School of Physiotherapy and Exercise Science

The effect of preterm birth on the physiological response to exercise testing in school aged children

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This thesis is presented for the Degree of
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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Human Ethics The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number # HR126/2012

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Abstract

Background

Children delivered very preterm (≤32 w completed gestational age (GA)) are born prior to the full development of the respiratory system and often require postnatal respiratory support including mechanical ventilation and supplemental oxygen. These adverse exposures may damage the developing lungs and lead to a chronic lung disease, bronchopulmonary dysplasia (BPD). Advances in neonatal care, including the widespread introduction of exogenous surfactant and more gentle forms of mechanical ventilation, have increased survival of children born at lower GA and birth weight. Contemporary BPD is characterized by altered peripheral lung structures, failed alveolarization and an abnormal pulmonary vascular architecture. The long-term sequelae of contemporary BPD are unclear and very few studies have investigated the effect of contemporary BPD on the physiological responses to exercise. Some studies have suggested no difference in response to exercise compared to healthy controls, while others have suggested children with contemporary BPD demonstrate a decreased peak oxygen consumption and an altered ventilatory response to exercise. To date there are no studies that include a comprehensive assessment of lung structure, peripheral lung function and respiratory morbidity or examined the influence of neonatal history on the long-term outcomes of contemporary BPD.

This thesis aimed to assess the physiological response to a maximal cardiopulmonary exercise test; including ventilatory response and early $\dot{V}O_2$ recovery, and the impact of peripheral lung structure and function and respiratory morbidity on children born prematurely with and without BPD and relate these outcomes to neonatal history.

Methods

School aged children (9-12yrs), born preterm (< 32 GA) were recruited as well as healthy term born controls. Preterm children were classified as having BPD if they had required at least 28 days of supplemental oxygen as assessed at 36 weeks postmenstrual age as per international guidelines. Children underwent complex lung function assessment (clinical forced oscillation technique (FOT), spirometry, lung volume measurements by multiple breath nitrogen washout and gas transfer capacity), parentally reported symptom questionnaire and a maximal exercise test on a treadmill, with exercise tidal flow volume loops. Preterm children also underwent a high-resolution computed tomography scan of the chest while controls did not.

Differences between groups were assessed by paired T-test, Mann-Whitney U test, one-way ANOVA or the Kruskal Wallis test, as appropriate. Bonferroni correction was applied to account for possible type 1 errors due to multiple testing. Chi-square analysis was used for differences in proportion between groups.

Hierarchal linear regression models were created; multi-collinearities between the neonatal predictors were identified and adjusted for by using residuals of independent regressions of the collinear variables.

Results

Two hundred and twenty-one (126 male) children, (99 BPD, 64 non-BPD and 58 healthy term controls) were enrolled in the study. One hundred and forty-nine (92 male) children successfully performed the flow volume loop manoeuvres required for assessment of expiratory flow limitation (64 BPD, 42 Non-BPD and 43 controls). One hundred and four (51 male) children, (38 BPD, 35 non-BPD children and 31 controls) completed a valid peak exercise test and 94 (47 male) children (33 BPD, 34 non-BPD children and 27 healthy term controls) had recovery data recorded for at least 1 min following exercise cessation. Preterm children had increased parentally reported exertional symptoms (46 %).

The flow volume loop analysis showed the majority of preterm children with BPD (53 %) had expiratory flow limitation (EFL) compared to preterm children without BPD (26 %) or controls (28 %) ($p<0.05$). The presence of EFL was independently associated with decreased FEV_1/FVC z-score and lower gestational age ($p<0.05$). In children who completed a peak exercise test there was no difference in peak $\dot{V}O_2$ between preterm children with BPD and controls (47.1 vs 47.7 mL/kg/min, $p=0.407$). Children born preterm with BPD had an elevated breathing frequency to tidal volume ratio compared to controls (76 vs 63 %, $p=0.002$). There were no other differences in exercise outcomes. Structural lung disease was not associated with any changes in exercise outcomes in this preterm population. Elevated breathing frequency to tidal volume ratio was associated with decreased birth weight z-score.

There was no difference in early $\dot{V}O_2$ recovery as assessed by the time to 50 % peak $\dot{V}O_2$ between children with BPD and controls (54 vs 54 sec $p=0.717$), or children with or without EFL (54 vs 48 sec $p=0.920$). Delayed $\dot{V}O_2$ recovery was associated with increased supplemental oxygen use and the presence of decreased pulmonary attenuation on expiration on CT.

Conclusions

Despite decreased pulmonary function and significant structural lung disease, aerobic exercise capacity is preserved in school-aged children born preterm. These children have an increased prevalence of EFL which is related to worse lung function and a lower gestational age. The alterations in ventilatory response to exercise appear to be associated with lower birth weight z-score suggesting that prematurity with intrauterine growth restriction may alter the ventilatory response to exercise in later childhood. These ventilatory responses to exercise may be an adaptation to assist preterm children to reach peak exercise.

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List of Abbreviations

ANOVA	Analysis of variance
AT	Anaerobic threshold
AX	Area under the reactance curve
BPD	Bronchopulmonary dysplasia
CPAP	Continuous positive airways pressure
CT	Computed tomography
T_LCO	Transfer factor of the lung for carbon monoxide
EELV	End expiratory lung volume
EFL	Expiratory flow limitation
EFL%VC	Percentage of tidal volume meeting or exceeding the maximum flow-volume loop
EILV	End inspiratory lung volume
FEV_1	Forced expiratory volume in 1 second
FOT	Forced oscillation technique
f_R	Respiratory frequency
FRC	Functional residual capacity
F_{res}	Resonant frequency
FVC	Forced vital capacity
GA	Gestational age
HR	Heart rate
HRCT	High resolution computed tomography
MV	Mechanical ventilation
MVV	Maximum voluntary ventilation
O_2	Oxygen
PCr	Phosphocreatine
PMA	Post menstrual age
RER	Respiratory exchange ratio, respiratory quotient
Rrs8	Resistance of the respiratory system at 8hz
RV	Residual volume

TLC	Total lung capacity
V_A	Alveolar volume
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
$\dot{V}O_2$	Oxygen uptake
V_T	Tidal volume
Xrs8	Reactance of the respiratory system at 8hz

Chapter 1 Introduction/ Hypotheses

Approximately 14.9 million babies per year worldwide are born preterm (<37 weeks gestational age) and approximately 2.4 million of these are born very preterm (<32 weeks completed gestational age) (1) equating to approximately 1.8 % of live births. Children born prior to 32 weeks completed gestational age are born prior to the full development of the respiratory system when the respiratory airways, arteries and veins are still forming (2). There is insufficient surfactant production, few or no alveoli and an inefficient gas exchange area that has not yet thinned out into a single layer (2). Following preterm birth, the lungs are forced to develop in an unnatural environment, that is a high oxygen environment rather than the relatively hypoxic fluid filled environment of the uterus (3). Post-natal events which may further contribute to the disruption to normal lung development are the injury to the lungs inflicted by ventilation pressures, infections, fluid overload, and nutritional deficiencies (3).

Bronchopulmonary dysplasia (BPD), a complication of preterm birth, is the most common form of chronic lung disease in infancy (4). Advances in neonatal care such as the introduction of exogenous surfactant have led to the survival of children to a lower gestational age and a change in the pathology associated with preterm birth (5). Contemporary BPD is characterised by immature airways, arteries and veins, few or no alveoli and an inefficient gas exchange area that has not yet thinned out into a single layer (6).

The long-term cardiopulmonary outcomes following very preterm birth during the surfactant era and subsequent neonatal care remain unclear as these children are now only reaching adulthood. Survivors of preterm birth have increased respiratory morbidity and exertional symptoms are reported frequently (7, 8). There is some evidence to suggest these children are at greater risk of cardiometabolic disease later in life (9).

Despite the high prevalence of respiratory symptoms especially on exertion in this population the physiological response to exercise is poorly understood and there are no obvious associations between exercise symptoms and exercise capacity. Given the high prevalence of exertional symptoms and increased cardiometabolic risk the use of a cardiopulmonary exercise test may provide insight into the aetiology and possible therapeutic interventions.

This research project was designed to investigate the impact of preterm birth on the physiological response to exercise in school aged children. This chapter will outline the aims and hypotheses as well as the significance and novelty of the research project.

1.0 **Aims**

1. To determine the response to a maximal cardiopulmonary exercise test (CPET); in particular the ventilatory response, in school aged children born preterm, with and without BPD.
2. To assess the importance of the relative effects of prematurity, neonatal lung disease and other perinatal factors on alterations in the response to CPET.
3. To assess the effects of peripheral lung function on the ventilatory response to exercise using tests that are sensitive to the pathophysiological changes encountered in children with BPD, namely the forced oscillation technique (FOT) and gas transfer factor (T_LCO).
4. To obtain novel information regarding lung structure in preterm children with and without a history of BPD using High Resolution CT scanning (HRCT) of the chest and how lung structure relates to the exercise response.
5. To investigate the oxygen uptake ($\dot{V}O_2$) recovery following a peak exercise test in preterm children and identify any factors that influence this.

1.1 **Aim 1**

To determine the response to a maximal cardiopulmonary exercise test (CPET); in particular the ventilatory response, in school aged children born preterm, with and without BPD.

1.1.1 **Hypothesis**

Children born preterm will have abnormal cardiopulmonary responses to exercise, including:

- An altered ventilatory response characterised by expiratory flow limitation which is more pronounced in children with BPD.
- Decreased peak $\dot{V}O_2$ which is more pronounced in children with BPD.
- Alterations in the heart rate response and oxygen pulse, secondary to changes in the pulmonary vasculature.
- Decreased gas exchange during exercise as expressed by elevated ventilatory equivalents for oxygen and carbon dioxide.

1.2 **Aim 2**

To assess the importance of the relative effects of prematurity, neonatal lung disease and other perinatal factors on alterations in the response to CPET.

1.2.1 **Hypothesis**

The abnormal CPET response in children born preterm will be related to disrupted lung growth and development due to gestational age and subsequent need for ventilatory support in the neonatal period.

1.3 **Aim 3**

To assess the effects of peripheral lung function on the ventilatory response to exercise using tests that are sensitive to the pathophysiological changes encountered in children with BPD, namely the forced oscillation technique (FOT) and T_LCO.

1.3.1 **Hypothesis**

The abnormal CPET responses in children born preterm will be related to alterations in peripheral lung function.

1.4 **Aim 4**

To obtain novel information regarding lung structure in preterm children with and without a history of new BPD using High Resolution CT scanning (HRCT) of the chest and how this relates to the exercise response.

1.4.1 **Hypothesis**

Children born preterm will have demonstrable structural lung abnormalities and these will be related to exercise outcomes. These structural abnormalities will be more sensitive to exercise outcomes compared to pulmonary function assessment.

1.5 **Aim 5**

To investigate the $\dot{V}O_2$ recovery following a peak exercise test in preterm children and identify any factors that influence this.

1.5.1 **Hypothesis**

Children born preterm will have delayed early $\dot{V}O_2$ recovery following a peak exercise test that will be associated with worsening neonatal lung disease.

1.6 **Novelty and significance**

1.6.1 **Novelty**

The research presented in this thesis is the first to describe:

- The interaction between structural lung disease and exercise capacity in preterm children in the surfactant era.
- The influence of lung function and neonatal factors leading to an altered ventilatory response to exercise.
- Early $\dot{V}O_2$ recovery following a peak exercise test in children born preterm.

1.6.2 **Significance**

The inclusion of cardiopulmonary exercise testing (CPET) provides novel information into the complex interactions of body systems in these children. CPET assesses the integrative response of the cardiovascular, pulmonary and skeletal muscle systems to exercise, providing clinical information that may not be apparent at rest. Indeed, health status correlates more strongly with exercise responses than with resting measurements across a variety of different pathologies (10). Accordingly, CPET may offer valuable insight into the long-term cardiopulmonary sequelae of new BPD.

Derangements in lung structure or cardiac dysfunction may underlie potential abnormalities in the exercise ventilatory response in BPD. Pulmonary hypertension and cor pulmonale frequently complicate the course of severe BPD in infancy, with structural abnormalities of the pulmonary vascular bed demonstrated in some patients (11, 12). Ventilatory inefficiency (V_E/VCO_2 slope) correlates with a range of clinical cardiopulmonary diseases (13, 14) and is linked to poor prognosis (15, 16). Similarly, peak oxygen consumption ($\dot{V}O_2$ peak) derived from CPET, also has a strong independent prognostic value (17, 18). In pulmonary arterial hypertension, elevated $\dot{V}_E/\dot{V}CO_2$ slopes can provide valuable diagnostic information, especially early in the disease, when pulmonary pressures are normal at rest but increase markedly during exercise, reflecting an inability of the pulmonary vasculature to vasodilate, manifesting in ventilatory abnormalities (19).

There will be significant benefits associated with an improved understanding of the pathophysiology of lung development in children born preterm such as:

- More targeted treatment of children with a history of preterm birth to manage respiratory morbidity.
- Planning for appropriate follow up of this group to identify early disease prior to symptom progression and counselling parents of preterm children.
- Identification of perinatal factors associated with subsequent respiratory sequelae is an important first step in the prevention of lung disease in children born preterm. Identification of these factors would enable the development of possible targeted intervention in the neonatal period.

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Chapter 2 Methodology

This chapter describes the general methodology for the entire project. Each chapter will describe specific methodological factors as relevant.

2.1 Study population

School aged children were recruited between the ages of 9 and 12 years. Three groups of children were recruited, preterm children with BPD, preterm children without BPD and healthy term controls.

Preterm children were recruited from a prospectively maintained neonatal database from King Edward Memorial Hospital of all preterm births within Western Australia.

Healthy term controls were recruited via email distribution lists for Perth hospitals, universities and local research groups. Fliers and basic study information were also sent to community groups and schools. Healthy friends and family members of preterm participants were also recruited.

Children were included in the study if they met the following criteria:

BPD - Premature birth with <32 weeks completed gestational age and >28 days of supplemental O_2 assessed at 36 weeks post menstrual age.

Non-BPD - Premature birth with <32 weeks completed gestational age and <28 days of supplemental O_2 assessed at 36 weeks post menstrual age; and

Healthy controls - Birth at ≥ 37 weeks completed gestational age and birth weight appropriate for gestational age (10^{th}-90^{th} centiles).

Children were excluded from the study if they had any of the following:

BPD and non-BPD group - Major congenital abnormalities (e.g. congenital heart disease) or significant neuro-developmental disability which would make clinical exercise testing unsafe.

Healthy controls - History of wheeze and/or recurrent cough, doctor diagnosis of respiratory disease or any neonatal respiratory disease.

2.2 **Ethics**

Approval for the study was obtained from the Princess Margaret Hospital for Children ethics committee (approval 170EP) and Curtin University Human Research ethics committee (approval HR126/2012). Written informed consent was received from parents and written assent from the child was obtained prior to the commencement of the study.

2.3 **Protocol**

The children attend the laboratory over 1 visit or 2 separate visits. Height was measured to the nearest centimetre. The child was measured without shoes with their back towards the wall looking forward, feet together; heels, calf, buttock and back were touching the stadiometer. The lower orbital level and the external auditory meatus were level. Weight was measured wearing indoor clothing without shoes to the nearest 0.1 kg.

Respiratory function tests occurred in the following order: Forced oscillation technique, multiple breath nitrogen washout, T_LCO and spirometry. A parentally reported respiratory symptom questionnaire was administered through the testing during test wait periods, current and recent (within the preceding 3 months) use of asthma medications were recorded during the questionnaire. A treadmill cardiopulmonary exercise test was the last assessment performed. In addition, the preterm participants underwent a high-resolution scan of the chest (HRCT) prior to the commencement of the exercise test; heathy controls did not undergo HRCT.

For the preterm children neonatal variables including gestational age, days of supplemental oxygen and ventilatory support (mechanical ventilation and continuous positive airway pressure (CPAP)) were extracted from a prospectively maintained neonatal database, missing data was extracted from the child's medical record.

2.4 Forced oscillation technique

The forced oscillation technique is a non-invasive measure of respiratory mechanics. The forced oscillation technique method involves the superimposing of pressure oscillations (from a loudspeaker) over the normal respiratory cycle and the measurement of response of the respiratory system. This response is the respiratory impedance (Zrs). Zrs is composed of respiratory system resistance (Rrs), compliance and inertance. The respiratory compliance and inertance components of Zrs are referred to as the respiratory reactance (Xrs) and reflect the energy storage properties of the respiratory system (20). The respiratory resistance is composed of chest wall, lung tissue, and airway resistance.

The study used pressure oscillations applied in a frequency range of 4 Hz to 40 Hz. In this frequency range, the healthy respiratory system exhibits a largely frequency-independent respiratory resistance (Rrs) whose major component is airway resistance (Raw) (20). Respiratory reactance (Xrs) undergoes the transition from negative values (when the elastic reactance dominates) to positive values increasing with f (the dominance of inertial reactance) (20).

For this study the clinical forced oscillation technique was performed using a commercially available system (i2M, Chess Medical, Chess Medical, Ghent Belgium). FOT measurements were performed to the ERS task force guidelines (20) . The participant performed stable tidal breathing through a bacterial/viral filtered mouthpiece while wearing a nose clip to prevent nasal breathing. A research assistant supported the cheeks and chin to minimize shunting of the oscillatory signal. The participant performed relaxed tidal breathing for at least 15 seconds. Measurements were removed from analysis if there was a swallow, jaw/mouth movement, air leak around mouthpiece, talking/other vocalisation or irregular breathing. At least 3 acceptable measurements were obtained, the standard deviation of Rrs6 and Rrs8 were less than 10 % of the average value. The average values of Rrs8, Xrs8, resonant frequency and area under the reactance curve were reported and converted to z-scores using the Calogero et al reference values (21).

2.5 **Spirometry**

Forced spirometry measurements were performed in all participants. Forced spirometry involves the measurement of flows and volumes during a maximal forced expiration from total lung capacity (TLC) to residual volume (RV). The largest volume that can be exhaled in a maximal forced breath is the Forced Vital Capacity (FVC); the amount of air that can be forcefully exhaled in the first second is the Forced Expiratory Volume in One Second (FEV_1) (22).

Maximal air flow depends primarily on the elastic recoil of the lungs and the compliance and calibre of the airways. During forced expiration from TLC, airflow limitation begins in the large airways (trachea and main stem bronchi) with the development of turbulent flow. As the forced expiration continues, the site of airflow limitation moves distally (i.e. towards the alveoli) to smaller airways where flow is laminar. Flow is limited by the compression of the airways proximally (i.e. closer to the airway opening) from the "equal pressure point". The equal pressure point is the point at which the intra-airway (lumen) and extra-airway (pleural) pressure is equal, the extra-airway pressure below this point is greater than the intra-airway (lumen) pressure leading to flow limitation (23). As the lungs empty, the equal pressure point moves into smaller airways (23). Measurements of flow at different lung volumes allow assessment of the status of the airways. Loss of elastic recoil (as in emphysema) results in increased compression of the airways and markedly reduced flows at all lung volumes. A decrease in the calibre of the airways (as in asthma or bronchitis) directly limits flow developed for a given driving pressure.

All spirometry was performed on a commercially available spirometer (Vmax 29 Encore, Sensormedics, Yorbalinda Ca). Spirometry was performed to the ATS/ERS standardisation of spirometry guidelines (24). In short, the participant was seated upright, and started with tidal breathing through a bacterial/viral filtered mouthpiece, after 2-3 tidal breaths the participant was instructed to inspire maximally to TLC, once full the participant was instructed to forcefully exhale without hesitation with maximal force. The participant was encouraged to continue exhalation until no further air was able to be exhaled (<25mL in the final second) as observed on the volume time graph.

Acceptability was per the ATS/ERS spirometry guidelines (24). A test was deemed acceptable if they were free from artefacts such as cough or mouthpiece occlusion, had a rapid start of the test and met the end of test criteria. At least 3 technically acceptable results were obtained with the highest FEV_1 and FVC being within 150mL of the next highest. The best value for FEV_1 and FVC were reported. The FEV_1, FVC and FEV_1/FVC were reported as BTPS and converted to z-scores using the GLI reference values (25).

2.6 **Lung volume measurements**

Lung volume measurements were assessed using a commercially available multiple breath nitrogen washout system (Vmax 29 encore, Sensormedics, Yorbalinda CA). For this method of measuring lung volume measurements, the participant breathes 100 % oxygen which washes out the nitrogen from the lungs. The concentration of N_2 in the lungs is presumed to be in equilibrium with the atmosphere (≈ 80 %). When the patient breathes 100 % O_2 for several minutes most of the N_2 is washed out of the lungs. The values for each breath are summed to provide the total volume of N_2 that has been washed out.

The measurements of lung volumes followed the ATS/ERS lung volume measurement guidelines (26). The participant was seated upright and started with tidal breathing through a bacterial/viral filtered mouthpiece wearing a nose clip. Following 4-5 stable tidal breaths with an even end tidal lung volume (assumed to be FRC) the participant was switched in to breathing 100 % Oxygen. The participant was asked to continue steady tidal breathing until N_2 dropped below 1.5 % for at least 3 breaths. Measurements were repeated at least twice with a wait period of double the washout time between trials. Results were considered acceptable if the respiratory rate was between 10-12 breaths per minute, tidal breaths were not excessively large, and the washout curved showed a steady decrease in N_2 concentration without abrupt changes or increases suggesting leak. Following the washout procedure, the participant was asked to perform a

slow vital capacity. They commenced breathing through the mouthpiece, once a stable end tidal volume was recorded, they were asked to inspire fully to TLC and then to exhale in a steady breath until the spirometry end of test criteria was met. From this slow vital capacity, the inspiratory capacity and vital capacity were recorded.

Lung volumes were calculated and total lung capacity (TLC), functional residual capacity (FRC) and residual volume (RV) were derived. Results were reported as BTPS and converted to z-scores using the predicted equations from Cook and Hamann (27) except for RV/TLC and RV/FRC which were presented as a percentage.

2.7 Transfer factor of the lung for carbon monoxide (T_LCO)

The transfer factor of the lung for carbon monoxide (T_LCO), also referred to as the diffusing capacity of the lung for carbon monoxide (D_LCO), is used to evaluate the transfer of gas from the distal air spaces into the pulmonary capillaries (22). It can be measured when known and very low concentrations of carbon monoxide (CO) are inspired. The rate of CO uptake is calculated from the ratio of the CO concentrations of the inspired and expired gas and then expressed as a function of the driving pressure.

The technique used in this study the participant inhaled a volume of test gas containing 0.3 % methane, 0.3 % CO, 21 % O_2 and the balance N_2. The test gas was held in the lungs for approximately 10 seconds, and then during exhalation the methane and CO concentrations were analysed continuously by a rapid response gas analyser.

The T_LCO was performed in this study using the Vmax 29 encore system (Sensormedics, Yorba Linda CA). The participant was rested and seated upright, lips were sealed around a bacterial/viral filtered mouthpiece, and a nose clip was applied. Following 2-3 tidal breaths the participant was asked to exhale fully. At or near RV the participant was switched into the test gas circuit and asked to fill rapidly to TLC. The participant was then asked to hold their breath for approximately 10 seconds, after the breath hold the participant was asked to exhale through the mouthpiece. A gas sample was

collected immediately following the washout of anatomical dead space to give an alveolar sample.

Tests were acceptable if they met the ATS/ERS 2005 single-breath T_LCO Criteria (28). The inspiration of the test gas to TLC occurred within 2.5 seconds (or 4 seconds if the patient showed airflow obstruction on spirometry), the volume of inspired gas (IVC) was >85 % of the best pre-recorded VC and the breath-hold was between 8-12 seconds. At least 2 acceptable measurements were performed with the results repeatable to within 10 % or 3 mL CO.min^{-1}.mmHg^{-1}, whichever was greater.

The average of the acceptable T_LCO and V_A measurements were recorded and converted to z-scores using the GLI reference values (29).

2.8 Respiratory symptoms

Parentally reported symptoms during exercise or physical activity were recorded using a respiratory symptom questionnaire (see appendix A) (30). Children were classified as having current exercise-induced symptoms if parents reported cough, wheeze or shortness of breath on exertion, or symptoms that limited their child's physical activity within the preceding three months. Current and recent (within the preceding 3 months) use of inhaled medications were recorded.

2.9 High resolution computed tomography scan (HRCT) of the chest

Computed tomography (CT) scans of the chest were performed during inspiration and expiration in children born preterm but were not performed in controls. Inspiratory images were taken at 10 mm intervals spanning from the lung apex to diaphragm. Three expiratory images were collected at the anatomical points of the tracheal carina and the midpoints between the tracheal carina and the lung apex, and the tracheal carina and diaphragm (Philips Brilliance 64; Philips Medical Systems, Netherlands). The scans were consensus scored by a specialist paediatric thoracic radiologist and a

paediatric respiratory physician using the scoring system described by Aukland *et al (31)*. Scorers were blinded to neonatal classification of BPD.

2.10 **Cardiopulmonary exercise test**

During a CPET it is possible to assess the complex interaction of the respiratory, cardiovascular, haematopoietic and metabolic systems (32). The CPET involves measurement of gas exchange and ventilation ($\dot{V}O_2$, $\dot{V}CO_2$, V_E), heart rate and, oxygen saturation (SpO_2), during a symptom limited, progressive maximal exercise test.

The most commonly measured variable from a CPET is the peak $\dot{V}O_2$ which is a measure of aerobic fitness. Peak $\dot{V}O_2$ can be limited by a variety of physiological processes involved in the oxygen pathway from the atmosphere to the mitochondria including pulmonary diffusing capacity, cardiac output, oxygen carrying capacity of the blood and the ability for the muscles to extract and use the available oxygen (33).

The pulmonary diffusing capacity relies on alveolar ventilation, ventilation/perfusion matching, gas diffusion, and haemoglobin affinity for oxygen (33). Cardiac output is predominantly influenced by heart rate and left ventricular stroke volume. Oxygen transport involves the oxygen carrying capacity of the blood which includes the availability of adequate haemoglobin, arterial oxygen saturation, and the impact of temperature, pH and CO_2 concentration on the oxygen dissociation curve. The extraction and use of available oxygen is reliant upon capillary density, muscle perfusion, and availability of active mitochondria to meet the energy requirements of the exercising muscles.

The Fick equation (33) describes the impact of these factors on $\dot{V}O_2$:

$\dot{V}O_{2\ max} = CO_{max} \times (CaO_2 - CvO_2)_{\ max}$

Where:

$\dot{V}O_{2\ max}$ = maximal oxygen uptakes (mL/min)

CO_{max} = Maximal cardiac output (mL/min)

$(CaO_2 - CvO_2)_{\ max}$ = the maximal arteriovenous difference in oxygen content.

Using the Fick equation, it is possible to identify the limitation to exercise, cardiac output is reliant upon heart rate (as measured by ECG) and left

ventricular stroke volume. (CaO_2 – CvO_2) is reliant upon the pulmonary diffusing capacity and the ability of the muscles to utilise the transported oxygen.

The underlying principles of the cardiopulmonary exercise test require the accurate measurement of heart rate, $\dot{V}O_2$ and $\dot{V}CO_2$. Heart rate was measured via a 3 lead ECG, for this study lead II was used for the assessment of the interval between the R waves. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated as the difference from the inspired gas concentration to expired gas concentration. $\dot{V}O_2$ was calculated using the following (33):

$\dot{V}O_2 = [(VI – Fi_{O2}) – (\dot{V}_E \times F_{\bar{E}\,O2})] / t$

Where VI = inspired volume

Fi_{O2}= Fraction of inspired O_2

$\dot{V}_E$ = minute ventilation

$F_{\bar{E}\,O2}$ = fraction of O_2 in mixed expired gas

t = time period for gas volume analysis

The measurement of $\dot{V}CO_2$ assumes there is no CO_2 in the inspired air and was calculated as:

$\dot{V}CO_2 = (\dot{V}_E \times F_{\bar{E}\,CO2}) /t$

$\dot{V}_E$ = minute ventilation

$F_{\bar{E}\,CO2}$ = fraction of CO_2 in mixed expired gas

t = time period for gas volume analysis

For this study the following procedure was used for the performance of the CPET.

The participant performed the exercise test with a face mask and flow sensor attached, breath by breath metabolic measurements occurred using the Vmax 29 Encore system. A 3 lead ECG was placed to measure heart rate. The skin was prepared using an abrasive skin preparation gel to remove surface oils and reduce electrical impendence. Electrodes were placed for the limb leads. Right arm and left arm electrodes were placed slightly below

the right and left clavicle in the mid clavicular line. Right leg and left leg electrodes were placed at the level of the umbilicus at the mid clavicular line. A finger oximeter probe was attached to the third finger with the cable taped to the wrist to reduce motion artefact and possible loss of the probe.

During the exercise test the participant breathed through a face mask (Hans Rudolf, Kansas City, MO) which was connected to a calibrated gas analyser system (Vmax 29 encore, sensor medics, Yoba Linda, Ca). Inspiratory and expiratory gas was passed through a heated wire anemometer to measure flow and integrated to generate volumes. A continuous gas sample was obtained through a permapure sample line that was connected to the gas analyser. Breath by breath analysis of V_E, $\dot{V}O_2$, and $\dot{V}CO_2$ were recorded and averaged at 20 second intervals.

Once the participant was breathing through the facemask and all equipment was attached the participant remained standing on the treadmill in a stationary position. A 5-minute baseline measurement period occurred, during this time the operator explained the procedure. The participant was advised to relax and not speak during this baseline period. Following the baseline measurement, the treadmill was slowly increased through walking speed to a comfortable jogging speed over a 1-minute warm up period. Following the warmup period, the exercise test continued using a modified Balke protocol (34). The treadmill speed was set to a comfortable jogging pace based on the participant's height and ability on the treadmill. The treadmill was set at a 0 % incline; the treadmill was increased to a 4 % incline following 2 minutes of exercise and a further 2 % every 2 minutes of exercise until volitional exhaustion.

A test was considered peak if there were physical signs of peak performance (inability to maintain running speed) and either peak heart rate >90 % age-predicted (35) or peak respiratory exchange ratio (RER) ≥ 1.0. Predicted peak heart rate was calculated using the following equation : HR = (226 – 0.78 X age) – (0.253 X weight) (35). Peak exercise was set at the final 20 seconds prior to exercise cessation, peak $\dot{V}O_2$, $\dot{V}CO_2$, respiratory exchange ratio (RER), $\dot{V}_E$, tidal volume, respiratory frequency, heart rate and oxygen

pulse were reported as measured values. Large normative datasets of exercise testing in children (in particular on a treadmill) are scarce and with the variation in exercise methodology it is hard to directly compare studies. To allow for the lack of normative data the pre-term cohort was compared to a healthy term control cohort to act as an appropriate control group. Breathing reserve was calculated by maximum minute ventilation – maximum voluntary ventilation (MVV) as a percentage of MVV. MVV can be measured by direct assessment which is a technically demanding measurement and the success rate in children with coordination issues as seen in the preterm population is likely low. The MVV can be estimated as from the measured FEV_1 and both FEV_1 X 35 and FEV_1 X 40 have been reported as estimates of MVV (10). A recent study from Salamon et al (36) reported FEV_1 X 40 was a more accurate estimate and this approach has been used in this thesis to estimate MVV

2.10.1 **Anaerobic threshold assessment**

The anaerobic threshold (AT) was assessed using the V-slope method The V-slope method involves plotting the $\dot{V}CO_2$ and $\dot{V}O_2$ relationship (33). The point at which the linear relationship between the 2 changes (break point) this point was taken as the AT (Figure 1). If this method was inconclusive the ventilatory equivalent method was used, for this method the ventilatory equivalents for $\dot{V}O_2$ ($\dot{V}_E/\dot{V}O_2$) and $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$) were plotted. AT occurs when the $\dot{V}_E/\dot{V}O_2$ curve inflects upwards indicating the compensation for metabolic acidosis while the $\dot{V}_E/\dot{V}CO_2$ remains constant due to isocapnic buffering. The point the $\dot{V}_E/\dot{V}O_2$ inflects away from the $\dot{V}_E/\dot{V}CO_2$ point is where AT occurs (33). If AT was not clearly defined by either method the AT was not recorded.

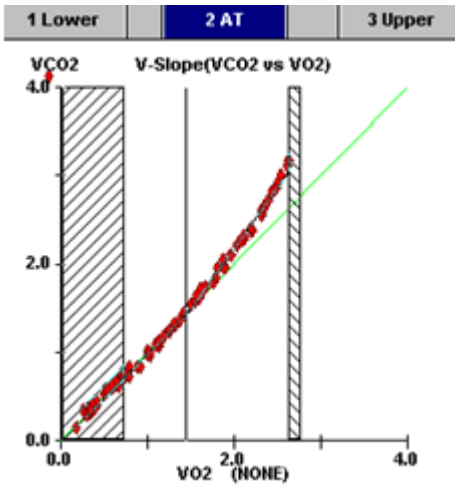

Figure 2-1 V-Slope method for the assessment of anaerobic threshold

2.10.2 **Slope analysis**

As per international guidelines (37) the slopes of the relationships between $HR/\dot{V}O_2$ and $V_E/\dot{V}CO_2$ were calculated for the entire period of exercise. The slope of the relationship between V_E/VO_2 was assessed up to AT.

2.10.3 **Exercise tidal flow volume loops**

Tidal flow volume loops were assessed at baseline prior to commencement of exercise, a practice measurement was performed to ensure the participant could perform the manoeuvre appropriately. 3-5 tidal breaths were recorded; the participant was not coached about how to breathe during the data collection. The operator ensured a stable end expiratory lung volume (dynamic FRC) was obtained. Once 3-5 even breaths were recorded the participant was instructed to perform a maximal inspiratory capacity (IC) manoeuvre to total lung capacity (TLC) and return to normal breathing. This process was performed in the final 30 seconds of each exercise stage and prior to exercise cessation. Placement of the tidal flow volume loop relative to TLC was determined from the IC manoeuvre at the end of each exercise stage. Tidal flow volume loops were set within the maximal flow volume loop obtained during baseline spirometry based on IC. TLC was assumed to remain constant throughout the exercise.

Expiratory flow limitation was defined as 5 % or more of the tidal flow volume loop tracking or exceeding the maximum flow volume loop obtained prior to exercise (Figure 2)

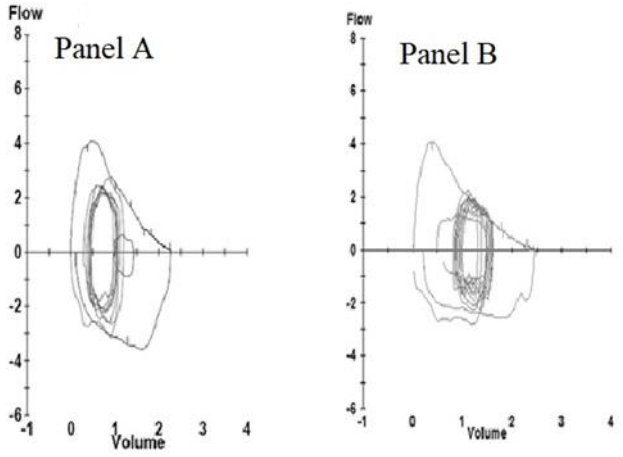

Figure 2-2 Assessment of expiratory flow limitation.
Demonstration of the assessment of expiratory flow limitation (EFL). Panel A shows no EFL, while panel B shows a subject with EFL >5 %.

2.10.4 **Recovery**

Following exercise cessation, the participant was brought to a walking speed and a gradual stop over 30 seconds. The participant remained standing on the treadmill breathing through the mouthpiece until 5 minutes post exercise cessation. Breath by breath analysis continued until the end of the 5-minute recovery period.

2.11 **Statistical analysis**

All data were assessed for normality using the Shapiro–Wilk test. Data are reported as means and standard deviations for normally distributed data and medians and interquartile range for non-normally distributed data. Differences between groups were assessed by paired T-test, Mann-Whitney U test, one-way ANOVA or the Kruskal Wallis test, as appropriate. Bonferroni correction was applied to account for possible type 1 errors due to multiple testing. Chi-square analysis was used for differences in proportion between groups.

Hierarchal linear regression models were created; multi-collinearities between the neonatal predictors were identified and adjusted for by using residuals of independent regressions of the collinear variables.

The statistical methods used in this thesis were guided by an independent statistician to ensure appropriate analysis methods were used to avoid statistical errors, and followed similar statistical methods has previously reported studies in this population (8).

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Chapter 3 Literature Review

3.1 Introduction

Preterm births (less than 37 weeks completed gestational age (GA)) make up approximately 11 % of births worldwide (38), with an increased rate of children born preterm reported from 1990 until 2010 (38). Preterm birth results in disruption of lung growth and development, the impact of which may be felt through life (4) Bronchopulmonary dysplasia (BPD), a complication of preterm birth is the most common form of chronic lung disease in infancy (4). Improvements in neonatal care have allowed for the survival of infants of increasing lower gestational age resulting in a change of the pulmonary pathology associated with preterm birth and BPD from that originally described in the 1960's (3). Contemporary BPD is characterised by immature airways, arteries and veins, few or no alveoli and an inefficient gas exchange area (3). The long-term cardiopulmonary outcomes following preterm birth during the surfactant era and the impact of neonatal care remain unclear as these children are now only reaching adulthood. Recent evidence suggests that infants born preterm are at risk of the development of cardiometabolic disease in adulthood (9) and increased respiratory morbidity and exertional dyspnoea (8, 39). Respiratory symptoms, particularly exertional symptoms are common and as such cardiopulmonary exercise testing may provide insights into the aetiology and possible therapeutic interventions. The aim of this review is to outline the current knowledge of cardiopulmonary exercise testing in the assessment of pulmonary outcomes in children born preterm in the surfactant era.

3.2 **Lung growth and development in the preterm newborn**

Preterm infants are delivered prior to the full development of the respiratory system, especially those born very (28-32 w GA) or extremely (<28 w GA) preterm. In infants born after 24 w GA the conducting airways and the related blood vessels are formed (3). However, it is not until completion of the saccular stage of lung development (from 24 to 38w GA) that the alveolar ducts and sacs form allowing for efficient gas exchange (3) During this time, there is also rapid expansion of the pulmonary vascular network and thinning of the air-blood barriers and the formation of a capillary bilayer in the septa. From around 36 weeks gestation to approximately 3 years postnatally the alveoli are formed, the bilayer fuses into a single layer and there is accelerated vascular growth during the phase termed alveolarization (4).

Delivery prior to complete development of the respiratory system has functional consequences for the preterm newborn (4). The large simplified alveoli and the lack of adequate surfactant production leads to decreased pulmonary compliance and the inability to maintain operating lung volumes, resulting in the need for mechanical ventilation (4). Further, decreased quantity and quality of surfactant causes ventilation heterogeneity leading to areas that are over inflated and other areas of atelectasis (4). As a result increased positive pressures and volumes during mechanical ventilation results in over inflation of the alveolar saccules injuring the immature lung resulting in inflammation; this may increase the impact of any pre-existing lung damage (4).

The thickened air-blood barrier in the lung of a preterm child results in impaired gas exchange, which may result in hypoxaemia and the need for supplemental oxygen. The introduction of supplemental oxygen leads to further decrements in the development of the pulmonary vasculature and alveolar (40). In addition exposure to supplemental oxygen may lead to increased oxidative damage to the developing lung (40). Further inflammatory damage may occur secondary to infection as well as increased ventilatory support required during infection.

Children born preterm in the surfactant era are now reaching adolescents and adulthood, the long-term consequences of the impaired lung growth and development on lung function, structure and exercise capacity is yet to be fully detailed.

3.3 Long term respiratory outcomes resulting from preterm birth

3.3.1 Pulmonary function

Lung function measures, such as spirometry, increase during childhood (with growth) and reach maximum in early adulthood after which lung function is shown to have a steady decline in healthy individuals (physiological aging) (41). Impairments of lung function in childhood have been reported to lead to a lower peak lung function, an increased rate of decline and an increased risk of the development of chronic obstructive pulmonary disease (41).

Children born preterm have reduced forced expiratory flows and the Forced Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC) ratio on spirometry at school age suggesting an increased incidence of obstructive airways disease (8, 42-48). Recent evidence has shown that preterm children have a reduced lung function trajectory which results in worse lung function throughout childhood (48, 49) and into adulthood (48). Quantification of lung volumes (total lung capacity, functional residual capacity and residual volume) in survivors of preterm birth suggest the FVC may be reduced due to air trapping (50) which is consistent with the structural abnormalities reported (8, 51, 52). Respiratory mechanics have been shown to be altered in preterm children indicating changes in peripheral airway function consistent with the small airways obstruction on spirometry (7, 53).

Given the disruption to normal lung development associated with preterm birth and the reported changes in alveolar growth the measurement of the gas transfer (T_LCO) has been assessed in a variety of studies, with most of these studies(42, 46, 47, 50, 54) but not all (8, 55) showing a reduction in the T_LCO. Simpson et al (8) report no changes in the T_LCO despite the high

prevalence of structural lung abnormalities. They suggest this may indicate there is no alteration in the diffusing capacity of the alveolar membrane, although there was a significant proportion of the preterm population unable to perform this measurement. However, the preterm cohort is heterogeneous with a range of gestational ages and neonatal treatments and the long-term impact of preterm birth on respiratory gas exchange at rest remains unclear.

There is conflicting evidence if survivors of preterm birth exhibit airway reactivity. The EPIcure study (45) showed that approximately a third of extremely preterm children demonstrated a significant degree of bronchodilator responsiveness. Suursalmi et al (53) reported a significant difference in the degree of reversibility using FOT between preterm and term children. Lum et al (46) reported increased bronchial hyper responsiveness to methacholine; and Kriemler et al (56) showed approximately half of preterm children exhibit exercise induced bronchoconstriction; however Ronkainen et al (54) showed no significant bronchoconstriction following exercise.

Studies that have assessed fraction of exhaled nitric oxide (FeNO) (a marker of eosinophilic airway inflammation) have shown no differences between preterm children and term controls (46, 50). Given the variability in bronchodilator responsiveness and airway hyperresponsiveness in the presence of normal FeNO it is likely a different mechanism results in airway dysfunction rather than the traditional eosinophilic inflammation associated with asthma.

3.3.2 **Lung structure**

Preterm birth results in arrested development of the respiratory system and lung damage may occur due to infection, inflammation and ventilatory pressures. Previous data in infants with BPD, prior to the introduction of surfactant and gentle ventilation, showed gross abnormalities of the chest radiograph including lung collapse, hyperinflation and emphysema (57). There are only a limited number of studies investigating structural changes in preterm children born in the surfactant era. These report that the majority of

children born preterm had abnormalities on chest CT (8, 51, 52). Both Simpson et al (8) and Aukland et al (51) showed a relationship between spirometric parameters and the total CT score suggesting that worsening lung function is associated with worse structural lung disease. Indeed, longitudinal analysis by Simpson et al (49) showed that that the presence of structural lung changes are related to continued decline and worse lung function trajectory throughout childhood. There are however no longitudinal imaging studies, so whether structural lung disease progresses remains unknown.

3.3.3 **Respiratory symptoms**

There is growing evidence that children born preterm have an increased prevalence of respiratory symptoms both at rest and on exertion as well as increased respiratory morbidity during infancy with increased reports of wheeze, cough, inhaled medication use, and hospital readmission (58). Hennessey et al (59) identified increased respiratory morbidity in the first years of life with increased night-time symptoms and increased use of inhaled medications within the previous 12 months in children with BPD.

Respiratory symptoms remain prevalent in preschool children with increased parentally reported wheeze and cough and a third of all preschool children born preterm having significant parentally reported symptoms on exertion (7). Further reports of increased respiratory symptoms and use of inhaled medication have been reported from early to mid-childhood (8, 45-47, 52, 60). The EPIcure study showed significantly increased parentally reported night time wheeze, wheeze with colds and exercise, as well as increased symptoms on exertion (45). While there was an increase in current use of inhaled medication there was no difference in current use of inhaled medication (45, 46).

Maclean et al (47) found a greater prevalence of parentally reported exercise induced symptoms and doctor diagnosed asthma in a cohort of 8-12 year old children born preterm, but no difference in the current use of asthma medication. Recently, Simpson et al (49) reported the continuation of

symptoms in preterm children from early to mid-childhood, with 70 % of children having parentally reported wheeze at some stage in their life and almost half of all children being given a doctor diagnosis of asthma by mid childhood. Been et al (61) performed a meta-analysis of studies in children born preterm born after 1995 and found children born less than 32 w GA had a 2.81 greater risk of development of wheezing disorders. Taken together these data suggest that preterm birth results in increased respiratory morbidity particularly with increased exertional symptoms that persist throughout childhood. To date there have been no reported associations between exercise induced symptoms and outcomes related to exercise testing in this population.

3.4 **Cardiopulmonary exercise testing**

3.4.1 **Peak aerobic exercise capacity ($\dot{V}O_2$)**

Peak $\dot{V}O_2$ is an independent predictor of all-cause mortality (62) and in lung diseases such as cystic fibrosis, peak $\dot{V}O_2$ and the ventilatory equivalents ($\dot{V}_E/\dot{V}CO_2$, $\dot{V}_E/\dot{V}O_2$) have been shown to independently predict hospitalisations and future exacerbations (63).

Children born preterm are at higher risk of cardio-metabolic diseases (9) such as increased vascular resistance and blood pressure (64), alterations in fat distribution (65) and impaired glucose regulation. Evidence in term born adolescents and young adults show that there is a significant association between gestational age and aerobic exercise capacity with poorer fitness in children delivered early term compared to those delivered at full or late term (66). Understanding the aerobic exercise capacity of children born preterm and any alteration to exercise outcomes may improve the understanding of any deterrents to physical activity in this population and help promote programmes to reduce this metabolic risk.

There is conflicting evidence of the aerobic capacity of preterm children born in the surfactant era, with some studies showing a lower peak $\dot{V}O_2$ (39, 42, 47) while others have shown no difference (44, 56, 67, 68). Comparisons of these studies are difficult due to the variability the cohorts studied such as

GA, birth weight, the presence and severity of BPD, and neonatal care. There are also differences in the methods of exercise testing, which include cycle ergometers (39, 47, 56, 67-70), treadmills (44, 71) or shuttle run tests (42, 43, 60, 72). Generally most treadmill exercise test studies have reported no difference in aerobic capacity (44), while all studies involving shuttle runs and most studies involving cycle ergometer have shown lower peak $\dot{V}O_2$ in the preterm population. The varied methodologies of exercise testing make comparisons difficult due to the different advantages and limitations of each method.

The assessment of peak $\dot{V}O_2$ from shuttle runs involves physical movements that are familiar to children, however, this is demanding from a cognitive perspective and may be challenging for preterm children who are at higher risk of poorer neurodevelopmental performance (38). Burns et al (72) reported that motor impairment was the only predictor of estimated peak $\dot{V}O_2$ assessed by shuttle tests, which may suggest the reductions reported are due to poor coordination rather than cardiopulmonary limitation. Unfortunately, during a shuttle run test other factors that may limit exercise such as workload, ventilatory response, or gas exchange during exercise cannot be assessed limiting the utility of these studies in understanding the underlying limitation to exercise.

The majority of exercise studies in preterm children in the surfactant era have used a cycle ergometer with some (39, 47, 70) but not all (56, 67-69) studies showing a reduced peak $\dot{V}O_2$. There have been reported reductions in workload (39) and $\dot{V}O_2$/work (56, 70) in the preterm population even with normal peak $\dot{V}O_2$ (68, 69). These differences in workload and $\dot{V}O_2$/work suggest peripheral muscle alteration could lead to reduced exercise capacity with the $\dot{V}O_2$/Work slope related to lean muscle mass (70) . The assessment of peak outcomes in cycle ergometer relies on the muscle endurance of the legs, in particular the quadriceps, and given the low workload and VO_2/work relationship it is plausible that these changes may be due to changes in peripheral muscle mass.

Children born preterm have a persistent reduction in body mass and it has been shown this reduction in body mass also results in a proportional reduction of fat mass (65). Preterm children have reduced lower body strength but normal upper body strength suggesting alteration in body composition (73). Alterations in the body composition and reduction of lean muscle mass of the legs may make it more difficult for preterm children to continue cycling at higher workloads resulting in a reduced peak $\dot{V}O_2$ and $\dot{V}O_2$/work.

Preterm children have been shown to have a normal peak $\dot{V}O_2$ when assessed using a treadmill-based exercise protocol (44). Exercise using a treadmill has the advantage that it mimics the daily activities such as running and walking. Treadmill exercise involves the recruitment of a larger muscle mass to achieve peak $\dot{V}O_2$ compared to the predominately lower body recruitment of cycle-based exercise, resulting in a larger peak $\dot{V}O_2$ and higher peak heart rate when measured by treadmill compared to cycle ergometry in healthy subjects (74) which may explain the differences in the outcomes of the studies between cycle and treadmill protocols. However, treadmill exercise testing relies on the child's cognitive ability to run to maximal exertion, preterm children who have coordination issues or gait impairment may terminate the exercise test early resulting in a healthy selection bias.

The impact of neonatal factors on peak $\dot{V}O_2$ is poorly understood, neonatal care such as mechanical ventilation and supplemental oxygen is closely related to gestational age and identifying the independent influence of each is difficult. Most studies have included all neonatal factors and the neonatal classification of BPD (an interaction of both gestational age and oxygen) within multivariate analyses leading to collinearity and reducing the power to detect the individual effect of each variable. As such to date no study has shown an independent effect of gestational age, oxygen use or mechanical ventilation on the aerobic exercise capacity of children born preterm (39, 47).

Structural lung disease has been shown to account for 42 % of variability in peak $\dot{V}O_2$ in other chronic chest diseases such as cystic fibrosis and total CT

score is a stronger predictor than lung function or body mass of exercise limitation (75). To date there have been no published data that explore the relationship between structural lung abnormalities in preterm children and exercise capacity, given the high prevalence of exertional symptoms and structural lung disease it is plausible there may be an association between exercise capacity and structural lung disease in children born preterm.

3.4.2 **Ventilatory response to exercise**

Exertional dyspnoea in preterm born individuals has been attributed to an altered ventilatory response to exercise, which may be associated with worsening lung function. There is a growing body of evidence suggesting that children born preterm mount an altered ventilatory response to exercise which is characterised by a rapid breathing pattern (39, 56, 68, 70), and some reports of a shallow breathing pattern as identified by a reduced tidal volume (39, 47, 56, 70). Two studies in extremely preterm children showed a reduction in maximal minute ventilation (39, 47), however, studies in very preterm children have shown no alterations (67, 70) suggesting that gestational age may play a role.

The assessment of $\dot{V}_E/\dot{V}CO_2$ during exercise as a measurement of ventilatory efficiency has shown conflicting results with two studies (47, 68) showing an increased slope suggesting inefficient ventilation and another showing no difference (70). Novais et al (68) showed that the $\dot{V}_E/\dot{V}CO_2$ was reduced up until the ventilatory threshold and was associated with a reduction in end tidal CO_2, however, peak exercise ventilatory responses were normal. They hypothesized this was either due to an altered CO_2 set point or inspiratory muscle fatigue, although given the normal peak exercise ventilatory outcomes the plausibility of a significant alteration in CO_2 set point is unclear. Combined with the reported reductions in maximal minute ventilation; a measure of respiratory muscle fatiguability, respiratory muscle fatigue is a more likely contributor to the altered ventilatory response, however the mechanism behind this remain unknown. One factor potentially contributing to altered ventilatory responses during exercise may be expiratory flow

limitation (EFL). EFL is associated with an impaired ventilatory response to exercise in asthma, cystic fibrosis and chronic obstructive pulmonary disease (76-79). To date only one study has assessed the prevalence and impact of EFL on exercise in preterm children born in the surfactant era (47). This study showed approximately half of all preterm children developed significant EFL during exercise compared to 25-30 % of term born controls. Unlike the results in other obstructive lung diseases there was no alteration in the inspiratory capacity during exercise or the operational lung volumes suggesting that gas trapping and airway collapse is not a likely cause. It is suggested this increased EFL without changes in operating lung volumes may be due to reduced pulmonary compliance increasing the elastic load of breathing. Preterm children may have respiratory muscle weakness which may lead to an inability to overcome the increased work of breathing leading to EFL; this increased work of breathing may also lead to dyspnoea. However, Maclean et al found no association with EFL and alterations in exercise or ventilatory outcomes in preterm children (47). EFL in healthy adults is largely explained by alterations in lung and airway size, or dysanapsis (smaller airway size relative to lung volume), which limits the capacity to generate the flows and volumes required during exercise (80). Given the disrupted lung growth and development associated with preterm birth, further investigations into the mechanisms of the altered ventilatory response such as respiratory muscle weakness/fatigue, increased work of breathing or dysanapsis are needed as potential pathways for reducing respiratory morbidity.

3.4.3 **Recovery following maximal exercise**

Exertional dyspnoea in preterm children is still poorly understood, despite reduced lung function there is no obvious correlation with exercise outcomes (39). In adults with congestive heart failure, dyspnoea is related to respiratory muscle strength and endurance and respiratory muscle strength is related to early recovery oxygen kinetics (81). Early recovery following maximal exercise is characterised by rapid payback of oxygen debt and resynthesis of phosphocreatine. This process is dependent on the transport and utilisation

of oxygen (82). In children with chronic chest diseases such as cystic fibrosis and bronchiectasis the $\dot{V}O_2$ recovery following maximal exercise is delayed as measured by the time to return to 50 % of peak $\dot{V}O_2$ and is associated with disease severity, however, there is a large variability (82) . The recovery following a maximal exercise test is not only dependant on the size of the oxygen debt (larger debt, faster recovery) but also on the ability of cardiovascular system to transport oxygen, therefore may be a measure of cardiovascular fitness (83) or the ability of the skeletal muscles to uptake the delivered oxygen due to alterations to structure or function (84). Currently there is no evidence regarding $\dot{V}O_2$ recovery in children born preterm; however, given the altered ventilatory response which may be related to altered respiratory muscle strength, assessment of early $\dot{V}O_2$ recovery may identify differences which could help explain increased exertional symptoms.

3.5 **Conclusion**

Children born preterm have disrupted lung growth and development which results in significant respiratory symptoms, structural changes and a reduction in lung function. There is variable evidence regarding the impact of such structural and functional deficits on peak exercise capacity at school age. However, a significant alteration in ventilatory response to exercise has been reported, although poorly understood. Given the high prevalence of structural lung disease in the preterm population, describing the association between with poorer pulmonary outcomes, increased reported respiratory morbidity, and the impact of structural lung disease on exercise outcomes in children born preterm may provide further understanding of ongoing pathology.

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Chapter 4 Increased prevalence of expiratory flow limitation during exercise in children with bronchopulmonary dysplasia

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Please note that this chapter's introductory and methods sections have been amended slightly from their published forms to avoid excessive repetition between chapters.

4.1 Introduction

A limited number of studies report an altered ventilatory response to exercise in children born preterm, including reduced tidal volume and increased respiratory frequency (39, 47, 68, 85). However, the mechanisms underlying altered ventilatory responses to exercise in children with BPD remain unknown. One factor potentially contributing to altered ventilatory responses during exercise may be expiratory flow limitation (EFL). Expiratory flow limitation is associated with an impaired ventilatory response to exercise in asthma, cystic fibrosis and chronic obstructive pulmonary disease (76-79). The single study describing EFL after preterm birth reports an increased prevalence of EFL in children born < 29 weeks GA (47). However, this study did not assess the impact of EFL on exercise capacity or explore risk factors

associated with the presence of EFL and thus the broader consequence of their finding is unclear.

We aimed to investigate the ventilatory response to a peak exercise test in school aged children born very preterm with and without a neonatal diagnosis of BPD. We also aimed to determine the prevalence of EFL and assess any contribution from neonatal exposures on the prevalence of EFL in these children. We hypothesised that children with a neonatal classification of BPD would exhibit an altered ventilatory response to exercise characterized by EFL and expiratory hyperinflation. Further, we hypothesised that the magnitude of the altered ventilatory response would be related to the severity of neonatal lung disease.

4.2 **Methods**

Refer to Chapter 2 for full methodological details

4.2.1 **Participants**

Children were recruited to the study if they were aged nine to 12 years and either born preterm (≤32 w completed gestational age (GA)) or were healthy term born children as previously described (8).

4.2.2 **Pulmonary function testing**

Spirometry and lung volume measurements by multiple breath nitrogen washout (Sensormedics Encore 21-1A, Yorba Linda, CA) were performed in accordance with international guidelines (24, 26), and are reported as predicted z-scores (25, 27).

4.2.3 **Peak exercise test**

Participants performed an incremental treadmill exercise test (Marquette, Sensormedics, Yorba Linda, CA) in accordance with a modified Balke protocol (86) (10) The testing was performed at ambient conditions within a laboratory and all results are reports at BTPS. A peak exercise test was defined as peak heart rate >90 % predicted and physical signs of peak performance (sweating, flushed face and inability to maintain running speed). Peak metabolic ($\dot{V}O_2$, $\dot{V}CO_2$) and ventilatory data (tidal volume (V_T) and breathing frequency) were recorded continuously using breath-by-breath analysis (SensorMedics 229 Metabolic Cart, SensorMedics, Yorba Linda, CA). Breathing reserve was calculated by maximum minute ventilation – maximum voluntary ventilation (MVV) as a percentage of MVV, MVV was calculated at FEV_1 X 40 (87).

4.2.4 **Tidal flow volume loops**

Tidal flow volume loops were assessed as reported previously (88) and adapted by our group (89). Briefly, 3-5 tidal breaths during exercise were recorded followed by a maximal inspiratory capacity (IC) manoeuvre to total lung capacity (TLC) at the end of each exercise stage. Placement of the tidal flow volume loop relative TLC was determined from the IC manoeuvre at the end of each exercise stage. Tidal flow volume loops were set within the maximal flow volume loop obtained during baseline spirometry based on IC. TLC was assumed to remain constant throughout the exercise. Expiratory flow limitation was determined if 5 % or more of the tidal flow volume loop tracked or exceeded the maximum flow volume loop obtained prior to exercise (88).

4.2.5 **Breathing strategy during exercise**

End expiratory and inspiratory lung volume (EELV and EILV, respectively) were assessed at each stage as a measure of dynamic functional residual capacity (FRC) (89, 90) and expressed as a change from baseline (e.g. ΔEELV) and as a percentage of TLC (e.g. EELV%TLC).

4.2.6 **Neonatal data and exercise symptoms**

Neonatal variables including GA, days of supplemental oxygen and ventilatory support (mechanical ventilation (MV) and continuous positive airway pressure (CPAP)) were extracted from medical records and a prospectively maintained neonatal database. Parentally reported exercise symptoms within the preceding three months were recorded using a respiratory symptom questionnaire (30). Children were classified as having current exercise-induced symptoms if parents reported cough, wheeze or shortness of breath on exertion, or symptoms that limited their child's physical activity within the preceding three months.

4.2.7 **Statistical analysis**

The relationships between neonatal factors, lung function and expiratory flow limitation were initially assessed using univariate regressions using EFL as a binary (yes/no) outcome. Factors with a significant univariate association ($p<0.05$) with the presence of EFL were included in subsequent stepwise binary logistic regressions. Multi-collinearities between the neonatal predictors were identified and adjusted for by using residuals of independent regressions of the collinear variables. For example, the independent impact

of mechanical ventilation on EFL was determined from the residual of the regression between GA and mechanical ventilation.

The effect of lung development (GA and birth weight z-score), neonatal lung disease (days of supplemental oxygen, days of mechanical ventilation and days on CPAP), age, sex, height, weight and lung function (FEV_1, FVC, TLC, RV and FRC z-scores) at the time of the exercise test were included in the logistic regression. Statistical analysis was performed using SPSS Version 22.0 (SPSS Inc, Chicago IL).

4.3 **Results**

4.3.1 **Participants**

Two hundred and twenty-one (126 male) children were enrolled in the study including 99 with BPD, 64 without BPD and 58 healthy term controls. This cohort is representative of the broader preterm population in Perth during the same birth period, with no differences in neonatal characteristics of the recruited cohort, as reported by our group previously (8). A valid maximal exercise test (n=171) was completed by 68 preterm children with BPD, 55 preterm children without BPD and 48 healthy term controls. Exercise tests (n=49) were determined as invalid if there was a leak in the mask during testing (n=12), equipment malfunction (n=12), early termination of exercise (n=6), physical inability to perform exercise testing (n=13), poor baseline lung function (exercise testing deemed clinically inappropriate) (n=3) or consent not given (n=3). Of those 171 children completing a valid exercise test, 149 children successfully performed the flow volume loop manoeuvres required for assessment of EFL (64 BPD, 42 Non-BPD and 43 Healthy) and this

population will form the basis of all analyses herein (figure 4-1). Table 4-1 shows the demographic details of the subjects who successfully completed the exercise test with matched flow volume loops measurements.

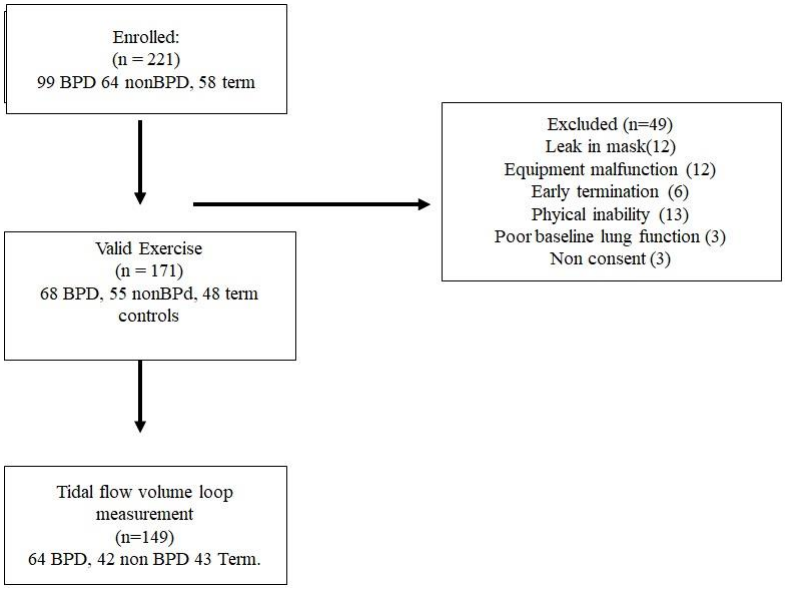

Figure 4-1 Flow diagram of enrolment for the study.

Children without a valid exercise test and matched flow volume loop measurements had a lower FEV_1 z-score (-0.78 vs -0.30) and had a higher prevalence of parentally reported exercise symptoms (65 % vs 39 %) (see Table 4A-1 in appendix 4A). Children who performed acceptable exercise flow volume loops had similar exercise outcomes as those who could not, although they had a lower peak tidal volume (0.88 L vs 1.03 L) (see Table 4A-1 in appendix 4A).

Table 4-2 shows the spirometry, lung volume and maximal exercise test results for the participants that performed a successful exercise test and flow volume loop measurements. Preterm children with a neonatal diagnosis of BPD had a lower absolute $\dot{V}O_2$ peak, however, $\dot{V}O_2$ peak was not reduced when expressed relative to bodyweight (Table 4-2). Children with BPD had a

lower tidal volume (mean difference = -27 mL/Kg; 95% CI = -49, -5; p<0.001) at peak exercise compared to the healthy, term born controls. Similarly, children with BPD had an increased respiratory rate (7 breaths/min; 2, 12; p<0.001), but minute ventilation and tidal volume at peak exercise were not different from preterm children without BPD. Preterm children without BPD demonstrated no differences at peak exercise compared to term born controls (Table 4-2).

Similarly, static lung volumes (TLC, FRC and RV) at rest and the change in EILV or EELV during exercise (expressed as either an absolute change or as a percentage of TLC), did not differ between preterm children with or without BPD and term born controls (Table 4-2). Children with BPD had a significantly lower FEV_1 z-score compared to healthy controls (-0.98; -1.5, -0.46; p<0.001) and non-BPD preterm children (-0.70; -1.23, -0.17; p=0.005). The FEV_1/FVC z-score of preterm children with (-1.06; -1.61, -0.51; p<0.001) and without BPD (-0.71; -1.31, -0.12; p=0.012) was lower compared to term born controls.

Table 4-1 Neonatal and demographic details of the study population

	BPD	Non-BPD	Term
	(n = 64)	(n = 42)	(n =43)
Male (%)	42 (66) *‡	32 (76) *	18 (43)
Gestational age (PMA)	26.0 (25, 27.5)‡	30 (29.1, 31.0)	-
Birth weight (g)	843 (709, 993) ‡	1420 (1238, 1615)	-
Birth weight z-score	-0.15 (0.82)	-0.12 (0.82)	-
Mechanical ventilation (d)	15.3 (4.4, 32.8) ‡	0.0 (0.0, 1.0)	-
CPAP (d)	14.5 (6.6, 24.0) ‡	0.7 (0.0, 3.6)	-
Supplemental O2 (d)	86.5 (57.5, 980) ‡	1 (0.0, 3.0)	-
Recent exercise symptoms n (%)	29 (46)	15 (38)	-
Doctor Diagnosed Asthma ever	24 (35) ‡	17 (44)	-
Current Asthma medication	4 (6) ‡	10 (29)	-
Age at test (y)	10.8 (0.6)	10.9 (0.6)	10.6 (0.6)
Height at test (cm)	141 (136, 146) ‡	142 (138, 148)	145.5 (138, 153)
Weight at test (kg)	32.4 (28.5, 37.6) ‡	36.5 (30.5, 41.3)	34.7 (0.0 42.2)

*BPD: bronchopulmonary dysplasia. PMA: postmenstrual age CPAP: continuous positive airway pressure. Exercise symptoms included parentally reported wheeze, cough. Shortness of breath during exertion. Data presented as mean (SD), median (IQR) or number (percent). * p<0.05 compared to healthy ; ‡ p<0.05 compared to non-BPD Note: Term controls did not have any neonatal intervention, nor respiratory symptoms.*

Table 4-2 Lung function and exercise variables for children who completed a successful maximal exercise test.

	BPD (n=64)	Non-BPD (n=42)	Term (n=43)
FEV_1 z-score	-0.83 (-1.57, -0.17)* ǂ	0.09 (-0.91, 0.37)	0.04 (-0.57, 0.61)
FVC z-score	-0.09 (-0.65, 0.87)*	0.37 (-0.25, 0.97)	0.24 (-0.65. 0.86)
FEV_1/FVC z-score	-1.35 (-2.59, -0.80)*	-0.87 (-1.52, -0.41)*	-0.42 (-1.06, 0.48)
TLC z-score	-0.28 (-0.92, 0.53)	0.25 (-0.50, 0.78)	-0.12 (-1.06, 0.48)
FRC z-score	0.29 (-0.64, 0.87)	0.71 (-0.19, 1.91)*	-0.07 (-0.68, 0.36)
RV z-score	-0.21 (-1.05, 0.30)	0.01 (-0.85, 0.77)	-0.33 (-1.16, 0.71)
$\dot{V}O_2$ peak (L/min)	1.53 (1.40, 1.76)* ǂ	1.78 (1.49, 1.95)	1.69 (1.45, 2.17)
$\dot{V}O_2$ peak (mL/kg/min)	47.7 (42.8, 53.2)	46.1 (42.5, 51.7)	48.1 (45.5, 52.4)
$\dot{V}O_2$ at (mL/kg/min)	26.6 (13.9, 47.2)	31.0 (15.8, 38.8)	34.7 (13.6, 45.3)
Peak RER	1.01 (0.98, 1.02) *	1.03 (1.01, 1.06)	1.04 (1.02, 1.06)
Peak HR (beats/min)	196 (187, 205)	195 (185, 202)	200 (195, 206)
Peak V_T (mL/kg)	24 (21, 27)	27 (23, 30)	28 (24 31)
Peak f_R (breaths/min)	64 (54, 72) ǂ	54 (49, 63)	58 (54, 68)
Peak V_E (L/min/kg)	1.53 (1.36, 1.72)	1.40 (1.30, 1.68)	1.55 (1.42, 1.81)
Breathing Reserve (%)	34.0 (28.2, 35.8)	37.2 (27.0, 38.3)	34.4 (30.0, 37.1)
EFL (n, %)	34 (53)* ǂ	11 (26)	12 (28)
EFL%VT	27.5 (0.0, 60.0)* ǂ	0.0 (0.0, 26.5)	0.0 (0.0, 25.0)
ΔIC (mL)	25 (-83, 193)	110 (-78, 225)	25 (-90, 203)
ΔEELV (mL)	-30 (-145, 200)	-50 (-180, 170)	-15 (-175, 137)
ΔEILV (mL)	397 (146, 557)	371 (238, 648)	368 (157, 723)
ΔEELV%TLC rest	32.0 (27.5, 37.4)	32.1 (28.0, 35.1)	30.1 (225.8, 34.9)
ΔEELV%TLC peak	31.8 (28.4, 35.9)	31.9 (27.6, 36.6)	29.9 (27.0, 33.7)

*BPD: bronchopulmonary dysplasia, AT: anaerobic threshold, V_T: tidal volume, EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow volume loop. EELV: end expiratory lung volume, EILV: end inspiratory lung volume, IC: Inspiratory capacity, V_T: tidal volume, f_R: respiratory frequency, V_E: minute ventilation, HR: Heart Rate, RER: Respiratory exchange ratio. Data presented as median (IQR). * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

4.3.2 **Tidal flow volume loop assessment**

Approximately half of the children with BPD (53 %) exhibited EFL during maximal exercise testing, which was significantly more prevalent than in the non-BPD and healthy term control groups (Chi Square analysis; $p<0.01$; Figure 3). The prevalence of EFL was not significantly different between the healthy term controls and the preterm children without BPD (26 % and 28 %, respectively).

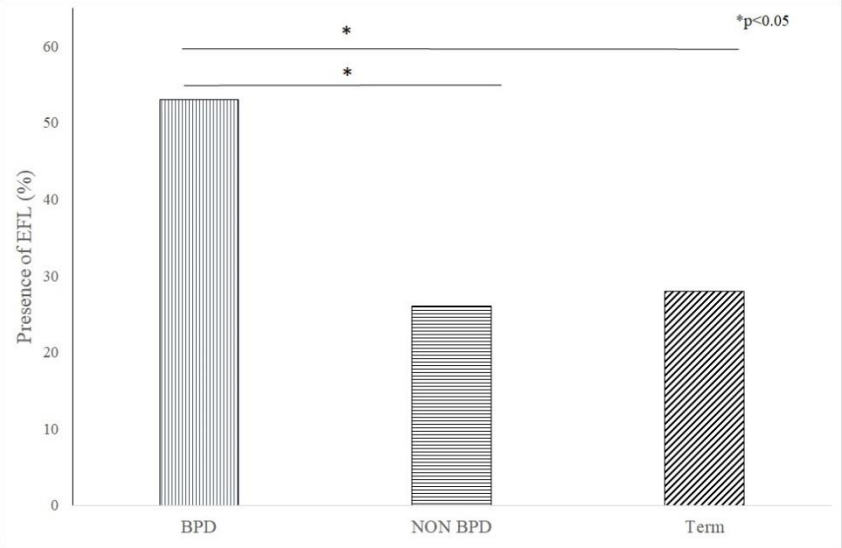

Figure 4-2 Prevalence of expiatory flow limitation
BPD: bronchopulmonary dysplasia, EFL: expiratory flow limitation. Vertical lines children with BPD, horizontal lines: preterm children without BPD, diagonal lines term born controls. *p<0.05.

Differences in neonatal, spirometry and maximal exercise test outcomes in preterm children with and without EFL are presented in Table 4-3. Preterm children with EFL had a significantly longer duration of supplemental oxygen and mechanical ventilation as well as a significantly worse baseline FEV_1 and FEV_1/FVC z-score compared to preterm children without EFL. Tidal volume, breathing frequency and breathing strategy at peak exercise and parentally reported frequency of symptoms during exercise were not significantly different between preterm children with EFL and those that did not

experience EFL during exercise. Days of supplemental oxygen, FEV_1 z-score and FEV_1/FVC z-score were significantly associated with the presence of EFL on univariate analysis (see Table 4A-2 located in appendix 4A). FEV_1/FVC z-score and days of supplemental oxygen after accounting for gestational age were subsequently included in the multivariate analysis; gestational age was also included to assess the impact of lung development. Binary logistic regression analysis showed that a reduced FEV_1/FVC z-score and lower gestational age were independent predictors of EFL developing during a maximal exercise test. (Table 4-4).

Table 4-3 Differences between preterm participants with and without EFL

	With EFL	Without EFL
	n = 45	n = 61
BPD n (%)	34 (75.5 %)*	30 (49.1 %)
Male n (%)	28 (62.2 %)	43 (70.4 %)
Gestation (PMA)	27.0 (25.0, 29.2)	28.6 (25.3, 30.2)
Mechanical Ventilation (d)	5.0 (1.3, 30.1)	2.0 (0.0, 13.5)
CPAP (d)	6.5 (1.0, 24.9)	5.6(0.6, 15.7)
Supplemental O_2 (d)	74.0 (28.0, 94.5) *	22.0 (1.0, 83.5)
FEV_1 z-score	-1.31(0.96) *	-0.12 (0.83)
FVC z-score	-0.40 (-0.57, 0.78)	0.22 (-0.51, 1.13)
FEV_1/FVC z-score	-1.85 (0.89) *	-0.71 (0.83)
$\dot{V}O_2$ peak (L/min)	1.54 (1.40, 1.78)	1.63 (1.42, 1.86)
$\dot{V}O_2$ peak (mL/kg/min)	49.6 (43.2, 52.3)	47.5 (42.4, 52.3)
Peak RER	1.03 (1.01, 1.05)	1.01 (0.99, 1.02)
Maximum HR (beats/min)	197 (187, 207)	195 (186, 202)
V_T (L/kg)	25 (22, 28)	25 (22, 28)
f_R (breaths/min)	62 (53, 70)	60 (52, 69)
Maximum V_E (L/min/kg)	1.55 (1.36, 1.74)	1.43 (1.30, 1.67)
Breathing Reserve (%)	31.1 (26.4, 35.6)	38.8 (29.3, 38.5)
Exercise symptoms n (%)	21 (47.7%)	23 (39.7%)
Dr Diagnosed Asthma ever	23 (52%)*	17 (29%)
Current Asthma Medication	4 (9%)	10 (17%)*

*All data are presented as mean (SD) or median (IQR) unless stated otherwise. *p<0.05, BPD, bronchopulmonary dysplasia; PMA, postmenstrual Age; EFL, expiratory flow limitation; CPAP, continuous positive airway pressure; V_T: tidal volume, f_R: respiratory frequency, V_E: minute ventilation, HR: Heart Rate, RER: Respiratory exchange ratio.*

Table 4-4 Binary logistic regression for neonatal and spirometry variables for presence of expiratory flow limitation

Variable	OR	95 %CI	P Value	R^2
FEV_1/FVC z-score	0.184*	0.084, 0.401	<0.001	0.477
Gestational Age (PMA)	0.799*	0.640, 0.997	0.047	
Supplemental oxygen (d)	1.22	0.674, 2.200	0.514	

OR, odds ratio; 95 %CI, 95 % confidence interval; PMA, postmenstrual age
**p<0.05*

4.4 Discussion

We investigated the impact of very preterm birth on the ventilatory response to exercise in school aged children and determined the factors associated with presence of expiratory flow limitation in these children. We report that children with bronchopulmonary dysplasia have an altered breathing pattern during exercise, characterised by rapid, shallow breathing. Furthermore, we show that half of children born very preterm with BPD exhibit expiratory flow limitation during a maximal exercise test. We found that the presence of expiratory flow limitation was independently predicted by poorer lung function and a lower gestational age.

This study shows that preterm children with a neonatal classification of BPD have a reduced minute ventilation due to a markedly decreased tidal volume with an increased breathing frequency at peak exercise. These observations are consistent with a rapid and shallow breathing pattern and are in keeping with previous exercise studies in children born preterm (39, 47, 68, 85). We hypothesized that this rapid and shallow breathing pattern would be associated with an increased prevalence of EFL. However, the absence of

significant differences in tidal volume, minute ventilation and breathing frequency between those with and without EFL during exercise suggests that expiratory flow limitation is not contributing to the altered breathing response to exercise.

Our study confirms that EFL is highly prevalent in children with BPD (47). Our observations of the prevalence of EFL in preterm children with (53%) and without (26%) BPD are similar to the prevalence reported by MacLean et al (47% and 33% in children with and without BPD, respectively). Our study adds to the current literature in that we report this prevalence over a wider gestational age (up to 32 w GA compared with < 29 w GA). Our data suggests that flow limitation during exercise is not limited to those children surviving extreme preterm birth. Selection of appropriate predicted values for lung function and exercise testing in a paediatric population is hampered by the lack of large normative datasets. We have previously confirmed that the Global Lung Function Initiative reference equations are valid for an Australian population (91). While we cannot confirm that the predicted values for lung volumes are valid in our population our inclusion of healthy term controls increases our ability to interpret our data and it is unlikely that the differences between groups reported here are associated with differences in group demographics.

The presence of EFL in adults with obstructive lung disease is often linked to an increase in operating lung volumes (EELV and EILV) and dynamic hyperinflation during exercise (76) (78). However, preterm children who developed EFL in this study did not show dynamic hyperinflation or changes in operating lung volumes. Preterm children with EFL were more likely to have had a doctor diagnosis of asthma in the past compared to those without, however, less likely to be currently using asthma medication. While the current use of asthma medication may blunt the ventilatory response to exercise all children were assessed without the use of a short acting bronchodilator medication prior to exercise and given the lack of dynamic hyperinflation during it exercise it suggests that current asthma may not be significantly contributing to the presence of EFL during peak exercise. We hypothesise the role of prematurity *per se*, rather than severity of neonatal lung disease or recent symptoms is the primary cause of EFL in children born very preterm. This is confirmed in the multivariate analysis demonstrating that the prevalence of EFL was significantly associated with lower gestational age and not markers of the severity of BPD, such as supplemental oxygen use (Table 4-4).

In support of our hypothesis, Novais et al (68) identified that very low birth weight children had a rapid breathing pattern during exercise and reduced inspiratory muscle strength, suggesting that an increased inspiratory resistive load may lead to the early onset of inspiratory muscle fatigue (68). Inspiratory muscle load, inspiratory muscle fatigue and/or impaired contractile function of the respiratory muscles may result in the inability of preterm children to increase the operating lung volumes during maximal exercise rather than

hyperinflation and hence to a higher prevalence of EFL. Furthermore, the damage associated with injurious tidal volumes during mechanical ventilation and altered peripheral lung development affects the mechanical properties of the lung (6). Reduced pulmonary compliance increases elastic load on breathing. Increased load (work of breathing) that exceeds the ability of the respiratory muscles to generate sufficient force to increase the operating lung volumes when higher flows are required may result in EFL. In addition, the higher elastic work of increasing the lung volumes may not be tolerated in children born prematurely, preventing the maintenance of a higher operating lung volume. We reported a similar mechanism in obese children with an increased load on the chest wall and diaphragm resulting in a similar response to exercise to the preterm population i.e. increased prevalence of EFL without any change in operating lung volumes (89).

Alternatively, the increased prevalence of EFL could be secondary to reduced lung and/or airway size. Children (88, 92) and women (80, 93) have increased EFL compared to adult males; both groups have a lower FEV_1 and FEV_1/FVC compared to adult males due to smaller lung volumes and airway size, a factor associated with increased prevalence and severity of EFL (80, 93). However, as the preterm children with EFL did not exhibit differences in measured static lung volumes (FVC, TLC, FRC and RV; Table 4-3 and 4A-2); it is unlikely that the EFL observed in this study is associated with inherent differences in lung size. Lung volumes in this study were assessed with multiple breath washout and it is known that this technique can underestimate actual FRC in the presence of significant airway obstruction leading to trapped gas (26). It is therefore feasible that our

assessments of FRC (and hence TLC) may be underestimated, exploration of this mechanism using whole body plethysmography to measure static lung volumes may be of value. However, given that there were no changes in measures of dynamic lung volume (i.e. inspiratory capacity during exercise) we do not feel that this is a significant limitation to our study. The repeatability of EFL in the paediatric population has not been well described and is difficult to assess due to the need to repeat the exercise test. However, the measurement of EFL is dependent upon accurate IC we did not see any changes in the measurement of IC in this study we are confident this measurement is valid.

Children with BPD had an increased prevalence of EFL in the presence of a normal response to exercise and no differences in exercise symptoms, the clinical implications of this increased EFL is unclear. While we report no differences in exercise symptoms in children with EFL we were unable to record rated perceived exertion scores or symptom scores at peak exercise as many children were unable to provide a clear answer, as such we are unable to identify if there are differences in symptoms in children with EFL at peak exercise. We suggest, children born preterm may maintain normal physical activity by moderating their ventilatory response or work in shorter exercise bouts before EFL becomes clinically significant. Further investigation into the mechanisms driving and the functional effects of EFL is necessary to fully identify its impact on exercise of different modes, intensities and durations and the long-term impact of EFL as these children continue to grow.

4.5 **Conclusion**

We investigated the impact of expiratory flow limitation on aerobic capacity and the ventilatory response to a maximal exercise test in children born preterm with and without a neonatal diagnosis of BPD. We show that children born preterm have a higher prevalence of EFL than term born controls, and this was not associated with an altered ventilatory response to exercise. The prevalence of expiratory flow limitation in children born before 32 weeks gestation associated with a lower gestational age and reduced lung function.

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Appendix 4A

Table 4A-1 Demographic, neonatal and exercise outcomes for subjects who performed successful exercise flow volume loops and those who did not

	Successful tidal FVL (n =149)	Unsuccessful tidal FVL (n =73)
Pre-term, n (%)	106 (71%)	57 (79%)
Male sex, n (%)	88 (59%)	38 (52)
Gestational age (PMA)	28.0 (25.0, 30.0)	27.0 (25.0, 29.1)
Birth weight (g)	1010 (788, 1356)	890 (740, 1205)
Birth weight z-Score	-0.46 (-0.66, 0.42)	-0.33 (-0.80, 0.45)
Mechanical ventilation (days)	40 (0, 24.7)	5.0 (0.1, 40.7)
CPAP (days)	6.4 (1.0, 19.3)	4.0 (0.5, 18.0)
Supplemental O_2 (days)	48.0 (2.0, 93.0)	46.0 (1.8, 89.0)
Recent exercise symptoms, n (%)	44 (39%)	32 (65%)*
Age at test (y)	10.8 (10.3, 11.3)	10.9 (10.2, 11.4)
Height at test (cm)	142 (137, 148)	144 (137, 151)
Weight at test (kg)	33.4 (29.5, 39.8)	32.2 (29.6, 42.3)
FEV_1 z-score	-0.30 (-1.04, 0.31	-0.78 (-1.47, -0.11) *
FVC z-Score	0.11 (-0.54, 0.86)	-0.05 (-0.79, 0.76)
FEV_1/FVC z-score	-0.98 (-1.67, -0.30)	-1.23 (-0.186, -0.50)
TLC z-score	-0.13 (-0.64, 0.55)	-0.26 (0.69, 0.31)
FRC z-score	0.3 (-0.41, 0.90)	-0.09 (-0.70, 0.87)
RV z-score	-0.21 (-1.01, 0.4)	-0.35 (-1.05, 0.33)
Inspiratory Capacity (L)	1.49 (1.26, 1.80)	1.41 (0.91, 1.76)
$\dot{V}O_2$ peak (L/min)	1.62 (1.42, 1.84)	1.76 (1.57, 1.96)
$\dot{V}O_2$ peak (ml/kg/min)	47.6 (43.1 52.3)	47.2 (37.7, 51.4)
Peak V_T (L)	0.88 (0.71, 1.04)	1.05 (0.90, 1.18) *
Peak f_R (breaths/min)	60 (53, 69)	56 (48, 61)
Peak V_E (L/min)	52.8 (44.8, 60.8)	55.6 (49.5, 63.0)

All data are presented as median (IQR) unless otherwise stated. *p<0.05. PMA, postmenstrual age; CPAP, continuous positive airway pressure; fR, respiratory frequency; VE, minute ventilation.

Table 4A-2 Univariate regression analysis for neonatal and lung function variables and the incidence of expiratory flow limitation in preterm children

	OR	95% CI	P
Gestation (PMA)	0.872	0.748- 1.015	0.078
Supplemental Oxygen (d)	1.011	1.002 - 1.020	0.019*
Mechanical Ventilation (d)	1.014	0.993 - 1.037	0.199
Birth Weight z-score	0.827	0.525 - 1.303	0.412
CPAP (d)	1.031	0.998 - 1.065	0.063
Age at test (y)	0.703	0.376 - 1.314	0.269
Height at test (cm)	0.981	0.933 - 1.031	0.451
Weight at test (cm)	0.952	0.902 - 1.005	0.073
Sex (Female)	1.45	0.41 - 3.280	0.372
FEV_1 z-score	0.244	0.128 -0.463	<0.001*
FVC z-score	0.758	0.481 - 1.193	0.231
FEV_1/FVC z-score	0.179	0.081 -0.394	<0.001*
FRC z-score	1.007	0.753 - 1.346	0.962
TLC z-score	0.696	0.660 - 1.424	0.874
RV z-score	1.24	0.893 - 1.722	0.198

PMA, postmenstrual age; CPAP, continuous positive airway pressure

Table 4A-3 Co-author attribution statement

	Conception and Design	Acquisition of Data	Data Analysis	Interpretation and Discussion	Final Approval
Dr Karla Logie		X	X		
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Maiorana	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Wilson	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Prof Jane Pillow	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Georgia Banton		X			
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Shannon Simpson		X	X	X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Prof Graham Hall	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					

Chapter 5 Lung abnormalities do not influence aerobic capacity in school children born preterm

Airway obstruction (7, 8, 45), air trapping (42, 94), and increased respiratory symptoms (7, 8, 59) are all observed in children with contemporary BPD. However, the impact of these factors on exercise capacity through childhood remains unclear; some studies show a reduction in aerobic fitness (39, 42, 72) while others show no effect (44, 56, 67). Children born preterm also have a reduced tidal volume and minute ventilation with an increased respiratory frequency, and increased expiratory flow limitation (39, 47, 68, 85). The underlying cause of these changes are poorly understood. We have previously shown that children born very preterm have altered lung structure on computed tomography (CT) scan which is associated with decreased lung function at school age (8) and declining lung function throughout childhood (49). In cystic fibrosis, structural lung abnormalities are associated with a reduced aerobic capacity (95), but no published studies report on the relationship between structural lung abnormalities and aerobic capacity in children born preterm.

This study aimed to investigate the relationship between structural and functional abnormalities of the lung and aerobic capacity in school-aged children born very preterm, with and without a neonatal diagnosis of BPD. We hypothesized that children born preterm would exhibit a reduced exercise capacity compared to controls. Further, we hypothesized that exercise capacity would be associated with the severity of neonatal lung disease and abnormal lung structure and function in mid-childhood.

5.1 **Methods**

See Chapter 2 Methods for full methods.

5.1.1 **Pulmonary function testing**

Spirometry, lung volume measurements by multiple breath nitrogen washout, T_LCO (Sensormedics Encore 21-1A, Yorba Linda, CA) and the Forced Oscillation Technique (FOT) (i2M Chess, Ghent, Belgium) were performed according to international guidelines (24, 26) and reported as predicted z-scores (21, 25, 27, 29) with the exception of FRC/TLC and RV/TLC.

5.1.2 **Chest computed tomography**

Computed tomography (CT) scans of the chest were performed during inspiration and expiration in children born preterm but were not performed in controls. The scans were consensus scored by a specialist paediatric thoracic radiologist and a paediatric respiratory physician using the scoring system described by Aukland *et al (31)*. Scorers were blinded to neonatal classification of BPD.

5.1.3 **Peak exercise test**

Participants performed an incremental treadmill exercise test (Marquette, Sensormedics, Yorba Linda, CA) in accordance with a Modified Balke Protocol (10, 34). A peak exercise test was defined as a peak respiratory exchange ratio (RER) ≥ 1.0.

5.1.4 **Statistical analysis**

Hierarchal linear regression models were created to assess the influence of neonatal factors (GA, birth weight z-score, supplemental oxygen use and ventilatory support), and functional and structural lung disease on peak exercise outcomes ($\dot{V}O_2$, ventilatory, and gas exchange parameters). Multi-collinearities between the neonatal predictors were identified and adjusted for using residuals of independent regressions of the collinear variables. For example, the independent impact of MV on $\dot{V}O_2$ was determined from the

residual of the regression between GA and MV. The effect of development (GA and birth weight z-score), neonatal lung disease (days of supplemental oxygen and ventilatory support), current demographics (age, height, weight and gender), lung function and the presence and extent of structural lung abnormalities at the time of the exercise test were included in the model building process. A p-value less than 0.05 was considered significant. Sample size was based on the power to identify a mean difference in the primary outcome ($\dot{V}O_2$ peak) of 0.15 L/min between controls and children with BPD, given a standard deviation of 0.25 L/min, based on pilot data. Assuming 80% power and 5% significance level we estimated a sample size for each group of n = 32. Statistical analysis was performed using SPSS Version 22.0 (SPSS inc, Chicago IL).

5.2 **Results**

5.2.1 **Participants**

Two hundred and twenty-one (126 male) children, (99 BPD, 64 non-BPD and 58 controls) were enrolled. The cohort is representative of the very preterm population in Perth during the same birth period, with no differences in neonatal characteristics of the recruited cohort, as reported previously (8, 49). One hundred and ninety-eight participants attempted the exercise test. Reasons for non-attempt were equipment issues (8), physical or mental impairment (7), poor baseline lung function that was deemed a contraindication to exercise testing (3), or no consent or non-attendance for the exercise test (5).

One hundred and four (51 male) children, (38 BPD, 35 non-BPD and 31 controls) completed a valid exercise test. Exercise tests were determined as being invalid due to a leak in the mask during testing (12), equipment malfunction (4), early termination of exercise (73) and physical inability to perform a maximal exercise test (5). These data form the basis of all analyses herein (Figure 5-1). Children who did not complete a peak exercise test had a lower GA, a longer duration of supplemental oxygen and MV (see Table 5A-1 in Appendix 5A)

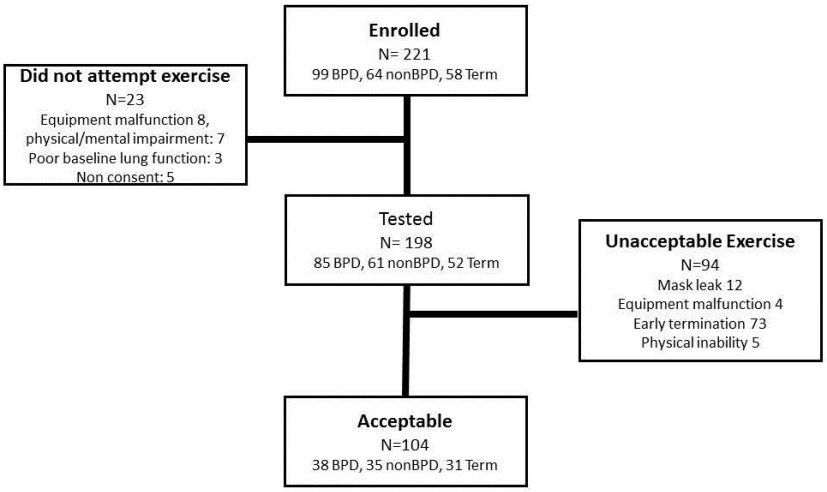

Figure 5-1 Flow diagram of enrolment for the study

Table 5-1 presents the neonatal and demographic information for the participants who completed a successful peak exercise test. Children with BPD had a lower GA and a higher number of days on supplemental oxygen and ventilatory support than the non-BPD children. They were shorter and weighed less than both the controls and the non-BPD participants at the time of testing. There were no differences in the proportion of obese children between groups suggesting obesity will not impact on the results.

Table 5-1 Neonatal and demographic data of children who completed a peak exercise test

	BPD (n =38)	Non-BPD (n =35)	Healthy (n =31)
Male n (%)	18 (47) *	23 (66) *	10 (32)
Gestation (w PMA)	26.0 (24.9, 28.0) ǂ	30.0 (29.0, 31.0)	-
Birth weight (g)	843 (676, 945) ǂ	1430 (1290, 1585)	-
Birth weight z-score	-0.37 (0.090)	0.03 (1.00)	-
Mechanical ventilation (d)	14.5 (3.9, 32.3) ǂ	0.0 (0.0, 0.6)	-
CPAP (d)	14.0 (6.4, 24.0) ǂ	0.3 (0.0, 1.0)	-
Supplemental O_2 (d)	85 (48.0, 103.2) ǂ	0.0 (0.0, 3.5)	-
Recent exercise symptoms	16 (42)	13 (38)	-
Asthma ever	14 (37)	15 (47)	-
Current asthma medication	3 (27)	8 (25)	-
Age at test (y)	11.0 (0.7)	11.0 (0.5)	10.7 (0.9)
Height at test (cm)	143 (7) ǂ *	145 (8)	146 (11)
Weight at test (kg)	32.0 (28.8, 37.6) ǂ *	37.2 (31.7, 41.5)	33.2 (29.9, 42.2)
BMI Centile	27.6 (14.5, 69.4)	56.2 (26.9, 88.1)	38.8 (9.3, 62.2)
Obese n (%)	1 (2)	4 (11)	0 (0)

*BPD: bronchopulmonary dysplasia; PMA: postmenstrual age; CPAP: continuous positive airway pressure; Exercise symptoms included parentally reported wheeze, cough, shortness of breath during exertion; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

Table 5-2 Lung function data of children who completed a peak exercise test

	BPD (n =38)	Non-BPD (n =35)	Healthy (n =31)
FVC z-score	-0.22 (1.11)	0.39 (0.88)	-0.06 (0.89)
FEV_1/FVC z-score	-1.24 (1.06) *	-0.87 (-0.81)	-0.28 (0.93)
TLC z-score	-0.14 (-0.87, 0.55)	-0.26 (-0.58, 0.45)	0.70 (-0.62, 0.55)
FRC z-score	0.15 (-0.65, 0.73)	0.08 (-0.63, 0.80)	0.02 (-0.38, 0.36)
RV z-score	-1.5 (-0.97, 0.36)	-0.35 (-1.26,0.38)	-0.18 (-0.92, 0.23)
FRC%TLC	52.8 (7.9)	52.0 (6.5)	51.8 (5.1)
RV%TLC	24.2 (7.1)	20.8 (7.9)	22.5 (6.1)
T_LCO z-score	-0.68 (0.79)	-0.39 (0.44)	-0.39 (0.64)
T_LCO/VA z-score	-0.31 (0.63)	-0.21 (0.73_	-1.42 (0.60)
VA z-score	-0.49 (0.90)	-0.29 (0.80)	-0.37 (0.76)
Rrs8 z-score	0.22 (1.06)	0.13 (0.22)	-0.32 (0.75)
Xrs8 z-score	-0.14 (-0.94, -0.12) *	0.14 (-0.40, 0.40)	0.35 (-0.07, 0.61)
AX z-score	0.19 (-0.53, 1.31) *	-0.33 (-0.95, 0.58)	-0.74 (-1.27, 0.00)
Fres z-score	0.73 (1.14) *	0.31 (1.61)	-0.49 (1.11)

*BPD: bronchopulmonary dysplasia; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

Children who completed a successful exercise test had better lung mechanics as measured by FOT; there were no differences in other lung function parameters compared to participants that did not complete the exercise test (see Table 5A-1 in Appendix 5A). Table 5-2 reports the lung function data of children who completed a peak exercise test. Children born very preterm with BPD had a lower FEV_1 z-score (-0.71; -1.24, -0.18; p<0.01) compared non-BPD, and a lower FEV_1/FVC z-score (-0.72; -1.64, -0.29; p<0.01) and FEV_1 z-score (-0.72; -2.24, -0.20; p<0.001) compared to controls. There were no other differences in static lung volume measurements or gas transfer measurements. Children with BPD had a

higher z-score for area under the reactance curve (0.93; 0.06, 1.79; p<0.05) and elevated resonant frequency z-score (1.23; 0.18, 2.28; p<0.05), compared to controls, indicative of worse lung mechanics. The majority (88 %) of preterm children had abnormalities identified on CT scan (see Table 5A-3 in Appendix 5A). The most common abnormalities were sub-pleural opacities (94 %), decreased pulmonary attenuation on inspiration (24 %) and peribronchial thickening (48 %). Children with BPD were more likely to have lobar collapse (10 % vs 0 %) on CT scan while there were no other differences between the preterm groups for the presence of abnormalities or the total CT score.

5.2.2 **Exercise testing data**

Preterm children with BPD had a lower absolute peak $\dot{V}O_2$ compared to non-BPD (-0.21 l/min; -0.4, -0.001; p<0.05) but no difference in absolute peak $\dot{V}O_2$ compared to controls. There were no differences between groups when peak $\dot{V}O_2$ was expressed relative to bodyweight (Table 2). The RER at peak exercise was not different between the preterm groups, but children with BPD had a lower RER compared to the controls (-0.04; -0.07, -0.01; p<0.01). Preterm children with BPD had a higher breathing frequency to VT ratio compared to non-BPD (24 %; 7, 41; p=0.002). There were no other differences in exercise outcomes between the BPD, non-BPD or controls (Table 5-3).

Table 5-3 Exercise outcomes

Exercise Outcome	BPD (n=38)	Non-BPD (n=35)	Healthy (n=31)
$\dot{V}O_2$ peak (L/min)	1.53 (1.38, 1.45) ǂ	1.79 (1.49 1.99)	1.57 (1.42, 1.99)
$\dot{V}O_2$ peak (mL/kg/min)	47.1 (6.8)	45.6 (6.9)	47.7 (5.7)
$\dot{V}O_2$ at % Peak	90 (81, 95)	81 (72, 93)	84 (73, 93)
RER at peak	1.03 (1.01, 1.07) *	1.05 (1.03, 1.09)	1.06 (1.04, 1.11)
Peak HR (bpm)	199 (9)	195 (11)	199 (10)
Oxygen pulse at peak	7.9 (7.2, 8.8) ǂ	8.9 (7.8, 10.2)	8.0 (7.1, 10.0)
Peak V_T (mL)	830 (645, 930) ǂ	1008 (831, 1145)	958 (782, 1228)
Peak f_R	63 (11) ǂ	56 (8)	59 (9)
f_R: V_T peak	76 (58.1, 111.3) ǂ	53 (43.8, 69.8)	63 (44.7, 86.8)
Peak V_E (L/min)	52.3 (9.7)	56.0 (11.8)	57.3 (11.2)
$\dot{V}_E/\dot{V}CO_2$ slope	29.5 (26.0, 31.9)	27.7 (26.1, 29.3)	28.8 (28.2, 30.9)
$\dot{V}_E/\dot{V}O_2$ slope	27.8 (25.5, 30.0)	25.9 (23.5, 27.9)	27.0 (21.0, 30.9)
Breathing reserve (%)	30.4 (22.1, 37.0)	37.1 (22.5, 42.3)	33.7 (26.7, 40.1)
Heart rate reserve (bpm)	10 (9)	14 (11)	10 (10)

*AT: Anaerobic threshold; V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio; * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

Table 5-4 reports the independent predictors of peak $\dot{V}O_2$ and the ventilatory responses derived from the multivariate regression analysis. After accounting for height, weight and gender there were no associations between neonatal factors, structural abnormalities on CT or lung function for $\dot{V}O_2$, V_T or breathing frequency at peak exercise. Minute ventilation at peak exercise, $\dot{V}_E/\dot{V}CO_2$ and $\dot{V}_E/\dot{V}O_2$ slopes were inversely related to T_LCO/V_A z-score. Breathing frequency to V_T ratio was inversely related to birth weight z-score.

Table 5-4 Multivariate analysis

	Beta	95% CI	P	R^2
$\dot{V}O_2$ (L/min)				
Height (cm)	0.246*	0.003, 0.017	0.018	0.689
Weight (Kg)	0.662*	0.014, 0.024	<0.001	
Peak minute ventilation (L)				
Height (cm)	0.24*	0.00, 0.91	0.002	0.380
Weight (kg)	2.33*	0.04, 0.55	0.03	
Peak tidal volume (L)				
Height (cm)	0.289*	-0.14, 0.846	0.058	
Weight (Kg)	0.449*	0.125, 0.638	0.005	0.38
T_LCO/V_A (z-score)	-0.249*	- 7.926, -0.022	0.049	
Peak breathing frequency (breaths/min)				
Weight (kg)	-0.464*	-0.627, -0.230	<0.001	0.2
$\dot{V}_E/\dot{V}O_2$ Slope				
T_LCO/V_A (z-score)	-0.38*	-3.4.52, -0.62	0.011	0.124
$\dot{V}_E/\dot{V}CO_2$ Slope				
T_LCO/V_A (z-score)	-0.457*	-23.319, -0.813	0.002	0.190
Breathing frequency: tidal volume				
Weight (kg)	-0.535*	- 1.930, -0.884	<0.001	0.353
Birth weight (z-score)	-0.238*	- 14.519, - 1.297	0.02	

95 %CI, 95 % confidence interval; PMA, postmenstrual age; T_LCO: transfer factor of the lung for carbon monoxide; V_A: alveolar volume $\dot{V}_E$: minute ventilation. *p<0.05

5.3 **Discussion**

This is the first study to assess the impact of neonatal factors, and the interrelationship between lung function and structural lung disease on the aerobic exercise capacity of school-aged children born very preterm in the surfactant era. We observed that while most preterm children have structural lung abnormalities, the presence of these abnormalities does not impact their aerobic capacity or ventilatory response to exercise at school age. We also observed that there was no relationship between either respiratory function or neonatal factors and exercise capacity in school aged children born very preterm. Preterm children had a slightly lower aerobic exercise capacity than children born at full-term; however, no difference was evident when $\dot{V}O_2$ peak was expressed relative to bodyweight.

The observation that preterm children with BPD had a higher breathing frequency to V_T ratio compared to preterm children without BPD is consistent with recently published data showing children born preterm exhibit a rapid shallow breathing pattern (39, 47, 68). The observed inverse association between the breathing frequency to V_T ratio and birth weight z-score suggests there may be a plausible additive effect of intrauterine growth restriction on prematurity for breathing patterns in school-aged children. The association of breathing frequency to V_T ratio with birth weight z-score but not ventilatory support suggests that prematurity, rather than lung damage may underlie these differences.

While the majority of preterm children had structural abnormalities on CT, we found no association between structural abnormalities and exercise outcomes. This finding contrasts with the recent observation that structural abnormalities observed on CT in children with cystic fibrosis (CF) were associated with impaired outcomes on a peak exercise test (95). A key difference between these two studies is that the children with BPD in the current study did not demonstrate significant bronchiectasis or lung collapse, while both bronchiectasis and lung collapse were associated with impaired exercise capacity in CF (95). The most common CT abnormality in the current preterm cohort were subpleural opacities; these opacities were present in almost all preterm children, and the presence of peribronchial

thickening present in nearly half of the preterm children and may be an indicator of ongoing airway inflammation rather than changes in airway function. Neither of these structural changes contributed to impaired exercise response in preterm children. The total CT score, an indicator of the extent of structural lung disease was not associated with any changes in the response to exercise in this preterm population. This finding is also in contrast to a CF population, in whom exercise capacity and total CT score are correlated (95). Since the completion of this study the role of spirometry guided CT scans have been reported to improve the ability to identify structural abnormalities in children with lung disease (96). Our study used the reporting system described by Auckland et al (31) and utilities that methodology. Given the high incidence of structural abnormalities we are confident that we gained reliable insights into the structural changes seen in a preterm population.

The observed abnormalities in pulmonary structure and function in children born preterm did not translate to a difference in peak $\dot{V}O_2$ expressed relative to body weight, compared to healthy age-matched controls. This finding is not surprising given the relatively mild impairment in pulmonary structure that was present. The respiratory system does not limit aerobic exercise performance in healthy children, due to a large respiratory reserve that typically exceeds 30% (97, 98). The physiological limit to maximal aerobic exercise in healthy individuals is predominantly cardiovascular in nature, such as an inability to increase cardiac output, due to attainment of maximal heart rate and/or a plateau in stroke volume (99, 100) . The lack of association between structural lung changes or lung function and aerobic capacity in preterm participants in the present study supports this premise, despite our previous observations that structural lung abnormalities were associated with impaired lung function and more severe respiratory symptoms (8). In adults with chronic diseases (i.e. heart failure or chronic obstructive pulmonary disease), aerobic capacity is also limited by the ability to extract and utilize oxygen at the cellular level. Such limitation in cellular oxygen extraction and utilization is due to impairments in peripheral blood flow (101) and skeletal muscle oxidative metabolism (102) that develop as sequelae of the underlying disease. We did not directly assess whether

peripheral physiological maladaptation's are present in school-age children born preterm in the current study, but the lack of difference in peak $\dot{V}O_2$ between groups would suggest not. Future research is warranted to investigate whether peripheral maladaptation's develop in adulthood and impact on aerobic capacity later in life.

Previous studies report conflicting findings regarding the aerobic capacity of children born in the surfactant era, with some studies showing a lower peak $\dot{V}O_2$ compared with age-matched controls (39, 42, 47) while others have shown no difference (44, 56, 67, 68). Comparisons of these studies is difficult due to the variability in the definition of preterm birth, differences in neonatal care and the different exercise modalities employed during testing, which include cycle ergometers (39, 47), treadmills with varying protocols (44), or shuttle runs (42). Generally, treadmill exercise test studies report no difference in aerobic capacity, while shuttle runs and studies involving cycle ergometer show lower peak $\dot{V}O_2$ in the preterm population. Advantages of a treadmill exercise test include the similarity to everyday activity and recruitment of a larger muscle mass to achieve peak $\dot{V}O_2$ compared to a cycle ergometer. Peak exercise capacity during cycle ergometry is more commonly limited by lower limb muscle fatigue than treadmill exercise (103). It has been reported that preterm children have reduced lower body strength (quadriceps strength) (104), compared with matched controls. This may translate to an impaired ability to continue cycling at higher workloads leading to a lower measured peak $\dot{V}O_2$ during cycle ergometry and may explain the difference in findings between studies that have used a cycling versus treadmill protocol.

Almost half the children assessed in the current study had parentally reported respiratory symptoms on exertion; it is intriguing that this finding did not translate to a reduction in aerobic capacity compared to age-matched healthy children. The number of preterm children using current asthma medication was low despite an increased prevalence of parentally reported symptoms. The low utilization of asthma medications suggests that these parentally reported respiratory symptoms were not sufficient to require medical therapy. Long term outcomes of these respiratory symptoms and structural changes

on exercise capacity are poorly understood at present. While Clemm *et al* (71) showed a normal trajectory of peak $\dot{V}O_2$ in preterm children after a 6 year period, the children in that study did not report symptoms and had an FEV_1 > 80 % predicted throughout the study, which may mask changes in children with more significant respiratory morbidity.

A limitation of the current study is the exclusion of children who were unable to perform a peak treadmill exercise test, therefore eliminating those with gait issues or cerebral palsy. This limitation may bias our results to a relatively healthy subset of the population and blunt any potential differences. We also excluded approximately a third of participants who underwent the peak exercise test but were excluded from the peak $\dot{V}O_2$ analysis because they were unable to achieve the study's criteria for a peak exercise test.

5.4 **Conclusion**

While children born preterm have significantly impaired lung function, and a high prevalence of structural lung abnormalities, these abnormalities did not impact on the aerobic exercise capacity at school age, in a cohort of children born preterm. Future studies are warranted to determine whether the observed abnormalities in lung structure and function manifest in an impairment in cardiorespiratory fitness at later stages in life.

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Appendix 5A

Table 5A-1 Demographic and neonatal outcomes for subjects who performed a successful peak exercise test and those that did not

	Successful (n =104)	Unsuccessful (n =117)
Preterm, n (%)	73 (70 %)	90 (77 %)
Male n (%)	51 (49 %)*	75 (64 %)
Gestational age (PMA)	28.3 (26.0, 30.0)	27.0 (25.0, 29.1)
Birth weight (g)	1050 (813, 1420)	905 (750, 1195)
Birth weight Z-Score	-0.18 (0.97)	-0.10 (0.87)
Mechanical ventilation (days)	2.0 (0.0, 21.0)	6.9 (1.0, 32.2)
CPAP (days)	5.2 (0.4, 14.5)	6.9 (1.0, 19.2)
Supplemental O_2 (days)	30.0 (0, 87.5)	65.5 (4.8, 95.0)
Recent exercise symptoms	29 (37 %)*	47 (57 %)
Asthma ever	28 (36)	39 (43)
Current asthma medication	19 (21)	11(14)
Age at test (y)	10.9 (0.7)	10.8 (0.7)
Height at test (cm)	144 (9)	142 (9)
Weight at test (kg)	33.7 (30.1, 40.9)	34.4 (29.0, 40.8)

**p<0.05; BPD: bronchopulmonary dysplasia. PMA: postmenstrual age CPAP: continuous positive airway pressure. Exercise symptoms included parentally reported wheeze, cough. Shortness of breath during exertion. Data presented as mean (SD), median (IQR) or number (percent).*

Table 5A-2 Lung function outcomes for subjects who performed successful peak exercise test and those that did not

	Successful (n =104)	Unsuccessful (n =117)
FEV_1 z-score	-0.4 (0.9)	-0.6 (1.3)
FVC z-Score	0.09 (0.99)	0.19 (1.05)
FEV_1/FVC z-score	-0.83 (1.07)	-1.17 (1.05)*
TLC z-score	-0.15 (-0.6, 0.48)	-0.24 (-0.78, 0.51)
FRC z-score	0.06 (-0.54, 0.70)	0.17 (-0.48, 0.93)
RV z-score	-0.23 (-1.02, 0.31)	-0.33 (-1.04, 0.46)
FRC%TLC	52 (7)	52 (8)
RV%TLC	23 (7)	22 (7)
T_LCO z-score	-0.47 (0.77)	-0.47 (0.73)
T_LCO/V_A z-score	-0.21 (0.66)	-0.26 (0.73)
VA z-score	-0.37 (0.81)	-0.31 (0.86)
Rrs8 z-score	0.06 (0.99)	0.44 (1.02)*
Xrs8 z-score	0.07 (-0.95, 0.24)	-0.23 (-0.55, 0.43)*
AX z-score	-0.33 (-0.92, 0.65)	0.25 (-0.64, 1.16)*
Fres z-score	0.29 (1.41)	0.66 (1.22)

**p<0.05; BPD: bronchopulmonary dysplasia, Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz, AX: area under the reactance curve, Fres: resonant frequency Data, T_LCO: transfer factor of the lung for carbon monoxide, V_A: Alveolar volume, presented as mean (SD) or median (IQR).*

Table 5A-3 Presence of structural changes on computed tomography scan of the chest for children who completed a peak exercise test

	BPD (n=30)	Non-BPD (n=28)
Presence Liner and triangular subplueral opacities	28 (93)	21 (75)
Presence Decreased pulmonary attenuation on inspiration	8 (26)	5 (18)
Presence Decreased pulmonary attenuation on expiration	11 (37)	13 (46)
Presence of Decreased bronchial arterial ratio	0 (0)	1 (4)
Presence Bronchiectasis	3 (10)	2 (7)
Presence Peribronchial thickening	15 (50)	13 (46)
Presence of Bullae	0 (0)	0 (0)
Presence of Emphysema	0 (0)	0 (0)
Presence of Lobar collapse	3 (10)	0 (0)

**p<0.05. all result report n(%), BPD= Bronchopulmonary dysplasia*

Table 5A-4 Univariate analysis of peak $\dot{V}O_2$

Peak $\dot{V}O_2$	Beta	95% CI	p
Gestation (PMA)	0.228	0.000, 0.058	0.052
Supplemental oxygen (d)	-0.366	-0.004, -0.001	0.001*
Mechanical Ventilation (d)	-0.295	-0.009, -0.001	0.011*
Birth weight z-score	0.372	0.052, 0.200	0.001*
CPAP (d)	-0.208	-0.012, 0.001	0.078
Age (y)	0.422	0.109, 0.334	0*
Height (cm)	0.656	0.020, 0.035	0*
Weight (kg)	0.815	0.020, 0.028	0*
Sex (female)	-0.236	0.305 -0.004	0.044*
FEV1 z-score	0.28	0.006, 0.194	0.038*
FVC z-score	0.193	-0.024, 0.141	0.163
FEV1/FVC z-score	0.065	-0.063, 0.102	0.64
FRC z-score	-0.2	-0.111, 0.010	0.102
TLC z-score	-0.167	-0.146, 0.027	0.173
RV z-score	-0.376	-0.163, -0.040	0.002*
T_LCO z-score	-0.071	-0.152, 0.095	0.645
T_LCO/VA z-score	-0.034	-0.164, 0.131	0.826
RV%TLC	-0.3	-0.025, 0.005	0.004*
FRC%TLC	-0.385	-0.036, -0.008	0.002*
Rrs8 z-score	-0.279	-0.172, -0.009	0.031*
Xrs8 z-score	0.247	-0.003, 0.206	0.057
AX z-score	-0.259	-0.145-0.001	0.046*
Fres z-score	-0.079	-0.075, 0.038	0.51
Presence Liner and triangular subplueral opacities	0.228	0.000, 0.058	0.052
Presence Decreased pulmonary attenuation on inspiration	-0.366	-0.004, -0.001	0.001*
Presence Decreased pulmonary attenuation on expiration	-0.295	-0.009, -0.001	0.011*
Presence Decreased bronchial arterial ratio	0.372	0.052, 0.200	0.001*
Presence Bronchiectasis	-0.208	-0.012, 0.001	0.078
Presence Peribronchial thickening	0.422	0.109, 0.334	0*
Total CT Score	0.656	0.020, 0.035	0*

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: Respiratory system resistance at 8hz, Xrs8: Respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-5 Univariate analysis of peak minute ventilation

Peak minute ventilation	Beta	95% CI	p
Gestation (PMA)	0.154	-0.342, 1.643	0.196
Supplemental oxygen (d)	-0.245	-0.114, -0.003	0.038*
Mechanical Ventilation (d)	-0.203	-0.253, 0.018	0.088
Birth weight z-score	0.241	0.112,5.294	0.041*
CPAP (d)	-0.184	-0.364, 0.443	0.121
Age (y)	0.476	4.643, 11.935	0*
Height (cm)	0.644	0.642. 1.155	0*
Weight (kg)	0.603	0.396, 0.761	0*
Sex (female)	-0.102	- 7.388, 2.949	0.395
FEV1 z-score	0.171	-0.750, 5.095	0.143
FVC z-score	0.157	-0.900, 4.436	0.191
FEV1/FVC z-score	0.074	- 2.184, 3.750	0.598
FRC z-score	-0.207	- 3.695, 0.287	0.092
TLC z-score	-0.215	- 5.292, 0.308	0.08
RV z-score	-0.386	- 5.458, -1.378	0.001*
T_LCO z-score	-0.313	- 8.057, -0.221	0.039*
T_LCO/VA z-score	-0.183	- 7.727, 1.944	0.234
RV%TLC	-0.273	-0.732, -0.103	0.01*
FRC%TLC	-0.527	-0.870, -0.001	0.05
Rrs8 z-score	-0.325	- 6.142, -0.815	0.011*
Xrs8 z-score	0.252	-0.033, 6.894	0.052
AX z-score	-0.25	- 4.722, 0.043	0.054
Fres z-score	-0.22	- 3.709, 0.282	0.091
Presence Liner and triangular subplueral opacities	0.072	- 6.643, 11.148	0.614
Presence Decreased pulmonary attenuation on inspiration	0.105	- 4.561, 10.080	0.453
Presence Decreased pulmonary attenuation on expiration	0.291	0.729, 12.431	0.028*
Presence Decreased bronchial arterial ratio	-0.246	- 40.704, 2.269	0.078*
Presence Bronchiectasis	0.159	- 4.431, 15.963	0.261
Presence Peribronchial thickening	0.216	- 1.319 10.573	0.124
Total CT Score	0.143	-0.303, 0.929	0.312

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-6 Univariate analysis of peak tidal volume

Peak tidal volume	Beta	95% CI	p
Gestation (PMA)	0.248	0.002, 0.050	0.034*
Supplemental oxygen (d)	-0.375	-0.004, -0.001	0.001*
Mechanical Ventilation (d)	-0.297	-0.008, -0.001	0.011*
Birth weight z-score	0.38	0.046, 0.168	0.001*
CPAP (d)	-0.258	-0.011, -0.001	0.028*
Age (y)	0.483	0.119, 0.299	0*
Height (cm)	0.672	0.017, 0.029	0*
Weight (kg)	0.798	0.016, 0.023	0*
Sex (female)	-0.089	-0.175, 0.079	0.453
FEV1 z-score	0.265	0.00, 0.153	0.05
FVC z-score	0.166	-0.027, 0.108	0.232
FEV1/FVC z-score	0.074	-0.049, 0.084	0.596
FRC z-score	-0.207	-0.092, 0.007	0.09
TLC z-score	-0.166	-0.119, 0.022	0.175
RV z-score	-0.407	-0.141, -0.040	0.001*
T_LCO z-score	-0.054	-0.129, 0.091	0.727
T_LCO/VA z-score	0.042	-0.144, 0.149	0.787
RV%TLC	-0.338	-0.021, -0.005	0.001*
FRC%TLC	-0.261	-0.023, 0.000	0.044*
Rrs8 z-score	-0.178	-0.117, 0.022	0.174
Xrs8 z-score	0.25	-0.001, 0.172	0.054
AX z-score	-0.233	-0.115, 0.005	0.073
Fres z-score	-0.223	-0.094, 0.006	0.086
Presence Liner and triangular subplueral opacities	-0.046	-0.225, 0.158	0.731
Presence Decreased pulmonary attenuation on inspiration	0.077	-0.121, 0.213	0.581
Presence Decreased pulmonary attenuation on expiration	0.225	-0.025, 0.256	0.105
Presence Decreased bronchial arterial ratio	0.249	-0.005, 0.268	0.059
Presence Bronchiectasis	0.132	-0.127, 0.355	0.345
Presence Peribronchial thickening	0.1	-0.091, 0.192	0.476
Total CT Score	-0.052	-0.017, 0.011	0.698

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-7 Univariate analysis of peak respiratory frequency

Peak respiratory frequency	Beta	95% CI	p
Gestation (PMA)	-0.248	-1.969, -0.079	0.034*
Supplemental oxygen (d)	0.37	0.035, 0.138	0.001*
Mechanical Ventilation (d)	0.283	0.032, 0.289	0.015*
Birth weight z-score	-0.339	-6.150, 1.273	0.003*
CPAP (d)	0.218	-0.011, 0.382	0.064
Age (y)	-0.22	-7.677, 0.149	0.059
Height (cm)	-0.31	-0.725, -0.112	0.008*
Weight (kg)	-0.466	-0.635, -0.241	0*
Sex (female)	0.048	-3.977, 6.023	0.685
FEV1 z-score	-0.113	-4.631, 1.931	0.413
FVC z-score	-0.088	-3.741, 1.931	0.525
FEV1/FVC z-score	0.008	-2.714, 2.881	0.952
FRC z-score	0.096	-1.202, 2.753	0.437
TLC z-score	0.035	-2.409, 3.217	0.775
RV z-score	0.194	-0.425, 3.809	0.115
T_LCO z-score	-0.188	-6.401, 1.526	0.221
T_LCO/VA z-score	-0.219	-8.076, 1.316	0.154
RV%TLC	0.225	0.026, 0.620	0.034*
FRC%TLC	0.145	-0.184, 0.647	0.269
Rrs8 z-score	-0.102	-3.484, 1.394	0.396
Xrs8 z-score	-0.124	-4.676, 1.472	0.302
AX z-score	0.096	-1.261, 2.964	0.424
Fres z-score	0.096	-1.041, 2.429	0.428
Presence Liner and triangular subplueral opacities	0.076	-6.333, 11.079	0.587
Presence Decreased pulmonary attenuation on inspiration	-0.036	-7.862, 6.076	0.798
Presence Decreased pulmonary attenuation on expiration	-0.029	-6.620, 5.374	0.836
Presence decreased bronchial arterial ratio	-0.248	-40.186, 1.917	0.074
Presence Bronchiectasis	-0.03	-11.184, 9.03	0.832
Presence Peribronchial thickening	0.022	-5.451, 6.374	0.876
Total CT Score	0.172	-0.219, 0.939	0.218

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-8 Univariate analysis of breathing frequency to tidal volume ratio at peak exercise

Breathing frequency to tidal volume ratio at peak	Beta	95% CI	p
Gestation (PMA)	-0.323	-6.379, - 1.014	0.008*
Supplemental oxygen (d)	0.481	0.173, 0.459	0*
Mechanical Ventilation (d)	0.38	0.241, 0.972	0.001*
Birth weight z-score	-0.389	-20.497, 5.337	0.001*
CPAP (d)	0.29	0.124, 1.243	0.17
Age (y)	-0.378	-29.823, -7.281	0.002*
Height (cm)	-0.497	-2.737, -1.075	0*
Weight (kg)	-0.602	-2.102, -1.063	0*
Sex (female)	0.112	-8.142, 21.677	0.368
FEV1 z-score	-0.204	-17.654, 2.914	0.156
FVC z-score	-0.144	-12.765, 4.318	0.325
FEV1/FVC z-score	-0.014	-9.146, 8.29	0.923
FRC z-score	0.153	-2.287, 9.160	0.234
TLC z-score	0.134	-3.907, 12.559	0.297
RV z-score	0.316	1.674, 13.736	0.013*
T_LCO z-score	-0.007	-7.519, 7.45	0.96
T_LCO/VA z-score	-0.075	-16.778, 10.376	0.636
RV%TLC	0.281	0.289, 2.107	0.01*
FRC%TLC	0.154	-0.561, 2.027	0.261
Rrs8 z-score	-0.053	-8.619. 5.615	0.675
Xrs8 z-score	-0.137	-14.262, 4.108	0.274
AX z-score	0.104	-3.648, 8.895	0.406
Fres z-score	0.085	-3.354, 6.835	0.497
Presence Liner and triangular subplueral opacities	0.117	-18.033, 41.250	0.434
Presence Decreased pulmonary attenuation on inspiration	-0.056	-26.301, 17.958	0.706
Presence Decreased pulmonary attenuation on expiration	-0.71	-29.171, 7.777	0.25
Presence Decreased bronchial arterial ratio	-0.114	-87.596, 39.110	0.445
Presence Bronchiectasis	-0.096	-39.263, 20.152	0.52
Presence Peribronchial thickening	-0.058	-21.9939, 14.816	0.698
Total CT Score	0.125	-1.150, 2.819	0.402

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-9 Univariate analysis of $V_E/\dot{V}O_2$ slope

$V_E/\dot{V}O_2$ slope	Beta	95% CI	p
Gestation (PMA)	-0.007	-0.408, 0.383	0.952
Supplemental oxygen (d)	0.119	-0.011, 0.34	0.324
Mechanical Ventilation (d)	-0.001	-0.054, 0.054	0.992
Birth weight z-score	-0.196	-1.902, .176	0.102
CPAP (d)	0.012	-0.077, 0.085	0.922
Age (y)	-0.167	-2.776, 0.476	0.163
Height (cm)	-0.262	-0.274, 0.016	0.028*
Weight (kg)	-0.381	-0.224, -0.059	0.001*
Sex (female)	-0.056	-2.517, 1.570	0.645
FEV1 z-score	-0.016	-1.424, 1.267	0.908
FVC z-score	-0.004	-1.210, 1.179	0.979
FEV1/FVC z-score	0.019	-1.153, 1.318	0.894
FRC z-score	0.135	-0.361, 1.232	0.279
TLC z-score	0.041	-0.952, 1.331	0.741
RV z-score	0.143	-0.367, 1.348	0.257
T_LCO z-score	-0.352	-3.649, -0.343	0.019*
T_LCO/VA z-score	-0.38	-4.520, .624	0.011*
RV%TLC	0.18	-0.023, 0.235	0.106
FRC%TLC	0.135	-0.087, 0.273	0.307
Rrs8 z-score	-0.17	- 1.793, 0.386	0.201
Xrs8 z-score	0.065	-1.06, 1.741	0.628
AX z-score	0	-1.218, 0.70	0.6
Fres z-score	-0.034	-0.906, 0.700	0.799
Presence Liner and triangular subplueral opacities	-0.046	-4385, 3.158	0.745
Presence Decreased pulmonary attenuation on inspiration	0.016	-2.876, 3.222	0.91
Presence Decreased pulmonary attenuation on expiration	-0.008	-2.682, 2.535	0.955
Presence Decreased bronchial arterial ratio	-0.019	-10.001, 8.763	0.895
Presence Bronchiectasis	-0.181	-7.091, 1.507	0.198
Presence Peribronchial thickening	0.099	-1.671, 3.459	0.487
Total CT Score	0.035	-0.223, 0.285	0.806

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-10 Univariate analysis of $V_E/\dot{V}CO_2$ slope

V_E/VCO_2 slope	Beta	95% CI	p
Gestation (PMA)	-0.114	-0.433, 0.153	0.344
Supplemental oxygen (d)	0.162	-0005, 0.028	0.177
Mechanical Ventilation (d)	0.106	-0.022, 0.058	0.38
Birth weight z-score	-0.147	-1.264, 0.299	0.222
CPAP (d)	0.065	-0.044, 0.077	0.588
Age (y)	-0.032	-1.391, 1.068	0.794
Height (cm)	0	-0.120, 0.079	0.679
Weight (kg)	-0.25	-0.134, -0.005	0.036
Sex (female)	0.013	-1.446, 1.607	0.917
FEV1 z-score	0.046	-0.807, 1.123	0.743
FVC z-score	-0.044	-0.986, 0.723	0.758
FEV1/FVC z-score	0.104	-0.555, 1204	0.463
FRC z-score	0.049	-0.646, 0.690	0.697
TLC z-score	-0.013	-0.863, 0.780	0.919
RV z-score	0.035	-0.533, 0.706	0.781
T_LCO z-score	-0.405	-2.616, -0.458	0.006*
T_LCO/VA z-score	-0.457	-3.319, -0.813	0.002*
RV%TLC	0.023	-0.091, 0.112	0.837
FRC%TLC	0.093	-0.085, 0.179	0.482
Rrs8 z-score	-0.19	-1.377, 0.221	0.153
Xrs8 z-score	0.031	-0.912, 1.152	0.817
AX z-score	-0.027	-0.784, 0.638	0.362
Fres z-score	0.017	-0.554, 0.629	0.9
Presence Liner and subplueral opacities	0.138	-1.408, 4.099	0.331
Presence Decreased pulmonary attenuation on inspiration	0.083	-2.891, 1.574	0.556
Presence Decreased pulmonary attenuation on expiration	-0.075	-2.309, 1.302	0.578
Presence of Decreased bronchial arterial ratio	-0.242	-12.582, 0.827	0.084
Presence Bronchiectasis	0.035	-2.819, 3.614	0.805
Presence Peribronchial thickening	-0.01	-1.965, 1.831	0.944
Total CT Score	0.097	-0.132, 0.250	0.495

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; *p<0.05*

Table 5A-11 Co-author attribution statement

	Conception and Design	Acquisition of Data	Data Analysis	Interpretation and Discussion	Final Approval
Dr Karla Logie		X	X		
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Wilson	X		X	X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Prof Jane Pillow	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Conor Murray	X		X	X	
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Georgia Banton		X			
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Dr Shannon Simpson		X	X	X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Prof Graham Hall	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Maiorana	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					

Chapter 6 Early oxygen uptake recovery in school children born very preterm

6.1 Introduction

At peak exercise anaerobic glycolysis is the primary process for energy production required for the contracting muscles (8). Following exercise, this process ceases and oxidative phosphorylation resynthesises phosphocreatine to restore muscular ATP, an important fuel source for anaerobic metabolism (9). This process relies on oxygen transport and utilization by contracting skeletal muscles, including the respiratory muscles (9, 10) and is associated with excess post-exercise oxygen consumption (EPOC). During early recovery the rate of phosphocreatine resynthesis is inversely proportional to the duration of $\dot{V}O_2$ recovery (9). In adult patients with congestive heart failure, impaired respiratory muscle strength and endurance contribute to dyspnoea and are associated with a delay in early recovery oxygen kinetics (10). Recovery of $\dot{V}O_2$ debt following exercise in children with chronic chest diseases, such as cystic fibrosis and bronchiectasis, is delayed but this is not associated with other lung function outcomes (11).

We hypothesized that children born preterm will demonstrate a delay in $\dot{V}O_2$ recovery following peak exercise that will be related to exertional dyspnoea and EFL.

6.2 Methods

See Chapter 2 Methods for full methodological details

6.2.1 **Pulmonary function testing**

Spirometry, lung volume measurements by multiple breath nitrogen washout, gas transfer capacity (T_LCO) (Sensormedics Encore 21-1A, Yorba Linda, CA) and the Forced oscillation technique (FOT) (i2M Chess, Ghent, Belgium) were performed according to international guidelines (24, 26) and are reported as predicted z-scores (21, 25, 27, 29) with the exception of FRC/TLC and RV/TLC.

6.2.2 **Chest computed tomography**

Computed tomography (CT) scans of the chest were performed during inspiration and expiration in children born preterm, as previously described (Philips Brilliance 64; Philips Medical Systems, Netherlands) (8, 49), but were not performed in controls. The scans were consensus scored by a specialist paediatric thoracic radiologist and a paediatric respiratory physician using the scoring system described by Aukland *et al (31)*. Scorers were blinded to neonatal classification of lung disease.

6.2.3 **Peak exercise test**

Participants performed an incremental treadmill exercise test (Marquette, Sensormedics, Yorba Linda, CA) employing a Modified Balke protocol (10, 86). A peak exercise test was defined as physical signs of peak performance (inability to maintain running speed) and peak respiratory exchange ratio (RER) ≥ 1.0. Peak metabolic ($\dot{V}O_2$, $\dot{V}CO_2$) and ventilatory data (tidal volume (V_T) and breathing frequency) were recorded continuously via a mask using breath-by-breath analysis (SensorMedics 229 Metabolic Cart, SensorMedics, Yorba Linda, CA).

6.2.4 **Recovery**

Following peak exercise the treadmill was rapidly slowed to a moderate walking pace based on the participant's height and ability. Following 30 seconds of slowing the treadmill came to a complete stop and the participants remained standing. Children were asked to remain standing to minimise any further movement following exercise and to enable standardisation of the level of movement in early recovery. Early $\dot{V}O_2$ recovery was set as the time in seconds from exercise cessation to when $\dot{V}O_2$ returned to 50 % of peak, as previously described (105). Additionally, $\dot{V}O_2$ was assessed at 1 min and 2 min after exercise cessation and expressed as a percentage of peak $\dot{V}O_2$. Early $\dot{V}O_2$ recovery was assessed to ensure the measurement reflected the alactic phase of oxygen debt repayment (106).

6.2.5 **Statistical analysis**

Hierarchal linear regression models were created to assess the influence of lung function, structural lung disease and neonatal data on early $\dot{V}O_2$ recovery. Multi-collinearities between the neonatal predictors were identified and adjusted for by using residuals of independent regressions of the collinear variables. For example, the independent impact of MV on $\dot{V}O_2$ recovery was determined from the residual of the regression between GA and MV. The effect of development (GA and birth weight z-score), neonatal lung disease (days of supplemental oxygen and ventilatory support), current demographics (age, height, weight and sex), the presence and extent of structural lung abnormalities, lung function at the time of the exercise test and peak exercise outcomes ($\dot{V}O_2$, HR, extent of EFL) were included in the model building process. A p-value less than 0.05 was considered significant. Sample size was calculated on the ability to detect a mean difference in $\dot{V}O_2$ recover at 1 min of 15 % between healthy term born controls and preterm children with BPD assuming a standard deviation of 7 %. Assuming 80 % power and a 5 % significance level we estimated a sample size of each group n=41. Statistical analysis was performed using SPSS Version 22.0 (SPSS inc, Chicago IL).

6.3 **Results**

6.3.1 **Participants**

Two hundred and twenty-one (126 male) children, (99 BPD, 64 non-BPD and 58 healthy term controls) were enrolled. The cohort is representative of the very preterm population in Perth during the same birth period, with no differences in neonatal characteristics of the recruited cohort, as reported previously (8, 49). One hundred and ninety-eight participants attempted the exercise test. Reasons for non-attempt were equipment issues (8), physical or mental impairment (7), poor baseline lung function that was deemed a contraindication to exercise testing (3), or no consent or non-attendance for the exercise testing assessment (5).

One hundred and four (51 male) children, (38 BPD, 35 non-BPD and 31 healthy term controls) completed a valid peak exercise test. Exercise tests were determined as being invalid due to a leak in the mask during testing (12), equipment malfunction (4), early termination of exercise (RER <1.0) (73) and physical inability to perform a maximal exercise test (5). Ninety-four participants (47 male) had recovery data recorded for at least 1 min following exercise cessation; the remaining children were unable to tolerate the mask once exercise was terminated. These data form the basis of all analysis in this study. Children that did not complete the recovery data analysis had a lower gestational age, increased ventilatory support during the neonatal period and longer duration of supplemental oxygen use (see Table 6A-1 located in appendix 6A). They were more likely to have parentally reported respiratory symptoms (see Table 6A-1 located in appendix 6A) and worse respiratory mechanics as measured by the forced oscillation technique (see Table 6A-2 located in appendix 6A). In children who performed a peak exercise there were no differences in peak exercise outcomes between those who had recovery data and those who did not (see Table 6A-3 located in appendix 6A). Table 6-1 shows the demographic and neonatal data in the children that completed a maximal exercise test and subsequent recovery analysis. Preterm children with a neonatal classification BPD had a lower GA and had greater duration of supplemental oxygen use and ventilatory support.

Table 6-1 Neonatal and demographic data of children who completed recovery assessment

	BPD (n =33)	Non-BPD (n =34)	Healthy (n =27)
Male	16 (48)	22 (64)	9 (33)
Gestation (w PMA)	26.6 (2.1) ǂ	29.9 (1.3)	-
Birth weight (g)	867 (192) ǂ	1433 (281)	-
Birth weight z-score	-0.31 (0.88)	0.04 (1.01)	-
Mechanical ventilation (days)	10 (4, 32) ǂ	0 (0,1)	-
CPAP (days)	14.0 (6.3, 24.0) ǂ	0.4 (0.0, 3.5)	-
Supplemental O_2 (days)	77 (48, 97) ǂ	0 (0,3)	-
Recent exercise symptoms	13 (39)	13 (39)	-
Asthma ever	12 (36)	14 (45)	-
Current asthma medication	3 (10)	8 (25)	-
Age at test (y)	11.1 (10.4, 11.7)	11.0 (10.7, 11.8)	10.8 (9.8, 11.5)
Height at test (cm)	144 (7.1)	144 (7.8)	144 (9.7)
Weight at test (kg)	33.1 (28.9, 37.7)	37.0 (31.7, 41.2)	32.9 (29.9)

*BPD: bronchopulmonary dysplasia; PMA: postmenstrual age; CPAP: continuous positive airway pressure; Exercise symptoms included parentally reported wheeze, cough, shortness of breath during exertion; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

Children with BPD had a lower FEV_1/FVC z-score and worse respiratory mechanics as measured by FOT compared to term controls (Table 6-2). Preterm children had a normal peak $\dot{V}O_2$ compared to term controls (Table 6-3), although preterm children with BPD had a slightly lower RER compared to term controls. Children with BPD were significantly more likely to exhibit EFL and have more severe EFL as a percentage of tidal volume during exercise than term controls and preterm children without BPD. There were no other alterations in peak exercise outcomes.

Table 6-2 Lung function data of children who completed recovery assessment

	BPD (n =33)	Non-BPD (n =34)	Healthy (n =27)
FEV_1 z-score	-0.81(0.78) ‡*	-0.20 (0.86)	-0.02 (0.82)
FVC z-score	0.14 (1.07)	0.40 (0.90)	-0.12 (0.91)
FEV_1/FVC z-score	-1.35 (-2.14, -0.76) *	-0.82 (-1.50, -0.44)	-0.78 (-1.05, 0.59)
TLC z-score	-0.20 (-0.9, 0.77)	-0.25 (-0.58, 0.45)	0.11 (-0.44,0.60)
FRC z-score	0.23 (-0.66, 0.95)	0.2 (0.54, 1.18)	0.07 (-0.23, 0.36)
RV z-score	-0.17 (-1.01, 0.64)	-0.33 (-1.18, 0.39)	-0.12 (-0.77,0.29)
FRC%TLC	53 (8)	52 (7)	53 (5)
RV%TLC	24 (7)	21 (8)	23 (6)
T_LCO z-score	-0.74 (0.77)	-0.35 (0.82)	-0.38 (0.66)
T_LCO/VA z-score	-0.36 (0.61)	-0.20 (0.75)	-0.07 (0.60)
VA z-score	-0.51 (0.93)	-0.24 (0.78)	-0.44 (0.78)
Rrs8 z-score	0.22 (1.06)	0.13 (1.04)	-0.40 (0.71)
Xrs8 z-score	-0.14 (-0.94, 0.12) *	0.15 (-0.41, 0.40)	0.35 (-0.06, 0.61)
AX z-score	0.19 (-0.53,1.31) *	-0.35 (-0.96, 0.59)	-0.76 (-1.27, -0.30)
Fres z-score	0.73 (1.14) *	0.30 (1.63)	-0.07 (0.09)
CT Abnormality	26 (93)	24 (86)	-

*BPD: bronchopulmonary dysplasia; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

.

Table 6-3 Peak exercise data in children who completed recovery assessment

Exercise Outcome	BPD (n=33)	Non-BPD (n=34)	Healthy (n=27)
$\dot{V}O_2$ peak (L/min)	1.54 (1.43, 1.77)	1.78 (1.49, 1.97)	1.57 (1.42, 1.81)
$\dot{V}O_2$ peak (mL/kg/min)	47.3 (7.1)	45.9 (6.8)	48.2 (5.9)
$\dot{V}O_2$ at % Peak	90 (83, 95)	84 (73, 94)	84 (3, 93)
RER at peak	1.03 (1.01, 1.07) *	1.05 (1.03, 1.09)	1.06 (1.04, 1.11)
Peak HR (bpm)	200 (10)	195 (11)	200 (9)
Oxygen pulse at peak	7.9 (7.4, 9.0)	8.9 (7.8, 10.1)	7.9 (7.1, 10.0)
Peak V_T (mL)	881 (664, 996)	990 (828, 112)	875 (762, 1144)
Peak f_R	62 (11) ǂ	57 (8)	60 (8)
Peak V_E (L/min)	53.7 (9.5)	55.4 (11.5)	56.1 (11.0)
$\dot{V}_E/\dot{V}CO_2$ slope	29.5 (26.8, 31.9)	27.6 (26.1, 29.2)	28.8 (28.2, 30.8)
$\dot{V}_E/\dot{V}O_2$ slope	27.7 (25.3,29.7)	26.0 (23.5, 28.0)	26.2 (5.1, 30.1)
Breathing reserve (%)	31 (18, 37)	37 (22, 43)	34 (27, 40)
Heart rate reserve (bpm)	10 (10)	14 (11)	9 (9)
$HR/\dot{V}O_2$ slope	2.6 (0.4)	2.4 (0.6)	2.3 (0.4)
EFL (%)	19 (63) ǂ*	7 (25)	10 (40)

*AT: Anaerobic threshold; VT: tidal volume; ƒR: respiratory frequency; V̇E: minute ventilation; HR: heart rate; RER: respiratory exchange ratio; EFL: expiatory flow limitation * p<0.05 compared to healthy; ǂ p<0.05 compared to non-BPD*

Following peak exercise there was no significant difference in early recovery between preterm children with BPD and term born controls (Table 6-4). Children who exhibited EFL during exercise did not recover differently following peak exercise to those without (Table 6-5).

Table 6-4 Recovery outcome data on children who completed recovery assessment

Recovery Outcome	BPD (n=33)	Non-BPD (n=34)	Healthy (n=27)
Time to 50% of Peak $\dot{V}O_2$ (s)	54 (42, 63)	48 (42, 54)	54 (48, 62)
$\dot{V}O_2$ recovery 1min (% peak)	42.7 (34.8, 51.3)	37.9 (34.0, 42.0) *	42.5 (38.6, 48.6)
$\dot{V}O_2$ recover 2 min (% peak)	22.6 (18.6, 26.7)	22.5 (17.5, 26.4)	23.3 (19.9, 26.8)
$\dot{V}O_2$ recover 5 min (% peak)	16.4 (13.5, 19.8)	17.8 (12.2, 20.7)	17.3 (13.5,21.7)

** p<0.05 compared to healthy BPD: bronchopulmonary dysplasia*

Table 6-5 Recovery data in children with and without expiratory flow limitation during exercise

Recovery Outcome	EFL (n=47)	no EFL (n=32)
Time to 50% of Peak $\dot{V}O_2$ (s)	54 (48, 60)	48 (42, 60)
$\dot{V}O_2$ recovery 1min (% Peak)	42.7 (38.3, 48.7)	39.8 (34.8, 47.0)
$\dot{V}O_2$ recovery 2 min (% peak)	24.8 (7.9)	22.2 (5.4)
$\dot{V}O_2$ recovery 5 min (% Peak)	18.9 (7.7)	17.0 (6.3)

EFL: expiratory flow limitation

Table 6-6 reports the independent predictors for early $\dot{V}O_2$ recovery following a peak exercise test. Time to 50 % peak $\dot{V}O_2$ was related to increased duration of supplemental oxygen and decreased pulmonary attenuation on expiration. T_LCO was inversely related to $\dot{V}O_2$ recovery at 1min. While there was a univariate correlation between peak $\dot{V}O2$ and $\dot{V}O2$ recovery at 2 minutes (see table 6A-4) there was no independent impact of peak $\dot{V}O2$ on the early $\dot{V}O2$ recovery (Table 6-6).

Table 6-6 Multivariate analysis

	Beta	95% CI	P	R^2
Time to 50 % peak $\dot{V}O_2$				
Supplemental oxygen (d)	0.374*	0.038, 0.190	0.004	0.192
Presence decreased pulmonary attenuation on expiration	0.274*	0.575, 12.632	0.032	
$\dot{V}O_2$ 1 min recovery (% peak)				
T_LCO (z-score)	-0.314*	-8.959, -0.149	0.043	0.076
$\dot{V}O_2$ 2 min recovery (% peak)				
Sex (Female)	0.283*	0.506, 7.189	0.025	0.065
$\dot{V}O_2$ 5 min Recovery (% peak)				
No significant associations				

*95 %CI, 95 % confidence interval; T_LCO: transfer factor of the lung for carbon monoxide; *p<0.05*

6.4 **Discussion**

This is the first study to assess the impact of preterm birth on the early oxygen uptake recovery following a peak exercise test in school-aged children. Time to 50 % of peak $\dot{V}O_2$ was associated with duration of supplemental oxygen and lung structural abnormalities, suggesting that children with worse neonatal lung disease may have reduced capacity to replenish phosphocreatine following bouts of high intensity exercise, however, oxygen kinetics during early recovery were not significantly different to controls, highlighting that any impact of preterm birth is relatively mild.

$\dot{V}O_2$ recovery can be used as a measure of the oxidative capacity for the resynthesis of PCr. The rate of $\dot{V}O_2$ recovery following maximal exercise has been shown to be decreased in subjects with heart failure (81, 105, 107), bronchiectasis (82) and cystic fibrosis (82). In our study, children born preterm exhibited a normal peak $\dot{V}O_2$ and normal $\dot{V}O_2$ recovery despite significant lung structural and functional changes. This finding suggests that these abnormalities are not severe enough to compromise the significant respiratory reserve that exists at peak exercise in healthy children (97, 98).

Forty percent of the preterm children assessed in the current study had parentally reported respiratory symptoms on exertion however this did not translate to a reduction in aerobic capacity or impaired recovery compared to age-matched healthy children. The number of preterm children currently using asthma medication was also low, suggesting that the symptoms reported by parents were not sufficiently severe to impact on the child's ability to perform exercise nor warrant treatment in many cases.

Although there were no between group differences in aerobic capacity, the early $\dot{V}O_2$ recovery responses in this study were variable, indicating a heterogeneity in early $\dot{V}O_2$ recovery in children consistent with previous observations (82). Despite normal early recovery responses, multivariate analysis revealed a statistically significant relationship between early $\dot{V}O_2$ recovery and both increased supplemental oxygen use and the presence of decreased pulmonary attenuation on expiration, a possible maker of air trapping or pre-emphysematous changes (8) in preterm children. There was

also an association between T_LCO and $\dot{V}O_2$ recovery at 1min, which suggests that reduced gas transfer capacity may result in slower $\dot{V}O_2$ recovery. However, most children in the current study had a T_LCO within the normal range indicating normal gas transfer capacity. The association with impaired gas transfer is suggestive of an impaired oxygen delivery to the skeletal muscles following exercise, however, there were no alteration in peak exercise outcomes. This suggests that despite the association of structural and function lung disease the significant respiratory reserve present at peak exercise preserves exercise capacity and early $\dot{V}O_2$ recovery. The long-term impacts of these structural and functional abnormalities found in preterm children on peak $\dot{V}O_2$ and early $\dot{V}O_2$ is unclear. We have previously shown that lung function in preterm children continues to decline throughout childhood (49) but the long-term impact of this decline on remains unclear.

A limitation of this study is the exclusion of children who were unable to perform a peak exercise test eliminating those with gait issues or cerebral palsy. One third of participants were excluded from the analysis as they were unable to meet the criteria for a peak exercise test. Furthermore, 10 % of children who achieved peak exercise were unable tolerate the mask following exercise cessation for the measurement of recovery and were also excluded from the analysis. These children may have exhibited altered recovery that led to this intolerance, although they all reached similar peak exercise outcomes compared to those who completed recovery data collection which suggest significant differences are unlikely. The children who were not included in the recovery analysis had a lower GA and increased ventilatory support in the neonatal period as well as having greater parentally reported exertional symptoms. This limitation may bias our results to a relatively healthy subset of the population and blunt any potential differences.

6.5 **Conclusion**

Children born preterm have a normal peak $\dot{V}O_2$ and early oxygen uptake recovery despite an increased prevalence of respiratory symptoms and EFL. EFL was not associated with any alterations in early $\dot{V}O_2$ recovery suggesting there is a minimal impact on cardiopulmonary fitness. While early $\dot{V}O_2$ recovery was normal there was an inverse relationship between early $\dot{V}O_2$ recovery and structural and functional changes at mid-childhood. Further research into the significance of these structural and functional changes on early $\dot{V}O_2$ recovery in adulthood will provide an understanding of any longer-term impact of preterm birth on physiological processes.

6.6 Chapter reference list

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All tables are listed in the following order: Neonatal factors, current demographics, current lung function, structural lung abnormalities and current exercise data

Appendix 6A

Table 6A-1 Neonatal and demographic details for children who successfully completed recovery assessment and those who did not

	Successful (n =94)	Unsuccessful (n =127)
Preterm n (%)	64 (71)	95 (75)
Male n (%)	47 (50)	79 (92)
Gestational age (PMA)	28.6 (26.1, 30.0)	27.0 (25.0, 29.1) *
Birth weight (g)	1150 (845, 1430)	882 (741, 1189) *
Birth weight Z-Score	-0.13 (0.96)	-0.14 (0.88)
Mechanical ventilation (days)	1.6 (0.0, 10.0)	7.0 (1.0, 33.0) *
CPAP (days)	4.8 (0.3, 14.0)	7.8 (1.0, 20.6)
Supplemental O_2 (days)	22.0 (0.0, 77.0)	67.5 (6.5, 95.8) *
Recent exercise symptoms n (%)	26 (39)	50 (60)
Asthma ever n (%)	26 (41)	41 (46)
Current asthma medication n (%)	11 (17)	19 (21)
Age at test (y)	11.2 (0.6)	11.1 (0.6)
Height at test (cm)	145 (7.8)	142 (8.8)
Weight at test (kg)	36.8 (31.6, 45.5)	34.5 (28.9, 40.7)

*BPD: bronchopulmonary dysplasia; PMA: postmenstrual age; CPAP: continuous positive airway pressure; Exercise symptoms included parentally reported wheeze, cough, shortness of breath during exertion; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05*

Table 6A-2 Lung function data for children who successfully completed recovery assessment and those who did not

	Successful (n =94)	Unsuccessful (n =127)
FEV_1 z-score	-0.42 (0.85)	-0.59 (1.29)
FVC z-Score	0.15 (0.98)	0.14 (0.10)
FEV_1/FVC z-score	-0.97 (1.08)	-1.10 (1.06)
TLC z-score	-0.02 (-0.58, 0.53)	-0.28 (-0.85, 0.41)
FRC z-score	0.18 (-0.43, 0.77)	0.11 (-0.68, 0.91)
RV z-score	-0.18 (-1.00, 0.37)	-0.33 (-1.15, 0.33)
FRC%TLC	52.6 (6.8)	51.8 (7.8)
RV%TLC	22.8 (7.3)	21.8 (6.7)
T_LCO z-score	-0.46 (0.77)	-0.47 (0.72)
T_LCO/VA z-score	-0.20 (0.67)	-0.26 (0.71)
VA z-score	-0.35 (0.83)	-0.30 (0.93)
Rrs8 z-score	0.04 (0.91)	0.35 (1.0) *
Xrs8 z-score	0.12 (-0.43, 0.44)	-0.16 (-0.98, 0.35) *
AX z-score	-0.34 (-0.94, 0.55)	0.03 (-0.77, 1.18) *
Fres z-score	0.23 (1.46)	0.46 (1.25)

*BPD: bronchopulmonary dysplasia; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; Data presented as mean (SD), median (IQR) or number (percent). * p<0.05*

Table 6A-3 Exercise outcomes in children who completed recovery assessment and those that did not

Exercise Outcome	Successful (n=94)	Unsuccessful (n=10)
$\dot{V}O_2$ peak (L/min)	1.67 (1.49, 1.83)	1.40 (1.30, 1.91)
$\dot{V}O_2$ peak (mL/kg/min)	47.2 (7.0)	45.0 (6.2)
$\dot{V}O_2$ AT % Peak	87 (75, 94)	77 (67, 85)
RER at peak	1.05 (1.03, 1.09)	1.04 (1.02, 1.08)
Peak HR (bpm)	197 (191, 206)	196 (186, 203)
Oxygen pulse at peak	8.4 (7.7, 9.9)	7.5 (6.4, 9.9)
Peak V_T (mL)	963 (776, 1092)	788 (646, 1438)
Peak f_R	60 (10)	58 (10)
Peak $\dot{V}_E$ (L/min)	56.1 (10.8)	52.1 (13.7)
$\dot{V}_E/\dot{V}CO_2$ slope	29 (27, 31)	28 (27, 31)
$\dot{V}_E/\dot{V}O_2$ slope	26 (25, 28)	28 (25, 28)
Breathing reserve (%)	34.6 (24.6, 30.5)	34.3 (28.1, 39.8)
Heart rate reserve (bpm)	9.8 (10.9)	1.5 (7.9)
$HR/\dot{V}O_2$ slope	2.4 (0.5)	2.6 (0.7)
EFL (%)	36 (43)	3 (30)

*AT: Anaerobic threshold; V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio; EFL: expiatory flow limitation * p<0.05*

Table 6A-4 Univariate analysis time to 50 % $\dot{V}O_2$

Time to 50 % peak $\dot{V}O_2$	Beta	CI	P
Gestation (w PMA)	-0.2	-2.5, 0.24	0.104
Supplemental O_2 (days)	0.30	0.22, 0.18	0.013*
Mechanical Ventilation (days)	0.044	-0.192, 0.274	0.726
Birth Weight z-score	0.007	-3.363, 3.571	0.953
CPAP (Days)	0.26	0.26, 0.55	0.031*
Age at test (y)	-0.098	-7.540, 3.240	0.428
Height at test (cm)	0.109	-0.252, 0.649	0.382
Weight at test (kg)	-0.009	-0.320, 0.291	0.943
Gender	0.26	0.56, 13.49	0.034*
FEV_1 z-score	-0.046	-4.659, 3.389	0.752
FVC z-score	-0.281	-6.885, 0.002	0.05
FEV_1/FVC z-score	0.225	-0.718, 5.982	0.121
FRC z-score	-0.231	-4.793, 0.206	0.071
TLC z-score	-0.174	-6.096, 1.142	0.176
RV z-score	-0.059	-3.426, 2.153	0.65
T_LCO z-score	-0.172	-7.272, 2.133	0.276
T_LCO/VA z-score	0.156	-2.806, 8.300	0.323
Rrs8 z-score	-0.067	-3.745, 2.228	0.613
Xrs8 z-score	-0.055	-4.581, 3.017	0.682
AX z-score	0.075	-1.865, 3.350	0.571
Fres z-score	0.102	-1.323, 3.002	0.44
Presence Linear/ triangular subpleural opacities	0.082	-0.114, 0.206	0.569
Presence Decreased pulmonary attenuation inspiration	0.1	-5.241, 11.152	0.473
Presence Decreased pulmonary attenuation on expiration	0.287	0.436, 13.392	0.037*
Peribronchial thickening	0.041	-1.809 2.423	0.772
Total CT Score	0.068	-0.522, 0.855	0.629
Exertional Symptoms	-0.148	-10.915, 3.723	0.235
$\dot{V}O_2$ peak (L/min)	-0.157	-17.461, 3.831	0.206
RER at peak	-0.142	-125.610, 33.658	0.253
EFL%VT	0.013	-0.127, 0.140	0.923
Peak V_E (L/min)	-0.039	-0.372, 0.272	0.758
Peak HR (bpm)	0.131	-0.148, 0.485	0.293
Peak V_T (L)	-0.134	-19.549, 5.771	0.281
Peak f_R	0.2178	-0.032, 0.603	0.077
$\dot{V}_E/\dot{V}CO_2$ slope	0.236	-0.026, 1.513	0.058
$\dot{V}_E/\dot{V}O_2$ slope	0.234	-0.045, 2.007	0.06
Breathing reserve (%)	0.065	-0.165, 0.277	0.612
Heart rate reserve (bpm)	-0.124	-0.469, 0.156	0.32

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow volume loop V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio *p<0.05*

Table 6A-5 Univariate analysis $\dot{V}O_2$ 1 min recovery

$\dot{V}O_2$ 1 min recovery	Beta	CI	P
Gestation (w PMA)	-0.156	-1.833, 0.405	0.207
Supplemental O_2 (days)	0.222	-0.005, 0.126	0.071
Mechanical Ventilation (days)	0.125	-0.092, 0.281	0.313
Birth Weight z-score	0.027	-2.487, 3.100	0.827
CPAP (Days)	0.216	-0.023, 0.401	0.08
Age at test (y)	-0.015	-4.633, 4.097	0.903
Height at test (cm)	0.125	-0.19, 0.546	0.315
Weight at test (kg)	0.015	-0.234, 0.263	0.907
Gender	0.107	-3.040, 7.694	0.39
FEV_1 z-score	-0.168	-6.268, 1.630	0.243
FVC z-score	-0.241	-6.399, 0.397	0.082
FEV_1/FVC z-score	0.078	-2.458, 4.247	0.594
FRC z-score	-0.16	-3.375, 0.775	0.215
TLC z-score	-0.23	-5.610, 0.243	0.072
RV z-score	-0.056	-2.779, 1.789	0.666
T_LCO z-score	-0.314	-8.959, -0.149	0.043*
T_LCO/VA z-score	0.042	-4.746, 6.171	0.793
Rrs8 z-score	-0.101	-3.739, 1.662	0.444
Xrs8 z-score	0.023	-3.151, 3.747	0.863
AX z-score	0.051	-1.914, 2.823	0.702
Fres z-score	0.063	-1.500, 2.436	0.636
Presence Linear/ triangular subpleural opacities	0.078	-5.240, 9.292	0.578
Presence Decreased pulmonary attenuation inspiration	0.12	-3.371, 8.585	0.386
Presence Decreased pulmonary attenuation on expiration	0.263	-0.138, 9.428	0.057
Peribronchial thickening	0.184	-1.620, 8.083	0.187
Total CT Score	0.287	0.031, 1.000	0.037*
Exertional Symptoms	-0.073	-7.159, 3.930	0.563
$\dot{V}O_2$ peak (L/min)	-0.015	-9.220, 8.155	0.903
RER at peak	-0.183	-111.706, 15.776	0.138
EFL%VT	0.031	0.095, 0.120	0.815
Peak V_E (L/min)	0.062	0.1914, 0.318	0.62
Peak HR (bpm)	-0.012	0.269, 0.245	0.925
Peak V_T (L)	-0.008	-10.637, 9.955	0.947
Peak f_R	0.041	-0.218, 0.306	0.74
$\dot{V}_E/\dot{V}CO_2$ slope	0.112	-0.352, 0.924	0.374
$\dot{V}_E/\dot{V}O_2$ slope	0.103	-0.500, 1.202	0.413
Breathing reserve (%)	-0.066	-0.223, 0.131	0.606
Heart rate reserve (bpm)	0.017	-0.236, 0.270	0.894

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow volume loop V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio *p<0.05*

Table 6A-6 Univariate analysis $\dot{V}O_2$ 2 min recovery

$\dot{V}O_2$ 2 min recovery	Beta	CI	P
Gestation (w PMA)	-0.078	-0.966, 0.512	0.542
Supplemental O_2 (days)	0.112	-0.024, 0.063	0.383
Mechanical Ventilation (days)	0.119	-0.067, 0.184	0.352
Birth Weight z-score	0.062	-1.371, 2.256	0.627
CPAP (Days)	0.048	-0114, 0.166	0.709
Age at test (y)	0.088	-1.058, 2.583	0.407
Height at test (cm)	-0.04	-0.20, 0.13	0.715
Weight at test (kg)	-0.15	-0.22, 0.04	0.159
Gender	0.279	0.938, 6.026	0.008*
FEV_1 z-score	-0.186	-2.846, 0.349	0.124
FVC z-score	-0.276	-2.926, -0.217	0.024*
FEV_1/FVC z-score	0.13	-0.61, 2.00	0.293
FRC z-score	-0.075	-1.419, 0.704	0.505
TLC z-score	-0.121	-2.164, 0.637	0.281
RV z-score	0.083	-0.687, 1.489	0.465
T_LCO z-score	-0.003	-0.189, 2.138	0.981
T_LCO/VA z-score	0.235	-0.230, 4.600	0.075
Rrs8 z-score	0.041	-1.135, 1.602	0.735
Xrs8 z-score	-0.09	-2.34, 1.06	0.454
AX z-score	0.114	-0.599, 1.715	0.34
Fres z-score	0.128	-0.436, 1.464	0.284
Presence Linear/ triangular subpleural opacities	0.25	-0.67, 10.56	0.083
Presence Decreased pulmonary attenuation inspiration	-0.04	-6.192, 4.698	0.784
Presence Decreased pulmonary attenuation on expiration	-0.007	-4.192, 4.009	0.964
Peribronchial thickening	-0.057	-4.844, 3.261	0.696
Total CT Score	-0.072	-0.575, 0.348	0.623
Exertional Symptoms	0.219	-0.271, 5.942	0.073
$\dot{V}O_2$ peak (L/min)	-0.228	-7.791, -0.384	0.031*
RER at peak	0.051	-18.897, 31.014	0.631
EFL%V_T	0.104	-0.030, 0.081	0.36
Peak V_E (L/min)	-0.133	-0.205, 0.046	0.21
Peak HR (bpm)	0.27	0.039, 0.285	0.01*
Peak V_T (L)	-0.146	-8.771, 1.581	0.171
Peak f_R	0.082	-0.082, 0.195	0.418
$\dot{V}_E/\dot{V}CO_2$ slope	0.106	-0.223, 0.644	0.337
$\dot{V}_E/\dot{V}O_2$ slope	0.134	-0.163, 0.688	0.223
Breathing reserve (%)	0.018	-0.091, 0.107	0.869
Heart rate reserve (bpm)	-0.271	-0.285, -0.039	0.01*

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: respiratory system resistance at 8hz; Xrs8: respiratory system reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow volume loop V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio *p<0.05*

Table 6A-7 Univariate analysis $\dot{V}O_2$ 5 min recovery

$\dot{V}O_2$ 5 min recovery	Beta	CI	P
Gestation (w PMA)	0.082	-0.519, 0.970	0.546
Supplemental O_2 (days)	-0.143	-0.072, 0.022	0.293
Mechanical Ventilation (days)	-0.124	-0.195, 0.072	0.361
Birth Weight z-score	0.246	-0.112, 3.189	0.067
CPAP (Days)	-0.036	-0.196, 0.150	0.79
Age at test (y)	-0.019	-3.118, 2.715	0.891
Height at test (cm)	0.056	-0.188, 0.284	0.687
Weight at test (kg)	0.175	-0.050, 0.236	0.197
Gender	-0.102	-4.598, 2.094	0.456
FEV_1 z-score	-0.052	-1.972, 1.415	0.742
FVC z-score	0.136	-0.8631, 2.060	0.395
FEV_1/FVC z-score	-0.202	-2.216, 0.493	0.206
FRC z-score	0.065	-1.107, 1.609	0.652
TLC z-score	0.076	-1.314, 2.260	0.597
RV z-score	-0.052	-1.699, 1.180	0.718
T_LCO z-score	0.123	-1.603, 3.382	0.473
T_LCO/VA z-score	-0.051	-3.299, 2.456	0.768
Rrs8 z-score	-0.004	-1.534, 1.493	0.979
Xrs8 z-score	-0.108	-2.607, 1.146	0.438
AX z-score	0.116	-0.744, 1.827	0.402
Fres z-score	0.125	-0.580, 1.547	0.366
Presence Linear/ triangular subpleural opacities	0.007	-0.040, 0.042	0.969
Presence Decreased pulmonary attenuation inspiration	0.028	-4.860, 5.837	0.855
Presence Decreased pulmonary attenuation on expiration	0.048	-3.351, 4.590	0.754
Peribronchial thickening	0.103	-2.577, 5.174	0.503
Total CT Score	0.006	-0.450, 0.466	0.971
Exertional Symptoms	-0.071	-4.325, 2.540	0.604
$\dot{V}O_2$ peak (L/min)	0.104	-3.175, 7.118	0.446
RER at peak	-0.069	-48.994, 29.140	0.613
Presence EFL	0.035	-3.409, 4.308	0.815
EFL%V_T	0.031	-0.059, 0.073	0.837
Peak V_E (L/min)	-0.001	-0.158, 0.157	0.994
Peak HR (bpm)	0.124	-0.083, 0.223	0.363
Peak V_T (L)	0.041	-5.330, 7.2222	0.764
Peak f_R	-0.035	-0.193, 0.149	0.799
$\dot{V}_E/\dot{V}CO_2$ slope	-0.251	-1.069, 0.037	0.067
$\dot{V}_E/\dot{V}O_2$ slope	-0.112	-0.755, 0.321	0.421
Breathing reserve (%)	0.173	-0.039, 0.170	0.216
Heart rate reserve (bpm)	-0.083	-0.002, 0.001	0.486

*95 %CI, 95 % confidence interval, PMA: postmenstrual age; CPAP: continuous positive airway pressure; T_LCO: transfer factor of the lung for carbon monoxide; VA: alveolar volume; Rrs8: resistance at 8hz; Xrs8: reactance at 8hz; AX: area under the reactance curve; Fres: resonant frequency; EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow volume loop V_T: tidal volume; f_R: respiratory frequency; $\dot{V}_E$: minute ventilation; HR: heart rate; RER: respiratory exchange ratio *p<0.05*

Table 6A-8 Co-author attribution statement

	Conception and Design	Acquisition of Data	Data Analysis	Interpretation and Discussion	Final Approval
Dr Karla Logie		X	X		
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Wilson	X		X	X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Prof Jane Pillow	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Conor Murray	X		X	X	
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Georgia Banton		X			
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Dr Shannon Simpson		X	X	X	X
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Prof Graham Hall	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					
Dr Andrew Maiorana	X			X	X
Co Author Acknowledgment: I acknowledge that these represent my contribution to the above research output					

Chapter 7 Summary, clinical implications and future research direction

This thesis examined the impact of preterm birth and associated neonatal factors as well as current structural and functional lung disease on the physiological response to a maximal exercise test. This was the first study investigating cardiorespiratory fitness in preterm children born in the surfactant era to include measures of peripheral lung function and structural assessment of lung disease via HRCT.

This thesis is presented as a series of manuscripts (chapters 4 – 6). The first manuscript (chapter 4) demonstrated an increased prevalence of expiratory flow limitation during exercise in children born preterm with a neonatal classification of BPD. The second manuscript (chapter 5) identified a normal aerobic exercise capacity in children born preterm and was the first study to show no impact of structural lung disease on the exercise capacity in school aged survivors of preterm birth. The third manuscript (chapter 6) was the first study to document early $\dot{V}O_2$ recovery following a cardiopulmonary exercise test in children born preterm during the surfactant era, showing a normal early $\dot{V}O_2$ recovery in children born preterm.

This chapter summarises the findings of this thesis, discusses the broader clinical implications of these findings and possible areas of future research.

7.1 Ventilatory response to exercise

My work has shown that children born preterm mount an altered ventilatory response to exercise, which is characterised by a rapid shallow breathing pattern, shown by an elevated respiratory frequency to tidal volume ratio, and an increased prevalence of expiratory flow limitation (EFL). I observed that the prevalence of EFL is associated with airway obstruction on spirometry (reduced FEV_1/FVC) and a lower gestational age (GA), and that the rapid shallow breathing pattern is associated with a lower birth weight z-score. These results taken together suggest that children born preterm exhibit an

altered ventilatory response to exercise that is related to obstructive airways disease that is most likely a result of disrupted lung growth due to prematurity rather than insults that occur following preterm birth.

A small number of previous studies reported an altered ventilatory response to exercise, including reduced tidal volume and increased respiratory frequency (39, 47, 68, 85), with only a single study assessing EFL in preterm children (47). While these studies identified the presence of an altered breathing pattern in children born preterm there was no exploration of the potential mechanisms behind this finding. I hypothesised that the altered ventilatory response would be driven by EFL associated with dynamic hyperinflation, as seen in other obstructive lung diseases such as cystic fibrosis (76), asthma (79), and chronic obstructive pulmonary disease (COPD) (77, 78). Children born preterm did not show any changes consistent with dynamic hyperinflation, which suggests that unlike other obstructive lung diseases bronchoconstriction and gas trapping are not an important mechanism leading to the increased incidence of EFL in children born preterm. The measurement of EFL requires a deep inspiration to total lung capacity during exercise, there is some evidence to suggest that deep inspiration may induce transient bronchodilation and provide a broncho-protective effect inhibiting bronchoconstriction and that this may be less pronounced in subjects with asthma (108). It is possible that the deep inspiration performed during the test may over report EFL in this population if they have asthma. However, all children were assessed without the use of a short acting bronchodilator prior to exercise and given the lack of dynamic hyperinflation, I hypothesise that the impact of airway hyper responsiveness on this finding is low. Children were also assessed after complex lung function measurements which involved repeated deep inspirations. It is unlikely that deep inspirations would significantly alter the incidence of EFL in this study.

Possible mechanisms behind increased EFL include increased inspiratory load due to decreased lung compliance in preterm children, inspiratory muscle weakness/fatigability, or alterations in airway size to lung size (dysanapsis).

Preterm children in this study had increased respiratory reactance suggesting decreased lung compliance. Decreased lung compliance may result in difficulty in overcoming the mechanical inspiratory load resulting in increased work of breathing and possibly the inability to increase the operating lung volumes required at the higher ventilation during exercise and therefore lead to EFL. It has been suggested children born preterm may have impaired inspiratory muscle strength (68) leading to an inability to overcome the increased resistive load. Increased resistive load, respiratory muscle weakness, or increased fatiguability may result in the inability to generate the force required to increase lung volumes resulting in an inability to overcome the development of EFL. This is consistent with data from obese children who have a similar increased load leading to increased EFL (89).

Children born preterm may have been born at different embryological stages of development (i.e. canalicular vs saccular stages) resulted in disrupted lung growth and development. Different lung compartments may grow at different rates, a process known as dysanaptic lung growth, this may lead to alterations in lung size (and lung volume) compared to airway size (109). Children and women have an increased incidence of EFL compared to adult males and this is attributed to their smaller airways for a given lung size as assessed by a lower FEV_1/FVC. The presence of EFL in this study was associated with a lower FEV_1/FVC and a lower gestational age, which suggests a possible influence of dysanapsis and disrupted lung growth in the development of EFL.

The increased prevalence of EFL surprisingly was not associated with any changes in ventilatory response or increased respiratory symptoms. This leads to a possibility that there are two separate pathologies occurring in this population or that EFL may be a consequence of altered ventilatory response as opposed to the cause.

This study did not find any association between altered ventilatory response to exercise and the high incidence of parentally reported symptoms. Despite the high prevalence of symptoms in this group (~40 %) there was a low proportion of children using current asthma medications (25 %). This may

suggest that while the parents report symptoms these were not clinically severe enough to impact on the child's ability to perform exercise or not severe enough to warrant treatment. Indeed, the EPICURE study reports no alterations in physical activity in extremely preterm children compared to term born controls (39). At this age physical activity may remain the same, however, school aged children tend to exercise in short bouts of high intensity exercise (110), which may enable children born preterm to moderate their exercise intensity, or work in shorter bouts of exercise, limiting the impact of these alterations in ventilation on respiratory symptoms and/or aerobic fitness. To date there are no data assessing the differences in physical activity in children born preterm with EFL and the impact of EFL on physical activity is not clear. The future impact of the ventilatory alterations, as children start to exercise in more organised sporting events with increased duration, is unknown and may provide further information.

I identified an altered ventilatory response to exercise which was characterised by a rapid shallow breathing pattern as documented by high respiratory frequency: tidal volume ratio. This result is consistent with previous findings (39, 47, 68, 85) which all showed an altered ventilatory pattern characterised by a lower tidal volume and increased respiratory rate. The altered ventilatory response to exercise does not appear to exceed the ventilatory reserve seen in healthy children. Indeed, in this study there was no difference in the breathing reserved as assessed from baseline spirometry. This suggests that despite the altered ventilatory pattern these children are able to maintain the required ventilatory demand. This rapid shallow breathing pattern is inversely associated with birth weight z-score, suggesting those children born small for GA are more likely to exhibit ventilatory changes to exercise.

The assessment of the ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}O_2$) and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) provides insight into the efficacy of ventilation and gas exchange and individual time points during exercise while the $\dot{V}_E/\dot{V}CO_2$ slope provides the average ventilatory efficacy during exercise. The $\dot{V}_E/VCO_2$ slope is comprised of the efficacy of ventilation (measure of dead space to total ventilation) as well as the $PaCO_2$ set point (32). Despite the altered

ventilatory response in this study there was no alteration in the $\dot{V}_E/\dot{V}CO_2$ slope or ventilatory equivalent measured at anaerobic threshold. This suggests that despite the altered breathing strategy employed the ventilation was in keeping with the metabolic demand of the exercise intensity.

Taken together it is clear that school aged children born preterm mount an altered ventilatory response to exercise characterised by a rapid shallow breathing pattern and are more likely to develop EFL. These altered responses to exercise are significantly associated with reduced gestational age, being small for gestational age and the presence of airway obstruction. This suggests that arrested lung development associated with prematurity rather than neonatal lung disease may lead to this response. It appears that despite this altered ventilatory response the ventilation is in keeping with the metabolic demand for exercise as demonstrated by a normal $\dot{V}_E/\dot{V}CO_2$ value. It is plausible that this alteration in ventilatory response is a beneficial adaptation rather than a pathological finding. The long-term impacts of this altered ventilatory response are yet to be assessed and it is unclear if these alterations are progressive or associated with worse cardiopulmonary outcomes.

7.2 **Structural and functional impairments and exercise capacity**

This thesis is the first to examine the impacts of structural lung abnormalities on the physiological response to a maximal exercise test in school aged survivors of very preterm birth. Despite most preterm children (88 %) having structural abnormalities as assessed by a high-resolution CT scan I observed there was no independent association with any peak exercise outcomes. The lack of association between exercise outcomes and lung structure is in contrast to studies in children with cystic fibrosis (CF) (95) where total CT score, bronchiectasis and lung collapse were associated with impaired peak $\dot{V}O_2$. In the preterm population I studied, no child showed lung collapse on CT and less than 10 % exhibited bronchiectasis. The most common structural abnormalities were linear and triangular sub pleural opacities (85 %

of preterm children) and peribronchial thickening (48 %). The structural abnormalities seen in preterm children may not be severe enough to limit the capacity of the respiratory system during exercise. In healthy children the respiratory system does not limit aerobic performance due to a large respiratory reserve which normally exceeds 30 % (98).

Children with non-CF bronchiectasis have also been shown to have no association between CT abnormalities and exercise capacity (111). Edwards et al (111) identified that children with bronchiectasis present with heterogeneous disease due to a variety of underlying causes of their chest disease. Similarly, the preterm population in this study had variation of disease severities despite similar gestational ages, the neonatal course of infants born preterm is varied and the care received within a neonatal intensive care unit can vary depending on the practitioner (112). Given the heterogeneity of the impact of prematurity, neonatal lung disease and subsequent lung health during childhood may be difficult to identify an association between outcomes and disease markers.

I hypothesized that children born preterm would exhibit a reduced exercise capacity; however, there were no differences between the preterm children and term controls. There have been conflicting reports on the impact of preterm birth on the aerobic exercise capacity at mid-childhood, with some (39, 42, 47) but not all (44, 67, 68) studies showing a reduction in peak $\dot{V}O_2$. Given the known variability in neonatal care between centres and between countries (112), the variability in results may be due to the heterogeneity of this population. Due to this variability in disease treatment it is possible there is variability in the long-term consequences of preterm birth on the exercise capacity. My study included a wider range of gestational ages (<32 wk GA) compared to other studies such as the EPICURE study (39) which investigated extremely preterm birth (<26 wk GA), the inclusion of a wider GA range may blunt any differences seen, although there was no significant association with GA which makes this unlikely. There was also a large variability in peak $\dot{V}O_2$/kg (coefficient of variation 25 %) in the healthy population within my study, a result similar to that reported by Edwards et al (111). With such a large variability in the control group the magnitude of any

impairment in our preterm population would need to be large to reach statistical significance. Unfortunately, there are no robust predicted values for treadmill exercise tests in a paediatric population to compare these results to.

The modality of exercise testing in this population may also result in alterations of results with previous studied using different exercise modalities employed including cycle ergometers (39, 47), treadmills with varying protocols (44), or shuttle runs (42). Generally, treadmill exercise test studies report no difference in aerobic capacity, while shuttle runs and studies involving cycle ergometer show lower peak $\dot{V}O_2$ in the preterm population. Advantages of a treadmill exercise test include the similarity to everyday activity and recruitment of a larger muscle mass to achieve peak $\dot{V}O_2$ compared to a cycle ergometer. The preterm children in this study were shorter and lighter than the healthy term controls which may suggest changes in fat free mass. Fat free mass was not measured in this study, however it has been shown children born at a very low birth weight have a reduction in fat free mass as measured by DEXA scan, however, the percentage of fat free mass remains similar (65). Peak exercise capacity during cycle ergometry is more commonly limited by lower limb muscle fatigue than treadmill exercise (103). It has been reported that preterm children have a normal upper body strength, but do exhibit a reduced lower body strength (quadriceps strength) (104), compared with matched controls. This may translate to an impaired ability to continue cycling at higher workloads leading to a lower measured peak $\dot{V}O_2$ during cycle ergometry. There have been reported reductions in workload (39) and $\dot{V}O_2$/work (56, 70) in the preterm population even with normal peak $\dot{V}O_2$ (68, 69). These differences in workload and $\dot{V}O_2$/work are consistent with my hypothesis of altered peripheral muscle leading to the exercise limitation (70) . However, I was unable to accurately assess workload in this cohort, while each child was encouraged to run without holding the handrail a large proportion of children needed to hold the handrail for safety and confidence. Without being able to assess for the amount weight placed on the handrails I was unable to calculate work rate accurately and is a limitation of this study.

My work has shown no association between measurements of peripheral respiratory mechanics as measured by FOT and the aerobic capacity or ventilatory response in children born very preterm. I had hypothesized that alteration in peripheral respiratory mechanics would be common in children born preterm and would result in an altered ventilatory response leading to a reduction in peak $\dot{V}O_2$. Previous studies have shown preterm children to have a reduction in respiratory mechanics as measured by the FOT (7, 8), however, in this study most children presented with FOT measurements within the normal range. This may indicate a healthy selection bias in this cohort, although there were children included in this study that had altered respiratory mechanics as measured by FOT there was no association between these outcomes and exercise responses. Simpson et al showed that children with respiratory symptoms have worse lung mechanics compared to those without (8). In a longitudinal analysis they showed that children with respiratory symptoms showed greater decline in FOT measurements than those without suggesting that FOT measurements are an important marker of respiratory morbidity (49). The lack of association between FOT measurements, CT outcomes and exercise outcomes suggests that peripheral lung structure is not impacting on the aerobic exercise capacity or ventilatory response to exercise in this cohort. Given the mild alterations seen in this population it is possible that these alterations were mild in nature and not severe enough to impact on the large ventilatory reserve in children. Simpson et al showed that FOT measurements were associated with peribronchial thickening which is a possible marker of post inflammatory changes or ongoing airway inflammation (49). There have been reports of ongoing airway inflammation in preterm children (113, 114) and this might suggest that the alterations with the peripheral airway function are associated with airway inflammation, leading to bronchoconstriction which may explain why there is no association with exercise outcomes as assessed with a cardiopulmonary exercise test.

My work has shown that despite alterations in lung function and structure there is no associated impairment of the aerobic exercise capacity in preterm children. It is likely that these changes are mild in nature and are not

significant enough to impair the exercise response or result in ventilatory limitation to exercise. Given the differences between cycle and treadmill exercise the role of a pulmonary rehabilitation program to improve fitness and lower limb strength on ongoing cardio-metabolic disease risk might be beneficial.

There is some evidence to suggest that the ẆO2/work or the work rate may be reduced in children born preterm as seen in children with CF.

7.3 **Respiratory symptoms and exercise capacity**

Children born preterm exhibited an increased prevalence of parentally reported exertional symptoms. Approximately 40 % of preterm children had parentally reported symptoms of cough, wheeze or dyspnoea on exertion or symptoms that limited physical activity. Despite this I did not observe any alterations in peak $\dot{V}O_2$ or peak ventilatory data between preterm children or children with and without symptoms. This may suggest that these parentally reported symptoms were not severe enough to limit the child's physical activity. The EPICURE study reported no alterations in the physical activity in extremely preterm children compared to term controls (39). This may be due to the type of physical activity children perform, school aged children tend to exercise in short bursts of activity compared to longer aerobic exercise seen in older populations (110). This may prevent these symptoms from limiting physical activity at this age, however, this may become a limiting factor as the child's physical activity domains change from less structured to more organised physical activity (115). A limitation of this study was the inability to assess the reason for exercise cessation, when asked why they ceased exercise many children were unable to provide a clear answer as to what limited their exercise capacity, future work assessing subjective exercise limitation may provide further insights.

Children born preterm are at a higher risk of metabolic diseases such as alterations in adipose distribution, fatty liver, insulin resistance and high blood pressure as they reach adolescence and adulthood (116) which is associated with low birth weight. Physical fitness and physical activity have been

reported to attenuate any impact that low birth weight has on the metabolic changes (9). In adults who were physically inactive and unfit the impact of preterm birth on metabolic outcomes is worse which suggests physical activity is extremely important in this population (9). While the children in my study appeared to have similar fitness levels to term controls it is unclear what the long-term implications are for physical fitness and metabolic risk factors. Clemm et al (71) showed that children born preterm had a normal trajectory of peak $\dot{V}O_2$ from mid-childhood through adolescence, however, in that study, lung function assessed using spirometry was within the normal limits and there were no reports regarding respiratory morbidity, in particular exertional symptoms. Exertional symptoms have been shown to be persistent throughout mid-childhood and associated with worsening lung function (49), it is possible that these symptoms will limit future physical activity. Clemm et al also showed that participation in physical activity became a stronger influence of aerobic exercise capacity throughout adolescence (71). Kemp et al (115) reported that children deemed “unskilled” are more likely to reduce participation in organised physical activity during adolescents, given the known increased motor coordination difficulties seen in preterm children (72) they may be at greater risk of decreased activity in adolescents which may enhance any impact of respiratory symptoms and lead to impaired cardio metabolic outcomes.

7.4 **Recovery**

This thesis is the first to report early $\dot{V}O_2$ recovery following exhaustive exercise in a preterm population. I observed that children born preterm exhibit similar early $\dot{V}O_2$ recovery to term born controls. Early $\dot{V}O_2$ recovery has been studied in adult cardiac disease patients with delayed $\dot{V}O_2$ recovery associated with exercise intolerance (117). The evidence surrounding early $\dot{V}O_2$ recovery in children is limited. Singh et al (83) and Stevens et al (82) have shown that $\dot{V}O_2$ recovery is strongly correlated with peak $\dot{V}O_2$ suggesting that $\dot{V}O_2$ recovery is associated with fitness and may be a suitable adjunct in assessing cardiopulmonary health in children. In children with cystic fibrosis and bronchiectasis early $\dot{V}O_2$ recovery is associated with

greater aerobic fitness, and in children with cystic fibrosis the rate of $\dot{V}O_2$ recovery was significantly correlated with structural lung disease as assessed by CT scan (82). In contrast, there was no independent association with early $\dot{V}O_2$ recovery and peak $\dot{V}O_2$ in the cohort I studied. There was also no difference in early $\dot{V}O_2$ recovery between the preterm population and term controls, which may be due to the preserved peak $\dot{V}O_2$ outcomes suggesting that the preterm children have a normal aerobic capacity. The time taken for $\dot{V}O_2$ recovery in children have been reported to be varied (82). This may indicate heterogeneity of the response and possible different phenotypes. Steven et al (82) suggested that children with a low $\dot{V}O_2$ peak but a fast recovery are probably fitter and have better outcomes compared to those with a maintained $\dot{V}O_2$ a delayed recovery. The clinical relevance of these different phenotypes is unclear.

I have shown that $\dot{V}O_2$ recovery is independently associated with supplemental oxygen use, decreased pulmonary attenuation on expiration, and the gas transfer capacity. Children born preterm have lung morphological changes associated with large simplified alveolar (4) which may be due to alterations in the expression of vascular endothelial growth factor (VEGF) due to supplemental oxygen (40). VEGF is important for angiogenesis and alveolarization in the immature lung (40). The use of supplemental oxygen may reduce the expression of VEGF and then impair the development of the alveolar and pulmonary capillary bed (40). Following exercise, oxidative phosphorylation commences to allow resynthesis of phosphocreatine (PCr) to restore muscular ATP, an important fuel source for anaerobic metabolism (105). During early recovery the rate of PCr resynthesis is inversely proportional to the duration of $\dot{V}O_2$ recovery (105). PCr resynthesis relies on oxygen transport and utilization by contracting skeletal muscles (81, 105). Although delayed $\dot{V}O_2$ recovery was not associated with respiratory symptoms it is possible that recovery is an early indication of pulmonary vascular derangement and an important tool for assessment of long-term cardiopulmonary outcomes.

I had hypothesised that the increased prevalence of EFL in the preterm population was likely related to respiratory muscle weakness and/or

fatiguability of the respiratory muscles. Rideau Batista Novais et al (68) showed that children born preterm had a reduction in respiratory muscle strength, which may indicate an inability to overcome the inspiratory resistive load. The use of early $\dot{V}O_2$ recovery has been shown to be associated with respiratory muscle weakness in adults with cardiac diseases and was an indicator of exercise intolerance (81). In this cohort there was no association between the prevalence of EFL or alterations in the ventilatory pattern. It is likely the altered ventilatory response and EFL is an adaptive change due to the altered lung structure and decreased lung compliance observed.

7.5 **Clinical significance**

My work is the first comprehensive study to assess the implications of preterm birth, neonatal care, lung function and lung structure on the physiological response to exercise in children born during the surfactant era. I have shown that despite a high incidence of structural lung abnormalities, respiratory symptoms and reduced lung function; children born preterm have a similar peak aerobic exercise capacity to term born controls.

Using the statistical approach taken in this analysis I have been able to assess the independent effects of neonatal factors including lung development (GA and birth weight z-score) and neonatal ventilatory support (mechanical ventilation, CPAP, supplemental oxygen). The impact of prematurity (lung development) and ventilatory support on subsequent lung health are highly collinear which makes the independent assessment difficult. By using the standardised residual, it was possible to assess the independent effect of each. Previous studies have included collinear values within the multivariate analysis reducing the ability to identify independent effects or included the classification of BPD which is a combination of lung development and ventilatory support. The importance of this is the ability to identify potential areas of therapeutic intervention. Using this approach, I was able to show:

- Children born preterm exhibit an altered ventilatory response to exercise characterised by a rapid shallow breathing pattern and

expiratory flow limitation that is associated with disruption of lung development, however, it was not associated with any impairment of cardiopulmonary fitness and may be a beneficial adaptation

- Children born preterm show similar early $\dot{V}O_2$ recovery following maximal exercise, however, delayed $\dot{V}O_2$ recovery is associated with increased supplemental oxygen, pre-emphysematous structural changes on CT and decreased gas transfer. This suggests that children with worse neonatal lung disease may have a reduced capacity to replenish phosphocreatine following bouts of high intensity exercise

The evidence provided in my thesis can be used to reassure parents of children born preterm that despite the presence structural and functional abnormalities and increased parentally reported symptoms it appears that school aged children that were born preterm are safe to exercise and maintain normal physical activity. The longer-term impact of the altered ventilatory response, exertional symptoms, and ongoing decline in lung function on aerobic exercise capacity is unclear and further research into the long-term outcomes needs further investigation.

7.6 **Future research**

Despite evidence of ongoing respiratory symptoms and impaired lung function, there was no association with alteration in the exercise response at school age. Further areas of research should include:

- Long term consequences of EFL on exercise capacity, metabolic disease and involvement in physical activity
- The longitudinal impact of structural and functional changes on $\dot{V}O_2$ peak trajectory
- The role of pulmonary rehabilitation and increased physical activity and exercise on improving the ventilatory response to exercise.
- The impact of supplemental oxygen and other neonatal treatments on subsequent cardiopulmonary fitness

It is unclear why there appears to be a difference in peak $\dot{V}O_2$ when aerobic capacity is assessed using a cycle ergometer compared with a treadmill. One

possible explanation is differences lower body strength making it difficult to maintain cycling cadence at higher workloads resulting in lower peak outcomes. Further investigation into the mechanisms of the muscle strength and body composition differences, and the implications for peak exercise, may provide useful diagnostic and therapeutic information.

7.7 **Conclusion**

This thesis has documented that despite increased respiratory symptoms, decreased pulmonary function and significant structural lung disease; aerobic exercise capacity is preserved in school aged children born preterm. These children have an increased prevalence of EFL which is related to worse lung function and a lower gestational age. The alterations in ventilatory response to exercise appear to be associated with lower birth weight z-score suggesting that prematurity with intrauterine growth restriction may alter the ventilatory response. It appears these ventilatory responses to exercise are an adaptation to assist preterm children to reach peak exercise.

7.8 Chapter reference list

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Appendix A Respiratory Symptom Questionnaire

Study Number | | | | | |

Occasion No. | 0 | |

BPD Follow-up. Questionnaire –Subsequent visits. DO NOT USE FOR FIRST VISIT.

QUESTIONNAIRE –subsequent visits

A. WHEEZE & ASTHMA

1. Has you child had wheezing or whistling in the chest in the last 12 months? — Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 7

2. How many attacks of wheezing has you child had in the last 12 months? — None ☐ 1 to 3 ☐ 4 to 12 ☐ > 12 ☐

3. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?
   Never woken with wheezing ☐
   Less than one night per week ☐
   One or more nights per week ☐

4. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? — Yes ☐ No ☐

5. In the last 12 months, has your child's chest sounded wheezy during or after exercise? — Yes ☐ No ☐

6. In the last 12 months, has your child had wheeze **not** associated with a cold or chest infection? — Yes ☐ No ☐

7. Has your child ever had asthma? — Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 11

8. Was this diagnosed by a doctor? — Yes ☐ No ☐

9. If yes what age was asthma first diagnosed? _____

10. If the asthma has stopped what age did it stop? _____

11. Has your child ever taken asthma medication? — Yes ☐ No ☐
    Medication: _____

12. Has your child taken asthma medication in the last 3 months? — Yes ☐ No ☐
    Medication: _____

13. Is you child currently on asthma medication? — Yes ☐ No ☐
    Medication: _____

Study Number | | | | | |

Occasion No. | 0 | |

BPD Follow-up. Questionnaire –Subsequent visits. DO NOT USE FOR FIRST VISIT.

B. COUGH

14. Has your child had cough when he/she does NOT have a cold in the last 12 months? — Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 23

15. Is your child's cough worse with exertion? — Yes ☐ No ☐

16. Is your child's cough worse at any time of day? — Yes ☐ No ☐

17. If yes, what time? — First thing in the morning ☐ During the day ☐ At night ☐

18. Is your child's cough worse in any particular weather? — Yes ☐ No ☐

19. If yes, what type of weather? — Cold ☐ Warm ☐

20. Is this cough accompanied by a rattle? — Always ☐ Sometimes ☐ Never ☐

21. Is this cough accompanied by phlegm? — Always ☐ Sometimes ☐ Never ☐

22. If the cough has stopped, what age was your child when cough ceased to be a problem? _____

C. HAYFEVER

23. In the past 12 months, has you child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu? — Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 27

24. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes? — Yes ☐ No ☐

Study Number | | | | | |

Occasion No. | 0 | |

BPD Follow-up. Questionnaire –Subsequent visits. DO NOT USE FOR FIRST VISIT.

25. In which of the past 12 months did this nose problem occur? (please tick any which apply)

January	☐	February	☐	March	☐	April	☐
May	☐	June	☐	July	☐	August	☐
September	☐	October	☐	Nov	☐	Dec	☐

26. In the past 12 months, how much did the nose problem interfere with your child's daily activities?
- Not at all ☐
- A little ☐
- A moderate amount ☐
- A lot ☐

27. Has your child ever had hayfever? Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 31

28. Was this diagnosed by a doctor? Yes ☐ No ☐

29. What age was your child when they first had hayfever? _____

30. If the hayfever has stopped what age did it stop? _____

D. ECZEMA

31. Has you child ever had an itchy rash which was coming and going for at least six months? Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 37

32. Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees, ankles, under the buttocks or around the neck, ears or eyes? Yes ☐ No ☐

33. Has your child had this itchy rash in the last 12 months? Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 37

34. Has the rash cleared completely any time during the last 12 months? Yes ☐ No ☐

35. In the last 12 months, how often , on average, has your child been kept awake by this itchy rash?
- Never woken ☐
- Less than one night per week ☐

Study Number | | | | | | |

Occasion No. | 0 | |

BPD Follow-up. Questionnaire –Subsequent visits. DO NOT USE FOR FIRST VISIT.

One or more nights per week ☐

36. If the rash has cleared, what age was your child when it cleared?_____

37. Has your child ever had eczema? Yes ☐ No ☐

IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 39

38. Was this diagnosed by a doctor? Yes ☐ No ☐

F. RESPIRATORY IRRITANTS

39. Does anyone smoke in the family?
Mother ☐ Father ☐ Other ☐ No ☐

40. Does anyone smoke inside the home? Yes ☐ No ☐

41. Does your family own any pets? (please indicate where they spend their time)

Type	Y/N (and number)	Inside/Outside/Both
Cat		
Dog		
Bird		
Rabbit		
Guinea-pig		
Other (please specify		

Study Number | | | | | |

Occasion No. | 0 | |

BPD Follow-up. Questionnaire –Subsequent visits. DO NOT USE FOR FIRST VISIT.

G. HOSPITAL ADMISSIONS

42. Has your child been admitted to hospital in the last 12 months ?
Yes ☐ No ☐

43. Month of admission Reason for admission

_____
_____
_____
_____
_____
_____
_____
_____
_____

H. OTHER HEALTH ISSUES

44. Does your child have any other health issues not asked about previously?
Yes ☐
No ☐

IF YOU HAVE ANSWERED "YES" PLEASE CONTINUE TO NEXT QUESTION

45 lease give brief details.

_____
_____
_____

46 s your child currently on any medications not listed previously
Yes ☐
No ☐

Please list:_____
_____

47 Does your child regularly see a health professional for these issues?
Yes ☐
No ☐

Appendix B ERJ Open Research Manuscript

ERJ open research

ORIGINAL ARTICLE
PAEDIATRIC PULMONOLOGY

Increased prevalence of expiratory flow limitation during exercise in children with bronchopulmonary dysplasia

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ABSTRACT Evidence regarding the prevalence of expiratory flow limitation (EFL) during exercise and the ventilatory response to exercise in children born preterm is limited. This study aimed to determine the prevalence of EFL as well as contributing factors to EFL and the ventilatory response to exercise in preterm children with and without bronchopulmonary dysplasia (BPD).

Preterm children (⩽32 weeks gestational age) aged 9–12 years with (n=64) and without (n=42) BPD and term controls (n=43), performed an incremental treadmill exercise test with exercise tidal flow–volume loops.

More preterm children with BPD (53%) had EFL compared with preterm children without BPD (26%) or term controls (28%) ($p<0.05$). The presence of EFL was independently associated with decreased forced expiratory volume in 1 s/forced vital capacity z-score and lower gestational age ($p<0.05$). There was no difference in peak oxygen uptake between preterm children with BPD and term controls (48.0 *versus* 48.4 $mL \cdot kg^{-1} \cdot min^{-1}$; $p=0.063$); however, children with BPD had a lower tidal volume at peak exercise (mean difference -27 $mL \cdot kg^{-1}$, 95% CI -49–-5; $p<0.05$). Children born preterm without BPD had ventilatory responses to exercise similar to term controls.

Expiratory flow limitation is more prevalent in children born preterm with BPD and is associated with airway obstruction and a lower gestational age.

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Children born preterm have an increased prevalence of expiratory limitation during exercise associated with reduced lung function and lower gestational age http://ow.ly/jLsk30leOVI

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Introduction

Preterm birth (<37 weeks gestational age) constitutes ~11% of live births worldwide [1]. Children born preterm, and in particular those born very preterm (<32 weeks gestational age), are born with an immature respiratory system characterised by few to no alveoli, insufficient surfactant production and gas exchange, and are at increased risk of developing bronchopulmonary dysplasia (BPD) [2]. Advances in neonatal care, including the widespread introduction of exogenous surfactant and more gentle forms of mechanical ventilation, have increased survival of children born at lower gestational age and birth weight [3]. Contemporary neonatal lung disease, termed "new BPD", is characterised by peripheral lung abnormalities, failed alveolarisation and abnormal pulmonary vascularisation [3], which contribute to long-term respiratory sequelae including airway obstruction [4–6], impaired gas transfer [7], gas trapping [7, 8] and increased respiratory morbidity [5, 6, 9] throughout childhood.

The exercise capacity of children born preterm during the surfactant era is unclear; with some [8, 10–12], but not all [13–15], studies reporting reduced peak exercise capacity. In addition, a limited number of studies report an altered ventilatory response to exercise, including reduced tidal volume (V_T) and increased respiratory frequency [10, 12, 16, 17]. However, the mechanisms underlying altered ventilatory responses to exercise in children with BPD remain unknown. One factor potentially contributing to altered ventilatory responses during exercise may be expiratory flow limitation (EFL). EFL is associated with an impaired ventilatory response to exercise in asthma, cystic fibrosis and chronic obstructive pulmonary disease [18–21]. The single study describing EFL after preterm birth reports an increased prevalence of EFL in children born at <29 weeks gestational age [12]. However, this study did not assess the impact of EFL on exercise capacity or explore risk factors associated with the presence of EFL and thus the broader consequence of their finding is unclear.

We aimed to investigate the ventilatory response to a maximal exercise test in school aged children born very preterm with and without a neonatal diagnosis of BPD. We also aimed to determine the prevalence of EFL and assess any contribution from neonatal exposures on the prevalence of EFL in these children. We hypothesised that children with a neonatal diagnosis of BPD would exhibit an altered ventilatory response to maximal exercise characterised by EFL and dynamic hyperinflation. Further, we hypothesised that the magnitude of the altered ventilatory response would be related to the severity of neonatal lung disease.

Methods

Full methodological details are provided in the online supplementary material.

Participants

Children were recruited to the study if they were aged 9–12 years and either born preterm (⩽32 weeks completed gestational age) or were healthy term-born children as previously described [6]. Preterm children were classified as having BPD if they required at least 28 days of supplemental oxygen before 36 weeks postmenstrual age as per international guidelines [22]. Written informed consent from parents and assent from the child were obtained prior to study enrolment. Ethics approval was obtained from the Princess Margaret Hospital for Children (Perth, Australia) Human Ethics Committee (approval 1760EP).

Pulmonary function testing

Spirometry and lung volume measurements by multiple breath nitrogen washout (Sensormedics Encore 21–1A; Sensormedics, Yorba Linda, CA, USA) were performed in accordance with international guidelines [23, 24], and are reported as predicted z-scores [25, 26].

Peak exercise test

Participants performed an incremental treadmill exercise test (Marquette; Sensormedics, Yorba Linda, CA, USA) in accordance with a modified Balke protocol [27, 28] The testing was performed at ambient conditions within a laboratory and all results are reports at BTPS (body temperature, ambient pressure, saturated with water vapour). Briefly, baseline observations were obtained over 5 min. Subsequently, children ran at a comfortable pace on a gradient of 0% for 2 min after which the gradient was increased to 4% and then by 2% increments every 2 min until volitional exhaustion. A peak exercise test was defined as peak heart rate >90% predicted and physical signs of peak performance (sweating, flushed face and inability to maintain running speed). Peak metabolic (oxygen uptake ($V'O_2$), carbon dioxide production) and ventilatory data (V_T and breathing frequency) were recorded continuously using breath-by-breath analysis (SensorMedics 229 Metabolic Cart; SensorMedics). Breathing reserve was calculated as maximum minute ventilation − maximum voluntary ventilation (MVV), expressed as a percentage of MVV; MVV was calculated as forced expiratory volume in 1 s (FEV_1) × 40 [29].

Tidal flow–volume loops

Tidal flow–volume loops were assessed as reported previously [30] and adapted by our group [31]. Briefly, 3–5 tidal breaths during exercise were recorded followed by a maximal inspiratory capacity (IC) manoeuvre to total lung capacity (TLC) which was recorded at the end of each exercise stage. Placement of the tidal flow–volume loop relative TLC was determined from the IC manoeuvre at the end of each exercise stage. Tidal flow–volume loops were set within the maximal flow–volume loop obtained during baseline spirometry based on IC. TLC was assumed to remain constant throughout the exercise. Dynamic flow limitation was determined if 5% or more of the tidal flow–volume loop tracked or exceeded the maximum flow–volume loop obtained prior to exercise (figure 1).

Breathing strategy during exercise

End expiratory and inspiratory lung volume (EELV and EILV, respectively) were assessed at each stage as a measure of dynamic functional residual capacity (FRC) [31, 32] and expressed as a change from baseline (*e.g.* ΔEELV) and as a percentage of TLC (*e.g.* EELV%TLC). Full details are described in the online supplementary material.

Neonatal data and exercise symptoms

Neonatal variables including gestational age, days of supplemental oxygen and ventilatory support (mechanical ventilation and continuous positive airway pressure (CPAP)) were extracted from medical records and a prospectively maintained neonatal database. Parentally reported exercise symptoms within the preceding 3 months were recorded using a respiratory symptom questionnaire [33]. Children were classified as having current exercise-induced symptoms if parents reported cough, wheeze or shortness of breath on exertion, or symptoms that limited their child's physical activity within the preceding 3 months.

Statistical analysis

Data are reported as mean±SD for normally distributed data and median (interquartile range) for non-normally distributed data. Differences between groups were assessed by Mann–Whitney U-test or Kruskal–Wallis, as appropriate. Bonferroni correction was applied to account for possible type 1 errors due to multiple testing. Chi-squared analysis was used for differences in proportions between groups. This study was powered to detect a 25% difference in EFL to 80% power at a 0.05 significance level.

The relationships between neonatal factors, lung function and EFL were initially assessed using univariate regressions with EFL as a binary (yes/no) outcome. Factors with a significant univariate association ($p<0.05$) with the presence of EFL were included in subsequent stepwise binary logistic regressions. Multi-collinearities between the neonatal predictors were identified and adjusted for by using residuals of independent regressions of the collinear variables. For example, the independent impact of mechanical ventilation on EFL was determined from the residual of the regression between gestational age and mechanical ventilation.

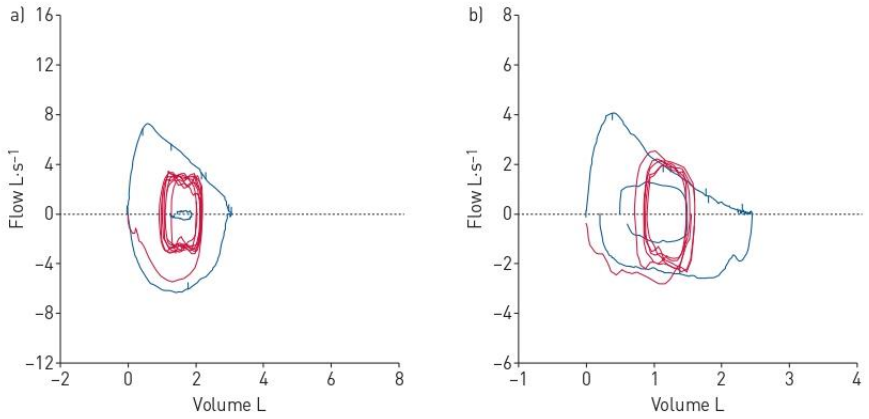

FIGURE 1 Demonstration of the assessment of expiratory flow limitation (EFL). a) A subject with no EFL; b) a subject with EFL >5%.

The effect of lung development (gestational age and birth weight z-score), neonatal lung disease (days of supplemental oxygen, days of mechanical ventilation and days on CPAP), age, sex, height, weight and lung function (FEV_1, forced vital capacity (FVC), TLC, residual volume and FRC z-scores) at the time of the exercise test were included in the logistic regression. Statistical analysis was performed using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

Participants

221 (126 male) children were enrolled in the study including 99 with BPD, 64 without BPD and 58 healthy term-born controls. This cohort is representative of the broader preterm population in Perth during the same birth period, with no differences in neonatal characteristics of the recruited cohort, as reported by our group previously [6]. A valid maximal exercise test (n=171) was completed by 68 preterm children with BPD, 55 preterm children without BPD and 48 healthy term controls. Exercise tests (n=50) were determined invalid if there was a leak in the mask during testing (n=13), equipment malfunction (n=12), early termination of exercise (n=6), physical inability to perform exercise testing (n=13), poor baseline lung function (exercise testing deemed clinically inappropriate) (n=3) or consent not given (n=3). Of the 171 children completing a valid exercise test, 149 children successfully performed the flow–volume loop manoeuvres required for assessment of EFL (64 preterm children with BPD, 42 preterm children without BPD and 43 healthy term controls) and this population will form the basis of all analyses herein (figure 2). Table 1 shows the demographic details of the subjects who successfully completed the exercise test with matched flow–volume loops measurements.

Children without a valid exercise test and matched flow–volume loop measurements had a lower FEV_1 z-score (−0.78 *versus* −0.30) and had a higher prevalence of parentally reported exercise symptoms (65% *versus* 39%) (table E1). Children who performed acceptable exercise flow–volume loops had similar exercise outcomes as those who could not, although they had a lower peak V_T (0.88 L *versus* 1.03 L) (table E1).

Table 2 shows the spirometry, lung volume and maximal exercise test results for the participants that performed a successful exercise test and flow–volume loop measurements. Preterm children with a neonatal diagnosis of BPD had a lower absolute V'_{O_2} peak; however, V'_{O_2} peak was not reduced when expressed relative to bodyweight (table 2). Children with BPD had a lower V_T (mean difference=−27 $mL{\cdot}kg^{-1}$; (95% CI −49–−5); p<0.001) at peak exercise compared with the healthy term-born controls. Similarly, children with BPD had an increased respiratory rate (7 $breaths{\cdot}min^{-1}$ (95% CI 2–12); p<0.001), but minute ventilation and V_T at peak exercise were not different from preterm children without BPD. Preterm children without BPD demonstrated no differences at peak exercise compared with term born controls (table 2).

Similarly, static lung volumes (TLC, FRC and residual volume) at rest and the change in EILV or EELV during exercise (expressed as either an absolute change or as a percentage of TLC), did not differ between preterm children with or without BPD and term-born controls (table 2). Children with BPD had a

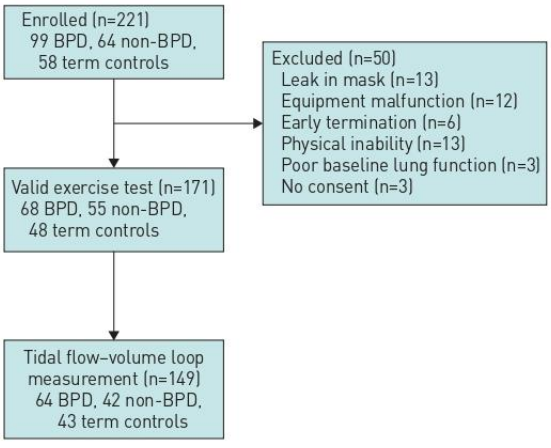

FIGURE 2 Flow diagram of enrolment for the study. BPD: bronchopulmonary dysplasia; non-BPD: preterm children without BPD.

TABLE 1 Neonatal and demographic details of the study population

	BPD	Non-BPD	Term controls
Subjects n	64	42	43
Male (%)	42 (66%)[#,¶]	32 (76%)[#]	18 (42%)
Gestational age (PMA) weeks	26.0 (25–27.5)[¶]	30 (29.1–31.0)	
Birth weight g	843 (709–993)[¶]	1420 (1238–1615)	
Birth weight z-score	−0.15±0.82	−0.12±0.82	
Mechanical ventilation days	15.3 (4.4–32.8)[¶]	0.0 (0.0–1.0)	
CPAP days	14.5 (6.6–24.0)[¶]	0.7 (0.0–3.6)	
Supplemental oxygen days	86.5 (57.5–98.0)[¶]	1 (0.0–3.0)	
Recent exercise symptoms[+]	29 (46%)	15 (38%)	
Doctor diagnosed asthma ever	23 (36%)[¶]	17 (44%)	
Current asthma medication	4 (6%)[¶]	10 (26%)	
Age at test years	10.8±0.6	10.9±0.6	10.6±0.6
Height at test cm	141 (136–146)[¶]	142 (138–148)	145.5 (138–153)
Weight at test kg	32.4 (28.5–37.6)[¶]	36.5 (30.5–41.3)	34.7 (30.0–42.2)

Data are presented as mean±SD, median (interquartile range) or n (%), unless otherwise stated. Note the term controls did not have any neonatal intervention or respiratory symptoms. Not all children completed every question in the symptom questionnaire. BPD: bronchopulmonary dysplasia; non-BPD: preterm children without BPD; PMA: postmenstrual age; CPAP: continuous positive airway pressure. [#]: p<0.05 compared with healthy term controls; [¶]: p<0.05 compared with non-BPD; [+]: exercise symptoms included parentally reported wheeze, cough and shortness of breath during exertion.

TABLE 2 Lung function and exercise variables for children who completed a successful maximal exercise test

	BPD	Non-BPD	Term controls
Subjects n	64	42	43
FEV_1 z-score	−0.83 (−1.57–−0.17)[#,¶]	0.09 (−0.91–0.37)	0.04 (−0.57–0.61)
FVC z-score	−0.09 (−0.65–0.87)[#]	0.37 (−0.25–0.97)	0.24 (−0.65–0.86)
FEV_1/FVC z-score	−1.35 (−2.59–−0.80)[#]	−0.87 (−1.52–−0.41)[#]	−0.42 (−1.06–0.48)
TLC z-score	−0.28 (−0.92–0.53)	0.25 (−0.50–0.78)	−0.12 (−1.06–0.48)
FRC z-score	0.29 (−0.64–0.87)	0.71 (−0.19–1.91)[#]	−0.07 (−0.68–0.36)
RV z-score	−0.21 (−1.05–0.30)	0.01 (−0.85–0.77)	−0.33 (−1.16–0.71)
$\dot{V}'O_2$ peak L·min^{-1}	1.53 (1.40–1.76)[#,¶]	1.78 (1.49–1.95)	1.69 (1.45–2.17)
$\dot{V}'O_2$ peak mL·kg^{-1}·min^{-1}	47.7 (42.8–53.2)	46.1 (42.5–51.7)	48.1 (45.5–52.4)
$\dot{V}'O_2$ at AT mL·kg^{-1}·min^{-1}	26.6 (13.9–47.2)	31.0 (15.8–38.8)	34.7 (13.6–45.3)
Peak RQ	1.01 (0.98–1.02)[#]	1.03 (1.01–1.06)	1.04 (1.02–1.06)
Peak heart rate beats·min^{-1}	196 (187–205)	195 (185–202)	200 (195–206)
Peak V_T mL·kg^{-1}	24 (21–27)	27 (23–30)	28 (24–31)
Peak f_R breaths·min^{-1}	64 (54–72)[¶]	54 (49–63)	58 (54–68)
Peak $\dot{V}'_E$ L·min^{-1}·kg^{-1}	1.53 (1.36–1.72)	1.40 (1.30–1.68)	1.55 (1.42–1.81)
Breathing reserve %	34.0 (28.2–35.8)	37.2 (27.0–38.3)	34.4 (30.0–37.1)
EFL n (%)	34 (53%)[#,¶]	11 (26%)	12 (28%)
EFL%V_T	27.5 (0.0–60.0)[#,¶]	0.0 (0.0–26.5)	0.0 (0.0–25.0)
ΔIC mL	25 (−83–193)	110 (−78–225)	25 (−90–203)
ΔEELV mL	−30 (−145–200)	−50 (−180–170)	−15 (−175–137)
ΔEILV mL	397 (146–557)	371 (238–648)	368 (157–723)
EELV %TLC rest	32.0 (27.5–37.4)	32.1 (28.0–35.1)	30.1 (25.8–34.9)
EELV %TLC peak	31.8 (28.4–35.9)	31.9 (27.6–36.6)	29.9 (27.0–33.7)

Data are presented as median (interquartile range), unless otherwise stated. BPD: bronchopulmonary dysplasia; non-BPD: preterm children without BPD; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; $\dot{V}'O_2$: oxygen uptake; AT: anaerobic threshold; RQ: respiratory quotient; V_T: tidal volume; f_R: respiratory frequency; $\dot{V}'_E$: minute ventilation; EFL%V_T: percentage of tidal volume assessed as meeting or exceeding the maximum flow–volume loop; IC: inspiratory capacity; EELV: end expiratory lung volume; EILV: end inspiratory lung volume. [#]: p<0.05 compared with healthy term controls; [¶]: p<0.05 compared with non-BPD.

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significantly lower FEV_1 z-score compared with healthy controls (−0.98 (95% CI −1.5–−0.46); p<0.001) and non-BPD preterm children (−0.70 (95% CI −1.23–−0.17); p=0.005). The FEV_1/FVC z-score of preterm children with (−1.06 (95% CI −1.61–−0.51); p<0.001) and without BPD (−0.71 (95% CI −1.31–−0.12); p=0.012) was lower compared with term-born controls.

Tidal flow–volume loop assessment

Approximately half of the children with BPD (53%) exhibited EFL during maximal exercise testing, which was significantly more prevalent than in the non-BPD and healthy term control groups (Chi-squared analysis p<0.01) (figure 3). The prevalence of EFL was not significantly different between the healthy term controls and the preterm children without BPD (26% and 28%, respectively).

Differences in neonatal, spirometry and maximal exercise test outcomes in preterm children with and without EFL are presented in table 3. Preterm children with EFL had a significantly longer duration of supplemental oxygen and mechanical ventilation as well as a significantly worse baseline FEV_1 and FEV_1/FVC z-score compared with preterm children without EFL. V_T, breathing frequency and breathing strategy at peak exercise and parentally reported frequency of symptoms during exercise were not significantly different between preterm children with EFL and those that did not experience EFL during exercise. Days of supplemental oxygen, FEV_1 z-score and FEV_1/FVC z-score were significantly associated with the presence of EFL on univariate analysis (table E2). FEV_1/FVC z-score and days of supplemental oxygen after accounting for gestational age were subsequently included in the multivariate analysis; gestational age was also included to assess the impact of lung development. The impact of each of these in the multivariate model can be seen in table E3. Binary logistic regression analysis showed that a reduced FEV_1/FVC z-score and lower gestational age were independent predictors of EFL developing during a maximal exercise test (table 4).

Discussion

We investigated the impact of very preterm birth on the ventilatory response to exercise in school-aged children and determined the factors associated with presence of EFL in these children. We report that children with BPD have an altered breathing pattern during exercise, which is characterised by rapid, shallow breathing. Furthermore, we show that half of children born very preterm with BPD exhibit EFL during a maximal exercise test. We found that the presence of EFL was independently predicted by poorer lung function and a lower gestational age.

This study shows that preterm children with a neonatal classification of BPD have reduced minute ventilation due to a markedly decreased V_T with an increased breathing frequency at peak exercise. These observations are consistent with a rapid and shallow breathing pattern and are in keeping with previous exercise studies in children born preterm [10, 12, 16, 17]. We hypothesised that this rapid and shallow breathing pattern would be associated with an increased prevalence of EFL. However, the absence of significant differences in V_T, minute ventilation and breathing frequency between those with and without EFL during exercise suggests that EFL is not contributing to the altered breathing response to exercise.

Our study confirms that EFL is highly prevalent in children with BPD [12]. Our observations of the prevalence of EFL in preterm children with (53%) and without (26%) BPD are similar to the prevalence reported by MacLean *et al.* [12] (47% and 33% in children with and without BPD, respectively). Our

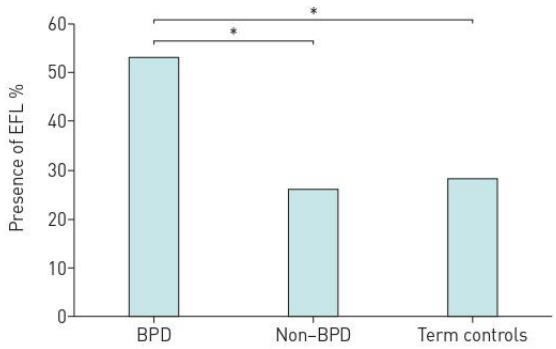

FIGURE 3 Prevalence of expiratory flow limitation (EFL). BPD: bronchopulmonary dysplasia; non-BPD: preterm children without BPD. *: p<0.05.

TABLE 3 Differences between preterm participants with and without expiratory flow limitation (EFL)

	With EFL	Without EFL
Subjects n	45	61
BPD	34 (76%)*	30 (49%)
Male	28 (62%)	43 (70%)
Gestation PMA weeks	27.0 (25.0–29.2)	28.6 (25.3–30.2)
Mechanical ventilation days	5.0 (1.3–30.1)	2.0 (0.0–13.5)
CPAP days	6.5 (1.0–24.9)	5.6 (0.6–15.7)
Supplemental oxygen days	74.0 (28.0–94.5)*	22.0 (1.0–83.5)
FEV_1 z-score	−1.31±0.96*	−0.12±0.83
FVC z-score	−0.40 (−0.57–0.78)	0.22 (−0.51–1.13)
FEV_1/FVC z score	−1.85±0.89*	−0.71±0.83
$\dot{V}O_2$ peak L·min^{-1}	1.54 (1.40–1.78)	1.63 (1.42–1.86)
$\dot{V}O_2$ peak mL·kg·min^{-1}	49.6 (43.2–52.3)	47.5 (42.4–52.3)
Peak RQ	1.03 (1.01–1.05)	1.01 (0.99–1.02)
Maximum heart rate beats·min^{-1}	197 (187–207)	195 (186–202)
V_T L·kg^{-1}	25 (22–28)	25 (22–28)
f_R breaths·min^{-1}	62 (53–70)	60 (52–69)
Maximum $\dot{V}E$ L·min^{-1}·kg^{-1}	1.55 (1.36–1.74)	1.43 (1.30–1.67)
Breathing reserve %	31.1 (26.4–35.6)	35.5 (29.3–38.5)
Exercise symptoms	21 (48%)	23 (40%)
Docter diagnosed asthma ever	23 (52%)*	17 (29%)
Current asthma medication	4 (9%)	10 (17%)*

Data are presented as mean±SD, median (interquartile range) or n (%), unless otherwise stated. Not all children completed every question in the symptom questionnaire. BPD: bronchopulmonary dysplasia; PMA: postmenstrual age; CPAP: continuous positive airway pressure; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $\dot{V}O_2$: oxygen uptake; RQ: respiratory quotient; V_T: tidal volume; f_R: respiratory frequency; $\dot{V}E$: minute ventilation. *: p<0.05.

study adds to the current literature in that we report this prevalence over a wider gestational age (up to 32 weeks gestational age compared with <29 weeks gestational age). Our data suggests that flow limitation during exercise is not limited to those children surviving extreme preterm birth. Selection of appropriate predicted values for lung function and exercise testing in a paediatric population is hampered by the lack of large normative datasets. We have previously confirmed that the Global Lung Function Initiative reference equations are valid for an Australian population [34]. While we cannot confirm that the predicted values for lung volumes are valid in our population, our inclusion of healthy term controls increases our ability to interpret our data and it is unlikely that the differences between groups reported here are associated with differences in group demographics.

The presence of EFL in adults with obstructive lung disease is often linked to an increase in operating lung volumes (EELV and EILV) and dynamic hyperinflation during exercise [18, 21]. However, the preterm children who developed EFL during this study (with reduced FEV_1/FVC; table 4), did not show dynamic hyperinflation or changes in operating lung volumes. Preterm children with EFL were more likely to have had a doctor diagnosis of asthma in the past compared with those without; however, they were less likely to be currently using asthma medication. While the current use of asthma medication may blunt the ventilatory response to exercise, all children were assessed without the use of a short-acting bronchodilator

TABLE 4 Binary logistic regression for neonatal and spirometry variables, for presence of expiratory flow limitation

Variable	Odds ratio (95% CI)	p-value	R^2
FEV_1/FVC z-score	0.184 (0.084–0.401)*	<0.001	0.477
Gestational age (PMA)	0.799 (0.640–0.997)*	0.047	
Supplemental oxygen days	1.22 (0.674–2.200)	0.514	

FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PMA: postmenstrual age. *: p<0.05.

medication prior to exercise. Given the lack of dynamic hyperinflation during exercise this suggests that current asthma may not be significantly contributing to the presence of EFL during peak exercise. We hypothesise the role of prematurity *per se*, rather than severity of neonatal lung disease or recent symptoms is the primary cause of EFL in children born very preterm. This is confirmed in the multivariate analysis demonstrating that the prevalence of EFL was significantly associated with lower gestational age and not markers of the severity of BPD, such as supplemental oxygen use (table 4).

In support of our hypothesis, Rideau Batista Novais *et al.* [17] identified that very low birth weight children had a rapid breathing pattern during exercise and reduced inspiratory muscle strength, suggesting that an increased inspiratory resistive load may lead to the early onset of inspiratory muscle fatigue. Inspiratory muscle load, inspiratory muscle fatigue and/or impaired contractile function of the respiratory muscles may result in the inability of preterm children to increase their operating lung volumes during maximal exercise rather than hyperinflation and hence to a higher prevalence of EFL. Furthermore, the damage associated with injurious tidal volumes during mechanical ventilation and altered peripheral lung development affects the mechanical properties of the lung [3]. Reduced pulmonary compliance increases elastic load on breathing. Increased load (work of breathing) that exceeds the ability of the respiratory muscles to generate sufficient force to increase the operating lung volumes when higher flows are required may result in EFL. In addition, the higher elastic work of increasing the lung volumes may not be tolerated in children born prematurely, preventing the maintenance of a higher operating lung volume. We reported a similar mechanism in obese children with an increased load on the chest wall and diaphragm resulting in a similar response to exercise to that seen in the preterm population, *i.e.* increased prevalence of EFL without any change in operating lung volumes [31].

Alternatively, the increased prevalence of EFL could be secondary to reduced lung and/or airway size. Children [30, 35] and women [36, 37] have increased EFL compared with adult males; both groups have a lower FEV_1 and FEV_1/FVC compared to adult males due to smaller lung volumes and airway size, a factor associated with increased prevalence and severity of EFL [36, 37]. However, as the preterm children with EFL did not exhibit differences in measured static lung volumes (FVC, TLC, FRC and residual volume; table 3 and table E2), it is unlikely that the EFL observed in this study is associated with inherent differences in lung size. Lung volumes in this study were assessed using multiple breath washout and it is known that this technique can underestimate actual FRC in the presence of significant airway obstruction leading to trapped gas. It is therefore feasible that our assessments of FRC (and hence TLC) may be underestimated, exploration of this mechanism using whole body plethysmography to measure static lung volumes may be of value. However, given that there were no changes in measures of dynamic lung volume (*i.e.* IC during exercise) we do not feel that this is a significant limitation to our study. The repeatability of EFL in the paediatric population has not been well described and is difficult to assess due to the need to repeat the exercise test. However, the measurement of EFL is dependent upon accurate measurement of IC and as we did not see any changes in the measurement of IC in this study we are confident this measurement is valid.

Children with BPD had an increased prevalence of EFL in the presence of a normal response to exercise and no differences in exercise symptoms; therefore, the clinical implications of this increased EFL are unclear. While we report no differences in exercise symptoms in children with EFL, we were unable to record rated perceived exertion scores or symptom scores at peak exercise as many children were unable to provide a clear answer. As such, we are unable to identify if there are differences in symptoms in children with EFL at peak exercise. We suggest that children born preterm may maintain normal physical activity by moderating their ventilatory response or work in shorter exercise bouts before EFL becomes clinically significant. Further investigation into the mechanisms driving EFL and the functional effects of EFL are necessary to fully identify its impact on exercise of different modes, intensities and durations, and the long-term impact of EFL as these children continue to grow.

Conclusion

We investigated the impact of EFL on aerobic capacity and the ventilatory response to a maximal exercise test in children born preterm with and without a neonatal diagnosis of BPD. We show that children born preterm have a higher prevalence of EFL than term-born controls, and that this was not associated with an altered ventilatory response to exercise. The prevalence of EFL in children born before 32 weeks gestation was associated with a lower gestational age and reduced lung function.

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Author contributions: C.A O'Dea performed data measurement and interpretation, conducted the statistical analysis and drafted the initial versions of the manuscript. K. Logie and G.L. Banton performed data measurement and revision of the manuscript. S.J. Simpson performed data measurement, assisted with data analysis and interpretation, and revision of manuscript. A. Maiorana, A.C. Wilson, J.J. Pillow and G.L. Hall designed the study, obtained funding, had oversight of data collection and interpretation, and reviewed the manuscript.

Conflict of interest: K. Logie has nothing to disclose. A. Maiorana has nothing to disclose. G.L. Banton has nothing to disclose. S.J. Simpson has nothing to disclose. G.L. Hall has nothing to disclose. C.A. O'Dea has nothing to disclose. J.J. Pillow has nothing to disclose. A.C. Wilson has nothing to disclose.

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