appropriate. As I demonstrate in Chapter 5, the characteristics of these children as a group are similar whether TLC was above or below the LLN.

Using the POLD and PnOLD grouping, I have shown that children with obstructive lung disease have particularly low lung function compared to those with non-obstructive lung disease, something that has not been identified in this population before. Interestingly, in this small population, there were few differences seen between the POLD and PnOLD children from the neonatal period, except a greater number of POLD children required invasive ventilation. Birth weights, gestation and even frequency of IUGR were similar between the groups. It is difficult to know the significance of the invasive ventilation; is there a direct link to invasive ventilation causing later obstruction, or is it related to the fact these children were likely to be more unwell, hence required ventilation, and whether it is the underlying disease process responsible? Interestingly, the rates of doctor-diagnosed asthma were similar in both groups, despite greater frequency of wheeze in the POLD group. Children with prematurity-associated lung disease are often (mis)classified as having asthma, with higher rates diagnosed in preterm-born children (Been et al., 2014). The frequency of wheeze was greatest in the PT_{low} group – it stands to reason why these children are then most likely to receive a diagnosis of asthma. It could then be inferred that, given POLD have the greatest frequency of symptoms, that this is then the group of children who account for these asthma diagnoses. Asthma is a multifactorial inflammatory process, a combination of genetic factors and environmental exposures, linked with other atopic conditions such as eczema and hayfever (Naja et al., 2018). Yet rates of asthma in preterm children exceed diagnoses of other atopic conditions, suggesting that, while symptomology may overlap, there is a different disease process going on in the preterm children (Edwards et al., 2016). Identifying this group of children is paramount, as the underlying pathological process may be different, and as such treatments. Identification of preterm children

with lung function deficits, who have evidence of obstructive lung disease, may be a way of identifying this particular group of children.

FE_{NO} is now commonly used in assessment of childhood asthma, forming part of the NICE recommended diagnosis and monitoring pathway, with a cut-off of 35ppb advised as potentially diagnostic (National Institute for Health and Care Excellence (NICE), 2017 (Last updated: 12 February 2020.)). While a significantly greater proportion of POLD has FE_{NO} greater than this cut off value, possibly representing "classical" asthma, approximately 60% of this group did not. This 60% may be the children in whom a true diagnosis of prematurity-associated obstructive lung disease can be made. The underlying pathology of this disease process is likely to be different to asthma. There have been previous links to ongoing inflammation in preterm-born children (Teig et al., 2012). In this instance, sputum profiles from preterm children showed neutrophil predominance, along with raised IL-8 in sputum supernatant. This suggests a separate process to the eosinophil-driven atopic asthma. Oxidative damage has also been suggested as a possible mechanism (Filippone et al., 2012). With an alternative pathological process likely at play, it is unknown whether standard asthma treatments are suitable in these cases. This is something that needs to be urgently assessed to ensure correct treatment is offered to the appropriate children. The RCT aspect of the study may be able to offer an answer to this, with all the PT_{low} children included in this chapter enrolled onto the trial.

What is known, is that regardless of the underlying diagnosis, either asthma or POLD, a significant post-exercise bronchodilator response is seen in these children. Despite over 80% of these children with obstructive disease having positive response to bronchodilator, only one-third were on salbutamol treatment. This may suggest that treatment with a LABA may have benefit for those with symptoms or functional impairment. The latter of these is difficult to assess, however the in-depth lung testing performed as part of this study may be helpful in informing this.

For instance, term controls showed little evidence of hyperinflation or air trapping on lung volume testing, with low rates of RV/TLC ratio >0.30 (5% and 6% for helium dilution testing and plethysmography respectively). This is compared to 27% and 36% respectively in POLD children. This may have an impact on exercise capacity, with

hyperinflation in children with cystic fibrosis linked to diminished exercise performance (Sovtic et al., 2013). While the exercise outcomes in the POLD and PnOLD groups were similar, the composite group of children with prematurity-associated lung disease were achieving poorer outcomes in exercise as a whole compared to the controls. It is unlikely that the lung disease in the PnOLD group, if largely of a fixed, restrictive nature, would respond to. However, the POLD children in particular used up a larger proportion of their ventilatory reserve, compared to the PnOLD and control groups. If one were to calculate a child's MVV from their post-exercise bronchodilator FEV_1, and re-calculate the proportion of their ventilatory reserve used during the exercise based on this revised MVV, these children would not need to use as much of their respiratory reserve. This suggests that treatment with bronchodilator may improve exercise capacity if these children do not need to use as much of their ventilatory reserve. This could then be expanded to whether longer acting medication may have the same benefit for functional impairments. It is possible that the majority of these children are unaware they have exercise limitation, unless they are attaining towards a higher level in sport, as they may exercise within their capabilities. Indeed, preterm children, particularly boys, may perform less moderate to vigorous activity compared to term-born children (Lowe et al., 2016b), meaning any impairments are not noticed. Alternatively there is the possibility that impaired exercise capacity may actually be driving a more sedentary lifestyle in preterm groups (Lowe et al., 2016a).

2.4.3 Strengths and limitations

The main strength of this study is assessing functional outcomes based on a clinically-relevant current measure (lung function) rather than on a diagnosis made several years prior that may have limited bearing for current health. Additionally, while it is common with other lung disease to assess whether it is obstructive or not in nature, this has not been the case in children with respiratory sequelae of preterm birth. This should be an ongoing clinical, as well as academic, focus. Probably the biggest strength of this study will be the associations that will be made with the large volume of data that has been collected as part of the larger RHiNO study.

Some limitations that are important to note. The main potential criticism may be of how the groups, particularly the obstructive/non-obstructive groups, were defined. I hope this is suitably addressed in my later chapter.

Exercise testing was performed on a cycle ergometer. These are known to be less successful at achieving max V̇O2 compared to treadmill testing (Edwards et al., 2015b). This is due to the limiting factor of muscle strength on a bike compared to a treadmill. The $\dot{V}O_2$ obtained from this study are lower than seen in other studies, especially those using a treadmill. This may explain the lack of exercise-induced bronchoconstriction seen in this study. However, the fact that all groups achieved similar peak heart rates suggest that all reached a similar degree of exertion, making their results comparable to each other.

Some of the perinatal and respiratory data were collected from parents. Recall inaccuracies mean this will not be as reliable as if it were collected from a contemporaneous source. It also limits being able to assess outcomes accurately against quantitative influences such as duration of invasive ventilation or oxygen therapy.

As with all research, there is potential for various sources of bias. I will address these in my final discussion as they apply to all my studies.

2.5 Conclusion

In summary, preterm-born children with low lung function have greater functional impairment compared to healthy children. Additionally, there are different phenotypes of preterm children with lung impairment (obstructive versus non-obstructive disease), both of whom are functionally affected in terms of exercise, but children with lung obstruction are more likely to benefit from bronchodilator treatment.

3 OSCILLOMETRY

This chapter covers the use of a lung function test called oscillometry to assess mechanical properties of the airways in preterm-born children. Understanding airway mechanics is vital for recognizing how the lungs work in the healthy and diseased states. Airway resistance, inertance and compliance can be affected by structural changes, inflammatory processes, or lung fibrosis. Being able to identify changes in the mechanical properties of the lungs can help with diagnosis and progression of lung pathology. There are several forms of oscillometry that have been used. Conventional oscillometry/Forced Oscillation Technique (FOT), impulse oscillometry (IOS), and single frequency oscillometry. An extension of this latter includes temporal-oscillometry (T-oscillometry) and will be covered in Chapter 4.

Nomenclature in this area can cause some confusion, and terms are sometimes used interchangeably when they actually mean different things. Additionally, there is ongoing evolution in these terms. Forced oscillation technique (FOT) is felt to be a misleading name due to the fact the technique does not involve any forced manoeuvres; this technique should simply be called oscillometry.

3.1 Introduction

3.1.1 Airway mechanics

Airway mechanics is the term used to explain the physical elements and physics of getting air in and out of the lungs. This comprises multiple factors including, but not limited to, the size and structure of the airways, muscles of respiration, elastic properties of the lungs, resistance within and outside of the airways as well as pathological factors. Of particular interest are the forces that need to be overcome to allow air movement.

Air flow in inspiration occurs when atmospheric air flows down a pressure gradient into the airways. This pressure gradient occurs due to the balance between the inward elastic recoil of the lungs versus the outward recoil of the chest wall. During inspiration, centrally-initiated contraction of the muscles of respiration occurs

(including the diaphragm and intercostal muscles), which causes distension of the alveoli (terminal air sacs of the airways which are responsible for gas exchange). The expansion of the alveoli causes a drop in alveolar pressure to below that of atmospheric pressure, which allows air movement into the lungs. As the inspiratory muscles finish contracting, the elastic recoil of the lungs come into play, causing the alveoli to start to compress, resulting in an increase in alveolar pressure. Once this reaches above atmospheric pressure, expiratory air flow occurs (Levitzky, 2018).

However, this relationship between pressure changes and airflow is affected by various factors, which can be classified as impedance. Impedance are the forces that need overcoming for air movement into the lungs to occur, in particular 3 main forces. These are resistance, elasticity and inertance (Kaminsky, 2012).

Pulmonary resistance is comprised of two elements. There is the resistance within and between the lung tissues, and the resistance within the airways, which is the greater contributor to overall pulmonary resistance (Levitzky, 2018). Airway resistance is the relationship between pressure and flow (Resistance = Pressure / Flow), and so is flow dependent, i.e., is not a factor at zero flow (end inspiration, end expiration). Airway calibre plays a large role in airway resistance due to Poiseuille's law, which essentially states that if a tube doubles in length, the resistance will double, however if a radius is halved, the resistance will increase to the power of 4 (i.e. multiplied by a factor of 16) (West, 2015). Therefore, a small change in airway diameter can result in a large change in airway resistance. This is clinically significant in conditions where there may be hyperreactive smooth muscle surrounding the airways (asthma, and potentially CLD), with constriction of the muscle causing a decrease in airway calibre resulting in symptoms from increased airway resistance.

Elasticity also plays a significant role in the airway mechanics, although it is often considered in terms of its inverse measure, which is compliance. The compliance relates to the volume change that occurs in relation to a given pressure change, and results from the compliance of the lung tissue and that of the chest wall. An increased compliance results in a greater change in volume for a given pressure compared to if the compliance was decreased. Increased compliance might occur if the lung tissue

loses some of its elastic properties (i.e.in emphysema); decreased compliance can occur in respiratory diseases such as pulmonary fibrosis (Levitzky, 2018).

Inertance plays a smaller role but relates partly to the mass effect from the column of gas within the airways and extrathoracic structures with inertial mass. This can affect how easily any change in airflow direction can occur, and is reflective of the relationship between pressure and acceleration (Lumb, 2016).

These forces of impedance may affect the ability of the lungs to function normally, and being able to quantify them can help with identifying and assessing pathology within the lungs.

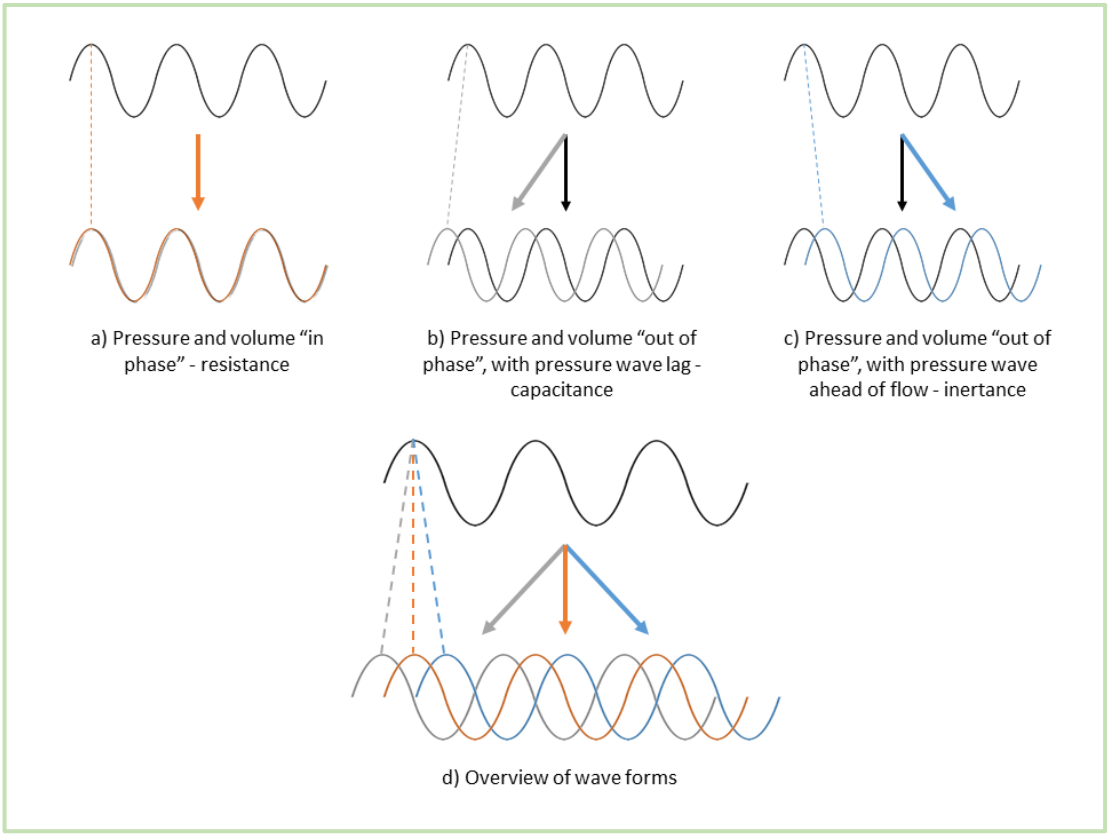
3.1.2 Basics of oscillometry

Oscillometry is one such method of assessing airway mechanics. Oscillometry works by superimposing a soundwave at a given frequency onto normal (tidal) breathing. The signal needs to be large enough to provoke a response from the airways but not to affect normal respiration (Frey, 2005). Oscillometry utilises the concept of pseudorandom noise. This is where the oscillatory signal is rapidly generated randomly at various frequencies and applied to the respiratory system.

A signal will travel into the lungs and reflect off the tissues. The rebounding signal is then measured in terms of pressure and flow at the airway opening, and this relationship establishes impedance of the respiratory system (Zrs). Signals are generated at multiple frequencies, typically within the range of medium frequencies (4-20Hz) (Frey, 2005), although sometimes up to 48Hz. The returning signal is assessed in 2 distinct ways based on whether it is ‘in-phase’ or ‘out-of-phase’ using mathematical transformations (Skylogianni et al., 2016). The ‘in-phase’ signal travels back with pressure and flow waves in-sync, and this signal response will calculate respiratory system resistance (Rrs). The ‘out-of-phase’ signal measures airway reactance (Xrs), comprising capacitance and inertance. Where elastic components of the lung distend as a result of flow, this delays the pressure wave causing it to lag behind flow. This creates a negative reactance value for capacitance (as the inverse of the elastic component). Inertial mass, however, requires a build-up in pressure to

overcome this force, and as such the pressure wave travels ahead of the flow wave, leading to positive values (Goldman, 2001). This is illustrated in Figure 3.1. The point at which capacitance and inertance cancel each other out results in a reactance value of 0, which is known as the resonant frequency (*f*Res).

Figure 3.1 Illustration of the relationship between pressure and flow waves during oscillometry and their response to a) resistance, b) capacitance, and c) inertance, and d) an overview of all mechanical properties impact on waves.



Different frequencies of signal will give information about different parts of the lung. The higher the frequency, the less distance the signal can travel into the airways. A signal at 20Hz will reflect impedance of the more proximal airways. A lower frequency signal will travel further into the smaller airways. As such a disease of the proximal airways will affect the impedance at all frequencies, whereas disease in the smaller airways will be isolated to the lower frequencies (Brashier and Salvi, 2015). This means oscillometry is a useful tool for identifying the location of pathology in the lungs. It is particularly helpful in paediatric populations, including preterm-born children, due to requiring only passive breathing rather than more complicated, dynamic manoeuvres as in spirometry.

3.1.3 Oscillometry in preterm children

Oscillometry/FOT, along with the impulse oscillometry system (IOS), has been previously used to assess respiratory mechanics in different preterm populations. IOS is a variant of oscillometry which uses pulses of square waves 5 times per second (rather than sine waves as in oscillometry) at a fixed frequency of 5Hz, superimposed on passive breathing. From this frequency, other multitudes of 5Hz can be derived (Komarow et al., 2011).

As discussed above, the utility of oscillometry for younger children have allowed preschool-aged children to be assessed. Vrijlandt *et al* assessed preschool children (mean age 57 months) including 77 preterm children with or without chronic lung disease of prematurity (CLD) and 73 term controls. Lower reactance averaged across 4-24Hz (Xrs_{4-24}) and higher *f*Res were seen in children with CLD compared to those preterm children without CLD. Children with CLD had worse outcomes across all reported parameters compared to the term controls. Resistance at 6Hz (Rrs_6) and average Rrs_{4-24} were higher in the preterm group with no CLD compared to term controls (Vrijlandt et al., 2007). These results suggest that children with CLD may be more affected than preterm children without CLD, however they were also born at a younger gestation which may be a confounding factor that was not investigated.

An alternative study of a similar age group from a research group in Perth, Australia reported similar results when comparing 49 young children (mean age 5.2 years) with a history of CLD to a previous cohort of 158 healthy term-born children of a similar age. Results of resistance and reactance at 6/8/10Hz were transformed into Z-scores calculated from the healthy control group. All of the impedance values in the CLD group were impaired compared to the healthy population. In this instance, only duration of oxygen was associated with impaired impedance in a multivariate regression model of neonatal factors (Udomittipong et al., 2008). The results of reversibility testing were published separately in comparison with children with other disease processes. A bronchodilator response compared to the healthy controls was seen in the resistance at 8 and 10Hz when considered as both absolute and relative changes, although when adjusted for baseline lung function as a covariate, these differences were lost (Thamrin et al., 2007). A further Perth-based study with greater

numbers (74 CLD, 44 no CLD, 32 term controls) was published more recently. The children studied were of a slightly older age (median 5.8-6 years). Z-scores for Rrs_8 and Xrs_8 were again higher in both preterm groups compared to term controls, and reactance was higher in the CLD group compared to the non-CLD group (Verheggen et al., 2016).

Other studies have assessed children from older age groups and found varying impedance parameters were worse in preterm-born, especially those children who had CLD (Suursalmi et al., 2015, Thunqvist et al., 2018). This includes lung function assessments of adolescents using IOS: Um-Bergström and colleagues studied teenagers with a mean age of 14.5 years. Of 51 children born very preterm, including 28 with CLD (classified by severity), when assessing the frequency dependence of resistance (i.e. the difference between Rrs_5 and Rrs_{20}), children with severe CLD had a significantly worse outcome than the non-CLD children suggesting peripheral airway involvement of disease. However, the numbers in this study were small, only 4 children were present in the severe CLD group (Um-Bergström et al., 2017).

Findings from studies looking at oscillometry in preterm-born children have consistently reported worse outcomes in this population, in particular in those children with a history of CLD, however there are limited data regarding response to bronchodilator, and no data regarding whether exercise has any impact on this patient group.

3.1.4 Aims of study

This part of my study has the following aims:

1) Establish baseline data for impedance in a population of preterm-born children with evidence of lung dysfunction, and compare their results to preterm- and term-born controls;
2) Assess the impact of exercise on airway mechanics in this preterm population;
3) Assess the response of oscillometry parameters to post-exercise bronchodilator therapy;

4) Review aims 1-3 with respect to preterm children with and without obstructive airway disease secondary to preterm birth.

3.2 Methods

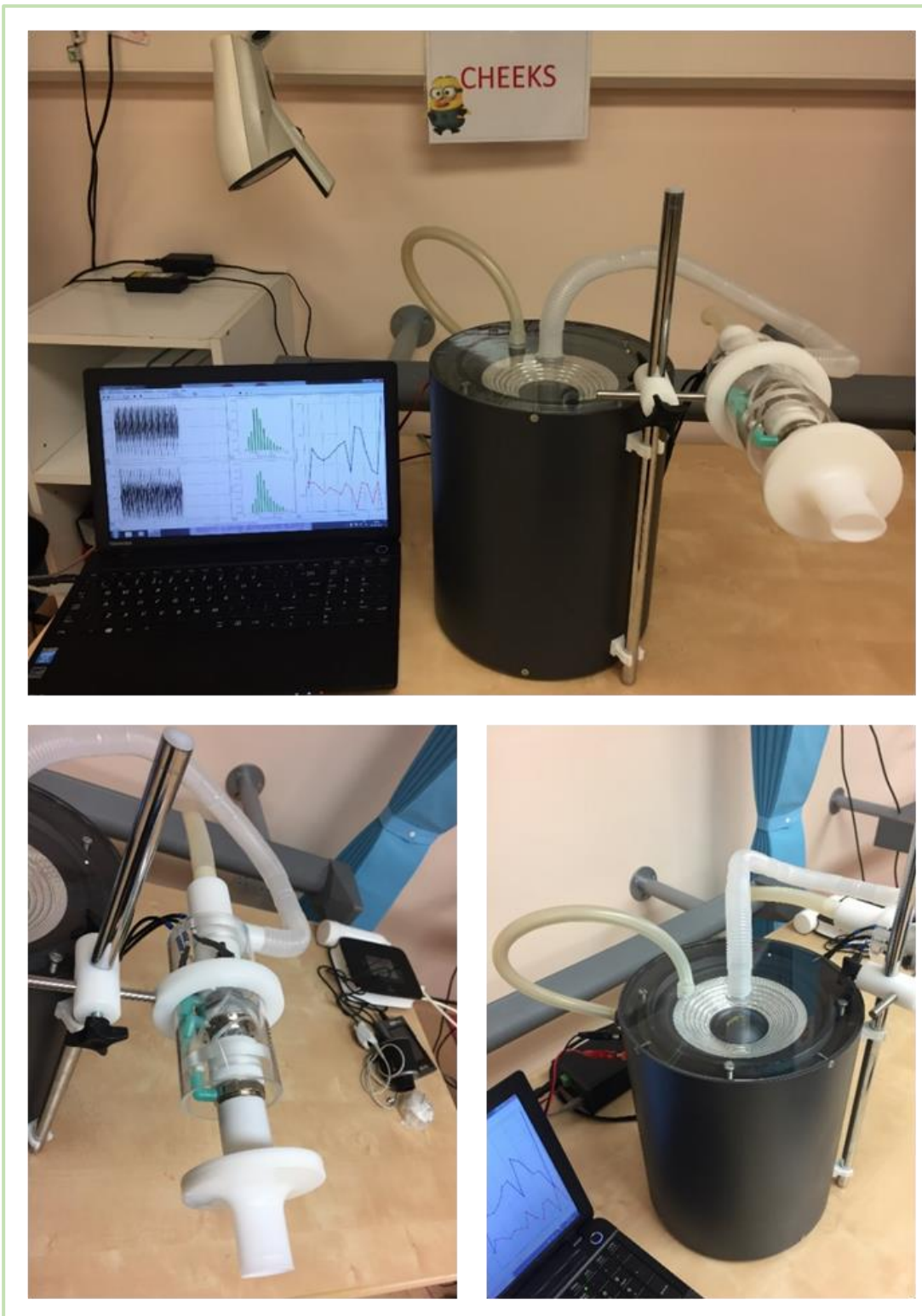
3.2.1 Population

The population and recruitment for participants is the same as described in Chapter 2, Section 2.2.1, as are the demographic, perinatal, and respiratory details reported in the results (Chapter 2, Section 2.3.1).

3.2.2 Oscillometry testing

Oscillometry testing was performed using a custom-built set-up and computer programme (NDAQ) developed by a team at University of Szeged in Hungary (Czövek et al., 2016, Lorx et al., 2017). The set-up comprised of a loudspeaker connected to a wave-tube. Within the wave-tube were sensors to measure pressure and flow at the airway opening. Due to the potential of increased breathing frequency and pressure from exercise affecting the soundwaves, the set-up was modified to negate this effect. The loudspeaker was encased within a larger, sealed cylinder with a 'blow-off' tube connecting the top of the speaker to the cylinder below the speaker, essentially allowing pressure to equalise above and below the speaker diaphragm (Figure 3.2).

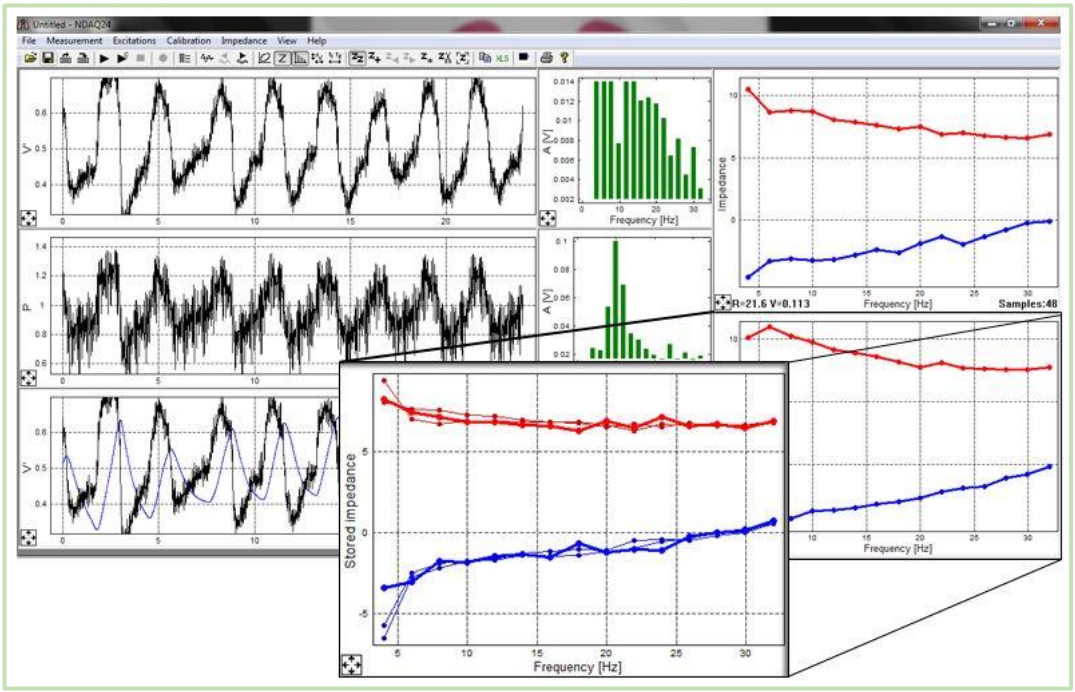
Figure 3.2 Pictures of the FOT set-up showing the encased loudspeaker with the blow-off tube, and the wave-tube.



Prior to testing, an explanation was given to the children of what the test was for and how it should be performed. The test was performed with the child sat upright in a chair and attached to the system via a Microgard II microbial filter (Vyaire, Germany). A nose clip was worn throughout testing and the participant was instructed to firmly hold their cheeks with their fingers and palms of hands to stop any soft tissue vibrations affecting the results.

Via the NDAQ system, the loudspeaker then generated soundwaves at random, at even frequencies between 4 and 32Hz. Impedance was measured at the mouth using the pressure and flow sensors, for each of the individual frequencies. This impedance was measured 'in-phase' with the soundwaves (representing airway resistance) and 'out-of-phase' from the input (representing airway reactance). Results from the individual frequencies were displayed in the form of a spectra. A minimum of 3 recordings were obtained from each child, aiming for spectra that appeared very similar, representing an accurate result of airway impedance (Figure 3.3).

Figure 3.3 Screenshot of the NDAQ software showing flow and pressure readings over time (left side of picture), and the computed spectra from the readings (right side of screen). Inset shows 3 spectra superimposed, representing satisfactory testing.



Oscillometry was performed at 3 time points:

- An initial baseline reading was obtained.
- A second test was performed at 20 minutes following maximal exercise testing.
- A final test was performed 15 minutes after administration of 400µg Salbutamol, a β2-agonist (Salamol, Teva UK Limited), given ~45 minutes after end of exercise test.

I performed blinded, post-acquisition analysis of the oscillometry data to obtain results. I assessed each of the recordings for quality and selected artefact-free segments from the 3 recordings (of at least 16 seconds), compiling results from these using the NDAQ software. The average resistance and reactance results at each individual frequency across the 3 recordings were calculated, plus an average of the resistance measured from all the frequencies from 6-32Hz. The capacitance (a surrogate for lung compliance) was calculated by the NDAQ software using a linear function applied to the negative reactance values.

3.2.3 Spirometry and cardiopulmonary exercise testing (CPET)

Spirometry testing and CPET were performed as described in Chapter 2, sections 2.2.2.3 and 2.2.2.7.

3.2.4 Terms and definitions

Figure 3.4 displays how some of the below parameters are derived from the oscillometry spectra.

$Rrs_{:}$ Respiratory system resistance. This is positive in sign and represents the in-phase part of impedance. It is usually denoted by the frequency(ies) at which the resistance was measured, i.e. Rrs_6 for the resistance at 6 Hz, or Rrs_{6-20} for resistance averaged across those frequencies.

Xrs: Respiratory system reactance. This can be positive or negative in sign and represents the out-of-phase part of impedance. Reactance negative in sign is associated with the compliance of the respiratory system and when positive in sign represents the inertance. It is usually denoted by the frequency at which the resistance was measured, i.e. Xrs_6 for the reactance at 6 Hz.

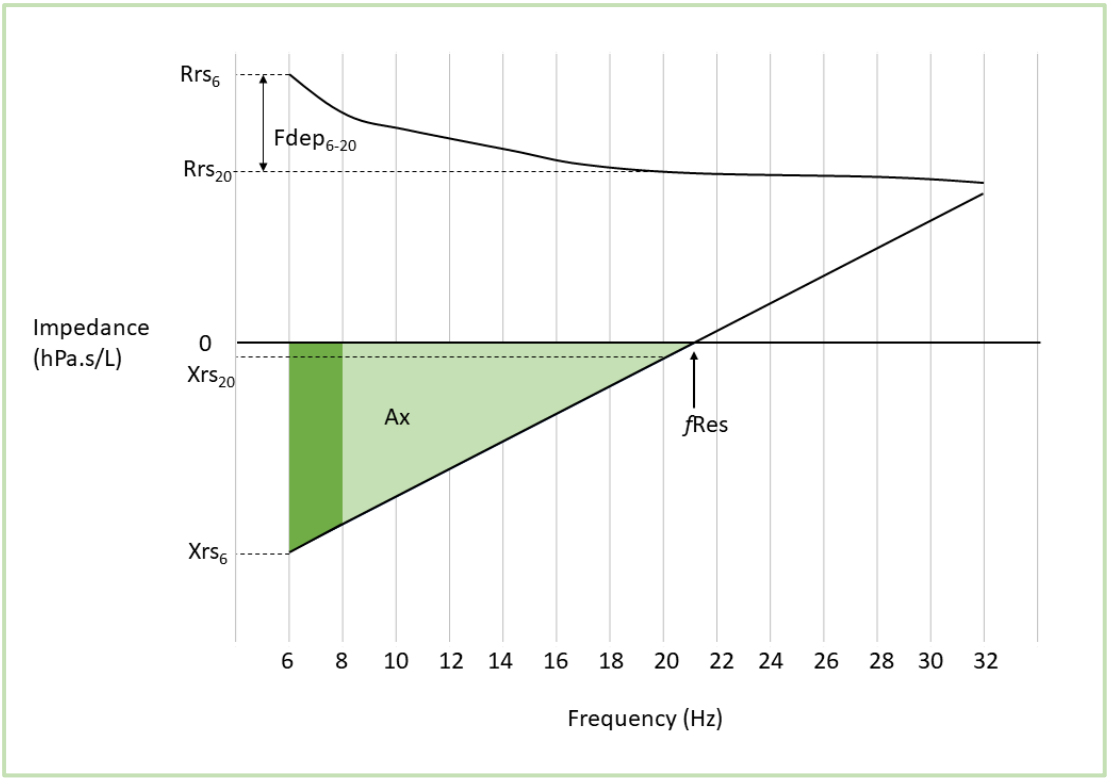
(r)Fdep: Frequency dependence of resistance, usually measured across 6 to 20 Hz. This represents the difference between resistance at 6 Hz and the resistance at 20 Hz (Rrs_6 - Rrs_{20}). The preceding "r" denotes the Fdep used in the form of a ratio to account for differences in baseline resistance between individuals ((Rrs_6 - $Rrs_{20)}$/Rrs_{20}). This represents small/distal airway resistance by removing the proximal part of the resistance (at the higher frequency).

*f*Res: Resonant frequency. The point at which the reactance is 0, i.e. where compliance and inertance cancel each other out. At this point, the entire impedance is accounted for by the resistance.

C: Compliance. Calculated by fitting a linear model to the negative reactances. Represents the inverse of airway elasticity.

Ax: Area under the reactance curve. A calculation of the entire area from the reactance at 6 Hz up to resonant frequency, calculated by applying the trapezoid rule to the reactance for every block of 2 Hz, and the sum of these areas equals the Ax (see Figure 3.4).

Figure 3.4 Graphic displaying various parameters derived from oscillometry spectra, as well as how the area under the reactance curve (Ax) is calculated. The trapezoid rule is applied to every block of 2 Hz from 6 Hz up to fRes, and the sum equates to the Ax.



3.2.5 Ethical approval

As outlined in Chapter 2, ethics approval for the RHiNO study was granted by the South West-Central Bristol Ethics Committee (Ref 15/SW/0289). Parents and children (where possible) provided written consent/assent.

3.2.6 Statistical analysis

Comparisons of continuous data between two groups were made using independent t-tests. Multi-group comparisons of continuous data were performed using one-way ANOVA with Bonferroni correction. Categorical data were assessed using Pearson's χ^2 tests. Within-group and between group comparisons across time points were measured with two-way repeated measures ANOVA, with Bonferroni correction. Change scores (i.e. difference from baseline to post-exercise, and post-exercise to post-exercise bronchodilation) between groups were compared using one-way

ANOVA with Bonferroni correction. Change scores were calculated either as an absolute value or relative to the starting point of comparison, for example for Rrs_6 between post-exercise to post-exercise bronchodilator (BD) the absolute change score was calculated [$Rrs_{6(exercise)}$ - $Rrs_{6(BD)}$] and for relative change score as a percentage [(($Rrs_{6(exercise)}$ - $Rrs_{6(BD)}$) / $Rrs_{6(exercise)}$) * 100]. Relative change scores were not performed in all cases. Due to reactance values crossing zero, this can greatly affect the magnitude or sign of the relative change scores, affecting interpretation.

No formal a priori power calculation was performed due to an exploratory nature of the study, however using G*Power (University of Düsseldorf, Germany) to see an effect size (f) of 0.4 (or η^2 of 0.14) in a 3 group ANOVA with an α of 0.05 and power of 0.8, would require a total sample size of 66. This effect size would be achieved with an overall mean of 5hPa.s/L for a given parameter, a difference of 0.5hPa.s/L between groups, and SD of 1 in each group.

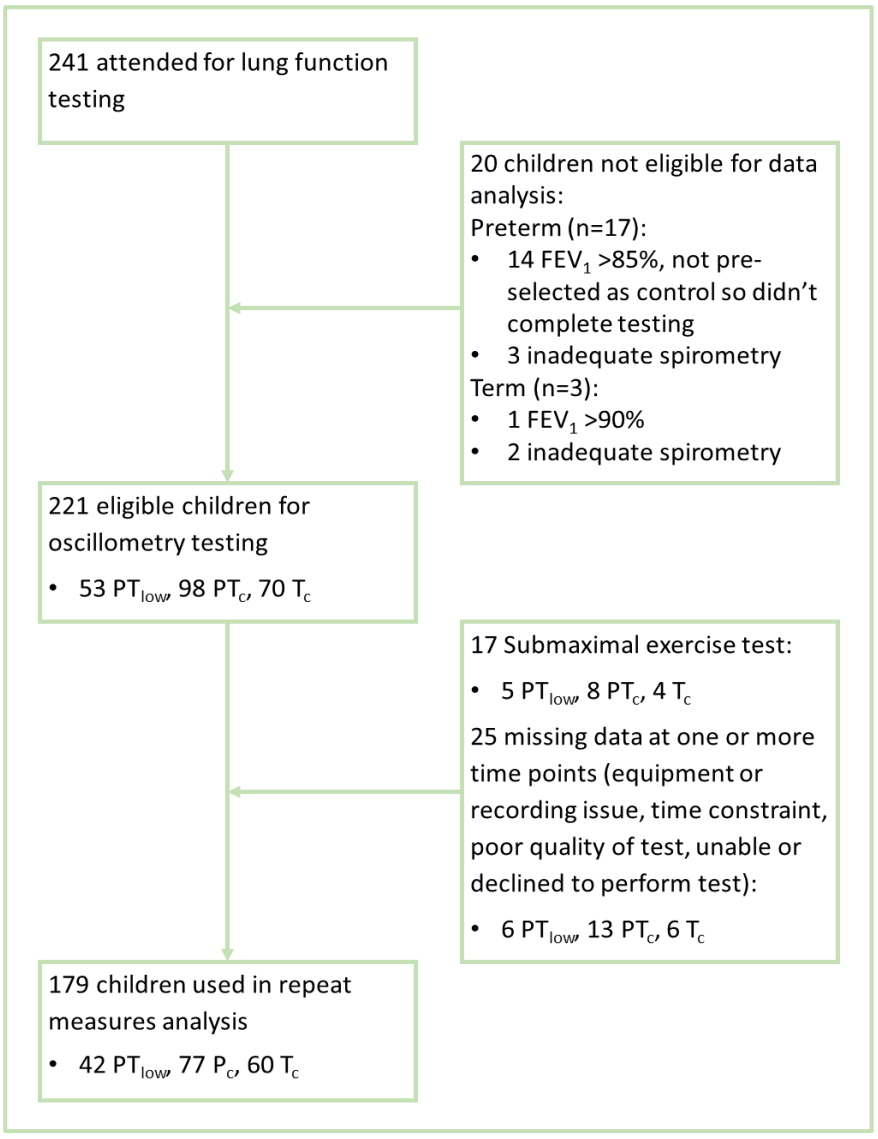
Statistical analysis was performed using SPSS version 26 (IBM, USA).

3.3 Results

3.3.1 Participant numbers

Figure 3.5 summarises participant flow through testing. Of the 221 eligible children who attended for lung function testing, 179 children were included in repeated measures analysis of oscillometry data. 17 children were excluded as they did not reach maximal exercise, and 29 were excluded due to missing data at 1 or more time points. The reasons for missing data included equipment or recording issues (n=7), time constraint (n=7), being unable or declining to perform the test (n=12), or unacceptable quality of recording (n=3).

Figure 3.5 Flow diagram showing recruitment and attrition for oscillometry testing.



3.3.2 Lung function groups

Oscillometry outcomes were first assessed in the original lung function groups (preterm with low lung function, PT_{low}; preterm controls, PT_c; term controls,T_c).

3.3.2.1 Participant characteristics

Participant characteristics were reviewed in Chapter 2 (Tables 2.1 and 2.2) and are summarised below. Current anthropometric measurements (both actual and standardised) were similar between groups including, importantly for the oscillometry given the association between height and impedance, no height difference seen between the groups. PT_{low} children were born earlier (29.7 vs 31.1 vs 40.0 weeks' gestation) and were smaller (1392 vs 1729 vs 3528 grams) than PT_c and T_c groups. CLD rates were higher in the PT_{low} children compared to PT_c. Higher rates of ever having wheezed, asthma diagnosis and salbutamol use were seen in PT_{low} compared to T_c (wheeze ever also greater compared to PT_c). In line with how the groups were divided, $\%FEV_1$ was lowest in PT_{low} group (76 vs 99 vs 105 %). Additionally, they also had lower %FVC (90 vs 103 vs 108 %) and FEV_1/FVC (0.74 vs 0.84 vs 0.85).

3.3.2.2 Resistance parameters

As per Tables 3.1 and 3.2, at baseline, $Rrs_{(6-20)}$, Rrs_6 and Rrs_{20} were all significantly greater in PT_{low} group compared to both control groups. There was a significantly greater Fdep seen in PT_{low} compared to both controls (1.51 vs 0.43 vs 0.37 hPa.s/L), including when accounting for the baseline resistance (1.27 vs 1.09 vs 1.07), suggestive of peripheral lung disease.

Following exercise there were few changes seen in resistance parameters, with an improvement for PT_{low} in Rrs_{20} but a resulting increase in $rFdep_{6-20}$, and no difference in change scores between groups.

Following post-exercise bronchodilator therapy, all groups saw a significant improvement in their resistance parameters on within group repeated measures, with the exception of the frequency dependence parameters for term controls. The greatest improvements on absolute change scores were seen in the PT_{low} children for those parameters affecting peripheral airways, with significantly greater differences seen in absolute $Rrs_{(6-20)}$ and Rrs_6 for the PT_{low} group compared to both controls. For

Rrs_6 there were a 56% and 54% greater absolute decrease in Rrs_6 compared to PT_c and T_c children. Additionally, the frequency dependences had ~threefold greater differences for PT_{low} against both controls. Following bronchodilator therapy there was a loss in significance for the differences seen between the PT_{low} children and controls for the resistance values, suggesting a graduation towards normality following medication.

Table 3.1 Oscillometry resistance results for study group (PT_{low}) compared to preterm (PT_c) and term (T_c) controls at baseline, post-exercise and post-exercise bronchodilator.

		Baseline	Post-exercise	Post-exercise BD
Resistance parameters (PT_{low}, n=42; PT_c, n=77; T_c, n=60)				
Rrs_{6-20}, hPa.s/L	PT_{low}	**6.40 (5.90 to 6.82)** *** ††	**6.16 (5.72 to 6.60)** ** †	∂∂∂ 5.09 (4.64 to 5.54)
	PT_c	5.20 (4.89 to 5.52)	5.25 (4.93 to 5.58)	∂∂∂ 4.68 (4.32 to 5.01)
	T_c	5.34 (4.98 to 5.69)	5.40 (5.04 to 5.77)	∂∂∂ 4.76 (4.39 to 5.13)
Rrs_6, hPa.s/L	PT_{low}	**7.26 (6.75 to 7.76)** *** †††	**7.28 (6.72 to 7.84)** *** †††	∂∂∂ 5.59 (5.05 to 6.12)
	PT_c	5.48 (5.11 to 5.85)	5.63 (5.22 to 6.05)	∂∂∂ 4.90 (4.50 to 5.29)
	T_c	5.55 (5.13 to 5.97)	5.71 (5.24 to 6.17)	∂∂∂ 4.92 (4.47 to 5.36)
Rrs_{20}, hPa.s/L	PT_{low}	**5.75 (5.37 to 6.13)** *	∂ 5.44 (5.04 to 5.83)	∂∂∂ 4.75 (4.35 to 5.15)
	PT_c	5.06 (4.77 to 5.34)	4.98 (4.69 to 5.27)	∂∂∂ 4.55 (4.25 to 4.84)
	T_c	5.18 (4.86 to 5.50)	5.14 (4.81 to 5.47)	∂∂∂ 4.63 (4.30 to 4.97)
$Fdep_{6-20}$, hPa.s/L	PT_{low}	**1.51 (1.24 to 1.78)** *** †††	**1.85 (1.51 to 2.18)** *** †††	**∂∂∂ 0.84 (0.55 to 1.13)** * †
	PT_c	0.43 (0.23 to 0.63)	0.65 (0.40 to 0.90)	∂ 0.35 (0.14 to 0.57)
	T_c	0.37 (0.14 to 0.60)	0.57 (0.29 to 0.85)	0.28 (0.04 to 0.53)
$rFdep_{6-20}$	PT_{low}	**1.27 (1.22 to 1.32)** *** †††	**∂ 1.35 (1.29 to 1.42)** *** †††	**∂∂∂ 1.17 (1.11 to 1.23)** * †
	PT_c	1.09 (1.05 to 1.13)	∂ 1.14 (1.09 to 1.19)	∂ 1.08 (1.03 to 1.12)
	T_c	1.07 (1.02 to 1.11)	1.10 (1.05 to 1.16)	1.06 (1.01 to 1.11)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **Rrs_{6-20}** – average respiratory system resistance 6 -20 Hz; **R / $Xrs_{6\ /\ 20}$** – respiratory system resistance (R) / reactance (X) at 6 / 20 Hz; **$Fdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz; **r$Fdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz with Rrs_{20} as denominator.

PT_{low} vs P_c: * p<0.05; ** p<0.01; *** p<0.001.

PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

P_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise BD: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Table 3.2 Oscillometry resistance change scores from baseline to post-exercise, and post-exercise to post-exercise bronchodilator for study group (PT_{low}) compared to preterm (PT_c) and term (T_c).

		PT_{low}, n=42	PT_c, n=77	T_c, n=60
Resistance parameters baseline to post-exercise				
Rrs_{6-20}	Absolute (hPa.s/L)	0.24 (-0.06 to 0.54)	-0.05 (-0.22 to 0.13)	-0.07 (-0.32 to 0.19)
	Relative (%)	3.8 (-1.0 to 8.7)	-1.8 (-5.2 to 1.6)	-2.7 (-7.4 to 2.0)
Rrs_6	Absolute (hPa.s/L)	-0.03 (-0.46 to 0.41)	-0.15 (-0.40 to 0.10)	-0.16 (-0.48 to 0.16)
	Relative (%)	-0.6 (-6.3 to 5.2)	-4.5 (-9.3 to 0.3)	-4.4 (-10.5 to 1.7)
Rrs_{20}	Absolute (hPa.s/L)	0.31 (0.05 to 0.58)	0.07 (-0.09 to 0.24)	0.04 (-0.20 to 0.28)
	Relative (%)	5.5 (0.6 to 10.4)	0.7 (-2.3 to 3.8)	-0.6 (-5.1 to 3.8)
$Fdep_{6-20}$, hPa.s/L (absolute)		-0.34 (-0.64 to -0.04)	-0.22 (-0.42 to -0.02)	-0.2 (-0.47 to 0.06)
$rFdep_{6-20}$	Absolute	-0.08 (-0.14 to -0.03)	-0.05 (-0.10 to -0.01)	-0.03 (-0.09 to 0.02)
	Relative (%)	-7.0 (-11.3 to -2.7)	-5.6 (-9.4 to -1.7)	-4.2 (-8.9 to 0.4)
Resistance parameters post-exercise to post-exercise BD				
Rrs_{6-20}	Absolute (hPa.s/L)	**1.07 (0.75 to 1.39)** ** †	0.57 (0.37 to 0.77)	0.64 (0.45 to 0.84)
	Relative (%)	15.8 (11.2 to 20.3)	11.7 (8.2 to 15.1)	11.3 (7.8 to 14.8)
Rrs_6	Absolute (hPa.s/L)	**1.70 (1.16 to 2.23)** ** ††	0.74 (0.46 to 1.01)	0.79 (0.47 to 1.11)
	Relative (%)	21.3 (15.4 to 27.2)	13.1 (8.7 to 17.5)	12.0 (7.0 to 17.0)
Rrs_{20}	Absolute (hPa.s/L)	0.68 (0.42 to 0.94)	0.44 (0.27 to 0.60)	0.51 (0.33 to 0.69)
	Relative (%)	10.5 (5.9 to 15.2)	9.3 (6.1 to 12.5)	9.4 (5.9 to 12.8)
$Fdep_{6-20}$, hPa.s/L (absolute)		**1.01 (0.58 to 1.44)** ** ††	0.30 (0.10 to 0.50)	0.29 (0.05 to 0.53)
$rFdep_{6-20}$	Absolute	**0.18 (0.10 to 0.26)** ** ††	0.06 (0.02 to 0.10)	0.04 (0.00 to 0.09)
	Relative (%)	**11.7 (6.3 to 17.1)** * †	4.3 (1.1 to 7.5)	3.1 (-0.5 to 6.8)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **Rrs_{6-20}** – average respiratory system resistance 6 -20 Hz; **R / $Xrs_{6\ /\ 20}$** – respiratory system resistance (R) / reactance (X) at 6 / 20 Hz; **$Fdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz; **$rFdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz with Rrs_{20} as denominator.

PT_{low} vs P_c: * p<0.05; ** p<0.01; *** p<0.001.

PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

P_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

3.3.2.3 Reactance parameters

As per Tables 3.3 and 3.4, similar to resistance parameters, reactance values were significantly worse in PT_{low} children compared to both controls. Compliance appeared particularly affected, approximately 40% lower than either of the control groups. Additionally, *f*res was significantly higher in PT_{low} children (29.5 vs 21.4 vs 21.7 Hz).

Following exercise, the only change seen on repeated measures ANOVA was an increase in negative value in T_c.

Following post-exercise bronchodilator therapy, improvements were seen in all groups for Xrs_{20}, compliance and *f*res; however, only the PT_{low} group showed improvement in Xrs_6, and PT_{low} and T_c for Ax. The change scores showed a greater improvement for PT_{low} children compared to controls in all reactance parameters except for compliance.

Table 3.3 Oscillometry reactance results for study group (PT_{low}) compared to preterm (PT_c) and term (T_c) controls at baseline, post-exercise and post-exercise bronchodilator.

		Baseline	Post-exercise	Post-exercise BD
Reactance parameters (PT_{low}, n=42; PT_c, n=77; T_c, n=60)				
Xrs_6, hPa.s/L	PT_{low}	**-3.68 (-4.01 to -3.35)** *** †††	**-3.87 (-4.30 to -3.44)** *** †††	‡‡‡ **-2.46 (-2.82 to -2.09)** **
	PT_c	-1.99 (-2.23 to -1.74)	-2.08 (-2.40 to -1.77)	*-1.77 (-2.03 to -1.50)*
	T_c	-2.06 (-2.34 to -1.78)	-2.21 (-2.57 to -1.85)	*-1.92 (-2.23 to -1.62)*
Xrs_{20}, hPa.s/L	PT_{low}	**-1.35 (-1.61 to -1.09)** *** †††	**-1.28 (-1.58 to -0.98)** *** †††	‡‡‡ -0.31 (-0.57 to -0.04)
	PT_c	-0.20 (-0.39 to 0.00)	-0.31 (-0.53 to -0.09)	‡‡‡ 0.07 (-0.13 to 0.27)
	T_c	-0.21 (-0.43 to 0.00)	∂∂ -0.48 (-0.73 to -0.24)	‡‡‡ -0.03 (-0.25 to 0.19)
C, ml/hPa	PT_{low}	**7.12 (5.85 to 8.39)** *** †††	**7.09 (5.75 to 8.43)** *** †††	‡‡‡ **10.45 (8.78 to 12.11)** **
	PT_c	11.91 (11.00 to 12.85)	11.48 (10.49 to 12.46)	‡‡‡ 13.86 (12.64 to 15.09)
	T_c	11.88 (10.82 to 12.94)	11.21 (10.10 to 12.33)	‡‡‡ 13.09 (11.70 to 14.48)
*f*Res, Hz	PT_{low}	**29.5 (27.5 to 31.6)** *** †††	**27.9 (25.8 to 30.1)** *** ††	‡‡‡ 22.2 (20.1 to 24.3)
	PT_c	21.4 (19.9 to 23.0)	22.2 (20.6 to 23.8)	‡‡‡ 19.1 (17.5 to 20.7)
	T_c	21.7 (20.0 to 23.4)	23.3 (21.5 to 25.1)	‡‡‡ 20.0 (18.3 to 21.8)
Ax, ml/hPa	PT_{low}	**43.8 (37.9 to 49.6)** *** †††	**43.9 (37.3 to 50.6)** ** †††	‡‡‡ **20.2 (15.4 to 25.0)** †
	PT_c	15.8 (11.5 to 20.2)	18.5 (13.6 to 23.5)	13.2 (9.6 to 16.8)
	T_c	16.1 (11.2 to 20.9)	20.4 (14.8 to 26.0)	‡ 14.7 (10.7 to 18.8)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **C** – compliance; ***f*res** – resonant frequency; **Ax** – area under the reactance curve.

PT_{low} vs P_c: * p<0.05; ** p<0.01; *** p<0.001.

PTl_{ow} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

P_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise BD: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Table 3.4 Oscillometry reactance change scores from baseline to post-exercise, and post-exercise to post-exercise bronchodilator for study group (PT_{low}) compared to preterm (PT_c) and term (T_c).

		PT_{low}, n=42	PT_c, n=77	T_c, n=60
Reactance parameters baseline to post-exercise				
Xrs_6, hPa.s/L (absolute)		0.19 (-0.32 to 0.71)	0.10 (-0.10 to 0.30)	0.16 (-0.05 to 0.36)
Xrs_{20}, hPa.s/L (absolute)		**-0.07 (-0.27 to 0.13)** †	0.12 (-0.03 to 0.26)	0.27 (0.08 to 0.46)
C, ml/hPa	Absolute (ml/hPa)	0.03 (-0.49 to 0.55)	0.44 (-0.33 to 1.20)	0.67 (-0.28 to 1.61)
	Relative (%)	-0.4 (-8.4 to 7.6)	0.6 (-5.5 to 6.7)	3.3 (-3.9 to 10.4)
*f*Res, Hz	Absolute (ml/hPa)	**1.6 (0.3 to 2.9)** †	-0.8 (-2.2 to 0.6)	-1.6 (-3.2 to 0.1)
	Relative (%)	**4.8 (0.8 to 8.8)** †	-6.7 (-13.4 to 0.0)	-10.8 (-19.3 to -2.3)
Ax, ml/hPa, Hz	Absolute (ml/hPa)	-0.2 (-5.2 to 4.9)	-2.6 (-5.4 to 0.1)	-4.3 (-7.9 to -0.7)
	Relative (%)	0.3 (-10.8 to 11.4)	-47.1 (-82.2 to -12.1)	-69.8 (-118.0 to -21.5)
Reactance parameters post-exercise to post-exercise BD				
Xrs_6, hPa.s/L (absolute)		**-1.42 (-1.99 to -0.84)** *** †††	-0.32 (-0.52 to -0.12)	-0.29 (-0.50 to -0.08)
Xrs_{20}, hPa.s/L (absolute)		**-0.97 (-1.28 to -0.66)** *** ††	-0.38 (-0.53 to -0.24)	-0.45 (-0.59 to -0.32)
C, ml/hPa	Absolute (ml/hPa)	-3.36 (-4.25 to -2.47)	-2.39 (-3.26 to -1.52)	-1.87 (-2.77 to -0.98)
	Relative (%)	**-61.3 (-80.0 to -43.7)** *** †††	-24.5 (-32.3 to -16.6)	-19.0 (-26.4 to -11.5)
*f*Res, Hz	Absolute (ml/hPa)	5.8 (4.1 to 7.4)	3.1 (2.1 to 4.2)	3.3 (2.0 to 4.5)
	Relative (%)	**19.1 (14.3 to 24.0)** * †	14.0 (9.8 to 18.2)	12.8 (7.3 to 18.2)
Ax, ml/hPa, Hz	Absolute (ml/hPa)	**23.8 (15.2 to 32.4)** *** †††	5.4 (2.4 to 8.3)	5.7 (3.3 to 8.0)
	Relative (%)	**46.0 (34.9 to 57.1)** †	27.1 (15.8 to 38.4)	21.2 (6.8 to 35.6)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **C** – compliance; ***f*res** – resonant frequency; **Ax** – area under the reactance curve.

PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

P_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

3.3.3 Obstructive & restrictive preterm lung disease groups

3.3.3.1 Participant characteristics

The baseline oscillometry characteristics of the two subgroups POLD and PnOLD are described in Chapter 2, Tables 2.6 and 2.7. The main differences between POLD and PnOLD were for rates of invasive ventilation and wheeze ever, as well as PnOLD having lower %FVC, and POLD having lower FEV_1/FVC and $\%FEF_{25-75\%}$.

3.3.3.2 Resistance parameters

As shown in Tables 3.5 and 3.6, for baseline oscillometry, POLD children had significantly higher resistance compared to PT_c at Rrs_{6-20} and Rrs_{20}, and higher than PnOLD and PT_c for $Fdep_{6-20}$ as absolute value (1.88 vs 0.85 vs 0.43) and ratio (1.33 vs 1.16 vs 1.09), as well as Rrs_6 (7.76 vs 6.34 vs 5.48) for POLD, PnOLD and PT_c respectively, suggesting a greater degree of peripheral airway disease in children with an obstructive picture of airway disease compared to non-obstructive lung disease. There were no differences found between PnOLD and PT_c children.

There were no changes seen post-exercise in either children with POLD or PnOLD. No group showed a greater difference in change score from baseline to post-exercise.

Following bronchodilator, all groups showed improvement on repeated measures analysis for all parameters except PnOLD for both frequency dependence, suggesting a lack of modifiable peripheral airway disease in this group. Following bronchodilator, POLD had a greater change compared to PT_c for absolute Rrs_{6-20} and Rrs_6 but not Rrs_{20}, again suggesting peripheral airways were affected by bronchodilation. Greater absolute (compared to PT_c: $Fdep_{6-20}$ 1.22 vs 0.30 hPa.s/L, $rFdep_{6-20}$ 0.21 vs 0.06) and relative (compared to PnOLD: $rFdep_{6-20}$ 13.4 vs 8.6 %) improvements were seen in the frequency dependence of resistances.

Table 3.5 Oscillometry resistance results for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c), for comparison at baseline, post-exercise and post-exercise bronchodilator.

		Baseline	Post-exercise	Post-exercise BD
Resistance parameters (POLD, n=27; PnOLD, n=15; PT_c, n=77)				
Rrs_{6-20}, hPa.s/L	POLD	**6.70 (6.17 to 7.22) ¥¥¥**	**6.55 (6.00 to 7.10) ¥¥¥**	∂∂∂ 5.36 (4.77 to 5.95)
	PnOLD	5.87 (5.17 to 6.57)	5.45 (4.72 to 6.57)	∂∂ 4.60 (3.82 to 5.39)
	PT_c	5.20 (4.89 to 5.52)	5.25 (4.93 to 5.58)	∂∂∂ 4.68 (4.33 to 5.03)
Rrs_6, hPa.s/L	POLD	**7.76 (7.14 to 8.39) § ¥¥¥**	**7.91 (7.23 to 8.59) §§ ¥¥¥**	**∂∂∂ 5.99 (5.29 to 6.68) ¥**
	PnOLD	6.34 (5.50 to 7.18)	6.16 (5.25 to 7.07)	∂∂ 4.87 (3.94 to 5.80)
	PT_c	5.48 (5.11 to 5.85)	5.63 (5.23 to 6.04)	∂∂∂ 4.90 (4.49 to 5.31)
Rrs_{20}, hPa.s/L	POLD	**5.89 (5.41 to 6.37) ¥**	5.64 (5.14 to 6.14)	∂∂ 4.94 (4.42 to 5.46)
	PnOLD	5.49 (4.85 to 6.14)	5.07 (4.40 to 5.74)	∂∂ 4.41 (3.72 to 5.11)
	PT_c	5.06 (4.77 to 5.34)	4.98 (4.69 to 5.28)	∂∂∂ 4.55 (4.24 to 4.85)
$Fdep_{6-20}$, hPa.s/L	POLD	**1.88 (1.52 to 2.23) §§ ¥¥¥**	**2.27 (1.86 to 2.68) §§ ¥¥¥**	**∂∂∂ 1.05 (0.69 to 1.41) ¥¥**
	PnOLD	0.85 (0.37 to 1.32)	1.09 (0.54 to 1.64)	0.46 (-0.03 to 0.94)
	PT_c	0.43 (0.22 to 0.64)	0.65 (0.41 to 0.90)	∂ 0.35 (0.14 to 0.56)
$rFdep_{6-20}$	POLD	**1.33 (1.26 to 1.40) § ¥¥¥**	**1.42 (1.33 to 1.50) § ¥¥¥**	**∂∂∂ 1.21 (1.13 to 1.28) ¥**
	PnOLD	1.16 (1.07 to 1.26)	1.24 (1.13 to 1.35)	1.11 (1.01 to 1.21)
	PT_c	1.09 (1.05 to 1.13)	∂ 1.14 (1.09 to 1.19)	∂ 1.08 (1.03 to 1.12)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **Rrs_{6-20}** – average respiratory system resistance 6 -20 Hz; **R / $Xrs_{6/20}$** – respiratory system resistance (R) / reactance (X) at 6 / 20 Hz; **$Fdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz; **$rFdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz with Rrs_{20} as denominator.

POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.

POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

PnOLD vs PT_c: ϕ p<0.05; ϕϕ p<0.01; ϕϕϕ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise BD: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Table 3.6 Oscillometry resistance change scores from baseline to post-exercise, and post-exercise to post-exercise bronchodilator for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c).

		POLD, n=27	PnOLD, n=15	PT_c, n=77
Resistance parameters baseline to post-exercise				
Rrs_{6-20}, hPa.s/L	Absolute (hPa.s/L)	0.15 (-0.24 to 0.53)	0.42 (-0.11 to 0.94)	-0.05 (-0.22 to 0.13)
	Relative (%)	2.0 (-4.3 to 8.3)	7.1 (-1.0 to 15.2)	-1.8 (-5.2 to 1.6)
Rrs_6, hPa.s/L	Absolute (hPa.s/L)	-0.14 (-0.72 to 0.44)	0.18 (-0.52 to 0.89)	-0.15 (-0.40 to 0.10)
	Relative (%)	-2.8 (-10.4 to 4.8)	3.5 (-5.8 to 12.7)	-4.5 (-9.4 to 0.3)
Rrs_{20}, hPa.s/L	Absolute (hPa.s/L)	0.25 (-0.06 to 0.55)	0.43 (-0.12 to 0.98)	0.07 (-0.09 to 0.24)
	Relative (%)	4.1 (-1.7 to 10.0)	7.9 (-2.0 to 17.8)	0.7 (-2.3 to 3.8)
$Fdep_{6-20}$, hPa.s/L		-0.39 (-0.80 to 0.01)	-0.24 (-0.72 to 0.23)	-0.22 (-0.42 to -0.02)
$rFdep_{6-20}$	Absolute	-0.09 (-0.16 to -0.01)	-0.08 (-0.17 to 0.02)	-0.05 (-0.10 to -0.01)
	Relative (%)	-7.5 (-13.0 to -2.0)	-6.1 (-14.0 to 1.7)	-5.6 (-9.4 to -1.7)
Resistance parameters post-exercise to post-exercise BD				
Rrs_{6-20}, hPa.s/L	Absolute (hPa.s/L)	**1.19 (0.73 to 1.66)** ¥¥	0.85 (0.50 to 1.20)	0.57 (0.37 to 0.77)
	Relative (%)	16.1 (9.4 to 22.8)	15.2 (10.0 to 20.4)	11.7 (8.2 to 15.1)
Rrs_6, hPa.s/L	Absolute (hPa.s/L)	**1.92 (1.21 to 2.64)** ¥¥	1.29 (0.46 to 2.12)	0.74 (0.46 to 1.01)
	Relative (%)	22.5 (14.3 to 30.6)	19.2 (10.4 to 27.9)	13.1 (8.7 to 17.5)
Rrs_{20}, hPa.s/L	Absolute (hPa.s/L)	0.70 (0.34 to 1.06)	0.65 (0.27 to 1.04)	0.44 (0.27 to 0.60)
	Relative (%)	10.1 (3.3 to 16.8)	11.4 (5.5 to 17.2)	9.3 (6.1 to 12.5)
$Fdep_{6-20}$, hPa.s/L		**1.22 (0.65 to 1.79)** ¥¥	0.63 (-0.01 to 1.28)	0.30 (0.10 to 0.50)
$rFdep_{6-20}$	Absolute	**0.21 (0.10 to 0.33)** ¥¥	0.13 (0.01 to 0.25)	0.06 (0.02 to 0.10)
	Relative (%)	**13.4 (6.0 to 20.8)** §	8.6 (0.4 to 16.8)	4.3 (1.1 to 7.5)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **Rrs_{6-20}** – average respiratory system resistance 6 -20 Hz; **$Xrs_{6/20}$** – respiratory system resistance (R) / reactance (X) at 6 / 20 Hz; **$Fdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz; **$rFdep_{6-20}$** – Frequency dependence of resistance between 6 – 20 Hz with Rrs_{20} as denominator.

POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.

POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

PnOLD vs PT_c: ᶲ p<0.05; ᶲᶲ p<0.01; ᶲᶲᶲ p<0.001.

3.3.3.3 Reactance parameters

As per Tables 3.7 and 3.8, group comparison results for Xrs_6 and Xrs_{20} showed a stepwise difference, with POLD most affected, followed by PnOLD compared to PT_c, (Xrs_6: -4.18 vs -2.77 vs -1.99 hPa.s/L; Xrs_{20}: -1.64 vs -0.84 vs -0.20 hPa.s/L respectively). Compliance was significantly lower in POLD and PnOLD than PT_c (6.18 vs 8.82 vs 11.91 ml/hPa). *f*res and Ax were highest in POLD children (*f*res: 31.6 vs 25.8 vs 21.4 Hz; Ax: 53 vs 27.1 vs 15.8).

Post-exercise saw no within-group changes on repeated measures or change score differences.

All groups showed an improvement for all parameters following bronchodilator, except in the PnOLD and PT_c groups for Xrs_6. Change scores for Xrs_6, relative compliance and absolute Ax were greater in POLD compared to both PnOLD and PT_c. Xrs_{20} and absolute *f*res were greater for POLD than PT_c. Change score in Xrs_6 for POLD was in fact ~threefold greater compared to PnOLD and ~sixfold greater compared to PT_c.

Table 3.7 Oscillometry reactance results for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c), for comparison at baseline, post-exercise and post-exercise bronchodilator.

		Baseline	Post-exercise	Post-exercise BD
Reactance parameters (POLD, n=27; PnOLD, n=15; PT_c, n=77)				
Xrs_6, hPa.s/L	POLD	**-4.18 (-4.60 to -3.76)** §§§ ¥¥¥	**-4.39 (-4.98 to -3.80)** § ¥¥¥	∂∂∂ **-2.52 (-3.01 to -2.03)** ¥
	PnOLD	**-2.77 (-3.33 to -2.20)** ϕ	-2.94 (-3.73 to -2.15)	-2.34 (-3.00 to -1.68)
	PT_c	-1.99 (-2.24 to -1.74)	-2.08 (-2.43 to -1.74)	-1.77 (-2.06 to -1.47)
Xrs_{20}, hPa.s/L	POLD	**-1.64 (-1.97 to -1.31)** § ¥¥¥	**-1.60 (-1.97 to -1.23)** § ¥¥¥	∂∂∂ **-0.50 (-0.83 to -0.18)** ¥
	PnOLD	**-0.84 (-1.28 to -0.40)** ϕ	-0.70 (-1.20 to -0.21)	∂∂∂ 0.05 (-0.39 to 0.48)
	PT_c	-0.20 (-0.39 to 0.00)	-0.31 (-0.53 to -0.09)	∂∂∂ 0.07 (-0.12 to 0.26)
C, ml/hPa	POLD	**6.18 (4.66 to 7.70)** ¥¥¥	**5.82 (4.31 to 7.33)** § ¥¥¥	∂∂∂ **9.54 (7.59 to 11.49)** ¥¥
	PnOLD	**8.82 (6.78 to 10.86)** ϕ	9.37 (7.34 to 11.40)	∂ 12.09 (9.47 to 14.70)
	PT_c	11.91 (11.01 to 12.81)	11.48 (10.58 to 12.37)	∂∂∂ 13.86 (12.71 to 15.02)
*f*Res, Hz	POLD	**31.6 (29.1 to 34.2)** § ¥¥¥	**30.2 (27.6 to 32.8)** § ¥¥¥	∂∂∂ **24.0 (21.5 to 26.5)** ¥¥
	PnOLD	25.8 (22.3 to 29.2)	23.9 (20.3 to 27.4)	∂∂∂ 18.9 (15.5 to 22.2)
	PT_c	21.4 (19.9 to 22.9)	22.2 (20.7 to 23.8)	∂∂∂ 19.1 (17.6 to 20.6)
Ax, ml/hPa	POLD	**53.0 (45.3 to 60.6)** §§§ ¥¥¥	**53.7 (45.0 to 62.4)** §§§ ¥¥¥	∂∂∂ 23.0 (16.8 to 29.2)
	PnOLD	27.1 (16.9 to 37.4)	26.4 (14.8 to 38.1)	∂ 15.1 (6.8 to 23.4)
	PT_c	15.8 (11.5 to 20.2)	18.5 (13.6 to 23.5)	∂ 13.2 (9.6 to 16.8)

Results expressed as mean and 95% confidence intervals for continuous data (two-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **C** – compliance**.** ***f*res** – resonant frequency. **Ax** – area under the reactance curve.

POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.

POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

PnOLD vs PT_c: ϕ p<0.05; ϕϕ p<0.01; ϕϕϕ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise BD: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Table 3.8 Oscillometry reactance change scores from baseline to post-exercise, and post-exercise to post-exercise bronchodilator for obstructive (POLD) and non-obstructive (PnOLD) preterm lung disease groups compared with preterm controls (PT_c)

		POLD, n=27	PnOLD, n=15	PT_c, n=77
Reactance parameters baseline to post-exercise				
Xrs_6, hPa.s/L		0.21 (-0.28 to 0.69)	0.17 (-1.10 to 1.44)	0.10 (-0.10 to 0.30)
Xrs_{20}, hPa.s/L		-0.04 (-0.30 to 0.23)	-0.14 (-0.49 to 0.21)	0.12 (-0.03 to 0.26)
C, ml/hPa	Absolute (ml/hPa)	0.35 (-0.20 to 0.91)	-0.55 (-1.65 to 0.54)	0.44 (-0.33 to 1.20)
	Relative (%)	2.6 (-7.3 to 12.5)	-5.9 (-20.8 to 9.0)	0.6 (-5.5 to 6.7)
*f*Res, Hz	Absolute (ml/hPa)	1.4 (-0.4 to 3.2)	1.9 (0.0 to 3.8)	-0.8 (-2.2 to 0.6)
	Relative (%)	3.5 (-1.6 to 8.7)	7.0 (0.1 to 14.0)	-6.7 (-13.4 to 0.0)
Ax, ml/hPa, Hz	Absolute (ml/hPa)	-0.7 (-7.5 to 6.1)	0.7 (-7.5 to 8.9)	-2.6 (-5.4 to 0.1)
	Relative (%)	-5.4 (-19.4 to 8.5)	10.7 (-8.7 to 30.0)	-47.1 (-82.1 to -12.1)
Reactance parameters post-exercise to post-exercise BD				
Xrs_6, hPa.s/L		**-1.87 (-2.68 to -1.06)** §§ ¥¥¥	-0.6 (-1.19 to 0.00)	-0.32 (-0.52 to -0.12)
Xrs_{20}, hPa.s/L		**-1.10 (-1.56 to -0.64)** ¥¥¥	-0.75 (-1.02 to -0.47)	-0.38 (-0.53 to -0.24)
C, ml/hPa	Absolute	-3.72 (-4.93 to -2.51)	-2.71 (-4.05 to -1.38)	-2.39 (-3.26 to -1.52)
	Relative (%)	**-77.1 (-103.1 to -51.2)** §§ ¥¥¥	-32.8 (-51.3 to -14.2)	-24.5 (-32.3 to -16.6)
*f*Res, Hz	Absolute (ml/hPa)	**6.2 (3.8 to 8.6)** ¥	5.0 (3.0 to 7.0)	3.1 (2.1 to 4.2)
	Relative (%)	18.3 (11.5 to 25.1)	20.6 (13.5 to 27.7)	14.0 (9.8 to 18.2)
Ax, ml/hPa, Hz	Absolute (ml/hPa)	**30.7 (18.2 to 43.1)** §§ ¥¥¥	11.3 (5.3 to 17.4)	5.4 (2.4 to 8.3)
	Relative (%)	46.2 (30.0 to 62.5)	45.5 (32.2 to 58.8)	27.1 (15.8 to 38.4)

Results expressed as mean and 95% confidence intervals for continuous data (one-way ANOVA with Bonferroni correction). **Abbreviations: BD –** bronchodilator; **C** – compliance**.** ***f*res** – resonant frequency. **Ax** – area under the reactance curve.

POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.

POLD vs PT_c: ¥ p<0.05; ¥¥ p<0.01; ¥¥¥ p<0.001.

PnOLD vs PT_c: ᶲ p<0.05; ᶲᶲ p<0.01; ᶲᶲᶲ p<0.001.

Figure 3.6 Graphic displaying the oscillometry spectra for resistance and reactance at a) baseline, b) post-exercise and c) post-exercise bronchodilator (BD) for preterm obstructive (POLD) and non-obstructive (PnOLD) lung disease groups, and preterm (PT_c) and term (T_c) controls.

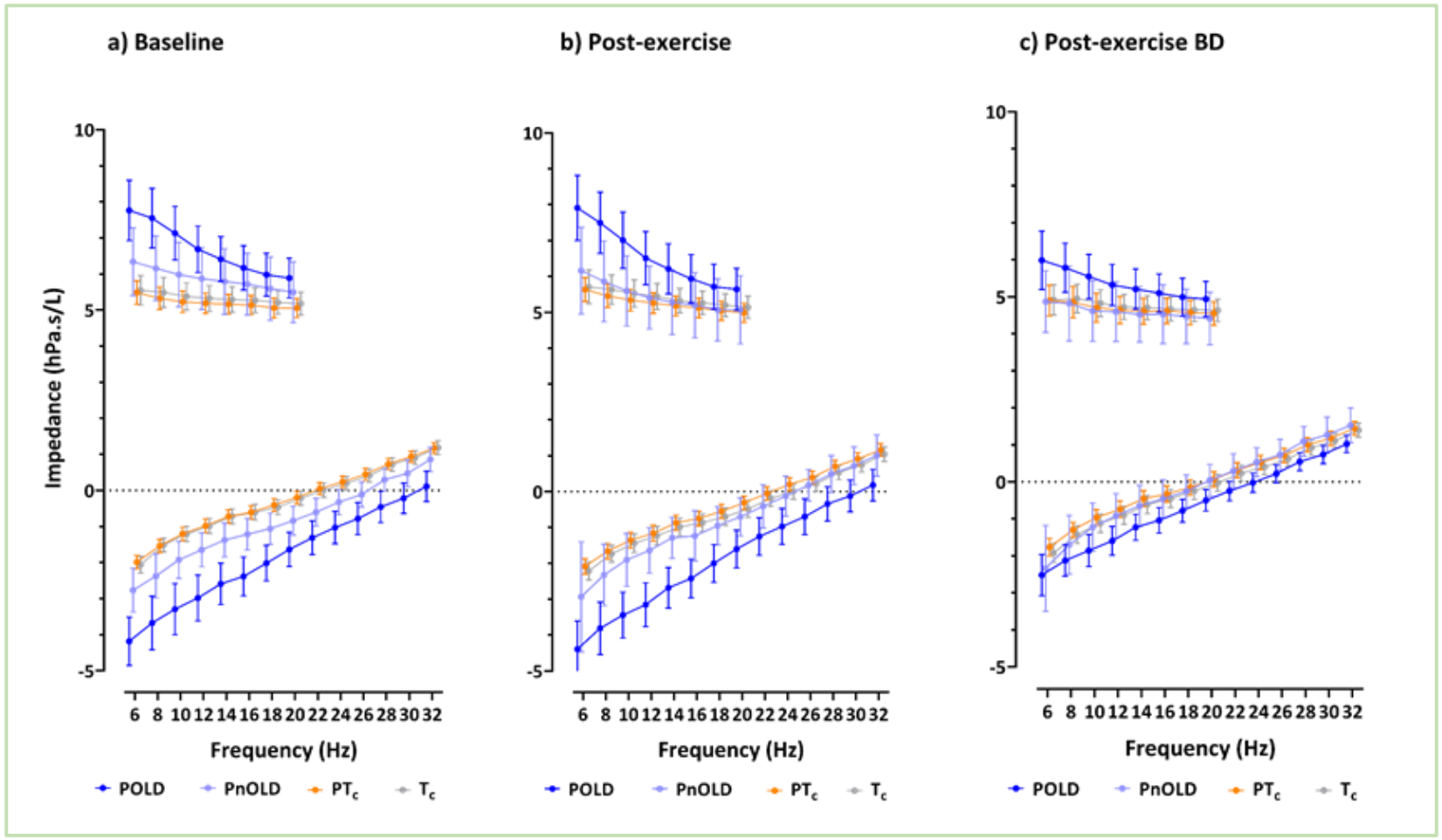
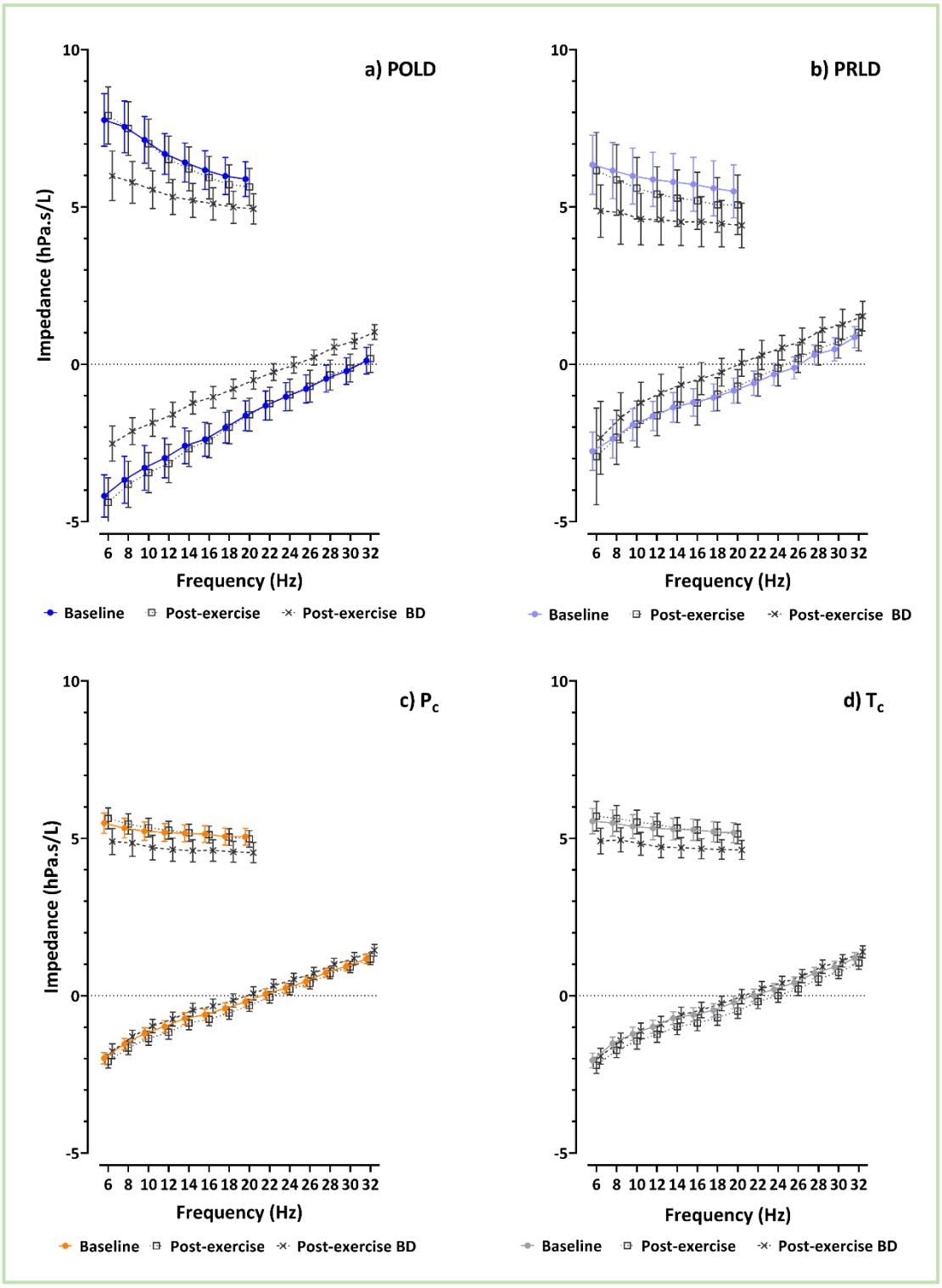


Figure 3.7 Graphic displaying the oscillometry spectra for resistance and reactance for a) preterm obstructive (POLD) and b) non-obstructive (PnOLD) lung disease groups, and c) preterm (PT_c) and d) term (T_c) controls at baseline, post-exercise and post-exercise bronchodilator (BD).



3.4 Discussion

This chapter has assessed the mechanical properties of the airways using oscillometry, including responses to exercise for the first time in this population, and following post-exercise bronchodilator. I have demonstrated that preterm-born children, when stratified by current lung function, have impaired baseline respiratory mechanics when compared to term controls. I have found that exercise has no major effect on oscillometry parameters in either preterm or term children, however both preterm and term children show varying degrees of bronchodilator response. The greatest response was seen in preterm children with current lung function deficits on spirometry.

Additionally, these results are amplified when assessing children with low lung function base on whether they have obstructive or non-obstructive lung disease. The results show the peripheral airways were greatest affected, with resistances and reactances at the lower frequencies showing greater differences between groups, and greater responses in affected children following bronchodilator.

Stratifying children by current lung function, as opposed to potential risk factors for later disease such as CLD, is potentially an important reframing of how we should follow-up children born preterm. This is a change from the traditional focus on those children with a historical diagnosis of CLD (Um-Bergström et al., 2017), or those born at extremes of gestation such as with the EXPRESS study (Thunqvist et al., 2018) or EPICure group (Lum et al., 2011). Given lung function status is associated with aerobic fitness (Hancox and Rasmussen, 2018), stratifying children by current lung function may be a more important way of assessing for differences that may impact on a child clinically. These data show that preterm children with low lung function in fact have widespread differences across oscillometry parameters compared to both 'healthy' preterm and term children.

Spirometry is known to correlate with oscillometry (Broström et al., 2010, Malmberg et al., 2000), and so identifying impaired respiratory mechanics in a group of preterm children stratified by lung function is unsurprising. However, as spirometry cannot be

performed reliably in younger children, oscillometry findings may be able to identify those children who have impaired lung function at an earlier stage.

The baseline oscillometry revealed that while the PT_{low} group, expectedly, had impaired oscillometry, of interest was that the lower frequencies were affected greater than the medium frequencies, suggesting that peripheral lung disease is a major factor for airway disease in preterm-born children. This has been shown to be the case in uncontrolled asthma (Heijkenskjöld Rentzhog et al., 2017), however this cohort does not match with my description clinically, suggesting a different disease mechanism. Reactance, particularly at lower frequencies, i.e., elastic properties of the respiratory system, appeared to be impaired; indeed this was reflected in the low compliance values observed in the PT_{low} group.

Of particular interest were the outcomes following exercise. While exercise is known to potentially cause bronchoconstriction in preterm children (Joshi et al., 2013), its impact on airway mechanics has not been reported previously. My data show that exercise does not appear to affect airway mechanics in either preterm or term children. There are some possible explanations behind these findings. Oscillometry was performed at 20 minutes after exercise when exercise-induced bronchoconstriction (EIB) is at its peak (Joshi et al., 2013). It is possible that any effects of exercise on the lung mechanics were present at an earlier stage after the cycle test, however the corresponding spirometry results also showed a lack of EIB.

Another possible explanation is that any bronchoconstrictive effects of exercise were countered by an adrenaline surge resulting from exercise. Adrenaline can act on the β-2 receptors in the airway smooth muscle and may reverse any increased tone from exercise. Dynamic hyperinflation has been found to occur in people with asthma during exercise (Kosmas et al., 2004), causing an increase in FRC. Given airway resistance decreases with increasing lung volume (Levitzky, 2018), if 'splinting' of airways occurs as a result of exercise, the increased airway size could again counteract any bronchoconstriction resulting in static impedance values. Airway resistance has been found to improve during exercise in a study of children with asthma (Mansfield et al., 1979), so a similar response may occur in children with lung pathology from preterm birth.

Bronchodilator use has previously been assessed in a range of respiratory conditions using oscillometry (Thamrin et al., 2007). While our preterm cohort's relative change in impedance may be lower than some of the recommended changes required for a positive response (King et al., 2020), there is still a greater change compared to controls. Reactance values, in particular at lower frequencies (i.e., 6 Hz) showed the greatest difference compared to controls, with three-fold greater decreases. Additionally, a greater drop in Fdep was observed, potentially suggesting that there is a bronchodilator action on the peripheral airways, possibly a result of smooth muscle extension distally as a result of remodelling from preterm birth (Hislop and Haworth, 1990, Margraf et al., 1991).

The greatest effects were observed when investigating the POLD and PnOLD groups. There was a clear step down in terms of impaired oscillometry, with POLD worst affected, followed by PnOLD, preterm controls and term controls in that order. Again, low frequencies were particularly involved, in both baseline results, and in post-exercise bronchodilator response. This reinforces the need to identify these children and assess whether their disease process is potentially modifiable with medications.

3.4.1 Strengths and Limitations

The main strength of this study is assessing functional outcomes based on a clinically-relevant current measure (lung function) rather than on a diagnosis made several years prior that may have limited bearing for current health. Additionally, while it is common with other lung disease to assess whether it is obstructive or restrictive in nature, this has not been the case in children with respiratory sequelae of preterm birth. This should be an ongoing clinical, as well as academic, focus.

One limitation regarding oscillometry is use of raw values rather than Z-scores from a reference range. While several studies have developed reference values within their own populations (Calogero et al., 2013), these do not always fit when applied to other populations (Shackleton et al., 2012). This results in individual studies developing normal values using regression equations to account for demographic factors within individual populations. As such, this limits comparisons to between

groups from within a given study, rather than a comparison with 'normality'. Standardised references are recognised to be necessary, however are still in the process of being developed (King et al., 2020).

Bias will be discussed in detail in the final discussion chapter.

3.5 Conclusions

In summary, preterm-born children with low lung function had greater impairment of airway mechanics compared to healthy children. This was particularly noticeable in children with obstructive lung disease. The oscillometry results showed peripheral airway disease appeared to be present in these children, and that this disease is responsive to post-exercise bronchodilator therapy. Although the exact mechanism behind lung dysfunction (structural versus inflammation) still needs to be clarified, it is unlikely to be a single pathology, and is dependent on a variety of individual perinatal and postnatal exposures.

4 INTRA-BREATH OSCILLOMETRY

4.1 Introduction

As outlined in the previous chapter, standard oscillometry using pseudorandom noise is an extremely useful tool for identifying differences in airway mechanics between populations. It has also been demonstrated that its use can be beneficial for improving understanding of disease pathology, including the peripheral airway disease identified in this cohort of preterm-born children. However, oscillometry use has advanced from its standard form and novel ways of analysing airway mechanics have been developed.

4.1.1 Technical aspects to test

Standard oscillometry is useful for looking across frequencies (which gives the information about locality of disease within the airway), however does not show what happens within each breath. This information may be able to further differentiate between pathological entities (Sly and Hantos, 2018). As such a modified form of oscillometry has been developed, known as intra-breath or temporal oscillometry (Czövek et al., 2016, Lorx et al., 2017). This differs from standard oscillometry and uses a single frequency to assess respiratory impedance, again delivered as a superimposed sine wave on top of normal respiration. In this case though, by measuring the wave reflections at frequent intervals throughout the breath cycle, changes in impedance during this time can be identified. This may reveal variations from normal in disease processes affecting the respiratory system. This approach is unique to oscillometry – there is currently no other tool that can assess this.

Underlying this technique is firstly being able to identify what happens to the impedance of the respiratory system during each breath cycle, and an understanding of how disease may then affect normality. This can be postulated based on knowledge of the respiratory cycle. As previously outlined, resistance is partly reflective of lung volume. As such, it would be expected that due to increased airway size at the end of inspiration, the resistance would be lower than at the end of expiration. Expiration itself can be limited by airway disease, particularly that of

airway obstruction, therefore in disease processes consistent with airway obstruction such as asthma, a slower rise to peak expiratory flow would potentially be expected, and the impedance at this point of peak flow would be considered flow-dependent, when compared to peak flow during inspiration (Sly and Hantos, 2018). Additionally, resistance in the airways would be expected to be greater during expiration in healthy children, and effect potentially amplified in obstructive disease processes, when compared to inspiration.

4.1.2 Use of test in other populations

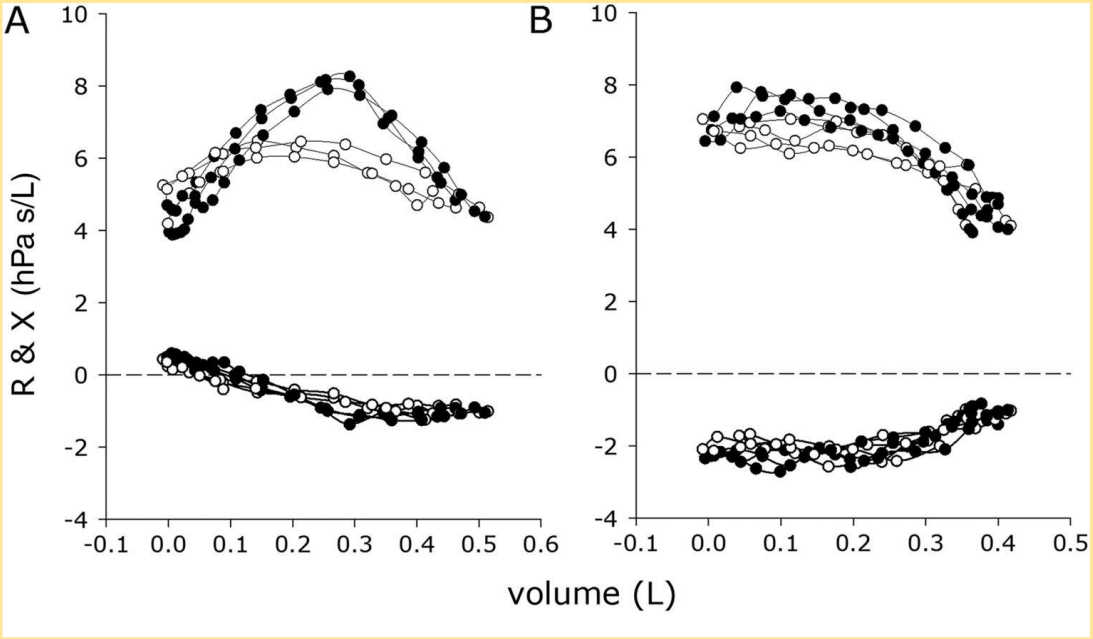
The use of intra-breath oscillometry has been limited so far. It is still to some degrees an exploratory technique, and as such there is yet to be any written consensus on how this should be performed. Indeed, the terminology itself can be confusing, with overlapping techniques using the similar names, but applying the test to differing extents. Examples of the names include within-breath, intra-breath or temporal (T-) oscillometry; however, where "within-breath" oscillometry has largely been used, this technique has grossly looked at the differences between expiratory or inspiratory impedance as a whole, rather than looking at the changes that occur within these components of respiration (Sol et al., 2019, Zannin et al., 2019). However, it is feasible to apply the same principles to be able to track the impedance throughout respiration, including at the points of volume or flow dependence.

Whilst this technique has not been explored in a preterm population, it has been utilised successfully in other populations. This includes a cohort of over 600 healthy infants in South Africa, where volume dependence of resistance and reactance (principle components of the airway impedance) were associated with increased risk of respiratory morbidity such as lower respiratory tract infection or wheezing during the first year of life (Gray et al., 2019). Use of this intra-breath impedance tracking has been used in a population of adults with Chronic Obstructive Pulmonary Disease (COPD) (Lorx et al., 2017), classically known as an obstructive form of lung disease. One of the potential applications of this test was demonstrated in this cohort study of 55 adults with COPD compared to 20 controls. It is possible to plot the impedance (in terms of resistance or reactance) against the volume in both inspiration and

expiration (as seen in Figure 4.1). This can be used to visually demonstrate and mathematically calculate an area within the impedance-volume loop, for example the area between the resistance in expiration vs volume and compared to the resistance in inspiration vs volume, which then gives an idea about the overall difference in resistance between the two stages of respiration. This same principle can also be applied to reactance. The main finding in this study by Lorx *et al* found an increased area within the reactance–volume loop, which was felt to be suggestive of expiratory flow limitation, consistent with airway obstruction. This flow limitation was then demonstrated to be improved with the use of continuous positive airway pressure (CPAP) (Lorx et al., 2017).

Preterm-born children are clearly a very different population group compared to the above COPD population, with disease that bears clinical similarities, but differing pathophysiology, to that of other childhood wheeze disorders, namely asthma. Therefore, comparing intra-breath parameters for similarities or differences to those seen in term-born children with wheeze or asthma is important. Tracked impedance measures in a pre-school population of 31 children with acute wheeze identified various potential impedance markers of disease that differed from 75 healthy children. Cut-offs for disease within these parameters were determined using ROC curves and were then used in a subgroup of 20 children with recurrent wheeze, but symptom free at time of testing. This showed that the ΔR_e measurement (difference in resistance at end-expiration compared to end-inspiration) had the best sensitivity/specificity for disease (Czövek et al., 2016). Given the overlap of symptoms in preterm-associated lung disease and wheeze or asthma, it would be reasonable to assume that if it were the same underlying disease process, preterm-born children would also exhibit the same increased ΔR_e as seen in wheeze.

Figure 4.1 Demonstration of appearance of impedance-volume loops, as tracked throughout the respiratory cycle, with open circles representing inspiration and closed circles representing expiration (Czövek et al., 2016). End-expiration is seen at 0 L volume, with end-inspiration at the highest volume. The impedance difference between these two points can be calculated. Additionally, the area with the loop can also be calculated. Image reproduced with permission of the rights holder.



4.1.3 Hypotheses and aims

Hypotheses:

1) Preterm-born children with evidence of airway disease (i.e., FEV_1 ≤85% predicted) will show a different pattern of airway disease on intra-breath impedance tracking compared to impedance patterns identified in children with wheeze;
2) Preterm-born children with evidence of obstructive disease will be distinguished from their non-obstructive counterparts using intra-breath oscillometry.

Aims:

1) To identify normal intra-breath impedance patterns in a term-born population of school aged-children;

2) To compare resistance and reactance measurements at 10Hz between standard and intra-breath oscillometry;
3) To compare intra-breath oscillometry between lung function and obstructive lung disease groups for differences at baseline, post-exercise, and post-exercise bronchodilator.

4.2 Methods

4.2.1 Population

The same population from the Respiratory Health Outcomes in Neonates (RHiNO) trial was used for this section of the study. Recruitment has been described in greater depth in Chapter 2, section 2.2.1. Children were designated into groups based on spirometry performed during a visit to the Noah's Ark Children's Hospital for Wales in Cardiff. Preterm-born children were designated PT_{low} if their $\%FEV_1$ at this visit was ≤85%. Preterm-born children with $\%FEV_1$ >85% who were within the first 10 screening visits of a calendar month were eligible to attend as preterm controls. If a preterm child was invited as PT_{low} but their FEV_1 on the day of testing was >85% they were deemed eligible to continue as preterm controls (PT_c) if they had been within the first 10 screening visits of a given month. Term controls (T_c) were eligible if they had $\%FEV_1$ >90% at testing.

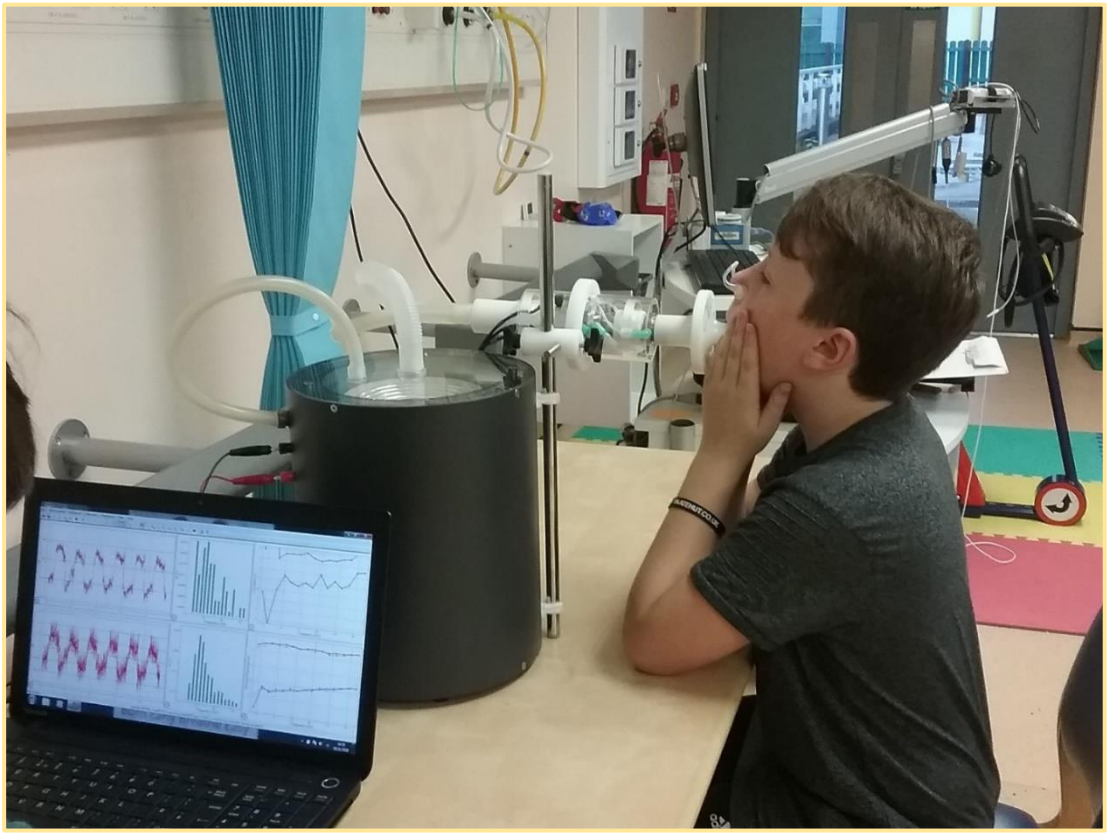
4.2.2 Intra-breath oscillometry testing

Intra-breath oscillometry testing was performed using the same custom-built set-up and computer programme (NDAQ) as per the standard oscillometry testing, as developed by the team at University of Szeged in Hungary. The set-up comprised of a loudspeaker connected to a wave-tube. Within the wave-tube were sensors to measure pressure and flow at the airway opening. Due to the potential of increased breathing frequency and pressure from exercise affecting the soundwaves, the set-up was modified to negate this effect. The loudspeaker was encased within a larger, sealed cylinder with a 'blow-off' tube connecting the top of the speaker to the cylinder below the speaker, essentially allowing pressure to equalise above and below the speaker diaphragm (see Figure 3.1 in Chapter 3).

Prior to testing I explained to the children what the test was for and how it would be performed. The test was performed with the child sat upright in a chair and attached to the system via a Microgard II microbial filter (Vyaire, Germany). They wore a nose clip during testing and were instructed to firmly hold their cheeks with their fingers and palms of hands to stop any soft tissue vibrations affecting the results. Figure 4.2

displays an example of a participant performing the test. Via the NDAQ system, the loudspeaker then generated a single frequency sine wave at 10 Hz, superimposed onto tidal breathing. Impedance was measured at the mouth using the pressure and flow sensors, at intervals of 0.1 seconds. This impedance was measured 'in-phase' with the soundwaves (representing airway resistance) and 'out-of-phase' from the input (representing airway reactance). A recording lasting 23.5 seconds was made and repeated 2 further times (or more in the event of poor recording quality), with the aim of obtaining artefact-free recordings with regular tidal breathing. Figure 4.3 shows a screenshot of the NDAQ display generated following the test (note the irregular breathing pattern at the start of this trace).

Figure 4.2: Photograph of oscillometry being performed by study participant. Consent obtained from parent for image and use in published works.



Intra-breath oscillometry was performed at 3 time points. An initial baseline reading was obtained. A second test was performed at 20 minutes following maximal exercise testing. A final test was performed 15 minutes after administration of 400µg of

Salbutamol, a β2 receptor agonist (Salamol, Teva UK Limited) , given at ~45 minutes post-exercise.

I performed blinded, post-acquisition analysis of the intra-breath oscillometry data to obtain results. I assessed each of the recordings for quality and selected artefact-free segments for analysis. Potential artefacts affecting quality of recording included lack of continuous breathing pattern (i.e., breath holding), coughing, blocking the filter with the tongue, increased force of tidal breaths, or leaks around the mouthpiece. Figure 4.4 displays a trace of the same test as Figure 4.3; however, the initial section of artefact has been removed. The results from the recording with the most regular respiratory pattern was then used for data analysis. Results were automatically generated by the software and imported into Excel (Version Excel 365, Microsoft, USA). For each parameter where a result was generated, this represented the mean value as averaged from all the individual breath cycles of that particular recording (for example, the resistance at end-expiration would be given for each breath cycle, and then the mean was calculated).

Figure 4.3 Screenshot of the NDAQ software display showing flow and pressure readings over time (left side of picture), and the impedance over time, with red representing resistance measurements and blue showing reactance measurements (right side of

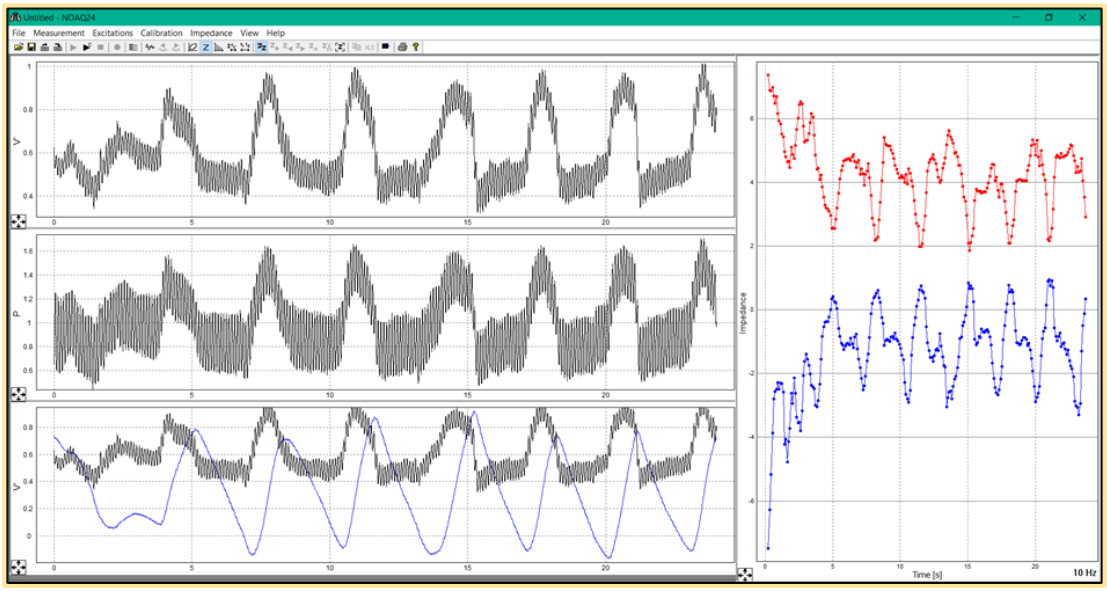
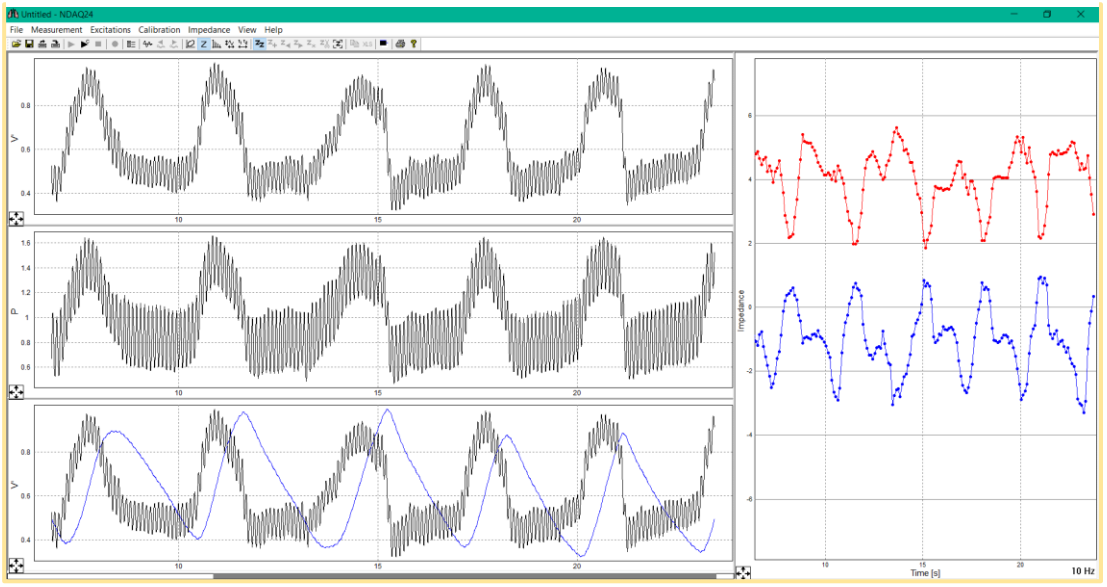


Figure 4.4 Screenshot of the NDAQ software displaying same test, however with the initial artefact removed to obtain flow-pressure and impedance traces with regular respiratory pattern.



4.2.3 Parameters

4.2.3.1 Respiratory cycle

Table 4.1 outlines the parameters of interest for the respiratory cycle, i.e., duration of breath, tidal volume, and respiratory rate.

Table 4.1 Outline of parameters of interest concerning the respiratory cycle

Parameter (units)	Abbreviation
Expiration duration (sec)	T_E
Expiration duration as proportion of full breath duration	T_E/T_{Tot}
Time to maximum expiratory flow as proportion of expiratory time	$T_{V'maxE}/T_E$
Tidal volume (L)	TV
Respiratory rate (breaths per minute, bpm)	F_{br}

Abbreviations: **T_E** – Expiration time**; T_E/T_{Tot}** – Expiration time as proportion of total breath time; **$T_{V'maxE}/T_E$ –** Time to maximum expiratory flow as proportion of expiratory time; **TV** – Tidal volume; **F_{br}** – breathing frequency.

4.2.3.2 Resistance parameters

Table 4.2 outlines the parameters of interest for resistance during inspiration, expiration, and the differences between the two stages of the respiratory cycle.

Table 4.2 Parameters under investigation for resistance

Parameter (unit)	Abbreviation		
	Inspiration	Expiration	Difference between expiration and inspiration
Mean resistance (hPa.s/L)	R_{meanI}	R_{meanE}	ΔR_{mean}
Resistance at maximum flow (hPa.s/L)	$R_{V'maxI}$	$R_{V'maxE}$	$\Delta R_{V'max}$
Resistance at end of breath cycle (hPa.s/L)	R_{eI}	R_{eE}	ΔR_e
Range of resistance from maximum to minimum (hPa.s/L)	ΔR_I	ΔR_E	
Difference between resistance at end of breath cycle and resistance at maximum flow rate in next breath cycle (hPa.s/L)	$\Delta R_{eE:V'maxI}$	$\Delta R_{eI:V'maxE}$	
Area within resistance-volume loop (hPa.s)			ARV
Area within resistance-flow loop			ARV'

Abbreviations: **E** – Expiration; **I** – Inspiration; **Δ** – Difference; **R_{meanI}** – Mean resistance; **$R_{V'max}$** – Resistance at maximum flow; **ARV** – Area within resistance-volume loop; **ARV'** – Area within resistance-flow loop.

4.2.3.3 Reactance parameters

Table 4.3 outlines the parameters of interest for reactance during inspiration, expiration, and the differences between the two stages of the respiratory cycle.

Table 4.3 Parameters under investigation for reactance

Parameter (unit)	Abbreviation		
	Inspiration	Expiration	Difference between expiration and inspiration
Mean reactance (hPa.s/L)	X_{meanI}	X_{meanE}	ΔX_{mean}
Reactance at maximum flow (hPa.s/L)	$X_{V'maxI}$	$X_{V'maxE}$	$\Delta X_{V'max}$
Reactance at end of breath cycle (hPa.s/L)	X_{eI}	X_{eE}	ΔX_{e}
Range of resistance from minimum to maximum (hPa.s/L)	ΔX_{I}	ΔX_{E}	
Difference between reactance at end of breath cycle and reactance at maximum flow rate in next breath cycle (hPa.s/L)	$\Delta X_{eE:V'maxI}$	$\Delta X_{eI:V'maxE}$	
Area within reactance-volume loop (hPa.s)			AXV
Area within reactance-flow loop			AXV'

Abbreviations: E – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{X_{meanI}}$ – Mean reactance; $\mathbf{X_{V'max}}$ – Reactance at maximum flow; **AXV** – Area within reactance-volume loop; **AXV'** – Area within reactance-flow loop.

4.2.4 Spirometry and cardiopulmonary exercise testing (CPET)

Cardiopulmonary exercise testing was performed on a Paediatric Cycle Ergometer (Lode, Netherlands) linked to a MasterScreen CPX system (Vyaire Medical, Germany) and is described in greater depth in Chapter 2, section 2.2.2.7. A test was deemed to be 'maximal' if it met ≥2/4 of the following criteria:

- Respiratory Exchange Ratio (RER) >1.00;
- Heart rate (HR) ≥80% predicted (220 bpm – age);
- ≥9/10 on OMNI scale (pictorial scale for rating of perceived exertion (Barkley and Roemmich, 2008));
- Peak oxygen uptake ($\dot{V}O_2$) plateau based on visual analysis.

4.2.5 Ethical approval

Ethics approval for the RHiNO study was granted by the South West-Central Bristol Ethics Committee (Ref 15/SW/0289). Parents and children (where possible) provided written consent/assent.

4.2.6 Statistical analysis

Independent t-tests were used for two group comparisons and one-way ANOVA with Bonferroni correction for multiple groups comparisons. Categorical data were assessed using Pearson's χ^2 tests. Within-group (baseline, post-exercise, and post-exercise bronchodilator) and between group comparisons across time points were compared using two-way mixed ANOVA which included repeated measures across time points, with Bonferroni correction. Change scores (differences from baseline to post-exercise, and from post-exercise to post-exercise bronchodilator) between groups were compared using one-way ANOVA with Bonferroni correction. Statistical analysis was performed using SPSS version 26 (IBM, USA). p-value of <0.05 was considered significant.

4.3 Results

4.3.1 Normal (term) children – changes during the breath cycle

4.3.1.1 Demographic details

70 term children attended for in-depth lung function testing, including the intra-breath oscillometry. These children comprised of 37 (53%) males, mean age 10.5 decimal years, height 143.9 cm, height z-score 0.48, and weight 37.9 kg. Included were 5 patients with a doctor's diagnosis of asthma, although not all of these were currently using salbutamol. Slightly over a quarter had experienced wheeze at some point. Mean spirometry scores for these children were greater than 100% predicted for $\%FEV_1$ (104.6%) and %FVC (108%), while mid-flows were 94.7%. Demographic details are summarised in table 4.4.

Table 4.4 Demographic details of term-born children performing intra-breath oscillometry.

Demographic	**Term control (T_c)**
Age, years	10.5 (10.2 to 10.7)
Male, n (%)	37 (53%)
Height, cm	143.9 (141.6 to 146.1)
Height, z-score	0.48 (0.25 to 0.72)
Weight, kg	37.9 (35.4 to 40.4)
Doctor-diagnosed asthma, n (%)	5 (7%)
Wheeze ever, n (%)	19 (27%)
Recent wheeze, n (%)	9 (13%)
Current salbutamol use, n (%)	4 (6%)
FEV_1 baseline, % predicted	104.6 (102.5 to 106.7)
FVC baseline, % predicted	108.0 (105.6 to 110.3)
FEV_1/FVC, ratio	0.85 (0.83 to 0.86)
$FEF_{25-75\%}$ baseline, % predicted	94.7 (90.2 to 99.2)
Completed baseline intra-breath oscillometry	68 (97%)

For continuous data, means and 95% CI of mean are displayed. **Abbreviations: FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** – forced expiratory flow between 25-75% of FVC.

From these 70 children, 68 successfully completed baseline intra-breath oscillometry. Of the 2 children in whom oscillometry was not obtained, one was due to poor quality of test, and the other due to an equipment issue.

4.3.1.2 Respiratory cycle overview

Expiration comprised just over half of the entire breath cycle, lasting under 2 seconds on average, with a respiratory rate of ~20 bpm. Tidal volumes were slightly high at 600 mls. Maximum flow was reached one-third into expiration, and there was similar flow rate in inspiration and expiration. These results are summarised in Table 4.5.

Table 4.5 Respiratory parameters of term-born children performing intra-breath oscillometry.

Parameter	Expiration
T_E (seconds)	1.74 (1.60 to 1.89)
T_E/T_{Tot}	0.52 (0.51 to 0.53)
$T_{V'maxE}/T_E$	0.34 (0.32 to 0.36)
V'_I (L/s)	0.60 (0.54 to 0.66)
V'_E (L/S)	-0.60 (-0.67 to -0.52)
TV (litres)	0.60 (0.54 to 0.66)
F_{br} (bpm)	19.9 (18.3 to 21.5)

Means and 95% CI of mean are displayed. **Abbreviations:** **T_E** – Expiration time; **T_E/T_{Tot}** – Expiration time as proportion of total breath time; **$T_{V'maxE}/T_E$** – Time to maximum expiratory flow as proportion of expiratory time; V'_I - Inspiratory flow rate; V'_E - Expiratory flow rate; **TV** – Tidal volume; **F_{br}** – breathing frequency.

4.3.1.3 Resistance parameters

Resistance was greater throughout and at the end of expiration in these term-born children, compared to inspiration, as shown in Table 4.6. Overall (mean) resistance was 5.70 hPa.s/L in expiration compared to 4.94 hPa.s/L in inspiration. This was a difference of 0.76 hPa.s/L between mean inspiratory and expiratory resistance. Resistance at zero flow (i.e., volume dependent) states was greater at end-expiration than end-inspiration, as would be expected due to the lower lung volume at these respective times. The mean difference between the resistance at end-expiration (R_{eE}) and at end-inspiration (R_{eI}), or ΔR_e, was 0.70 hPa.s/L.

There was an increase in resistance from the zero-flow state to when flow was maximal in both stages of the respiratory cycle. In inspiration (i.e., following end-expiration), resistance at max flow was 5.35 hPa.s/L. This compares to a peak flow

resistance of 6.17 hPa.s/L in expiration. However, as expiratory flow follows end-inspiration, this means there was an increase in resistance of 1.84 hPa.s/L (42% increase from zero-flow resistance), compared to an increase of 0.32 hPa.s/L from end-expiratory to peak inspiratory flow resistance (6% increase from zero-flow resistance).

Table 4.6 Resistance parameters of term-born children performing intra-breath oscillometry.

Parameter	Inspiration	Expiration	ΔE:I
R_{mean} (hPa.s/L)	4.94 (4.58 to 5.29)	5.70 (5.26 to 6.13)	0.76 (0.58 to 0.94)
$R_{V'max}$ (hPa.s/L)	5.35 (4.95 to 5.75)	6.17 (5.69 to 6.64)	0.81 (0.61 to 1.02)
R_e (hPa.s/L)	4.33 (4.03 to 4.62)	5.03 (4.68 to 5.38)	0.70 (0.50 to 0.89)
ΔR (hPa.s/L)	1.75 (1.51 to 1.99)	2.40 (2.08 to 2.72)	
$\Delta R_{e:}R_{V'max}$ (hPa.s/L)	0.32 (0.13 to 0.52)	1.84 (1.56 to 2.11)	
ARV (hPa.s)	-0.53 (-0.67 to -0.39)		
ARV'	1.71 (1.37 to 2.06)		

Means and 95% CI of mean are displayed. **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{R_{mean}}$ – Mean resistance; $\mathbf{R_{V'max}}$ – Resistance at maximum flow; **ARV** – Area within resistance-volume loop; **ARV'** – Area within resistance-flow loop.

4.3.1.4 Reactance parameters

Table 4.7 shows there was less difference seen comparing inspiration and expiration for reactance. At this frequency of 10 Hz in this age group, reactance remained negative, consistent with compliance having the predominant effect at this frequency. As such, a 'more negative' reactance was a move further away from 'normal'. Similar trends were also partly seen in reactance as were seen for resistance. Mean reactance in expiration was more negative than compared to inspiration (-1.63 vs -1.18 hPa.s/L), as were the reactances seen at max flow (-2.03 vs -1.54 hPa.s/L). However, there was little difference in the reactance at zero-flow (-0.86 hPa.s/L at end-inspiration compared to -0.89 hPa.s/L at end-expiration).

Table 4.7 Reactance parameters of term-born children performing intra-breath oscillometry.

Parameter	Inspiration	Expiration	ΔE:I
X_{mean} (hPa.s/L)	-1.18 (-1.39 to -0.98)	-1.63 (-1.91 to -1.36)	-0.45 (-0.62 to -0.28)
$X_{V'max}$ (hPa.s/L)	-1.54 (-1.77 to -1.3)	-2.03 (-2.34 to -1.72)	-0.49 (-0.68 to -0.31)
X_e (hPa.s/L)	-0.86 (-1 to -0.72)	-0.89 (-1.07 to -0.7)	-0.03 (-0.15 to 0.09)
ΔX (hPa.s/L)	1.23 (0.98 to 1.47)	1.86 (1.54 to 2.18)	
$\Delta X_{e:}X_{V'max}$ (hPa.s/L)	-0.65 (-0.82 to -0.48)	-1.17 (-1.4 to -0.95)	
AXV	0.34 (0.22 to 0.46)		
AXV'	-0.42 (-0.61 to -0.22)		

Means and 95% CI of mean are displayed. **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{X_{meanI}}$ – Mean reactance; $\mathbf{X_{V'max}}$ – Reactance at maximum flow; **AXV** – Area within reactance-volume loop; **AXV'** – Area within reactance-flow loop.

4.3.2 Agreement between standard and intra-breath oscillometry

The children who performed intra-breath oscillometry also performed standard oscillometry, with the latter also recording the resistance and reactance at 10 Hz. With standard oscillometry, this was done by pseudorandom noise – i.e., 10 Hz frequency sine waves were superimposed onto tidal breathing at random intervals, and impedance at this frequency was therefore not measured at such repeated intervals (every 0.1 seconds in temporal oscillometry). However, it would be expected that in the case of reliable testing, these results should still generate similar values to overall mean impedance measured by the intra-breath technique.

Strong correlation was noted between standard and intra-breath oscillometry for both resistance and reactance at 10 Hz using Pearson's correlation (0.936, $p < 0.001$; 0.928, $p < 0.001$ respectively), as summarised in Table 4.8. When looking at the individual study groups, strong correlation remained for resistance at 10 Hz, particularly the PT_{low} group (PT_{low} 0.959; P_c 0.898; T_c 0.922; all $p < 0.001$). PT_{low} group's reactance results also correlated stronger than that of PT_c and T_c, although all still showed strong correlation (PT_{low} 0.962, P_c 0.856, T_c 0.850, all $p < 0.001$).

Interclass correlation coefficient (ICC) was performed to ensure that this positive correlation was due to agreement between the results.

Table 4.8 Interclass correlation coefficient showing agreement levels for resistance and reactance measurements obtained at 10 Hz between standard and intra-breath oscillometry.

Group	Interclass correlation coefficient for resistance at 10Hz	Interclass correlation coefficient for reactance at 10Hz
Combined	0.966 (0.956 to 0.974) ***	0.950 (0.922 to 0.966) ***
PT_{low}	0.979 (0.964 to 0.988) ***	0.972 (0.949 to 0.985) ***
PT_c	0.943 (0.914 to 0.962) ***	0.873 (0.794 to 0.920) ***
T_c	0.955 (0.927 to 0.972) ***	0.893 (0.800 to 0.940) ***

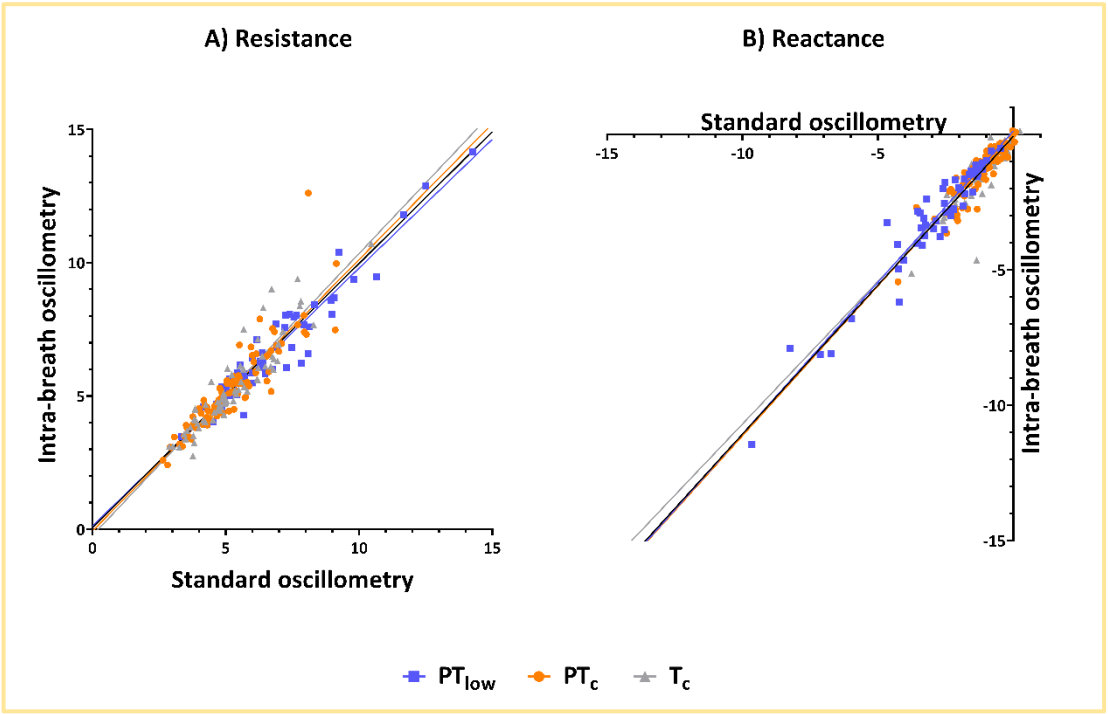
Abbreviations: **PT_{low}** - Preterm low lung function group; **PT_c** - Preterm control; **T_c** - Term controls
Statistical significance: p value <0.001: ***

A similar pattern was observed with the ICC as with the Pearson's correlation. While there was strong agreement between standard and intra-breath oscillometry throughout, there was better agreement for resistance results than were observed

for reactance. For the individual groups, the greatest agreement was seen in the PT_{low} group (Table 4.8).

These results reassure as to the validity of the data obtained, with the correlation between spectral and intra-breath oscillometry displayed in Figure 4.5.

Figure 4.5 Graphs showing the relationship between A) resistance and B) reactance at 10 Hz between standard oscillometry and intra-breath oscillometry.



4.3.3 Comparisons between lung function groups

As described in greater detail in previous chapters (see section 2.3.2), preterm- and term-born children were divided into the following groups:

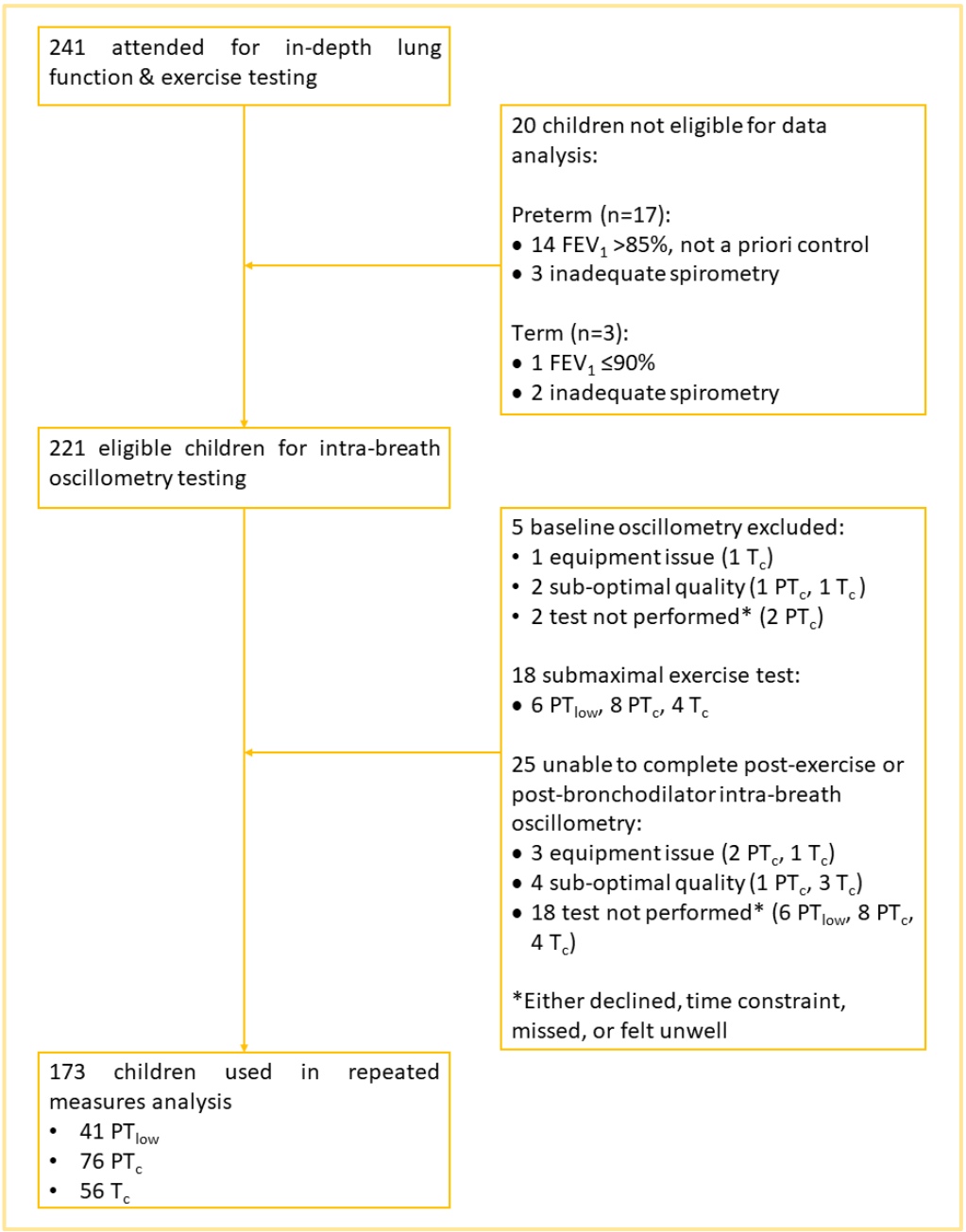
- PT_{low}: Preterm-born children with $\%FEV_1$ ≤85%;
- PT_c: Preterm-born children with $\%FEV_1$ >85%;
- T_c: Term-born children with $\%FEV_1$ >90%.

Demographic comparisons are previously detailed in Tables 2.1 and 2.2 (Chapter 2). In terms of current demographics, there were small differences between the three study groups. The preterm control group were slightly older than their term control counterparts; however, current height and weight were similar. As discussed previously, the PT_{low} children were born earlier and were smaller than their preterm control comparisons, with a higher rate of CLD. Wheeze ever was highest in the PT_{low} group, and they had greater rates of asthma diagnosis and salbutamol use than the term controls. As per their designation, $\%FEV_1$ was lower in the PT_{low} group as were the %FVC, FEV_1/FVC ratio and mid-expiratory flow rates.

4.3.3.1 Missing data

Not all children had valid intra-breath oscillometry at all 3 time points of testing (baseline, post-exercise, and post-exercise bronchodilator). Comparison of data across time points was done with two-way repeated measures ANOVA, using listwise comparisons. Therefore, if data were missing at any single time point, or a valid exercise test was not performed, these children were excluded from the analysis. Figure 4.6 summarises the excluded participants.

Figure 4.6 Flow diagram showing where, why and grouping of children excluded from repeated measures analysis.



4.3.3.2 Respiratory parameters

4.3.3.2.1 Baseline

Baseline respiratory parameters between the three study groups were similar, as shown in Table 4.9. Duration of expiration and respiratory rate showed no differences. PT_{low} children had lower tidal volumes compared to preterm controls (0.48 vs 0.61 L), and their expiration was a greater proportion of the total breath cycle compared to preterm controls (0.54 vs 0.51).

4.3.3.2.2 Changes post-exercise

Following exercise, there were few changes on repeated measures analysis, as per Table 4.9. There was a drop of expiratory duration for T_c (1.75 to 1.55 seconds). Otherwise, exercise did not have a perceivable effect on the other respiratory parameters.

4.3.3.2.3 Changes post-exercise bronchodilator

PT_{low} children demonstrated a decrease in expiratory and total respiratory duration, corresponding with an increase in respiratory rate (23.7 to 25.7 bpm), as seen in Table 4.9. No other changes in respiratory pattern were seen following post-exercise bronchodilator.

Table 4.9 Respiratory parameters across time-points of testing for the children divided by preterm and lung function status (preterm children with low lung function – PT_{low}, preterm controls – PT_c, and term controls – T_c).

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
T_E (seconds)	Ba	1.63 (1.46 to 1.79)	1.67 (1.55 to 1.79)	1.75 (1.61 to 1.89)
	Ex	1.54 (1.37 to 1.71)	1.56 (1.43 to 1.69)	∂∂ 1.55 (1.40 to 1.70)
	BD	₴ 1.37 (1.21 to 1.53)	1.55 (1.43 to 1.67)	1.54 (1.40 to 1.68)
T_E/T_{Tot}	Ba	**0.54 (0.52 to 0.55)** **	0.51 (0.5 to 0.52)	0.52 (0.51 to 0.53)
	Ex	0.53 (0.52 to 0.54)	0.52 (0.51 to 0.53)	0.52 (0.51 to 0.53)
	BD	0.53 (0.51 to 0.54)	0.53 (0.52 to 0.54)	0.52 (0.51 to 0.53)
$T_{V'maxE}/T_E$	Ba	0.33 (0.30 to 0.36)	0.33 (0.31 to 0.35)	0.34 (0.31 to 0.37)
	Ex	0.32 (0.29 to 0.35)	0.34 (0.32 to 0.36)	0.35 (0.32 to 0.38)
	BD	0.35 (0.32 to 0.39)	0.36 (0.33 to 0.38)	0.36 (0.33 to 0.39)
VT (L)	Ba	**0.48 (0.41 to 0.56)** *	0.61 (0.56 to 0.67)	0.59 (0.52 to 0.66)
	Ex	0.46 (0.39 to 0.54)	0.57 (0.52 to 0.63)	0.57 (0.51 to 0.64)
	BD	0.50 (0.43 to 0.57)	0.61 (0.56 to 0.66)	0.58 (0.52 to 0.64)
F_{br} (bpm)	Ba	21.6 (19.8 to 23.5)	20.2 (18.9 to 21.6)	19.7 (18.1 to 21.3)
	Ex	23.7 (21.5 to 25.9)	∂∂ 22.4 (20.8 to 24.0)	∂∂ 22.3 (20.4 to 24.2)
	BD	₴ 25.7 (23.3 to 28.0)	23.2 (21.5 to 25.0)	22.2 (20.2 to 24.2)

Results expressed as mean and 95% confidence intervals for continuous data (two-way repeated measures ANOVA with Bonferroni correction). **Abbreviations:** **T_E** – Expiration time**;** **T_E/T_{Tot}** – Expiration time as proportion of total breath time; **$T_{V'maxE}/T_E$ –** Time to maximum expiratory flow as proportion of expiratory time; **TV** – Tidal volume; **F_{br}** – breathing frequency; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.

PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.

PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ∂ p<0.05; ∂∂ p<0.01; ∂∂∂ p<0.001.

Post-exercise vs Post-exercise bronchodilator: ₴ p<0.05; ₴₴ p<0.01; ₴₴₴ p<0.001.

4.3.3.3 Resistance parameters

4.3.3.3.1 Baseline

4.3.3.3.1.1 Inspiration

Resistance parameters were greater throughout inspiration for PT_{low} children compared to both controls, as per Table 4.10a and illustrated in Figure 4.7. Greater resistance was seen in PT_{low} compared to PT_c and T_c for R_{meanI} (6.06 vs 4.92 vs 4.97 hPa.s/L respectively), $R_{V'maxI}$ (6.56 vs 5.27 vs 5.41 hPa.s/L respectively) and R_{eI} (5.34 vs 4.37 vs 4.35 hPa.s/L respectively). There were no differences between the two control groups. The pattern of resistance across the preterm groups was similar to that as described above with the term children in isolation, with an increase in resistance from end-expiration to at maximum flow; however, there was no difference between groups for the extent of this increase. The gap between maximum and minimum resistance in inspiration was similar across the 3 groups (ΔR_I: 1.89 vs 1.56 vs 1.80 hPa.s/L for PT_{low}, PT_c and T_c respectively).

4.3.3.3.1.2 Expiration

There was a similar pattern of differences between PT_{low}, PT_c and Tc for resistance in expiration as seen in inspiration, as seen in Table 4.10b and displayed in Figure 4.7, with higher R_{meanE} (6.75 vs 5.56 vs 5.77 hPa.s/L respectively), $R_{V'maxE}$ (7.17 vs 5.91 vs 6.25 hPa.s/L respectively) and R_{eE}: 6.31 vs 5.04 vs 5.08 hPa.s/L respectively) for PT_{low} compared to PT_c and T_c. An increase was seen from end-inspiration to at maximum expiratory flow, of a greater magnitude in all groups than the difference from end-expiration to maximum inspiratory flow resistance (PT_{low} 1.83 vs 0.25, PT_c 1.54 vs 0.22, T_c 1.9 vs 0.33); however, again the magnitude of increase was no different across the study groups. Again, there were no differences between preterm and term controls. The overall range of resistance (from maximum to minimum resistances measured, ΔR_E) was similar between all three groups, but appeared greater than the resistance range seen in inspiration (PT_{low}: 2.46 vs 1.89, PT_c 2.06 vs 1.56, T_c 2.48 vs 1.80 hPa.s/L), suggesting more variability in resistance during expiration than inspiration.

4.3.3.3.1.3 Differences between inspiration and expiration

The differences between inspiration and expiration were consistent across the three groups. At all stages of the respiratory cycle, expiratory resistance was higher than inspiratory resistance (for mean resistance, resistance at max flow and the resistance at end of respiratory cycle), as per Table 4.10c. However, all 3 study groups showed a similar degree of difference between inspiration and expiration for these parameters.

The area within the resistance-volume curve (ARV), representing the changing resistance with volume, showed a smaller area for the PT_{low} group compared to T_c children (0.33 vs 0.55 hPa.s/L). There was a trend towards a bigger area of the resistance-flow (ARV') curve, not reaching statistical significance (2.0 vs 1.79 vs 1.71 hPa.L/s for PT_{low}, PT_c, and T_c respectively).

4.3.3.3.2 Post-exercise changes

Repeated measures analysis did not show any differences after exercise for resistance in inspiration or expiration, or for the differences between these two parts of the respiratory cycle, as displayed in Table 4.10a-c and illustrated in Figure 4.8.

4.3.3.3.3 Post-exercise bronchodilator changes

4.3.3.3.3.1 Inspiration

Resistance at all stages of inspiration showed a statistically significant improvement for all three study groups on repeated measures ANOVA between post-exercise and post-exercise bronchodilator testing, shown in Table 4.10a and illustrated in Figure 4.8. These changes resulted in a loss of statistical significance compared to both control groups (R_{meanI}, $R_{V'maxI}$) or compared to term controls only (R_{eI}). This suggests a greater improvement seen in PT_{low} resistance compared to the improvement seen in the 2 control groups. There was also a change in the range of inspiratory resistance (ΔR_I) for PT_{low} children (1.67 to 1.4 hPa.s/L), suggesting a lower variability in resistance following the post-exercise bronchodilator across inspiration, a change not seen in the control groups.

4.3.3.3.3.2 Expiration

A very similar pattern was seen in expiration as with inspiration for all the study groups, including the loss of significance as described above between the groups following post-exercise bronchodilator administration, again suggesting the greatest improvements were seen in the PT_{low} children (Table 4.10b and Figure 4.8). There was no change in ΔR_E for the PT_{low} children, suggesting less reduction in resistance variability in expiration than seen in inspiration.

4.3.3.3.3.3 Differences between inspiration and expiration plus resistance loops

There were no significant changes seen from post-exercise to post-exercise bronchodilator for the differences between inspiration and expiration, although there was a trend in all groups for a higher resistance difference from baseline to post-exercise to post-exercise bronchodilator. For example, the change in ΔR_{mean} for PT_{low} was 0.68 hPa.s/L (baseline) to 0.82 hPa.s/L (post-exercise) to 0.95 hPa.s/L (post-exercise bronchodilator). This change would result from a greater improvement in inspiratory resistance compared to expiratory resistance (hence a widening of the difference). A similar pattern was seen in PT_c and T_c children for ΔR_{mean}, as well as for all three groups for $\Delta R_{V'max}$. There was a non-significant trend for reduced difference between zero flow states (ΔR_e) in all three groups, in this case suggesting the greater fall for this parameter was seen at end-expiration than end-inspiration. Following post-exercise bronchodilator, the difference in resistance from end-expiration to maximal flow in inspiration was only 0.05 hPa.s/L for PT_{low} children. The areas within the resistance-volume and resistance-flow loops showed no changes. These data are displayed in Table 4.10c.

Table 4.10 Resistance parameters across time points for the children divided by preterm and lung function status (preterm children with low lung function – PT_{low}, preterm controls – PT_c, and term control – T_c), during a) inspiration, b) expiration, and c) differences between inspiration and expiration results, and independent parameters.

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
a) Inspiration				
R_{meanI} (hPa.s/L)	Ba	**6.06 (5.61 to 6.52)** *** †††	4.92 (4.59 to 5.25)	4.97 (4.58 to 5.36)
	Ex	**6.1 (5.65 to 6.55)** *** †††	4.95 (4.62 to 5.28)	5.15 (4.77 to 5.54)
	BD	‡‡‡ 4.66 (4.24 to 5.08)	‡‡‡ 4.03 (3.72 to 4.33)	‡‡‡ 4.37 (4.01 to 4.73)
$R_{V'maxI}$ (hPa.s/L)	Ba	**6.56 (6.08 to 7.05)** *** †††	5.27 (4.91 to 5.62)	5.41 (4.99 to 5.82)
	Ex	**6.39 (5.91 to 6.86)** *** †	5.22 (4.87 to 5.57)	5.54 (5.14 to 5.95)
	BD	‡‡‡ 4.93 (4.47 to 5.39)	‡‡‡ 4.26 (3.92 to 4.60)	‡‡‡ 4.67 (4.27 to 5.06)
R_{eI} (hPa.s/L)	Ba	**5.34 (4.94 to 5.74)** *** †††	4.37 (4.08 to 4.66)	4.35 (4.01 to 4.69)
	Ex	**5.63 (5.22 to 6.03)** *** †††	4.50 (4.20 to 4.80)	4.64 (4.29 to 4.99)
	BD	**‡‡‡ 4.36 (4.01 to 4.71)** **	‡‡‡ 3.64 (3.38 to 3.89)	‡‡‡ 3.92 (3.62 to 4.22)
ΔR_I (hPa.s/L)	Ba	1.89 (1.62 to 2.16)	1.56 (1.36 to 1.76)	1.8 (1.57 to 2.03)
	Ex	1.67 (1.38 to 1.96)	1.51 (1.3 to 1.73)	1.82 (1.57 to 2.06)
	BD	‡ 1.4 (1.1 to 1.71)	1.51 (1.29 to 1.73)	1.8 (1.55 to 2.06)
$\Delta R_{eE}:R_{V'maxI}$ (hPa.s/L)	Ba	0.25 (0.01 to 0.49)	0.22 (0.05 to 0.40)	0.33 (0.12 to 0.54)
	Ex	0.08 (-0.18 to 0.34)	0.13 (-0.06 to 0.32)	0.26 (0.04 to 0.48)
	BD	0.05 (-0.26 to 0.37)	0.06 (-0.17 to 0.29)	0.17 (-0.10 to 0.44)

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
b) Expiration				
R_{meanE} (hPa.s/L)	Ba	**6.75 (6.21 to 7.28)** ** †	5.56 (5.17 to 5.95)	5.77 (5.31 to 6.22)
	Ex	**6.92 (6.36 to 7.48)** **	5.69 (5.27 to 6.1)	6.01 (5.53 to 6.49)
	BD	~~‡‡‡~~ 5.6 (5.09 to 6.12)	~~‡‡‡~~ 4.89 (4.51 to 5.27)	~~‡‡‡~~ 5.34 (4.9 to 5.79)
$R_{V'maxE}$ (hPa.s/L)	Ba	**7.17 (6.61 to 7.72)** ** †	5.91 (5.50 to 6.31)	6.25 (5.77 to 6.73)
	Ex	**7.24 (6.65 to 7.82)** **	6.07 (5.64 to 6.50)	6.41 (5.91 to 6.92)
	BD	~~‡‡‡~~ **6.00 (5.47 to 6.53)** *	~~‡‡‡~~ 5.19 (4.80 to 5.58)	~~‡‡‡~~ 5.76 (5.31 to 6.21)
R_{eE} (hPa.s/L)	Ba	**6.31 (5.81 to 6.81)** *** †††	5.04 (4.68 to 5.41)	5.08 (4.65 to 5.50)
	Ex	**6.31 (5.81 to 6.80)** *** ††	5.09 (4.73 to 5.46)	5.28 (4.86 to 5.71)
	BD	~~‡‡‡~~ 4.88 (4.43 to 5.33)	~~‡‡‡~~ 4.21 (3.87 to 4.54)	~~‡‡‡~~ 4.50 (4.11 to 4.88)
ΔR_E (hPa.s/L)	Ba	2.46 (2.13 to 2.79)	2.06 (1.82 to 2.30)	2.48 (2.20 to 2.76)
	Ex	2.29 (1.91 to 2.68)	2.12 (1.83 to 2.40)	2.45 (2.12 to 2.78)
	BD	2.29 (1.89 to 2.68)	2.31 (2.01 to 2.60)	2.56 (2.22 to 2.90)
ΔR_{eI}:$R_{V'maxE}$ (hPa.s/L)	Ba	1.83 (1.53 to 2.12)	1.54 (1.32 to 1.76)	1.9 (1.64 to 2.15)
	Ex	1.61 (1.30 to 1.92)	1.58 (1.35 to 1.80)	1.77 (1.51 to 2.04)
	BD	1.63 (1.33 to 1.94)	1.56 (1.33 to 1.78)	1.84 (1.57 to 2.10)

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
c) Differences between inspiration and expiration				
ΔR_{mean} (hPa.s/L)	Ba	0.68 (0.49 to 0.88)	0.64 (0.5 to 0.79)	0.79 (0.63 to 0.96)
	Ex	0.82 (0.55 to 1.08)	0.73 (0.54 to 0.93)	0.86 (0.63 to 1.09)
	BD	0.95 (0.67 to 1.23)	0.87 (0.66 to 1.07)	0.98 (0.74 to 1.22)
$\Delta R_{V'max}$ (hPa.s/L)	Ba	0.61 (0.36 to 0.86)	0.64 (0.45 to 0.82)	0.85 (0.63 to 1.06)
	Ex	0.85 (0.52 to 1.18)	0.85 (0.61 to 1.09)	0.87 (0.59 to 1.16)
	BD	1.07 (0.75 to 1.38)	0.93 (0.7 to 1.16)	1.1 (0.83 to 1.36)
ΔR_e (hPa.s/L)	Ba	0.97 (0.71 to 1.22)	0.68 (0.49 to 0.86)	0.72 (0.51 to 0.94)
	Ex	0.68 (0.43 to 0.93)	0.60 (0.41 to 0.78)	0.64 (0.43 to 0.86)
	BD	0.52 (0.26 to 0.77)	0.57 (0.38 to 0.76)	0.57 (0.35 to 0.79)
ARV (hPa.s)	Ba	**0.33 (0.20 to 0.47)** †	0.42 (0.32 to 0.52)	0.55 (0.43 to 0.66)
	Ex	0.46 (0.29 to 0.63)	0.47 (0.35 to 0.60)	0.56 (0.42 to 0.71)
	BD	0.57 (0.35 to 0.78)	0.62 (0.46 to 0.78)	0.69 (0.51 to 0.88)
ARV' (hPa.L/s)	Ba	2.00 (1.55 to 2.45)	1.79 (1.46 to 2.12)	1.71 (1.33 to 2.10)
	Ex	1.93 (1.48 to 2.38)	1.80 (1.46 to 2.13)	2.02 (1.63 to 2.41)
	BD	1.89 (1.40 to 2.37)	1.78 (1.42 to 2.13)	1.86 (1.44 to 2.28)

Results expressed as mean and 95% confidence intervals for continuous data (two-way repeated measures ANOVA with Bonferroni correction). **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{R_{meanI}}$ – Mean resistance; $\mathbf{R_e}$ – Resistance at end of respiratory cycle; $\mathbf{R_{V'max}}$ – Resistance at maximum flow; **ARV** – Area within resistance-volume loop; **ARV'** – Area within resistance-flow loop; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.

PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.

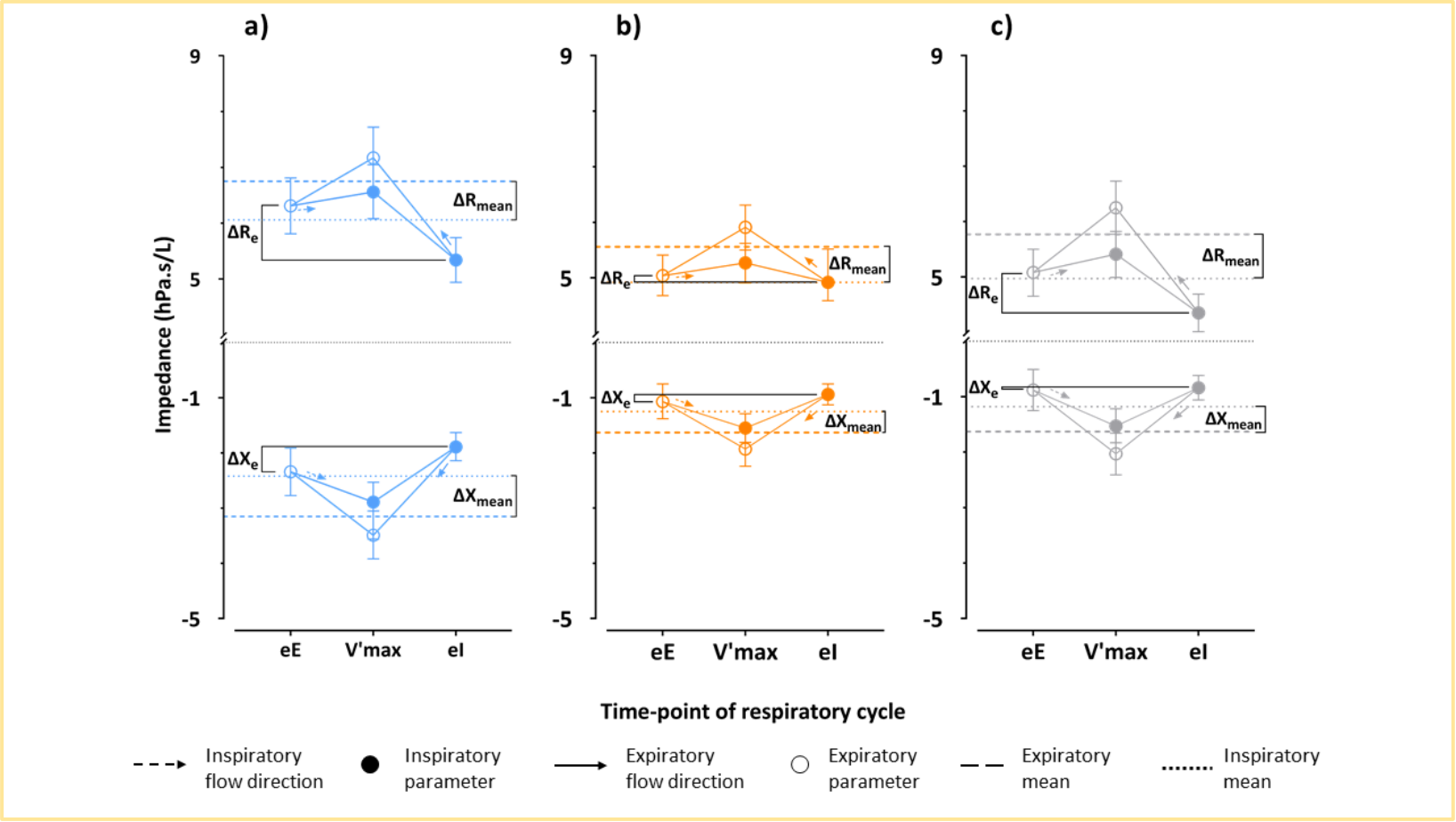
PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ^{ð} p<0.05; ^{ðð} p<0.01; ^{ðððð} p<0.001.

Post-exercise vs Post-exercise bronchodilator: ^{ƨ} p<0.05; ^{ƨƨ} p<0.01; ^{ƨƨƨ} p<0.001.

Figure 4.7 Illustration of impedance at different time points of respiratory cycle (end expiratory/inspiratory: eE/I; at maximal flow rate of expiration/inspiration: V'maxE/I), with annotations displaying the mean expiratory and inspiratory impedance, plus their difference, and the difference between end expiratory and end inspiratory impedance, for a) preterm low lung function group (PT_{low}); b) preterm controls (PT_c); and c) term controls (T_c).



4.3.3.4 Reactance parameters

Reactance parameter data are displayed in Tables 4.9 a-c.

4.3.3.4.1 Baseline

4.3.3.4.1.1 Inspiration

Baseline differences were seen for reactance in inspiration, as per Table 4.11a and illustrated in Figure 4.7, between PT_{low} children compared to both controls, similar to those seen with inspiratory resistance. In this case, the PT_{low} children had worse (more negative reactance) compared to PT_c and T_c children for mean inspiratory reactance (X_{meanI}: -2.42 vs -1.25 vs -1.18 hPa.s/L respectively), the reactance at maximum inspiratory flow ($X_{V'maxI}$: -2.89 vs -1.55 vs -1.53 hPa.s/L respectively) and end-inspiratory reactance (X_{eI}: -1.89 vs -0.94 vs -0.84 hPa.s/L respectively). Again, as with resistance in inspiration, there were no differences between the two controls groups for reactance parameters in inspiration. The PT_{low} had a wider gap between min and max reactances compared to PT_c (1.6 vs 1.15 hPa.s/L).

4.3.3.4.1.2 Expiration

Expiratory reactance followed a similar pattern to the other baseline results, as shown in Table 4.11b and illustrated in Figure 4.7, with significant differences between PT_{low} and PT_c/T_c children. Mean expiratory reactance (X_{meanE}: -3.15 vs -1.63 vs -1.63 hPa.s/L respectively), the reactance at maximum expiratory flow ($X_{V'maxE}$: -3.49 vs -1.93 vs -2.03 hPa.s/L respectively) and end-expiratory reactance (X_{eE}: -2.34 vs -1.07 vs -0.88 hPa.s/L respectively). There was a greater variability of expiratory reactance from minimum (most negative reactance) to maximum (least negative/most positive reactance) for PT_{low} compared to both preterm- and term controls (2.63 vs 1.64 vs 1.88 hPa.s/L respectively). Additionally, the PT_{low} children had a greater drop in reactance from the end of inspiration to reactance at maximum expiratory flow (-1.6 vs -0.99 hPa.s/L) compared to PT_c group.

4.3.3.4.1.3 Differences between inspiration and expiration plus reactance loops

Any differences in reactance parameters between expiration and inspiration were similar across all three study groups. However, compared to term controls, the PT_{low} children have a greater area within the reactance-flow loop (AXV' 1.15 vs 0.42).

4.3.3.4.2 Post-exercise changes

Unlike resistance, there were some changes in reactance following exercise seen on repeated measures, as summarised in Tables 4.11a-c and illustrated in Figure 4.8. Both PT_{low} and T_c children had more negative reactance at end-inspiration compared to at baseline. More differences were seen for expiratory reactance. This included an increasingly negative reactance for PT_{low} children for X_{meanE} (-3.15 to -3.75 hPa.s/L) and $X_{V'maxE}$ (-3.49 to -4.1 hPa.s/L). T_c did also show a worsening reactance at max flow (-2.03 to -2.43 hPa.s/L). No reactance changes were identified for PT_c children. Additionally, there were no changes for any of the groups for the differences between inspiration and expiration; however, a significant difference had evolved for AXV' for PT_{low} compared to PT_c, a reversal of the difference seen at baseline, where T_c showed a difference with PT_{low}.

4.3.3.4.3 Post-exercise bronchodilator changes

4.3.3.4.3.1 Inspiration

Following bronchodilator therapy, changes were observed for several of the inspiratory reactance parameters, as shown in Table 4.11a and displayed in Figure 4.8. PT_{low} children showed improvements in reactance at all stages of inspiration, and had a decrease in variability of reactance (max to min reactance difference). Similar changes were seen in PT_c children at all stages of the inspiratory cycle, but no change in variability of reactance during inspiration. T_c children did not show statistically significant improvement in their minimum inspiratory reactance. The greatest absolute changes in reactance were seen in the PT_{low} group with 55-64% greater improvements (for several parameters) compared to PT_c and 69-76% greater improvements compared to T_c. Following post-exercise bronchodilator, there were still some inter-group differences remaining, particularly between PT_{low} and PT_c children.

4.3.3.4.3.2 Expiration

Following post-exercise bronchodilator therapy, expiratory reactance also showed improvements, particularly within the PT_{low} children, as shown in Table 4.11b and illustrated in Figure 4.7. All parameters again showed a less negative reactance, as well as lower variability between minimum and maximum reactance. PT_c children

also showed improvements in all but variability. The only improvement seen in T_c children following post-exercise bronchodilation was for end-expiratory reactance. Again, the PT_{low} children showed the greatest degree of improvements of 61-73% when compared to PT_c and 76-89% improvements when compared to T_c children, depending on the variable. Again, as with the inspiratory results, following post-exercise bronchodilator, there were still some inter-group differences remaining, particularly between PT_{low} and PT_c children.

4.3.3.4.3.3 Differences between inspiration and expiration plus reactance loops

PT_{low} children demonstrated some changes post-exercise bronchodilator. ΔX_e, the difference between end-expiratory and end-inspiratory reactance, showed a decrease from -0.36 to 0 hPa.s/L, i.e., following bronchodilator, end-expiratory and end-inspiratory reactance were the same. Additionally, there was a significant reduction in the drop from end-inspiratory to maximal expiratory flow reactance (ΔX_{eI}:$X_{V'maxE}$) from -1.87 to -1.23 hPa.s/L. The AXV' showed improvements with a decrease from 1.18 to 0.59 (approximately a 50% decrease).

Table 4.11 Reactance parameters across time points for the children divided by preterm and lung function status (preterm children with low lung function – PT_{low}, preterm controls – PT_c, and term control – T_c), during a) inspiration, b) expiration, and c) differences between inspiration and expiration results, and independent parameters.

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
a) Inspiration				
X_{meanI} (hPa.s/L)	Ba	**-2.42 (-2.76 to -2.08)** *** †††	-1.25 (-1.50 to -1.00)	-1.18 (-1.47 to -0.89)
	Ex	**-2.78 (-3.19 to -2.37)** *** †††	-1.36 (-1.66 to -1.06)	-1.52 (-1.87 to -1.17)
	BD	‡‡‡ **-1.38 (-1.69 to -1.07)** *	‡‡‡ -0.81 (-1.04 to -0.59)	‡‡ -1.16 (-1.43 to -0.90)
$X_{V'maxI}$ (hPa.s/L)	Ba	**-2.89 (-3.25 to -2.53)** *** †††	-1.55 (-1.81 to -1.29)	-1.53 (-1.83 to -1.22)
	Ex	**-3.12 (-3.56 to -2.69)** *** †††	-1.63 (-1.94 to -1.31)	-1.82 (-2.19 to -1.45)
	BD	‡‡‡ **-1.65 (-1.99 to -1.31)** **	‡‡‡ -0.99 (-1.24 to -0.74)	‡‡ -1.37 (-1.66 to -1.08)
X_{el} (hPa.s/L)	Ba	**-1.89 (-2.14 to -1.63)** *** †††	-0.94 (-1.13 to -0.75)	-0.84 (-1.06 to -0.62)
	Ex	∂∂ **-2.23 (-2.51 to -1.94)** *** †††	-1.04 (-1.25 to -0.83)	∂ -1.11 (-1.35 to -0.87)
	BD	‡‡‡ **-1.22 (-1.44 to -1.00)** ***	‡‡‡ -0.64 (-0.80 to -0.47)	‡ -0.87 (-1.06 to -0.68)
ΔX_I (hPa.s/L)	Ba	**1.60 (1.31 to 1.90)** *	1.15 (0.93 to 1.37)	1.26 (1.00 to 1.51)
	Ex	**1.93 (1.51 to 2.36)** *	1.25 (0.94 to 1.56)	1.57 (1.21 to 1.94)
	BD	‡‡‡ 1.16 (0.86 to 1.47)	1.07 (0.84 to 1.29)	1.42 (1.16 to 1.68)
ΔX_{eE}:$X_{V'maxI}$ (hPa.s/L)	Ba	-0.55 (-0.82 to -0.29)	-0.48 (-0.68 to -0.29)	-0.64 (-0.87 to -0.42)
	Ex	-0.54 (-0.85 to -0.23)	-0.57 (-0.8 to -0.34)	-0.67 (-0.94 to -0.41)
	BD	-0.43 (-0.7 to -0.17)	-0.42 (-0.61 to -0.22)	-0.56 (-0.78 to -0.33)

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
b) Expiration				
X_{meanE} (hPa.s/L)	Ba	**-3.15 (-3.62 to -2.68)** *** †††	-1.63 (-1.98 to -1.28)	-1.63 (-2.03 to -1.23)
	Ex	∂∂ **-3.75 (-4.24 to -3.25)** *** †††	-1.78 (-2.14 to -1.41)	-2.00 (-2.42 to -1.57)
	BD	∂∂∂ **-2.14 (-2.56 to -1.71)** **	∂∂∂ -1.30 (-1.61 to -0.99)	-1.75 (-2.11 to -1.38)
$X_{V'maxE}$ (hPa.s/L)	Ba	**-3.49 (-3.92 to -3.05)** *** †††	-1.93 (-2.24 to -1.61)	-2.03 (-2.41 to -1.66)
	Ex	∂∂ **-4.10 (-4.60 to -3.60)** *** †††	-2.08 (-2.45 to -1.72)	∂ -2.43 (-2.86 to -2.00)
	BD	∂∂∂ **-2.45 (-2.90 to -2.00)** **	∂∂∂ -1.52 (-1.85 to -1.19)	-2.18 (-2.57 to -1.79)
X_{eE} (hPa.s/L)	Ba	**-2.34 (-2.77 to -1.91)** *** †††	-1.07 (-1.38 to -0.75)	-0.88 (-1.25 to -0.51)
	Ex	**-2.58 (-2.97 to -2.19)** *** †††	-1.06 (-1.34 to -0.77)	-1.14 (-1.48 to -0.81)
	BD	∂∂∂ **-1.22 (-1.51 to -0.92)** **	∂∂∂ -0.57 (-0.79 to -0.36)	∂ -0.81 (-1.07 to -0.56)
ΔX_E (hPa.s/L)	Ba	**2.63 (2.18 to 3.08)** ** †	1.64 (1.31 to 1.97)	1.88 (1.5 to 2.27)
	Ex	**3.06 (2.56 to 3.56)** ** †	1.87 (1.5 to 2.23)	2.2 (1.78 to 2.63)
	BD	∂∂∂ 2.16 (1.69 to 2.64)	1.76 (1.41 to 2.1)	2.25 (1.85 to 2.65)
ΔX_{el}:$X_{V'maxE}$ (hPa.s/L)	Ba	**-1.6 (-1.87 to -1.33)** **	-0.99 (-1.19 to -0.79)	-1.2 (-1.43 to -0.97)
	Ex	**-1.87 (-2.21 to -1.54)** *** †	-1.05 (-1.29 to -0.8)	-1.32 (-1.6 to -1.04)
	BD	∂∂∂ -1.23 (-1.56 to -0.91)	-0.88 (-1.12 to -0.65)	-1.31 (-1.59 to -1.04)

		PT_{low} (n=41)	PT_c (n=76)	T_c (n=56)
c) Differences between inspiration and expiration				
ΔX_{mean} (hPa.s/L)	Ba	-0.73 (-0.96 to -0.49)	-0.38 (-0.55 to -0.21)	-0.45 (-0.65 to -0.25)
	Ex	**-0.97 (-1.29 to -0.65)** *	-0.42 (-0.66 to -0.18)	-0.48 (-0.75 to -0.20)
	BD	-0.75 (-1.04 to -0.46)	-0.49 (-0.70 to -0.27)	-0.58 (-0.83 to -0.34)
$\Delta X_{V'max}$ (hPa.s/L)	Ba	-0.60 (-0.85 to -0.34)	-0.38 (-0.56 to -0.19)	-0.51 (-0.73 to -0.29)
	Ex	-0.98 (-1.33 to -0.62)	-0.46 (-0.72 to -0.19)	-0.61 (-0.92 to -0.31)
	BD	-0.80 (-1.11 to -0.49)	-0.53 (-0.76 to -0.30)	-0.81 (-1.07 to -0.55)
ΔX_e (hPa.s/L)	Ba	-0.45 (-0.71 to -0.20)	-0.13 (-0.32 to 0.06)	-0.04 (-0.26 to 0.18)
	Ex	-0.36 (-0.60 to -0.11)	-0.02 (-0.20 to 0.16)	-0.04 (-0.24 to 0.17)
	BD	₴₴ 0.00 (-0.18 to 0.18)	0.06 (-0.07 to 0.19)	0.06 (-0.10 to 0.21)
AXV (hPa.s)	Ba	0.41 (0.27 to 0.55)	0.24 (0.14 to 0.35)	0.33 (0.21 to 0.46)
	Ex	0.58 (0.37 to 0.79)	0.28 (0.12 to 0.43)	0.36 (0.18 to 0.54)
	BD	0.47 (0.28 to 0.66)	0.36 (0.22 to 0.5)	0.43 (0.27 to 0.59)
AXV' (hPa.L/s)	Ba	**1.15 (0.73 to 1.58)** †	0.51 (0.20 to 0.83)	0.42 (-0.78 to -0.05)
	Ex	**1.18 (0.79 to 1.57)** **	0.42 (0.14 to 0.71)	0.62 (-0.95 to -0.28)
	BD	₴₴ 0.59 (0.29 to 0.88)	0.21 (0 to 0.43)	0.38 (0.13 to 0.63)

Results expressed as mean and 95% confidence intervals for continuous data (two-way repeated measures ANOVA with Bonferroni correction). **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{X_{meanI}}$ – Mean reactance; $\mathbf{X_e}$ – Reactance at end of respiratory cycle; $\mathbf{X_{V'max}}$ – Reactance at maximum flow; **AXV** – Area within reactance-volume loop; **AXV'** – Area within reactance-flow loop; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.

PT_{low} vs PT_c: * p<0.05; ** p<0.01; *** p<0.001.

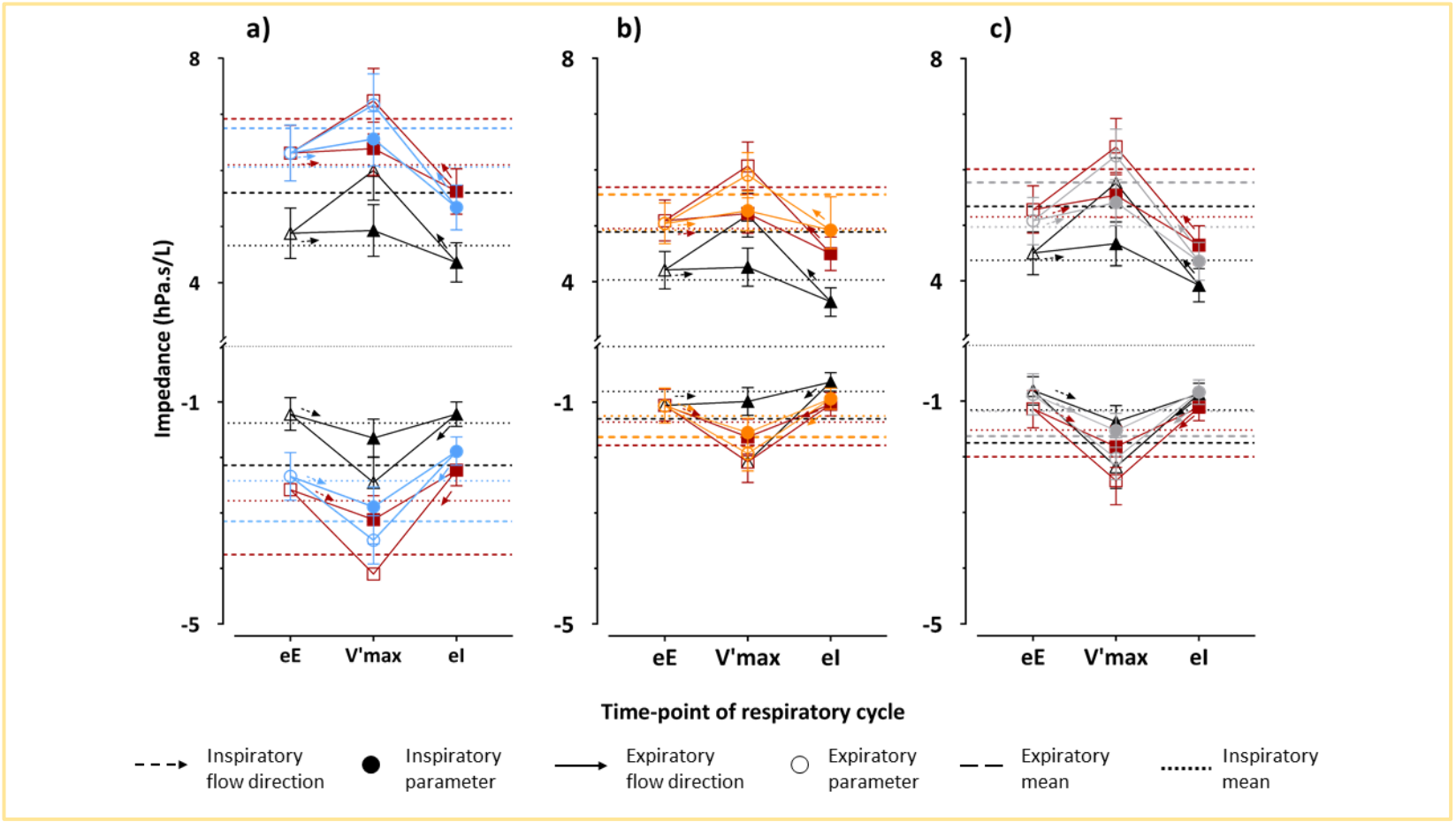
PT_{low} vs T_c: † p<0.05; †† p<0.01; ††† p<0.001.

PT_c vs T_c: ‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001.

Baseline vs Post-exercise: ₫ p<0.05; ₫₫ p<0.01; ₫₫₫ p<0.001.

Post-exercise vs Post-exercise bronchodilator: ₴ p<0.05; ₴₴ p<0.01; ₴₴₴ p<0.001.

Figure 4.8 Illustration of impedance at different time points of respiratory cycle (end expiratory/inspiratory: eE/I; at maximal flow rate of expiration/inspiration: V'maxE/I), at baseline (circle), post-exercise (maroon square) and post-exercise bronchodilator (black triangle), for a) preterm low lung function group (PT_{low}); b) preterm controls (PT_c); and c) term controls (T_c).



4.3.4 Comparisons between obstructive and non-obstructive groups

PT_{low} children from this cohort have been, as described in Chapter 2 (section 2.3.3), further classified as obstructive or non-obstructive disease. This division has been made pragmatically by spirometry outcomes, namely based on the FEV_1/FVC ratio and whether it is <0.8 (prematurity-associated obstructive lung disease or POLD) or ≥0.8 (prematurity-associated non-obstructive lung disease or PnOLD), in addition to $\%FEV_1$ ≤85%.

Of the 53 children in the PT_{low} group, 37 meet the criteria for POLD and 16 for PnOLD. Of the PnOLD children, one child did not complete testing at all three time points. Of the POLD children, 6 did not reach maximal exertion on cardiopulmonary exercise testing, and 5 did not complete intra-breath oscillometry at all three time points of testing.

4.3.4.1 Respiratory parameters

There were no differences seen between POLD and PnOLD children for any of the respiratory parameters at baseline, as shown in Table 4.12. Post-exercise the POLD children reached peak expiratory flow quicker than PnOLD children, with peak flow occurring at 0.28 compared to 0.38 in terms of proportion of total expiratory duration. There were no differences seen post-exercise bronchodilator.

Table 4.12 Respiratory parameters across time-points of testing for the PT_{low} children divided by obstructive (POLD) or non-obstructive (PnOLD) lung disease status.

		POLD (n=26)	PnOLD (n=15)
Duration (seconds)	Ba	1.66 (1.47 to 1.85)	1.57 (1.32 to 1.82)
	Ex	1.56 (1.32 to 1.80)	1.50 (1.18 to 1.82)
	BD	1.36 (1.17 to 1.55)	1.38 (1.13 to 1.63)
Expiration as proportion of total breath cycle	Ba	0.54 (0.53 to 0.56)	0.52 (0.5 to 0.54)
	Ex	0.54 (0.52 to 0.55)	0.52 (0.5 to 0.54)
	BD	0.53 (0.52 to 0.55)	0.51 (0.49 to 0.54)
Time to max exp flow as proportion of expiratory time	Ba	0.32 (0.27 to 0.36)	0.36 (0.3 to 0.42)
	Ex	0.28 (0.25 to 0.32) §§	0.38 (0.33 to 0.42)
	BD	0.33 (0.29 to 0.38)	0.39 (0.32 to 0.45)
Tidal volume (L)	Ba	0.50 (0.44 to 0.57)	0.45 (0.37 to 0.54)
	Ex	0.49 (0.42 to 0.56)	0.41 (0.32 to 0.51)
	BD	0.52 (0.45 to 0.59)	0.47 (0.37 to 0.56)
Respiratory rate (bpm)	Ba	21.75 (19.28 to 24.23)	21.42 (18.16 to 24.68)
	Ex	23.52 (20.43 to 26.61)	24.00 (19.93 to 28.07)
	BD	25.85 (22.78 to 28.92)	25.37 (21.33 to 29.41)

Results expressed as mean and 95% confidence intervals for continuous data (two-way repeated measures ANOVA with Bonferroni correction). **Abbreviations:** $\mathbf{T_E}$ – Expiration time**;** $\mathbf{T_E/T_{Tot}}$ – Expiration time as proportion of total breath time; $\mathbf{T_{V'maxE}/T_E}$ **–** Time to maximum expiratory flow as proportion of expiratory time; **TV** – Tidal volume; $\mathbf{F_{br}}$ – breathing frequency; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
Baseline vs Post-exercise: ð p<0.05; ðð p<0.01; ðððp<0.001.
Post-exercise vs Post-exercise bronchodilator: ƶ p<0.05; ƶƶ p<0.01; ƶƶƶ p<0.001.

4.3.4.2 Resistance parameters

4.3.4.2.1 Inspiration

A trend towards higher resistance in POLD children compared to PnOLD was seen across inspiration at baseline, however this did not reach statistical significance, as shown in Table 4.13a and presented in Figure 4.9.

There were no post-exercise changes on repeated measures for either group.

Resistance across inspiration improved for both groups from post-exercise to post-exercise bronchodilator; however, the magnitude of change was greater for the POLD group compared to for the PnOLD group (26% vs 18% reduction for R_{meanI}, 27% vs 14% reduction for $R_{V'maxI}$, 23 vs 19% for R_{eI}). There was a decrease in the ΔR_I (1.78 to 1.42 hPa.s/L) for POLD children, suggesting a reduction in variability of resistance during resistance. Post-exercise and post-exercise bronchodilator results are also demonstrated in Figure 4.10.

4.3.4.2.2 Expiration

As with inspiration, resistance across expiration showed no statistically significant differences between POLD and PnOLD at baseline, but a similar trend for greater resistance was noted in POLD children, as shown in Table 4.13b and illustrated in Figure 4.9.

While repeated measures did not show any changes from baseline to post-exercise results, mean resistance (7.48 vs 5.95 hPa.s/L) and end-expiratory resistance (6.83 vs 5.4 hPa.s/L) were significantly higher in POLD compared to PnOLD at the post-exercise time point. This appeared to be due to an increase in resistance in expiration for POLD and a decrease in resistance for PnOLD creating this increased difference after exercise.

Following post-exercise bronchodilator therapy there was, as with inspiration, an improvement in resistance throughout the expiratory cycle for POLD children (21% vs 13% reduction for R_{meanE}, 20% vs 11% reduction for $R_{V'maxE}$, 26 vs 15% for R_{eE}). With the exception of end-inspiratory/expiratory resistance, this was a smaller reduction compared to that seen during inspiration, suggesting bronchodilator has greater effect on the inspiratory component of respiration in the POLD children. Post-

exercise and post-exercise bronchodilator results are also demonstrated in Figure 4.10.

4.3.4.2.3 Differences between inspiration and expiration

POLD and PnOLD showed similar degrees of difference between inspiration and expiration for all the parameters, and showed no change across the time points, as per Table 4.13c. Following post-exercise bronchodilator, a difference for ARV was then observed for POLD (0.71 hPa.s) compared to PRLD (0.32 hPa.s).

Table 4.13 Resistance parameters across time points for the PT_{low} children divided by obstructive (POLD) or non-obstructive (PnOLD) lung disease status during a) inspiration, b) expiration, and c) differences between inspiration and expiration results, and independent parameters.

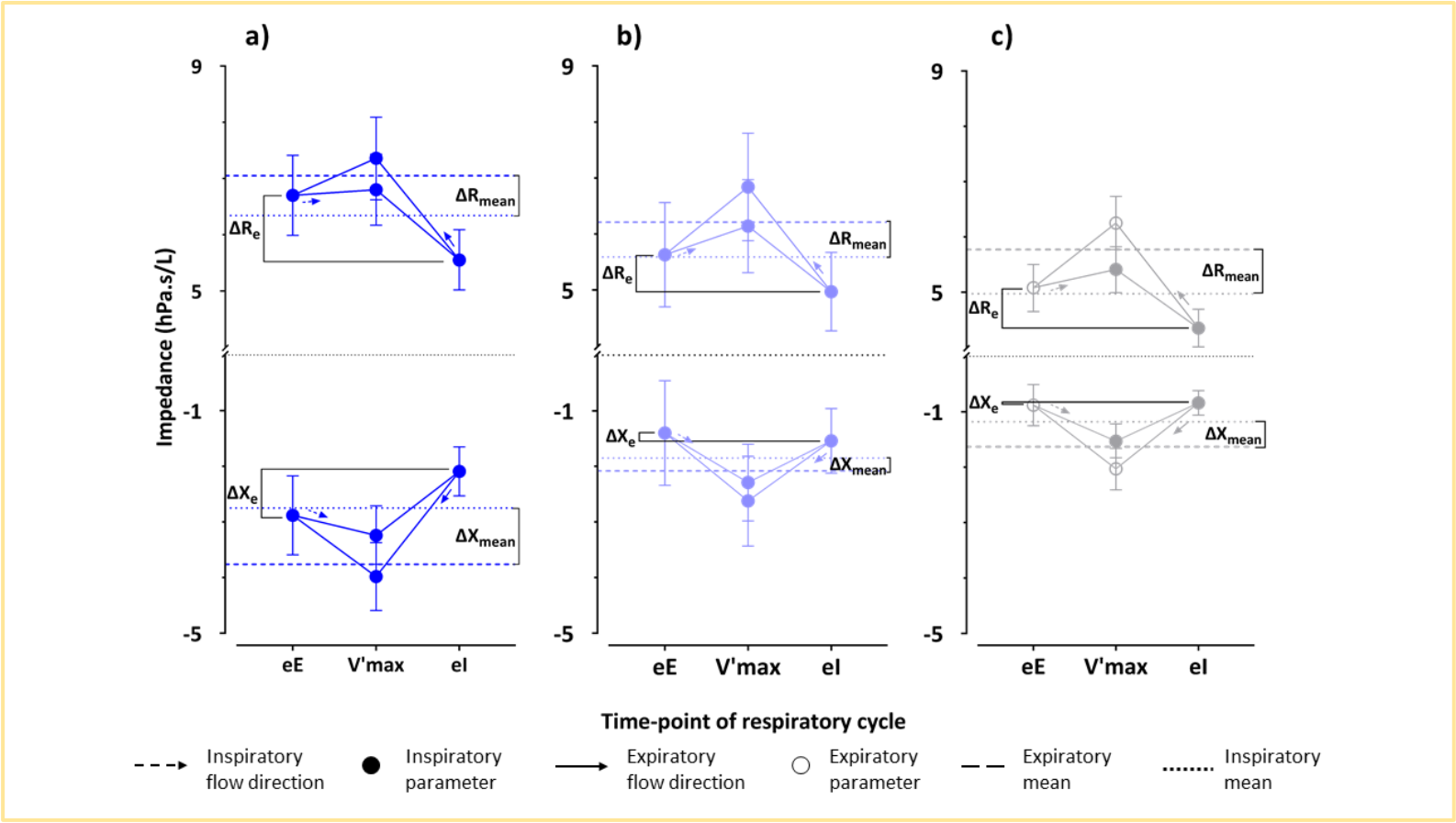
		POLD (n=26)	PnOLD (n=15)
a) Inspiration			
R_{meanI} (hPa.s/L)	Ba	6.34 (5.77 to 6.91)	5.59 (4.84 to 6.34)
	Ex	6.45 (5.79 to 7.12)	5.49 (4.61 to 6.37)
	BD	⁂ 4.75 (4.19 to 5.30)	⁑ 4.50 (3.77 to 5.23)
$R_{V'maxI}$ (hPa.s/L)	Ba	6.80 (6.17 to 7.43)	6.14 (5.31 to 6.97)
	Ex	6.74 (6.05 to 7.43)	5.77 (4.86 to 6.67)
	BD	⁂ 4.92 (4.32 to 5.52)	⁋ 4.95 (4.15 to 5.74)
R_{eI} (hPa.s/L)	Ba	5.55 (5.02 to 6.09)	4.97 (4.27 to 5.67)
	Ex	5.98 (5.39 to 6.57)	5.01 (4.24 to 5.79)
	BD	⁂ 4.56 (4.08 to 5.04)	⁑ 4.03 (3.39 to 4.66)
ΔR_I (hPa.s/L)	Ba	2.04 (1.69 to 2.38)	1.64 (1.18 to 2.09)
	Ex	1.78 (1.43 to 2.14)	1.47 (1 to 1.94)
	BD	⁋ 1.42 (1.13 to 1.7)	1.39 (1.01 to 1.76)
$\Delta R_{eE}:R_{V'maxI}$ (hPa.s/L)	Ba	0.10 (-0.23 to 0.43)	0.51 (0.08 to 0.94)
	Ex	-0.09 (-0.42 to 0.24)	0.37 (-0.06 to 0.81)
	BD	-0.13 (-0.43 to 0.17)	0.37 (-0.03 to 0.76)

		POLD (n=26)	PnOLD (n=15)
b) Expiration			
R_{meanE} (hPa.s/L)	Ba	7.05 (6.35 to 7.76)	6.21 (5.28 to 7.14)
	Ex	**7.48 (6.64 to 8.32)** §	5.95 (4.84 to 7.05)
	BD	²²² 5.88 (5.21 to 6.55)	5.13 (4.24 to 6.01)
$R_{V'maxE}$ (hPa.s/L)	Ba	7.36 (6.62 to 8.09)	6.84 (5.88 to 7.80)
	Ex	7.71 (6.86 to 8.56)	6.42 (5.30 to 7.53)
	BD	²²² 6.18 (5.53 to 6.83)	5.69 (4.83 to 6.54)
R_{eE} (hPa.s/L)	Ba	6.70 (5.99 to 7.41)	5.63 (4.70 to 6.56)
	Ex	**6.83 (6.07 to 7.60)** §	5.40 (4.39 to 6.40)
	BD	²²² 5.05 (4.41 to 5.69)	4.58 (3.73 to 5.42)
ΔR_E (hPa.s/L)	Ba	2.59 (2.18 to 3.01)	2.23 (1.68 to 2.78)
	Ex	2.54 (2.03 to 3.05)	1.86 (1.19 to 2.54)
	BD	2.42 (1.94 to 2.90)	2.05 (1.43 to 2.68)
$\Delta R_{eI}:R_{V'maxE}$ (hPa.s/L)	Ba	1.80 (1.37 to 2.23)	1.87 (1.31 to 2.44)
	Ex	1.73 (1.32 to 2.14)	1.41 (0.87 to 1.94)
	BD	1.62 (1.27 to 1.97)	1.66 (1.20 to 2.12)

		POLD (n=26)	PnOLD (n=15)
c) Differences between inspiration and expiration			
ΔR_{mean} (hPa.s/L)	Ba	0.72 (0.48 to 0.95)	0.63 (0.32 to 0.94)
	Ex	1.03 (0.65 to 1.4)	0.46 (-0.04 to 0.95)
	BD	1.13 (0.77 to 1.49)	0.63 (0.16 to 1.1)
$\Delta R_{V'max}$ (hPa.s/L)	Ba	0.55 (0.24 to 0.86)	0.70 (0.29 to 1.11)
	Ex	0.97 (0.56 to 1.37)	0.65 (0.12 to 1.18)
	BD	1.25 (0.80 to 1.71)	0.74 (0.15 to 1.34)
ΔR_e (hPa.s/L)	Ba	1.15 (0.79 to 1.51)	0.66 (0.19 to 1.13)
	Ex	0.85 (0.51 to 1.19)	0.38 (-0.06 to 0.83)
	BD	0.50 (0.21 to 0.79)	0.55 (0.17 to 0.93)
ARV (hPa.s)	Ba	0.35 (0.23 to 0.47)	0.30 (0.14 to 0.46)
	Ex	0.59 (0.36 to 0.81)	0.24 (0.06 to 0.53)
	BD	**0.71 (0.48 to 0.93)** §	0.32 (0.02 to 0.62)
ARV' (hPa.L/s)	Ba	2.28 (1.71 to 2.84)	1.52 (0.78 to 2.27)
	Ex	2.20 (1.62 to 2.78)	1.47 (0.70 to 2.23)
	BD	2.01 (1.44 to 2.57)	1.67 (0.93 to 2.42)

Results expressed as mean and 95% confidence intervals for continuous data (two-way mixed ANOVA including repeated measures with Bonferroni correction). **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{R_{meanI}}$ – Mean resistance; $\mathbf{R_e}$ – Resistance at end of respiratory cycle; $\mathbf{R_{V'max}}$ – Resistance at maximum flow; **ARV** – Area within resistance-volume loop; **ARV'** – Area within resistance-flow loop; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
Baseline vs Post-exercise: ð p<0.05; ðð p<0.01; ðððp<0.001.
Post-exercise vs Post-exercise bronchodilator: ª p<0.05; ªª p<0.01; ªªª p<0.001.

Figure 4.9 Illustration of impedance at different time points of respiratory cycle (end expiratory/inspiratory: eE/I; at maximal flow rate of expiration/inspiration: V'maxE/I), with annotations displaying the mean expiratory and inspiratory impedance, plus their difference, and the difference between end expiratory and end inspiratory impedance, for a) preterm obstructive (POLD) and b) non-obstructive (PnOLD) lung disease groups; and c) term controls for comparison (T_c).



4.3.4.3 Reactance parameters

4.3.4.3.1 Inspiration

POLD children had more negative (worse) reactance compared to PnOLD children for X_{meanI} (-2.75 vs -1.85 hPa.s/L respectively), and $X_{V'max}$ (-3.24 vs -2.29 hPa.s/L respectively) during inspiration, as shown in Table 4.14a and Figure 4.9.

Baseline to post-exercise did not show any statistically significant difference, although reactance did seem to show a worsening trend throughout inspiration, as displayed in Figure 4.10.

Post-exercise bronchodilator resulted in an improved reactance throughout inspiration for both groups, but with greater absolute change for POLD compared to PnOLD (X_{meanI} 1.59 vs 1.04, $X_{V'maxI}$ 1.75 vs 0.99, X_{eI} 1.13 vs 0.78 hPa.s/L reductions for POLD vs PnOLD groups respectively).

4.3.4.3.2 Expiration

As with inspiration, POLD children had worse reactance compared to PnOLD for all parameters – X_{meanE} (-3.76 vs -2.08 hPa.s/L respectively), $X_{V'maxE}$ (-3.98 vs -2.62 hPa.s/L respectively) and X_{eE} (-2.88 vs -1.4 hPa.s/L respectively), as per Table 4.14b and Figure 4.9. There was a greater change from end-inspiration reactance to reactance at max expiratory flow (ΔX_{eI}:$X_{V'maxE}$) seen in POLD than PnOLD at baseline and post-exercise (-1.9 vs 1.08 hPa.s/L and -2.39 vs -0.98 hPa.s/L at these time points respectively).

There were no significant changes from baseline to post-exercise, but again, a trend for worsening reactance was seen throughout expiration. There remained significant difference between POLD and PnOLD at the post-exercise time point.

There were significant improvements on repeated measures seen post-exercise bronchodilator for the POLD group throughout expiration. Again, there were greater absolute reductions for POLD children compared to PnOLD (X_{meanE} 2.07 vs 0.81, $X_{V'maxE}$ 2.16 vs 0.75, X_{eE} 1.7 vs 0.78 hPa.s/L absolute decreases for POLD vs PnOLD groups respectively). This resulted in a loss of statistical significance for differences between POLD and PnOLD at the post-exercise bronchodilator time point. Post-exercise and post-exercise bronchodilator results are also demonstrated in Figure 4.10.

4.3.4.3.3 Differences between inspiration and expiration

Baseline differences were seen between POLD and PnOLD for the change in reactance from inspiration to expiration as seen in Table 4.14c. A greater negative reactance difference between expiration and inspiration was seen for the POLD than PnOLD groups for ΔX_{mean} (-1.01 vs -0.23) and ΔX_e (-0.79 vs 0.14), i.e., worse reactance seen during/at end of expiration compared to inspiration.

This difference persisted to post-exercise. Area within reactance-volume and reactance-flow curves both demonstrated differences between POLD and PnOLD. For AXV this remained throughout the time points (baseline: 0.54 vs 0.18, post-exercise: 0.88 vs 0.07, post-exercise bronchodilator: 0.64 vs 0.18) and for AXV' at baseline (1.63 vs 0.34) and post-exercise (1.59 vs 0.48) for POLD and PnOLD groups respectively.

AXV' demonstrated a decrease for POLD group from post-exercise to post-exercise bronchodilator (1.59 to 0.78). The other significant change post-exercise bronchodilator was a drop in the difference between end-inspiratory and end-expiratory reactance from -0.64 to -0.07 hPa.s/L, in line with a greater improvement in X_{eE} compared to X_{eI}.

Table 4.14 Reactance parameters across time points for the PT_{low} children divided by obstructive (POLD) or non-obstructive (PnOLD) lung disease status during a) inspiration, b) expiration, and c) differences between inspiration and expiration results, and independent parameters.

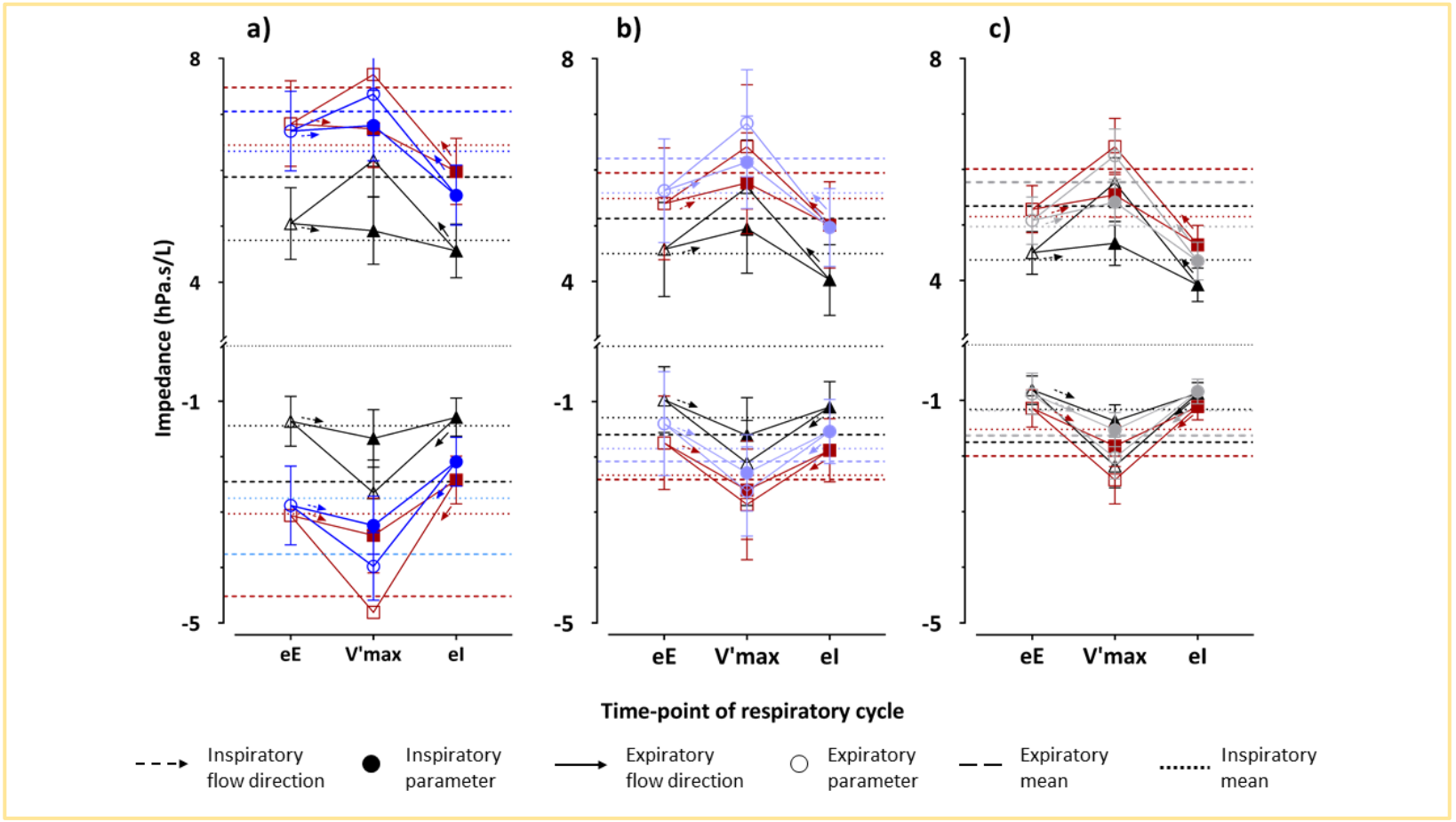
		POLD (n=26)	PnOLD (n=15)
a) Inspiration			
X_{meanI} (hPa.s/L)	Ba	**-2.75 (-3.23 to -2.26) §**	-1.85 (-2.49 to -1.21)
	Ex	-3.03 (-3.68 to -2.38)	-2.33 (-3.19 to -1.48)
	BD	ƂƂƂ -1.44 (-1.88 to -1.00)	ƂƂ -1.29 (-1.86 to -0.71)
$X_{V'maxI}$ (hPa.s/L)	Ba	**-3.24 (-3.76 to -2.71) §**	-2.29 (-2.98 to -1.60)
	Ex	-3.42 (-4.10 to -2.75)	-2.60 (-3.49 to -1.71)
	BD	ƂƂƂ -1.67 (-2.19 to -1.15)	Ƃ -1.61 (-2.30 to -0.93)
X_{eI} (hPa.s/L)	Ba	-2.09 (-2.53 to -1.65)	-1.54 (-2.12 to -0.96)
	Ex	-2.42 (-2.85 to -1.99)	-1.88 (-2.45 to -1.31)
	BD	ƂƂƂ -1.29 (-1.63 to -0.94)	ƂƂ -1.10 (-1.55 to -0.64)
ΔX_I (hPa.s/L)	Ba	1.78 (1.46 to 2.10)	1.29 (0.87 to 1.72)
	Ex	2.01 (1.38 to 2.65)	1.80 (0.96 to 2.64)
	BD	ƂƂ 1.18 (0.86 to 1.50)	1.13 (0.71 to 1.55)
$\Delta X_{eE}:X_{V'maxI}$ (hPa.s/L)	Ba	-0.36 (-0.80 to 0.08)	-0.89 (-1.47 to -0.31)
	Ex	-0.36 (-0.87 to 0.15)	-0.86 (-1.52 to -0.19)
	BD	-0.31 (-0.65 to 0.03)	-0.65 (-1.09 to -0.20)

		POLD (n=26)	PnOLD (n=15)
b) Expiration			
X_{meanE} (hPa.s/L)	Ba	**-3.76 (-4.50 to -3.01)** §§	-2.08 (-3.06 to -1.10)
	Ex	**-4.52 (-5.28 to -3.76)** §§	-2.41 (-3.41 to -1.40)
	BD	¢¢¢ -2.45 (-3.06 to -1.84)	-1.60 (-2.40 to -0.79)
$X_{V'maxE}$ (hPa.s/L)	Ba	**-3.98 (-4.59 to -3.37)** §§	-2.62 (-3.43 to -1.82)
	Ex	**-4.81 (-5.57 to -4.06)** §§	-2.86 (-3.86 to -1.86)
	BD	¢¢¢ -2.65 (-3.23 to -2.06)	-2.11 (-2.88 to -1.34)
X_{eE} (hPa.s/L)	Ba	**-2.88 (-3.59 to -2.17)** §	-1.40 (-2.34 to -0.46)
	Ex	**-3.06 (-3.71 to -2.42)** §	-1.75 (-2.59 to -0.90)
	BD	¢¢¢ -1.36 (-1.81 to -0.91)	-0.97 (-1.56 to -0.37)
ΔX_E (hPa.s/L)	Ba	**3.13 (2.42 to 3.84)** §	1.76 (0.83 to 2.70)
	Ex	**3.70 (2.92 to 4.49)** §§	1.96 (0.92 to 2.99)
	BD	¢¢ 2.49 (1.86 to 3.13)	1.59 (0.75 to 2.43)
ΔX_{eI}:$X_{V'maxE}$ (hPa.s/L)	Ba	**-1.90 (-2.22 to -1.57)** §§	-1.08 (-1.52 to -0.65)
	Ex	**-2.39 (-2.91 to -1.87)** §§	-0.98 (-1.67 to -0.30)
	BD	-1.36 (-1.74 to -0.98)	-1.01 (-1.52 to -0.51)

		POLD (n=26)	PnOLD (n=15)
c) Differences between inspiration and expiration			
ΔX_{mean} (hPa.s/L)	Ba	**-1.01 (-1.38 to -0.64) §**	-0.23 (-0.72 to 0.26)
	Ex	**-1.49 (-2.00 to -0.98) §§§**	-0.07 (-0.74 to 0.60)
	BD	**-1.01 (-1.42 to -0.60) §**	-0.31 (-0.85 to 0.24)
$\Delta X_{V'max}$ (hPa.s/L)	Ba	-0.75 (-1.07 to -0.42)	-0.33 (-0.76 to 0.10)
	Ex	**-1.39 (-1.96 to -0.81) §**	-0.26 (-1.02 to 0.50)
	BD	-0.98 (-1.43 to -0.53)	-0.50 (-1.09 to 0.10)
ΔX_e (hPa.s/L)	Ba	**-0.79 (-1.19 to -0.40) §§**	0.14 (-0.38 to 0.66)
	Ex	**-0.64 (-0.99 to -0.29) §**	0.13 (-0.33 to 0.60)
	BD	ᵉᵉ -0.07 (-0.30 to 0.16)	0.13 (-0.17 to 0.44)
AXV (hPa.s)	Ba	**0.54 (0.34 to 0.74) §**	0.18 (-0.09 to 0.44)
	Ex	**0.88 (0.55 to 1.21) §§**	0.07 (-0.37 to 0.50)
	BD	**0.64 (0.39 to 0.88) §**	0.18 (-0.15 to 0.51)
AXV' (hPa.L/s)	Ba	**-1.63 (-2.35 to -0.91) §**	-0.34 (-1.28 to 0.61)
	Ex	**-1.59 (-2.21 to -0.96) §**	-0.48 (-1.31 to 0.34)
	BD	ᵉ -0.78 (-1.16 to -0.41)	-0.25 (-0.74 to 0.25)

Results expressed as mean and 95% confidence intervals for continuous data (two-way mixed ANOVA including repeated measures with Bonferroni correction). **Abbreviations: E** – Expiration; **I** – Inspiration; **Δ** – Difference; $\mathbf{X_{meanI}}$ – Mean reactance; $\mathbf{X_e}$ – Reactance at end of respiratory cycle; $\mathbf{X_{V'max}}$ – Reactance at maximum flow; **AXV** – Area within reactance-volume loop; **AXV'** – Area within reactance-flow loop; **Ba** – Baseline; **Ex** – Post-exercise; **BD** – Post-exercise bronchodilator.
POLD vs PnOLD: § p<0.05; §§ p<0.01; §§§ p<0.001.
Baseline vs Post-exercise: ᵟ p<0.05; ᵟᵟ p<0.01; ᵟᵟᵟ p<0.001.
Post-exercise vs Post-exercise bronchodilator: ᵉ p<0.05; ᵉᵉ p<0.01; ᵉᵉᵉ p<0.001.

Figure 4.10 Illustration of impedance at different time points of respiratory cycle (end expiratory/inspiratory: eE/I; at maximal flow rate of expiration/inspiration: V'maxE/I), at baseline (circle), post-exercise (maroon square) and post-exercise bronchodilator (black triangle), for a) preterm obstructive (POLD) and b) non-obstructive (PnOLD) lung disease groups; and c) term controls for comparison (T_c).



4.4 Discussion

This chapter is the first to explore in-depth intra-breath oscillometry in a preterm population, in comparison to a well-matched term control population, with a focus on preterm-born children with evidence of lung dysfunction, as identified on spirometry testing. The aim was to explore the mechanics or discern any patterns underpinning the lung pathology.

Firstly, I was able to demonstrate in a population of term-born children, the normal pattern of airway mechanics using this single frequency oscillometry. A signal at 10Hz superimposed onto tidal breathing in a child revealed characteristics of airway mechanics to a reasonable depth of the airways as opposed to a higher frequency that would be limited to more proximal airways, and at a frequency where energy storage (capacitance, analogous to compliance) predominates over inertance, and likely to reflect better airway disease.

I have also demonstrated that there was good agreement between both standard oscillometry and intra-breath oscillometry for both preterm and term children.

This chapter has then looked in depth at the differences between preterm-born children with and without evidence of lung disease, against term controls, and assessed changes in impedance throughout the respiratory cycle between these groups, as well as the effects of exercise and post-exercise bronchodilator on these airway mechanics.

Finally, I have investigated whether children with evidence of lung dysfunction show any differences depending on whether they were classified as having obstructive or non-obstructive lung disease.

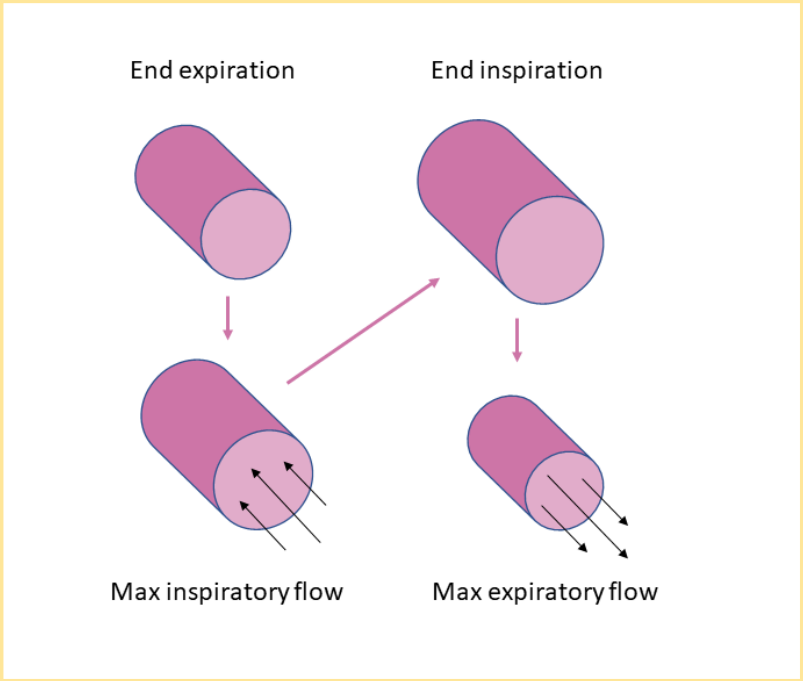
4.4.1 Term population

The term-born population was investigated to show normal intra-breath oscillometry measurements. Only one term child was unable to perform the test sufficiently to obtain a result, which indicates that it is easy to perform successfully. The breathing pattern observed in this term population was only slightly different to what would be expected from normal tidal breathing. While respiratory rate was normal, there were higher tidal volumes than would be expected. Normal tidal volumes for children are

approximately 6 - 8 mls/kg (Venkataraman, 2006). This would equate to approximately 230 – 300 mls based on the average weight of this population. The average tidal volume for the term children was 600 mls, so approximately double normal, suggesting the children were breathing deeper than would normally. The most likely reason for this is when breathing through the filter and wave tube, the dead space is increased (i.e., volume of airway not involved in gas exchange). In normal respiration this would extend from outside the alveoli though to oro- or naso-orifice; however, in the case of oscillometry, also includes the filter and wave tube, thus introducing greater dead space. This could promote, particularly in children, the need to breath more deeply than usual. However, flow rates were not excessively high.

As expected, resistance was greater throughout expiration compared to inspiration. Resistance is dependent on airway size or volume, and due to the decreasing thoracic volume during expiration, there would be a decrease in airway calibre resulting in the higher resistance. At the end of expiration, the lungs are at their smallest, hence the resistance is higher than at the end of inspiration. Following end-expiration when resistance is high, there is only a relatively small increase then to the resistance at peak inspiratory flow, compared to the large increase from the zero flow at end-inspiration to peak expiratory flow resistance. This is likely due to a greater change in airway calibre between these two time points, as illustrated in Figure 4.11.

Figure 4.11 Diagram illustrating the potential changes in airway calibre from end-expiration (eE) to maximum inspiratory flow (V'maxI), and from end-inspiration (eI) to maximum expiratory flow (V'maxE), that explain the greater increase in resistance from eI:V'maxE compared to eE:V'maxI.



Reactance also followed a similar but inverse pattern to resistance, with more negative reactance during expiration compared to during inspiration. While reactance is less volume dependent, due to compliance being related to degree of lung inflation (i.e., the greater the lung inflation, the greater the elastic recoil of the lungs), as well as related to airway resistance, the combination of recoil and greater resistance during expiration will likely explain the more negative reactance during expiration. At zero flow states, which reflect static compliance and hence elastic properties of the lung, the reactance for term children is very similar between end-inspiration and end-expiration. This similar negative, and hence reflective of compliance, reactance at both zero flow states suggests that there is not increased elastic recoil, potentially that could suggest structural disease, in these healthy children.

Understanding the normal pattern of impedance during the respiratory cycle of a healthy population enables identification and understanding of any abnormalities when compared to any group of interest.

4.4.2 Standard versus intra-breath oscillometry

I have also been able to demonstrate that there is good agreement between standard oscillometry and intra-breath oscillometry. Due to the nature of standard oscillometry using pseudorandom noise, it is variable between tests how often a specific single frequency will be superimposed onto breathing. Thus, it would not be able to give in depth information about the mechanical response to that individual frequency across the respiratory cycle. However, it would be expected that the overall mean of the impedance (resistance and reactance) at that frequency would be similar to that generated by the in-depth single frequency intra-breath oscillometry. Using intraclass coefficient I was able to confirm a strong agreement for both resistance and reactance between these two oscillometry techniques. The resistance showed slightly better agreement than reactance. Interestingly, the PT_{low} group demonstrated the strongest agreement. Interclass correlation coefficient was used over Bland-Altman plot, as the latter would be better suited for looking for systemic differences between the two. The greater concern for this, given the same equipment was used, was a user difference (i.e., the candidate) rather than an in-built error with the test, hence the choice of test. It is reassuring that the test seems to have been performed similarly for both across the candidates.

4.4.3 Intra-breath analysis

4.4.3.1 Respiratory parameters

Similar patterns for respiratory parameters were generally seen across the study groups except for tidal volume, which was lower in the PT_{low} children. This is in keeping with findings from Chapter 2, sections 2.3.2.2-3, with PT_{low} children having lower TLC on static lung testing, lower FVC on spirometry and lower minute volume on exercise testing. There was a statistically significant difference in the proportion for expiration in the total breath cycle, which could represent a degree of air trapping, again consistent with earlier findings of air trapping and prior knowledge surrounding hyperinflation in preterm populations (Welsh et al., 2010). Otherwise breathing parameters were similar across baseline.

After exercise, there was some potential evidence of persisting respiratory effects of exertion with a slightly raised respiratory rate seen in all groups. This increased respiratory rate was then sustained, and actually increased in PT_{low} children, after post-exercise bronchodilator therapy. Interestingly exercise and bronchodilation did not affect tidal volume, even though flow rates improved after bronchodilation, suggesting that flow may not be the only limiting factor in PT_{low} children for changes in tidal breathing volume. However, as shown from post-exercise bronchodilator spirometry, this improvement in airflow and its effect on lung volumes are better demonstrated on forced manoeuvres.

Obstructive and non-obstructive groups were similar for the breathing parameters compared to each other. There was also little change following exercise and bronchodilator, except the change in flow rate seen in the PT_{low} group as a whole, likely reflecting an increase in flow seen largely in the obstructive group.

4.4.3.2 Impedance

Little is definitively known about impedance changes in preterm-born children within the respiratory cycles. Forced oscillation had been used previously to compare overall impedance (resistance and reactance) during inspiration and expiration as a whole in a group of 7 year old ex-preterm children and term controls, finding raised inspiratory resistance compared to controls, as well as higher resistance in expiration compared to inspiration (Hamon et al., 2013). However, this was not looking at the changes that occur throughout these parts of the respiratory cycle. As would be expected from the standard oscillometry findings, resistance was greater, and reactance more negative throughout both inspiration and expiration, for PT_{low} children compared to controls. Again, at every stage of the respiratory cycle (including at end of inspiration or expiration, at max flow, and for the maximum and minimum recorded values) resistance was greater and reactance more negative for expiration compared to inspiration.

For resistance, this could be partly volume-related as outlined in section 4.4.1 on the isolated term results, and exacerbated by the fact this PT_{low} group did have evidence of lower lung volumes. Height in theory is a factor and ideally values would be standardised, but as there are no reference values for this lung function technique at

present, normalising for this is not possible. The heights across the lung function groups were largely the same suggesting this may not have a significant impact in my population. However, given that the preterm children with normal lung function also have smaller lungs on spirometry (FVC) and static lung volume testing (TLC) compared to term controls, yet there were no differences between these two groups on this oscillometry testing, suggests there is another mechanism to explain the higher resistance. The findings from the standard oscillometry suggest that these effects were seen at the lower frequencies to a greater extent. As the 10 Hz frequency reflects the distal airways, this may suggest an issue with the smaller distal airways, possibly related to distal extension of smooth muscle surrounding the airways. The upper airways could account for some of the changes seen, particularly at maximal flow when there was likely to be greater turbulent flow through the vocal cords. The impedance at end respiration points was free from any extra-airway factors contributing to this however (Czövek et al., 2016).

Similarly, the reactance parameters showed similar (but opposite in sign) trends to the resistance. As outlined above, the negative reactance values are representing energy storage due to compliance. An increasingly negative reactance value in the PT_{low} group suggests lower compliance, again this could be due to a variety of reasons. Airway size may play a part but is unlikely to be fully explanatory for the differences seen. Other causes of reduced respiratory system compliance include factors affecting chest wall compliance (structural abnormalities, unlikely in this cohort) or lung compliance itself including decreased elasticity or fibrosis (Edwards and Annamaraju). Changes in elasticity and fibrosis are possible in a preterm population, as seen on imaging of lungs following preterm birth (Wong et al., 2008).

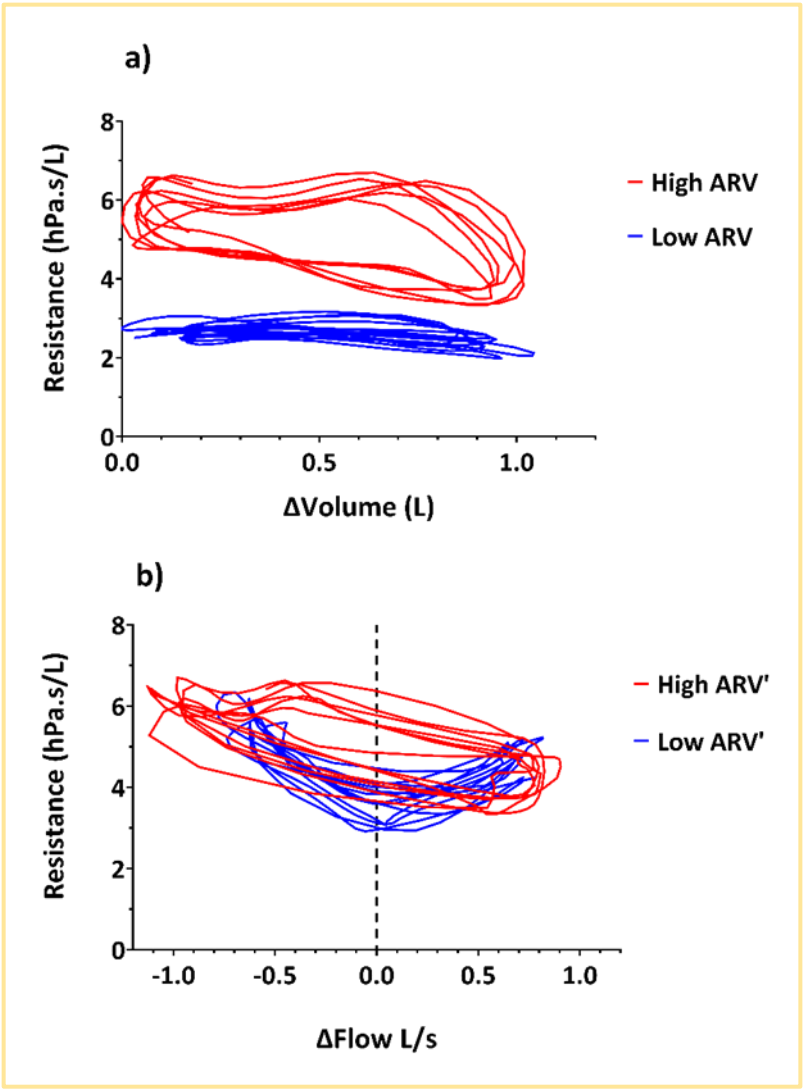
While there were differences between inspiration and expiration for resistance and reactance, these seem to be similarly noted across the groups, with no parts of the respiratory cycle particularly greater affected in the PT_{low} when compared to controls. This included the resistance at end-expiration when compared to end-inspiration. This differs somewhat in findings for preschool aged children with a history of wheeze (Czövek et al., 2016). Using the same intra-breath oscillometry technique, in children aged approximately 5 years old, a difference between resistance at end-expiration

(R_{eE}) and end-inspiration (R_{eI}), called ΔR_e, was present in children with acute wheeze, and a cut-off of 1.42 hPa.s/L was determined using ROC for identifying airway obstruction. This was subsequently applied to a group of slightly younger children with recurrent wheeze and controls: 80% had a ΔR_e greater than the cut off, with 96% of controls having a value below.

In my group, PT_{low} did not show the same difference between zero flow resistances as seen in the preschool wheeze population, although the POLD group in isolation did have the highest ΔR_e, suggesting this is a different disease process to childhood wheeze disorders. There was even less difference seen when comparing ΔX_e between the lung function groups – which had also been identified to a lesser extent as being a marker of wheeze in the preschool wheeze children (Czövek et al., 2016). However, the POLD children again had the most negative ΔX_e. Interestingly, the non-obstructive group actually had a positive ΔX_e, consistent with there being a more negative reactance at end-inspiration compared to end-expiration.

Adults with COPD had flow limitation identified by examining the area within the impedance-volume and impedance-flow loops (see Figure 4.12 for example). The area within an impedance-flow loop can be calculated, and a population of adults with COPD were found to have larger areas within the reactance-flow loop (AXV'), with AXV' improving after applying Continuous Positive Airway Pressure (CPAP) at increasing pressures. In my population, there was a slightly higher AXV' in the PT_{low} group compared to T_c, with improvement seen after bronchodilator therapy. Figure 4.12 gives an example of the ARV and ARV' loops from participants from my study, with an example of each with higher ARV/ARV' and lower ARV/ARV'. An increase in the area within these loops may be suggestive of flow limitation between expiration and inspiration, especially the AXV loop (Lorx et al., 2017).

Figure 4.12 a) resistance-volume loops (ARV) and b) resistance-flow loops (ARV') demonstrating examples of area within the curve for participants with higher (red) and lower (blue) a) ARV and b) ARV' values.



4.4.3.3 Exercise and post-exercise bronchodilator effects

Exercise did not have any significant effect on PT_{low} children's resistance; however, reactance worsened following exercise, especially during the expiratory phase, and in particular in the POLD group. This suggests that there is something about their airway compliance that is affected by exercise. While diminished spirometry was not noted in post-exercise in my study, bronchoconstriction can occur following exercise (Joshi et al., 2013), and this change in reactance post-exercise may be a more sensitive marker than spirometry. However, a change in resistance would also be expected in the presence of bronchoconstriction due to airway calibre change,

leaving the possibility of another mechanism behind this change in reactance post-exercise. A reactance change in the absence of a change in resistance suggests a direct impact on compliance as a result of exercise. A deterioration in reactance and compliance was not noted in standard oscillometry, thus suggests that the intra-breath technique may be better at identifying subtle changes in respiratory mechanics. Post-exercise bronchodilator had a positive effect on all groups, but in particular the PT_{low} group. While the results did not normalise to the same extent as seen in standard oscillometry, there was a widespread improvement across impedance. Additionally, the AXV' improved significantly, and this is an example where it did normalise.

4.4.4 Limitations

There are some limitations present. Recruited numbers, particularly in the low function group, and hence in the obstructive/non-obstructive groups, were low, thus potentially underpowered to detect changes, particularly between the POLD and PnOLD groups. Regardless, I still identified a number of differences between groups but these need to be replicated across other studies.

While this is exciting exploratory, hypothesis-generating work, it does mean there is a lack of standardised values to be able to compare the results to. There is a chance other factors may be affecting the results, i.e., height, that would be accounted for in standardised values.

4.5 Conclusion

In summary, preterm-born children with low lung function, especially those with evidence of obstructive lung disease, had evidence of impaired impedance throughout the respiratory cycle. Exercise appeared to have a direct impact on the reactance in children with evidence of obstructive lung disease, the mechanism of which needs to be explored. Post-exercise bronchodilator had a positive impact on impedance, particularly improving both resistance and reactance in those with obstructive airway disease, and to a greater extent in expiration than inspiration. Smaller differences in resistance and reactance at zero-flow states of end-expiration and end-inspiration were observed compared to previous reports in children with childhood wheeze disorders, suggesting preterm-born children have an alternative underlying pathology. Further studies need to confirm these findings and explore potential mechanisms.

5 INTERPRETATION OF LUNG FUNCTION TESTING

5.1 Introduction

Lung function testing encompasses a range of techniques, most of which are well established particularly in the research domain and in adult clinical practice. Consensus guidelines for performing testing are available for many of these applications, although with a greater focus on the adult population. Guidance is also available for interpretation strategies, however again the predominant emphasis is on adult populations. Interpretation of testing is limited by availability of reference ranges. The Global Lung Function Initiative (GLI) have developed reference equations for spirometry across several ethnic groups, covering ages from 3 to 95, by compiling normal results from countries across the world. This is an evolving resource which originally developed from >160,000 data points from almost 75,000 individuals (Quanjer et al., 2012). Population groups were divided into several ethnicity groups (Caucasian, African-American, North-East Asian, South-East Asian, and "Other" where individuals did not fit into one of the other groups) due to systematic differences found from various centres globally. While these original reference values were developed several years ago, there is ongoing effort to either add to the data resource or evaluate lesser represented populations against the current references for validity (Arigliani et al., 2017).

However, while effort is ongoing to build similar references for other methods of lung function testing (Stanojevic et al., 2017, Hall et al., 2021), this remains incomplete for others (Hall et al., 2021). As such reference values for lung function tests outside of spirometry are based on small numbers, particularly for paediatric populations. This makes interpretation more difficult, as without knowing "normal" values, identify abnormal is more difficult. While spirometry and lung volume testing have clear guidance for performing (Miller et al., 2005, Graham et al., 2019, Wanger et al., 2005) and interpreting (Pellegrino et al., 2005) the tests, sometimes their use in clinical practice could potentially should differ to recommended practice (Murray et al., 2017).

5.1.1 Interpretation of spirometry

Spirometry has very clear guidance on how to perform and interpret testing. This includes equipment specifications and calibration requirements, within test and between test acceptability criteria, as well as what the results mean (Miller et al., 2005, Pellegrino et al., 2005). Typically, this involves using a stepwise approach to the spirometry, with or without additional information from lung volume testing. The ERS guidance (Pellegrino et al., 2005) uses results at their lower limit of normal (LLN), starting with the FEV_1/VC (it does not specify a forced vital capacity should be used, and that a slow vital capacity is equally, if not more, useful), and then FVC, before using TLC at LLN to confirm restrictive deficits. A similar approach is suggested by Johnson et al (Johnson and Theurer, 2014). One difference between these interpretative strategies is the used of z-scores for LLN in the ERS methodology, and a mixture of LLN and absolute cut-offs of % predicted or ratio depending on age of the patient. Use of z-scores is widely encouraged due to their ability to remove variability around the mean across the correction factors, which %predicted cannot do (Stanojevic et al., 2013). There is also discouragement to using absolute cut-offs for FEV_1/(F)VC ratio, particularly in children due to discrepancy between lung growth and overall growth, termed dysanapsis (Quanjer et al., 2010).

However, there is a case to be made that using defined cut-offs such as LLN may not apply to identifying pathology such as that described by Murray et al (Murray et al., 2017). In their review of children with a diagnosis of asthma, they found that using different cut-offs, including %predicted or absolute ratios, had better sensitivity and specificity for identifying a confirmed diagnosis than those suggested by NICE. This has not been applied to the preterm population in terms of identifying lung dysfunction, and is complicated by the fact there is not a single underlying diagnosis in those ex-preterm children who have respiratory issues, whether symptoms or impaired lung function.

5.1.2 Spirometry comparison

Consideration should also be paid to how and where spirometry is performed. Taking a standardised approach as outlined above is important, however variables such as

equipment used are not always the same, particularly between community and lung function lab settings. A question should be asked whether different equipment all perform the test to the same standard, even in the event of all equipment specifications and calibration being performed as per the consensus guidelines. This is a particularly pertinent question for several reasons in both clinical practice and research domains. For instance, if performing longitudinal spirometry, then knowing whether the device or setting may affect the results is important. Alternatively, the presence of a systematic difference between methods of obtaining results may affect whether a referral for testing or meeting diagnostic criteria for a disease (i.e., GOLD criteria for COPD).

Indeed, a comparison of spirometry results between a General Practice setting and a lung function lab setting over 2 years identified a small (69 mls for FEV_1 and 81 mls for FVC as a pooled pre- and post-bronchodilator in year 1; 58 mls and 79 mls respectively for year 2) but statistically significant difference between these settings in over 300 patients with COPD, with greater volumes obtained in the General Practice setting (Schermer et al., 2003). This was using the same spirometer in both settings, which were performed <30 days apart. Pre-bronchodilator results had greater differences than post-bronchodilator differences, which is interesting, given that the values would likely be greater post-bronchodilation, hence more possibility for variance. Instead, a conclusion of variability between the 4 lung function testing labs was hypothesised as an explanation, although the larger number (n=61) of General Practices would suggest a greater potential for variability. However, their conclusion that spirometry from different settings should not be used interchangeably seems valid.

However, a community versus laboratory setting comparison in a group of ex-preterm children and term controls (n=50 total, 37 in the ex-preterm group) aged ~11 years from the EPICure study found little difference in spirometry z-scores. In this instance a different spirometer was used at the two visits, although both were pneumotachograph spirometers. For FEV_1, FVC FEV_1/FVC and $FEF_{25-75\%}$ z-scores, bias was approximately 0 z-score in all, with limits of agreement +/- 1 z-score for FEV_1 and FVC, compared to +/- 1.5 z-scores between community vs lab settings. Similarly, a

comparison of two other pneumotachograph spirometers reached similar conclusions for lack of systematic difference between different spirometers, in this case specifically for FEV_1 and FVC (Swart et al., 2003). These studies do not answer the question of what happens if a different type of spirometer is used, as frequently occurs in clinical practice.

Other studies have explored this question with comparisons between ultrasonic flow and pneumotachograph flow meters (Milanzi et al., 2019) and turbine and pneumotachograph flow meters (Caras et al., 1999), although the latter may have been affected by the methodology, with both types of meters being tested during the same expiration for all readings, with the turbine spirometer placed in series past the pneumotachograph. Given the heavy reliance on flow, this may have caused the systematic difference found, a point acknowledged by the authors. Both found systematic differences in favour of the pneumotachograph compared to the alternative. Additionally it is possible that updated versions of the same spirometer may produce differences in results (Künzli et al., 2005).

5.1.3 Aims and hypothesis

I hypothesise that:

1) Using thresholds identified in the course of this research to identify children with obstructive lung disease will be comparative to other published methodology;
2) Comparison of spirometry performed by the same participant on two different types of spirometry will yield systematic differences in results.

Aims:

1) With regards to defining obstructive and non-obstructive I aimed to compare our method of defining obstructive lung disease with that of two published methods, including those from the European Respiratory Society standards for interpreting lung function tests.

2) I aimed to compare two types of spirometers (turbine and pneumotachograph) to assess whether there are any systematic differences in the results that are obtained.

5.2 Methods

5.2.1 Population and testing

Recruitment and participant testing have been described in detail elsewhere (Chapter 2.2) in the thesis. For the purposes of this chapter, the relevant tests include spirometry and reversibility testing, fractional exhaled nitric oxide (FE_{NO}), and lung volume testing with helium dilution and body plethysmography methods.

The two types of spirometers used were: 1) MicroLoop spirometer with turbine (Carefusion, Germany), and 2) MasterScreen Body and PFT systems with pneumotachograph (Vyaire Medical, Germany). The method of performing the test was identical for both pieces of equipment. All participants had performed spirometry at the screening visit and so were familiar with the technique; however, all underwent further explanation and tutoring prior to performing the test to ensure adequate quality was obtained. Calibration, test performance and criteria for adequate testing were in line with ERS recommendations (Miller et al., 2005). All participants performed MicroLoop spirometry prior to MasterScreen spirometry.

5.2.2 Defining groups

Participants were grouped according to whether they are classed as having obstructive, restrictive/non-obstructive or mixed disease based on pathways suggested by Johnson et al and Pellegrino et al, as well as how these terms have been defined in the course of the thesis. Figures 5.1 and 5.2 show the algorithms by Johnson and Pellegrino to classify into the above groups. VC in the Pellegrino methodology is considered as FVC for the purposes of making this classification.

Figure 5.1 Algorithm from Johnson et al for identifying different types of lung disease (Johnson and Theurer, 2014). Image reproduced with permission of the rights holder.

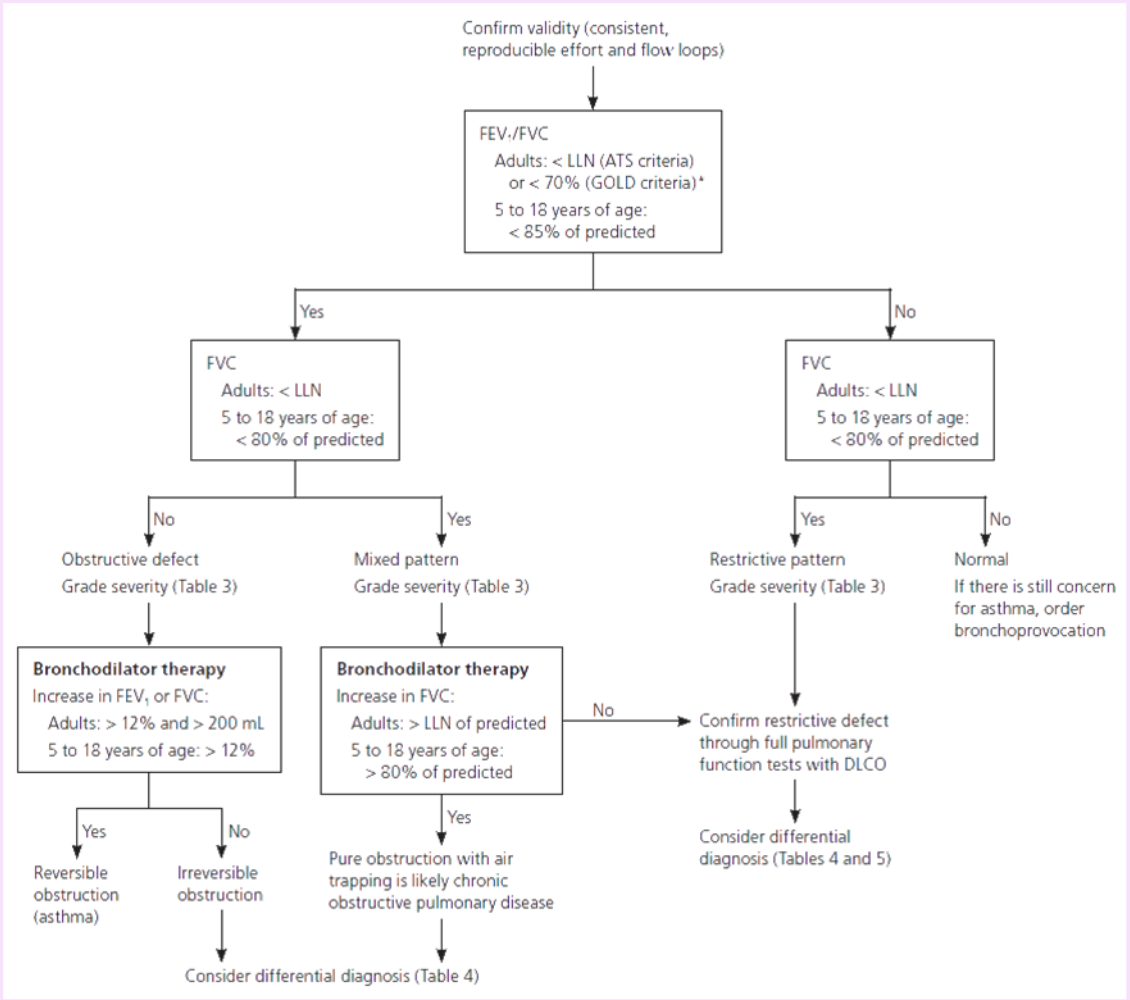
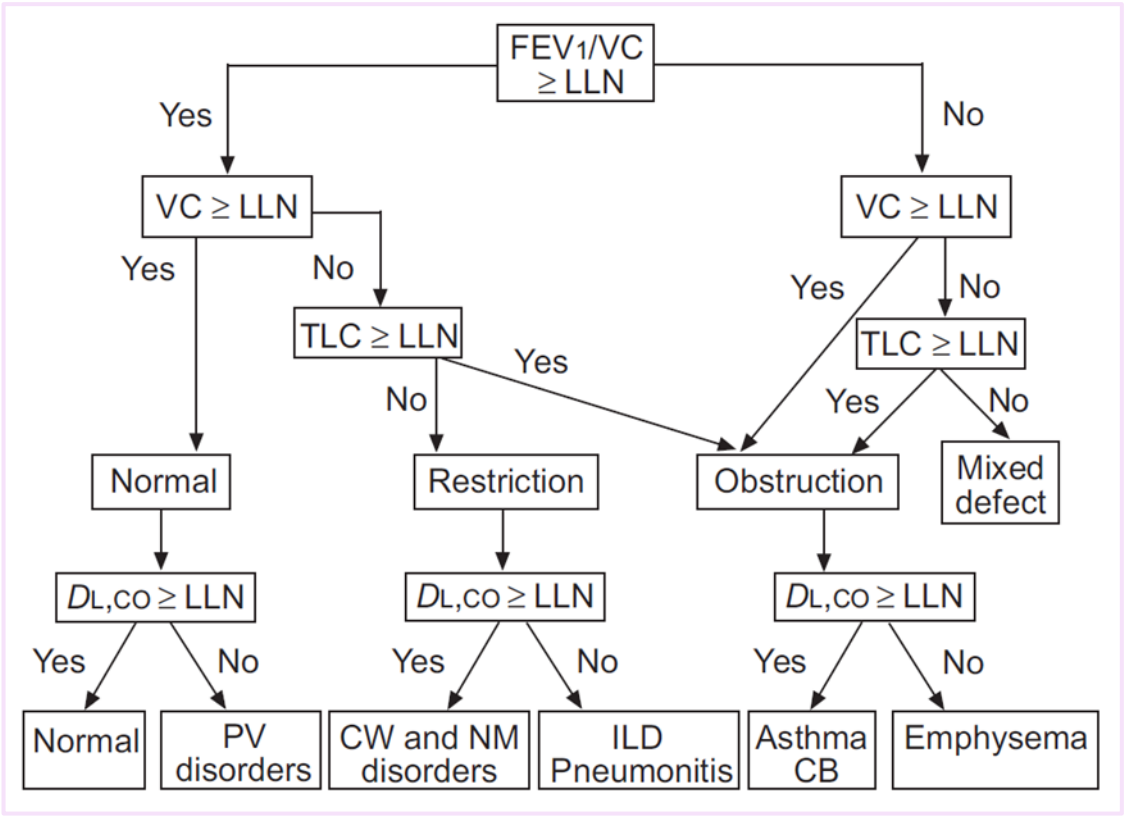


Figure 5.2 Algorithm from Pellegrino et al for identifying different types of lung disease (Pellegrino et al., 2005). Image reproduced with permission of the rights holder.



Children were assigned as low lung function (PT_{low}: $\%FEV_1$ ≤85%), preterm controls (PT_c: $\%FEV_1$ >85%) and term controls (T_c: $\%FEV_1$ >90%) from within the RHiNO study. The children defined as low lung function were subsequently divided into whether their lung function abnormality was due to an obstructive (POLD: FEV_1/FVC <0.8) or non-obstructive (PnOLD: FEV_1/FVC ≥0.8) deficit.

Children in the PT_{low} group were further classified based on their total lung capacity (TLC) on both helium dilution testing and whole-body plethysmography testing, using LLN from the GLI equations as a cut off. Groups were defined as follows:

- Obstructive: POLD group + TLC > LLN
- Mixed obstructive/restrictive: POLD; TLC < LLN
- Restrictive: PnOLD; TLC < LLN
- Pseudorestrictive: PnOLD; TLC < LLN

5.2.3 Statistics

Descriptive statistics were used for comparison of data.

Receiver operating characteristic curves were used to determine potential cut offs for identifying bronchodilator responsiveness and true lung restriction.

Pearson's correlation was used to assess correlation between and Bland-Altman plots with mean difference and limits of agreement at 95% were used to assess for systematic differences within spirometry testing.

5.3 Results

5.3.1 Obstructive restrictive further analysis

221 patients performed spirometry and were assigned to an original group of study based on their FEV_1. Their demographic details are detailed in full in Chapter 2. 53 children were assigned as low lung function (PT_{low}), 98 as preterm controls (PT_c) and 70 as term controls (T_c). Table 5.1 shows the numbers in of children defined as low lung function after being divided into whether their lung function abnormality was due to an obstructive (POLD) or non-obstructive (PnOLD) deficit.

Table 5.1 Numbers in each of the original group defined by lung function (FEV_1) and obstructive/non-obstructive (FEV_1/FVC) status.

Group	POLD	PnOLD	PT_c	T_c
Number	37	16	98	70
FE_{NO} >35ppb	13/34 (38%)	1/16 (6%)	17/97 (18%)	8/68 (12%)
%FEV_1	73.2 (70.0 to 76.5)	80.1 (77.7 to 82.5)	99.3 (97.4 to 101.1)	104.3 (102.6 to 106.0)
FEV_1 <LLN	27/37 (73%)	8/16 (50%)	0/98 (0%)	0/70 (0%)
%FVC	94.4 (91.8 to 97.0)	82.8 (80.4 to 85.1)	102.8 (100.7 to 104.9)	107.6 (105.5 to 109.7)
FVC <LLN	2/37 (5%)	4/16 (25%)	1/98 (1%)	0/70 (0%)
FEV_1/FVC ratio	0.68 (0.65 to 0.70)	0.85 (0.83 to 0.87)	0.84 (0.83 to 0.85)	0.84 (0.83 to 0.86)
FEV_1/FVC <LLN	30/37 (81%)	0/16 (0%)	12/98 (12%)	4/70 (6%)
Increase %FEV_1 post-exercise BD	+15.8 (+12.3 to +19.3)	+4.2 (+2.5 to +5.9)	+5.7 (+4.8 to +6.5)	+6.3 (+5.0 to +7.6)
Post-exercise BD increase >10%	26/31 (84%)	1/16 (6%)	15/87 (17%)	11/66 (17%)
$\%TLC_{(He)}$	96.5 (91.8 to 101.3)	79.3 (75.6 to 83.1)	96.9 (94.8 to 99.0)	102.2 (99.4 to 103.8)
$\%TLC_{(Pleth)}$	97.2 (93.3 to 101.1)	80.5 (76.8 to 84.2)	97.2 (95.2 to 99.2)	101.6 (99.4 to 103.8)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

5.3.1.1 Addition of lung volume testing

Children in the PT_{low} group were further classified based on their total lung capacity (TLC) on both helium dilution testing and whole-body plethysmography testing, using LLN from the GLI equations as a cut off, as outlined above.

5.3.1.1.1 Results with TLC from Helium dilution

Six children within the POLD group were unable to perform adequate Helium dilution testing. All children within PnOLD were successful. Of the 31 POLD children who had available lung volume results, 29 of these remained defined as obstructive disease, with 2 having low TLC suggesting mixed obstructive/restrictive disease. 9 of the children originally defined as restrictive disease based on low FEV_1 and normal FEV_1/FVC also had TLC < LLN, with 7 having normal TLC. This latter group have been defined as pseudorestrictive as they suggest potential restriction on spirometry but have normal TLC. This is summarised in Table 5.2.

Within these defined groups, children in the obstructive lung disease group appear different to the other 3 groups, with a greater bronchodilator response and response rate, and higher %TLC, as shown in Table 5.3. The mixed, restrictive and pseudorestrictive all have similar values for these. Interestingly, there was little difference between TLC obtained by plethysmography and by helium for the obstructive, restrictive and pseudorestrictive group, whereas the mixed group had a lower %TLC on helium compared to plethysmography. This suggests air trapping; however, it would be expected to be seen in the obstructive group as well. However, this is only a small number (n=2) so conclusions cannot be firmly drawn.

Table 5.2 Comparison between obstructive groups using only FEV_1/FVC (as used in RHiNO study) and re-defined groups when using LLN of TLC from Helium dilution testing – numbers present in each group.

		RHiNO classification	
		POLD	**PnOLD**
Re-defined groups based on addition of lung volume testing	**Obstructive**	29	0
	Mixed	2	0
	Restrictive	0	9
	Pseudorestrictive	0	7

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease.

Table 5.3 Numbers in each of the original group defined by lung function (FEV_1), and obstructive/restrictive/mixed disease status (FEV_1/FVC and TLC He).

Group	Obstructive	Mixed	Restrictive	Pseudorestrictive
Number	29	2	9	7
FE_{NO} >35ppb	10/28 (36%)	0/1 (0%)	1/9 (11%)	0/7 (0%)
%FEV_1	75.2 (72.0 to 78.3)	76.5 (6.62 to 146.4)	78.2 (74.5 to 82.0)	82.4 (80.0 to 84.9)
FEV_1 <LLN	20/29 (69%)	1/2 (50%)	6/9 (67%)	2/7 (29%)
%FVC	96.0 (93.2 to 98.8)	87.0 (-1.9 to 175.9)	80.7 (78.1 to 83.2)	85.4 (81.5 to 89.4)
FVC <LLN	0/29 (0%)	1/2 (50%)	3/9 (33%)	1/7 (14%)
FEV_1/FVC ratio	0.68 (0.65 to 0.71)	0.78 (0.50 to 1.04)	0.85 (0.83 to 0.88)	0.84 (0.80 to 0.89)
FEV_1/FVC <LLN	24/29 (83%)	0/2 (0%)	0/9 (0%)	0/7 (0%)
Increase %FEV_1 post-exercise BD	+16.5 (+12.7 to +20.4)	+5.5 (-0.8 to +11.9)	+3.8 (+1.4 to +6.1)	+4.7 (+1.3 to +8.1)
Post-exercise BD increase >10%	24/26 (92%)	0/2 (0%)	0/9 (0%)	1/7 (14%)
$\%TLC_{(He)}$	98.1 (93.6 to 102.6)	73.4 (26.6 to 120.2)	74.3 (71.0 to 77.6)	85.9 (83.1 to 88.6)
$\%TLC_{(Pleth)}$	98.1 (93.9 to 102.3)	83.8 (64.6 to 103.0)	76.9 (72.0 to 81.8)	84.6 (80.1 to 89.1)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

5.3.1.1.2 Results with TLC from Plethysmography

3 children within the POLD group and 1 from the PnOLD were unable to perform adequate plethysmography testing. Of the 34 POLD children who had available lung volume results, all had normal TLC on plethysmography suggesting no evidence of restrictive lung disease in addition to obstructive disease. Only 6 of the plethysmography group had true restrictive disease if based on TLC, and 9 of the original restrictive group had normal TLC.

While the children are classified into slightly different groups based on plethysmography compared to helium, with loss of any children within the mixed obstructive/restrictive group, the results within the group remain fairly similar, with greatest bronchodilator responsiveness and greatest %TLC seen in the obstructive group. These numbers are shown in Table 5.4.

There are few differences seen between the groups when split by this method, although the proportion in the obstructive group who have a positive bronchodilator response is higher when classified by helium, suggesting that this may be a more sensitive way to identify reversible airway obstruction. Table 5.5 summarises these data.

Table 5.4 Comparison between obstructive groups using only FEV_1/FVC (as used in RHiNO study) and re-defined groups when using LLN of TLC from body plethysmography – numbers present in each group.

		RHiNO classification	
		POLD	**PnOLD**
Re-defined groups based on addition of lung volume testing	**Obstructive**	34	0
	Mixed	0	0
	Restrictive	0	6
	Pseudorestrictive	0	9

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease.

Table 5.5 Numbers in each of the original group defined by lung function (FEV_1), and obstructive/restrictive/mixed disease status (FEV_1/FVC and TLC Pleth).

Group	Obstructive	Mixed	Restrictive	Pseudorestrictive
Number	34	0	6	9
FE_{NO} >35ppb	11/32 (34%)		1/6 (17)	0/9 (0%)
%FEV_1	74.2 (70.9 to 77.4)		77.0 (72.1 to 81.9)	82.1 (79.4 to 84.8)
FEV_1 <LLN	24/34 (71%)		5/6 (83%)	2/9 (22%)
%FVC	94.5 (91.7 to 97.3)		79.7 (75.8 to 83.6)	84.7 (81.6 to 87.7)
FVC <LLN	2/34 (6%)		3/6 (50%)	1/9 (11%)
FEV_1/FVC ratio	0.68 (0.66 to 0.71)		0.84 (0.81 to 0.87)	0.85 (0.82 to 0.89)
FEV_1/FVC <LLN	27/34 (79%)		0/6 (0%)	0/9 (0%)
Increase %FEV_1 post-exercise BD	+15.8 (+12.3 to +19.3)		+3.2 (+0.2 to +6.3)	+5.1 (+2.5 to +7.8)
Post-exercise BD increase >10%	26/31 (84%)		0/6 (0%)	1/9 (11%)
$\%TLC_{(He)}$	96.5 (91.8 to 101.3)		73.8 (67.4 to 80.2)	83.4 (79.5 to 87.3)
$\%TLC_{(Pleth)}$	97.2 (95.2 to 99.2)		74.1 (69.5 to 78.8)	84.7 (81.9 to 87.6)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

5.3.1.2 Johnson et al interpretation

Using Johnson et al's interpretation of lung function testing involves using set cut-offs of FEV_1/FVC and percent predicted FVC as a way to divide by obstructive restrictive status. Applying these criteria to our population gives a comparison as seen in Table 5.6.

Of the 37 POLD children, 10 would be reclassified as normal, despite having a $\%FEV_1 \leq 85\%$. Only 4 of the PnOLD children would meet criteria for restrictive. There would also be 6 children (4 PT_c and 2 T_c) who meet criteria for obstructive lung disease despite having normal FEV_1 on spirometry. These numbers are displayed in Table 5.6.

Of the children defined as POLD based on our RHiNO criteria, 36% of those also classified as obstructive based on the Johnson criteria had raised FE_{NO} compared to 44% of those defined by normal by Johnson. Additionally of these children with potential obstruction defined as normal by Johnson, 56% still have reversible airways based on >10% predicted $\%FEV_1$ increase after bronchodilator. While this is a smaller proportion than the children that both classifications would define as obstructive (96%), it remains higher than the restrictive or control groups whichever way they are classified. Table 5.7 shows these comparisons.

Table 5.6 Comparison between obstructive groups using only FEV_1/FVC (as used in RHiNO study) and re-defined groups when using Johnson et al interpretation – numbers present in each group.

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
Re-defined groups based on Johnson et al	**Obstructive**	27	0	4	2
	Mixed	0	0	0	0
	Restrictive	0	4	0	0
	Normal	10	12	94	68

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control.

Table 5.7 Comparison between RHiNO classification and re-defined group designations using Johnson et al interpretation – lung function (continued over page).

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
FE_{NO} >35ppb	Obstructive	9/25 (36%)	-	1/4 (25%)	0/2 (0%)
	Restrictive	-	0/4 (0%)	-	-
	Normal	4/9 (44%)	1/12 (8%)	16/93 (17%)	8/66 (12%)
%FEV1	Obstructive	70.8 (66.8 to 74.8)	-	94.8 (86.7 to 102.8)	93.5 (87.2 to 99.9)
	Restrictive	-	76 (66.5 to 85.5)	-	-
	Normal	79.7 (76.7 to 82.8)	81.4 (79.4 to 83.4)	99.5 (97.5 to 101.4)	104.6 (102.9 to 106.3)
FEV_1 <LLN	Obstructive	21/27 (78%)	-	0/4 (0%)	0/2 (0%)
	Restrictive	--	3/4 (75%)		-
	Normal	6/10 (60%)	5/12 (42%)	0/94 (0%)	0/68 (0%)
%FVC	Obstructive	96.2 (93 to 99.4)	-	113.0 (103.0 to 123.0)	116 (39.8 to 192.2)
	Restrictive	-	77 (74.8 to 79.3)	-	-
	Normal	89.5 (86.5 to 92.5)	84.7 (82.7 to 86.7)	102.3 (100.2 to 104.5)	107.3 (105.2 to 109.4)
FVC <LLN	Obstructive	1/27 (4%)		0/4 (%)	0/2 (0%)
	Restrictive		4/4 (100%)		
	Normal	1/10 (10%)	0/12 (0%)	1/94 (1%)	0/68 (0%)
FEV_1/FVC ratio	Obstructive	0.64 (0.61 to 0.67)	-	0.74 (0.73 to 0.75)	0.7 (0.42 to 0.98)
	Restrictive	-	0.87 (0.79 to 0.95)	-	-
	Normal	0.77 (0.76 to 0.79)	0.84 (0.82 to 0.86)	0.85 (0.84 to 0.86)	0.85 (0.84 to 0.86)
FEV_1/FVC <LLN	Obstructive	27/27 (100%)	-	4/4 (100%)	2/2 (100%)
	Restrictive	-	0/4 (0%)	-	-
	Normal	3/10 (30%)	0/12 (0%)	8/94 (9%)	2/68 (3%)
Increase $\%FEV_1$ post-exercise BD	Obstructive	+18.2 (+13.7 to +22.8)	-	+8.0 (+1.4 to +14.6)	+13.5 (-43.7 to +70.7)
	Restrictive	-	+2.8 (-3.1 to +8.6)	-	-
	Normal	+10 (+7.1 to +12.9)	+4.7 (+2.7 to + 6.6)	+5.6 (+4.7 t +6.4)	+6.1 (+4.8 to +7.4)
Post-exercise BD increase >10%	Obstructive	21/22 (96%)	-	1/3 (33%)	1/2 (50%)
	Restrictive	-	0/4 (0%)	-	-
	Normal	5/9 (56%)	1/12 (8%)	14/84 (17%)	10/64 (16%)

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
$\%TLC_{(He)}$	Obstructive	101.8 (97.1 to 106.5)	-	106.1 (99.3 to 112.9)	111.3 (90.7 to 131.8)
	Restrictive	-	74.6 (58.2 to 91)	-	-
	Normal	83.6 (77.1 to 90.1)	80.9 (77.7 to 84.2)	96.5 (94.3 to 98.6)	101.9 (99.5 to 104.3)
$\%TLC_{(Pleth)}$	Obstructive	101.2 (96.7 to 105.7)	-	104.6 (98.5 to 110.7)	110.5 (66.2 to 154.7)
	Restrictive	-	74.6 (62.3 to 86.9)	-	-
	Normal	87.6 (83.9 to 91.2)	82.6 (79.4 to 85.9)	96.9 (94.9 to 98.9)	101.4 (99.1 to 103.6)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

5.3.1.3 Pellegrino et al interpretation

Pellegrino's interpretation for distinguishing between obstructive and restrictive disease involves using vital capacity rather than specifically a forced vital capacity, both in isolation and as part of a FEV_1/VC ratio, with the cut-off as the LLN to distinguish normality. For certain participants, the addition of TLC at the LLN is used within this classification. This results in 4 potential disease classifications of obstruction, restriction, mixed disease or normal.

Due to data collection, a vital capacity other than from a forced manoeuvre was not recorded from spirometry during this testing. The rationale behind the use of a slow inspiratory or expiratory vital capacity is FVC may be lower due to airway obstruction (Pellegrino et al., 2005). For the purposes of this comparison between classification methods, the FVC has been used as the VC. Additionally, it should be noted that the GLI spirometry reference ranges are for FVC rather than VC, and as such, it could be argued that use of FVC is best to compare to 'normality'.

5.3.1.3.1 Helium dilution

Using helium dilution testing for lung volumes, and the LLN for TLC to divide into disease type, of 36 children defined as POLD by RHiNO, 29 of these still fell into the obstructive lung disease group; however, 1 would be classed as restrictive and 6 as normal. Of the PnOLD children, only 3 of the 16 able to do helium testing would be classed as true restrictive. 13 PT_c children would get redefined as obstructive lung disease, as would 4 term children, as shown in Table 5.8.

Table 5.9 shows comparisons for results between the groups. The children defined as POLD by RHiNO definition but normal by Pellegrino had the highest proportions of FE_{NO} >35ppb (60%) whereas proportion with bronchodilator response >10% was greatest in the POLD-obstructive group (87%), as well as overall %FEV_1 increase following post-exercise bronchodilator (17.6%). While post-exercise bronchodilator response was similar in POLD-normal (11.5), PT_c-obstructive (9.8) and T_c-obstructive (10.8) groups, the proportion with post-exercise bronchodilator response was greatest in POLD-normal (83 vs 50 vs 25%), suggesting that calling this group normal is not entirely accurate given the presence of reversible airways disease.

Table 5.8 Comparison between old and re-defined group designations using Pellegrino (Helium) et al interpretation – numbers present in each group.

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
Re-defined groups based on Pellegrino	**Obstructive**	29	1	13	4
	Mixed	0	0	0	0
	Restrictive	1	3	0	0
	Normal	6	12	85	66

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control.

Table 5.9 Comparison between RHiNO classification and re-defined group designations using Pellegrino (Helium) et al interpretation – lung function (continued over page).

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
FE_{NO} >35ppb	Obstructive	10/27 (37%)	0/1 (0%)	2/12 (17%)	1/3 (33%)
	Restrictive	0/1 (0%)	0/3 (0%)	-	-
	Normal	3/5 (60%)	1/12 (8%)	15/85 (18%)	7/65 (11%)
%FEV1	Obstructive	72.2 (68.5 to 75.9)	84 (N/A)	95.9 (92.1 to 99.8)	96 (88.5 to 103.5)
	Restrictive	71 (N/A)	73.3 (65.4 to 81.3)	-	-
	Normal	81.2 (77.6 to 84.8)	81.4 (79.4 to 83.4)	99.8 (97.7 to 101.9)	104.8 (103.1 to 106.5)
FEV_1 <LLN	Obstructive	22/29 (76%)	0/1 (0%)	0/13 (0%)	0/4 (0%)
	Restrictive	1/1 (100%)	3/3 (100%)	-	-
	Normal	3/6 (50%)	5/12 (100%)	0/85 (0%)	0/66 (0%)
%FVC	Obstructive	96.3 (93.5 to 99.1)	79 (N/A)	109.3 (102.7 to 116)	114.8 (105.4 to 124.1)
	Restrictive	80 (N/A)	76.3 (74.9 to 77.8)	-	-
	Normal	89.8 (87.1 to 92.6)	84.7 (82.7 to 86.7)	101.8 (99.6 to 104)	107.1 (105 to 109.3)
FVC <LLN	Obstructive	0/29 (0%)	1/1 (100%)	1/13 (8%)	0/4 (0%)
	Restrictive	1/1 (100%)	3/3 (100%)	-	-
	Normal	0/6 (100%)	0/12 (0%)	0/85 (0%)	0/66 (0%)
FEV_1/FVC ratio	Obstructive	0.65 (0.63 to 0.68)	0.94 (N/A)	0.77 (0.74 to 0.8)	0.74 (0.67 to 0.81)
	Restrictive	0.8 (N/A)	0.85 (0.77 to 0.94)	-	-
	Normal	0.78 (0.76 to 0.8)	0.84 (0.82 to 0.86)	0.85 (0.84 to 0.86)	0.85 (0.84 to 0.86)
FEV_1/FVC <LLN	Obstructive	29/29 (100%)	0/1 (0%)	12/13 (92%)	0/4 (0%)
	Restrictive	0/1 (0%)	0/3 (0%)	-	-
	Normal	0/6 (0%)	0/12 (0%)	0/85 (0%)	0/66 (0%)
Increase $\%FEV_1$ post-exercise BD	Obstructive	+17.6 (+13.1 to +22.1)	-2.0 (N/A)	+9.8 (+7.2 to +12.3)	+10.8 (+3.0 to +18.5)
	Restrictive	+5.0 (N/A)	+4.3 (-1.4 to +10.1)	-	-
	Normal	+11.5 (+7.7 to +15.3)	+4.7 (+2.7 to +6.6)	+5.0 (+4.2 to +5.8)	+6.0 (+4.7 to +7.3)
Post-exercise BD increase >10%	Obstructive	20/23 (87%)	0/1 (0%)	6/12 (50%)	1/4 (25%)
	Restrictive	0/1 (0%)	0/3 (0%)		
	Normal	5/6 (83%)	1/12 (8%)	9/75 (12%)	10/62 (16%)

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
$\%TLC_{(He)}$	Obstructive	100.9 (96.3 to 105.5)	89.4 (N/A)	102.8 (97.8 to 107.9)	107.3 (99.0 to 115.6)
	Restrictive	69.7 (N/A)	69.6 (60.8 to 78.5)	-	-
	Normal	83.4 (79.3 to 87.5)	80.9 (77.7 to 84.2)	95.9 (93.6 to 98.2)	101.8 (99.3 to 104.3)
$\%TLC_{(Pleth)}$	Obstructive	100.9 (96.7 to 105)	85.0 (N/A)	102.9 (96.6 to 109.2)	110.6 (102.9 to 118.3)
	Restrictive	82.3 (N/A)	71.2 (60.4 to 81.9)	-	-
	Normal	85.5 (81.6 to 89.4)	82.6 (79.4 to 85.9)	96.4 (94.3 to 98.4)	101.1 (98.8 to 103.3)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

5.3.1.3.2 Plethysmography

When using the LLN for TLC taken from body plethysmography, the numbers are very similar to when using Helium dilution TLC. One child from POLD that helium would define as restrictive was classed obstructive, and 1 child from PT_c classed as obstructive on helium was in the restrictive group, as outlined in Table 5.10.

Results were very similar to when using helium to classify types of lung disease, as per Table 5.11. This suggests that using either would be suitable for interpretation of Pellegrino's methods of classifying obstructive or restrictive lung disease.

Table 5.10 Comparison between RHiNO classification and re-defined group designations using Pellegrino (Plethysmography) et al interpretation – numbers present in each group.

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
Re-defined groups based on Pellegrino et al	**Obstructive**	31	1	12	4
	Mixed	0	0	0	0
	Restrictive	0	3	1	0
	Normal	6	12	85	66

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control.

Table 5.11 Comparison between RHiNO classification and re-defined group designations using Pellegrino (Plethysmography) et al interpretation – lung function (continued over page).

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
FE_{NO} >35ppb	Obstructive	10/29 (35%)	0/1 (0%)	2/11 (18%)	1/3 (33%)
	Restrictive	-	0/3 (0%)	0/1 (0%)	-
	Normal	3/5 (60%)	1/12 (8%)	15/85 (18%)	7/65 (11%)
$\%FEV_1$	Obstructive	71.7 (68.1 to 75.3)	84 (N/A)	96.6 (92.6 to 100.5)	96 (88.5 to 103.5)
	Restrictive	-	73.3 (65.4 to 81.3)	88 (N/A)	-
	Normal	81.2 (77.6 to 84.8)	81.4 (79.4 to 83.4)	99.8 (97.7 to 101.9)	104.8 (103.1 to 106.5)
FEV_1 <LLN	Obstructive	24/31 (77%)	0/1 (0%)	0/12 (0%)	0/4 (0%)
	Restrictive	-	3/3 (100%)	0/1 (0%)	-
	Normal	3/6 (50%)	5/12 (42%)	0/85 (0%)	0/66 (0%)
%FVC	Obstructive	95.3 (92.3 to 98.2)	79 (N/A)	111.8 (107.4 to 116.1)	114.8 (105.4 to 124.1)
	Restrictive	-	76.3 (74.9 to 77.8)	80 (N/A)	-
	Normal	89.8 (87.1 to 92.6)	84.7 (82.7 to 86.7)	101.8 (99.6 to 104)	107.1 (105 to 109.3)
FVC <LLN	Obstructive	2/31 (7%)	1/1 (100%)	0/12 (0%)	0/4 (0%)
	Restrictive	-	3/3 (100%)	1/1 (100%)	-
	Normal	0/6 (0%)	0/12 (0%)	0/85 (0%)	0/66 (0%)
FEV_1/FVC ratio	Obstructive	0.66 (0.63 to 0.68)	0.94 (N/A)	0.75 (0.74 to 0.76)	0.74 (0.67 to 0.81)
	Restrictive	-	0.85 (0.77 to 0.94)	0.93 (N/A)	-
	Normal	0.78 (0.76 to 0.8)	0.84 (0.82 to 0.86)	0.85 (0.84 to 0.86)	0.85 (0.84 to 0.86)
FEV_1/FVC <LLN	Obstructive	30/31 (97%)	0/1 (0%)	12/12 (100%)	4/4 (100%)
	Restrictive	-	0/3 (0%)	0/1 (100%)	-
	Normal	0/6 (0%)	0/12 (0%)	0/85 (0%)	0/66 (0%)
Increase $\%FEV_1$ post-exercise BD	Obstructive	+16.9 (+12.6 to +21.2)	-2.0 (N/A)	+10.2 (+7.5 to +12.8)	+10.8 (+3.0 to +18.5)
	Restrictive	-	+4.3 (-1.4 to +10.1)	+5.0 (N/A)	-
	Normal	+11.5 (+7.7 to +15.3)	+4.7 (+2.7 to +6.6)	+5.0 (+4.2 to +5.8)	+6.0 (+4.7 to +7.3)
Post-exercise BD increase >10%	Obstructive	21/25 (84%)	0/1 (0%)	6/11 (55%)	1/4 (25%)
	Restrictive	-	0/3 (0%)	0/1 (0%)	-
	Normal	5/6 (83%)	1/11 (8%)	9/75 (12%)	10/62 (16%)

		Original RHiNO classification			
		POLD	**PnOLD**	**PT_c**	**T_c**
$\%TLC_{(He)}$	Obstructive	99.7 (94.5 to 104.8)	89.4 (N/A)	104.3 (100.1 to 108.6)	107.3 (99 to 115.6)
	Restrictive	-	69.6 (60.8 to 78.5)	84.8 (N/A)	-
	Normal	83.4 (79.3 to 87.5)	80.9 (77.7 to 84.2)	95.9 (93.6 to 98.2)	101.8 (99.3 to 104.3)
$\%TLC_{(Pleth)}$	Obstructive	99.7 (95.5 to 103.9)	85 (N/A)	105.3 (101.5 to 109.1)	110.6 (102.9 to 118.3)
	Restrictive	-	71.2 (60.4 to 81.9)	76.4 (N/A)	-
	Normal	85.5 (81.6 to 89.4)	82.6 (79.4 to 85.9)	96.4 (94.3 to 98.4)	101.1 (98.8 to 103.3)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution; **Pleth** – Plethysmography.

Table 5.12 Comparison of lung function data in between obstructive, restrictive and normal/controls when using RHiNO, Pellegrino, and Johnson methods of classifying lung disease in preterm children.

	Obstructive			Restrictive/Non-obstructive (RHiNO)			Normal		
	RHiNO	Johnson	Pellegrino	RHiNO	Johnson	Pellegrino	RHiNO	Johnson	Pellegrino
n=	37	31	44	16	4	4	98	116	103
FE_{NO} >35ppb	13/34 (38%)	10/29 (35%)	12/40 (29%)	1/16 (6%)	0/4 (0%)	0/4 (0%)	17/97 (18%)	24/114 (18%)	19/102 (19%)
FEV_1	73.2 (70.0 to 76.4)	73.9 (69.3 to 78.5)	78.8 (74.4 to 83.1)	80.1 (77.7 to 82.5)	76.0 (66.5 to 85.5)	77.0 (64.6 to 89.4)	99.3 (97.4 to 101.1)	95.9 (93.8 to 98.0)	96.5 (94.3 to 98.8)
FVC	94.4 (91.8 to 97.0)	98.4 (94.8 to 101.9)	99.4 (96.0 to 102.8)	82.8 (80.4 to 85.1)	77.0 (74.8 to 79.3)	77.3 (74.2 to 80.3)	98.6 (96.8 to 100.4)	99.4 (97.3 to 101.5)	99.1 (96.9 to 101.2)
FEV_1/FVC	0.68 (0.65 to 0.70)	0.65 (0.63 to 0.68)	0.69 (0.66 to 0.72)	0.85 (0.83 to 0.87)	0.87 (0.79 to 0.96)	0.87 (0.79 to 0.95)	0.84 (0.83 to 0.85)	0.84 (0.83 to 0.85)	0.85 (0.84 to 0.86)
Increase %FEV_1 post-exercise BD	15.8 (12.3 to 19.3)	17.0 (12.8 to 21.2)	14.4 (11.2 to 17.6)	4.2 (2.5 to 5.9)	2.8 (-3.1 to 8.6)	4.5 (1.5 to 7.5)	5.7 (4.8 to 6.5)	5.8 (5.7 to 6.6)	5.4 (4.6 to 6.2)
Post-exercise BD increase >10%	26/31 (84%)	22/25 (88%)	27/37 (73%)	1/16 (6%)	0/4 (0%)	0/4 (0%)	15/87 (17%)	20/105 (19%)	15/93 (16%)
%$TLC_{(He)}$	96.5 (91.8 to 101.3)	102.5 (98.4 to 106.5)	100.9 (97.7 to 104.1)	79.3 (75.6 to 83.1)	74.6 (58.2 to 91.0)	65.5 (65.0 to 79.5)	96.9 (94.8 to 99.0)	93.7 (91.6 to 95.8)	94.1 (92.1 to 96.1)

Abbreviations: POLD – Prematurity-associated obstructive lung disease; **PnOLD** – Prematurity-associated non-obstructive lung disease, **PT_c** – Preterm control, **T_c** – Term control; **FE_{NO}** – fractional exhaled nitric oxide; **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **LLN** – lower limit of normal; **BD** – bronchodilator; **TLC** – total lung capacity; **He** – helium dilution.

Table 5.12 shows direct comparisons in the numbers in each group as defined by RHiNO, Johnson, and Pellegrino, and a selection of their lung function results. There were small differences in the numbers seen in the obstructive groups, with fewest designated by Johnson (31), and largest by Pellegrino (43) with RHiNO classifying 37 as obstructive. Restrictive is where RHiNO has greater numbers, with 16 compared to 4 in the Pellegrino and Johnson. In the obstructive groups, Pellgrino has the lowest overall post-exercise bronchodilator response seen with total %predicted change of 14.4% and proportion with >10% repsonse of 72% compared to 15.8% and 17% increases and 84% and 88% response rate in RHiNO and Johnson methods of classifying obstruction. Conversely bronchodilator response was similarly low with all classifications, including RHiNO's description of restriction with only 1/16 responding >10% following post-exercise bronchodilators, with none in the Pellegrino or Johnson groups. However, 16-19% of normal preterm children had post-exercise bronchodilator response >10% predicted. This suggests that RHiNO's method of diagnosing restriction shows similar characteristics despite being a more liberal definition. Additionally, all methods of classification appeared to miss children with reversibility suggesting no method is entirely optimal for this purpose. Figures 5.3 and 5.4 represent comparisons between RHiNO and the Johnson and Pellegrino methods. In both cases, there were children who have reversibility who would be missed by those methods of disease classification. Conversely, there were children with reversibility missed by RHiNO classification also. The sensitivity, specificity, positive predictive value and negative predictive values are in Table 5.13.

Table 5.13 Table showing the sensitivity, specificity, positive predictive value, and negative predictive value for the various methods for identifying obstructive lung disease, with the binary outcome of reversible (>10% absolute response in %predicted FEV_1) airways disease as the disease.

	RHiNO	Johnson	Pellegrino
Sensitivity	62%	52%	64%
Specificity	95%	97%	89%
Positive predictive value	84%	88%	73%
Negative predictive value	84%	82%	84%

Figure 5.3 Scatter plot of change in %FEV$_1$ post-exercise bronchodilator vs FEV$_1$ z-score, grouped by obstructive /non-obstructive/ restrictive status as classified by Johnson et al methods vs RHiNO methods

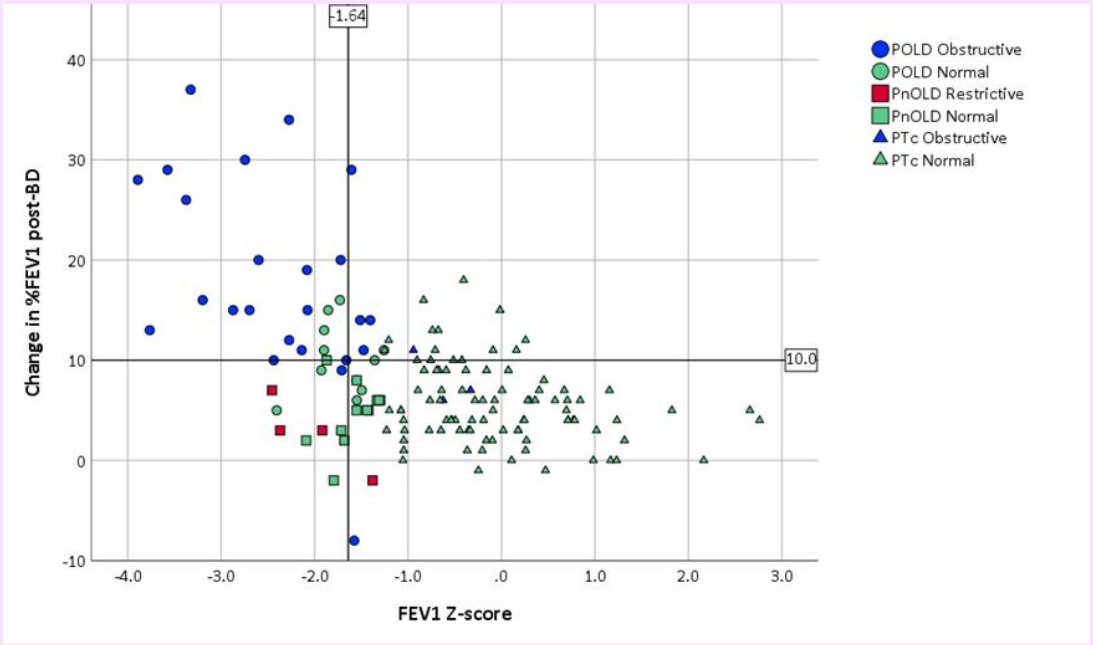
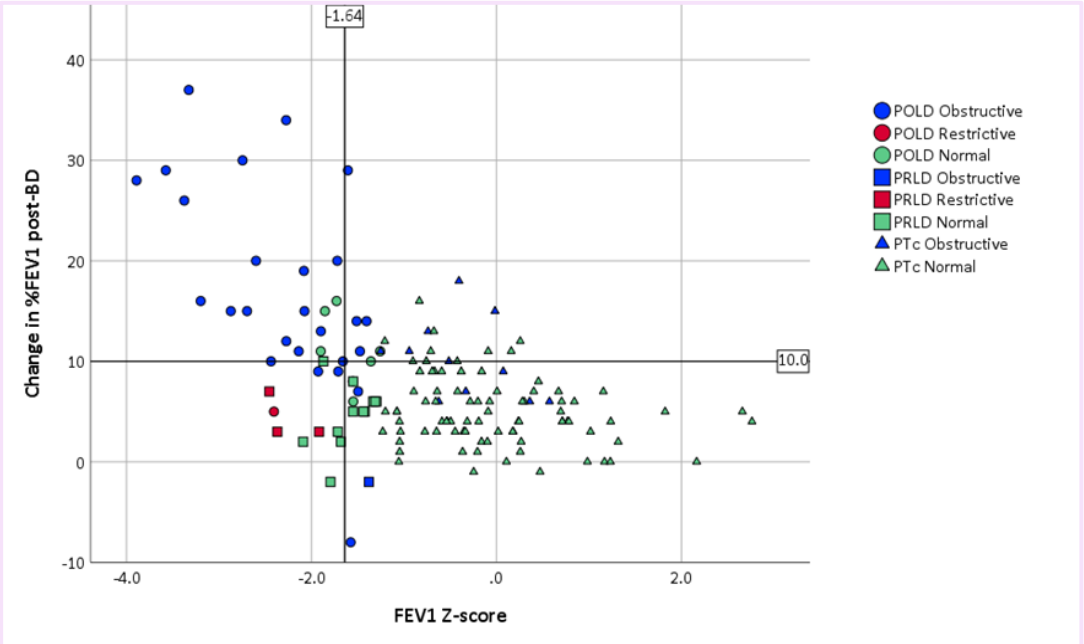


Figure 5.4 Scatter plot of change in %FEV$_1$ post-exercise bronchodilator vs FEV$_1$ z-score, grouped by obstructive /non-obstructive/ restrictive status as classified by Pellegrino et al methods vs RHiNO methods.



5.3.1.4 Receiver operating characteristic curves

5.3.1.4.1 Bronchodilator response

In order to identify bronchodilator responsiveness as defined by increase of %predicted FEV_1 following administration of 400 micrograms of salbutamol, a receiver operating characteristic curve was drawn, as shown in Figure 5.5. It included FEV_1 in %predicted and z-score and FEV_1/FVC ratio in both absolute number and z-score. Absolute FEV_1/FVC of 0.80 had the best sensitivity/specificity (see circle on Figure 5.5 and Table 5.14), showing a sensitivity of 0.844 and a specificity of 0.790. This was better than using a z-score or either FEV_1 parameters.

Figure 5.5 Receiver operating characteristic curves for %FEV_1 and FEV_1/FVC and their z-scores to identify bronchodilator responsiveness in the preterm children.

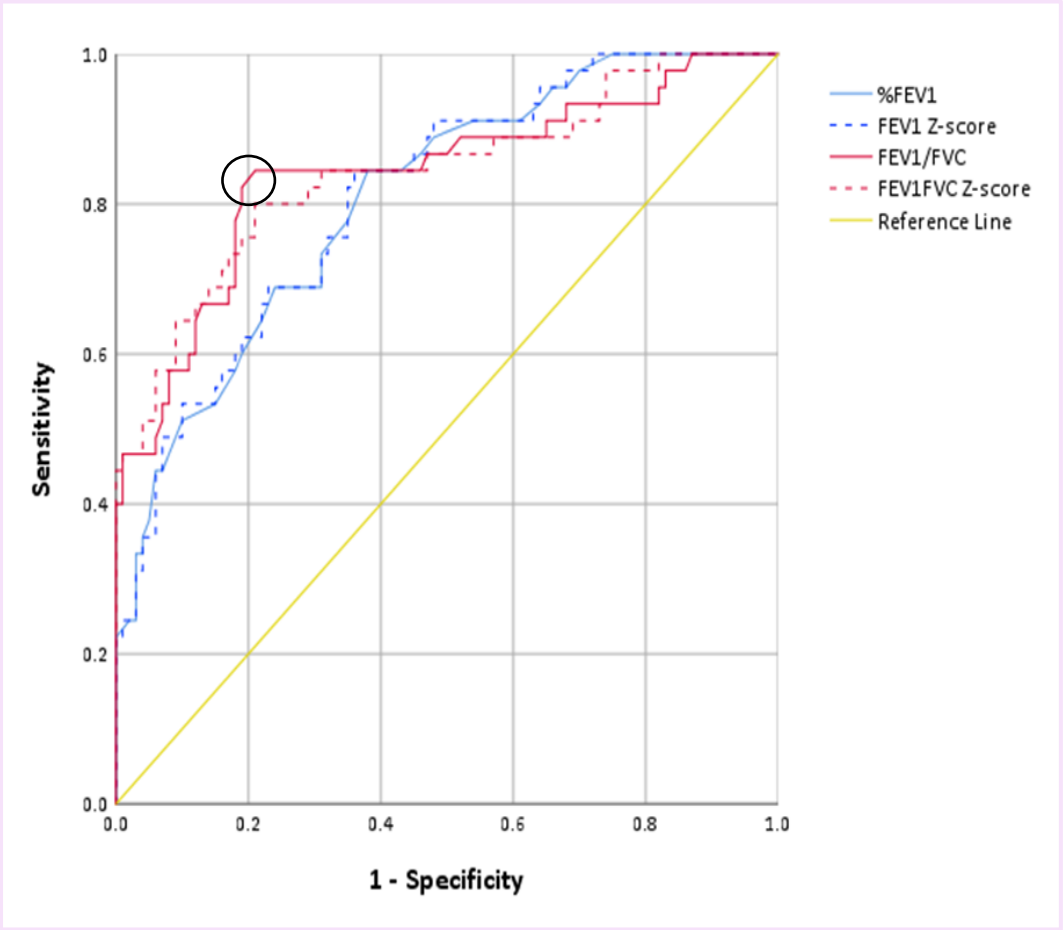


Table 5.14 Receiver operating characteristic curve measures for preterm population using change in FEV_1 ≥10% as outcome variable.

Increase in FEV_1 ≥10% post-exercise bronchodilator	**Valid N (listwise)**	
Positive	45	
Negative	100	
Test	**Area under the curve**	
%FEV_1	0.808	
FEV_1 z-score	0.810	
FEV_1/FVC	0.841	
FEV_1/FVC z-score	0.842	
Value	**Sensitivity**	**1 - Specificity**
(FEV_1/FVC):		
0.7970	0.844	0.210
(FEV_1/FVC z-score):		
-1.12	0.800	0.210

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity.

5.3.1.4.2 Restrictive lung disease

True restrictive lung disease is defined by total lung capacity less than LLN on lung volume testing, although FVC has been used as a surrogate. Using TLC <LLN as a target, FVC was used as %predicted and z-score to see what cut-off might predict restrictive disease. Only 10 restrictive cases were present on testing, however %predicted FVC of 86% or z-score of -1.3 both had sensitivities of 0.8, with specificities of 0.908 and 0.916 respectively (Figure 5.6 and Table 5.15). This potentially suggests that a higher cut off could be used to screen for restrictive disease, however this is limited by small numbers.

Figure 5.6 Receiver operating characteristic curve for %FVC and its z-score to identify restrictive lung disease based on total lung capacity at lower limit of normal on body plethysmography testing.

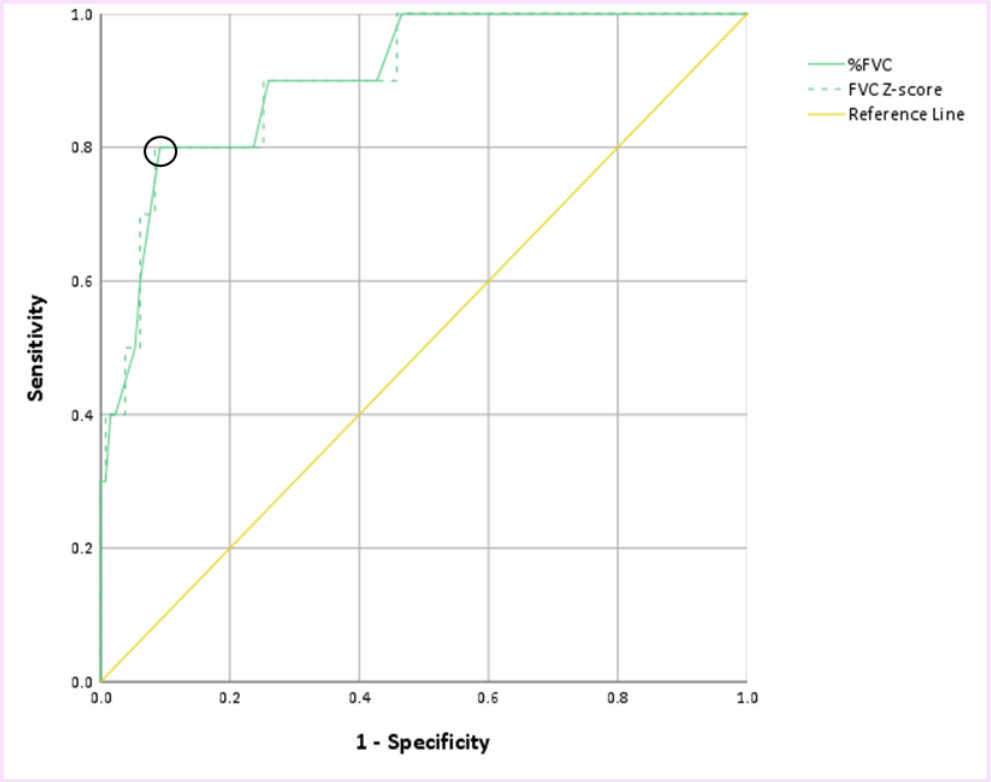


Table 5.15 ROC curve measures for preterm population using LLN for TLC on body plethysmography as outcome variable.

TLC <LLN	**Valid N (listwise)**	
Positive	10	
Negative	131	
Test	**Area under the curve**	
%FVC	0.905	
FVC z-score	0.904	
Positive if Less Than or Equal To	**Sensitivity**	**1 - Specificity**
%FVC:		
0.86	.800	.092
FVC z-score:		
-1.2680	.800	.084

Abbreviations: **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity.

5.3.2 Spirometry comparison

Baseline comparisons between MicroLoop and MasterScreen spirometry testing were performed for four parameters (FEV_1, FVC, FEV_1/FVC ratio and $FEF_{25-75\%}$) for three result outcomes (raw value, %predicted, z-score).

5.3.2.1 Correlation

All results from both types of spirometry correlated strongly with each other, with Pearson's correlation coefficient >0.9 for all, as shown in Table 5.16. The strongest correlation for raw results was seen for FEV_1, followed by FVC, $FEF_{25-75\%,}$ then FEV_1/FVC (0.987, 0.976, 0.954 and 0.921 respectively). For all except for the ratio, the raw value showed the strongest correlation between the three result-types. For FEV_1/FVC, the %predicted was the strongest correlation (0.922). There was generally little difference between the %predicted and z-score correlations (0 for FEV_1, 0.002 for FVC and $FEF_{25-75\%}$), however this was greater in the FEV_1/FVC with %predicted correlation 0.018 higher for %predicted, suggesting a less linear agreement between %predicted and z-score for this outcome, within this cohort.

Table 5.16 Mean results for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing, along with Pearson's correlation and its significance value between methods of testing.

	MicroLoop Mean	MasterScreen Mean	Correlation	Sig.
FEV_1 raw (litres)	1.990	2.113	0.987	<0.0001
$\%FEV_1$	89.7	95.1	0.966	<0.0001
FEV_1 z-score	-0.89	-0.41	0.966	<0.0001
FVC raw (litres)	2.395	2.586	0.976	<0.0001
%FVC	94.0	101.4	0.909	<0.0001
FVC z-score	-0.53	0.12	0.907	<0.0001
FEV_1/FVC raw	0.830	0.816	0.921	<0.0001
$\%FEV_1$/FVC	94.8	93.2	0.922	<0.0001
FEV_1/FVC Z-score	-0.61	-0.82	0.904	<0.0001
$FEF_{25-75\%}$ raw (L/sec)	2.024	2.192	0.954	<0.0001
$\%FEF_{25-75\%}$	74.9	80.9	0.935	<0.0001
$FEF_{25-75\%}$ z-score	-1.24	-0.95	0.937	<0.0001

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.2 Overall differences

FEV_1 raw values were 125 mls higher on average with MasterScreen (i.e., pneumotach) spirometry. This equated to a 5.5 %predicted or just under 0.5 z-score difference. FVC had a greater raw difference compared to FEV_1 with a difference of 12 mls in favour of the MasterScreen, equating to 7.5 %predicted and almost 2/3 of a z-score. There was little difference for FEV_1/FVC ratio with MicroLoop (i.e., turbine) giving a raw value of 0.014 (or 1.4%) higher than the MasterScreen. This equated to %predicted and z-score being 1.6% and 0.2 higher respectively for MicroLoop. This relates to the greater difference being seen in the denominator (i.e., the FVC). Raw values for $FEF_{25-75\%}$ were 0.17L/sec higher on MasterScreen testing compared to MicroLoop, equating to 6.1 and 0.29 of %predicted or z-score respectively. These data are shown on Bland-Altman plots in Figures 5.7 to 5.10 and in Table 5.17.

Table 5.17 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, for whole population (n=220).

	Mean difference	LLOA	ULOA
FEV_1 raw (litres)	0.125	-0.041	0.291
$\%FEV_1$	5.5	-1.8	12.7
FEV_1 z-score	0.48	-0.14	1.10
FVC raw (litres)	0.192	-0.049	0.434
%FVC	7.5	-2.3	17.3
FVC z-score	0.65	-0.20	1.50
FEV_1/FVC raw	-0.014	-0.08	0.052
$\%FEV_1$/FVC	-1.6	-9.1	5.9
FEV_1/FVC z-score	-0.20	-1.19	0.79
$FEF_{25-75\%}$ raw (L/sec)	0.170	-0.321	0.662
$\%FEF_{25-75\%}$	6.1	-12.0	24.1
$FEF_{25-75\%}$ z-score	0.29	-0.58	1.16

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.3 Lung Function Groups

When divided by lung function groups (preterm with $FEV_1 \leq 85\%$, preterm with $FEV_1 > 85\%$ and term), there was significant difference seen on one-way ANOVA for all FEV_1 spirometry differences between PT_{low} and P_c groups, with lower difference seen in the PT_{low} group (0.098 vs 0.146, 4.5 vs 6.1, 0.38 vs 0.54 for raw, %predicted and z-score respectively), as shown in Table 5.18. For FVC, the mean differences for raw values, %predicted and z-score were all higher in the PT_{low} group compared to T_c group. Raw values and %predicted mean differences for FEV_1/FVC raw and %predicted values for PT_{low} compared to both control groups were higher, again with MicroLoop spirometry giving high FEV_1/FVC ratios (i.e., more negative values in the PT_{low} group). PT_{low} had lower mean differences for all three $FEF_{25-75\%}$ parameters compared to both control groups.

Table 5.18 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by lung function status (preterm with FEV_1 ≤85%, PT_{low} - n=53, preterm controls, P_c – n=97, term controls, T_C – n=70).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	PT_{low}	**0.098 ****	-0.07	0.266
	PT_c	**0.146**	-0.029	0.321
	T_c	0.117	-0.022	0.256
%FEV_1	PT_{low}	**4.5 ***	4.4	4.7
	PT_c	**6.1**	6.0	6.3
	T_c	5.2	-2.9	13.3
FEV_1 z-score	PT_{low}	**0.38 ****	-0.28	1.04
	PT_c	**0.54**	-0.08	1.17
	T_c	0.47	-0.07	1.02
FVC raw (litres)	PT_{low}	**0.218 †**	-0.07	0.507
	PT_c	0.199	-0.046	0.443
	T_c	**0.164**	-0.021	0.349
%FVC	PT_{low}	**9.0 †**	8.8	9.3
	PT_c	7.3	7.1	7.5
	T_c	**6.5**	-7.2	20.3
FVC z-score	PT_{low}	**0.80 ††**	-0.42	2.01
	PT_c	0.64	-0.10	1.37
	T_c	**0.56**	-0.03	1.16
FEV_1/FVC raw	PT_{low}	**-0.030 *** ††**	-0.111	0.051
	PT_c	**-0.009**	-0.068	0.051
	T_c	**-0.009**	-0.064	0.046
%FEV_1/FVC	PT_{low}	**-3.4 ** ††**	-3.5	-3.4
	PT_c	**-1.0**	-1.0	-0.9
	T_c	**-1.0**	-10.2	8.2
FEV_1/FVC z-score	PT_{low}	-0.36	-1.38	0.67
	PT_c	-0.15	-1.15	0.84
	T_c	-0.15	-1.07	0.77
$FEF_{25-75\%}$ raw (L/sec)	PT_{low}	**0.044 *** ††**	-0.278	0.365
	PT_c	**0.222**	-0.311	0.754
	T_c	**0.195**	-0.284	0.675
%$FEF_{25-75\%}$	PT_{low}	**1.2 *** †††**	0.7	1.7
	PT_c	**7.8**	7.3	8.2
	T_c	**7.4**	-4.6	19.4
$FEF_{25-75\%}$ z-score	PT_{low}	**0.06 *** ††**	-0.63	0.75
	PT_c	**0.38**	-0.53	1.29
	T_c	**0.34**	-0.48	1.17

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.4 Sex

There were no differences for any of the mean differences between males and females for any of the parameters, as seen in Table 5.19.

Table 5.19 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by sex (male – n=109, female – n=111).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	Male	0.124	-0.044	0.293
	Female	0.126	-0.039	0.291
$\%FEV_1$	Male	5.3	-1.9	12.4
	Female	5.6	-1.7	13
FEV_1 z-score	Male	0.48	-0.16	1.12
	Female	0.49	-0.12	1.09
FVC raw (litres)	Male	0.192	-0.056	0.44
	Female	0.193	-0.044	0.43
%FVC	Male	7.1	-1.8	16
	Female	7.9	-2.7	18.4
FVC z-score	Male	0.62	-0.15	1.4
	Female	0.68	-0.24	1.6
FEV_1/FVC raw	Male	-0.013	-0.073	0.048
	Female	-0.015	-0.087	0.056
$\%FEV_1$/FVC	Male	-1.5	-8.5	5.6
	Female	-1.7	-9.7	6.3
FEV_1/FVC z-score	Male	-0.19	-1.05	0.68
	Female	-0.22	-1.32	0.89
$FEF_{25-75\%}$ raw (L/sec)	Male	0.167	-0.324	0.658
	Female	0.174	-0.32	0.667
$\%FEF_{25-75\%}$	Male	6.2	-12.8	25.2
	Female	6.0	-11.2	23.2
$FEF_{25-75\%}$ z-score	Male	0.29	-0.63	1.21
	Female	0.29	-0.53	1.12

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.5 Age groups

Children were split into age groups of 2 years. There were fairly even numbers of children who were 9 to 10 as there were 11 to 12 years, however there were only 20 in the youngest age group (7 to 8 years). The only difference seen was a higher difference between spirometry techniques in raw FEV_1 in the 11 to 12 compared to 9 to 10 years (0.144 vs 0.109 litres). This did not equate to a difference in %predicted or z-score. Table 5.20 summarises these results.

Table 5.20 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by age (7 to 8 years – n=20, 9 to 10 years – n=97, 11 to 12 years – n=103).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	7 to 8 years	0.105	-0.019	0.228
	9 to 10 years	**0.109 ‡**	-0.044	0.263
	11 to 12 years	**0.144**	-0.034	0.322
$\%FEV_1$	7 to 8 years	6.1	5.9	6.3
	9 to 10 years	5.3	5.1	5.5
	11 to 12 years	5.5	-2.6	13.5
FEV_1 z-score	7 to 8 years	0.52	-0.10	1.14
	9 to 10 years	0.47	-0.19	1.13
	11 to 12 years	0.49	-0.10	1.08
FVC raw (litres)	7 to 8 years	0.160	-0.119	0.439
	9 to 10 years	0.176	-0.057	0.408
	11 to 12 years	0.215	-0.023	0.452
%FVC	7 to 8 years	8.4	8.2	8.6
	9 to 10 years	7.5	7.2	7.7
	11 to 12 years	7.3	-9.3	23.8
FVC z-score	7 to 8 years	0.73	-0.74	2.20
	9 to 10 years	0.65	-0.21	1.51
	11 to 12 years	0.63	-0.04	1.31
FEV_1/FVC raw	7 to 8 years	-0.014	-0.096	0.069
	9 to 10 years	-0.015	-0.084	0.053
	11 to 12 years	-0.013	-0.074	0.048
$\%FEV_1$/FVC	7 to 8 years	-1.5	-1.6	-1.5
	9 to 10 years	-1.7	-1.8	-1.7
	11 to 12 years	-1.5	-10.6	7.7
FEV_1/FVC z-score	7 to 8 years	-0.16	-1.21	0.90
	9 to 10 years	-0.22	-1.22	0.78
	11 to 12 years	-0.19	-1.17	0.78
$FEF_{25-75\%}$ raw (L/sec)	7 to 8 years	0.166	-0.281	0.613
	9 to 10 years	0.146	-0.307	0.598
	11 to 12 years	0.195	-0.338	0.727
$\%FEF_{25-75\%}$	7 to 8 years	7.4	6.9	7.8
	9 to 10 years	5.7	5.2	6.2
	11 to 12 years	6.2	-13.2	25.6
$FEF_{25-75\%}$ z-score	7 to 8 years	0.36	-0.67	1.39
	9 to 10 years	0.27	-0.61	1.14
	11 to 12 years	0.30	-0.54	1.14

Abbreviations: **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.6 Height

Children were divided into two height groups (120 to <150 cm and 150 to <180 cm). Mean difference in raw values for FEV_1 and FVC were lower for the shorter children (0.108 vs 0.156 litres for FEV_1; 0.174 vs 0.226 litres for FVC). This did not equate to a systematic difference in %predicted or z-score, as per Table 5.21.

Table 5.21 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by height (120 to <150 cm – n=143, 150 to <180 cm – n=77).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	120 to <150 cm	**0.108 *****	-0.045	0.262
	150 to <180 cm	**0.156**	-0.017	0.329
$\%FEV_1$	120 to <150 cm	5.3	-2.3	13.0
	150 to <180 cm	5.7	-0.7	12.0
FEV_1 z-score	120 to <150 cm	0.47	-0.18	1.12
	150 to <180 cm	0.50	-0.05	1.06
FVC raw (litres)	120 to <150 cm	**0.174 ****	-0.059	0.407
	150 to <180 cm	**0.226**	-0.018	0.471
%FVC	120 to <150 cm	7.6	-3.1	18.3
	150 to <180 cm	7.2	-0.7	15.1
FVC z-score	120 to <150 cm	0.66	-0.28	1.6
	150 to <180 cm	0.63	-0.04	1.31
FEV_1/FVC raw	120 to <150 cm	-0.015	-0.085	0.055
	150 to <180 cm	-0.012	-0.071	0.047
$\%FEV_1$/FVC	120 to <150 cm	-1.7	-9.7	6.2
	150 to <180 cm	-1.4	-8.1	5.3
FEV_1/FVC z-score	120 to <150 cm	-0.20	-1.19	0.78
	150 to <180 cm	-0.20	-1.2	0.8
$FEF_{25-75\%}$ raw (L/sec)	120 to <150 cm	0.150	-0.322	0.622
	150 to <180 cm	0.208	-0.311	0.728
$\%FEF_{25-75\%}$	120 to <150 cm	5.9	-13.1	24.9
	150 to <180 cm	6.4	-9.9	22.8
$FEF_{25-75\%}$ z-score	120 to <150 cm	0.28	-0.64	1.19
	150 to <180 cm	0.32	-0.47	1.1

Abbreviations: **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.7 Preterm status

When grouped by preterm or term status, most parameters showed no difference, except raw, %predicted and z-score FVC were lower in term compared to preterm children (0.164 vs 0.206 litres, 6.5 vs 7.9 %, 0.56 vs 0.69 for raw values, %predicted and z-score respectively). Table 5.22 displays these results.

Table 5.22 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by preterm or term status (Term – n=70, Preterm – n=150).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	Term	0.117	-0.022	0.256
	Preterm	0.129	-0.049	0.306
$\%FEV_1$	Term	5.2	-1.1	11.5
	Preterm	5.6	-2.1	13.2
FEV_1 z-score	Term	0.47	-0.07	1.02
	Preterm	0.49	-0.17	1.14
FVC raw (litres)	Term	**0.164 ****	-0.021	0.349
	Preterm	**0.206**	-0.055	0.466
%FVC	Term	**6.5 ***	-0.3	13.4
	Preterm	**7.9**	-2.9	18.7
FVC z-score	Term	**0.56***	-0.03	1.16
	Preterm	**0.69**	-0.25	1.63
FEV_1/FVC raw	Term	-0.009	-0.064	0.046
	Preterm	-0.016	-0.087	0.054
$\%FEV_1$/FVC	Term	-1.0	-7.3	5.2
	Preterm	-1.8	-9.9	6.2
FEV_1/FVC z-score	Term	-0.15	-1.07	0.77
	Preterm	-0.23	-1.25	0.8
$FEF_{25-75\%}$ raw (L/sec)	Term	0.195	-0.284	0.675
	Preterm	0.159	-0.338	0.656
$\%FEF_{25-75\%}$	Term	7.4	-11.2	26.1
	Preterm	5.4	-12.3	23.2
$FEF_{25-75\%}$ z-score	Term	0.34	-0.48	1.17
	Preterm	0.27	-0.63	1.16

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.8 Preterm groups

Preterm children were divided into whether they were extremely preterm, very preterm, or moderate to late preterm. However, between these groups the differences between the methods of spirometry were similar for all parameters., as shown in Table 5.23.

Table 5.23 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by gestation band (moderately to late preterm – n=75, very preterm – n=48, extremely preterm – n=27).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	Mod to late preterm	0.131	-0.063	0.324
	Very preterm	0.129	-0.023	0.281
	Extremely preterm	0.124	-0.057	0.305
$\%FEV_1$	Mod to late preterm	5.6	5.4	5.7
	Very preterm	5.4	5.2	5.5
	Extremely preterm	5.9	-1.9	13.7
FEV_1 z-score	Mod to late preterm	0.49	-0.20	1.19
	Very preterm	0.47	-0.08	1.03
	Extremely preterm	0.50	-0.22	1.22
FVC raw (litres)	Mod to late preterm	0.202	-0.042	0.446
	Very preterm	0.212	-0.076	0.5
	Extremely preterm	0.204	-0.059	0.468
%FVC	Mod to late preterm	7.5	7.2	7.8
	Very preterm	7.9	7.6	8.1
	Extremely preterm	9.0	0.2	17.8
FVC z-score	Mod to late preterm	0.66	-0.09	1.41
	Very preterm	0.70	-0.27	1.66
	Extremely preterm	0.79	-0.52	2.10
FEV_1/FVC raw	Mod to late preterm	-0.015	-0.079	0.048
	Very preterm	-0.016	-0.093	0.062
	Extremely preterm	-0.020	-0.098	0.058
$\%FEV_1$/FVC	Mod to late preterm	-1.7	-1.8	-1.7
	Very preterm	-1.8	-1.8	-1.7
	Extremely preterm	-2.3	-9.5	5.0
FEV_1/FVC z-score	Mod to late preterm	-0.26	-1.36	0.84
	Very preterm	-0.18	-1.14	0.79
	Extremely preterm	-0.22	-1.13	0.68
$FEF_{25-75\%}$ raw (L/sec)	Mod to late preterm	0.194	-0.306	0.693
	Very preterm	0.106	-0.402	0.615
	Extremely preterm	0.156	-0.294	0.606
$\%FEF_{25-75\%}$	Mod to late preterm	6.7	6.2	7.2
	Very preterm	3.6	3.1	4.0
	Extremely preterm	5.4	-12.1	22.9
$FEF_{25-75\%}$ z-score	Mod to late preterm	0.32	-0.52	1.16
	Very preterm	0.17	-0.75	1.09
	Extremely preterm	0.28	-0.69	1.25

Abbreviations: FEV_1 – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

5.3.2.9 CLD

No differences were seen between preterm children with or without a CLD diagnosis, in the mean differences between MicroLoop and MasterScreen spirometry, as per Table 5.24.

Table 5.24 Mean difference for raw, percent predicted and z-scores of FEV_1, FVC and FEV_1/FVC from MicroLoop and MasterScreen methods of testing with limits of agreement, grouped by CLD status (no CLD – n=112, CLD – n=38).

		Mean difference	LLOA	ULOA
FEV_1 raw (litres)	No CLD	0.128	-0.06	0.317
	CLD	0.130	-0.014	0.274
%FEV_1	No CLD	5.4	-2.4	13.2
	CLD	6.1	-1.2	13.3
FEV_1 z-score	No CLD	0.48	-0.2	1.16
	CLD	0.52	-0.06	1.09
FVC raw (litres)	No CLD	0.204	-0.063	0.47
	CLD	0.212	-0.035	0.459
%FVC	No CLD	7.5	-2.3	17.3
	CLD	9.1	-4.1	22.3
FVC z-score	No CLD	0.66	-0.18	1.5
	CLD	0.80	-0.37	1.97
FEV_1/FVC raw	No CLD	-0.016	-0.087	0.056
	CLD	-0.019	-0.088	0.051
%FEV_1/FVC	No CLD	-1.8	-9.9	6.3
	CLD	-2.1	-9.9	5.7
FEV_1/FVC z-score	No CLD	-0.23	-1.32	0.87
	CLD	-0.22	-1	0.57
$FEF_{25-75\%}$ raw (L/sec)	No CLD	0.163	-0.363	0.689
	CLD	0.146	-0.258	0.549
%$FEF_{25-75\%}$	No CLD	5.6	-13	24.2
	CLD	4.9	-10.2	20
$FEF_{25-75\%}$ z-score	No CLD	0.27	-0.63	1.18
	CLD	0.25	-0.61	1.12

Abbreviations: **FEV_1** – forced expiratory volume in 1 second; **FVC** – forced vital capacity; **$FEF_{25-75\%}$** - forced expiratory flow between 25-75% of FVC.

Figure 5.7 Bland-Altman plots comparing MicroLoop and MasterScreen spirometers for FEV_1 for a) raw value, b)%predicted, and c) z-score, including mean bias (solid line) and limits of agreement (dashed line) , divided by lung function group (preterm with low lung function: PT_{low}, preterm controls: PT_c and term controls: T_c).

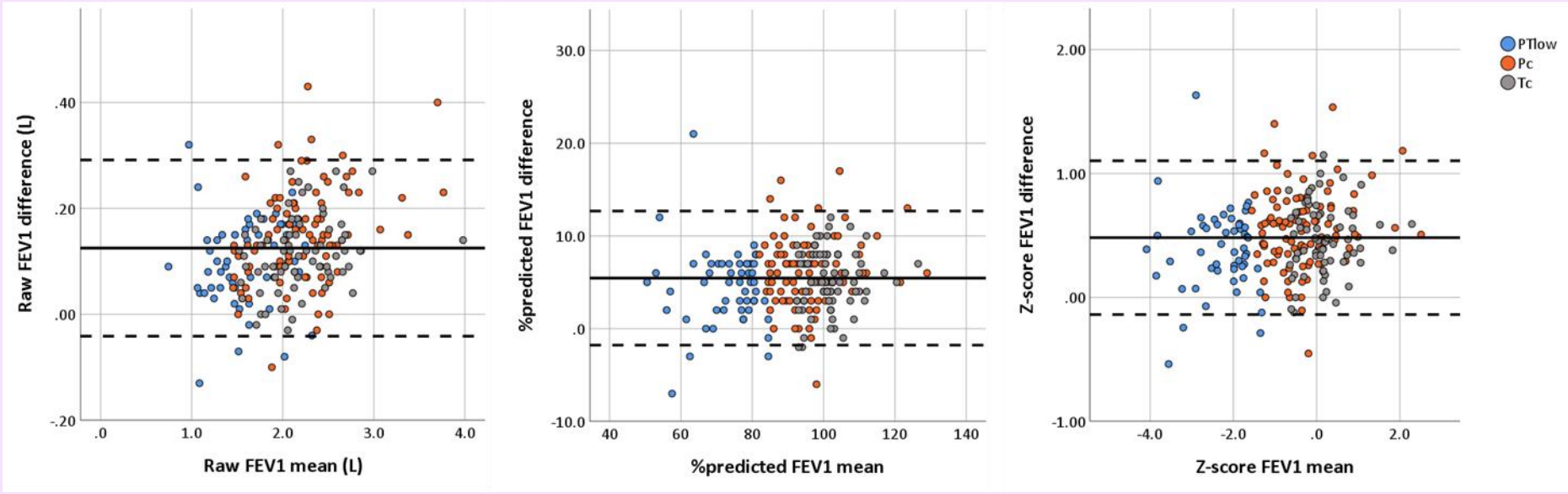


Figure 5.8 Bland-Altman plots comparing MicroLoop and MasterScreen spirometers for FVC for a) raw value, b)%predicted, and c) z-score, including mean bias (solid line) and limits of agreement (dashed line), divided by lung function group (preterm with low lung function: PT_{low}, preterm controls: PT_c and term controls: T_c).

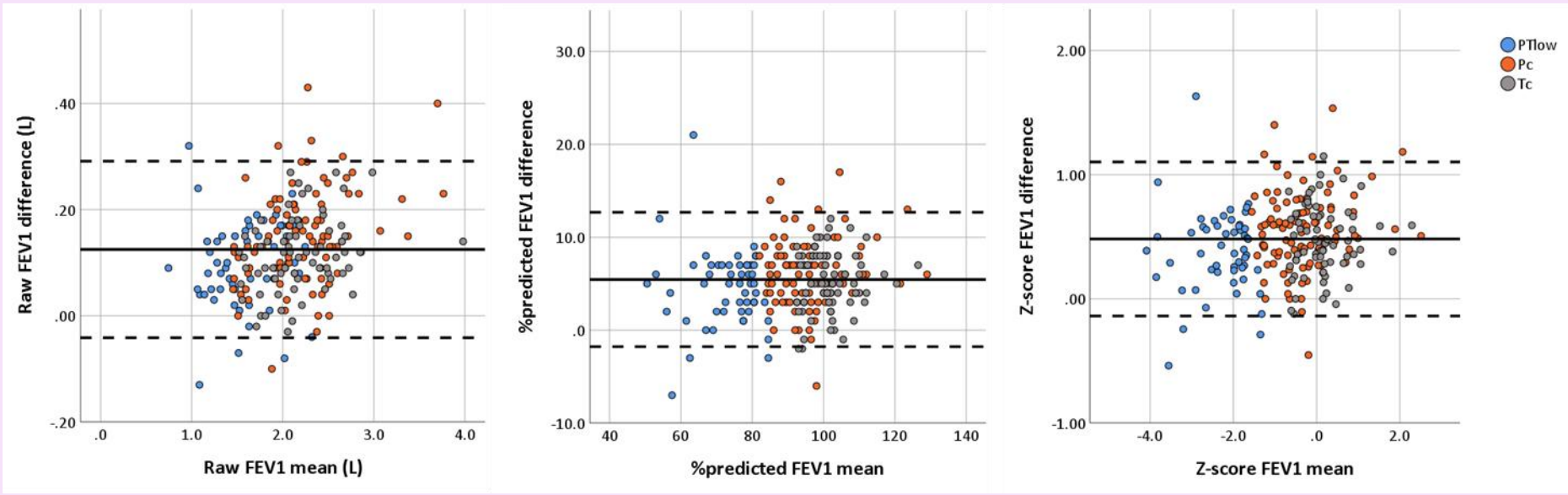


Figure 5.9 Bland-Altman plots comparing MicroLoop and MasterScreen spirometers for FEV_1/FVC for a) raw value, b)%predicted, and c) z-score, including mean bias (solid line) and limits of agreement (dashed line), divided by lung function group (preterm with low lung function: PT_{low}, preterm controls: PT_c and term controls: T_c).

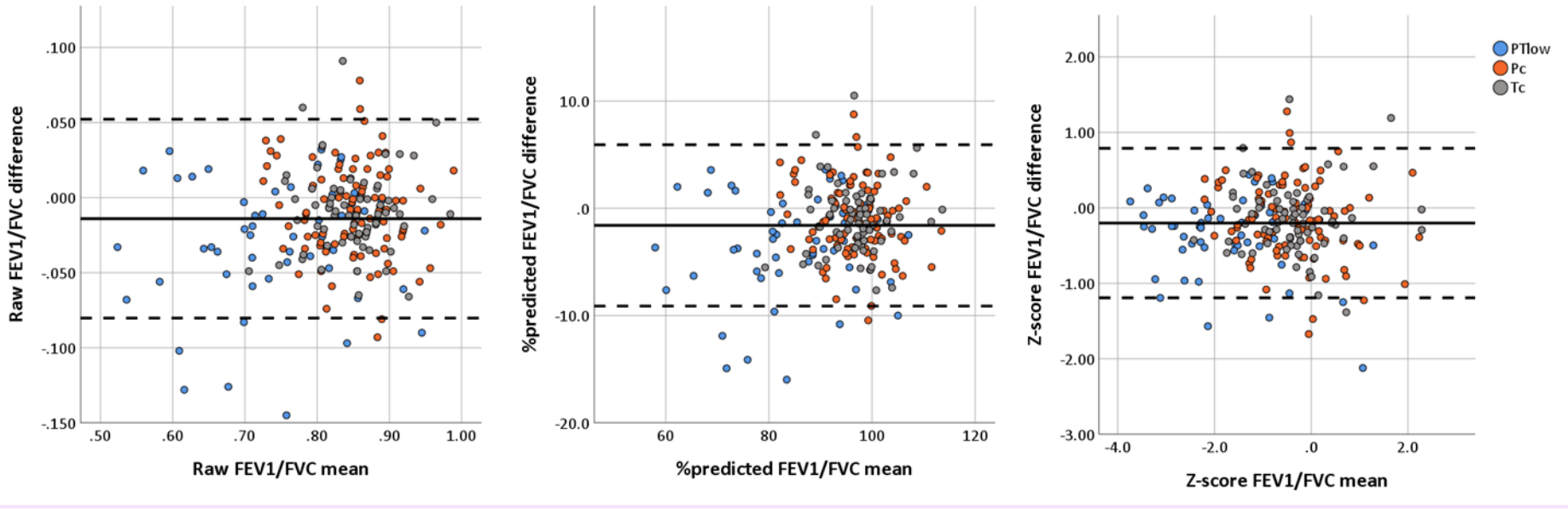
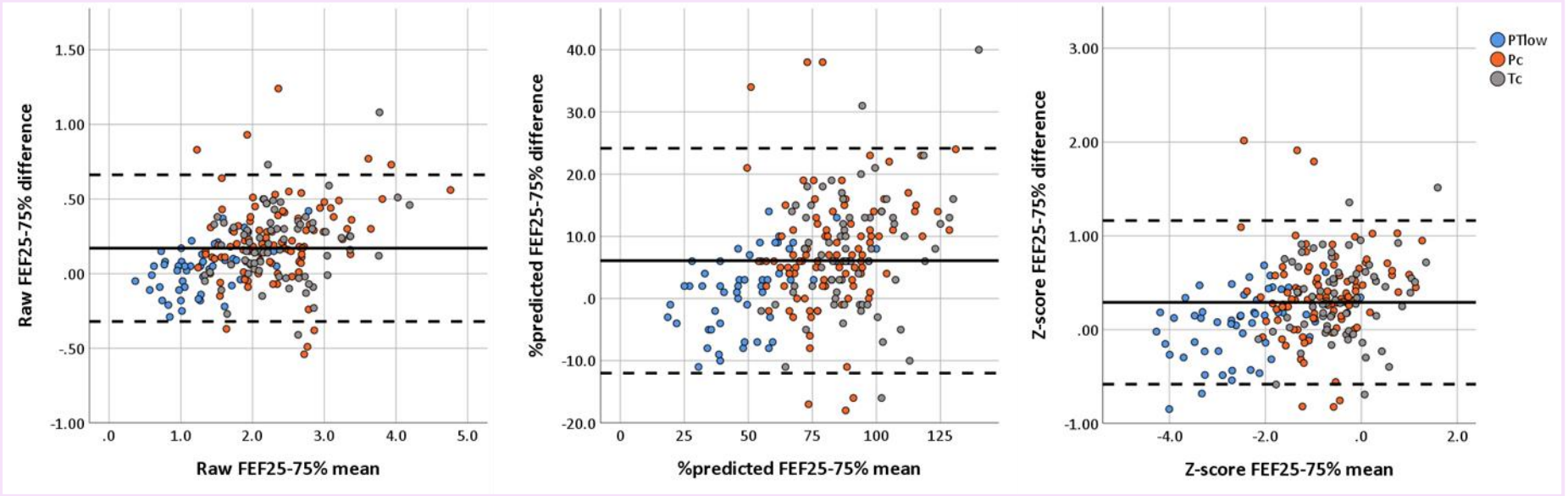


Figure 5.10 Bland-Altman plots comparing MicroLoop and MasterScreen spirometers for $FEF_{25-75\%}$ for a) raw value, b)%predicted, and c) z-score, including mean bias (solid line) and limits of agreement (dashed line), divided by lung function group (preterm with low lung function: PT_{low}, preterm controls: PT_c and term controls: T_c).



5.4 Discussion

In this chapter I have been able to show that there are different methods in published literature for classifying obstructive and restrictive lung disease. These methods, such as those described by Pellegrino et al from the ATS/ERS task force for standardisation of spirometry (Pellegrino et al., 2005) and Johnson et al (Johnson and Theurer, 2014), use spirometry as the basis for classifying lung disease with further interpretation and classification using other tests including lung volumes in the case of Pellegrino. These methods for classification of obstructive and restrictive lung disease are based primarily around the (F)VC and FEV_1/(F)VC ratio obtained from spirometry, with FEV_1 used primarily for disease severity classification. These two distinct approaches; however, may not be applicable to all populations. Obstructive lung disease classification in a preterm population is not something that has been widely examined before, with an emphasis on identifying neonatal characteristics (e.g. birth weight, IUGR, gestation, CLD) and comparing long-term outcomes within these populations with either preterm and/or term controls (Lista et al., 2014, Korhonen et al., 2004, Kotecha et al., 2010, Fawke et al., 2010, Landry et al., 2011). Conversely, in recruiting for the RHiNO study, I had a cohort of children who were defined by their respiratory outcome following preterm birth, in this case defined by FEV_1 ≤85% and was able to use this group to examine their perinatal and phenotypic characteristics. Additionally, I was able to explore alternative methods of designating obstructive lung disease with a focus on a particular outcome. In this case, my characteristic of interest was bronchodilator reversibility, on the basis this is an outcome that may be of most importance to identify, i.e., something that is potentially modifiable with treatment.

In the first instance I divided the low lung function group ($PT_{low,}$ FEV_1 ≤85%) into prematurity-associated obstructive (POLD) and non-obstructive (PnOLD) lung disease based on FEV_1/FVC ratio cut off of 0.8. The striking thing about this was the high degree of bronchodilator reversibility seen within this group (15.8% change, 84% of the group with reversibility >10% absolute predicted value). This contrasted with the low rates seen in the PnOLD group (4.2% mean change, 6% with significant reversibility). This was slightly lower than that seen in either control group.

5.4.1 Use of lung volumes

I was interested in whether lung volumes could be implemented into a further classification within this group of PT_{low} participants, in order to identify true restrictive lung disease. As such, using total lung capacity (TLC) at the lower limit of normal (LLN), applied to the original obstructive/non-obstructive groups, I was able to divide the PT_{low} group into four distinct groups: obstructive disease (low ratio, normal TLC), mixed disease (low ratio, low TLC), restrictive disease (normal ratio, low TLC), and non-obstructive disease (normal ratio, normal TLC). There were small differences when using the TLC obtained from helium dilution as opposed to body plethysmography. There was a small difference in absolute numbers of 47 vs 49 (out of the total 53 within PT_{low} group) respectively due to unsuccessful tests. Using helium dilution identified more children who were classed as having low TLC (2 within mixed disease group and 9 within the restrictive group), compared to only 6 within the restrictive group using plethysmography. Conversely, plethysmography identified 34 children with obstructive disease compared to 29 with helium testing plus the 2 with mixed disease. It seems that plethysmography is more likely to identify those with obstructive disease which may appear to be restrictive in origin using helium testing (Dahlqvist and Hedenstierna, 1985), as air trapping would result in regions of ventilation heterogeneity where helium gas would not reach, falsely lowering functional residual capacity (FRC) and TLC. This would suggest that some of those within the restrictive group identified on helium testing would potentially have an obstructive lung disease, however the lack of bronchodilator response seen within this group suggests that any lung obstruction identified would be fixed airway disease. This suggests that while plethysmography may be a better method of truly distinguishing restrictive vs obstructive disease, that helium dilution testing may have better specificity for identifying reversible obstructive disease.

5.4.2 Comparisons with other methodology

5.4.2.1 Johnson's et al

I applied other methods of classifying obstructive/restrictive/mixed lung disease using the methods suggested by Johnson et al and Pellegrino et al to explore the

differences with the classification I used. Johnson's criteria for lung disease uses a combination of FEV_1/FVC ratio (cut-off 85% predicted rather than absolute value) followed by FVC (with cut-off 80% predicted), with FEV_1 used for determining severity of disease. This contrasts with the method used by myself of FEV_1 as the starting point for identifying lung disease.

As such, of the children I had defined as obstructive lung disease based on FEV_1 and FEV_1/FVC ratio that Johnson's methodology redefined as normal, 56% of these children had significant reversibility. This means that while their absolute FEV_1/FVC ratio was less than 0.8, their %predicted ratio was actually >85%, potentially suggesting that either the 85% predicted cut-off is too high to identify these children, or using an absolute figure for this ratio is a reasonable approach for identifying reversible lung disease in this population. Combining these methods resulted in agreement for 22 children as obstructive by both definitions, with 96% of these children having reversibility.

Conversely, Johnson's method would classify 10 children (27%) from the POLD and 12 (75%) from the restrictive groups as normal, while calling 4 (4%) from the PT_c and 2 (3%) from the T_c group as actually having restrictive lung disease. Looking at the characteristics of these groups, the children that Johnson classify as obstructive disease that come from the control groups had particularly high %FVC (113% from the PT_c and 116 from the T_c) relative to their FEV_1 (94.8% vs 93.5%). This results in a high FEV_1/FVC ratio. One explanation for this may be that these children have dysanapsis (i.e., lung volume out of proportion with airway size). However, these children did have a greater degree of reversibility (8% and 13.5% from preterm and terms respectively) compared to those remaining in the controls (5.6% and 6.1% respectively), albeit from a small sample size. This suggests that actually there is a degree of reversible lung obstruction, despite normal appearing lungs based on FEV_1 and FVC predicted values alone. This suggests the need to put more weight on the FEV_1/FVC ratio to identify potential reversibility.

5.4.2.2 Using Pellegrino et al methodology

When using Pellegrino's methodology for identifying obstructive/restrictive lung disease (based on LLN for initially FEV_1/VC and then VC, with subsequent addition of

TLC at LLN), a similar pattern was identified with children from my obstructive/restrictive groups re-classified as normal, and several controls redefined as obstructive disease. Pellegrino's method is interesting as obstructive lung disease is defined in several ways: 1) normal FEV_1/VC ratio, low VC, normal TLC; 2) low FEV_1/VC ratio, normal VC; and 3) low FEV_1/VC ratio, low VC, normal TLC. I ran this comparison having used both helium dilution and plethysmography methods to define the Pellegrino groups. As per tables 5.8 and 5.10 in the Results (section 5.3.1.3.), there were few differences in the numbers between the groups. As per the earlier discussion regarding helium dilution potentially mis-diagnosing restrictive lung disease my discussion below focuses on the data obtained from the plethysmography methodology.

Of the 31 children that both Pellegrino and RHiNO define as obstructive disease (out of a total of 37 from the POLD group), 84% show bronchodilator reversibility >10% with a mean increase in FEV_1 of 16.9%. However, the children I defined as obstructive disease, but were defined as normal by Pellegrino, still had a mean reversibility of 11.5% with 83% of them having significant reversibility. This suggests that using Pellegrino methodology has its flaws in identifying the group of interest, i.e., those with bronchodilator reversibility.

A greater number of preterm controls (12; 12%) and term controls (4; 6%), as designated by RHiNO classification, were defined as obstructive disease by Pellegrino's method when using plethysmography, compared to when classifying by Johnson's criteria (4 PT_c, 2 T_c). Similar to Johnson's methodology, the children who were redefined as obstructive by Pellegrino from the RHiNO control groups did have greater mean response to post-exercise bronchodilators (10.2% vs 5% in preterm and 10.8% vs 6% for term in the Pellegrino obstructive vs normal respectively) and proportion with response >10% absolute predicted value (55% vs 12% in preterm and 25% vs 16% in term for obstructive vs normal).

5.4.3 Comparison of all 3 methods

Overall, it could be argued that there are benefits from the various methods of defining types of lung disease. Ultimately the area of interest is several-fold – recognising who has lung function deficits earlier in life to potentially be aware if later lung disease, finding those who are functionally affected, and identifying those who may respond to therapies. In this instance, my interest was within those who may have modifiable lung disease, with a simple, pragmatic approach that could be easily utilised in an outpatient clinic setting.

I found there was a variation in the numbers defined as having obstructive lung disease between different methodology, with the greatest number of children with obstructive disease being identified by Pellegrino, and fewest by Johnson. Conversely, Pellegrino had the lowest percentage for post-exercise bronchodilator response within the obstructive group, with Johnson having the highest. However, Pellegrino detected more children who had a post-exercise bronchodilator response than either other method suggesting potentially a better sensitivity but lower specificity for this. Additionally, this would require lung volume testing which as discussed is difficult in clinical paediatric practice. Conversely Johnson's method of delineating obstructive/restrictive disease potentially misses more preterm children with reversible disease by up to a third, albeit in this small cohort.

Identifying true restrictive disease is difficult without lung volume testing, and so using a cut off of 85% predicted FEV_1 along with a normal FEV_1/FVC ratio overestimates those children with true restrictive disease. However, the children that I identified in the non-obstructive preterm lung disease group do appear potentially different to those children who I defined as normal, with lower rates of FE_{NO}, lower %FVC, lower post-exercise bronchodilator response rates/post-exercise bronchodilator change scores, which appear more similar to those with true restrictive disease. It suggests that these children may still have a degree of fixed structural deficit somewhere on a spectrum from normal lungs to restrictive disease.

However, the downside of using this method is that it requires the use of lung volume testing which in a clinical setting is often difficult to obtain, compared to just using spirometry which is more widely available, including in primary care.

5.4.4 Use of %predicted vs absolute vs z-score values

A potentially controversial aspect of how these children were divided would be viewed as the use of percent predicted values (for FEV_1) and raw value (for FEV_1/FVC) compared to z-scores. There has a been a drive, correctly, to the use of z-scores, which has largely been alongside the switch to the use of GLI reference values within spirometry and championed by the experts behind this. While %predicted within GLI for spirometry account for age/sex/height/ethnicity, they do not account for the difference in distribution within these. As a result, the %predicted for a given value may not correlate with the z-score across a population. Additionally, despite FEV_1/FVC changing across childhood, likely related to separation between overall and lung growth, However, while z-scores are now widely used in research, their translation to clinical practice has been slower, and this is important. If the clinicians are not using and interpreting the z-scores, then familiarity is not present making it harder to implement ideas and practices. There is also no evidence that a particular z-score is of any definitive meaning within a given population. For instance, in this cohort, identifying those with reversible lung obstruction was my aim, and as such the ROC performed to find a potential cut off within FEV_1 and FEV_1/FVC ratio for identifying a bronchodilator response, which resulted in finding an FEV_1/FVC absolute ratio of 0.8 as having the best combined sensitivity and sensitivity. This result equated to a random z-score rather than any limits of normal. As such it would likely be easily implemented in practice, although with awareness that it could only apply to this age group and disease.

The method of defining obstructive lung disease has similar or better sensitivity, specificity, positive and negative predictive values compared to when using the Johnson or Pellegrino methods.

5.4.5 MicroLoop comparisons

I have shown that systematic bias is seen in the readings obtained when comparing spirometers with a turbine flow-meter and a pneumotachograph. Over 5% predicted

systematic difference was seen for FEV_1, FVC and FEV_1/FVC ratios, which for FEV_1 equates to almost half a standard deviation (z-)score, and larger for FVC. This explains some of the attrition rate seen during recruitment for children whose spirometry ended up being too good for inclusion into the RCT part of the RHiNO study, and hence limiting numbers to study defined as low lung function.

The MicroLoop spirometer was used at the screening visit and then performed again at the in-depth lung function visit, prior to definitive spirometry. Although the order of testing (turbine and then pneumotachograph) was the same for all, as the participants had already performed acceptable spirometry, this systematic difference was unlikely to be due to having had greater amount of practice at the time of performing pneumotachograph spirometry. Instead, what is more likely is the turbine is less likely to be able to detect low flow rates (Caras et al., 1999). This fits with the FVC being greatest affected; the low flow at the end of expiration may not have been fast enough to move the turbine, hence an earlier cut off made for final FVC. This also fits with some of the group comparisons, with greater differences seen sometimes between groups that may have greater differences in spirometry, for instance the lung function groupings, by age or by height.

What is important to note, is due to the change in FEV_1 and FVC, there was little change in the overall FEV_1/FVC ratio, important as a function for defining obstructive disease.

These findings are important for clinicians and researchers to be aware of, since misdiagnosis could occur if lung function is below a certain threshold using a particular type of spirometer, when the threshold may not be met on another. Additionally, if monitoring a person over a time period, the same equipment should be used, otherwise the results are not comparable (Milanzi et al., 2019).

5.4.6 Strengths and limitations

The main strength of this study is the identification of a pragmatic, straightforward way to potentially identify children with reversible airways disease, using simple

spirometry parameters, which are comparable to other established methods for diagnosing types of lung disease.

At the same time, I have clearly demonstrated caution must be used when interpreting spirometry results, with respect to the type of spirometer used, and the potential effect this may have on the results.

A limitation of this study is that the use of the established thresholds discussed in this chapter are only currently applicable to this population, i.e., age and disease process. This would have to be validated in other cohorts of a similar age. It is likely these results would not be directly applicable to older populations, as there would be a different relationship between the %predicted results and z-scores that would make these thresholds invalid. However, I have demonstrated that use of lower limit of normal may not always be applicable to a particular disease process.

5.5 Conclusions

Children with preterm lung disease as defined by FEV_1 ≤85% can be further stratified into the presence of obstructive or non-obstructive lung disease. This can be done using a simple cut-off of FEV_1/FVC <0.80, which ROC analysis has shown to have the best combined sensitivity and specificity for identifying reversible airway obstruction. While the non-obstructive group would need further investigation (i.e., lung volume testing) to identify true restrictive disease, those identified as having obstructive lung disease can be strongly presumed to have reversible airways disease. This could be formally tested with a reversibility test using bronchodilator therapy, and potentially treatment indicated, although the best therapy is yet to be determined.

6 OVERALL DISCUSSION

6.1 Overview

My thesis has explored aspects of long-term respiratory consequences in preterm-born children. I have used well-established approaches (spirometry, lung volume, cardiopulmonary exercise testing) and newer approaches (oscillometry, including novel use of intra-breath oscillometry to track airway mechanics through the breathing cycle, which has yet to be reported in this population). I have also used an alternative approach in how to evaluate lung dysfunction in the form of obstructive and non-obstructive phenotypes of disease. I have also explored methodological considerations, including potential systematic biases within the use of different types of spirometers, as well how to define obstructive lung disease in this cohort of preterm-born children.

Below I have recapped my key findings, as well as summarising my conclusions from across the chapters.

6.2 Key findings

6.2.1 Population characteristics

- Children born preterm with low lung function (%FEV_1 ≤85%; PT_{low}) were born at earlier gestations and lower birth weights compared to preterm controls (PT_c), potentially suggesting risk factors for lung disease;
- Higher rates of intrauterine growth restriction (IUGR) and Caesarean-section delivery were seen in both preterm groups compared to term controls (T_c);
- PT_{low} had higher rates of chronic lung disease of prematurity (CLD), one or more of combination of retinopathy of prematurity (ROP)/intraventricular haemorrhage (IVH)/necrotising enterocolitis (NEC), and persistent ductus arteriosus (PDA) compared to $P_{c,}$ suggesting a more severe perinatal course;
- There were no differences for gestational age or birth weight between preterm children with obstructive (POLD) or non-obstructive (PnOLD) lung disease;
- POLD had higher rates of invasive ventilation compared to PnOLD children, suggesting more severe perinatal respiratory morbidity (i.e., respiratory distress syndrome).

6.2.2 Spirometry, lung volume and exercise testing

6.2.2.1 Spirometry

- PT_{low} had lower %FEV_1, %FVC and FEV_1/FVC compared to PT_c children, a result of group stratification;
- PnOLD had lower FVC than POLD, potentially in line with low FVC acting as a surrogate for restrictive lung disease;
- POLD had lower FEV_1/FVC compared to PnOLD, consistent with the group designation of obstructive disease;
- PT_{low}, PT_c and T_c all had similar response to exercise, with a small but significant decrease in %FEV_1 and FVC, with PT_{low} having a larger decrease in %FVC compared to $PT_{c,}$ possibly a result of increased air trapping in PT_{low};
- Similar response to exercise was seen in POLD and PnOLD;
- All groups had a significant increase in %FEV_1, %FVC and FEV_1/FVC post-exercise bronchodilator;

- PT_{low} had a significantly greater post-exercise bronchodilator response, and a significantly greater proportion with substantial bronchodilator response compared to both controls, consistent with reversible airways disease;
- POLD had a significantly greater degree of reversibility with the majority having significant increase in $\%FEV_1$, while PnOLD had minimal reversibility, suggesting these group designations were appropriate for distinguishing reversible airways disease.

6.2.2.2 Lung volume testing

- PT_{low} had lower total lung capacity (TLC) and higher residual volume (RV), with higher RV/TLC compared to both control groups on body plethysmography testing;
- PnOLD had lower functional residual capacity (FRC), TLC and RV than POLD with a significantly greater proportion having TLC < lower limit of normal (LLN), consistent with true restrictive disease, while POLD had the highest RV/TLC in line with hyperinflation.

6.2.2.3 Exercise testing

- PT_{low} had impaired minute ventilation compared to controls, using a greater extent of their ventilatory reserve;
- Both preterm groups reached lower relative (for weight) workloads and peak oxygen uptakes;
- Suggests functional impairment present in preterm, particularly PT_{low} children;
- Little difference seen between POLD and PnOLD during exercise suggesting impaired lung function rather than underlying mechanism/pathology of impairment more important.

6.2.3 Oscillometry

- PT_{low} had higher airway resistance than controls, particularly at lower frequencies, consistent with small airway disease;

- PT_{low} had worse airway reactance, particularly at the lower frequencies, along with reduced compliance;
- Exercise had negligible effect on airway mechanics in preterm children;
- Bronchodilator significantly improved resistance, particularly in PT_{low}, suggesting increased airway calibre after treatment;
- Improved reactance and compliance greatest in PT_{low}, likely due to improved muscle tone, following bronchodilator;
- Similar trends at baseline observed in POLD compared to PnOLD, with greatest effect seen in the negative reactance parameters, i.e., related to impaired compliance;
- Greatest improvements following reversibility found in POLD, particularly at lower frequencies/distal airways, consistent with improvement in smooth muscle tone in this region of the lung.

6.2.4 Intra-breath oscillometry

- Intra-breath oscillometry at single frequency (10 Hz) showed good agreement with isolated 10 Hz impedance from standard spectral oscillometry;
- PT_{low} have impaired resistance and reactance parameters throughout the respiratory cycle compared to both controls, with the greater impedances seen in expiration compared to inspiration for all groups;
- Few differences were seen between the groups for the difference in impedance between inspiration and expiration,
- Greater improvements were seen in PT_{low} throughout the respiratory cycle for resistance and reactance in both inspiration and expiration compared to controls following bronchodilator therapy;
- Area within the reactance-flow loop was greatest for PT_{low} children, which may represent greater dynamic changes during respiration relating to changing compliance affecting flow in these children;
- No baseline differences in resistance were seen in expiration between POLD and PnOLD;

- Due to a slight increase in expiratory resistances in POLD, and a slight decrease in PnOLD, a significant difference was seen for some expiratory resistance parameters between POLD and PnOLD at post-exercise timepoint, which may be a sign of subtle airway obstruction as a result of exercise;
- POLD and PnOLD showed similar inspiration-expiration differences in resistance;
- POLD had worse baseline reactances in both inspiration and expiration compared to PnOLD;
- The reactance gap at various points of the respiratory cycle between inspiration and expiration was greater in POLD compared to PnOLD, including at zero-flow state, which may represent an effect of elasticity having lesser effect on airway mechanics during inspiratory flow than expiratory flow.

6.2.5 Review of obstructive lung disease

- Cut offs from receiver operator characteristic (ROC) curves for identifying post-exercise bronchodilator response of >10% predicted FEV_1 showed similar area under the curves for z-score vs %FEV_1, and z-score vs absolute FEV_1/FVC;
- Highest combined sensitivity and specificity on ROC for post-exercise bronchodilator response was for an FEV_1/FVC absolute ratio of 0.80, which had a comparative z-score of -1.1;
- Use of FEV_1 ≤85% predicted and FEV_1/FVC of ≤0.80 showed similar or better sensitivity, specificity, positive predictive value, and negative predictive value compared to two alternative methods for defining obstructive lung disease for identifying post-exercise bronchodilator responsiveness.

6.2.6 Spirometer comparison

- There was strong correlation between spirometry performed on turbine and pneumotachograph spirometers;
- There were systematic differences between methods of spirometry, with pneumotachograph spirometer giving higher FEV_1, FVC and $FEF_{25-75\%}$, and lower FEV_1/FVC compared to turbine spirometry;

- This systematic difference was affected by lung function grouping and preterm status, and height with lower lung function and smaller height giving smaller differences in some parameters.

6.3 Discussion

6.3.1 Spirometer comparison

I shall address my final chapter first in this discussion, as the outcomes from this work determines the population, findings, and conclusion from the other chapters.

The primary aim of the Respiratory Health outcomes in Neonates (RHiNO) study was to address the clinical question of whether inhalers used in asthmatics (i.e., inhaled corticosteroid with or without long-acting beta-2 agonists) improved spirometry in children with low lung function (%FEV_1 ≤85%). The children were screened during a home visit performed by two trained research nurses, and those who met the above inclusion criteria using a turbine spirometer, along with the other inclusions as listed in my earlier chapters, were invited to attend for in-depth lung function testing, along with preterm and term controls. Definitive testing was performed using a pneumotachograph spirometer, after performing spirometry with the same turbine spirometry on the day if the in-depth lung function visit. The exploration of the comparison between methodology was inspired by the fact a number of children were ineligible for recruiting to the trial due to their higher FEV_1 at definitive testing. This raised a question early in the process of the RHiNO study as to whether there was systematic bias between types of spirometer in children.

Previous studies looking at community versus laboratory spirometry testing but using similar types of spirometer have not found systematic differences in results (Kirkby et al., 2008, Swart et al., 2003), while others have found that different types of spirometers may show systematic differences (Milanzi et al., 2019, Caras et al., 1999). This may be due to differences in how the different devices take their measurements, for instance a turbine may be less sensitive for detecting low flows (Caras et al., 1999). Alternatively there may be differences in resistance between devices, irrespective of whether they are the same type of flow meter (Lefebvre et al., 2014).

This can have several implications. For patients who are requiring longitudinal follow-up for either research or clinical purposes, inaccuracies may arise if performing spirometry using different equipment. A suggestion of using regression equations to correct for systematic differences has been suggested (Milanzi et al., 2019), although

this would likely be very difficult in practice. Additionally, for patients being assessed in, for instance, a primary care where non-pneumotachograph hand-held spirometers may be dominant, there may be a higher chance of misdiagnosis of a respiratory disease based on spirometry, which would not be made on a pneumotachograph spirometer.

In the case of my cohort of children, the impact of this was an attrition rate of ~20% of those children with low lung function on screening.

6.3.2 Defining obstructive lung disease

The other major methodological consideration in my work is surrounding the use of my definition for obstructive lung disease. As I have outlined previously in Chapter 5, section 5.2.2., obstructive and/or restrictive lung disease are usually defined by cut-offs in FVC and FEV_1/FVC, previously at defined absolute or %predicted values (Johnson and Theurer, 2014), but with a drive towards the use of z-scores at the lower limit of normal (LLN)(Pellegrino et al., 2005). In the case of my thesis, a more pragmatic approach was taken, with the idea that it may be a practical approach to use in a health care setting. While familiarity with use of z-scores and LLN, as well as ability to translate this into an understandable explanation when discussing with patients is presumed not to be an issue (Stanojevic et al., 2013), this may not be the case (Curtis et al., 2016). Additionally it is possible that using pre-defined cut-offs not specifically related to the disease process may lack sensitivity or specificity for detecting a particular condition (Murray et al., 2017).

While obstructive lung disease in preterm children is well described (Broström et al., 2010, vom Hove et al., 2014, Doyle et al., 2019b), as far as I am aware, there has not been a specific focus on identifying obstructive lung disease in preterm-born children, and assessing specifically their clinical characteristics compared to peers without obstructive disease. Instead, the spotlight remains on perinatal outcomes such as CLD (Moschino et al., Prenzel et al., 2020) or extremes of prematurity (Lundberg et al., 2020).

As such, I used ROC curves to try and determine an optimal cut-off for FEV_1/FVC that would distinguish obstructive lung disease. The outcome measure within the ROC analysis was to identify a positive post-exercise bronchodilator response, consistent with reversible airway obstruction. The logic of this was to identify children who may have modifiable disease. The cut-off I used for post-exercise bronchodilator response was an absolute increase in $\%FEV_1$ of 10%. While again there are published definitions of what constitutes a positive bronchodilator response, this lacks consistency across various respiratory societies (Guezguez et al., 2019), and may involve absolute or relative increases in raw or %predicted values, with or without minimal changes required in absolute values. In individuals with low baseline lung function, a small absolute change may result in a large relative change, while the converse of a larger absolute change in a higher baseline may have a lower relative change. This makes application and interpretation of bronchodilator response difficult. The widely used threshold of 12% change in raw FEV_1 from baseline is supported by the 95^{th} percentile of bronchodilator response seen in healthy individuals (Tan et al., 2012), a large population but comprising of adults. This corresponds to a relative change of 10% of the baseline predicted value. While the same value is often used in children, studies have demonstrated that its use in diagnosing obstructive diseases such as asthma has limitation, with lower bronchodilator response thresholds having better sensitivity in detecting asthma (Tse et al., 2013).

In my studies I used a set cut-off of absolute increase of 10% predicted FEV_1 value from post-exercise to post-exercise bronchodilator. This could potentially underestimate a positive bronchodilator response in my group of interest (low lung function). For instance, a participant with a pre-bronchodilator $\%FEV_1$ of 65%, to have a positive bronchodilator response would need to reach >75% predicted, a relative $\%FEV_1$ change of ~15%. This would be compared to an individual with a pre-bronchodilator $\%FEV_1$ of 95% who would need to reach >105% predicted, equivalent to a lower relative %predicted change of 10.5%. Additionally, what constitutes a positive response in numbers and what constitutes a clinical response may be separate (i.e., a smaller absolute increase in someone with low baseline lung function is likely to have a greater clinical response that a slightly larger increase in someone

with higher baseline lung function). The choice was again a pragmatic cut-off that may be easily applied to a clinical setting.

Using these various thresholds, I was able to compare my definitions of obstructive and non-obstructive disease with those including from the European Respiratory Society (Pellegrino et al., 2005), and demonstrated, at the least, parity with these other methods of defining lung disease in terms of sensitivity, specificity, positive and negative predictive value. While I have only been able to demonstrate these cut-offs' applicability in my population, it has allowed a specific assessment of obstructive lung disease in preterm-born children, not explored previously.

6.3.3 Lung function groups

Assessment of children as defined by their current respiratory status is a different approach to the standard follow-up of children born preterm. In this instance, I have demonstrated that along with the impaired spirometry, these children with low lung function have impairments in exercise capacity, differences in lung volumes with lower total lung capacity but higher residual volume consistent with air trapping as previously described (Cousins et al., 2018), as well as higher airway impedance. Additionally, I have explored the use of intra-breath impedance tracking through oscillometry and demonstrated that the airway impedance remains greater throughout the respiratory cycle in children with low lung function. Bronchodilator had a greater effect on the low lung function children, on spirometry, standard oscillometry and intra-breath oscillometry.

Exercise capacity is known to be impaired in children born preterm as demonstrated by a previous systematic review, with worse outcomes in those with than without CLD (Edwards et al., 2015b). While theoretically the use of exercise bike achieves lower peak oxygen consumptions, which is true in this study with the term children's $\dot{V}O_2$ of 38.1 mls/kg/min being lower than 15 of the 20 studies (predominantly treadmill) in the review, the preterm children in this study still had a greater difference of $\dot{V}O_2$ versus the term controls of 6.2mls/kg/min compared to an average of 2.2mls/kg/min in the review. This is most likely due to investigating children who

had low lung function rather than those with CLD, especially as a significant proportion of CLD children had normal lung function.

The oscillometry findings are largely consistent with those found previously, with higher resistances and lower (more negative reactances). Oscillometry is now fairly widely used in preterm populations, including in infancy, with differences in impedance already seen compared to term infants prior to discharge from the neonatal period (Travers et al., 2021). These changes appear to track through childhood with differences seen in pre-school age (Manti et al., 2021) to adolescence (Thunqvist et al., 2016), although airway mechanics may be less affected in adulthood compared to spirometry (Um-Bergström et al., 2019). My findings are consistent with others particularly with regards to peripheral airway disease being a significant clinical feature in these children, as represented by resistance at low frequencies and frequency dependence of resistance (Thunqvist et al., 2018). Exercise outcomes for oscillometry were surprisingly unremarkable. While there was little exercise-induced bronchoconstriction seen in this study on spirometry compared to previous studies (Joshi et al., 2013), I was expecting that oscillometry may be more sensitive than spirometry for detecting these changes following exercise. While post-exercise bronchodilator oscillometry responses were potentially lower than the defined thresholds (Calogero et al., 2013) in the low lung function preterm group, significantly greater responses were seen compared to control groups, plus a trend towards normal in these children were seen.

Tracking impedance through the respiratory cycle is an advancement of standard oscillometry, offering insight into the changes seen at various points within inspiration, expiration and at zero-flow states, where impedance is no longer affected by potential effects of changes in flow. Its use has been limited to identifying risk of lower respiratory infections in infants (Gray et al., 2019), distinguishing airway obstruction in young children (Czövek et al., 2016), and assessing patients with COPD (Lorx et al., 2017). The most relevant of these with regards to the current study is that of the children with wheeze, where differences in impedance, particularly resistance, at zero-flow states at end-expiration and end-inspiration was able to distinguish between those children with wheeze and healthy

children (Czövek et al., 2016). Due to the predominance of potential obstructive disease in this preterm cohort, potentially this could be attributed to asthma. While it is possible some of these children do have true atopic/eosinophilic asthma, the majority are likely to have a different disease process. In the overall preterm low lung function group, there were no differences noted at the zero-flow states compared to controls. Instead, impedance was greater throughout both parts of the respiratory cycle, with little effect from exercise, and generalised improvements seen after post-exercise bronchodilator. This suggests a different clinical phenotype, and an underlying pathology, to children who have specifically wheeze/asthma symptomology.

6.3.4 Obstructive lung disease groups

Overall, it is difficult to summarise some of these differences seen between low lung function preterm-born children and their controls, knowing that this is not a homogenous group of individuals, and that they are likely better defined by their obstructive or non-obstructive disease state.

As previously discussed, distinguishing children by whether they have obstructive lung disease is a novel approach in the preterm population. However, I have demonstrated that children with obstructive lung disease, and those preterm children without, appear to have differing characteristics to each other and to controls.

Firstly, the POLD and PnOLD children can be distinguished by their lung volume testing. POLD children have significant air trapping, as would be expected in the presence of airway obstruction (Welsh et al., 2010, Simpson et al., 2017). Additionally, the use of normal FEV_1/FVC may be a reasonable surrogate for restrictive lung disease, given the reasonable numbers of children with low TLC in this non-obstructive group. What is of interest is the lack of differences between POLD and PnOLD for exercise. Presence of airway obstruction could feasibly suggest greater impairment of exercise, although there are limited data comparing exercise outcomes in these two groups, except that maximal voluntary ventilation may predict

peak $\dot{V}O_2$ and $\dot{V}E$ in obstructive patients with COPD but not in restrictive disease (LoRusso et al., 1993). One finding from my study that may correlate is the obstructive group used a larger proportion of their ventilatory reserve (calculated from MVV) compared to preterm controls, whereas the non-obstructive group did not. The most significant finding with these group divisions is the large proportion in POLD group who have significant bronchodilator response. Additionally, minimal numbers in the non-obstructive group responded, showing a homogeneity to their clinical characteristics.

As outlined above, small airways disease seemed to predominate in the PT_{low} children, and this effect is amplified in the obstructive lung disease children, suggesting their disease is particularly driven by peripheral airways disease and, given both impairment in resistance and reactance. Frequency dependence of resistance, found in this study to be particularly greater in POLD children is supportive of peripheral obstructive airways disease, as is seen in eosinophilic asthma, particularly those who respond to treatments (Abdo et al., 2020), as well as preterm populations (Thunqvist et al., 2018). A previous systematic review assessing FE_{NO} in preterm populations did not find raised FE_{NO} in preterm-born children (Course et al., 2019); however, in my cohort of children with obstructive lung disease, over 1/3 had raised FE_{NO}. Potentially this could suggest a degree of atopy, however rates of atopy were similar between preterm-born children with obstructive disease compared to preterm controls. A number of these may have atopic asthma or may have an alternative inflammatory process (Teig et al., 2012). However, rates of reversibility to post-exercise bronchodilator were over double those with raised FE_{NO} suggesting reversible airway obstruction is not confined to those with potential inflammatory disease, and that several clinical and pathological phenotypes exist within this population and need to be better defined.

One potential outcome with intra-breath oscillometry was the potential for finding differences in the impedance difference between end-expiratory and end-inspiratory states. These, in particular the difference in resistance at these particular points of the respiratory cycle, have been found to distinguish young children with wheeze and healthy children (Czövek et al., 2016). My findings in the POLD children showed no

difference in the resistance at the zero-flow states, suggesting a different pathology to those children with acute wheeze. Even though both are diseases of airway obstruction, preterm-born children appear to have airway obstruction present throughout the respiratory cycle, as opposed to predominantly affecting expiration as seen in asthma. However, in POLD children I found that there were reactance differences between inspiration and expiration, with a greater difference for overall mean reactance, the minimum (i.e., lowest/worst) reactance and the reactance at end-expiration/inspiration.

Additionally, there was greater area within the reactance-volume and reactance flow-curves. All these suggest that there are dynamic changes between expiration and inspiration for the elastic properties of the lung. At the end of expiration, the increased negative reactance suggests a greater elasticity component to the airways (Czövek et al., 2016). The increase that then occurs suggests the elastic component has greater effect during expiration than inspiration possibly due to the elastic recoil of the lungs that drives the increase in airway pressure and hence expiratory flow. This higher reactance in elastic recoil appears to be greater in POLD children hence more negative reactance occurring compared to PnOLD children, potentially a result of increased muscle tone, which is then responsive to bronchodilator. This is potentially different to asthma where elastic recoil pressures are greater during inflation than deflation (An et al., 2007). It would be interesting to know whether there are differences in the connective tissue make-up of the lungs in preterm born children with obstructive lung disease, that may be answered with metabolomic studies using urine or exhaled breath condensate, which is also a part of the scope RHiNO.

As with standard oscillometry, the impedance improves to a greater degree in POLD than PnOLD, again affirming that these children need to be identified due to the reversible nature of their disease.

6.4 Strengths and limitations

6.4.1 Strengths

There are many strengths to this study. Firstly, there are several novel aspects. The main ones relate to a re-consideration of how to approach long-term follow-up of preterm-born children, with an emphasis on current lung function status rather than historical diagnoses. Additionally, this study raises the importance of considering lung function deficits in the context of obstructive or non-obstructive disease, an important concept as this may help identify children who may benefit from intervention. My method of classification, while potentially going away from recommendations, has been designed specifically to apply to this population, rather than being adapted from adult populations. While oscillometry has been used a lot in preterm populations, the effect of exercise on airway mechanics was unexplored in this group. A large novel aspect is the use of intra-breath oscillometry which has not been applied in preterm children previously. It is exciting to have been able to present these results. In addition, I studied a large number of healthy preterm and term-born subjects.

6.4.2 Limitations

There are some limitations to this work. Firstly, the numbers recruited, while reasonable overall, are lower than I would have liked in the group(s) of interest, particularly when assessing by obstructive disease status. This could have effect on both alpha- but more likely beta-errors, with potentially greater differences to be found if numbers were higher. The fact these children were recruited based on their outcome at screening visit means that I cannot draw any conclusions based on their characteristics, particularly their perinatal descriptors, in terms of trying to identify associations or causations with my findings.

More specific limitations to various parts of my work include the systematic finding of differences between the two forms of spirometry. Due to the design of the in-depth lung function visit, the MicroLoop (turbine) spirometer was used before the MasterScreen (pneumotachograph) for all the participants. This could be argued to

have been the cause for the difference noted. However, all children had performed spirometry previously to a standard acceptable to be recruited. They then underwent further education prior to starting the spirometry, and were able to perform acceptable spirometry on the MicroLoop before moving on to the MasterScreen. This would reduce the chance of it purely being a result of practice. The reverse argument of potential tiredness by the time of reaching the MasterScreen could be valid; however, a short break was offered between the tests.

Regarding the oscillometry, it is advised that oscillometry should be performed prior to spirometry due to potential residual effects of the dynamic spirometry manoeuvre on airway mechanics (Saadeh et al., 2015). In the case of the baseline spirometry this took place, however, the post-exercise oscillometry took place in between spirometry testing due to the need to fit in repeat spirometry for the purposes of the wider trial. Similarly, following post-exercise bronchodilator, the oscillometry was performed after the spirometry, as this was the more important test for the RCT and was prioritised. All participants from all groups performed the tests in the same order and timings, so any effects from this would apply to all participants and thus should be negated.

The timing of the oscillometry post-exercise, in hindsight, may not have been optimal. The rationale was partly to allow repeated spirometry, but also due to the Joshi et al findings of the peak time for exercise-induced bronchoconstriction being at ~20 minutes (Joshi et al., 2013). In this case little EIB was observed and so it is more likely the lack of oscillometry changes post-exercise were related to a lack of EIB.

6.4.3 Sources of bias

There are of course potential sources of bias in this study. The largest one is relating to selection bias. Being a study designed to investigate lung function, parents may have been more inclined to respond if their child had any respiratory impairment or previous respiratory problem. This potentially applies to all stages of the recruitment process. Bias may have resulted from attrition during the stages of recruitment. Of those parents who initially responded positively to the questionnaire, a number were

not able to be contacted. Additionally, following screening, some parents opted not to be involved in the in-depth lung function testing visit, for various reasons, but including not wanting their child to perform in the RCT.

Recruiting preterm control children involved offering an invite to any children who were from within the first 10 screening visits for a given month. This is not the best way for randomising these potential participants and could potentially introduce selection bias, as the research nurses could select candidates they think would be most appropriate to invite. However, in reality, this was not feasible due to the number of changing visits that occurred due to cancellations, rescheduled appointments, and last-minute appointments. The rationale behind this decision for selecting controls was due to recruitment starting at the upper end of the age spectrum and wanting to recruit controls with a spread of age, as well as being able to avoid recruitment occurring at one time of year.

6.5 Suggestions for future research and clinical application

6.5.1 Potential further analyses using current data

There are a number of areas within the current work which could be expanded on. Identifying preterm-born children who have a bronchodilator response, in the case of this population from post-exercise spirometry, is one of the main perspectives of this thesis, especially as outlined in Chapter 5 section 5.3.1, with comparison of methods for detection of obstructive lung disease. As such, one option to be explored is using regression models to determine whether any characteristics contribute to the likelihood of having reversible airway disease. This is work that I am currently contributing to with analysis of data from Part 1. The same methodology could be applied to Part 2 data, but would have to consider a couple of caveats, including that bronchodilator response in my data is from a post-exercise timepoint so smooth muscle tone may have been altered prior to administering salbutamol. Additionally, due to the population being selected based on lung function, results of any model may not be applicable to the preterm population in general.

The randomised control trial within RHiNO, published after my initial thesis submission, has found that inhaled corticosteroids combined with long-acting beta-2 agonist improve %FEV_1 by 14% after 12 weeks of treatment (Goulden et al., 2021). Combined with my findings concerning children with obstructive lung disease, it would be reasonable to assume that those children who responded are those within the obstructive lung disease group. The response to treatment within individual phenotypes including obstructive disease has not yet been analysed but may reveal specific groups more likely to benefit from treatment; however, results may be limited by low numbers for separate phenotypes within the individual treatment groups.

There are likely additional phenotypes of lung disease within the preterm population I studied, including within the preterm non-obstructive lung disease (i.e., low FEV_1, normal FEV_1/FVC ratio). This may encompass different aspects of lung disease from the lower end of the normal spectrum to restrictive lung disease. Additionally, the concept of preserved ratio, impaired spirometry (PRISM) has recently been discussed in the literature in adult populations (Schwartz et al., 2021), and its exploration in

paediatrics, in particular preterm-born children, is novel. Again, examining this data with Part 1 participants has been started, but it could be applied to my data to explore specifics of lung volumes and exercise capacity within a PRISM population.

In view of the absence of available oscillometry reference values, I would like to use the oscillometry data from my term population to develop z-scores for various parameters, normalising for aspects such as height and age. The oscillometry parameters could also be used to identify potential cut-offs for (post-exercise) bronchodilator response specific to this population.

6.5.2 Potential future directions for research

There are a number of directions I believe this research study can develop, both realistically and idealistically. Firstly, it is important to validate the methodology for identifying obstructive/reversible lung disease, in a different population of a similar age. This could either be done prospectively on a new population, or retrospectively on an alternative dataset, providing sufficient data are available. If the methodology is validated, then applying the same approach to preterm born populations of other ages (i.e. adolescents or adults) could be done. It is likely that differing cut-offs for alternative age groups would be required; however, receiver operator characteristic curves could be used to identify the relevant thresholds within any new population.

Another finding from the main RHiNO trial was that there was no impact from active treatments on exercise capacity (Goulden et al., 2021). It is still unknown whether children born preterm have impaired exercise capacity as a direct result of poor lung function, or whether there is a habitual detraining effect from either respiratory morbidity and/or neuromotor impact of preterm birth including differences in body habitus and muscle mass (Lowe et al., 2016a). Indeed, I believe impaired lung function may not be appreciable in day-to-day activity; however, may prevent children reaching their athletic potential as they may exercise within their limits. As such it may be that if lung function could be modified, this might allow improvement of exercise capacity with exercise intervention. I think it would be fascinating to be able to combine this hypothesis with our knowledge on effective treatment from the

RHiNO trial. An interventional study of combining ICS+LABA treatment with an exercise training programme versus regular activity over a prolonged treatment period (i.e. 12 weeks as per RHiNO study) may answer this question.

I believe there is a great deal more that could be done with the application of oscillometry in preterm populations. Assessing direct impact of bronchodilator on oscillometry would be important to assess whether any changes I identified on oscillometry testing at the post-exercise bronchodilator stage were a result of improvement from post-exercise or whether the same findings would be seen if reversibility testing was performed directly from baseline.

One limitation of the study with regards to oscillometry was its application post-exercise. The priority of the study was assessing spirometry parameters following exercise at different time points. This resulted in only a single post-exercise oscillometry test being performed. It would be interesting to assess whether timing of oscillometry post-exercise have an impact on airway mechanics, for instance whether greater changes are seen immediately post-exercise as opposed to at the 20-minute interval as used in my data collection.

I would be interested in exploring the intra-breath oscillometry in greater detail. Given I found a differential response at various frequencies using standard oscillometry, particularly in the obstructive preterm lung disease group (i.e. greater differences at lower frequencies compared to higher frequencies), it would exciting to see whether there are greater changes within the breath cycle if single-frequency intra-breath testing was performed at lower frequencies, including response to exercise and bronchodilator. This may reveal more about whether there are dynamic changes occurring in the peripheral lungs, and may confirm the hypothesis about distal smooth muscle extension contributing to disease in these children.

Given oscillometry can be performed at a younger age than spirometry, there is potential for it to be used to identify respiratory disease at a younger age in the preterm population. Ideally this could be done with a large-scale longitudinal study, starting in the neonatal period and tracking oscillometry parameters through infancy and childhood, with the addition of other modalities of lung function testing

introduced at appropriate ages. Children could then be stratified by respiratory morbidity and/or lung function outcomes, and consideration of whether oscillometry is able to identify at an early stage in life which children are at risk of later lung disease, and potentially subsequently exploring early intervention (i.e., with ICS+LABA) at a younger age and seeing whether this modifies their outcomes.

6.6 Overall messages and conclusion

- Preterm-born children are at risk of long-term lung disease which can be identified with spirometry;
- Preterm children with low lung function have reduced exercise capacity compared to their term counterparts;
- Those children with impaired lung function on spirometry can be grouped into those with obstructive lung disease based on FEV_1 and FEV_1/FVC ratio using simple cut-offs;
- These children likely have small airways disease with peripheral extension of smooth muscle into the peripheries, resulting in smaller airway calibre causing higher resistances, and increased elasticity causing lower (more negative) compliance;
- Children with obstructive lung disease have the greatest deficits in spirometry and greatest degree of response to bronchodilators, which will be a result of improvement in airway calibre and relaxation of the smooth muscles improving lung compliance;
- These children should be identified in order to assess potential benefit from treatment (i.e., bronchodilator);
- Use of oscillometry may be able to identify preterm-born children with lung disease at a younger age, as well as potentially distinguish them from other pathologies with further research.

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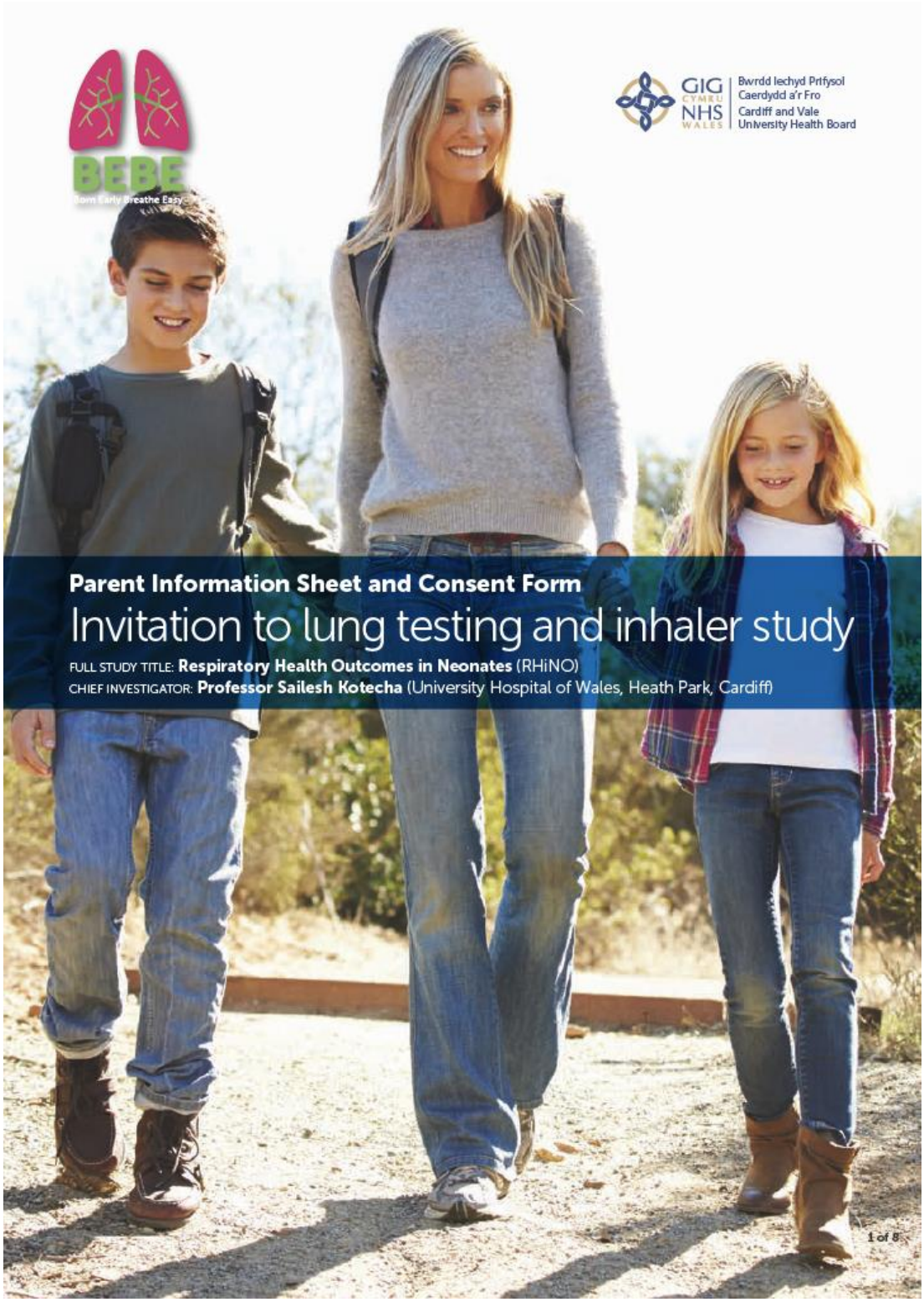
8 APPENDICES

8.1 Appendix 1: Example of Parent Information Sheet and Consent Form for study visit


BEBE
Born Early Breathe Easy
GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysol Caerdydd a'r Fro
Cardiff and Vale University Health Board
Parent Information Sheet and Consent Form
Invitation to lung testing and inhaler study
FULL STUDY TITLE: Respiratory Health Outcomes in Neonates (RHiNO)
CHIEF INVESTIGATOR: Professor Sailesh Kotecha (University Hospital of Wales, Heath Park, Cardiff)
1 of 8

This information sheet is divided into two parts:

PART ONE – tells you the purpose of the research and what will happen if you decide to take part.

PART TWO – gives you more detailed information about how the study will be organised.
Please ask us if there is anything that is not clear or if you would like any more information.

PART ONE

What is the purpose of the study?

You and your child kindly took part in the first part of the study and as we noticed that your child had lower values in their lung function (blowing tests), we would like to find out why this might be. We would also like to assess if these lower values respond to medicines commonly used for treating asthma. By studying the possible mechanism (reasons) for the low values and by using different treatments for breathing problems in premature children, our aim is to find the best treatment to use.

Does my child have to take part?

No, taking part is completely voluntary. It is up to you and your child (if they can) to decide whether or not to take part. Even if you do agree to join, you can stop at any time without giving a reason. A decision to leave the study, or a decision not to take part, will not change the standard of care you and your child receive now or in the future. If you do take part, you will be given this information sheet to keep and be asked to sign a consent form. The study doctor may also stop your child from taking the study treatments at any time if they feel it is best for them to do so. However, if this happens, they will still want to carry on collecting information from your child if you both agree this.

What will happen to my child if we agree to take part and how long will it take?

We will arrange for you to visit the RHiNO study clinic at the Paediatric Clinical Research Facility (CRF), Children's Hospital for Wales on two occasions. Each visit will take most of the morning or afternoon (3-4 hours). We will ensure your car parking (if necessary) is paid for and that you are provided with a voucher for a meal after the testing is finished for you and your child. We would like your child to remain in this part of the study for about 3 months.

VISIT 1

When you arrive, the nurse or doctor will explain this part of the research in detail. If you are satisfied with the explanations, you will be asked to sign a consent form. If your child is able to understand the research and is happy to take part, they will be asked to sign an assent form with you. You will be given a copy of this information sheet and your signed consent/assent forms to keep.

Once consent has been given, you and your child will be asked some questions to make sure that they are OK to join. They will ask some questions about your child's medical history (including any allergies), what other medicines they may be taking and do a quick examination, including taking their temperature, to make sure they are well enough to take part. A cardiovascular assessment will also be performed which is very similar to taking a blood pressure but also needs an extra cuff to go around your child's thigh as well as their upper arm - this will only take a few minutes. The nurse will then measure their height, weight and body composition (how much muscle and fat they have). We will also perform an allergy 'skin prick' test. For this test your child's skin is lightly pricked with substances to which they might be allergic. This is relatively painless – the skin is gently pricked but it is not punctured. If they are allergic to a given substance, the skin reacts with a small 'hives-like' reaction, and we will measure the size of the reaction.

Next, the nurse or doctor will ask your child to do some breathing and blowing tests and pedalling on an exercise bike to see if they have any signs of narrowing or 'redness' (inflammation) in their breathing tubes. We will demonstrate the tests to your child and let them practise until they are happy to do the tests. Some of the breathing tests will also involve collecting samples of water vapour from their breath and some of their sputum (phlegm). Collecting the water vapour involves simple breathing in and out of a cooled collection

tube. To collect the sputum, we will ask your child to breathe in vaporised (nebulised) salty water (saline) then cough to bring up the secretions. We would like to collect a urine sample during the visit to check your child's exposure to other peoples cigarette smoke and to measure substances that are increased in other lung diseases. All of these samples can be tested to tell us more about the health of the lungs and airways.

We would also like to collect a sample of your child's saliva (spit) so we can look at some of their genes (DNA) which we think are important for breathing problems and how they respond to different medicines. The saliva sample is optional and it is OK if you would prefer your child not to provide it - you can still take part in the rest of the study. More information about what will happen to the samples your child provides is given in part 2.

For some of the tests, your child will be asked to sit inside a cubicle with glass doors; the cubicle door will be closed for a few minutes but they will still be able to hear and see us. For the exercise test, your child will be asked to pedal a bike for as long as possible. We expect this to be around 15 minutes. We will closely monitor your child during the exercise test and will stop when your child is too tired to continue or if their heart rate goes too high. After the exercise test, the nurse will then give a small dose of a medicine called salbutamol using an inhaler, before repeating some of the breathing tests to see if the medicine has any effect. The most common side effects are an increased heart rate and headaches. We do not expect these to happen often, but we will check to make sure your child is OK.

We will ensure that your child is comfortable and that there is time for breaks; you will be able to stay with them throughout the tests. During a break period, we would like you to complete a short questionnaire about how your child's health affects their and your lifestyle (such as time taken off work and missing school) and how your child is feeling on the day of the visit.

If the doctor or nurse finds that your child has signs of narrowing of the airways, we would like you and your child to think about taking an inhaler for 3 months (12 weeks).We are testing the inhalers **fluticasone** (known as an 'inhaled corticosteroid' or 'ICS') and **salmeterol** (known as a 'long acting beta 2 agonist' or 'LABA') both work in asthma to help children breathe better and try to prevent them from having symptoms such as wheeze and tightness in the chest. They do this in different ways:

- **Fluticasone** makes it easier to breathe by reducing any inflammation (redness) in the airways
- **Salmeterol** relaxes muscles in the chest to widen the airways (tubes that let air into the lungs)
- Placebo (dummy inhaler) We need to use a placebo or dummy inhaler for some children so we can compare children who had the different medicines and those who did not.

All the inhalers have been specially made to look the same. Every child will get two inhalers:

- Fluticasone inhaler and placebo inhaler
- Fluticasone inhaler and salmeterol inhaler
- Placebo inhaler and placebo inhaler

No one including the nurses or doctors will know which inhalers your child will get. The chance that they will get any one is exactly the same for all of the options. You, your child, the doctors or nurses will not be able to choose or know which combination your child is given, however the study doctors and nurses can find out if they need to.

During the study, your child should not take any of the following medicines (inhalers or tablets) unless prescribed by your doctor (for example if the medicine prescribed by us is not working) or in an emergency:

- Inhaled corticosteroids (other than the trial treatment)
- Long-acting beta 2 agonists (other than trial treatment)
- Leukotriene receptor antagonists (such as montelukast e.g. Singulair)
- Beta-blockers
- Theophylline

You can ask your study doctor or nurse if you are unsure about any of these. Please inform your study doctor or nurse if your child is prescribed any new medicines or if any changes are made to their current medicines.

You will need to make sure that your child takes:

- **Two puffs** from **each** inhaler **twice a day** (in the morning and at night time)
- Before taking the inhalers in the morning and in the evening, we would like to test your child's breathing using a 'peak flow meter'. This is a simple device your child blows in to as hard and as fast as they can to see how quickly they can breathe out.

We will make sure both you and your child understand the best way to take the inhaler and use the peak flow meter before you leave. You will be given a diary to record the peak flows, if your child missed any inhaler doses, or if they have any other symptoms or problems. It is important that the treatments are stored safely and kept out of reach of younger children. **Please telephone us if you have any questions (contact details below).**

Your child will also be given the "reliever" inhaler (salbutamol) which he or she should use if they are wheezy even when taking the drug we prescribe. You should record why and when this is used.

We will ensure that your child is comfortable before we allow them home. Your child will be checked by the research team after they start taking the study medicine, usually by the research nurse making a telephone call; you will be asked about your child's health and about any symptoms they have had and will be reminded to fill in the symptom diary between visits.

VISIT TWO

When you return after 3 months of trying the inhaler, we will ask your child to repeat the test they did at the first visit to see if the medicine had an effect. We will not repeat the tests that the medicine will not change (allergy test and saliva samples).

You will need to return all of the study medicine packaging and unused medicine to your study nurse at the 2nd visit.

What does my child have to do if we agree to take part?

If you and your child decide to take part in this study, it is important that you both follow the instructions and advice given to you by the study doctor and research nurse. If you are unsure about anything, please ask us. Before taking part and throughout the study it is important that you tell the study doctor (or any of the staff) about any changes in your child's health that you have noticed. You must tell them if your child's symptoms seem to be any worse or if you are worried that they are not getting any better. If you are concerned at any time you should seek medical advice as you usually would (e.g. by visiting your GP). At each visit or phone call, you should also tell the research doctor or nurse about any other medicines your child is taking.

It is important to make sure that any other doctor your child visits knows that they are taking part in this study. Details of the contact people for this study and their telephone numbers will be in the diary which is issued to you at your first visit. The study doctor will write to your GP and let them know that you are taking part in a research study.

What are the alternatives for treatment?

There are a few different medicines used for children with breathing symptoms. If you were not taking part in the study, your child may have been given a medicine your doctor thought would work best for them. The medicines we are looking at are used to treat children with similar breathing symptoms anyway so your child may have received one or more of them even if they weren't taking part in the study.

What are the side effects of any treatment received when taking part?

The trial medicine might have some side effects, though these are not very common and are usually quite mild when they do happen. Please look out for the following signs and symptoms in your child and report them to the study doctor or nurse when you next see or speak to them:

- throat irritations
- chest infections
- hoarseness
- headaches
- muscle cramps
- fluttery feelings in the chest (palpitations)
- mild throat infections
- visual disturbances

What are the other possible disadvantages and risks of taking part?

Although the breathing tests involved in the study are straightforward, it can be tiring to get through the whole set; we will make sure there are opportunities to take breaks and that water is available to drink. Some children may not like the taste of the salty water used to encourage the sputum (phlegm) production, and in some rare cases the salty water can cause the airways to narrow. However, we will only do this test after the exercise bike so that your child will have had a rest and received the 'reliever' (salbutamol) inhaler to open their airways. We will do some blowing tests during the sputum collection to make sure this is OK.

Sometimes, the allergy test liquids can make the skin red, itchy and a bit swollen if the test is positive (a reaction). This wears off quickly and we will have some relief (antihistamine) cream available to make things more comfortable.

Some people might worry that if their child is put in the placebo inhaler group they will not be getting enough medicine to manage their child's symptoms. We will also give everyone a 'reliever' inhaler (salbutamol), which your child can take 'on the spot' if you think they need to.

Throughout the study we will check that all of the children are well at each study visit. If a child's symptoms gets worse at any time then the doctor will decide if they need to stop taking the trial medicines and might recommend they are put on a different medicine. You will need to make sure that you contact the study doctor or nurse, or your GP, at any time between visits if you think your child's symptoms have got any worse or if you are worried that they are not getting any better.

What are the possible benefits of taking part?

We are conducting this research so that we know how best to treat children born prematurely who have breathing problems and symptoms. Your child's symptoms may improve by taking the study treatments and with the extra help they receive from taking part in this research. However, we cannot promise that taking part will help your child personally. The information we get might help to improve the treatment of other children with similar symptoms in the future though.

What happens when the research study stops?

It will be some time after your child has completed the study before the results from all of the children taking part are known. At the end of the study, we shall write to you (with a copy to your GP) to inform you of your child's results including which drug your child was on and if the drug improved your child's lung function. In the meantime, before these results are known to us, if you have any questions about whether or not your child should have any ongoing treatment, please consult your usual doctor.

What if there is a problem?

If you have a concern about any aspect of this study you should contact the researchers who will do their best to answer any questions.
If you are still unhappy after you have spoken to them and wish to complain formally, you can do this through the NHS Complaints Procedure.

In the event that something goes wrong and your child is harmed during the research study, there are no special compensation arrangements. If your child is harmed due to someone's negligence then you may have grounds for legal action. However, you may have to pay your own legal costs. The normal NHS complaints mechanism will still be available to you.

Will my child's taking part be kept confidential?

Yes. All of the information about your child's participation in this study will be kept confidential. The details are included in Part Two.

Contact details:

Please do not hesitate to contact the RHiNO team on telephone 029 2074 4187 or by email (rhino@cardiff.ac.uk) if you have any questions. Further information is available at our website http://rhino-health.org.

This completes PART ONE of the Information Sheet.

If the information in PART ONE has interested you and you are considering participation, please continue to read the additional information in PART TWO before making any decisions.

PART TWO

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatments being studied. If this happens, your study doctor will tell you and your child about it and discuss whether you both want to, or should, continue in the study. If you or your child decides not to carry on, your research doctor will make arrangements for your child's care to continue. If you and your child decide to continue in the study you will be asked to sign a new consent form and your child (where appropriate) will be asked to sign an updated assent form. Alternatively, on receiving the new information your study doctor might consider it in your child's best interests to withdraw them from the study. They will explain their reasons and arrange for appropriate care for your child.

If the study is stopped for any other reason, you will be told why and your child's continuing care will be arranged.

What will happen if my child or I don't want to carry on with the research?

You or your child can withdraw at any time, if you wish. Your child will then be treated as per local clinical practice and procedures. All data collected up until the time of withdrawal will be anonymised (this means that a number will be used instead of your child's name so that no-one will know the information is about them) and included in the study analysis, unless you specifically state otherwise. If at any point you or your child decides to withdraw from the study, we will ask that you return all of their unused study medicine back to us. You can withdraw from treatment but please consider permitting us to follow up your child so we can collect information.

What if there is a problem?

If you have a concern about any aspect of this study you should contact the researchers who will do their best to answer any questions (contact numbers are in Part One). If you are still unhappy after you have spoken to them and wish to complain formally, you can do this through the NHS Complaints Procedure.

In the event that something goes wrong and your child is harmed during the research study, there are no special compensation arrangements. If your child is harmed due to someone's negligence then you may have grounds for legal action. However, you may have to pay your own legal costs. The normal NHS complaints mechanism will still be available to you.

Will my child's taking part be kept confidential?

Yes. As in the first part of the study, all of the information about your child's participation will be kept confidential. The paper files used to record information in this study will be labelled with an anonymous study number only. Giving information to someone else ('a third party') is not allowed. However, it will be necessary for authorised people from regulatory authorities, the study sponsor, or NHS bodies to check the study is being carried out correctly. Medical information may be also given to your child's doctor or appropriate medical personnel responsible for their welfare. By signing the consent form you are giving permission for this to happen. In the event of the results of the study being sent to Health Authorities or published, all of your child's records will be kept confidential and your child's name will not be disclosed to anyone outside of the study. All documents and files relating to the study will be stored confidentially for a maximum period of 25 years.

Involvement of the General Practitioner/ family doctor (GP)

With your consent, the study doctor will write to your child's GP to let them know that they are taking part in the study. The study doctor may ask your child's GP for further medical information about them if necessary.

What will happen to any samples my child gives?

All samples (saliva, urine) will be sent to the Department of Child Health at Cardiff University for testing in our laboratory. Some tests may be conducted by commercial companies or other university laboratories which have expertise to analyse the samples. The samples will have a code which means they will not be linked to information about your child.

With your permission any remaining samples, including DNA, may be stored for future research into comparing children who were born prematurely and those who were born at term. The samples will be anonymised before use in future studies and may be accessed by researchers in the UK and abroad; the research may include genetic (e.g. DNA) and commercial research. You may withdraw your consent for the storage and future use of your child's samples at any point. If you do withdraw your consent your child's samples will not be used in any subsequent studies and will be destroyed according to locally approved practices of Cardiff University. Any samples already distributed for use in research prior to the withdrawal of consent will continue to be used in that study and any samples remaining at the end of the study will be destroyed.

What will happen to the results of the research study?

The results will be published in medical journals and presented at medical conferences. Your child's confidentiality will be ensured at all times and they will not be identified in any publication. At the end of the study, the results can be made available to you (should you wish). They will also be published on the RHiNO website.

Who is organising and funding the research?

The study is sponsored by Cardiff University and funded by the Medical Research Council (MRC). Cardiff University have assigned the management of the study to the North Wales Organisation for Randomised Trials in Health (NWORTH) at Bangor University.

Who has reviewed the study?

The study was approved by the South West-Central Bristol Research Ethics Committee (Ref 15/SW/0289). It has been registered with the International Standard Randomised Controlled Trial Number ISRCTN14767962.

Thank you for reading this information sheet.

BEBE
Born Early Breathe Easy
University Hospital of Wales, Heath Park, Cardiff CF14 4XW Tel: 029 2074 7747

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Bwrdd Iechyd Prifysol Caerdydd a'r Fro
Cardiff and Vale University Health Board

Parent/Guardian Consent Form

RHiNO: Respiratory Health Outcomes in Neonates (Part 2)

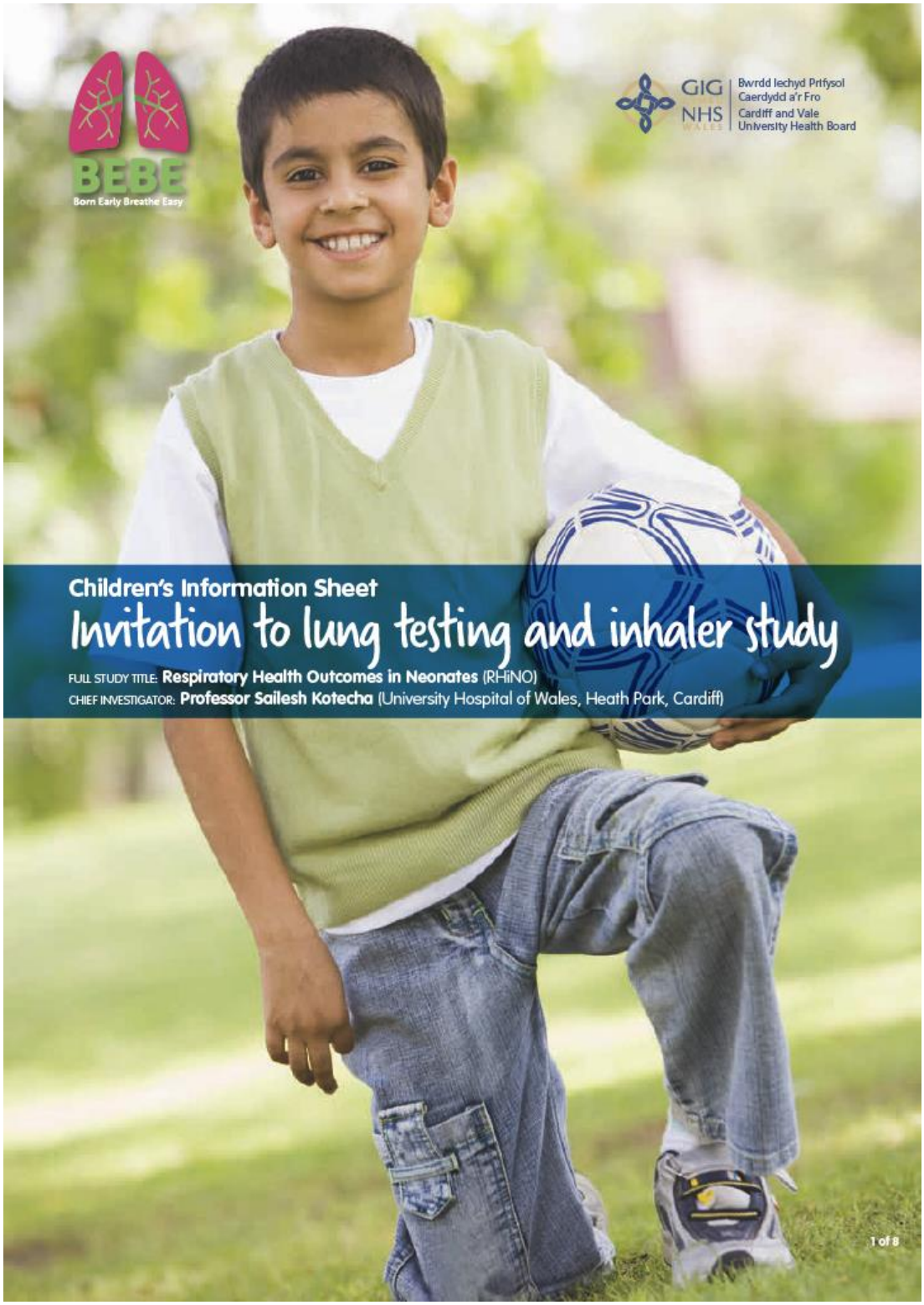
1 I confirm that I have read and understood the information sheet dated 'Version 7 13/02/2018' for the above study. I have had the opportunity to consider the information, ask questions, and have had these answered satisfactorily.		Initial:
2 I understand that my child's participation is voluntary and that we are free to withdraw at any time, without giving a reason, and without my child's present or future medical care or legal rights being affected.		Initial:
3 I understand that relevant sections of any of my child's records and data collected during the study relating to my child may be looked at by responsible individuals from the sponsor, funder, regulatory authorities or hospital. I give permission for these individuals to have access to these records where it is relevant to taking part in this research.		Initial:
4 I agree that personal identifiable information will be collected, stored and used to enable follow-up of my child. This is on the understanding that all information will be treated confidentially.		Initial:
5 I agree to my family doctor being informed of my child's participation in the study.		Initial:
6 I agree to allow my child to take part in the RHiNO study (part 2).		Initial:
7 Optional: I agree to allow a sample of my child's saliva to be taken for use in this study, which will include genetic (DNA) research.	YES NO Please circle	Initial:
8 Optional: I agree for any remaining samples to be used in future for research into children who were born prematurely in the UK and abroad which may include genetic (DNA) and commercial research. I understand I am free to withdraw my consent to future research at any point and that all samples will be destroyed as detailed in the information sheet.	YES NO Please circle	Initial:

Name of Child:

Name of Parent:	Signature:	Date:
Researcher:	Signature:	Date:

Original for case notes, 1 copy for parent/guardian, 1 copy for investigator site file

8.2 Appendix 2: Example of Children's Information Sheet and Assent Form for study visit


BEBE
Born Early Breathe Easy
GIG NHS WALES
Bwrdd Iechyd Prifysol Caerdydd a'r Fro
Cardiff and Vale University Health Board
Children's Information Sheet
Invitation to lung testing and inhaler study
FULL STUDY TITLE: Respiratory Health Outcomes in Neonates (RHiNO)
CHIEF INVESTIGATOR: Professor Sailesh Kotecha (University Hospital of Wales, Heath Park, Cardiff)
1 of 8

Why was I asked to take part?

You were chosen to take part because when we visited you at home, our nurses found out that your breathing might be improved by trying a medicine. This part of the research study will involve about 200 children like you in Wales.

What is a research study? Why is this study being done?

A research study is what you do when you want to learn about something or find out something new. It can help doctors and nurses and other people in the hospital find out which are the best medicines to use. This research study looks at two different medicines called 'Salmeterol' and 'Fluticasone'. Both of these medicines are already used to help children with breathing problems. What we don't know for certain though, is if Salmeterol and Fluticasone help the breathing problems of children who were born early (prematurely).

Did anyone else check the study is OK to do?

Before any study is allowed to happen, it has to be checked by a group of people called an Ethics Committee. The Ethics Committee is a group of experts and ordinary people who look at studies very carefully to decide whether they are OK to do. An Ethics Committee has looked at this study and decided it is OK.

Do I have to say yes?

No – not at all. It's up to you! Just say if you don't want to take part. Nobody will mind. If you do take part, you will need to write your name on a form called an 'assent form'. This form is to say that you understand the study and what will happen if you join. You will be given your own copy of this form to keep as well as this information sheet. Your Mum or Dad will also have a form to sign to say it's OK.

What will I need to do and how long will it take?

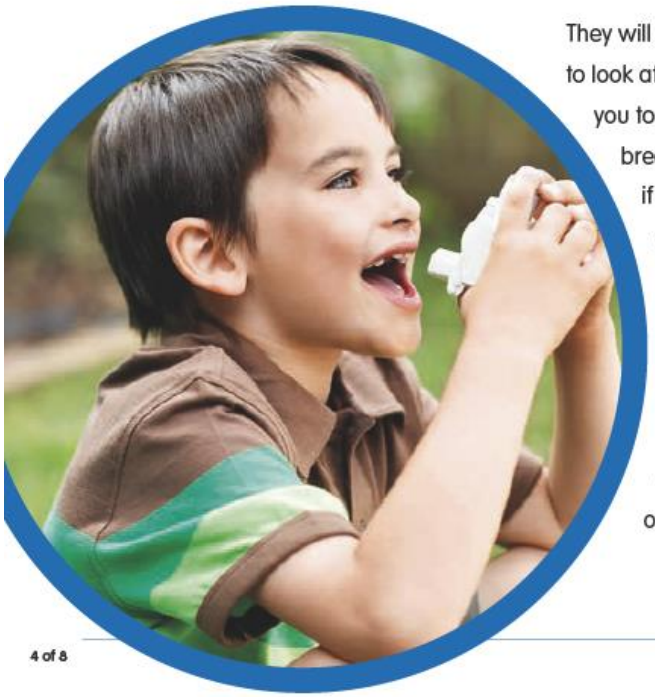
We would like to arrange for you and your parents or carers to come and see us for 2 visits. At your first visit you will see either the study doctor or nurse and they will talk to you about the research. If you say yes to joining the study, you will need to answer some questions and tell the doctor or nurse about how your breathing problems affect you (if they do). They will check you over to make sure that you are well enough to be in the study. They will also measure your growth, development, and how much muscle and fat you are made of. All of these tests need you to stand still and take just a few moments.

We would like to do an allergy 'skin prick' test. The nurse or doctor will drop small drops of liquid on your arm and gently 'prick' your skin. We do not break the skin or make it bleed, and it does not hurt. Sometimes your arm will be itchy, but this will go away quickly and the nurse or doctor will keep checking this is OK.

The doctor or nurse will also measure how much air you can breathe in and out of your lungs by asking you to do some blowing tests. For some of the tests you will need to blow the air out of your lungs in one big breath; for others you will just need to breathe quietly. Some of the tests are done while sitting inside a breathing box. For one of the tests we will ask you to peddle an exercise bike to measure how fit you are.

Last of all, the nurse or doctor will ask if you would mind us having a sample of your urine (wee). We would also like to take a sample of your saliva (spit), but only if your parents or carers think this is OK. These can be tested to help us understand more about reasons for breathing problems. You will have time to practise each test so you can do your best. All the tests might take about 3 to 4 hours, but don't worry, there will be plenty of time to take a break.

At the end of the tests, if the doctor and nurse find the same results as your blowing test at home we would like you to try taking some medicine that might help your lungs. This is called an 'inhaler'.



Some of the children in the study will be given different medicines. You will not be able to choose which ones you get or be told which ones you're taking. Your doctor and nurse will not know but they can find out if they need to.

From the two inhalers you will be given, you will need to take two puffs from each inhaler, twice a day, (morning and evening) for 12 weeks (3 months). Before taking the inhalers, your parents or carers will test your breathing by asking you to blow out as hard as you can in to a special meter. The nurse will give you and your parents or carers some advice about your inhaler and meter. You will have chance to practice to make sure you're using it properly.

They will give you a special diary that is yours to look after and keep. The nurse will ask you to write in it whenever you have any breathing problems. For example, if you were running at break time at school but had to stop because of your breathing, you should write that in. Your parents, carers or any other adult, like a teacher, can help you fill this in if you need them to. The study nurse will ask about the diary each time they speak to your or your parents or carers.

At the end of the 12 weeks you and your parents will visit the doctor or nurse for a second time to see if the inhaler has helped you. You will need to answer some questions and have your breathing measured again by doing the same tests. They will check that you are well, like they did at the beginning of the study.

Will the medicines upset me?

The medicines we are using have been given to lots of children with breathing problems before so we know they are safe to take. Some children who are given these medicines may get some side effects though. The most common ones are:

- itchy or sore throat
- headaches
- chest infections
- muscle cramps
- croaky voice
- fluttery feelings in the chest (called 'palpitations')
- shaky feeling
- eye problems

Will joining in the study help me?

We cannot promise that joining in the study will help you but we hope that it might do. In the future the information we get from this study might help other boys and girls with breathing problems who are born early.

Are there other sorts of treatment I could have had instead?

Yes. There are a few different medicines used for children with breathing problems. If you were not taking part in the study, you may have been given the medicine your doctor thought would work best for you. The medicines in this study are used to treat children with breathing problems anyway so you might have taken one of them even if you weren't taking part.

Who will know that I am in the study?

The study doctor and nurse who are taking care of you will know. So will the doctor who usually looks after you (your GP).

How will the information about me be kept private?

Everything you tell us is private. The only time we would ever tell somebody what you have said is if something made us worried about you. All information collected for this study will be kept safely on the computer or as paper records. Of course, you can tell your family and friends about the study if you want to.

What happens when the research stops?

When you have finished taking part in the study, it will be some time before we know which medication you were on. When the results are available, we shall write to you and your GP, to say which treatment you were on and if there was any improvement with the medication.

What happens if a better medicine comes along?

Sometimes during a research study, new things are found out about the research medicine. Your doctor will tell you about it if this happens. What is best for you might be:

- To carry on taking part in the study
- To stop taking part and have the medicine that the doctor usually uses instead, if they think you need it.

What happens if there is a problem with the study?

If you think there are any problems with the study or if you have any worries about it you can tell your parents or carers. You can also tell the study nurse or doctor. They will do their best to answer your questions. If you are still unhappy you can talk to someone else. Your parents or carers will probably be the best people to talk to.

What if I don't want to do the study anymore?

If you want to stop the study at any time, just tell your parents or carers, study doctor or nurse. They will not be cross with you. If you say no or want to stop the study at any time it will not change the way the doctors and nurses will look after you. Your doctor will choose which treatment is best to use instead.

What will happen to the results of the study?

We will write reports for the doctors and nurses who look after children with breathing problems. The results will also be written in special magazines (scientific journals). No-one will know that they are your results because your name will not be written on them. We will put the results on the study website so you can see them too.

What shall I do now?

Now you know about the study you need to think about whether you want to join or not.

Who can I talk to for more information?

If you have any questions at all, at any time, please contact:

The RHiNO team by
telephone (029 2074 4187)
or email (rhino@cardiff.ac.uk)

Further information is available at our website http://rhino-health.org.

Thank you for reading this information sheet. We hope you have found the information helpful.

BEBE
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University Hospital of Wales, Heath Park, Cardiff CF14 4XW Tel: 029 2074 7747

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysol Caerdydd a'r Fro
Cardiff and Vale University Health Board

Children's Assent Form

RHINO: Respiratory Health Outcomes in Neonates (RHiNO) (Part 2)

Young person (or, if unable, parent/guardian on their behalf) to circle all they agree with:

Have you read (or had read to you) the information about this study?	Yes	No
Has a doctor or nurse explained this study to you?	Yes	No
Do you understand what this study is about?	Yes	No
Have you asked all the questions you would like to?	Yes	No
Have all your questions been answered in a way you understand?	Yes	No
Do you know that it's OK to stop taking part at any time?	Yes	No
Are you happy to take part in this study?	Yes	No
Are you happy to give a sample of you saliva (spit)?	Yes	No

If the answer to any question is 'No', or you don't want to take part, don't sign your name.
If you DO want to take part, please write your name and today's date below:

Your name: Date:

Your parent/guardian must also write their name here too if they are happy for you to take part:

Sign: Print: Date:

The doctor or nurse who explained the study needs to sign as well:

Sign: Print: Date:

Original for case notes, 1 copy for parent/guardian, 1 copy for investigator site file

8.3 Appendix 3: Template of consent form for use of participant images

Parental consent for the use of images of children

Child's Name:-_____

D.O.B.:-_____

Address:-_____

Parent/Carer's Name:-_____

I give consent for the RHiNO research study team to use any photographs or videos of my child, named above, which are taken or have been taken during my child's visit.

I understand that:

- The images may be put onto the RHiNO Website or be used in future publications/papers/educational work. These may therefore be seen by the general public including images which may be identifiable.
- The images will be held in accordance with the Data Protection Act, 1998.
- My child's name will not appear with any of the images.
- I can ask the RHiNO team to stop using the images at any time. If this occurs any images of my child will be removed from the website and will not be used in any future publications, however if the image has already been published then it cannot be withdrawn.

Signed by Parent/Carer:- _____

Signed by Child:-_____

Date:-_____

Name, signature and designation of person taking consent:-_____

_____

RHiNO___Photograph Consent____Version1, 06/09/2016.

8.4 Appendix 4: Publications during PhD

Goulden N, **Cousins M**, Hart K, Jenkins A, Willets G, Yendle L, Doull I, Williams E M, Hoare Z, Kotecha S. 2021. (in press) Inhaled corticosteroids alone and in combination with long-acting β-2 receptor agonists to treat reduced lung function in preterm-born children; A randomized clinical trial. 2021 JAMA Pediatrics.

Hart K, **Cousins M,** Watkins W J, Kotecha S J, Henderson, A J, Kotecha S. 2021. Association of Early Life Factors with Prematurity-Associated Lung Disease: Prospective Cohort Study. European Respiratory Journal.

Cousins M, Hart K, Gallacher D, Palomino MA, Kotecha S. Long-term respiratory outcomes following preterm birth. 2018 Revista Médica Clínica Las Condes; 29(1):87-97.

Hart K, **Cousins M**, Kotecha S. Respiratory outcomes after preterm birth. 2017 Minerva Pneumologica; 56(2):139-51.

Lowe J, **Cousins M**, Kotecha SJ, Kotecha S. Physical activity outcomes following preterm birth. 2017 Paediatric Respiratory Reviews; 22:76-82.

8.5 Appendix 5: Presentations during PhD

Poster discussions

Cousins M, Hart K, Williams EM, Henderson AJ, Kotecha S. Comparison of two types of spirometers in preterm- and term-born children. European Respiratory Society International Congress, 01/10/2019.

Cousins M, Hart K, Williams EM, Henderson AJ, Kotecha S. Exercise capacity and response of exercise-induced bronchoconstriction to bronchodilator in preterm-born children with low lung function. European Respiratory Society International Congress, 16/09/2018.

Cousins M, Hart K, Radics B, Czovek D, Hantos H, Sly P, Henderson AJ, Kotecha S. Forced oscillation technique to assess exercise and post-exercise bronchodilator responses in preterm-born children. European Respiratory Society International Congress, 16/09/2018.

Thematic posters

Cousins M, Hart K, Hantos Z, Sly P, Henderson AJ, Kotecha S. Intra-breath oscillometry for assessing respiratory outcomes in preterm-born children. European Respiratory Society International Congress, 29/09/2019.