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# CONSEQUENCES OF PRETERM BIRTH ON LUNG FUNCTION, PHYSICAL ACTIVITY AND EXERCISE CAPACITY

Jenny Svedenkrans



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## **Consequences of preterm birth on lung function, physical activity and exercise capacity**

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To Axel, Elmer, Anna and Herman. You are my heroes.



## ABSTRACT

The incidence of preterm birth is increasing worldwide. Some of the survivors of preterm birth will be affected by varying degrees of disabilities like lower cognitive or respiratory function. Moreover, the survivors will encounter an increased risk of non-communicable diseases like hypertension, coronary heart disease, and diabetes, later in life. More knowledge is needed in order to prevent these adverse outcomes.

Physical activity (PA) and exercise have well-established positive effects on several non-communicable diseases. In addition, there is growing evidence that physical activity has a positive effect on cognitive function. In study I and II, we used information from the conscript register and linked it to birth characteristics in the medical birth register in order to associate preterm birth to later exercise capacity and cognitive function. The results revealed that young men born preterm have lower exercise capacity than men born at term, with a step-wise relation to gestational age. Furthermore, cognitive function was positively associated with increases in exercise capacity, across all gestational ages. Men born extremely preterm (<28 weeks gestational age) with the lowest exercise capacity, exhibited the lowest results on the cognitive function test.

To evaluate if a reduced exercise capacity in young adulthood could be a consequence of less physical activity in childhood, 71 children born extremely preterm and 87 controls born at term, wore an activity monitor on the wrist for seven days at 6.5 years of age. Extremely preterm boys were less active than term boys, which could be linked to severe brain injury during infancy, which was more prevalent in preterm boys. There was no statistically significant difference in physical activity when comparing all preterm born children with controls. From study I-III we conclude that preterm birth and morbidities during infancy are associated with level of PA in childhood. Furthermore, prematurity can be correlated to lower exercise capacity in young adulthood and exercise capacity is related to cognitive function. Further studies need to reveal if increased PA could mitigate these late outcomes of preterm birth.

Exercise capacity and physical activity could be affected by pulmonary function. Children born preterm may develop bronchopulmonary dysplasia (BPD) in infancy, a chronic lung disease which may affect respiratory function through childhood and into adult life. The diagnostic criteria for the disease lack objectivity and may not reflect the level of respiratory function. To test the utility of a physiological definition of BPD severity, 200 infants born very and extremely preterm had a modified oxygen reduction test at 36 weeks postmenstrual age. Values of shift (kPa), ventilation:perfusion ratio, and shunt (%) were derived from analysis of the shape and position of the saturation ( $SpO_2$ ) vs pressure of inspired oxygen curve, using a dedicated computer program. Shift was shown to be the most useful measure, approximately corresponding to the extra supplemental oxygen required for a sick infant to achieve the same  $SpO_2$  as a respiratory healthy infant. We conclude that shift could provide a physiological based, continuous outcome measure of BPD severity, with the possibility to increase objectivity of BPD diagnostics. More studies are needed to evaluate short-term repeatability and to understand the prognostic value.





## LIST OF SCIENTIFIC PAPERS

- I. SVEDENKRANS J, Henckel E, Kowalski J, Norman M, Bohlin K.  
Long-term Impact of Preterm Birth on Exercise Capacity in Healthy Young Men: A National Population-based Cohort Study.  
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- II. SVEDENKRANS J, Kowalski J, Norman M, Bohlin K.  
Low Exercise Capacity Increases the Risk of Low Cognitive Function in Healthy Young Men Born Preterm: A Population-Based Cohort Study.  
PLoS One. 2016 Aug 22;11(8):e0161314. doi: 10.1371/journal.pone.0161314.  
eCollection 2016
- III. SVEDENKRANS J, Ekblom Ö, Domellöf M, Fellman V, Norman M, Bohlin K.  
Physical Activity in 6.5 year old children born extremely preterm.  
*Manuscript*
- IV. SVEDENKRANS J\*, Stoecklin B\*, Jones J G, Gill A W, Doherty D, Pillow J J.  
Physiological basis of the NICHD BPD classification: a prospective observational study in very preterm infants. \*shared first authorship  
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## LIST OF ABBREVIATIONS

AGA	Appropriate for gestational age
BMI	Body mass index (m <sup>2</sup> /kg)
BPD	Bronchopulmonary dysplasia
BW	Birth weight
BWSDS	Birth weight standard deviation score
CHARM	Comprehensive heart and respiratory measurements
CP	Cerebral Palsy
CPAP	Continuous positive airway pressure
DCD	Developmental coordination disorder
DOHAD	Developmental origin of health and disease
EXPRESS	Extremely preterm infants in Sweden study
FSIQ	Full scale IQ
HHFNC	Humidified high flow nasal cannula
GA	Gestational age
IVH	Intraventricular hemorrhage
MVPA	Moderate to vigorous physical activity
MBR	Medical Birth Register
NDD	Neurodevelopmental disability
NEC	Necrotizing enterocolitis
NICHD	National Institute of Child and Health Development
ODC	Oxygen dissociation curve
ORT	Oxygen reduction test
PA	Physical activity
PDA	Patent ductus arteriosus
PIFCO	Preterm infant functional and clinical outcomes
PIO <sub>2</sub>	Pressure of inspired oxygen
PMA	Postmenstrual age
PHVD	Post hemorrhagic ventricular dilatation
PPV	Positive pressure ventilation
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity

SED	Sedentary physical activity
SGA	Small for gestational age
SpO <sub>2</sub>	Peripheral oxygen saturation
V <sub>A</sub> /Q	Ventilation:perfusion ratio
VO <sub>2</sub>	Oxygen consumption
W	Watt
W <sub>max</sub>	Maximal exercise capacity in Watt

# 1 INTRODUCTION

## 1.1 REFLECTIONS ON WRITING A THESIS

Neonatology was never on my list. Neither was research. As a child, I never dreamt of going to medical school or even to get an academic degree. In a home without academic tradition, these things were not discussed. It was only at the end of my high school years, that one of my best friends' father suggested that I should go into medical school. He thought it would suit me. It is one of the best advices that I have ever gotten and I'm quite sure it changed my life.

I really liked medical school, and I was glad to be able to do my training in pediatrics. But already on my first day at the neonatal unit at Karolinska Huddinge, I knew that neonatology was truly something for me. I loved it from the very first moment. I loved the intensity and the feeling that anything could happen. I was happy to do manual work. And, to be honest, I loved being able to save lives. Neonatology is engaging and close to life and death. You work extremely hard but at the same time, the rewards are fast and frequent. It's an adrenaline junkies' heaven.

Research, however, is not at all like that. The rewards come slowly after a lot of work. Sometimes the reward is more like a relief since you tried so hard to reach it and you desperately need it to be able to go on. The daily life in research is very far away from the intensity in neonatology. At times you get tired and bored, and slightly hyperactive personalities like me may sometimes lose focus and get nothing done. Nevertheless, I was curious, and wanted to know more. I also realized that I needed something that was not as intense all the time. Something that would take time and not force me to rush every day. The challenge, however, was to work with something for several years, before seeing any results.

As time went by I realized that I started to appreciate the small rewards in research. Like when you read an elegant study, when you can start analyzing your new results or when you have a really engaging scientific discussion. When you get a new idea!

Although including a lot of effort, I really enjoyed writing this thesis. I feel privileged that I got the opportunity. Lack of academic tradition may have prevented me from dreaming of a doctoral degree as a child, but the support and encouragement that I got from my family definitely helped me to finish it. It would never have been possible without you. The finalization of a thesis, however, is not the end but the beginning of something new, and I am very grateful to have found the perfect combination of intense clinical work in neonatology and more time for reflection in research. I hope I will be able to continue for many years to come.





## 2 BACKGROUND

### 2.1 PRETERM BIRTH

#### 2.1.1 Definitions

All infants born before 37 weeks of gestation are considered preterm. While infants being born at 35-36 weeks gestation usually can be cared for in the ordinary postnatal ward, with special attention on temperature and feeds, the need for medical interventions increase with decreasing gestational age (GA). Extremely preterm infants commonly need full intensive care with ventilator support, parenteral nutrition, intravascular catheters, and antibiotics due to bacterial infections. Furthermore, the extremely premature infants are at greater risk to suffer from life-long consequences of their preterm birth.

Prematurity can be further classified into extremely preterm (<28 weeks), very preterm (28-31<sup>6</sup> weeks), and moderately or late preterm (32-36<sup>6</sup> weeks). In this thesis, study I and II include the whole range of prematurity, study III a subgroup of extremely preterm infants (<26 w), whereas study IV includes very and extremely preterm infants (<32 weeks).

#### 2.1.2 Incidence and survival

The worldwide incidence of preterm birth has been estimated to 11.1%, ranging from 5% in northern Europe to 18% in Malawi.<sup>5</sup> Swedish data from the Medical Birth Register show that the incidence of preterm birth was 5.6% in 2015 (Central bureau of statistics, Sweden).

Worldwide, the incidence is increasing<sup>5</sup> and the consequences are large. Preterm birth accounts for approximately 14% of the mortality under five years of age and it is the major cause of neonatal deaths in the world.<sup>6</sup> For the survivors, preterm birth increases the risk of

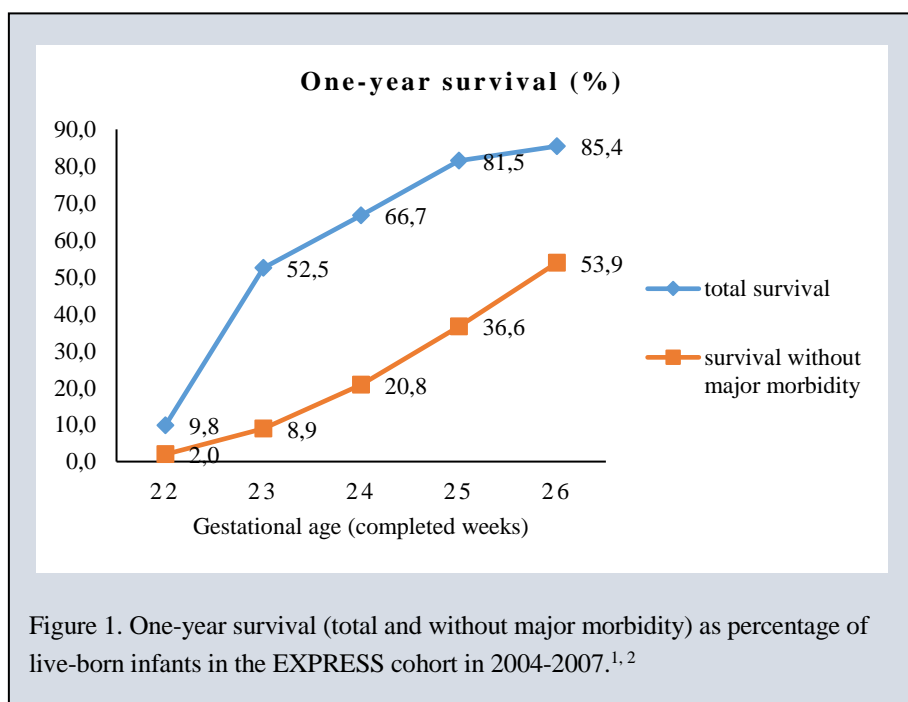


Figure 1. One-year survival (total and without major morbidity) as percentage of live-born infants in the EXPRESS cohort in 2004-2007.<sup>1,2</sup>

lower cognitive function, Cerebral Palsy (CP), lower academic achievement, impaired lung function, diabetes and cardiovascular diseases.<sup>7-15</sup> Still in young adulthood the risk of early death is increased after preterm birth.<sup>16</sup> The risk of neonatal death

increases with immaturity, as shown by the survival data from the Extremely Preterm Infants in Sweden Study (EXPRESS), a national population-based cohort of children born 2004-

2007.<sup>2</sup> The one-year survival rates following extremely preterm birth for children are shown in figure 1. Starting with 10% at 22 weeks, there was a rapid increase in survival for every week of gestational age, and for infants being born at 26 weeks, the survival was 85%.<sup>2</sup>

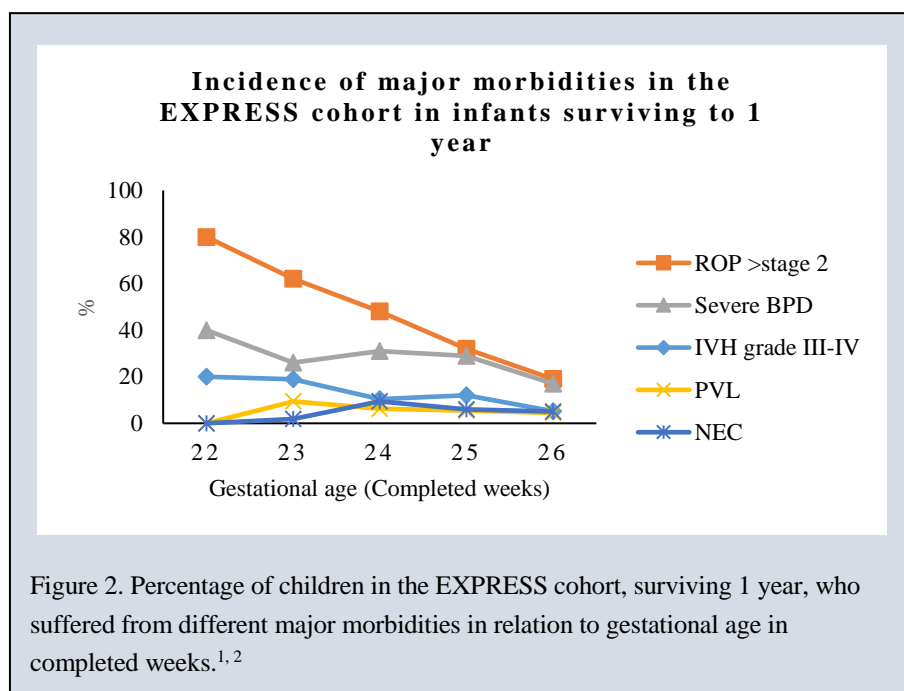
## 2.2 MORBIDITIES OF PRETERM BIRTH

Survival is not the only goal with neonatal care. Ideally, none of the surviving infants should suffer from major disabilities. One way to get an early indication of the long-term outcome is to measure major morbidities, often referred to as Intraventricular hemorrhage (IVH) grade III or more, periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP) stage 3 or more, and severe bronchopulmonary dysplasia (BPD). The risk of major morbidities will increase with lower GA as will the risk of a worse long term outcome.<sup>17</sup> In the EXPRESS cohort, the chance to survive one year without any major morbidity, was 2%

at 22 weeks GA compared to 54% at 26 weeks (figure 1).<sup>1,2</sup> The

incidence of the different major morbidities in relation to GA in completed weeks within the EXPRESS cohort is shown in figure 2. The major

morbidities are described further, later in this chapter.



### 2.2.1 Respiratory distress syndrome

Immaturity of the lungs imply one of the largest risks with preterm birth. Surfactant deficiency will cause low compliance of the lungs and collapsible airways. The consequence is respiratory distress syndrome (RDS), which without treatment may lead to respiratory failure and death. Before the introduction of antenatal steroids and the possibility to give exogenous surfactant, this was a very important contributor to mortality in preterm infants.<sup>18-21</sup> Between 1988 and 1991, when surfactant instillation became an established treatment for RDS in the United States, mortality from RDS was decreased by 28%.<sup>22</sup> CPAP treatment is enough for milder cases of RDS, whereas severely sick infants need mechanical ventilation. The risk of RDS increases with immaturity, nonetheless, most extremely preterm infants

suffer from RDS at some degree. RDS was traditionally considered as the first stage of the development into bronchopulmonary dysplasia (BPD).<sup>23</sup> Infants not recovering from the respiratory distress within 28 days were considered as having BPD, however the interpretation and definition of BPD has changed over time.

## 2.2.2 Bronchopulmonary Dysplasia

*Bronchopulmonary dysplasia was first described fifty years ago.<sup>23</sup>*

### 2.2.2.1 The classic presentation of BPD

In 1967 a new presentation of pulmonary disease in infants was described by Northway and colleagues.<sup>23</sup> It was suggested to be a consequence of severe RDS and related to low gestational age (GA), prolonged mechanical ventilation and high concentrations of supplemental oxygen (80-100%). Infants with respiratory difficulties after one month (chronic phase) had radiographic findings of irregular dense strands, large lucent areas and in some cases, cardiomegaly. In autopsy, irregularly aerated lungs were seen, with a combination of atelectasis and emphysema, hypertrophic peribronchiolar smooth muscles, diffuse fibroproliferation, hypertensive remodelling of pulmonary arteries and decreased alveolarisation and surface area. The disease was named bronchopulmonary dysplasia (BPD).<sup>23</sup> Explanations provided were oxygen toxicity, pulmonary healing from severe RDS or a combination of both. Later, high ventilator

*The infants dying from respiratory distress syndrome in the first paper on BPD had a median gestational age of 30 weeks.<sup>23</sup>*

pressures, barotrauma and alveolar rupture was added to the proposed explanatory factors.<sup>24</sup>

### 2.2.2.2 The “new” bronchopulmonary dysplasia

After the establishment of surfactant therapy in the 80s, antenatal steroids in the 90s and more gentle ventilation strategies, the presentation of BPD changed.<sup>18, 25, 26</sup> Most infants suffering from BPD today are still exposed to prolonged mechanical ventilation and oxygen supplementation, however to a much smaller extent than in the classic form, and a large proportion of the infants have a mild RDS to start with. The infants suffering from the “new” BPD are less mature and have lower birth weights than previously. The main findings in affected infants’ lungs are that the alveoli are larger and simplified in comparison to normal lungs and the arteries dysmorphic, suggesting a disruption of the distal lung growth. The “new” BPD is nowadays referred to as just BPD, which will also be the term used further on in this thesis.

### 2.2.2.3 Clinical presentation of BPD

The common contemporary BPD patient is an extremely preterm infant with initial RDS that usually responds promptly to surfactant treatment. Many infants will tolerate extubation, whereas others, despite a “honeymoon” period on ventilation with low pressure requirements and no or low oxygen supplementation, will need prolonged mechanical ventilation. The infant subsequently deteriorates with increased need of oxygen supplementation and/or

increased pressures on the ventilator. The deterioration can commonly be attributed to a patent ductus arteriosus (PDA) or be triggered by bacterial infection.<sup>27</sup> The severe cases will be dependent on supplemental oxygen or even ventilation for several months to years. In particular infants with severe BPD may develop pulmonary hypertension, which is associated with a poor outcome with mortality rates between six and thirty-eight percent.<sup>28</sup> BPD is a combined restrictive and obstructive disease which changes over time. The restrictive component (poor compliance) tend to normalize during the first 1-2 years, whereas the obstructive component tend to predominate later in life causing wheeze and asthma-like symptoms.<sup>29, 30</sup>

#### 2.2.2.4 Risk factors of BPD

In the early reports on BPD some of the affected infants were term or near term.<sup>23</sup> However, the contemporary BPD almost exclusively affect preterm infants, and the risk increases with decreasing GA, prolonged mechanical ventilation and oxygen therapy. Several contributory factors have been proposed (listed in Table 1). It is clear that BPD is a multifactorial disease and the risk of severe disease increases with more of these factors present. One risk factor may also be linked to another, for example, PDA increases the risk of edema, prolonged ventilation and need for more supplemental oxygen. Infection or inflammation may disturb alveolar development which may lead to increased need for respiratory support and increased oxygen supplementation.

<b>Table 1. Risk factors for the development of bronchopulmonary dysplasia (BPD)</b>	
Prematurity <sup>23, 26</sup>	Genetic predisposition <sup>31, 32</sup>
Mechanical ventilation <sup>23, 24, 26, 33</sup>	Early adrenal insufficiency <sup>34-36</sup>
Oxygen toxicity <sup>23</sup>	PDA <sup>27, 37, 38</sup>
Inflammation with or without Infection <sup>27, 39-41</sup>	Excessive fluid administration <sup>42-44</sup>
Nutritional deficiency <sup>45-47</sup>	
PDA - Persistent ductus arteriosus	

#### 2.2.2.5 Diagnostics of bronchopulmonary dysplasia

Bronchopulmonary dysplasia has had several definitions over the years, resulting in difficulties to compare the incidence of PBD over time and between studies. The workshop on bronchopulmonary dysplasia in 1978 first defined BPD as oxygen dependency past 28 days in addition to typical radiographic findings.<sup>48</sup> The definition then altered between oxygen dependency during all first 28 days, on day 28, in total 28 days or more, which gave different incidence numbers.<sup>26</sup> All definitions, however, had oxygen dependency as criteria. Below, the two most frequently used definitions are described, as well as the method which is further explored in this thesis.

### 2.2.2.5.1 NICHD definition

As BPD developed from the classic form into the new BPD, the need for a graded definition grew. Shennan and collaborators described that oxygen dependency for 28 days had a positive predictive value for reduced pulmonary function at two years of age of only 38%. If choosing 36 weeks PMA instead, the positive predictive value improved to 63% whereas the negative predictive value was 90%.<sup>49</sup> Subsequently, in 2000 the NICHD workshop<sup>50</sup> agreed

<b>Table 2. Definition of BPD according to NICHD criteria</b>		
Gestational age	<32 w	≥32 w
Time point for assessment	36 w PMA or discharge whichever comes first	>28 d but <56d postnatal age or discharge to home, whichever comes first
BPD at all	treatment with oxygen >21% for ≥28 d	
Mild BPD	breathing room air at assessment	
Moderate BPD	need for <30% oxygen at assessment	
Severe BPD	need for ≥30% oxygen and/or positive pressure (PPV or CPAP)	
w- weeks, d - days, PMA - postmenstrual age, PPV - positive pressure ventilation, CPAP - Continuous positive airway pressure. Jobe et al <sup>50</sup>		

on the definition that is still the most commonly used (table 2). The definition, however, has several problems. First, the diagnosis is dependent on the treatment that the infant is given. The choice to give oxygen supplementation is subjective, depending on

local guidelines for accepted peripheral oxygen saturation levels (SpO<sub>2</sub>) and individual physicians' decisions to treat. Second, oxygen dependency is affected by other intrinsic and external factors such as hemoglobin concentration and altitude. Third, the need for mechanical ventilation or CPAP at 36 weeks PMA does not necessarily reflect poor alveolar maturation, but could instead be a sign of immature respiratory drive, collapsible airways or poor diaphragmatic function. Consequently the diagnostic criteria lack objectivity and cannot reliably be used for comparisons of incidences nor within or between infants neither between clinical settings.

### 2.2.2.5.2 The oxygen reduction test according to Walsh

The oxygen reduction test (ORT)<sup>51</sup> was developed to meet the need of a more physiologic and objective definition of BPD. Infants with the need for positive pressure ventilation (PPV) or CPAP or ≥30% supplemental oxygen at 36 weeks PMA would be considered as having BPD, while infants breathing room air would be considered as not having BPD. The infants treated in < 30% oxygen would go through an oxygen reduction test, when oxygen was reduced by 2% every 10 minutes until reaching room air. Failure of the test was defined as SpO<sub>2</sub> 80-87% for five minutes or <80% for one minute. Passing was defined as either rapid pass (SpO<sub>2</sub>≥96% for 15 minutes) or monitoring for 60 minutes without failing. Infants passing the test were considered as having no BPD.<sup>51</sup> Later the SpO<sub>2</sub> level for passing was increased to 90% due to new guidelines on the recommended saturation levels in preterm infants.<sup>52</sup> The ORT solved the problem with different clinical guidelines and definitely added objectivity to the criteria. However, the dichotomous outcome makes the test insensitive to smaller

*According to the Walsh test, an infant with a saturation of 90% in room air, will be considered as disease free. Nonetheless, a healthy infants would have a saturation of >97% in air.<sup>51,53,54</sup>*

differences and improvements. Furthermore, while healthy infants have saturations >97% in room air<sup>53, 54</sup> infants with a SpO<sub>2</sub> of 90% will be considered as disease free.

#### 2.2.2.5.3 The Quine model

The Quine model was initially described in adults and used as a measure of pulmonary oxygen exchange.<sup>55, 56</sup> In 2001, Smith and colleagues applied the model on infants in order to assess the efficacy of treatment for neonates with pulmonary failure.<sup>57</sup> Subsequently, Quine suggested that the model could be used as an objective measure of disease severity in BPD.<sup>58</sup> The method, which is further described in the methods section of this thesis, uses the combined values of pressure of inspired oxygen (PIO<sub>2</sub>) and the corresponding oxyhemoglobin saturation (SpO<sub>2</sub>) in order to measure the efficacy of gas exchange. In contrast to the previously described definitions of BPD, hemoglobin concentration is taken into consideration and the model gives a continuous outcome of disease severity.<sup>58</sup> The method has showed very promising results, however, limitations on previous work in preterm infants include relatively small size of study cohorts ( $\leq 32$  infants),<sup>58-61</sup> studies heavily weighted to infants with moderate to severe BPD,<sup>58-60</sup> and use of an algorithm compensating for the effect of adult rather than fetal hemoglobin on the calculated value of shunt.<sup>58-61</sup> To increase the utility of the method it has to be validated across the whole spectrum of BPD severity.

### 2.2.3 Intraventricular hemorrhage

Intraventricular hemorrhage (IVH) is a hemorrhage from the germinal matrix located in the lateral ventricles in the brain. The germinal matrix is a highly vascularized structure from which neuronal and glial cells migrate to form the brain during development. The structure is only present in preterm infants. Changes in blood flow may induce bleeding in this sensitive structure. The hemorrhage can be graded I-IV, where grade I is a small hemorrhage in the subependymal area or in the matrix, grade II includes blood in the ventricle (<50% of the ventricle), but without dilatation of the ventricle, grade III includes intraventricular blood associated with ventricular dilatation whereas grade IV includes parenchymal engagement.<sup>62</sup> A grade IV hemorrhage can also be described as a periventricular hemorrhagic infarction but in this thesis it will be referred to as IVH grade IV, since it is the grading used within the EXPRESS study.<sup>1, 2</sup> The prognosis for IVH grade I-II is generally considered as good with few negative consequences. Nevertheless, IVH grade I-II has been associated with CP, cognitive impairment, neurosensory impairment and developmental delay. Some of these consequences however, may be explained by associated white matter lesions or periventricular leukomalacia (PVL).<sup>63</sup> The risk of IVH grade III-IV increases with lower GA and lower BW. The incidence in the EXPRESS cohort was 19% among 22-23 week infants and 5.2% among 26 week infants (Figure 2).<sup>1, 2</sup> Grade III-IV IVH increases the risk of poor neurological outcome.<sup>63, 64</sup> This is mainly mediated by three complications of IVH; posthemorrhagic ventricular dilatation (PHVD), destruction of the germinal matrix,<sup>65</sup> and associated white matter damage (PVL). PHVD will occur in 30-50% of infants with IVH grade III-IV. Some will resolve spontaneously whereas others need interventions such as

puncture through a reservoir or a later ventriculo-peritoneal shunt. The timing for interventions is under debate.<sup>63</sup> In different studies, CP rates differ from 7% in grade III IVH,<sup>64</sup> to 88% in bilateral grade IV.<sup>66</sup> Other complications described are lower cognitive function and impaired motor function.<sup>63</sup>

#### **2.2.4 Periventricular Leukomalacia**

Periventricular Leukomalacia (PVL) are ischemic lesions in the periventricular white matter. Hypotensive episodes may cause ischemia due to poor cerebrovascular autoregulation and fewer arterial anastomosis in the periventricular area in the preterm infants. The development of PVL has also been associated with sepsis, NEC and other inflammatory processes.<sup>67</sup> When grading PVL, grade 1 is referred to as increased echogenicity in the periventricular white matter remaining for at least seven days on ultrasound scans, whereas grade II-IV includes formation of cysts in the area.<sup>68</sup> Grade I lesions which resolves spontaneously will not be regarded as a major morbidity. In similarity to IVH, the risk of developing PVL is higher in extremely preterm infants. The incidence of PVL in the EXPRESS-cohort was 5.6% in all survivors at one year of age.<sup>68</sup> Infants developing PVL in the neonatal period have an increased risk to develop CP, and visual and cognitive impairments.

#### **2.2.5 Necrotizing enterocolitis**

Necrotizing enterocolitis (NEC) is an inflammatory condition in the gut affecting infants given enteral feeds. Incidence ranges from 5-10% in different populations and between different centers.<sup>2, 69, 70</sup> The Incidence in the EXPRESS cohort was 5.1%.<sup>1, 2</sup> The condition is more common in smaller (<1500g) and more immature infants (<32 weeks). Breast milk rather than formula and standardized feed regimens are considered as protective.<sup>71, 72</sup> Milder cases may be treated conservatively by withheld feeds and antibiotics. In the severe cases, accounting for 20-40%, the inflammatory process progress and cause necrosis and perforation of the gut which requires surgical intervention.<sup>73</sup> The condition is much-feared and mortality rates can be up to 50% in cases needing surgery.<sup>74</sup> Surviving infants may develop short bowel syndrome and failure to thrive. NEC is also associated with an increased risk of neurodevelopmental delay.<sup>75</sup>

#### **2.2.6 Retinopathy of Prematurity**

Retinopathy of Prematurity (ROP) is a vascular condition in the retina, which in severe cases may cause blindness. The disease was first described in the 1940s after the introduction of a new treatment, when preterm infants were kept in incubators with high concentrations of oxygen. Mortality rates improved to the cost of blindness.<sup>76</sup> Today, the incidence varies widely between studies, from 10% to 73%, partly dependent on grading and gestational age of subjects included.<sup>77</sup> In the EXPRESS cohort, 80% of infants born at 22 weeks had severe ROP, while only 17% of infants at 26 weeks were affected (figure 2).<sup>1, 2</sup> The strongest risk factors for development of ROP are low gestational age and treatment with high levels of supplemental oxygen.<sup>78-80</sup> Moreover, boys are more affected than girls.<sup>80</sup> The disease develops in two phases, with the first initiated at birth, when vascularization of the retina is

arrested due to hyperoxia and loss of nutritional and growth factors supplied from the mother in utero. The second phase, neovascularization of the retina, starts at around 30 weeks PMA.<sup>77</sup> The new vessels, however, are leaky and poorly perfuse the retina and the leakage leads to fibrous scars and in the worst cases, detachment of the retina.<sup>77</sup> ROP severity is graded in five stages.<sup>81</sup> Stage 1 is characterized by a demarcation line between the vascularized and non-vascularized retina, which in stage 2 has grown to become a ridge. Stage 1 and 2 are usually benign and likely to regress spontaneously. Stage 3 is characterized by extraretinal fibrovascular proliferation which may cause the retina to detach. In stage 4 the retina is partly detached whereas stage 5 is characterized by a complete retinal detachment. To further classify the disease, the retina is divided into three zones from central to the periphery, with affection of zone 1 (central) being the worst. In addition, occurrence of dilatation and tortuosity of posterior retinal blood vessels is a poor prognostic sign, classified as plus disease. In general, infants with ROP stage 1 and 2 will be followed whereas stage 3 or more will imply treatment. Stage 3 or more is classified as a major morbidity. Ablation of the non-vascularized retina by transpupillary laser is the most commonly used treatment.<sup>82</sup> The complication of ROP is very much dependent on severity of the disease. Infants with advanced stages of the disease are at high risk to develop visual impairment, and in worst case, blindness.<sup>83</sup> Laser treated children have 70-80% risk of myopia in later life<sup>84</sup> and about 40% of children with ROP will develop strabismus.<sup>85</sup> Severe ROP is also associated with lower academic performance and need for special education.<sup>86</sup> In addition, preterm birth increases the risk of astigmatism and hyperopia.<sup>87</sup>

### **2.2.7 Septicemia**

Septicemia, and other invasive bacterial infections, are common complications to preterm birth. The incidence in extremely preterm infants is 25-60% depending on gestational age.<sup>88</sup> In the EXPRESS study the overall incidence was 41%.<sup>1,2</sup> Preterm infants may be born with a bacterial infection due to chorioamnionitis or other maternal bacterial infections that may also trigger preterm birth.<sup>89,90</sup> Moreover, preterm infants have an increased risk to contract bacterial infections postnatally due to long term need for intravascular catheters which may facilitate entry of bacteria into the blood stream. A bacterial infection may be direct life threatening but may also imply long-term consequences. Septicemia has been associated with adverse neurodevelopmental outcome in a series of reports,<sup>88,91-96</sup> and has been correlated to hyperactivity at 4-6 years and attention deficit at 6-9 years of age.<sup>95,97</sup> Septicemia, and other inflammatory conditions, may trigger release of pro-inflammatory cytokines in the brain, also with the absence of a CNS infection, which in turn may induce damages to the white matter.<sup>67</sup> Furthermore, severe sepsis may be associated with hypotensive episodes, which may also trigger the development of PVL or IVH.<sup>90,98,99</sup>

### **2.2.8 Patent ductus arteriosus**

In fetal circulation, oxygenated blood from the placenta enters the fetus via the umbilical cord and the ductus venosus, into the right atrium of the heart. In order to by-pass the lungs, which are not in use, foramen ovale (a passage on atrial level) and the ductus arteriosus (a vessel



between the main pulmonary artery and the aorta), will be shunting the blood from right to left, straight into the systemic circulation. When the infant is born, the initiation of breathing will lower the pulmonary vascular resistance, allowing for blood to flow through the lungs. This will lower the pressure on the right side of the heart, which will cause the foramen ovale to close. The ductus arteriosus will close functionally in response to decreased levels of prostaglandin E<sub>2</sub> and increased oxygen tension in the blood,<sup>100, 101</sup> and later close structurally. In term healthy infants, this process starts immediately after birth, while the process in preterm infants may be delayed or absent.

When the closure of the ductus arteriosus is delayed it is referred to as patent ductus arteriosus (PDA). The incidence in the EXPRESS cohort was 61%, with decreased risk with advancing GA.<sup>102</sup> Treatment for PDA is either pharmacological or surgical (ligation). There are two common alternatives for pharmacological treatment, ibuprofen and indomethacin, the latter associated with more side-effects.<sup>103</sup>

With PDA, blood will flow backwards from the aorta into the main pulmonary artery and further through the lungs. This may cause over-circulation of the lungs leading to pulmonary edema and impaired pulmonary mechanics, resulting in need for mechanical ventilation or extubation failure. Consequently, PDA may increase the risk of future BPD.<sup>27</sup> Furthermore, blood flow through the gut and the brain may be reduced and increase the risk of NEC,<sup>104-106</sup> IVH,<sup>107</sup> and ROP.<sup>102</sup> In contrast, the relation between PDA and other morbidities is complicated. Although prophylactic indomethacin reduced PVL, IVH and need for surgical treatment, it was not associated with improved long-term outcomes.<sup>108-111</sup> Studies comparing early surgical ligation with late selective ligation showed no decrease in BPD<sup>112</sup> and neurodevelopmental outcome was worse.<sup>38</sup> Besides the association between PDA and NEC, the treatment with indomethacin has in itself been associated with NEC.<sup>103</sup> Different centers use different approaches and there is no agreed best way to handle PDA. Early treatment in case of a large PDA has been suggested.<sup>113</sup> The most common Swedish approach is to monitor the infant clinically and with echocardiography, and treat with ibuprofen in case of a hemodynamically significant PDA. In case of treatment failure and long-term need for mechanical ventilation, surgical ligation may be indicated.

## **2.3 LONG-TERM CONSEQUENCES OF PRETERM BIRTH**

### **2.3.1 The Barker hypothesis**

The intrauterine environment influences the development of cells and organs of the fetus. If the environment provides poor nutrition, hypoxia or severe stress, the fetus will adapt to these circumstances and prepare for a similar environment in future life. Nonetheless, the programming that was supposed to prepare the infant for life, will increase the risk for adult diseases when the extrauterine environment don't match.<sup>114</sup> The idea that intrauterine exposure may predispose diseases in adult life was first introduced by Barker and colleagues, as they correlated low birth weight to adult cardiovascular deaths.<sup>115</sup> The hypothesis of the developmental origin of health and disease (DOHaD), also called the Barker hypothesis, has

since been developed and discussed widely. Socioeconomic factors have to be taken into account when interpreting these relationships, because the same environmental factors affecting intrauterine life, may continue after birth and add to the risk factors of future diseases. In any case, today the epidemiological evidence for correlations between low birth weight and occurrence of chronic diseases in later life is substantial. Commonly, birth weight, and not gestational age (GA), has been used as exposure measure to determine if there is a relation to adult disease. The reason is simple, the registration of birth weights is in most cases more accurate than GA, while information about GA is more dependent on antenatal care. For that reason, infants with growth restriction are mixed with preterm infants in many reports, implying difficulties to discriminate between the two exposures. Nonetheless, besides coronary heart disease,<sup>115-119</sup> low birth weight and/or growth restriction, and prematurity have been correlated to hypertension,<sup>12</sup> stroke,<sup>118, 120</sup> diabetes type 1<sup>121, 122</sup> and type 2,<sup>15, 123-125</sup> and overweight.<sup>126, 127</sup>

### 2.3.2 Pulmonary outcome of preterm birth

The preterm infant is at risk to develop RDS and later BPD. Most infants however, breathe room air at the time of discharge and are seemingly lung healthy. If the infant passed the Walsh ORT test<sup>51</sup> before discharge the feeling of being disease free may be even stronger. Nevertheless, children growing up after preterm birth will be more likely to suffer from wheeze or to be hospitalized during childhood.<sup>29, 128, 129</sup> In a recent meta-analysis where FEV<sub>1</sub>% (forced expiratory volume at 1 sec, expressed as % of normal value) was compared between preterm born children with and without BPD and term controls. All preterm groups were shown to have significant impairments in pulmonary function. The largest differences were seen for children with oxygen dependency at 36 weeks PMA (moderate to severe BPD) where an average difference of -19% was seen (Figure 3). Furthermore, children with oxygen dependency at 28 days had an average difference of -16% and children born preterm but without BPD had a difference in -7%.<sup>130</sup> In the EPICure study, 66% and 32% respectively of

children born extremely preterm with and without prior BPD had an abnormal spirometry at 11 years of age.<sup>128</sup> Moderate to severe BPD at discharge

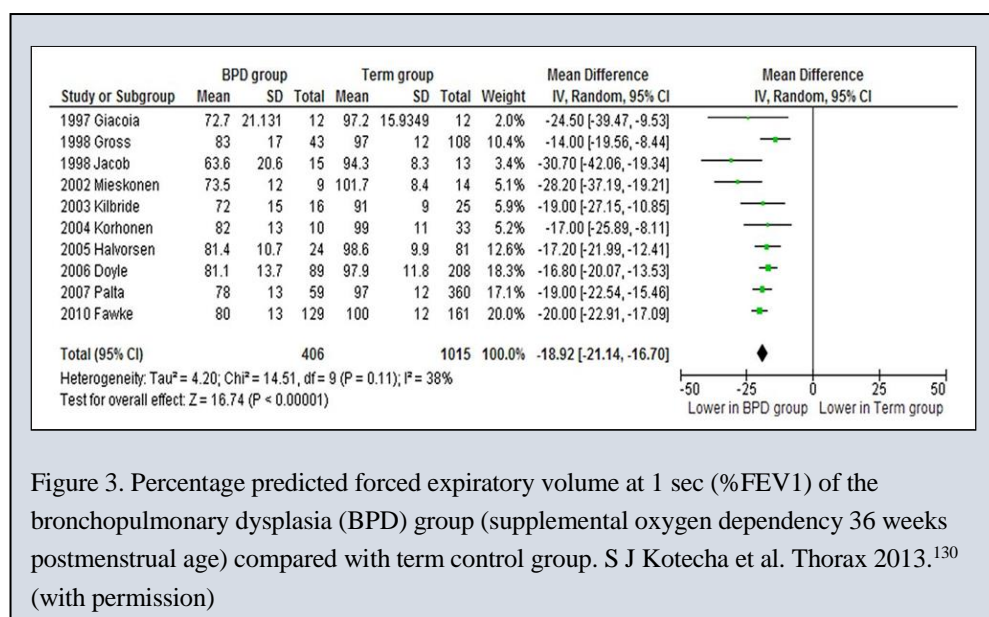


Figure 3. Percentage predicted forced expiratory volume at 1 sec (%FEV1) of the bronchopulmonary dysplasia (BPD) group (supplemental oxygen dependency 36 weeks postmenstrual age) compared with term control group. S J Kotecha et al. Thorax 2013.<sup>130</sup> (with permission)

is commonly used as a predictor of the pulmonary complications, however infants with mild or no BPD tend to have increased respiratory symptoms as well. In the paper by Shennan and collaborators, suggesting 36 weeks PMA as the best time point to define BPD disease severity, the positive predictive value of oxygen dependency for abnormal pulmonary outcome at two years of age, was 63%.<sup>49</sup> In a recent Swedish study, pulmonary outcome at 6 and 18 months for infants with moderate to severe BPD, compared to infants with mild BPD, was not statistically different, except for lower compliance at 6 months of age for the children with moderate to severe BPD.<sup>29</sup> There is also evidence that decreased pulmonary function may persist into adulthood.<sup>131, 132</sup> In summary, survivors of preterm birth are more likely to suffer from respiratory symptoms and decreased lung function than term peers. Survivors with moderate to severe BPD tend to be more affected, however survivors with mild or no BPD are at greater risk for respiratory symptoms than the general population.

### 2.3.3 Neurodevelopmental outcome of preterm birth

It is well-known that survivors of preterm birth have an increased risk of impaired neurological function, consequently it may be the complication most feared by expecting parents. High risk of severe neurodevelopmental disability (NDD) is also a reason for withdrawal of care. Severe NDD can be defined as a disability where the child is likely to be highly dependent on caregivers whereas a child with moderate NDD is likely to have some degree of independence.<sup>133</sup> The criteria used to define severity of NDD at the 6.5 year follow-up in the EXPRESS cohort can further illustrate the level of impairment according to the classification (table 3).<sup>134</sup>

<b>Table 3. Criteria for NDD severity classification.</b> <sup>134</sup> The child will be classified according to the disability with highest severity			
	Neurodevelopmental disability (NDD)		
	mild	moderate	severe
FSIQ score	-2SD to < -1SD	-3SD to < -2SD	< -3SD
CP, GMFCS level	1	2-3	≥4
Visual impairment*	<20/40 but ≥20/63	<20/63 but ≥20/400	<20/400 (blindness)
hearing	normal	hearing loss corrected with hearing aids	deafness

FSIQ-full scale IQ<sup>135</sup>, SD-Standard Deviation, CP-Cerebral palsy,<sup>136</sup> GMFCS-Gross Motor Function Classification System,<sup>137</sup> \*severity in the better eye, according to modified WHO criteria.<sup>138</sup>

The incidence of NDD differs widely between centers and countries, which could be related to survival rates in different gestational ages, testing tools used, and use of control group. The risk is usually described to increase with immaturity, nevertheless, smaller reports showed no difference between children born at different GA weeks within the extremely preterm group.<sup>139</sup> A recently published meta-analysis, including nine studies from eight different developed countries, revealed that the proportion of survivors with moderate to severe NDD, ranged from 43% among infants born at 22 weeks GA to 24% in infants born at 25 weeks gestation.<sup>133</sup> At the 6.5 year follow-up of the EXPRESS cohort (<27 weeks GA), 33.6% had moderate to severe NDD, of which 89.2% had a moderate to severe cognitive disability,

either in combination with another disability or alone.<sup>134</sup> The risk of severe disability decreased for every extra week of gestational age.

Infants born at later gestational ages have lower than the more preterm infants, but increased rates of CP and cognitive impairment compared to term peers. An historical Swedish cohort reported CP rates of 8.6%, 6.0%, 0.6% and 0.1% for children born extremely preterm, very preterm, moderately preterm and term respectively.<sup>140</sup>

Children born preterm have an increased risk of other mild disabilities that are not classified as NDD, including a condition called Developmental Coordination Disorder (DCD).<sup>141-146</sup> DCD was defined by Politajko in 1995 in order to describe children previously referred to as “clumsy”, “physically awkward” or “poorly coordinated”.<sup>147, 148</sup> DCD includes minor and gross motor dysfunction in the absence of CP and full scale IQ-score less than -2SD.<sup>144, 148</sup> Difficulties may include shoe tying, handwriting, bicycle riding or ball catching. The child may bump into things, have poor balance and have difficulties in doing activities requiring frequent adaptation to the environment (eg tennis). The increased difficulties in performing motor skills may cause the child to avoid participation in motor activities. In a study from 2007, the incidence of DCD among 8 year old children with BW<1000g or GA<28w, was 9.5% compared to 2% in the control group.<sup>144</sup> In addition, these children had an increased risk of mild cognitive impairment, poor academic progress and behavioral difficulties compared to children without DCD and it was shown to be more common among boys.<sup>144</sup> Other studies have described higher incidences of up to 51%,<sup>141</sup> which could be attributed to the fact that the children included were born before improvements in neonatal care, but also due to the fact that there is no agreed scale or cut-offs to define DCD.<sup>144</sup> Altogether, DCD implies motor disabilities which could prevent children from participation in physical activity. It affects children without major disabilities and the condition is more common in children born preterm, particularly boys.

### **2.3.4 Physical activity and exercise capacity after preterm birth**

An abnormal pulmonary function may affect the efficiency at exercise and the ability to be physically active. Survivors of preterm birth report increased levels of exercise-induced bronchoconstriction which may possibly limit exercise capacity and physical activity.<sup>149, 150</sup> Consequently, the question has been addressed whether decreased pulmonary function affect exercise capacity and physical activity. In most reports, lung function and exercise capacity has been tested objectively whereas physical activity, when measured, has been assessed using questionnaires. Previous observations have revealed conflicting results. Exercise capacity has in most studies been reported to be lower compared to term subject,<sup>8, 151-153</sup> however sometimes to a smaller degree. Clemm and colleagues emphasized the small differences and that exercise capacity was within the normal range at 25 years of age.<sup>154</sup> The levels of PA has been reported to be similar<sup>149, 155, 156</sup> or lower<sup>8, 150, 153</sup> compared to term peers, but no studies have shown elevated levels of PA in the preterm group. Three previous reports used objectively measured PA as outcome.<sup>9, 157, 158</sup> In the EPICure study, PA was measured in 31 extremely preterm infants (GA<25 w) and compared to 30 children born at term. The

children were 11 years at follow-up and no difference was found between preterm and term children in PA, although the preterm children had lower oxygen consumption at peak exercise, lower peak workload, increased tachypnea at exercise and reported more breathing difficulties during exercise.<sup>9</sup> In the ALSPAC cohort, PA was monitored at 11 and 15 years of age and no difference was found between children born preterm and children born at term and PA was not correlated to spirometry findings.<sup>157</sup> Both these studies reported levels of PA that were considerably lower than the recommended 60 minutes in moderate to vigorous physical activity (MVPA) per day. In the Millennium Cohort, PA was measured at in 6422 children at 7 years of age, of which 79 were born very preterm (<32 weeks). The main difference was that boys born very preterm spent less time in MVPA than term peers. The size of the cohort allowed adjustment for several socioeconomic factors which was also shown to be important for the level of PA.<sup>158</sup> Furthermore, most of the children included met the criteria for time in MVPA per day. Nonetheless, no correlation was found between pulmonary function and PA.

## 2.4 HEALTH ASPECTS OF PHYSICAL ACTIVITY

### 2.4.1 Benefits of physical activity

The benefits of an active life style can probably not be overestimated. There is well-established evidence that there is a dose-response relation between Physical activity (PA) and non-communicable diseases like cardiovascular diseases, stroke, hypertension, diabetes type 2, colon cancer, breast cancer, and osteoporosis.<sup>159, 160</sup> Low levels of PA has been associated with depressive and anxiety disorders<sup>161</sup> and exercise as treatment for depression has proven to be equal to or sometimes even better than psychological and pharmacological treatment for depression.<sup>162</sup> Furthermore, PA reduces all-cause mortality in a dose response manner (figure 4).<sup>163</sup> The relation between PA and overweight is arguable, although several studies support the hypothesis that PA has a beneficial effect on body weight.<sup>164-166</sup>

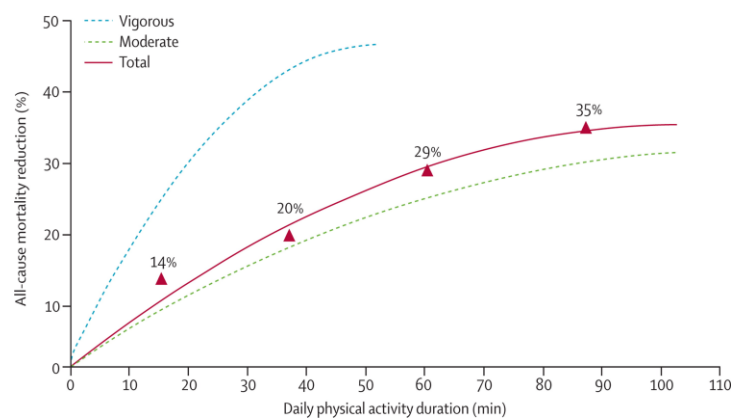


Figure 4. Reduction of all-cause mortality in relation to daily physical activity. Lee et al, Lancet 2012.<sup>163</sup> (With permission)

There is also increasing evidence that besides preventing diseases, PA may have positive effects on the cognitive function.<sup>167-169</sup> Physical activity and exercise capacity is positively associated with school achievement in childhood,<sup>170, 171</sup> but on the other hand, some argue that the evidence for a causative relationship is not strong enough.<sup>168</sup> In other respects, a recent review on the effect of school-based interventions with increased PA, revealed higher academic achievement and improved cognitive function in the intervention groups.<sup>172</sup> Studies with the intention to explain this relationship between PA and cognitive function has

suggested that the effect may be related to exercise-induced structural changes and growth of the hippocampus,<sup>173, 174</sup> or be mediated by brain-derived neurotrophic factor (BDNF) which is released with exercise and known to affect brain plasticity.<sup>175</sup> In summary, several studies show a positive effect of physical activity on the cognitive function. However there are no previous studies looking into the possible relationship between physical activity and cognitive function in survivors of preterm birth.

## **3 AIMS**

### **3.1 GENERAL AIMS OF THESIS**

The general aim of this thesis was to increase the knowledge about the consequences of being born preterm, with focus on pulmonary function, physical activity and exercise capacity,

### **3.2 SPECIFIC AIMS**

- I. To study the correlations of preterm birth and fetal growth restriction to exercise capacity in young adulthood.
- II. To study the correlation of cognitive function to exercise capacity in young adults born preterm.
- III. To objectively measure physical activity in 6.5 year old children born extremely preterm and compare it to children born at term. Secondly, to analyse the correlation of physical activity to perinatal morbidities.
- IV. To test the utility of shift, ventilation/perfusion ratio ( $V_A/Q$ ), and right to left shunt for determination of the severity of BPD in very preterm infants. Furthermore, to identify the contribution of explanatory variables to severity of impaired gas exchange.





## **4 METHODS**

### **4.1 STUDY DESIGN AND STUDY SUBJECTS**

#### **4.1.1 Exercise capacity and cognitive function in young adulthood (I, II)**

Study I and II are population-based retrospective register studies. Data regarding conscription results were collected from the Conscript Register and linked to the Medical Birth Register (MBR), the Population and Housing Census 1990 and the Multigeneration Register. Inclusion criteria were male sex, being born in Sweden 1973-1983 and conscription for military service in 1993-2001. Exclusion criteria were not performing the maximal exercise capacity test or the test for cognitive function (study II) or missing data in the medical birth register. The study cohort consisted of almost 220000 men and is further described in paper I and II.

#### **4.1.2 EXPRESS/CHARM (III)**

Study III is an observational follow-up study with participants recruited from the EXPRESS/CHARM (EXtremely PREterm infants in Sweden Study and Comprehensive Heart and Respiratory Measurements) cohort. EXPRESS is a population-based prospective observational study which includes all extremely preterm infants (GA <27 weeks) born between 1<sup>st</sup> of April 2004 and 31<sup>st</sup> of March 2007 in Sweden. CHARM is a sub-cohort to EXPRESS, with the inclusion criteria to be born in any of the regions Stockholm, Lund and Umeå. Exclusion criteria were cardiovascular or pulmonary congenital malformations or not coming for follow-up. Further description of the study cohort is found in paper III.

#### **4.1.3 The PIFCO study (IV)**

Study IV is a prospective observational cohort study and a part of the PIFCO (Preterm infant, functional and clinical outcome) study. Inclusion criteria were being born at King Edward Memorial Hospital in Perth, Western Australia, from 21<sup>st</sup> of July 2013 and 3<sup>rd</sup> of August 2016 and GA <32 weeks. Exclusion criteria were major congenital malformation and no parental consent. The study subjects are further described in paper IV.

### **4.2 DATA COLLECTION**

#### **4.2.1 Study I and II**

All data for study I and II were collected from registers. Four population based Swedish registers were used; The Conscript Register, The Medical Birth Register (MBR), The Population and Housing Census 1990 and the Multigeneration Register. Data was linked using the personal registration number assigned to each Swedish citizen at birth and was performed by the Central bureau of Statistics, Sweden. The anonymized dataset was released to the authors. The main outcome was maximal exercise capacity in Watt ( $W_{max}$ ) at the time of conscription in study I and its correlation to the results on the cognitive function test at conscription in study II. The cohort includes men conscripting for military service between 1993 and 2001. The period was chosen according to data availability from the medical birth

register (MBR) and the fact that mandatory conscription ended after 2001. The inclusion pathway is shown in the flowchart (figure 5). For study II, another 18 subjects were excluded due to missing data on the cognitive function test.

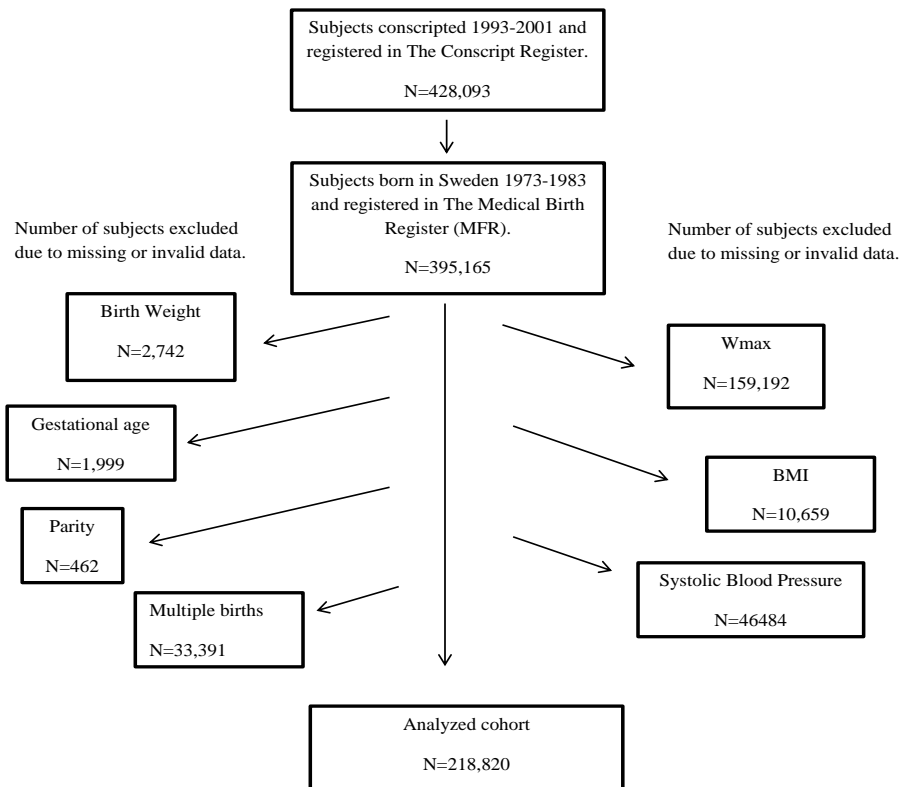


Figure 5. Flow chart for inclusion of patients in study I and II. Subjects who were born during another period of time, with missing data either in the MBR, the Multigeneration Register or the population and housing Census or missing data in the Conscript Register were excluded. One subject can have missing data in more than one category. Reproduced from paper I, Svedenkrans et al, PLoS ONE 2013.<sup>4</sup>

#### 4.2.1.1 The Conscript Register

Until 2001 conscription was mandatory for all Swedish men without severe handicaps. At conscription all recruits would have a physical examination by a physician and take the cognitive function test (further description below). Depending on health and type of expected service, some of the recruits would be tested for maximal exercise capacity on a cycle ergometer (further description below). All results from the tests and examinations were recorded into the Conscript Register, and the anonymized information is available for scientist after application and ethical approval. Data retrieved from the Conscript Register were the outcome variables; maximal exercise capacity and results from the cognitive function test, as well as other factors at conscription that could affect the outcome (listed in table 4)

Table 4. Factors and covariates included in the analyses in paper I and II		
Perinatal factors	Socioeconomic factors	Factors at conscription
Gestational age (GA)	Parental education	Body mass index (BMI)
Maternal age	Parental income	Systolic blood pressure (mmHg)
Parity	Parental socioeconomic index	Health status
Multiple birth		
Maternal country of birth		
Birth weight standard deviation score (BWSDS)		

#### 4.2.1.2 *The Medical Birth Register*

All birth units in Sweden report to the Medical Birth Register (MBR), which contains information about >99% of all births in Sweden. The information is collected prospectively during pregnancy on standardized forms and forwarded to the register. Validation of the MBR revealed high quality.<sup>176</sup> The MBR was used to retrieve information on perinatal factors (listed in table 4).

#### 4.2.1.3 *The Population and housing Census 1990*

At the Population and Housing Census in 1990 all Swedish citizens above 16 years of age mandatorily reported information about education, income, profession and family structure. The response rate was 97.5%.<sup>177</sup> The information was used to adjust for socioeconomic differences in parental education, income and socioeconomic index (table 4).

#### 4.2.1.4 *The Multigeneration Register*

In the multigeneration register blood relationships of all Swedish citizens are recorded. It was used to identify the parents of the recruits in order to be able to adjust for socioeconomic factors retrieved from the Population and Housing Census 1990.

#### 4.2.1.5 *The maximal exercise capacity test at conscription*

The maximal exercise capacity test was performed on cycle ergometer at conscription. Only conscripts with a normal echocardiogram were allowed to do the test. The initial workload was determined by weight (125W for 75 kg). After five minutes of cycling on the initial workload, with a pulse between 120 and 170 (submaximal test), the load was increased with 25 W every minute as long as the conscript would manage. The subject was instructed to perform his maximal capacity. Outcome used in the analyses was the maximal effort in Watt that the conscript could manage ( $W_{\max}$ ).

#### 4.2.1.6 *The cognitive function test*

The test for cognitive function used at conscription was developed by the Swedish military and includes 160 time limited multiple choice questions equally distributed to test verbal, spatial, theoretical/technical and logical/inductive skills. The results on the test were recalculated into a STANDARD NINE score (stanine), which is a statistical instrument to scale test results that follow a standard distribution. The scale is from 1-9, where average is 5 and

each unit change corresponds to +/- 0.5 SD. The questions are deemed as secrecy and were not revealed to the researchers. The stanine score for the whole test was used as outcome in study II.

## 4.2.2 Study III

### 4.2.2.1 Collection of data

For the index children, information about birth characteristics (BW, GA, sex) and perinatal morbidities (IVH, PVL, septicemia, ROP, NEC, and BPD) were collected within the EXPRESS-study in the perinatal period. Birth characteristics for the control children were collected from maternal medical records at the follow-up visit. Information about cerebral palsy, and measurements of height and weight were collected at the follow-up visit for all participants.

### 4.2.2.2 Measurement of Physical Activity

Physical activity was objectively measured using wrist-worn accelerometers (Actigraph GT3X+, Pensacola, USA) during seven consecutive days. Wrist-worn accelerometers were chosen since it has proven to be reliable and imply a better compliance.<sup>178, 179</sup> Registrations between 7 am and 8 pm were used for analysis. Data were analyzed using the Actilife software (version 6.13.3, Actigraph, Pensacola, USA).

#### 4.2.2.2.1 The function of an accelerometer

The Actigraph GT3X+ accelerometer is a small (45x33x15 mm), lightweight (19g) device developed to measure accelerations. The most common placement is the hip or the wrist. A simplified description would be that it is an advanced pedometer. The g-power from the movements is transformed from mechanical power to electronical signals by using microelectronic mechanical systems (MEMS). The amount of counts produced is dependent on frequency and velocity of the accelerations. Sampling of data is done in pre-determined intervals (Hz) and summarized over a selected time period (epochs). The unit used is counts.

The GT3X+ can measure accelerations in three different axes (x, y, z) and any of the axis, all three or the combined measure called the vector magnitude (VM, defined as  $\sqrt{x^2 + y^2 + z^2}$ ) can be used as outcome. A filter is applied on the measurement in order not to register too low (eg travelling by car, waves on a boat etc) or too high frequencies, as movements, since they are considered as not being caused by human activity.



Figure 6. Accelerometer GT3X+

#### 4.2.2.2.2 Accelerometer cut-points for activity levels

Very much effort has been spent to translate counts produced by accelerometers into activity levels. Study subjects have performed different activities, energy expenditure has been calculated (eg 4 metabolic equivalents (METs) for moderate intensity) and the corresponding counts has been used as cut-points for different activity levels.<sup>179, 180</sup> Nevertheless, there are no agreed cut-points to measure PA in children. This is besides different cut-points suggested by different studies also explained by the fact that accelerometers can be worn on wrists, hips or ankles and that cut-points will need to change as children grow. We chose to use the cut-points validated by Chandler and colleagues<sup>181</sup> since they are validated for wrist-worn accelerometers and the closest available in age to our study participants. The best model fit was seen when 1-axis measurements were used ( $R^2=0.77$ ). We chose to use vector magnitude which was almost as good ( $R^2=0.74$ ),<sup>181</sup> taking into account a suspicion of a different movement pattern in children born extremely preterm. In order to avoid that poorly chosen cut-points for PA would affect the comparison between index and control children, a 10-scale cut-off was created by dividing the total range of counts/minute into 10 equally large intervals. Percentage of time in each interval was compared between index and control children.

#### 4.2.2.2.3 Description of activity levels

In the Chandler validation study, the grading of the intensity of PA was based on the System for Observing Play and Leisure Activity in Youth's (SOPLAY),<sup>181, 182</sup> used in conjunction with heart rate monitoring. Resting and enrichment were classified as SED, walking as light, and playground play, swimming, and PACER run as MVPA. The Children's Activity Rating Scale (CARS)<sup>183</sup> is another established method that has been used in several calibration studies to rate PA in children.<sup>184</sup> Children are observed and the activity intensity is rated in level 1-5. According to this scale minor movements (level 1) is considered as SED, standing (level 2) and walking slowly (level 3) as light, walking briskly (level 4) as moderate PA, whereas running (level 5) is considered as vigorous PA.

#### 4.2.2.2.4 Limitations

Measurement of physical activity by using accelerometers have some important limitations. First, the same kind of activity will generate different amount of counts in different individuals even when performing simple activities like walking. Second, the same activity level may imply different effort for different people. An example is that for the obese person to walk in 5 km/h may be very effortful compared to for a normal weight person. The movement however, will be basically the same. Third, some physical activity will not be registered, such as bike riding, since it doesn't include any movement around the wrist or the hip. When reaching very high levels of activity, the amount of counts registered will reach a plateau. This may be due to a low-pass filter within the accelerometer but also due to the fact that when running very fast a hip-worn accelerometer will not move much more than when running at a slower pace.

## 4.2.3 Study IV

### 4.2.3.1 Data collection-possible predictors of the outcome

Data on perinatal factors, factors associated with disease severity, growth and nutrition were collected from the mother's and the infant's medical records (listed in table 5) and managed using Research Electronic Data Capture (REDCap) software hosted at The University of Western Australia<sup>185</sup>. Weight for gestational age (z-score) was calculated using the Fenton growth chart as reference<sup>186</sup>. All factors were tested for correlations to the outcomes, shift, Ventilation:perfusion ratio ( $V_A/Q$ ) and shunt.

### 4.2.3.2 Data collection-The modified oxygen reduction test

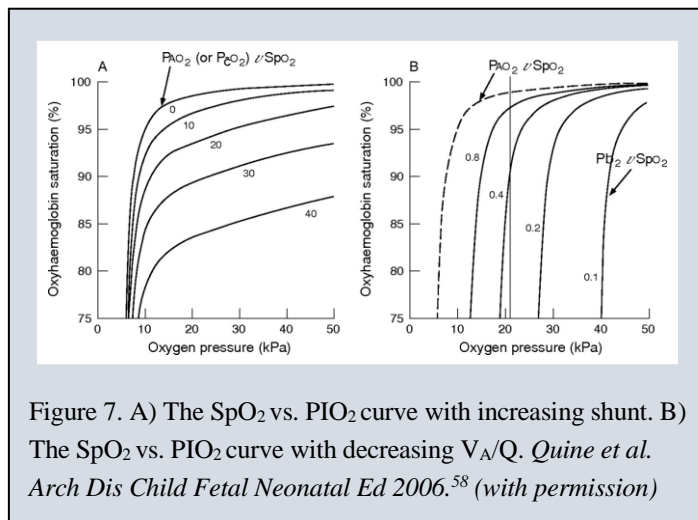
To derive a SpO<sub>2</sub> vs. pressure of inspired oxygen (PIO<sub>2</sub>) curve for each infant in study IV, a modified oxygen reduction test was performed. The infants were tested at 36w post menstrual age (PMA). Preductal peripheral oxyhemoglobin saturation (SpO<sub>2</sub>) were measured from the infant's right hand. The test was usually performed using a head box with a continuous fresh gas flow of 6 L/min at the prescribed PIO<sub>2</sub>. PIO<sub>2</sub> was reduced in decrements of 1-3 % at 5 min intervals in at least 4 to 5 steps, to achieve SpO<sub>2</sub> ranging from ~86-94%. Commencing PIO<sub>2</sub> for the test was 21% or the current prescribed PIO<sub>2</sub> if receiving supplemental oxygen. For infants with a SpO<sub>2</sub> of  $\geq 90$  % in air, PIO<sub>2</sub> was decreased below 21% by mixing air with a mixture of 14 % oxygen in nitrogen to achieve the target PIO<sub>2</sub>. A period of 4 min elapsed after each decrement in PIO<sub>2</sub> before signals were recorded for 1 min. SpO<sub>2</sub> were averaged, and paired values of SpO<sub>2</sub> and PIO<sub>2</sub> recorded. The lowest permissible PIO<sub>2</sub> and SpO<sub>2</sub> were 14% and 86 % respectively. Infants receiving CPAP or humidified high flow nasal cannula

<b>Table 5. Factors possibly affecting outcome, retrieved from mother's and infants medical records.</b>		
<b>Maternal Factors</b>	<b>Maturity</b>	<b>Growth and nutrition</b>
Maternal betamethasone	Gestational Age	Birth weight
Rupture of membranes > 72 h	<b>Disease severity</b>	Birth weight z-score
Fever > 38.0 C in labour	Mechanical ventilation (days)	Birth head circumference z-score
Histological chorioamnionitis	Non-invasive ventilation (CPAP or HHFNC, days)	36w PMA weight
Labour	Supplemental oxygen (days)	36w PMA weight z-score
<b>Birth and early postnatal treatment</b>	postnatal steroids	36w PMA head circumference z-score
Intubated in 1st 48h	hyaline membrane disease	36w PMA length
Multiple birth?	airleak	36w PMA length z-score
Male	pulmonary haemorrhage	Growth (weight z-score at test - birthweight z-score)
APGAR at 5 minutes	sepsis	Average fluid intake 1 <sup>st</sup> 28 d (ml/kg/d)
Admission temp	intraventricular haemorrhage	Average caloric intake 1 <sup>st</sup> 28 d (kcal/kg/d)
Surfactant given?	periventricular leukomalacia	Average protein intake 1 <sup>st</sup> 28 d (g/kg BW/d)
	necrotising enterocolitis	
CPAP-Continuous positive airway pressure, HHFNC-humidifies high flow nasal cannula, PMA-post menstrual age		

(HHFNC) with supplemental  $\text{PIO}_2$  above 25% were tested bedside using adjustments to the air/oxygen blender whilst continuing on the prescribed respiratory support. Infants also had capillary blood gas obtained for assessment of haemoglobin (Hb), if one was not available from clinical testing within three days of the oxygen reduction test.

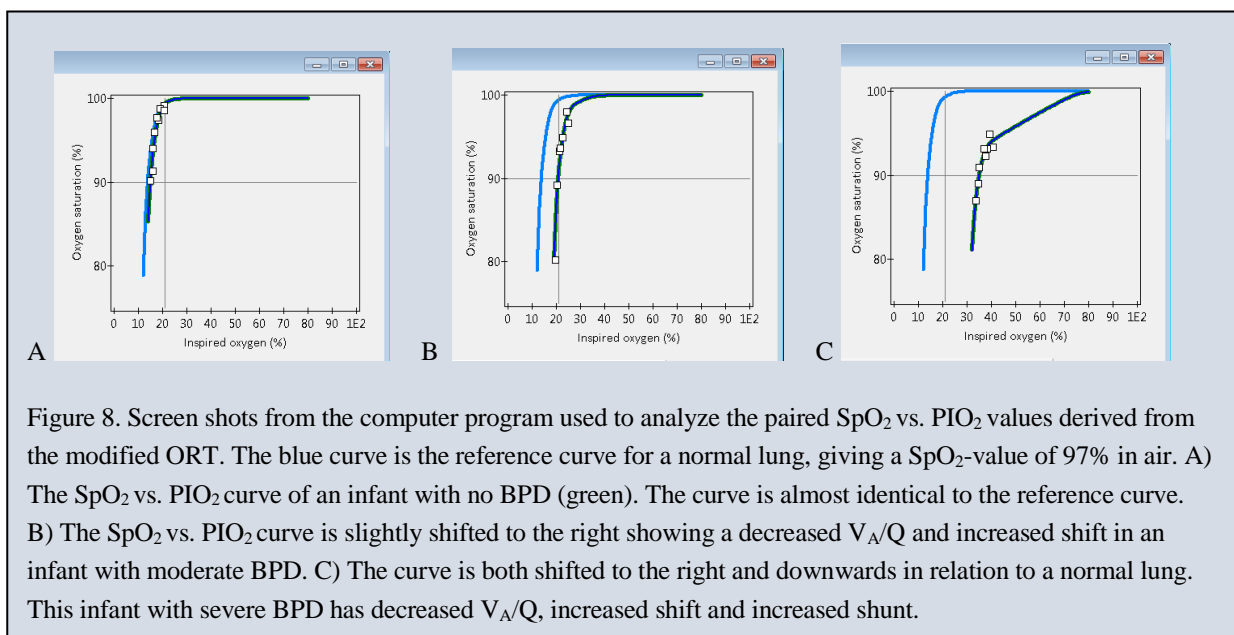
#### 4.2.3.3 Data analysis – Principles of the Quine model, two compartment model

Figure 7 describes the principles of the Quine model using a two compartment approach (2C).<sup>58</sup> By plotting an infant's  $\text{SpO}_2$  vs.  $\text{PIO}_2$  curve (derived from the modified ORT) and analyze its position in relation to the oxygen dissociation curve (ODC), values of shift (kPa), shunt (%) and ventilation:perfusion ratio ( $\text{V}_A/\text{Q}$ ) can be derived. In figure 7A the ODC is the



0-line, and in 7B the dashed line. In case of shunt (A), the upper part of the  $\text{SpO}_2$  vs.  $\text{PIO}_2$  curve will be shifted downwards, whereas a decreased ventilation:perfusion ratio ( $\text{V}_A/\text{Q}$ , figure 7B) will shift the curve to the right. The  $\text{V}_A/\text{Q}$  value will be dependent on the shift value. For analysis, the paired values of  $\text{SpO}_2$  vs.  $\text{PIO}_2$  derived from the modified ORT will be entered into the computer program

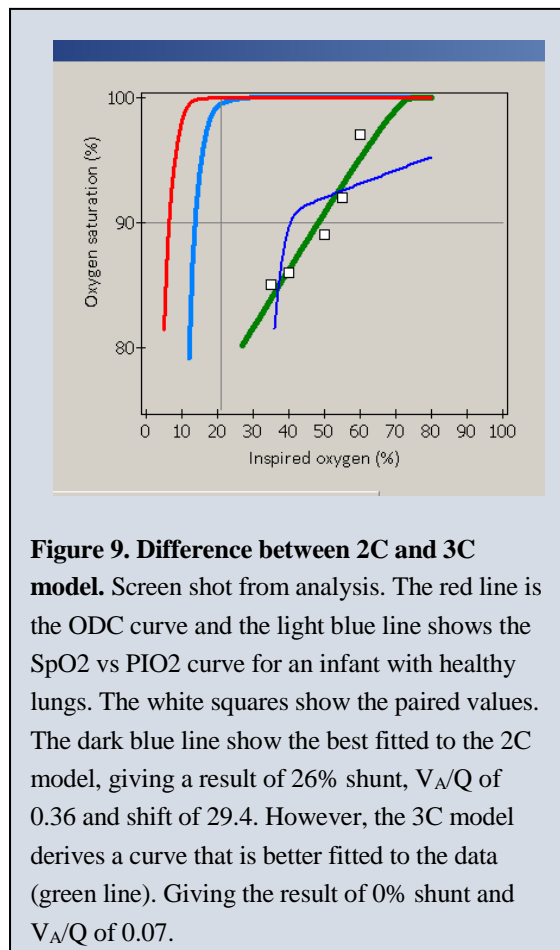
specifically developed for the purpose.<sup>187</sup> The program automatically derives a  $\text{SpO}_2$  vs.  $\text{PIO}_2$  curve and analyzes its position. Figure 8 shows examples of screen shots from analysis of infants with no BPD (A), moderate BPD (B) and severe BPD(C). The light blue curve is the reference for normal lungs (ODC plus the shift caused by mixture of air and  $\text{CO}_2$  in the lung), giving a  $\text{SpO}_2$ -value of 97% in air. The shift value will be approximately the difference in



supplemental oxygen supplied to an infant with reduced pulmonary function in comparison to an infant with normal lungs.

#### 4.2.3.4 The three compartment model (3C)

The two-compartment (2C) model described above is easy to understand and most infants' SpO<sub>2</sub> vs PIO<sub>2</sub> curves will fit to the model. However, the model presumes that the lungs are homogeneously ventilated, which is not always the case in sicker infants. The 2C model presumes one compartment with shunt and one compartment with V<sub>A</sub>/Q. Reduced V<sub>A</sub>/Q will cause a right shift of the SpO<sub>2</sub> vs PIO<sub>2</sub> curve. Nevertheless, in non-homogeneously ventilated lungs some parts may be well ventilated and others poorer ventilated. The analysis program will, when using the three-compartment model, (3C) analyze one fictive compartment with shunt, one with high V<sub>A</sub>/Q and one with low V<sub>A</sub>/Q.<sup>187</sup> Low V<sub>A</sub>/Q is close to shunt (poorly ventilated area that is well perfused) and the curve will be shifted downwards although no shunt is present. The screenshot in figure 9 will describe the difference between a curve derived by the 2C and the 3C model. All infants in study IV had their SpO<sub>2</sub> vs PIO<sub>2</sub> curve compared to the normal lung using both a 2C and 3C model.



### 4.3 STATISTICS

#### 4.3.1 Study I

All statistical analyses for study I were performed by a statistician and co-author of the paper (J Kowalski). Data were analysed using Analysis of Variance, ANOVA, with perinatal factors and cofactors as fixed factors in univariate models and multivariate models. Least square means was used to calculate the point estimates in the multivariate model and thereby control for co-variables and evaluate the independent contribution by each factor to the outcome.

#### 4.3.2 Study II

All statistical analyses for study II were performed by a statistician and co-author of the paper (J Kowalski). Results on the cognitive function test at conscription was used as outcome.



Ordinal regression was used to calculate the odds ratio (OR) for a higher results on the cognitive function test compared to the reference category (term, normal birth weight, average exercise capacity). The ordinal regression was adjusted stepwise in order to further understand the contribution of each independent factor. The results from three different models adjusted for an increased amount of variables, were used.

### **4.3.3 Study III**

The statistical analyses for study III were performed by the author of the thesis, and discussed with statistician to confirm the choice of method. All analyses were tested and judged to fulfill validity criteria for the chosen method. T-test,  $\chi^2$ , and Mann-Whitney U-test was used to compare groups. Linear regression was used in adjusted and unadjusted analyses to analyze the correlation between predictors and the outcome.

### **4.3.4 Study IV**

Primary descriptive and unadjusted statistical analyses for paper IV were performed by the author of the thesis. Adjusted analyses and regression models were performed by the senior author (J J Pillow) in cooperation with a statistician and co-author (D Doherty). The relation between the outcome variables (shift,  $V_A/Q$  and shunt) and the infant's NICHD BPD classification were determined using one way ANOVA and Kruskal-Wallis ANOVA. Receiver operating curve was constructed to identify the threshold level of shift for detection of mild, or moderate to severe BPD. Multiple imputation was used to complete the data set for analysis for potential confounders and predictive variables when individual data were not accessible and the percent of missing data for individual measures was less than 10 %.

Potential explanatory variables were examined for normal distribution and collinearity. Postnatal explanatory variables that were collinear with maturity at birth were regressed against gestation, and the unstandardized residuals as an independent linear measure of the variable of interest.

Potential explanatory variables for entry into regression analysis were identified from univariate correlations against continuous and binary variables. Principal component analysis was used to identify key variance factors within the cohort and to address and overcome residual multiple collinearity between potential explanatory variables. The factor with the highest score within each factor of the rotated component matrix and least overlap with other factors was selected for multiple linear regression using stepwise regression.

## **4.4 ETHICAL CONSIDERATIONS**

### **4.4.1 Register studies (I, II)**

Study I and II were approved by the ethical review board in Stockholm (2009/1186-31/5 and 2014/1070-32). In both studies data from registers were used and the study subjects did not have to do any extra tests for the studies. No discomfort or risks for the study subjects were involved. The ethical considerations will rather be related to the registration itself and the fact

that the study subjects were never asked individually if they wanted to be part of this study. Some people are uncomfortable with the fact that there's a lot of information about them available in registers. In order to diminish discomfort for the study subjects the linkage was done at central bureau of statistics, Sweden and all data were de-identified to the researchers. Consequently, no result can be linked to an individual study subject. The advantage to use the registers for research and the knowledge we can get from it was considered as more important.

#### **4.4.2 Measurement of physical activity (III)**

Study III was approved by the ethical review board in Lund (42/2004) and in Stockholm (2010/520-31/2). The discomfort related to wearing an accelerometer on the wrist is similar to wearing a watch. You could dislike the look of it or you may find it is itching. There were no risks included. The children could withdraw at any time of the study. The benefits include better knowledge about the physical activity level in children born extremely preterm and further on it may possibly lead to interventions which could improve outcome for children born preterm. The benefits were considered as much larger than the risks of discomfort for the children.

#### **4.4.3 Modified oxygen reduction test on preterm infants (IV)**

The study was approved by the Women and Newborn Health Service Human Research Ethics Committee (HREC, EC 00350) at King Edward Memorial Hospital in Perth, Western Australia (2013091 EW). The largest ethical consideration regarding the modified oxygen reduction test is the decrease in oxygen delivery and the risk of causing a desaturation for the infant. Desaturations could theoretically affect the brain. However, preterm infants have irregular breathing patterns, and even if not having a modified ORT they will have occasional drops in saturation. Furthermore, fraction of inspired oxygen (FIO<sub>2</sub>) will be reduced and increased in clinical practice in order to optimize the treatment level, which means that the ORT is similar to the clinical situation. During the modified ORT, the infants were more thoroughly monitored than in the clinical situation and if the infant would drop below 86-88%, oxygen level would be increased accordingly. The use of 14% oxygen was also being used in clinical practice for the flight-ready test,<sup>188, 189</sup> and the test was very well tolerated by the infants and there were no negative events during the study. The benefits of a possible improvement of BPD diagnostics was considered to override the minimal risks with the test.

## 5 RESULTS

### 5.1 POPULATION CHARACTERISTICS STUDY I AND II

During the period 1993 to 2001, 428 092 young men were conscripted for military service. After exclusion of subjects with missing or invalid data the cohorts for study I and II consisted of 218,820 and 218,802 men respectively (figure 5 in methods section). The most important reason for exclusion was not performing the maximal exercise capacity test. Comparison between the included and the excluded men revealed small but significant differences (table 1-3 in paper I), however the most striking difference was that the included men were healthier than those excluded.

### 5.2 EXERCISE CAPACITY IN YOUNG MEN BORN PRETERM (I)

#### 5.2.1 Exercise capacity in relation to gestational age and intrauterine growth

The young men with the lowest GA also exhibited the lowest average results on the maximal exercise capacity test ( $W_{max}$ ). Furthermore, being born small for gestational age (SGA,  $<-2SD$ ) was also associated with lower  $W_{max}$ . Calculated least squared means, adjusted for perinatal factors, socioeconomic factors and factors at conscription (listed in table 4 in methods section) revealed that both increases in GA and BWSDS was independently

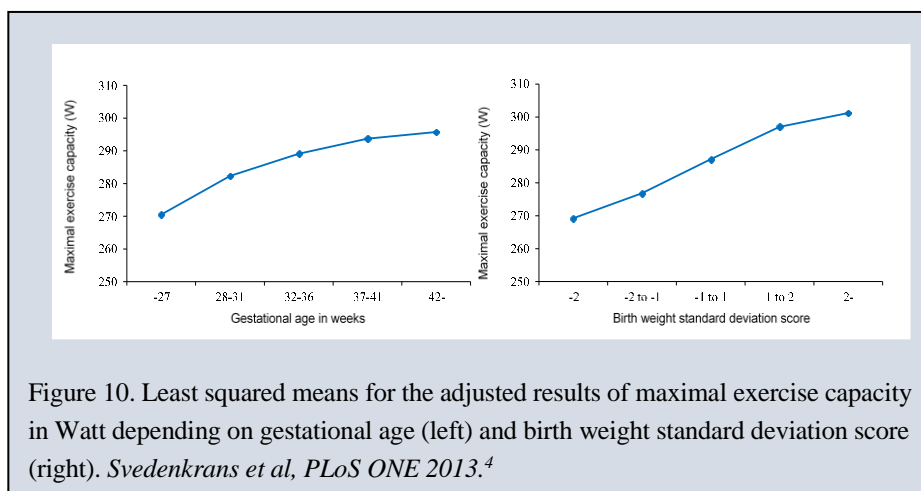


Figure 10. Least squared means for the adjusted results of maximal exercise capacity in Watt depending on gestational age (left) and birth weight standard deviation score (right). *Svedenkrans et al, PLoS ONE 2013.*<sup>4</sup>

associated with increase in  $W_{max}$  ( $p<0.001$ ). The difference in least square mean between extremely preterm ( $<28$  w) born young men and men born at term was 23W whereas the difference in

least square mean for BWSDS  $<-2SD$  and  $>+2SD$  was 32W (figure 10). The interactive effect of GA and BWSDS was tested and shown to be low. All results are shown in table 8, paper I.

#### 5.2.2 Exercise capacity in relation to other covariates

All factors and covariates (table 4 in methods section) were shown to be associated with  $W_{max}$  in unadjusted ANOVA analysis (table 4-7 in paper I) and were included in the adjusted analysis accordingly. Adjusted analysis to test the effect of body mass index (BMI) at conscription and parental education revealed that  $BMI<18$  and the lowest level of parental education were associated with the lowest level of  $W_{max}$ , whereas  $BMI 25-30$  and parental

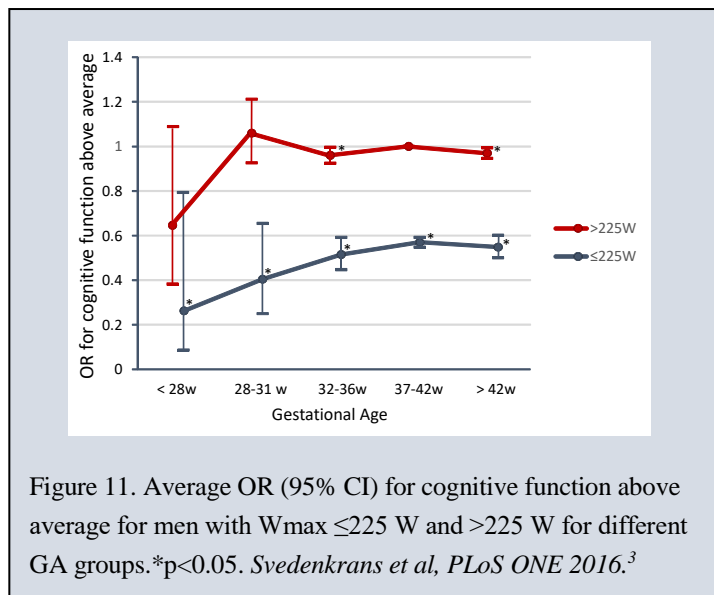
post-secondary education  $\geq 3$  years were associated with the highest average of  $W_{max}$ . All results are described further in paper I, table 4-8.

### 5.3 COGNITIVE FUNCTION IN RELATION TO GESTATIONAL AGE AND EXERCISE CAPACITY (II)

Yong men born extremely preterm and men with the lowest maximal exercise capacity ( $\leq 225$  W) had the lowest average results on the cognitive function test. In comparison to men born at term and men with average exercise capacity the OR for higher cognitive function were 0.51 (CI: 0.31-0.81) and 0.57 (CI: 0.55-0.59) respectively (table 6). Stepwise adjustment revealed that exercise capacity had the strongest effect on cognitive function which changed only marginally when adjusted for GA and BWSDS. GA was the second strongest predictor and the effect was modified mainly by  $W_{max}$  and BWSDS (table 6). To further explore the combined effect of GA and exercise capacity the data were stratified on low  $W_{max}$  ( $\leq 225$  W)

<b>Table 6. Mean stanine scores and Odds Ratios (OR) for cognitive function &gt;2.9 in relation to Gestational age (GA), Birth Weight Standard Deviation Score (BWSDS) and maximal exercise capacity (<math>W_{max}</math>).</b>					
GA	N	Mean Stanine score (SD)	OR (95% CI) for stanine score >2.9		
			crude	model 1	model 2
<28w	56	2.4 (1.4)	0.51 (0.31-0.81)*	0.57 (0.36-0.91) <sup>‡</sup>	0.60 (0.38-0.97) <sup>‡</sup>
28-31w	726	2.9 (1.4)	0.99 (0.87-1.12)	1.02 (0.90-1.16)	1.04 (0.91-1.18)
32-36w	9927	2.8 (1.4)	0.94 (0.91-0.98)*	0.95 (0.92-0.99)*	0.96 (0.93-1.00) <sup>‡</sup>
37-41w	182477	2.9 (1.4)	1.00	1.00	1.00
$\geq 42w$	25616	2.8 (1.4)	0.96 (0.94-0.98) <sup>#</sup>	0.97 (0.95-0.99)*	0.97 (0.94-0.99)*
<b>BWSDS</b>					
-2	7051	2.8 (1.4)	0.91 (0.88-0.95) <sup>#</sup>	0.96 (0.92-1.01)	0.96 (0.92-1.01)
-2 to <-1	35744	2.8 (1.4)	0.95 (0.93-0.96) <sup>#</sup>	0.97 (0.95-0.99)*	0.97 (0.95-0.99)*
-1 to <+1	147805	2.9 (1.4)	1.00	1.00	1.00
+1 to <+2	22970	2.9 (1.4)	1.05 (1.03-1.08) <sup>#</sup>	1.03 (1.01-1.06)*	1.03 (1.01-1.06) <sup>‡</sup>
$\geq +2$	5232	2.9 (1.4)	1.07 (1.02-1.13)*	1.05 (1.00-1.10) <sup>‡</sup>	1.05 (1.00-1.10)
<b><math>W_{max}</math></b>					
$\leq 225$	11726	2.4 (1.3)	0.57 (0.55-0.59) <sup>#</sup>	0.57 (0.55-0.59) <sup>#</sup>	0.56 (0.54-0.58) <sup>#</sup>
226-275	46171	2.8 (1.4)	0.92 (0.90-0.94) <sup>#</sup>	0.92 (0.90-0.94) <sup>#</sup>	0.91 (0.90-0.93) <sup>#</sup>
276-325	76384	2.9 (1.4)	1.00	1.00	1.00
326-375	67896	2.9 (1.4)	1.06 (1.04-1.08) <sup>#</sup>	1.05 (1.04-1.07) <sup>#</sup>	1.05 (1.03-1.07) <sup>#</sup>
$\geq 376$	16625	3.0 (1.4)	1.13 (1.09-1.16) <sup>#</sup>	1.12 (1.08-1.15) <sup>#</sup>	1.11 (1.08-1.14) <sup>#</sup>
<p>Model 1; OR adjusted for GA, BWSDS and <math>W_{max}</math>. Model 2; OR adjusted for GA, BWSDS, <math>W_{max}</math>, maternal age, maternal origin of birth, parity, singleton or multiple birth, parental income, parental educational level, socioeconomic index, BMI, blood pressure, health status. # <math>p &lt; 0.001</math>, * <math>p &lt; 0.01</math>, <sup>‡</sup> <math>p &lt; 0.05</math>. Modified from Svedenkrans et al PLoS ONE 2016.<sup>3</sup></p>					

and non-low  $W_{max}$  ( $>225$  W, figure 11). The sub-group of men who were born extremely preterm ( $<28$  w GA) and had low exercise capacity exhibited the lowest odds ratio (OR=0.26, 95% CI:0.09-0.82) of having a cognitive function above the mean for men born at term with normal birth weight (i.e. stanine score 2.9) (Figure 11).



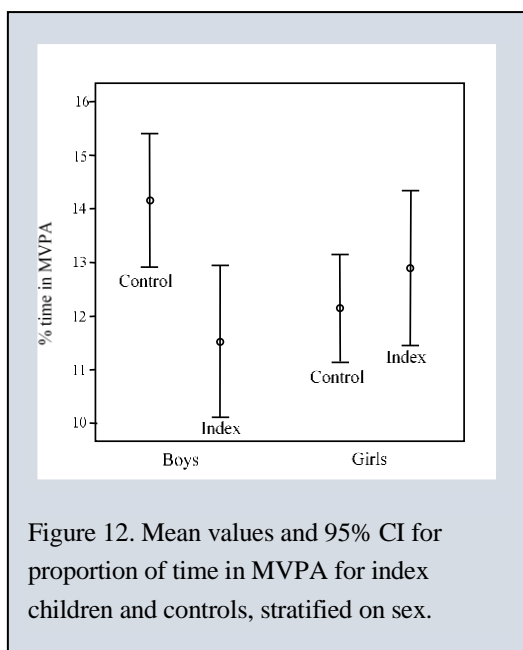
## 5.4 PHYSICAL ACTIVITY IN CHILDREN BORN EXTREMELY PRETERM (III)

### 5.4.1 Study population

Of 158 included children, 71 (39 boys) were born extremely preterm with a mean (SD) GA of 24.5 weeks (1.0), BW of 788g (160) and BWSDS of -0.77 (1.16). The 87 control children (53 boys) who were matched on sex, date of birth, and region of birth, had a mean (SD) GA of 39.8 weeks (1.2), BW of 3625g (463) and BWSDS of 0.18 (0.99). The children were 6.5 years old at follow-up.

### 5.4.2 Physical activity in extremely preterm children compared to term children

The average time per day in moderate to vigorous physical activity (MVPA) in the cohort was 96.5 minutes (SD 32.1), proportion of time in MVPA, 12.8% (SD 4.2) and proportion of time in sedentary physical activity (SED), 52.3% (SD 8.4). An overall comparison of the extremely preterm children (index) to term controls revealed no statistically significant difference in any of the levels of PA. However, when stratifying on sex, boys born extremely preterm spent 20 minutes less per day in MVPA and lower proportion of time in MVPA (2.6% difference) compared to boys born at term (Figure 12). Furthermore, index boys spent more time in sedentary physical activity (SED, 3.8% difference). No difference was seen between index and control girls. All results are presented in table 2, paper III.



### 5.4.3 Growth, neonatal morbidities and physical activity

Severe brain injury (IVH and/or PVL) was the strongest neonatal predictor for lower physical activity (PA) in childhood. Extremely preterm born children with a history of severe brain injury spent in average 35 minutes less in MVPA per day, which accounts for a difference of 4.4 percentage units in proportion of time, compared to children without severe brain injury (figure 13). In contrast, blood culture verified sepsis was associated with 21 minutes more time spent in

MVPA per day compared to children without sepsis (figure 13). There was also an association with BW when each kg increase was associated

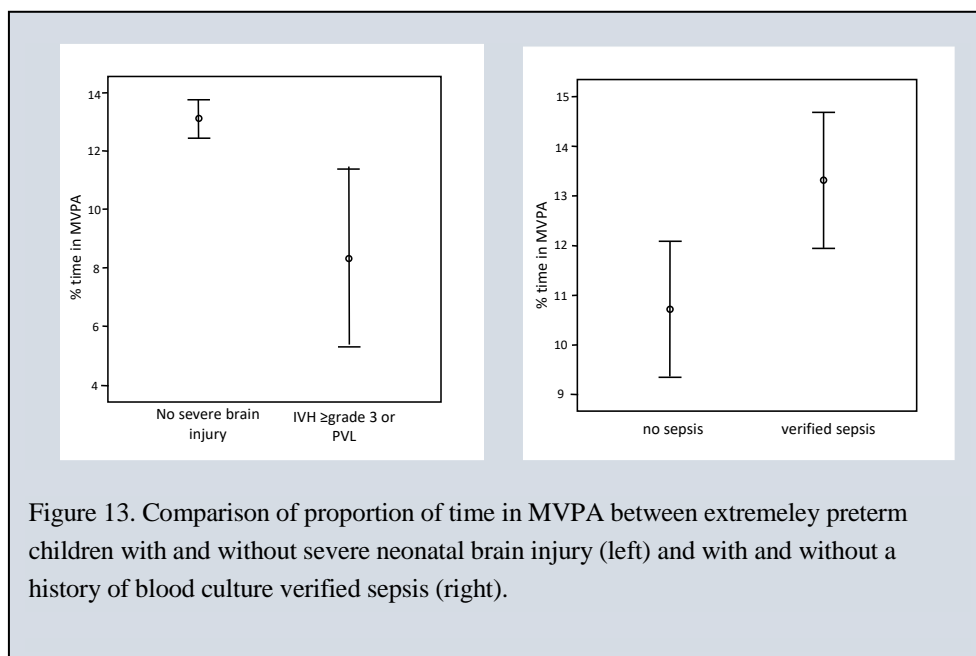


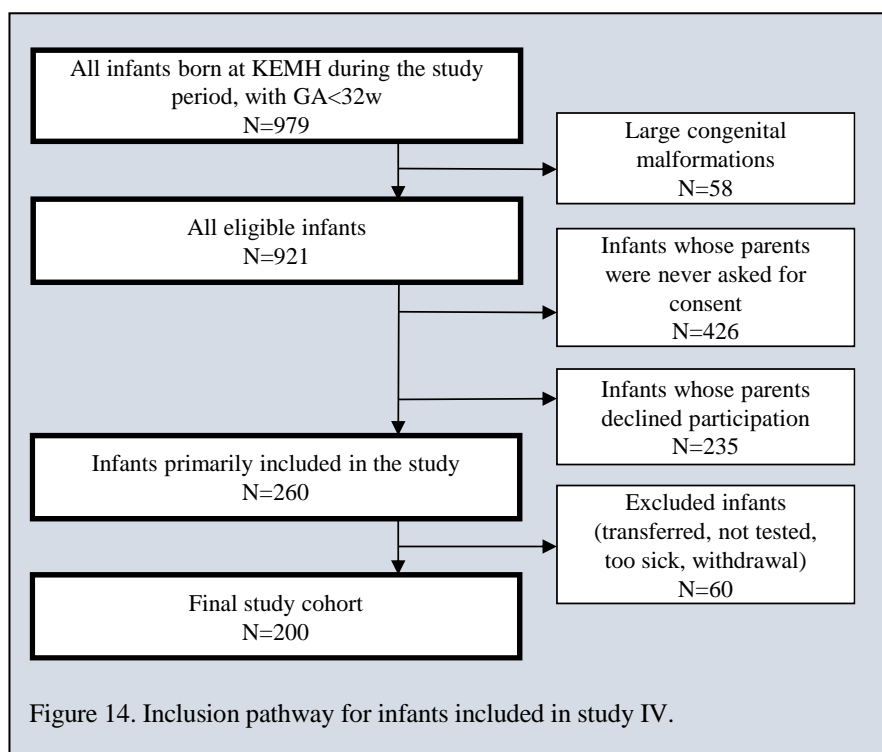
Figure 13. Comparison of proportion of time in MVPA between extremely preterm children with and without severe neonatal brain injury (left) and with and without a history of blood culture verified sepsis (right).

with 50 minutes less time in MVPA per day. However, the BW range was only 460-1161g, and the model fit was lower ( $R^2=0.06$ ), but significance and effect size increased when BW was adjusted for GA (BW vs GA US residuals). In a multiple linear regression model including statistically significant variables only severe brain injury and sepsis came out as significant variables (table 7).

Table 7. Multiple linear regression, proportion of time in MVPA (%), correlation to perinatal morbidities						
			95% CI			
	$\beta$	Std. Error	Lower bound	Upper bound	$R^2$	Sig.
corrected model					0.236	0.000
Intercept	11.31	0.70	9.91	12.71	0.795	0.000
Blood culture verified sepsis	2.34	0.91	0.53	4.15	0.090	0.012
IVH $\geq$ 3 and/or PVL	-3.53	1.39	-6.30	-0.76	0.088	0.013
BW vs GA (US resid)	-6.45	3.55	-13.53	0.64	0.047	0.074
US resid - unstandardized residuals						

## 5.5 PHYSIOLOGICAL BASIS OF BPD CLASSIFICATION (IV)

### 5.5.1 Description of study cohort



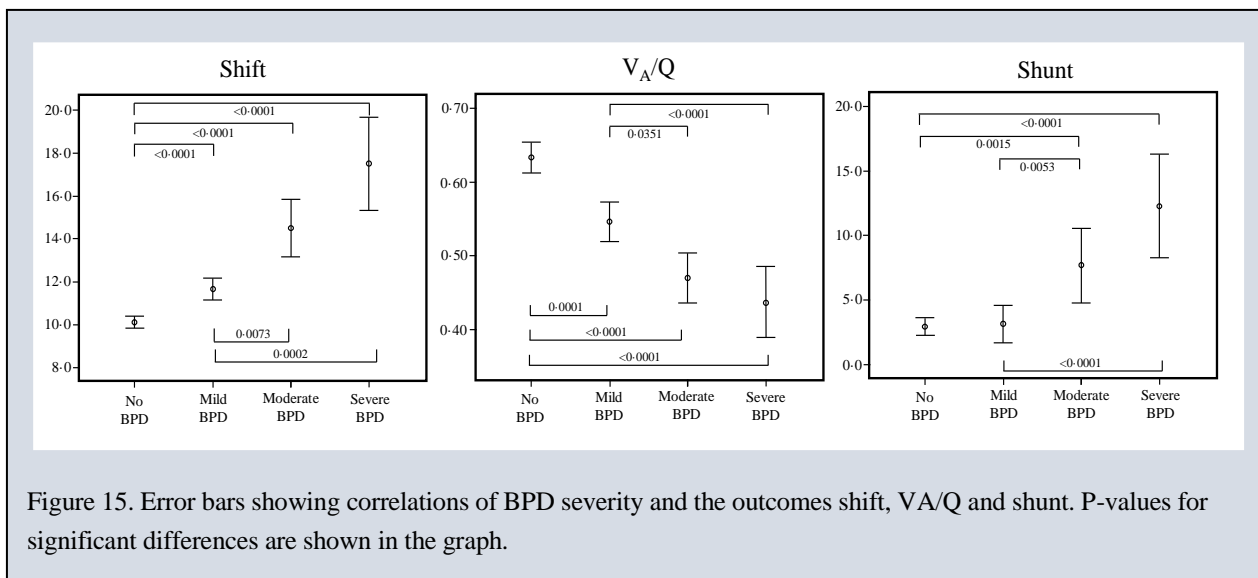
The inclusion flow chart is shown in figure 14. Of 921 eligible infants, 260 were included in the PIFCO study and 200 in the final study cohort. Most of the infants, included in PIFCO but not in the final study cohort were excluded due to need for inter-hospital transfers (n=44) caused by pressures on beds, and was biased towards more mature and healthier

infants being transferred. The main characteristics of the infants in the final study cohort are shown in table 8.

sex (n, % male)	127 (63.5)
GA (median, range)	27.9 (23.0-31.0)
BW (mean, SD)	1066 (311)
BW z-score (mean, SD)	0.02 (0.88)
Choriamnionitis (n, %)	96 (48.0)
Any maternal betamethasone (n, %)	194 (97.0)
Full course maternal betamethasone (n, %)	141 (70.5)
Any postnatal steroids (n, %)	13 (6.5)
Mechanical ventilation (d: median, range)	0.67 (0-81.1)
Moderate to severe BPD (n, %)	54 (27.0)
BW-birth weight, GA-Gestational Age, BPD-Bronchopulmonary Dysplasia	

### 5.5.2 The relation of shift, $V_A/Q$ and shunt to severity of BPD

Shift,  $V_A/Q$  and shunt were all significantly correlated to BPD severity. Shift and  $V_A/Q$  significantly separated no, mild and moderate BPD, whereas the difference between moderate and severe BPD was non-significant (figure 15). Infants with no or mild BPD had very little shunt, but there was a statistical difference to moderate-to-severe BPD.



Threshold values for BPD at all and moderate to severe BPD, detected by using receiver operating curve (ROC) analysis (table 3, paper IV), showed that shift was the best outcome to use to separate BPD severity. The threshold for moderate to severe BPD was identified as 12.22 kPa, with a sensitivity of 83.3% and a specificity of 87.0%.

Interestingly, when separating infants with severe BPD in two groups; infants with supplemental oxygen at 36w and infants without supplemental oxygen but with positive pressure ventilation (CPAP or mechanical ventilation) at 36w, the two groups had significantly different values of shift and  $V_A/Q$  (table 9). These results explain some of the overlaps between moderate and severe BPD and indicate that the pathophysiology behind the need for respiratory support may be different in these two groups.

<b>Table 9. Mean (CI) shift, <math>V_A/Q</math> and shunt for infants with severe BPD and respiratory support at 36w, with and without need for supplemental oxygen.</b>			
	Respiratory Support, no supplemental oxygen, (n=6)	Respiratory support and/or supplemental oxygen, (n=25)	Mean difference (95 %CI)
Shift	10.5 (10.3,10.8)	16.8 (15.4, 18.2)	8.2 (5.7, 10.6)
$V_A/Q$	0.61 (0.59, 0.63)	0.43 (0.40, 0.46)	-0.20 (-0.30, -0.11)
Shunt	3.1, (2.5, 3.8)	10.6 (7.9, 13.4)	7.7 (-1.7, 17.1)



### 5.5.3 Correlations of predictors and the outcomes of shift, $V_A/Q$ and shunt

Six factors were identified as key potential explanatory variables including; gestational age, duration of mechanical ventilation (unstandardized residuals), weight z-score at the time for modified ORT, intubation the first 48 h of life (unstandardized residuals), protein/kg/day the 1<sup>st</sup> 28 days, and postmenstrual age at test (suppl table 4, paper IV). When entered into a stepwise multiple linear regression model, GA came out as the major explanatory factor for shift and  $V_A/Q$ , (table 10). GA together with duration of mechanical ventilation explained 33.6% of the variability in shift and 23.6% of the variability in  $V_A/Q$  with other explanatory factors affecting the outcome only marginally. Duration of mechanical ventilation was the major factor explaining for variability in shunt and GA the second most important factor. The two factor's contribution to the variability in shunt was 18.3% (table 10). Duration of mechanical ventilation was identified as the most important factor describing disease severity. Consequently, the analysis does not show that it is the time on ventilator that is most important, but that infants who are severely sick have worse outcome. Running the analysis with postnatal steroids or air leak had the same effect.

Table 10. Multiple linear regression for key factors explaining shift, $V_A/Q$ and shunt.						
Outcome Variable	Explanatory Variables	Adjusted R <sup>2</sup>	Coefficient (B)	SE	95 % CI	p value
Shift	Intercept		31.6	1.5	28.6, 34.7	<0.0001
	Gestational age (w)	0.190	-0.712	0.051	-0.81, -0.61	<0.0001
	Duration of mechanical ventilation (d) (US Resid)	0.336	0.136	0.009	0.12, 0.15	<0.0001
	Length Z score at 36w PMA	0.340	0.318	0.119	0.08, 0.55	0.0078
	Surfactant treatment	0.343	0.694	0.259	0.19, 1.20	0.0074
	Shunt (%) (US Resid)	0.354	-0.070	0.017	-0.10, -0.04	<0.0001
$V_A/Q$	Constant		-0.162	0.044	-0.248, -0.076	0.0002
	Gestational age (w)	0.181	0.026	0.002	0.023, 0.029	0.0000
	Duration of mechanical ventilation (d) (US Resid)	0.236	-0.003	0.000	-0.004, -0.003	0.0000
	Shunt (%) (US Resid)	0.262	0.002	0.001	0.001, 0.004	0.0006
	Shift (kPa) (US Resid)	0.268	0.006	0.002	0.002, 0.010	0.0026
Shunt	Constant		8.44	8.58	-8.40, 25.3	0.3256
	Duration of mechanical ventilation (d) (US Resid)	0.137	0.23	0.02	0.20, 0.26	0.0000
	Gestational age (w)	0.183	-0.71	0.10	-0.91, -0.51	0.0000
	Surfactant	0.189	1.37	0.49	0.40, 2.34	0.0055
	PMA Test (w PMA)	0.193	0.43	0.22	0.00, 0.86	0.0514
	Shift (kPa) (US Resid)	0.231	-0.42	0.06	-0.54, -0.30	0.0000

W PMA – weeks postmenstrual age, US Resid – unstandardized residuals, CI – confidence interval



## 6 DISCUSSION

### 6.1 METHODOLOGICAL CONSIDERATIONS AND IMPLICATIONS OF RESULTS

#### 6.1.1 Exercise capacity in young men born preterm (I, II)

To use registers for research has the great advantage that large populations can be studied without having to organize follow-ups or test all the individuals. A lot of information can be retrieved without too much effort. In study I and II almost 220000 subjects were included. It gives enough power to adjust for several possibly confounding factors with excellent statistical significance. The population-based design also implicates good generalizability. However, there are some disadvantages with register studies in general. The large datasets makes it possible to identify even small differences that may not be of clinical relevance. Another limitation is that even though tests are standardized and should have been performed in the same way, there is always a risk that over the eight years that conscription data were collected, there may have been differences in how the recruits performed the tests. Furthermore, data may have been entered wrong. However, all these are random errors that would affect preterm born men and term born men equally. If something, the effects may be diluted. Moreover, register studies can never show a causal relationship, only give indications of such a relation.

##### 6.1.1.1 *The maximal exercise capacity test*

The maximal exercise capacity test used at conscription for military service has some limitations. Standardized maximal exercise capacity test usually starts on a low workload. According to American Heart Association recommendation a common starting point is 25W.<sup>190</sup> At conscription the test was started at 125W. Furthermore, the workload was increased every minute whereas the recommendations are to increase every 2-3 minutes.<sup>190</sup> Given that all the men were young and healthy it probably had minor effects on the results. The cycle ergometer has the advantage compared to a treadmill that body weight has little effect on the effort needed. However, quadriceps fatigue may be a limiting factor. Nonetheless, all these factors were equal for all men conscripting for military service. All men were instructed to try to achieve their very best. Given that the test was done when conscripting for military service, one has to consider that not all men did their best. Motivation to do the military service may have affected the outcome. Most likely these are random errors affecting all study subject equally. Another discussion relevant to the results is the choice not to express the maximal exercise capacity in W/kg instead of W. Exercise tests on treadmill always have to be expressed in W/kg since the effort will be dependent on the weight of the subject. But, when sitting on a cycle ergometer the effort will be the same independent of weight. Absolute value of W was chosen in order not to overestimate the exercise capacity of men with low weight. Nonetheless, in unadjusted analysis, men with low BMI (<18 kg/m<sup>2</sup>) had a lower W<sub>max</sub> than men with higher BMI. The men with BMI 25-30

kg/m<sup>2</sup> exhibited the best results. In adjusted analyses, the least squared means were adjusted for BMI and therefore the effect of weight has to be considered as minor.

Maximal oxygen consumption (VO<sub>2max</sub>) is the common variable to use as outcome when measuring exercise capacity. Preliminary analyses were done using VO<sub>2max</sub> giving basically the same results why we chose to use the plain value in Watt. Although, if repeating the study, VO<sub>2</sub> max would probably have been included for comparative reasons.

#### *6.1.1.2 The test for cognitive function*

The largest limitation to consider when interpreting the results from the cognitive test used at conscription is that it is not a standardized intelligence test. The results cannot be compared to other tests for cognitive function. The questions are regarded as secrecy and were not revealed for us to compare with other intelligence tests. In other respects, the test was developed to test cognitive function and all conscripts took the same tests in the same amount of time. Consistent with our results children born preterm has performed lower on IQ tests previously.<sup>134, 191</sup> Given the limitations, the results of the cognitive function test has to be considered reliable.

#### *Implication of results from exercise capacity testing*

In study I, we show that healthy male survivors of preterm birth exhibit lower levels of maximal exercise capacity at 18 years of age. The analysis is done on a large population-based cohort which indicates good generalizability, at least to the male population. These results suggest that preterm birth will have consequences still in young adulthood also for healthy survivors. The difference between an average young man born extremely preterm and a young man born at term is 23W. On cycle ergometer testing that accounts for approximately 1 metabolic equivalent (MET), which is approximately equal to an oxygen consumption of 3.5 ml/kg/min.<sup>190</sup> In unadjusted analysis, the difference is somewhat larger, 29W (I). All in all, the difference is not very big, and the results are within the normal range. One conclusion could be that healthy survivors of preterm birth has a normal exercise capacity. On the other hand, level of fitness has been correlated to risk factors for cardiovascular diseases and the lower level of fitness may be a mediator in the increased risk of cardiovascular diseases that a preterm birth imply.<sup>11-14, 192, 193</sup> Similarly, preterm survivors who are growth restricted would suffer an additive effect of 19W compared to a man born with a weight appropriate for gestational age. The difference would then be almost 2 METs. The difference in maximal exercise capacity between an average man and woman is 1-2 METs, indicating that this difference may affect these men in daily life. Furthermore, the group of preterm survivors is a diverse group, and the lower average exercise capacity imply a higher percentage of men with low fitness. The question arises whether these differences can be attributed to a difference in PA. Habitual PA is poorly correlated to exercise capacity in the youth, at least when measured by pulse monitors, nonetheless, exercise capacity can be increased by exercise.<sup>194</sup> A hypothesis could be that survivors of preterm birth have more difficulties at exercise and PA already in early childhood and consequently choose other

activities. Especially since exercise and sport, activities shown to increase exercise capacity, tend to include competition. Future studies have to shed light on the explanation for the difference, and more importantly, to find interventions that could improve the outcome.

#### *6.1.1.3 The level of cognition after preterm birth is associated with exercise capacity*

Cognitive impairment is a well-known consequence of preterm birth, which is confirmed by the findings in study II.<sup>134, 191</sup> We knew from study I that there was an association between preterm birth and lower exercise capacity. Åberg and collaborators previously revealed that there is an association between exercise capacity and cognitive function in young men at conscription.<sup>169</sup> From the results in study II we conclude that there is an association between low exercise capacity and low cognitive function also in men born preterm. The men with low GA and low exercise capacity exhibited the lowest results on the cognitive function test. The correlation could be explained by an association to neurodevelopment and both exercise capacity and cognitive function, but could also be an indication that exercise improves cognitive function (further discussed below). We can draw the conclusion that low exercise capacity is associated with lower cognitive function in healthy young men born preterm. This knowledge can be used as a background information for further studies on exercise and physical activity in children and adolescents born preterm.

### **6.1.2 Physical activity in children born preterm (III)**

#### *6.1.2.1 Measurement of physical activity with accelerometer*

As described in the methods section, measurement of PA using accelerometers imply several general limitations. When interpreting the results from study III these has to be taken into consideration. The general limitations, that accelerometry is a poor method to measure bike riding and that the amount of counts will be different in different study subjects would most likely imply random errors not affecting the outcome. It is also likely that few 6 year olds spend a lot of time bike riding. Nonetheless, preterm children have an increased risk of DCD, which at least theoretically could cause different movement patterns which may affect both how the counts are measured and the ability to ride a bike. To my knowledge no study have addressed this question. Accelerometers don't measure how much energy that is used when performing the activity and children born extremely preterm has previously been shown to find PA more effortful than term peers.<sup>9</sup> Consequently, even if the PA levels are in a normal range, children born preterm may require more effort to achieve these levels. This means that accelerometer results should be interpreted as the movement that happened, not how much effort that was needed to produce the activity.

More important when interpreting the results is the choice of cut-points for the activity levels. There are no validated cut-points for the age group using wrist-worn accelerometers. There are cut-points for 4-year olds and cut-points for 8-12 year-olds available. Our choice to use the older category was two-fold. First, 8-year-olds were closer in age to our 6.5-year-olds and second, we assumed that the movement pattern would change more between 4 and 6.5 than between 6.5 and 8. Nonetheless, the application of these cut-points will give an idea of a

comparison but it is very difficult to draw conclusions about whether the level of PA is enough from a health point of view.<sup>195</sup>

A last consideration was the choice to use 60 seconds epochs for the analysis of the accelerometer results. Generally it is recommended to use shorter epochs for children (5-15 seconds), since a lot of the activity will be performed in short time periods.<sup>180</sup> Although the data was collected in shorter epochs, we chose to analyze the results in 60 seconds epochs, assuming that an activity episode of less than 60 seconds may not add anything in relation to the health aspect. However, the data will be reanalyzed in 10 seconds epochs and the outcome will be compared, before submission of the paper.

#### *6.1.2.2 Placement of the accelerometer*

Most studies in the literature used hip-worn accelerometers.<sup>9, 157, 158</sup> and they have been validated more thoroughly. The choice to use wrist-worn accelerometers was based mainly on the need for a higher compliance while still giving valid measurements.<sup>178, 179</sup> In the Millennium Cohort study, where hip-worn accelerometers were used, almost 50% of the children accepting to wear the accelerometer did not produce valid data.<sup>158</sup> In study III, 82% of the children produced valid data. There may have been other differences between the studies regarding distribution of accelerometers and how motivated the study subject were, but placement of the accelerometer as a factor for compliance cannot be excluded. The comparison between index and control children and the association to perinatal morbidities, which were the main aims of the study, would not be affected.

#### *6.1.2.3 Implication of results from accelerometer measurements*

When comparing PA levels in children born extremely preterm to children born at term there were no differences. However, when stratifying on sex, extremely preterm boys are less physically active than boys born at term. Correlation of neonatal morbidities revealed that severe brain injury (IVH  $\geq$  grade 3 and/or PVL) was associated with less time in MVPA. Given that the boys were significantly more affected by severe brain injury (20% vs 3%,  $p < 0.05$ ), and that male sex was no longer significant in adjusted analyses, these findings are most likely explained by the fact the boys were more severely affected by their preterm birth. NDD or DCD may be possible explanatory factors for lower activity levels, however correlation of PA to these outcomes was not the aim of the study. Another interesting finding was that sepsis was associated with more time in MVPA. One possible explanation could be hyperactivity. There is an indication of such a relationship in previous studies,<sup>95, 97</sup> however this finding has to be further explored. Only one previous study have found differences in PA between children born preterm and children born at term.<sup>158</sup> They were measured at the same age and the difference was seen in boys only. Two studies reported no such differences.<sup>9, 157</sup> The inconsistent results between studies could be attributed to the fact that different age groups were measured, or to the limitations with accelerometer measurements.

From study III we conclude that there is a difference in time in MVPA between boys born extremely preterm and term peers, which could be attributed to the fact that the boys were

more severely affected by preterm birth as mirrored in a higher incidence of severe brain injury.

### 6.1.3 Exercise capacity and physical activity (I, II, III)

The health benefits of physical activity are well-known.<sup>163</sup> There is also growing evidence that PA is good for development of the cognitive function.<sup>167</sup> Children and adults born preterm constitute a risk population considering non-communicable diseases like overweight, hypertension and diabetes and have an increased risk of lower cognitive function.<sup>12, 121, 122, 124, 134, 196</sup> These facts raises the question whether the outcomes in terms of cardiovascular disease

*Could increased physical activity mitigate the long-term consequences of preterm birth?*

and lower cognitive function could be improved by increased PA.

From study I-III, we can conclude that being born preterm is associated with effects on physical activity and exercise capacity in later life.

Moreover, we demonstrate a correlation between low exercise capacity and low cognitive function. The results from these studies cannot prove that increased PA would be beneficial for survivors of preterm birth, but the results could provide clues to understand if such relationship exists. And they rather support than contradict the hypothesis.

Young men born preterm have lower exercise capacity at 18 years of age. Exercise capacity has been correlated to cardiovascular risk factors.<sup>160</sup> The lower exercise capacity may be a mediator to future cardiovascular diseases. Several reports suggest that the difference in exercise capacity is prevalent already in childhood.<sup>8, 9, 197</sup> The lower exercise capacity could be related to decreased lung function,<sup>9</sup> vascular growth,<sup>198-201</sup> or possibly due to lower levels of physical activity and exercise. However the relation between PA and exercise capacity is complicated. In a meta-analysis by Armstrong and collaborators, the correlation between PA in youth, measured predominantly by heart rate monitors, and exercise capacity, was poor.<sup>194</sup> When using accelerometers or self-reported PA more studies have managed to demonstrate such relationship.<sup>202, 203</sup> Some studies on adolescents and young adults indicate that preterm born are less physically active, which could imply a lower level of exercise capacity.<sup>153, 204</sup> Study III further supports that at least sub-groups of children born preterm may be less physically active. The correlation could also be the other way around that subjects born preterm are less physically active because of lower exercise capacity. Nonetheless, exercise capacity is related to exercise,<sup>194</sup> and it seems like preterm born adolescents can improve their capacity by training.<sup>202</sup> Survivors of preterm birth, in at least some cases, are; (1) less physically active, (2) exhibit lower levels of exercise capacity (3) have the possibility to improve their exercise capacity by training and (4) PA in the general population is protective against risk factors associated with preterm birth. This knowledge indicates that future studies aiming to increase PA in children and adolescents born preterm would be of high interest.

Young adults born extremely preterm achieving the lowest results on the exercise test also exhibited the lowest results on the cognitive function test. This correlation is known in the

general population,<sup>169</sup> but these findings indicate that this relationship is a reality also in subjects born preterm. There are several different explanations for the lower cognitive function after preterm birth. Some of the survivors have moderate to severe neurodevelopmental disabilities (NDD) which affects both the possibility to be physically active and the cognitive function. However, there is a large group of survivors with mild or no NDD which still may have lower exercise capacity and lower cognitive function. This is reflected by the results in study II, which only included healthy young men without moderate to severe disabilities. In the general population, exercise and PA was positively related to academic achievement and/or cognitive function, and interventions to increase PA has shown beneficial effects.<sup>168, 170-172</sup> If such an intervention would have a positive effect also for children born preterm, remains to be proven.

#### **6.1.4 Physiologic definition of BPD (IV)**

##### *6.1.4.1 The modified oxygen reduction test*

Most infants were tested in a head box by reducing  $PIO_2$  in a stepwise manner. However, some of the sicker infants, were tested on CPAP or HHFNC which could theoretically have improved gas exchange and affected the outcome. The effect of respiratory support on shift,  $V_A/Q$  and shunt needs to be further explored. The post menstrual age (PMA) at test ranged from 33<sup>5</sup> to 39<sup>2</sup> weeks. The well infants were tested earlier than the sicker infants. The well infants may have had better results if tested later and the sicker worse results. This may have affected the thresholds derived from the ROC analysis, causing smaller differences between infants with no BPD and moderate to severe BPD. Nevertheless, the threshold derived from the ROC curve may rather be used as starting point to define disease severity. When using the measure in more studies, and more importantly, relating shift value at discharge to respiratory function at follow-up, more accurate thresholds for disease severity can be defined.

##### *6.1.4.2 Analysis of shift, $V_A/Q$ and shunt using the Quine model*

Analysis of shift,  $V_A/Q$  and shunt by using the specially developed computer program has several pitfalls. First, preterm infants have irregular breathing patterns and occasional desaturations that can sometimes not be explained or predicted. To mitigate the effect of these irregular saturation patterns, we used averaged values from at least one minute recording of  $SpO_2$  for each level. In addition, the use of several data points also improved the accuracy of the  $SpO_2$  vs  $PIO_2$  curve. Nonetheless, these desaturations may still imply random errors in the analyses.

Second, the program utilizes a model to calculate  $V_A/Q$  and shunt from the  $SpO_2$  vs  $PIO_2$  curve. The program has been validated by using exact datasets with known values of  $V_A/Q$  and shunt.<sup>187</sup> Shunt values were usually clinically accurate in both the 2 compartment (2C) and the 3 compartment (3C) models (absolute error  $\pm 2\%$ ), whereas the 2C model systematically overestimated  $V_A/Q$ . The 3C model produced accurate values for the low  $V_A/Q$  compartment but there were large errors in  $V_A/Q$  values in the high  $V_A/Q$  compartment, and the proportion of the distribution between the high and the low  $V_A/Q$  compartment.



Nevertheless, the 3C model produced more accurate values in relation to the exact values and when data points were removed.<sup>187</sup> Overall, the program was judged as providing values that were clinically accurate.

The program provides results for both the 3C and the 2C model and recommends which one to use based on the best fit. In most cases (195/200) our data sets fitted the 2C model best and furthermore, most of the values were identical for the two models. The 3C model has been shown to be more accurate when testing sicker infants due to less homogeneously ventilated lungs. For infants with reasonable homogenous lungs, which seems to be the case in most preterm infants, the 2C would be enough. This is particularly important, since testing of homogeneously ventilated lungs, will produce a SpO<sub>2</sub> vs PIO<sub>2</sub> curve that is similar in shape to the ODC, from which a reliable shift value can be derived. The advantage with the shift value is that it is independent of the calculations of V<sub>A</sub>/Q and shunt and will be decided by the value on the x-axis that corresponds to 80-85% saturation. Consequently, in 195/200 infants, the shift value would be an accurate measure of pulmonary function, however not taking the shunt into consideration. How to use the shift, shunt, and V<sub>A</sub>/Q values in clinical practices needs to be further explored.

#### *6.1.4.3 Relation of predictors and BPD status to shift, V<sub>A</sub>/Q and shunt (IV)*

BPD is related to several perinatal factors, which we evaluated in order to analyze the relation to the outcome measures. One problem with these predictors are that they are interrelated which will cause multicollinearity in a regression model. We used principal components analysis in order to identify key variables to use in a regression model to avoid this problem. In multiple linear regression, gestational age and time on mechanical ventilation came out as the strongest predictive factors. Even though ventilation per se may have an effect on outcome, this variable should mainly be interpreted as a marker of disease severity. Basically all other predictors will be interrelated with time on mechanical ventilation eg GA, antenatal steroids, NEC, sepsis, nutrition, IVH, postnatal steroids and so on. These relationships have to be taken into consideration when interpreting the outcome in relation to time on mechanical ventilation. Moreover, some of the predictors, eg antenatal steroids and surfactant, were distributed to almost all sick infants which makes analysis of correlations to pulmonary function useless.

#### *6.1.4.4 Implications of results*

The results for shift, V<sub>A</sub>/Q and shunt are related to BPD severity according to the NICHD criteria, showing that shift increases and V<sub>A</sub>/Q decreases with increasing disease severity,

*Severity of BPD needs to be classified according to an objective and physiologic measure. Right shift of the SpO<sub>2</sub> vs PIO<sub>2</sub> curve has the potential to be that measure.*

whereas shunt is more common in moderate to severe BPD. The strongest predictor of shunt is disease severity with GA as the second most important, indicating that other factors than maturity are important for the development of

shunt. The outcomes were related to BPD severity in order to be able to compare the results to the clinical situation. Neonatologists have a relation to BPD severity according to the NICHD criteria,<sup>50</sup> but not to shift,  $V_A/Q$  and shunt.

The current NICHD definition of BPD lacks objectivity, and Walsh criteria risk to classify too many infants as disease free.<sup>50, 51</sup> BPD severity needs to be classified according to a more objective and physiologic measure than the methods used today.<sup>50</sup> The measurement of right shift of the SpO<sub>2</sub> vs PIO<sub>2</sub> curve has the potential to become this measure. There are several advantages of the method. First, it is an objective measure that is not dependent on local traditions of oxygen supplementation traditions or guidelines for saturation levels. Second, the shift value is related to factors known to cause severe pulmonary outcome. Third, being a continuous value it could be used to identify small changes in disease severity. Fourth, the shift in kPa is easy to relate to, since it is equal to the amount of supplemental oxygen that needs to be given to a sick infant in order to achieve the same level of saturation as an infant with healthy lungs. Finally, while providing a simple and intuitive value for disease severity, the same method can be used to understand the pathphysiological background in sicker infants. The method can be used bedside to understand whether an infant is shunting or if there is a pulmonary failure due to decreased  $V_A/Q$ . The method has the possibility to change BPD classification, nonetheless, it needs to be further explored to reach full utility.

## 7 CONCLUSIONS

Being born preterm implies increased risks of neurodevelopmental disabilities, lower cognitive function, reduced pulmonary function, and increased risks of cardiovascular diseases and early death. This knowledge could be used as arguments against the spending of resources and effort on neonatal intensive care. In contrast, two thirds of the children in the EXPRESS cohort had no or only mild neurodevelopmental disabilities at 6.5 years of age. There was no difference in physical activity levels when comparing all extremely preterm born children to children born at term and the men born preterm conscripting for military service achieved good results on the exercise capacity test. A lower average exercise capacity, some points lower on an IQ-test or lower FEV<sub>1</sub>% don't necessarily mean that you have a lower quality of life. The results presented in this thesis may add pieces to the puzzle we have to build to further improve the outcome for these children. The physiologic definition of BPD severity may help to evaluate treatments aiming to improve respiratory outcome in a more accurate way. This could help us to find the best way to handle our tiny patients in order to avoid poor respiratory outcomes. Furthermore, the studies on exercise capacity and physical activity motivates further investigations on physical activity and preterm birth. If such studies show positive results, we may develop ways to improve late outcomes after the neonatal period. Compared to neonatal care fifty years ago, when BPD was new, we have achieved almost miraculous improvements. The next big challenge is not only to increase survival, but to further increase survival without severe disabilities and to the best health possible.

## 8 FUTURE PERSPECTIVES

### 8.1 PHYSICAL ACTIVITY

The results from study I-III in this thesis demonstrate that preterm birth is associated with less physical activity in sub-groups of children born preterm, with lower exercise capacity, and with lower cognitive function. To increase the knowledge about the implications of these findings, further studies are needed. Such studies need to address the following questions:

- i. What are the relations between NDD and PA levels at 6.5 years of age?
- ii. What are the correlations between PA levels and neuropsychiatric disorders at 6.5 years of age?
- iii. What happens to the PA levels as the children grow?
- iv. Could increased PA improve the outcome of preterm birth?

Some of these studies are easy to conduct. The follow-up of the EXPRESS cohort at 6.5 years of age included neurodevelopmental and neuropsychiatric follow-up. To analyze the correlations to PA would imply a collaboration with those who conducted these parts of the study, which should be possible.

Moreover, the children in the EXPRESS cohort are now reaching 12 years of age, and the next follow-up has already started. This time all included children will be invited to

participate in the activity measurement. We will be able to collect data in a larger cohort, and for some children we will get longitudinal data.

It will be more challenging to test if outcome of preterm birth could be improved by increased PA. First, the ideal intervention has to be identified, second, the ideal target group needs to be identified, and third, an accurate outcome measure has to be chosen. Two possible approaches could be as follows:

- i. *Intervention to improve DCD symptoms.* Study subjects would be children born preterm without NDD, but with DCD symptoms. Control subjects would be children born preterm matched on sex and GA receiving conventional follow-up and care. Intervention would be coordination and motor training together with physiotherapist. Outcome measures would be improvement in DCD symptoms and level of PA after intervention. Ideally, these children would be followed-up for a long time to evaluate if any consistent change could be seen.
- ii. *Intervention to increase PA in schools.* By liaising with studies aiming to increase PA in schools and measure academic achievement, we could reach children born preterm without severe NDD. The intervention could be increased amount of PE lessons and the outcome grades in the later school years. Controls would be children born preterm on schools not included in the intervention.

## **8.2 FURTHER DEVELOPMENT OF THE PHYSIOLOGICAL DEFINITION OF BPD**

Our results on the physiological definition of BPD show promising results. Nevertheless further studies are needed to reach the full utility of the method. Future studies should aim to:

- i. Evaluate the short-term repeatability and reliability of the measures.
- ii. Evaluate the utility of one paired value measurements.
- iii. Assess the impact of non-invasive ventilation on the measurements of shift,  $V_A/Q$  and shunt.
- iv. Develop a simple look-up table to estimate shift in infants who are classified as having no BPD or mild BPD to avoid the need for hypoxic testing for screening purposes.
- v. Understand the prognostic value of shift,  $V_A/Q$  and shunt and subsequent respiratory well-being during infancy and beyond.

In earlier papers utilizing the Quine model, one single measurement of paired values of  $SpO_2$  vs  $PIO_2$  was shown to be enough to derive a shift value.<sup>58</sup> This was before the development of the 3C model and the knowledge about the impact on the  $SpO_2$  vs  $PIO_2$  curve of increased flow to the low  $V_A/Q$  compartment.<sup>187</sup> Nevertheless, only 5/200 infants in our cohort had a  $SpO_2$  vs  $PIO_2$  curve better fitted to the 3C model. Consequently, 97.5% of the infants had shift values that could be considered as reliable. To use single measurements to derive a shift

value would increase utility, but the reliability of this approach needs to be evaluated. One limitation may be that a single shift value will not be affected by a shunt and will consequently risk to overestimate the pulmonary function. If not possible in sicker infants, the one value approach would most likely be possible in infants who are without supplemental oxygen at discharge. A look-up table for these infants has been calculated on the same cohort, but has not yet been published.

Furthermore, the possibility to predict respiratory outcome needs to be evaluated. One-year follow-up of the PIFCO cohort is on-going, with no data available yet.



## 9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Allt fler barn föds för tidigt. Vissa av dessa barn kommer att drabbas av följder i form av olika funktionsnedsättningar. Exempel på sådana är nedsatt kognitiv förmåga och nedsatt lungfunktion. Dessutom har de som överlevt en för tidig födelse en ökad risk att drabbas av livsstilssjukdomar som hjärt-kärlsjukdomar och diabetes senare i livet. Mer kunskap behövs för att vi ska kunna förebygga eller lindra dessa komplikationer.

Fysisk aktivitet har positiv inverkan på hälsan och kan förebygga och minska effekterna av livsstilssjukdomar. Dessutom finns det alltmer forskning som visar att rörelse också kan påverka hjärnans funktion i positiv riktning. I studie I och II har vi använt resultat från cykelprovet och intelligenstestet vid månstringen till värnplik och kopplat det till uppgifter om graviditetslängd och födelsevikt i födelseregistret. Analyserna visade att unga, friska män som är för tidigt födda har en lägre fysisk arbetsförmåga än män som är födda i normal tid. Resultaten blev stegvis bättre med ökande graviditetslängd. Dessutom var resultatet på intelligenstestet bättre för de med högre fysisk arbetsförmåga. De som hade lägst resultat på intelligenstestet var de män som både var extremt för tidigt födda (< 28 veckor), och hade lägst resultat på cykelprovet. Vi kunde således se en koppling mellan för tidig födelse, lägre fysisk arbetsförmåga och lägre intelligens.

Vi ville också ta reda på om för tidigt födda, förutom en lägre fysisk arbetsförmåga, också rörde på sig mindre. Vi jämförde 71 extremt för tidigt födda barn (<27 veckor) med 87 barn födda i vanlig fullgången tid. Vid 6,5 års ålder mätte vi deras rörelseaktivitet i vardagen med en accelerometer, ett slags stegräknare, under en veckas tid. När vi jämförde alla barnen med varandra var det ingen skillnad i rörelse. När vi däremot jämförde pojkar och flickor var för sig, visade det sig att de för tidigt födda pojkarna rörde sig mindre än sina jämnåriga som var födda i fullgången tid. Vi såg att hjärnskada under nyföddhetsperioden var en möjlig förklaring till lägre fysisk aktivitet, och att det oftare drabbade pojkar.

Från studie I-III drar vi slutsatsen att nivån av fysisk aktivitet i barndomen kan ha ett samband med för tidig födelse och sjuklighet under nyföddhetsperioden. Vidare kan vi se en koppling mellan för tidig födelse och nedsatt fysisk arbetsförmåga och intelligens vid 18-års ålder. För att få veta om ökad fysisk aktivitet skulle kunna minska följsjukdomarna eller bidra till en bättre kognitiv utveckling hos för tidigt födda barn, måste flera studier göras.

Träningsförmåga och fysisk aktivitet skulle kunna påverkas av en försämrad lungfunktion. Barn som är för tidigt födda riskerar att drabbas av bronkopulmonell dysplasi (BPD) i nyföddhetsperioden. Det är en kronisk lungsjukdom som kan ge försämrad lungfunktion genom barnåren och upp i vuxen ålder. Den metod som vanligtvis används för att ställa diagnosen BPD utgår ifrån vilken behandling barnet får istället för att vara ett mått på hur barnets lungor fungerar. Syftet med studie IV var att testa en annan, nyare metod i en större grupp barn. Man varierar barnets syrgastillförsel stegvis, samtidigt som man registrerar vilken syremättnad barnet har vid varje nivå. Den kurva som skapas av dessa parade värden kan

sedan analyseras i ett speciellt datorprogram som genererar tre olika värden på lungfunktion. Vi visade att shift-värdet var det värde som fungerade bäst som ett mått på allvarlighetsgrad av BPD. Shift-värdet är ungefär lika stort som den extra mängd syrgas som måste ges till ett barn med sjuka lungor för att det ska få samma syremättnad i blodet som ett barn med friska lungor. Förhoppningen är att shift-värdet ska användas för att diagnostisera BPD i framtiden och att en förbättrad diagnostik ska göra det lättare och säkrare att veta vilken behandling som bäst förebygger BPD. Innan metoden är helt färdig att användas behöver vi göra fler studier för att ta reda på om resultatet blir detsamma om man gör om testet. Dessutom vill vi göra fler studier för att undersöka om vi med hjälp av värdet kan förutse hur lungfunktionen blir när barnet växer upp.



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