Long-term Respiratory Outcomes of Extreme Preterm Birth. A regional cohort study

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A Population-based Cohort Study

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“From such small beginnings…”

Julia Toivonen, L’il Aussie Prems Foundation

CONTENT

1. PREFACE

During the last 3 to 4 decades, major theoretical, medical and technological advances have been made in the treatment of prematurely born infants. Immediate and long-term survival has increased considerably. Large cohorts of these tiny infants are growing up and are becoming new fellow citizens of our community, and their public health importance is increasing.

Being born extremely preterm in the second trimester of pregnancy implies that the continuous development and maturation of human organ systems that should have taken place inside a sheltered uterus, now has to take place in a neonatal intensive care unit (NICU). The full consequences of this remain unknown, particularly for the smallest and most immature infants born at the limits of viability, since their high survival rates are recent history.

All organ systems are immature and vulnerable when born extremely preterm (EPB), and most of these infants require advanced intensive care treatment. Paradoxically, treatment measures required to save their lives in the short-term may also be potentially harmful in the long-term.

As borders of viability move downward, survival increases also for those born most immature. Improved outcomes for "all" preterm born children may thus be counteracted by worse outcomes for the potentially most vulnerable. Repeated studies over decades are needed to address these continuously evolving changes.

In this thesis, we have studied long-term respiratory outcomes in three consecutive population-based cohorts of extremely preterm born (EPB) subjects and matched term-born (TB) controls.

1.1 Scientific environment

The present work was carried out between 2010 and 2015 in collaboration with the Departments of Paediatrics at Haukeland and Stavanger University Hospitals.

The thesis originated from the PhD program of the Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway. The research was conducted within the framework of the 'Research Group for Paediatric Follow-up Studies'. The main research environment was the Department of Paediatrics at Haukeland University Hospital, where ideas and research questions were conceived and clinical examinations were performed.

The thesis research is based on three consecutive, population-based cohorts of children born extremely preterm. The first two regional cohorts were established in 2001 by my main supervisor, Professor Thomas Halvorsen, and the third, national cohort was established in 1999-2000 by my co-supervisor, Professor Trond Markestad, who is also the head of the 'Research Group for Paediatric Follow-up Studies'.

The Western Norway Regional Health Authority, Haukeland University Hospital and the University of Bergen, Bergen, Norway provided major funding.

Statistical analyses were carried out in collaboration with biostatistician and Professor Geir Egil Eide, Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway; and Department of Global Public Health and Primary Care, Lifestyle Epidemiology Research Group, University of Bergen, Bergen, Norway.

1.2 Acknowledgements

Meeting children and their parents is a major part of clinical life as a medical doctor and is truly a gift. I was therefore excited to start my research journey in 2010. Of course, I would miss the everyday clinical life, but being a researcher has been surprisingly much more fun and exciting than I had expected. After years in this business, I cannot imagine leaving it as I return to clinical work. There is so much more to do! I have had the opportunity to collaborate with and learn from so many inspiring and talented colleagues, and have climbed a steep mountain, learning the scientific crafts. The path has definitely been arranged while walking it!

First, I am grateful to all the children and parents who took part in the studies. Without their participation and patience, this work would have been impossible.

Thanks to Western Norway Health Authority for financial support.

Thomas Halvorsen, my supervisor, you are the pulmonary research environment at Barneklinikken (BKB). Your own doctoral thesis has grown, and provided the basis for subsequent research, leading to important new knowledge of the respiratory consequences of preterm birth. My thesis was possible only because of your work and your generosity. You are always eager, and your mind overflows continuously with new ideas and research hypotheses. The way you shared your knowledge, inspired, supported, and advised me through my thesis work could never have been realized by anyone else. It is always a pleasure to see you and to have your advice not only in professional life but also in private life, and to have discussions on lung function, research, sports, economics, politics, and family life—no subject escapes our conversations! I am grateful.

Trond Markestad, my highly valued co-supervisor and also the founder and main chief of the preterm research environment at BKB, your extensive knowledge of the field of preterm birth and child health spans decades, both from broad clinical experience gained nationally and internationally and from your deep insight into research in the field. Your ‘down-to-earth’ way of advising is always helpful and you are always prepared to help. Thank you both for inviting me to join the group!

Ola Røksund, my second co-supervisor and the lung function physiologist of the study, all this work has been possible because of your extensive dedication and interest in the field of lung medicine. Your high-quality standards for testing, and your constant presence and interest have always been useful to me and other members of the group. You are always honest, and I have never doubted your opinion, in practical or academic life!

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Thanks to my dear parents and brothers – our close relationship is a true daily gift. You always believe in me and challenge me.

Finally, I want to express my gratitude to my family. Bjørn, life companion and best friend; and Julia Helene, Oscar Johan, and Ludvig Andreas; the four of you are my beloved, joyful and chaotic all-and-everything, always!

1.3 Summary of thesis

Background: Survival after extremely preterm (EP) birth has increased considerably during the last three decades. The lifetime respiratory prospects for survivors are unknown.

Aim: To study long-term respiratory health following EP birth, here defined as being born before or at 28 weeks of pregnancy, or with a birth weight less than 1001 gram.

Methods: Three population-based cohorts born EP (EPB) in 1982-1985, 1991-1992, or 1999-2000, and individually matched term-born control subjects (TB) underwent clinical examinations, comprehensive tests of pulmonary function (PF) and surveys of respiratory symptoms. The first cohort was examined at 18 and 25 years of age, the second at 11 and 18 years of age, and the third at 11 years of age.

Results: Symptoms of lung disease resolved during the age span studied, but variables of bronchial airflow were lower in the EPB than the TB group, and most marked in subgroups with neonatal bronchopulmonary dysplasia (BPD). Airflow limitations tracked in parallel in EPB and TB groups through puberty (11-18 years) and into early adult life (18-25 years), with no relative improvement or deterioration. At 25 years of age, significant bronchial obstruction, resistance, and hyperresponsiveness were observed, and subgroups had pulmonary hyperinflation. Children born EP in 1999-2000 had less airflow limitation and less pulmonary hyperinflation compared to children born similarly preterm in 1991-1992, particularly those with a history of BPD. Improvements were statistically related to increased use of antenatal corticosteroids and treatment with surfactant in the 1999-2000 cohort.

Conclusions and implications: EP birth was associated with persistent bronchial obstruction and hyperresponsiveness from childhood to adulthood, with no indications of catch-up growth or early onset deterioration compared to TB subjects. Failing to reach peak predicted PF at early adulthood, combined with possible prospects of steeper age-related declines, suggest a potential for early onset chronic obstructive pulmonary disease (COPD) in subgroups. Less impairment among the most recently born 1999-2000 cohort indicates that better treatment not only improves survival but also respiratory outcome. Studies of these issues are few, hampering our understanding of long-term outcomes and ability to propose explanatory mechanisms.

1.4 List of papers

I. Vollsæter M, Roksund OD, Eide GE, Markestad T, Halvorsen T. Lung function after preterm birth: development from mid-childhood to adulthood. *Thorax. 2013 Aug; 68(8):767-76. doi: 10.1136/thoraxjnl-2012-202980.*

II. Vollsæter M, Clemm HH, Satrell E, Eide GE, Røksund OD, Markestad T, Halvorsen T. Adult respiratory outcomes of extreme preterm birth: a regional cohort study. *Ann Am Thorac Soc. 2015 12(3):313-22. doi: 10.1513/AnnalsATS.201406-285OC.*

III. Vollsæter M, Skromme K, Satrell E, Clemm HH, Røksund OD, Øymar K, Markestad T, Halvorsen T. Children born preterm at the turn of the millennium had better lung function than children born similarly preterm in the early 1990s. *PLoS One. 2015 Dec 7;10(12):e0144243. doi: 10.1371/journal.pone.0144243. eCollection 2015.*

1.5 Abbreviations

AGA	Appropriate for gestational age
ATS	American Thoracic Society
BHR	Bronchial hyperresponsiveness
BPD	Bronchopulmonary dysplasia
BTPS	Body temperature and pressure saturated; denotes a volume of gas saturated with water vapour at 37ºC and ambient barometric pressure; should be standardized with pulmonary function testing
BW	Birth weight
C_ANO	Alveolar concentration of nitric oxide
$C_{aw}NO$	Airway wall concentration of nitric oxide
CI	Confidence interval
CLD	Chronic lung disease of infancy
COPD	Chronic obstructive pulmonary disease
CP	Cerebral palsy
CV	Coefficient of variation
DL_{CO}	Diffusing capacity of the lung for carbon monoxide
DRS	Dose response slope; ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (µmol) of methacholine (%/µmol)
EIB	Exercise induced bronchoconstriction
ELBW	Extremely low birth weight (< 1000 g)
EPB	Extremely preterm born
EP_{1982}	EPB January 1982 through December 1985 in Hordaland and Sogn og Fjordane
EP_{1991}	EPB January 1991 through June 1992 in Hordaland and Sogn og Fjordane
EP_{1999}	EPB January 1999 through December 2000 in Western Norway Health Authority
ERS	European Respiratory Society
FEF_{25-75}	Forced expired flow at 25 to 75% of vital capacity
$FE_{NO\ Na}$	Fraction of expired NO, measured in the nose
$FE_{NO\ Sa}$	Fraction of expired NO, measured in the lungs, at a set expiratory flow rate of 50 ml/sec
FEV_1	Forced expired volume during the first second of expiration
FiO_2	Fraction of inspired O_2
FRC	Functional residual capacity
FVC	Forced vital capacity
GA	Gestational age
GLI	Global Lung Function Initiative

Hb	Blood haemoglobin
HUS	Haukeland University Hospital
ISAAC	International Study of Asthma and Allergy in Childhood
IUGR	Intrauterine Growth Restriction
$J_{aw}NO$	Bronchial flux of NO
K_{CO}	DL_{CO} corrected for VA
LBW	Low birth weight (< 2500 g)
LMP	Last menstrual period
LLN	Lower limit of normal
Log	The logarithm to base 10, the common logarithm
MBRN	Medical Birth Registry of Norway
MLM	Mixed linear model
NICU	Neonatal intensive care unit
O_2	Oxygen
PD20	The cumulative dose of inhaled methacholine (μmol) that induces a 20% fall in FEV_1 from baseline
PDA	Patent ductus arteriosus
PF	Pulmonary function
R^2	Coefficient of determination
R_{aw}	Airway resistance
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
RV	Residual volume
SD	Standard deviation
SGA	Small for gestational age
SPT	Skin Prick Test
SUS	Stavanger University Hospital
TLC	Total lung capacity
VA	Alveolar volume
VI	Volume inhaled
VLBW	Very low birth weight (< 1500 g)
β	Estimated regression coefficient

2. GENERAL INTRODUCTION

2.1 Preterm birth

Normal human pregnancy lasts 40 weeks, calculated from the first day of the mother's last menstrual period (LMP). Preterm birth is defined by the World Health Organization (WHO) as birth occurring before 37 completed weeks of pregnancy, or fewer than 259 days since the LMP, preceded or not by preterm labor[1,2]. Preterm birth is further sub-classified by gestational age (GA)[2] or birth weight (BW)[3] (Table 1).

Table 1: Prematurity, definitions by GA or BW.

	Gestational age *(weeks)*	**Birth weight** *(g)*
Extremely preterm (EP)	< 28	
Extremely low birth weight (ELBW)		< 1000
Very preterm (VP)	28-31	
Very low birth weight (VLBW)		< 1500
Moderate-to-late preterm	32-36	
Low birth weight (LBW)		< 2500
Preterm	< 37	
Term	37-42	≥ 2500
Post term	> 43	

Infants may also be classified by weight according to a certain GA. There is no uniform consensus on definitions, but for clinicians the most commonly used are[3]:

1. Small for GA (SGA): weight < 10 percentile
2. Appropriate for GA (AGA): weight 10-90 percentile
3. Large for GA (LGA): weight > 90 percentile

GA and BW are co-linear, but not interchangeable, measures of prematurity. It is debated which parameter is best for describing effects and prognosis of a short or abnormal intrauterine life[4,5]. Low BW for GA (SGA) introduces measures of growth retardation of the foetus, and low BW *per se* does not always imply preterm birth. Preterm babies are more often growth retarded[6]; and as many as 10-25% of preterm births are complicated by intra uterine growth restriction (IUGR), the proportion increases with decreasing GA[7].

2.1.1 The epidemiology of preterm birth

More than 80% of preterm births occur between 32 and 37 completed weeks of pregnancy and less than 10% occur before 28 weeks. There is a vast geographic difference in survival rates. In high-income countries more than 90% of babies born before 28 weeks of pregnancy survive, whereas in low-income countries most of these babies die[2].

Preterm delivery is the leading cause of *neonatal death* (i.e., death in the first 28 days of life), and globally the second leading cause of death for children under 5 years after pneumonia[2]. Worldwide, approximately 15 million babies are born prematurely annually (range: 12-18 million). That is, more than 1 in 10 live births are preterm (range: 5% in parts of Europe to 18% in parts of Africa)[2,8]. Neonatal deaths comprise 40% of all deaths among children, totalling more than one million deaths annually.

All newborn infants are vulnerable. Due to immaturity and/or the pathology that triggered early birth, preterm babies are biologically more susceptible to death or disease than term-born. Many of those who survive face a lifetime of morbidity and disability, imposing emotional and financial burdens on families and stressing the healthcare system[9]. Mortality, morbidity and disability rates, and need of advanced intensive care and costs to health care and society increase with decreasing GA and BW[10-14]. Females have less morbidity and mortality when born at a given GA or BW, suggesting that female gender is advantageous equivalent to one week more mature or 100 g higher BW[15]. Males have more adverse neurologic outcomes and higher mortality[16,17].

The increased incidence of preterm birth in most countries since the 1980s has been attributed to increasing rates of multiple births and greater use of assisted reproduction and obstetric intervention (e.g., induced labour and caesarean section)[8,18]. In Norway, the rate of preterm birth is approximately 5.9%, and the EP birth rate has been fairly stable since 1970 at approximately 0.5%. However, the number of surviving EP-born subjects is increasing (Medical Birth Registry of Norway, MBRN).

2.1.2 Pathophysiology and risk factors for preterm birth

Most preterm births occur spontaneously, but some occur because of selective induction[19]. The obstetric precursors leading to preterm birth principally fall into three categories[20]:

1. Elective delivery because of maternal or foetal indications (30-35%), in which labour is induced or the infant is delivered by pre-labour caesarean section.
2. Spontaneous preterm labour (40-45%), in which the membranes are intact.
3. Preterm premature rupture of membranes (PPROM) (25-30%), in which delivery is either vaginal or by caesarean section.

Several classes of risk factors have been identified for preterm birth. Genetic influences (individual or family history of preterm birth)[21], young or advanced maternal age, poor maternal nutritional status, tobacco smoking, alcohol abuse, and socioeconomic demographics are *maternal factors* that might lead to preterm labor[11]. Short inter-pregnancy intervals, maternal or foetal infection or inflammation, multiple gestations, vaginal bleeding, and maternal medical disorders (asthma, vascular disease, diabetes or hypertension) are *obstetric factors* known to increase risk[22,23]. Rates of preterm birth differ among ethnicities[22].

Risk factors preceding spontaneous preterm birth are often not acknowledged in follow-up studies. Wilcox et al. summarized it this way: 'Preterm babies carry the burden of whatever pathology triggered their early birth'[24].

2.1.3 Short-term complications of preterm birth

Due to anatomic and functional immaturity, and other factors related to early birth[24], preterm infants are at high risk of developing complications. The risk decreases as GA and BW increase[25]. Accurate knowledge of the GA therefore enables better assessment of the likelihood of both survival and complications developing. The most frequent complications reported for immature neonates are the following[12,26-28]:

1. Hypothermia
2. Respiratory abnormalities, specifically respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), and apnoea of prematurity

3. Cardiovascular abnormalities, specifically patent ductus arteriosus (PDA) and blood pressure lability
4. Neurological and brain abnormalities, specifically intraventricular haemorrhage, periventricular haemorrhage, and white matter abnormalities like periventricular leukomalacia
5. Abnormalities in glucose metabolism
6. Necrotizing enterocolitis
7. Infection, early (first week postnatal) or late (postnatal age > 6 days, before discharge from the neonatal intensive care unit (NICU)) onset sepsis
8. Retinopathy of prematurity (ROP)

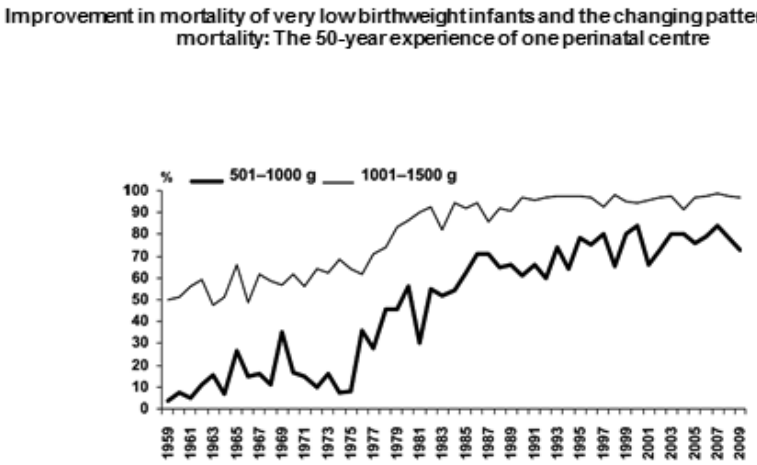
The reported frequency of complications varies among studies, possibly because of various forms of subject selection bias and study designs. Outcomes are reported based on either BW or GA, some studies are population based whereas many are from tertiary centres, and there are population differences among nations and ethnicities and within nations due to social inequalities. Differences may also relate to differences in the organization of care (i.e., centralized vs. decentralized care), to differences in attitudes towards resuscitation and limits of viability (starting or withholding treatment), or to differences in obstetric and neonatal treatment practices.

2.1.4 Neonatal mortality

Survival rates have changed fundamentally during the past 40 years, due to medical and technological advances[28,29]. Data from the Vermont Oxford Network indicate that a plateau in survival (and morbidity) may have been reached in the late 1990s[30], confirmed also in other studies[31] (Figure 1). The National Institute of Child Health and Human Development (NICHD) Neonatal network reported decreasing mortality in VLBW infants from 1987/88 to 1992/93 to 1999/2000[17]. Presently, one-year survival rates for EPB in high-income countries are in the range of 50-75%[5,15,32]. According to the MBRN, 57% of Norwegian infants born EP 2001 - 2010 survived their first week. A 2000 review article reported survival rates until discharge to home in the range of 4-38% for neonates with BWs less than 500 g[9].

Survival rates based on GA have been reported in numerous studies[12,15,26,33]. Survival rates range from 45% to more than 90% for infants born 24 to 28 weeks GA[26]. In the Norwegian national EP born cohort from 1999-2000, survival was explored on whether these babies were admitted to the NICU. Survival rates from 23 to 27 weeks GA increased for all births from 16% to 82%, and from 39% to 93% for NICU-admitted infants. Survival rates from BW < 500 g to > 750 g increased for all births from 10% to more than 78% and for NICU-admitted infants from 54% to more than 90%[12]. These rates were higher, compared to a Swedish national cohort born in 1991-1992, that is, a group born 8 years earlier with slightly different management options[34]. In a recent study, the survival rate was approximately 60% for infants with GA 24 weeks admitted to Norwegian NICUs during 2011-2014[35].

Figure 1: Improvements in mortality for preterm infants, based on BW[31].



2.1.5 Neonatal morbidity

Rates of neonatal morbidity are fairly similar across different reports[15,30,33,36]. In the Norwegian national EP cohort born 1999-2000, the number of days with mechanical ventilation decreased with increasing GA (from 37 to 3 days for GA from 23-27 weeks), the proportion needing supplemental oxygen (O_2) at 36 weeks GA decreased (from 67 to 26%), and treatment for ROP decreased (from 33 to 0% in infants born at 23 vs. > 25 weeks). The incidence of other morbidities were generally unrelated to GA, such as periventricular haemorrage > grade 2, which occurred in 6% of infants and periventricular leukomalacia, which occurred in 5%. Surgical closure of PDA was performed in 14%, surgery for necrotizing enterocolitis was required in 2%, and late onset sepsis was reported in 20%. From 23 to 27 weeks GA, the proportion that was free of neurosensory impairment or pulmonary morbidity (defined as no requirement for assisted ventilation or O_2 at 40 weeks GA) increased from 44 to 86%[12]. These morbidity rates were quite similar to those of the Swedish cohort born 8 years earlier[34], suggesting that increased survival did not lead to increased morbidity.

2.1.6 Ethics

Different attitudes exist on what constitutes the limits of viability, and ethical considerations are often conferred to the specialties working with EPB. In some countries, termination of pregnancy is possible up to the GA at which an infant is viable[37]. Based on outcome data, a consensus reached sets a threshold for 'compulsory' resuscitation at 25 weeks, a lower threshold of no resuscitation at < 22-23 weeks, and a 'grey zone' between these two limits[38,39].

As viability limits are pushed earlier, infants that are more immature survive. The cost of this downward trend remains debated, both in terms of morbidity and strain on resources and financial costs[40]. The American Academy of Pediatrics and the American College of Gynaecologists conclude that it is extremely challenging for families and health professionals to make decisions on the institution and continued life support in infants born at the threshold of viability[41], a conclusion confirmed by a Norwegian study[42]. Most agree that decisions regarding life support for infants at the lowest GAs should be discussed with the parents[41,43]. Fanaroff et al. states that if

viability is defined by a survival rate of ≥ 50%, infants delivered at GA 24 weeks and with a BW of 600 g are viable. This definition does not consider issues of long-term morbidity[15]. Research conducted on the long-term health consequences of EP birth and survival beyond the NICU stay is imperative to better balance this discussion. A recent Swedish study observed significant regional differences in survival of the most immature infants (GA 22-24 weeks) born 2004-2007[36], and also found that improved survival was not associated with increased morbidity, although the range of major neonatal morbidity was high (range: 50-88%)[36].

2.1.7 Development of neonatal intensive care

During the 1950s, neonatology emerged as a paediatric sub-specialty. In 1953, anaesthetist Victoria Apgar developed a score to rapidly assess the early vitality of the newborn based on heart frequency, respiratory effort, muscular tone, reaction to stimuli, and skin color[44]. Phototherapy for jaundice was introduced in 1958, drastically reducing psychomotor sequelae of hyperbilirubinemia in the newborn.

Among the most important advances in modern NICU medicine was the randomized trial of antenatal corticosteroids in the early 1970s; steroids were administered to pregnant mothers admitted for threatening premature delivery in order to accelerate foetal lung maturation[45]. The study led to widespread use of antenatal corticosteroids in the 1980s, and reduced the incidence and severity of neonatal RDS and intracranial haemorrhage, the two leading causes of neonatal morbidity and mortality in EPB[46,47]. New and better modes of mechanical ventilation and better strategies for non-invasive surveillance of O_2 and carbon dioxide (CO_2) tension led to more accurate information on and hence better titration of ventilation and oxygen supplementation[48]. The role of surfactant in RDS of preterm babies was discovered in 1959 by Avery and Mead[49], setting the scene for later surfactant replacement therapy, which was first described in 1980[50]. It was introduced clinically in the late 1980s and widely in use in the 1990s, initially synthetic and later derived from animal extracts. Surfactant replacement therapy aided immature airspaces in overcoming high surface tension and helped inflation and expansion when lungs filled with air. This treatment reduced both death and severity of chronic lung disease (CLD), but not the incidence of CLD[51,52]. Positive

effects of postnatal administration of corticosteroids were noted in infants with respiratory disease; however, caution was later warranted due to negative side effects, in particular, poorer neurodevelopmental outcome[53,54].

Other developments in neonatology also contributed to increased survival, e.g. improved nutritional strategies[55] and more aggressive treatment of symptomatic PDA by surgery or indomethacin which led to better treatment of over-perfusion of the pulmonary vasculature[56,57]. The importance of preventing neonatal bacterial infections, more aggressive use of antibiotics[58] and the use of better nursing procedures[59] were also acknowledged. The collaborative efforts of several medical specialties were important in these advances, such as neonatologists, specialized nurses, radiologists, obstetricians, and others working together in highly specialized NICUs. In 1989, the Vermont Oxford Network (*vtoxford.org*) was established as a non-profit collaboration with the aim to improve the quality and safety of medical care for newborns through education, research, and quality improvement[30].

With the provision of contemporary treatment, life and death expectancies of EPB have reversed in that previous high mortality rates have been replaced by similarly high survival rates, a trend that began in the 1970s[8,60,61]. The limits of viability have moved downwards[26,36]. Large cohorts of EPB are now for the first time entering childhood and subsequently adolescence and adulthood. Their impact on public health issues is thereby increased because of direct costs related to NICU care and indirect social and economic costs related to the disability burden[13,62]. Clinicians of most specialties will increasingly be exposed to survivors of preterm birth, also those working with adults[63]. Awareness of long-term health problems is imperative, for survivors, families, society, and healthcare systems[64,65].

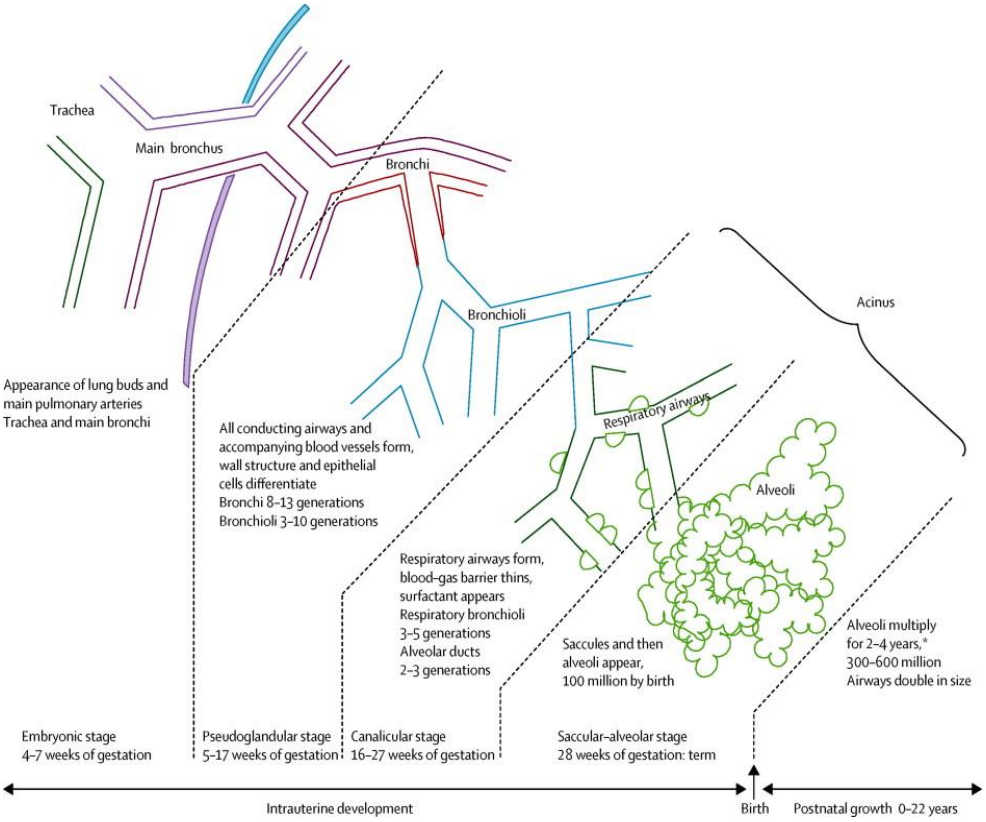
2.2 Development of the respiratory system

2.2.1 Intrauterine development

Antenatal development is an orderly process. Governed by genetic information, complex interactions among different cell groups result in the creation of organs. Most organs are created during the first trimester (first 12 weeks of pregnancy), followed by

further growth and development in the second (12-27 weeks) and third (28-40 weeks) trimesters. Birth before term pregnancy imposes a challenge, as it interrupts programmed and fine-tuned sequences of normal development that now must take place outside the uterus. Most organs risk being injured. The brain[66] and lungs[40] are particularly susceptible to damage and subsequent disabilities[67,68].

Figure 2: The different stages of lung development.



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Lung morphogenesis in humans occurs both prenatally and postnatally, beginning around 5 weeks of gestation. Thyroid hormones, respiratory movements (breathing), and the volume of amniotic fluid influence the rate of growth and maturation of the lungs and airways.

The development is typically divided into five stages: embryonic, pseudoglandular, canalicular, saccular, and alveolar stages (Figure 2). The GA of the transition from one stage to the next is not absolute[70,71].

During the *embryonic stage* (up to 7 weeks gestation), the lung bud appears as a ventral outgrowth from the endoderm cells of the primitive foregut. The bud expands and divides into the surrounding mesenchyme. Each bud is supplied by a pulmonary artery extending from the outflow tract of the embryonic heart. Pulmonary veins drain the blood via a mesenchymal capillary plexus. By approximately 6 weeks GA, the lungs can be distinguished as separate thoracic organs.

During the *pseudo-glandular stage* (5-17 weeks gestation), the airway buds divide successively to form the conductive epithelial tubes and the complete branching pattern of all pre-acinar airways. Smooth muscle, cartilage, submucosal glands, and connective tissue develop, and the epithelium starts to differentiate. Endothelial tubules surround each airway bud, and airway growth seems to act as a template for vessel growth, such that by 17 weeks gestation, pre-acinar branching of both arteries and veins is also completed. Gas exchange is not possible, and the foetus is not viable if born during this stage.

During the *canalicular* (16-26 weeks gestation) and *saccular stages* (26-36 weeks gestation), the peripheral airways continue to divide to form the later respiratory bronchioles and prospective alveolar ducts. The size of the pre-acinar airways increases. Arteries and veins continue to develop, establishing a three-dimensional capillary network in the mesenchyme alongside the airway. Thinning and flattening of the epithelium by underlying capillaries at the lung periphery leads to the formation of a thin blood-gas barrier sufficient to sustain life if the foetus is born at this stage. Specialized epithelial cells can be identified, and surfactant can be detected in the amniotic fluid around 26-28 weeks GA.

During the *alveolar stage* (36 weeks to post-term), the edges of primitive alveolar saccules elongate, forming simple alveoli. Secondary septation leads to a rapid increase in the number of alveoli of smaller size, dramatically expanding the area for

gas exchange, where blood and air is in intimate contact across a layer 1/50 the thickness of paper[72]. The lungs are filled with fluid excreted by epithelial cells lining the airways, though will expand when the infant takes its first breath in the extra-uterine environment. Studies have shown that only about 15-30% of alveoli (approximately 150 million) have formed at birth.

Autopsy studies that include lung histopathology of infants dying from BPD have described severely disrupted alveolar structures[71], and until recently, it has been assumed that this pathology represents lifelong traits. Narayanan et al. challenge this assumption, showing that growth of alveolar structures in EPB occurs beyond 3 years of age[73]. Recent studies indicate that alveolarization continues for years[74], suggesting that the human lung is a dynamic organ in terms of growth and differentiation, thereby introducing some optimism for the ability of the respiratory organs to recover from early injury[73,75-77]. Such continued development and refinement may imply that the vulnerability to external influences extends beyond childhood[78].

2.2.2 The respiratory system after birth

During pregnancy, the placenta provides foetal gas exchange. As the term newborn exits the birth canal, the central nervous system is triggered, causing the normal infant to take its first breath to inflate the lungs with air and then go on breathing spontaneously. Surfactant is critical to reduce air-liquid surface tension and allow the airspaces to expand. O_2 reaches the alveolar duct by ventilation, diffuses into the alveoli and through the alveolar-capillary membrane, after which red blood cells in the capillaries combine the O_2 with haemoglobin (Hb), and are carried within the arterial blood stream to the organs. CO_2 travels in the opposite direction through the venous blood stream to exchange at the alveolar-capillary membrane. Soon after normal term birth, the arterial CO_2 tension attains adult values, indicating that the lung is able to provide adequate gas exchange at a very early stage[79].

The number of airway generations and the branching pattern is complete at birth. The most peripheral airways have not attained full length. Several transitory ducts end in saccules and later transforms into alveoli by secondary septation. Remodelling occurs

within the blood vessels and capillary network in proportion to alveolar formation, with growth slowing alongside alveolar growth after 18 months of age.

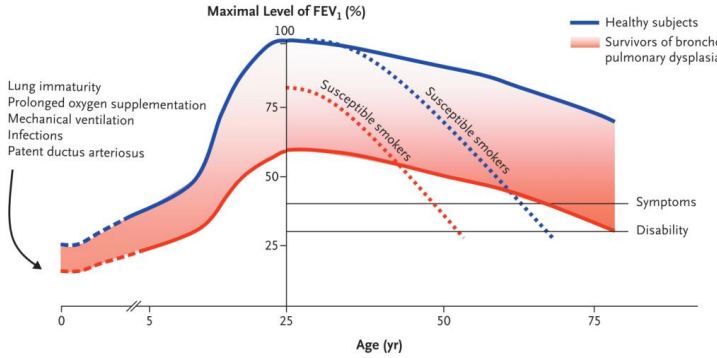
Birth implies a transition from foetal to postnatal circulation, and the blood flow that before birth shunted past the lungs, now flows through the pulmonary vessels. Intrauterine pulmonary vascular resistance is high in the foetus and falls immediately after birth, followed by an increase in blood flow. During the first year of life, the amount of bronchial wall muscle increases rapidly. Airway calibre is large relative to lung volume at birth. It increases approximately two- to threefold in diameter and length between birth and adulthood[80,81].

For all ages, boys have a larger number of alveoli[82,83]. Before the pubertal growth spurt (which on average occurs in girls at 10 years compared to boys at 12 years[84]), there is a linear relationship between age and pulmonary function (PF). The height-PF relationship shifts with age during puberty, introducing changes that are challenging to mathematical models[85]. The thorax changes both in size and shape, implying a greater fractional increase in thoracic volume than in height[85]. Girls have wider and shorter airways than boys during early childhood, whereas by adulthood, this is reversed[86]. This might explain the shift in incidence of reversible obstructive airways disease (asthma), which is most common in boys in childhood, but more common in girls after puberty[82,87,88]. Hormones or metabolic processes are involved in determining adult PF[89].

2.3 Lifetime course of pulmonary function in health and disease

In healthy children, lung volume and function increase steadily[86], reaching a maximum in late adolescence or early adulthood. Peak volume and function are reached in males about 5 years later than in females[90], and then both decline progressively with age after a brief plateau phase[91] (Figure 3). Despite the decline, and provided the absence of disease, the respiratory system is capable of sustaining adequate gas exchange throughout one's life span without ever attaining values associated with symptoms or disability[91]. Tracking studies of PF reveal that infants with lower PF are at increased risk of airflow obstruction as young adults[92], and the PF attained in young[93] and middle[94] adulthood determines the PF level attained 20 years later.

Figure 3: Theoretical model of lifetime progression of forced expiratory volume in one second (FEV_1) in healthy subjects and survivors of BPD.



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There is growing awareness that adult lung diseases may arise early in life. Prior exposures to adverse factors (infections, environmental pollutants, cigarette smoke) causes failure to achieve optimal peak PF[69,87,92,94,96], and further may induce maladaptive patterns in responses to harmful agents[97] (Figure 3). Given the finely tuned respiratory development, any factor influencing lung growth in the pre- or perinatal period or during childhood is likely to affect adult PF adversely[96,98]. This idea was proposed decades ago in the 'developmental origins of health and disease hypothesis' (DOHaD). Subsequent studies confirmed associations between respiratory infections in infancy and low BW. These combined lead to lower adult PF and COPD death[99-102]. Exposure to toxic agents may lead to an earlier and/or accelerated decline in PF from the highest-level attained[90,103], possibly more strongly affecting subjects with an already reduced peak PF.

2.4 Respiratory consequences for the foetus outside the uterus

Birth in the second trimester requires extra-uterine development of foetal lungs. Gas exchange takes place in developmentally immature lungs, which are unable to produce surfactant, and with ongoing growth and proliferation of complex gas exchanging units. The area for gas exchange is limited and inefficient, and the NICU environment is totally different from the dark, warm, and relatively hypoxic state inside the uterus.

Premature birth disturbs pre-set and fine-tuned patterns of development, resulting in immediate as well as long-term consequences for respiratory health. Structurally and

functionally immature alveoli combined with surfactant deficiency are key causes of RDS in EPB[104]. Major clinical interventions are required to sustain life, such as antenatal and postnatal corticosteroids, assisted ventilation, and O_2 supplementation. Paradoxically, these treatments are potentially injurious. Development of airways and vasculature can be profoundly disrupted by mechanical ventilation[80,105,106], possibly due to stretching and over-distension of fragile airways in poorly compliant lungs. Hyperoxia and subsequent toxic oxygen reactants can directly damage DNA and proteins, inducing lipid peroxidation and inflammation[107]. Immature neonates are particularly vulnerable, as anti-oxidative mechanisms are poorly developed[108].

Preterm birth is caused by some underlying pathology, which also may have the potential to further adversely influence development[24,109-111]. Polymorphisms for genes encoding endothelial growth factors in the vasculature, or altered expression of different surfactant proteins might be involved[40,112]. In the healthy foetus in utero, the arterial (umbilical vein) O_2 tension is about 4.7 kPa, and the arterial saturation (SaO_2) is 80-90%[113]. Room air (O_2 tension 11-13 kPa) is therefore relatively hyperoxic compared to in utero arterial O_2 tension. Additional O_2 must be administered to most EPB, as immature lungs are inefficient gas exchanging organs. Increasing evidence indicates that even low levels of supplemental O_2 can be harmful, and current practice is to use the lowest possible fraction of inspired O_2 (FiO_2) to maintain adequate SaO_2[114,115]. Resuscitation manoeuvres, nosocomial infections, fluid overload, left-to-right shunting through a PDA, and supplemental nutrients are among the factors shown to influence the development of PF[40,112,116,117].

We know that IUGR influences future respiratory health in the general population[99,118-121]; but we do not know the full impact of this in EPB. There is solid evidence that preterm born infants who received neonatal ventilatory support have impaired PF in childhood[122,123]; however, impaired PF is a characteristic also seen in preterms who did not receive any such support, indicating that preterm birth *per se* affects pulmonary outcome[124-126].

The importance of surfactant administration has been explored in multiple studies. Whether this should be administered as an early prophylactic or delayed selective

treatment remains a matter of debate[127]. Relationships between different modes of ventilation and later respiratory and neurocognitive outcomes have also been explored, with diverging results. Currently less invasive methods are favoured for both respiratory support and surfactant administration[128-130].

2.4.1 Bronchopulmonary dysplasia (BPD)

The term CLD refers to any pulmonary disease resulting from a neonatal respiratory disorder[116]. BPD is by far the most prominent cause of respiratory illness and abnormal, postnatal lung development in preterm infants, and leads to short and long-term morbidity and mortality[40,95,116,131]. The condition was first described by Northway et al. in 1967[132], who observed a disorder in infants who were born moderately preterm with severe RDS and were exposed to aggressive mechanical ventilation and supplementation of high fractions of O_2. Two decades later, the same authors showed that respiratory symptoms and functional abnormalities persisted into early adulthood [133]. This important study suggested lifelong consequences of neonatal lung injury.

Shennan et al. studied a population of very low birth weight (VLBW) infants[134]. In infants born at GA more than 30 weeks O_2 requirement at 28 postnatal days was a predictor of later abnormal pulmonary signs and symptoms (widely defined as death due to non-anomalous cause, oxygen requirement at 40 weeks GA, surgery in the respiratory tract, wheezing requiring medication, pathologic x-ray findings or persistent wheeze/tachypnoea /retractions alongside growth failure, neurodevelopmental delay or hypotonia). In infants born at GA less than 30 weeks, O_2 requirement at 36 weeks GA was a better predictor, with a positive predictive value for unfavourable outcome of 63%. For infants with no O_2 requirement at 36 weeks GA, the prediction of a normal outcome remained at 90%[134]. These observations led to the idea that O_2 requirement at certain time points should somehow reflect the extent of lung damage or lung disease following preterm birth.

The definition of BPD has continued to evolve, because of changes in care strategies and in the population at risk. Presently, the diagnosis is basically applied to preterm infants (GA < 32 weeks) with a prolonged need for supplemental O_2. Thus, premature infants who remain dependent on O_2 supplementation for 28 postnatal days are given

the diagnose BPD, the severity of which is further defined by the need for O_2 supplementation at near term (36 weeks GA). Mild BPD is assigned if the infant is breathing room air at that stage, moderate BPD is assigned if the infant needs a FiO_2 of < 0.30, and severe BPD is assigned if the infant needs a FiO_2 of ≥ 0.30, or if other means of supported ventilation is required[40]. For infants born at GA $\geq$ 32 weeks, the assessment is made at 56 postnatal days or at discharge, whichever comes first.

Paradoxically, O_2 most likely is a key part of the aetiology of BPD, O_2 supplementation defines the BPD diagnosis, and it is used to treat infants with BPD.

2.4.2 Pathophysiology of BPD

The pathophysiology of BPD is not properly understood. It is most probably a combination of complex injuries and interactions due to arrested lung development, alongside ongoing inflammation and consequent repair pathways[135]. There are few histopathological studies; most biopsies come from animals[136,137], or from subjects that subsequently died[71,105,112,138-140]. These studies suggest that acinar development is altered, manifested by larger, fewer and less complex structures, variable alveolar wall thickness, and disrupted vascularization[139]. As most subjects with BPD survive, current understanding of the condition remains mostly clinical, resting mainly on physiological studies. This means that management must be guided by these same data. Due to continuously changing NICU strategies and the survival of increasingly more immature infants, new mechanisms of lung injury have emerged, thereby changing the clinical course, and most likely, the pathological characteristics of pulmonary involvement.

2.4.3 'Old' and 'new' BPD

Notions of what constitutes BPD have changed. What is now considered to be 'old' BPD was characterized by major pathological changes in the lungs and airways of moderately preterm infants[132]. These changes reflected extensive postnatal disruptions related to barotrauma caused by simple ventilators applying high pressures[141] and to O_2 toxicity. Post-mortem histological preparations revealed diffuse airway damage, extensive parenchymal fibrosis, smooth muscle hypertrophy, and neutrophilic inflammation; resulting in atelectasis and variations in alveolar shape and size.

Modern and conservative treatment modalities now have given rise to a new pattern of lung injury, 'new' BPD[71,112,142]. This condition is observed in immature infants with often only minor, if any, RDS at birth, and is considered mainly a developmental disorder. The normal processes of alveolarization and vascularization are dysregulated by exposure to even minimal injurious factors at a very early developmental stage. New BPD is less characterized by inflammatory processes, cellular proliferation, and fibrosis; instead more by developmental arrest in the normal structural complexity of the lung. This results in a reduced surface area for gas exchange, due to decreased septation, fewer and larger alveoli, and disrupted pulmonary microvasculature[71,140].

In 2007, the Children's Interstitial Lung Disease Research Co-operative (ChILD) proposed a new biopsy-based classification of diffuse lung disease in infants and young children. 'New BPD' is not mentioned. Acinar dysplasia and congenital alveolar dysplasia are described as changes characterized by lung growth arrest in infants born in the early canalicular or late canalicular/early saccular phase of pregnancy, respectively. This, perhaps, acknowledge the imprecise pathologic description yielded by the symptom-based diagnosis of BPD[143].

In 2012, Bancalari and Jobe recently proposed a new diagnostic term to better describe the clinical course followed by many of today's EPB NICU dwellers; respiratory instability of prematurity[144]. They were challenged among others by Hjalmarson and Sandberg[60], arguing that lung disease in EPB reflects continuous and not dichotomous features, with no clear tendency for 'healthy' and 'diseased' subgroups[125,145].

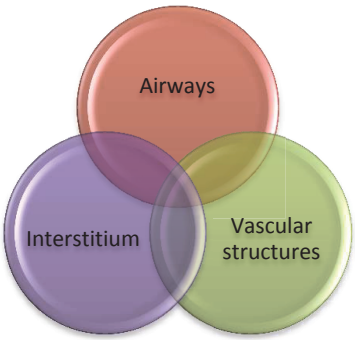
Clinically, preterm birth has been associated with increased respiratory symptoms and reduced PF throughout childhood, adolescence, and adulthood. The mechanisms behind the abnormalities remain unknown. Antenatal factors, the extent of immaturity and the duration of exposure to injurious extra-uterine conditions might determine the degree of subsequent impairment. The respiratory abnormalities might be remnants of structural sequelae, or expressions of an ongoing active metabolic process.

An important candidate mechanism is persistent airway inflammation. Studies of EPB display normal temperature[146] and low or normal levels of systemic markers of

eosinophilic inflammation[147] and of nitric oxide (NO) in exhaled air[148,149], indicating that eosinophilic inflammation is not an important part of lung impairments. Other studies have indicated increased oxidative stress and neutrophilic airway inflammation in exhaled breath condensates and induced sputum from EPB children and adolescents, suggesting the presence of some active process in the airways, thereby challenging the theory of 'inactive' sequelae[150-152].

Anatomically, the lung can be seen metaphorically, as a distended spring attached to the thorax. The framework for the spring is the airways, paralleled by vasculature. The parenchyma ties the framework together. Thus, a functional interdependence within the architecture exists, with lung parenchyma tethering the airways and serving as a framework for the gas-exchanging units. These complex interactions are difficult to separate from one another by means of physiological testing, making it difficult to disentangle the mechanisms behind the impairments (Figure 4).

Figure 4: The interdependent structures of the pulmonary system.



Airways *include the trachea (extrapulmonary), bronchi, bronchioles, and terminal bronchioles.* ***Interstitium*** *includes the supportive tissue, alveoli, and immune-active cells.* ***Vascular structures*** *include the bronchial and pulmonary arteries and veins.*

2.4.4 Incidence of BPD

The rate of BPD increases with the degree of prematurity. Of infants born in the NICHD network (US) in 1995-99, BPD (supplemental O_2 at 36 weeks GA) occurred in 52% of infants at BW 501-750 g, in 34% at BW 751-1000 g, and in 15% at BW 1001-1200 g[153]. In a nationwide Norwegian study, the incidence was 67% at GA 23-25 weeks and 37% at GA 26-30 weeks[12], and higher in males[154]. For cohorts born in the 1980s and the early 1990s, BPD incidence was in the range of 20%[155,156] to 50%[157] for

VLBW infants, rates that are difficult to compare due to varying definitions and inclusion criteria. BPD has apparently taken on a more benign clinical course, although the incidence seems to have been less influenced[12,26,158,159].

The proportion of surviving infants with BPD seems to be unchanged in the period between 1995 and 2006[27], although one study reported an increased incidence from 48% to 58% from 2001 to 2006 for infants born at GA 23-29 weeks. No survival differences were found to possibly explain this, but decreased use of surfactant alongside more non-invasive ventilation strategies could have contributed[160].

As reported by Hjalmarson and Sandberg, postnatal development of gas exchange in EPB infants is likely to reflect complex dynamic and continuous processes, a state of affair that may explain some of the variability of a dichotomous measure of neonatal lung disease, such as BPD [60,125,145].

2.4.5 EP birth - an economic burden

EP birth carries high costs[62]. BPD is (or at least reflects) a multisystem disorder often associated with other impairments, such as neurodevelopmental delay, sensory defects, psychosocial malfunction, and growth restriction. Multidisciplinary follow-up is often required[116,158]. Mangham et al. estimated the total costs of EP birth in England and Wales during an 18-year period to be £242 million; the incremental cost per EPB surviving to 18 years was estimated to be £94,740 greater than that of a TB child[161].

2.5 Long-term course of respiratory health after preterm birth

2.5.1 Cross-sectional studies

The bulk of studies on respiratory health after preterm birth use cross-sectional designs.

Childhood

Recurrent wheeze is common in infancy and early childhood. Up to 50% of EPB are readmitted to hospital due to respiratory tract infections in the first year of life[162-168]. Those with more severe neonatal lung disease generally fare worse. Avoidance of viral infections and exposure to cigarette smoking for this group is imperative in order to

maintain lung health in the vulnerable early years[116,169,170]. Studies have documented abnormal infant PF following EP birth and BPD[171-173], tests generally show lower forced expiratory volumes and flows and elevated residual volumes (RVs).

After the first year of life, rates of wheezing illnesses and hospital readmissions for respiratory diseases decline[174]. Still, in school-aged children, those with a history of EP birth might be at increased risk of respiratory morbidity[126], specifically more coughing, wheezing, asthma-like symptoms, and use of asthma medication. PF tests show abnormalities with lower forced expiratory flows and volumes—partially reversible with beta agonists—and increased RVs, indicating persistent airway obstruction and pulmonary hyperinflation, particularly in those with BPD [124,126,149,171,172,175-178]. The same findings apply to EPB infants born after the introduction of surfactant[13,126,149,179,180]. Some research groups have tried to find links between neonatal PF indices and subsequent PF (tracking), although no firm conclusions have been reached[124,125,181]. Impairments in the respiratory system are seemingly associated with impairments in the cardiovascular system of EPB[182].

Adolescence and early adulthood

The increased risk of hospitalization persists into early adulthood[183,184]. Adolescents and young adults who were EPB more often report coughing, wheezing, and asthma-like symptoms[175,185]. Forced expiratory flows and volumes remain lower and RVs remain higher compared to TB[124,133,156,157,175,185-187].

Few studies have explored long-term pulmonary outcomes in EPB beyond their early 20s. Wong et al. reported airway obstruction, elevated RV, and reduced diffusing capacities at age 19 in 21 of 133 (16%) BPD survivors; however, failed to include control subjects[187]. Northway et al. reported airway obstruction, elevated RV, increased total lung capacity (TLC), and bronchial hyperresponsiveness (BHR) in 26 selected adults born preterm with BPD in the 1960s. However, important limitations were that preterms were relatively mature (mean GA 33.2 weeks) and subjected to harsh interventions, and the control groups were biased[133]. More recently, Gough et al. found reduced spirometry parameters in 56 of 153 (37%) 24-year-old adult BPD survivors born 1978-1993, concluding that airway obstruction was present[157].

Taken together, a picture emerges that reveals clinically significant airway obstruction, with some heterogeneity regarding other pulmonary features. However, disagreements exist regarding changes in PF in EPB as they progress from childhood to the age when peak PF is expected, complicating predictions of what may be their future[188].

Prospects for later adulthood

Lifelong tracking of PF has been addressed in studies of elderly people from the general population in Great Britain and Sweden[101,189]. The social and medical conditions around which they were born are not comparable to those of today. The presence of airway obstruction in early adulthood seems to be predictive of airflow obstruction in middle age. Kalhan et al. assessed PF in 2496 young healthy, non-asthmatic adults (not EPB) aged 18 to 30 years and then again 20 years later[93]. Low forced expiratory volume in one second (FEV_1) and the ratio FEV_1 to forced vital capacity (FVC) and smoking at first assessment were highly predictive of airflow obstruction in middle age[93].

Airway obstruction appears to be a consistent feature in children and adolescents who were born EP. Thus, combined with the normal age-related decline, development of COPD has been a feared scenario, at least in subgroups; and this is an issue currently receiving broad attention[95,96,112,131 190]. The first large cohorts of EPB in the 1980s or later are now approaching their 30s, and valuable information on their respiratory health will be available in the years to come.

2.5.2 Longitudinal studies

Childhood

Two studies from the 1990s are difficult to interpret, as they did not include TB controls. Koumbourlis et al. assessed PF at 8 and 15 years in 17 EPB subjects born before 1980 (mean GA 29.1 weeks; mean BW 1120 g) in a hospital-based study[178]. The authors used radiological data to document CLD at 4 weeks of age. The study provided evidence of small airway obstruction, and the authors observed that the initial increased RV and decreased RV/TLC resolved over time, indicating that air trapping gradually resolved, although they had no TB controls for comparison[178]. Blayney et al.

followed 32 EPB with BPD from seven to 10 years of age. The 32 of 80 (40%) subjects were born 1977-80 (mean GA 29 weeks; mean BW 1228 g), and received O_2 supplementation at 28 days postnatal age[176]. BDP subjects had elevated RV and RV/TLC, but FEV_1 within normal range. Those with an initially low FEV_1 (below 80% predicted) showed increased FEV_1 during follow-up, interpreted as 'catch-up growth'. There were signs of BHR (PC20 < 10 mg/ml methacholine), which was reversible by salbutamol administration; resting diffusing capacity for carbon monoxide (DL_{CO}) was within normal range.

Adolescence

Doyle et al. studied a large cohort of 210 subjects born EP during 1977-1982; 147 of 210 (70%) were VLBW, and 33 had BPD[175]. Of the 147, 129 had PF data at 8 and 18 years of age, whereas 37 of 60 TB had longitudinal data. Persistent airway obstruction was present, and a significant fall in the FEV_1/FVC ratio was observed from 8 to 18 years in the BPD group. Developmental data for TB were not detailed. Filippone et al. studied the course of PF in childhood in a small cohort of 17 survivors with BPD (mean GA 28.1 weeks), along with a TB group and a preterm group without BPD, all born in the early 1990s[124]. Maximum flow at functional residual capacity (FRC) at 2 years of age[122] was measured, and FEV_1 was measured at 9 and 15 years of age[171]. The study revealed tracking, with consistent z-scores from 2 to 9 and 15 years of age, as well as a lower FEV_1 compared to TB and to subjects with no BPD.

Adulthood

Narang et al. followed a cohort (GA 31.5 weeks) into early adulthood[186,191]. Airway obstruction and BHR was present at 7-9 years but not at 21 years of age, leading the authors to conclude that catch-up development had occurred. A caveat is that only 20% of the original cohort participated at the 21-year-old assessment, and different TB groups were used for comparison at the two assessments. Trachsel et al. reported signs of progressive pulmonary hyperinflation and a decline of FVC between 18-38 years in 14 of 20 subjects born preterm at their institution during the very early era of NICU care, i.e., without access to surfactant and with application of high airway pressures[141]. Their findings contradicted the findings from the London group. Gibson et al. studied

47 preterm ELBW survivors born 1977-1982[155]. At 25 years of age preterms had more airway obstruction and pulmonary hyperinflation compared to TB, and a lower FEV_1 was observed in subjects with neonatal BPD compared to those without BPD[155]. Some subjects had been assessed earlier, and there were relationships between FEV_1 obtained in early childhood and at 25 years, particularly in the BPD group[175,192,193]. Few respiratory symptoms were observed in subjects assessed at 25 years of age.

2.5.3 Changes in outcome over time

Changes in treatment strategies and polices in the 1980s contributed to increased survival of EPB, especially the introduction of major medical innovations such as antenatal corticosteroids and exogenous surfactant and policies instituted to standardize and centralize perinatal care[10,29]. There have been no similar major therapeutic advances with comparable outcome consequences since the mid-1990s[15,29,30,61]. Survival did increase slightly, but the pattern of neonatal morbidity remained basically unchanged[27,36].

The follow-up studies on adults discussed above were undertaken with subjects born before access to surfactant and more advanced intensive care therapies, and thus may not be representative of future outcomes in premature infants born more recently. However, these pioneer subjects are likely to present themselves at adult pulmonary clinics in the years to come, in numbers approximating 500,000 in the US alone[194]. Outcome data are needed in order to provide adequate and evidence-based care[65]. Continually changing management strategies pose concerns for producing cohort effects that may be difficult to predict, as better treatments potentially benefit all NICU patients, but also contribute to increased survival in more immature individuals who are possibly more at risk for unfavourable outcomes[95]. This issue is a challenge to surveillance studies[29].

The first large cohorts of EPB from the 'surfactant era' have entered an age range in which complex tests of PF are possible. Recent studies indicate that respiratory ill health is still present[149,195]. Hacking et al. found similar airflow obstruction in 8-year-old EPB in two cohorts born in different periods, though observed significant improvements in FEV_1 and FVC for those without BPD born most recently[179]. In a

2013 review by Kotecha et al., evidence was presented of milder impairments in FEV_1 in subjects born preterm with neonatal BPD (defined by O_2 supplementation at 28 days) in the most recent published studies compared to earlier studies[196]. There were insufficient data to evaluate changes for those with BPD defined at 36 weeks of gestation.

2.6 Gaps in knowledge

From the studies above, one may conclude that there is solid evidence that airway obstruction and pulmonary hyperinflation is present in large proportions of EPB in childhood, whereas the findings are less consistent in adulthood. These gaps in current knowledge, and more specifically on the longitudinal course of pulmonary function in EPB as they grow older, need to be filled in. This will require larger studies where EPB are followed regularly during childhood and into adolescence and adulthood. Such studies may provide important data to determine whether childhood lung function impairments will persist, and whether lung function in EPB will decline during adulthood on the same or steeper trajectories as those of subjects born at term, and if lung function will reach a level eventually leading to early onset of symptoms of chronic obstructive pulmonary disease.

These issues are important to the individual EPBs and their caregivers, both in terms of personal and financial costs, as well as to the medical society that made their survival possible, and as regards current and future allocation of societal resources.

3. AIMS OF THE THESIS

The overall aim of this thesis was to provide new comprehensive data on respiratory health and pulmonary function in middle childhood, adolescence, and early adult life in EPB subjects. The special emphasis of this work was to acquire new data longitudinally across age spans and over times, and thus gain knowledge on determinants of long-term pulmonary function and attainable respiratory health in subjects born EP.

The overall null-hypothesis of the thesis was the following:

H_0: There is no difference in PF or PF trajectories between children, adolescents, and young adults born EP and at term during the period 1982-2000.

The following three main research areas were addressed:

1. *Tracking bronchial airflow variables from childhood to adulthood (Paper #I).* We specifically assessed trajectories for variables of bronchial airflow from childhood through puberty and into early adult life in two different cohorts of EP and matched TB subjects, with the research questions:
   a) Do abnormalities in spirometry parameters reported in studies of EPB children and adolescents performed at our institution in 2001-2002 persist 8 years later in the same subjects?
   b) Do trajectories for maximal bronchial airflow from middle childhood through puberty to early adult life differ between groups born EP and at term?
2. *PF at age 25 and tracking of PF data from age 18 to 25 years (Paper #II).* We specifically assessed respiratory health and comprehensive PF at the expected peak of PF in early adulthood in a cohort of EP and matched term-born subjects born in the early 1980s, with the research questions:
   a) Do respiratory symptoms in EPB persist into early adulthood?
   b) Do impairments in PF in adolescents born EP persist into adulthood?

3. *PF in children born EP in 1999-2000 vs. in 1991-1992 (Paper #III).*
   We specifically compared respiratory health and PF in 11-year-old subjects born EP in 1999-2000 and in 1991-1992, with the research questions:
   a) Does PF differ between 11-year-old children born EP in 1999-2000 and in matched controls born at term?
   b) Does PF differ between children born EP in 1999-2000 and comparable children born in 1991-1992?
   c) Do respiratory abnormalities after EP birth represent irreversible structural sequelae, or is there evidence of reversible airway obstruction and/or signs of active processes occurring, e.g., eosinophilic inflammation?

The main hypotheses of this thesis work were the following:

1. Variables of maximal bronchial airflow are reduced in EPB compared to TB, and deficits persist throughout puberty and into early adult life (Paper #I).
2. Tracking of variables of bronchial airflow from mid-childhood to adulthood is similar in EPB and TB groups (Paper #1)
3. Twenty-five-year-old EPB adults have poorer PF than matched TB subjects, but any changes that occur from 18-25 years of age will be similar (Paper #II).
4. Eleven-year-old children born EP in 1999-2000 have poorer PF compared to matched TB children. Compared to children born similarly EP in 1991-1992, pulmonary outcomes will be poorer due to increased survival rates of the most immature children. Alternatively, outcomes will be better due to improved perinatal care (Paper #III).
5. Airway abnormalities after EP birth may be irreversible and related to structural injuries, or reversible and related to bronchospasm and inflammatory processes, as reflected in positive responses to salbutamol (Papers #II and III) and increases in exhaled NO (Paper #III).

4. METHODS

4.1 Subjects

This thesis was based on three population-based cohorts of EPB, who were individually matched to randomly selected TB control subjects. The control subject-matching process is described in section 4.1.2.

4.1.1 EPB index subjects

The first two cohorts were born during two inclusion periods, between January 1982 and December 1985 (hereafter, referred to as EP_{1982} cohort) and between February 1991 and June 1992 (hereafter, referred to as EP_{1991} cohort). The protocol included all infants born at GA < 29 weeks or BW < 1001 g in Hordaland and Sogn og Fjordane, the two northern counties within the Western Norway Health Authority. Haukeland University Hospital (HUS) was the only institution in the region admitting and caring for EPB, serving a population of approximately 500,000, with an annual birth rate of approximately 6,700 at that time. Seventy-six of the 81 EPB (94%) were born at HUS. GA was determined according to the same algorithms, which were primarily based on the number of completed weeks since the mother's LMP. Ultrasound scans, if available, were performed before the 21st week of pregnancy. If delivery dates set by LMP and ultrasound scan assessments differed by more than 2 weeks, scans were given preference; however, if they differed by more than 3 weeks, paediatric postnatal assessment was preferred[197]. In cases of doubt, an uninvolved obstetrician was consulted before decisions were made (Professor T. Kiserud).

Subjects were considered enrolled in the study when they were admitted to the NICU at HUS. The senior staff, for the most part, comprised the same personnel during the two inclusion periods. Neonatal data were obtained from hospital charts, consistently and systematically recorded and prospectively tabulated. Other background data were obtained from questionnaires at the time of the study assessments. The decision to wean from O_2 administration was primarily based on transcutaneous measurements in the EP_{1982} cohort and by oximetry in the EP_{1991} cohort.

The third cohort was born between January 1999 and December 2000 (hereafter referred to as EP_{1999} cohort), and included all infants born at GA < 28 weeks or BW < 1000 g from mothers living within the area served by the Western Norway Health Authority, also including Stavanger University Hospital (SUS). The population comprises approximately 1.1 million residents, with an annual birth rate of 11,500. This was a regional selection of a national cohort[12]. GA was primarily determined based on early ultrasound scans. If scans were unavailable, GA was determined using the mother's LMP, if consistent with clinical findings.

4.1.2 TB control subjects

One individually matched control subject was recruited for each EPB at the time of the first follow-up visit. For the EP_{1982} and EP_{1991} cohorts, the TB who was delivered nearest in time to the corresponding EPB index subject of the same sex, and with a GA ≥ 37 weeks and a BW of 3000-4000 g (approximate Norwegian 10-90 percentiles), was identified from birth protocols in the same delivery unit. The parameters for matching index subjects in the EP_{1999} was GA ≥ 37 weeks and BW > 3000 g. After identification, the matching TB subjects were invited to participate by contacting their parents by postal letter. If he/she declined, the next TB delivered closest in time to the index subject was identified, and so on, until an appropriate match was found. There were no exclusion criteria for the controls, other than that they had to be able to perform PF tests. Also, it was our aim that transportation from their home address to the hospital would be under 1 hour, for financial and practical reasons.

4.2 Study design

The study of the EP_{1982} and EP_{1991} cohorts was designed as a longitudinal population-based controlled cohort study. The cohorts were identified in retrospect from neonatal protocols at HUS[168], after the event of interest (preterm birth) had happened, and with the neonatal data prospectively tabulated, before assessment of outcome. The study of the EP_{1999} cohort was designed as a prospective population based controlled cohort study.

4.3 Questionnaires

The study subjects and/or their parents answered different sets of questionnaires. The Child Health Questionnaire (CHQ)[198] was used to assess quality of life in the EP_{1982} and EP_{1991} cohorts, and the KIDSCREEN to evaluate quality of life and well-being[199] in the EP_{1999} cohort. These data were not considered in this thesis. The subjects completed a separate custom-made questionnaire specifically designed for these studies in order to obtain relevant demographic variables and other information on health status. To obtain information on respiratory symptoms, the questionnaire from the International Study on Asthma and Allergy in Childhood (ISAAC) was used[200]. This questionnaire is thoroughly validated and has been used in numerous studies[201]. The questionnaire records a history of asthma (hereafter referred to as 'asthma ever') and wheezing in the last 12 months; wheezing or respiratory symptoms during exercise, sleep, and talking in the last 12 months; recurrent coughing at night (apart from airway infections) in the last 12 months; and use of asthma medication in the last 12 months.

4.4 Definitions

BPD was defined as mild if O_2 treatment or assisted ventilation was required at 28 postnatal days and as moderate-severe if such treatment was still required at 36 weeks GA[40]. For the EP_{1999} cohort, the cut-off point for assigning BPD was administration of O_2 treatment at 36 weeks, since there were not sufficient formal data available for 28 postnatal days' age to determine whether the infant had mild BPD.

To minimize the risk of errors made in the definition of asthma, we combined the subjects' answers to two or more ISAAC questions[201]. For Papers #I and #II, current asthma was defined as, (a) a history of at least one episode of wheezing in the last 12 months, and (b) use of either asthma medication in that period or a positive answer to the question, 'have you ever experienced asthma?'[200]. In Paper #III, current asthma was defined as, (a) a physician's diagnosis of asthma with either respiratory symptoms or use of asthma medication in the last 12 months, or (b) use of asthma medication and presence of respiratory symptoms in the last 12 months, regardless of a physician's

prior diagnosis. Asthma medication included inhaled corticosteroids and short- or long-acting β_2 agonists (in separate or combined devices), anticholinergics, and oral leukotriene modifiers. Atopy was defined as a minimum of one positive skin prick test (SPT) in a panel of relevant airway allergens, or, for the EP_{1982} and EP_{1991} cohorts, a minimum of one positive SPT or specific serum-IgE (Immunoglobulin E) assay. Maternal smoking was defined as self-reported daily or occasional smoking by the subject's mother during pregnancy. A family history of asthma was defined as at least one positive report from first-degree relatives (parents or siblings).

4.5 Measurements and testing conditions

All subjects underwent comprehensive surveys, clinical examination, and PF tests. For the EP_{1982} cohort, these were performed at HUS at 18 and 25 years of age (in 2001-2002 and 2008-2009). For the EP_{1991} cohort, these were performed at HUS at 11 and 18 years of age (in 2001-2002 and 2008-2009). For the EP_{1999} cohort, these were performed at 11 years of age (in 2010-2012), at HUS for those born in Hordaland and Sogn og Fjordane and at SUS for those born in Rogaland. Standard and commercially available testing equipment were used. The same paediatrician and respiratory physiologist performed all examinations at both visits using similar equipment in the EP_{1982} and EP_{1991} cohorts. The same physiologist performed the PF tests also in those examined at HUS in the EP_{1999} cohort. For the EP_{1999} cohort, the equipment for examination and PF tests were identical and produced by the same manufacturers in Bergen and Stavanger for all measured variables; as were calibration routines and testing protocols. The manufacturer's representative in Norway visited yearly and on request to double-check software and hardware. The test-administration was routinely double-checked by the physiologist in Bergen. The same person (MV), who also ensured coordinated methodologies between centres, plotted data from both centres.

Testing began with standardized BTPS (body, temperature, pressure saturated) test conditions[83,202-207]. Technical calibration was performed according to the manufacturer's recommendations. Biological calibration was based partly on the control population, and additionally based on testing ourselves (physicians and physiologists or close relatives) regularly.

Participants of the EP_{1982} and EP_{1991} cohorts came to the laboratory twice on two different days, whereas participants of the EP_{1999} cohort visited three times, approximately 2 weeks apart for a period of 0.5 - 3.0 hours for each session. Clinical examinations and detailed anthropometric measurements (height, weight, and for the EP_{1999} cohort, abdominal circumference, triceps, and subscapular skinfold thickness) were performed, along with SPTs (and specific serum-IgE assays in the EP_{1982} and EP_{1991} cohorts), and collection of blood samples and morning urine samples. PF tests were performed, including fractional expired NO (FE_{NO}), alveolar NO (only the EP_{1999} cohort), spirometry, plethysmography, lung diffusion capacity, reversibility by salbutamol (on a separate day, only with the EP_{1999} cohort); EIA test with reversibility testing (on a separate day, only with the EP_{1982} and EP_{1991} cohorts); and BHR to methacholine. The EP_{1999} cohort also underwent dual-energy X-ray absorptiometry (DXA) scanning for bone mineral density and body composition (data not included in this thesis).

Subjects were rescheduled if respiratory symptoms suggestive of an obstructive exacerbation or a viral infection were suspected in the prior 2 weeks. Inhaled corticosteroids and short-acting β_2 agonists were stopped one day prior to testing, long-acting β_2 agonists and leukotriene antagonists were stopped 2 days prior to testing. Subjects were asked to abstain from drinking caffeine-containing drinks (e.g., coffee, tea, cola, etc.) in the 4 hours prior to the examination[204,205,207]. We requested that antihistamines should not be used, if possible, in the week prior to examination, because of their influence on the results of the SPTs (Appendix I).

4.5.1 Spirometry

Maximal expiratory flow volume loops were measured with Vmax equipment (SensorMedics, Anaheim, USA)[202,205]. Subjects were tested in seated position, wearing a nose clip. FEV_1, FVC, and forced expiratory flow at 25-75% of vital capacity (FEF_{25-75}) were recorded, and expressed as standardized z-scores and percentages of predicted using the all-age reference equations[208,209]. Measurements and calibration were performed according to standard quality criteria[202,205]. The test started with tidal breathing, and the test subject then performed a full inspiratory manoeuvre followed

by a forced expiratory manoeuvre. The two highest values of FEV_1 and FVC should be reproducible, exhalation should start instantly and without hesitation, the effort should be coded as being maximal by the technician, and the maximal expiratory flow-volume curve should pass visual quality inspection (the peak expiratory flow rate should be a clearly visible peak, and there should be no fluctuations in the curve). According to European Respiratory Society (ERS) and American Thoracic Society (ATS) recommendations, an FVC manoeuvre is finished at the time when the volume changes < 25 ml in 0.5 sec, and a plateau in the volume-time curve is apparent[205]. In children, most agree that an exhalation of at least 3 seconds is suitable if a simultaneous flattening of the volume-time curve occurs. We repeated recordings until three technically acceptable curves were obtained. According to guidelines, the best acceptable FEV_1 and FVC were recorded from individual curves, while flow values were obtained from the curve with highest sum of FEV_1 and FVC.

4.5.2 Body plethysmography

Static lung volumes were measured with an Autobox 6200 plethysmograph (SensorMedics, Anaheim, USA). The subject was seated, wearing a nose clip. Measurements and calibration were performed using standard quality criteria[202]. The following lung volume parameters were registered: inspiratory and expiratory reserve volume (IRV and ERV), FVC, RV, and TLC. Dynamic airway resistance against tidal respiration (R_{aw}) was determined. Lung volumes were standardized to percentages of predicted values, ratios, or z-scores[210,211].

The panting technique was used. At tidal breathing, the shutter occludes the mouthpiece at end expiration, and the subject is told to pant gently (frequency 1 Hz). At least four panting movements and corresponding pressure-volume loops are recorded. Shutter closure results in zero flow through the mouthpiece. Consequent pressure oscillations are recorded simultaneously at the mouthpiece and within the plethysmograph. By employing Boyle's law, which states that the product of pressure and volume of gases is constant inside a sealed container, the volume of thoracic gas (VTG) remaining in the chest at the end of a tidal volume breath is estimated from each acceptable pressure-volume loop, from which FRC is calculated[206]. Pressure-

volume loops were considered inadequate if the pressure gradient exceeded 1.5 kilopascal (kPa), or if major irregularities occurred, like obvious 'looping', open ends or nonlinear segments.

4.5.3 Pulmonary diffusing capacity for carbon monoxide (D_{LCO})

D_{LCO} was measured with Vmax equipment (SensorMedics, Anaheim, USA). The single breath method was used, with the subject seated and using a nose clip. Measurements and calibration were performed using standard quality criteria[203,207,212]. Measured values were adjusted for venous Hb concentration (g/dl) using ERS/ATS recommendations[207]. The following parameters were recorded: D_{LCO}, alveolar volume (VA); and D_{LCO}/VA (K_{CO}). The results were reported as raw data.

Criteria for an acceptable test were the following: inhaled volume (VI) of at least 90% of FVC and inspired within 2.5 sec, a breath-holding period of 10 ± 2 sec during the plateau phase, and exhalation within 4 sec. If two acceptable manoeuvres produced values with coefficients of variation (CVs) ≤ 10%, their mean was calculated. A maximum of four attempts were allowed, conducted at a minimum of 5-minute inter-trial intervals, to allow the test gas to be exhaled fully before the next manoeuvre. If only one acceptable manoeuvre was obtained, that value was recorded. Data from a few cases with smooth curves within time limits and VIs below but close to 90% of FVC were accepted[207,213].

The D_{LCO} test requires use of an insoluble and inert tracer gas (methane (CH_4)) in order to measure VA, as well as the diffusing test gas carbon monoxide (CO) mixed with O_2 and nitrogen (N_2). The test starts with relaxed tidal breathing. The subject exhales fully to RV within the limits of 6 (3 for children) seconds (the time limit for obtaining the FVC in the spirometry manoeuvre). At RV, the mouthpiece is connected to the test gas, a mixture of 0.3% CO, 0.3% CH_4, 0.3% acetylene (C_2H_2), and 21% O_2, balanced with N_2, and the subject inhales rapidly and fully. VI should be as similar to FVC as possible, as a submaximal VI might influence the results. Ninety % of VI should be inspired within less than 2.5 seconds, as slower lung filling decreases the amount of time at full inspiration and hence decreases CO uptake. At TLC, the subject holds his or her breath for 10 ± 2 seconds, before slowly exhaling without hesitation or

interruption. During the breath hold, the CO gas is allowed to diffuse over the membrane and into the red blood cells. A mid-expiratory sample of alveolar gas is then collected and D_{LCO} is calculated by analysing the amount of CO in this sample.

4.5.4 Exhaled nitric oxide

FE_{NO} from the lungs ($FE_{NO\,Sa}$) and the nose ($FE_{NO\,Na}$) was measured using an Exhalyzer CLD-88 (EcoMedics, Switzerland) equipped with a visual display. $FE_{NO\,Sa}$ was calculated using standard quality criteria[214]. The subject was seated, not wearing a nose clip. The test was conducted before spirometry and other PF tests in order to avoid artefacts that could influence NO levels associated with the forced exhalations during other PF tests. NO-free air was inhaled to near TLC, followed immediately by full exhalation at a constant flow of 50 ml/sec, or at multiple flows when evaluating two-compartment models of exhaled NO. Nasal NO ($FE_{NO\,Na}$) was measured at tidal breathing through a nasal prong. Expired NO was expressed as parts per billion (ppb), calculated as the mean value from three measurements that had CV within 10%, or if this was not possible, from the curve with the most stable and horizontal plateau phase of expiration[214]. $FE_{NO\,Sa}$ was considered log-normally distributed in the statistical analyses and was reported as geometric means. Alveolar concentration of NO (C_ANO) was calculated using three different flows (30, 100, and 300 ml/sec). Airway wall concentration of NO ($C_{aw}NO$), maximum flux of NO in the airways ($J_{aw}NO$), and the airway wall diffusing capacity of NO ($D_{aw}NO$) was recorded[215,216].

4.5.5 Bronchial hyperesponsiveness (BHR)

BHR was evaluated by methacholine provocation and was performed with an inhalation-synchronized dosimetry nebulizer (Spira Electra 2, Finland); this provided a baseline $FEV_1 \geq 65\%$ of the predicted values[204,217]. A baseline FEV_1 acquired after saline inhalation in the sitting position was followed by inhalation of doubling methacholine doses via the Spira dosimeter. This was an automated, inhalation-synchronized, dosimetry jet nebulizer with 2.0 bar driving pressure and an integrated flow turbine sensor. Nebulization time was 0.5 sec and set to start after the inhalation of 100 ml of air. The first dose was 0.05 μmol of methacholine and continued in

increasing concentration increments until a fall of ≥ 20% (PD20) was achieved compared to baseline FEV_1, or the maximum cumulative dose of 22.3 µmol methacholine was given. For the EP_{1999} cohort, the test continued until the maximum dose of 11.5 µmol was given (due to local procedures at SUS).

The dose-response slope (DRS) was calculated as the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (µmol) of methacholine (%/µmol). The data were log-normally distributed[218] and reported as geometric means. The cut-off point proposed for the normal range was DRS of 2.39 %/µmol or higher[219,220].

4.5.6 Airway reversibility to salbutamol and bronchial lability

Salbutamol reversibility was assessed in the EP_{1999} cohort after administering 0.1 mg/10 kg salbutamol (Ventoline®) from a metered dose inhaler via a spacer (Volumatic); this followed by a subsequent FEV_1 measurement after 10-15 minutes[83]. For the EP_{1982} and EP_{1991} cohorts, reversibility to salbutamol was assessed in conjunction with a maximal cardiopulmonary exercise test[221] and combined with a test for exercise-induced bronchoconstriction (EIB). After baseline FEV_1 was acquired, the subjects ran to exhaustion on an incremental treadmill (ELG 70; Woodway, Weil am Rhein, Germany), and simultaneous measurements of exercise flow volume loops and O_2 consumption were collected, applying a modified Bruce protocol[222]. Subjects wore a facemask connected to a Vmax29 spirometer and cardiopulmonary exercise unit. The primary criterion for test termination was exhaustion, confirmed by either a respiratory quotient (RQ) > 1.05, a heart rate exceeding 95% of predicted maximum level (220 minus age in years), or a plateau in O_2 consumption. FEV_1 was assessed before commencing the cardiopulmonary exercise test, and then 1, 3, 5, 10, and 15 minutes after its completion. The EIB test was positive if the largest decline in FEV_1 relative to baseline was ≥ 12%. The test subject then received salbutamol (Ventoline®) from a metered dose inhaler via a plastic spacer (Volumatic) at a dose of 0.1 mg/10 kg bodyweight if bodyweight was less than 50 kg and 0.6 mg if bodyweight was 50 kg or greater. The test subject had a final FEV_1 assessment after another 15 minutes. The test was considered to be positive if FEV_1 increased ≥ 12% relative to baseline[83].

Bronchial lability was calculated as the sum of the percentage changes in baseline FEV_1 induced by exercise and salbutamol[223].

4.5.7 Skin prick tests (SPT) and Immunoglobulin E (IgE) assay

SPTs for house dust mites (*D. Farinae* and *D. Pteronyssinus*); animal dander (cat, dog, horse); pollens (timothy, birch, and mug wort); and moulds (*Alternaria* and *Cladesporium*) were performed with standard extracts (Soluprick®SQ, ALK-Abello AS, Hørsholm, Denmark) in accordance with European guidelines[224]. Histamine (10 mg/mL) and the allergen diluent were used as positive and negative controls. If the mean of the two perpendicular weal diameters was at least 3.0 mm, the reaction was positive. The test preferably began after the PF tests in order to avoid influencing the results in case of allergic sensitization. In the EP_{1982} and EP_{1991} cohorts, serum allergen-specific IgE assays (CAP-FEIA, Pharmacia, Uppsala, Sweden) were performed. The cut-off point for a positive test was set at 0.35 E/ml.

4.5.8 Anthropometric measures

Height (cm) was measured with a stadiometer with the subject standing erect, shoes off, with the head in the horizontal plane. Weight (kg) was measured with the subject wearing no shoes and only light clothing (e.g., t-shirt/top, underwear, socks). Values were recorded to one decimal precision and were then converted to z-scores correcting the raw data for gender and age, using the Norwegian growth references for children aged 0-19 years[84]. Growth after 19 years of age is typically minimal, and this method is considered adequate for the age groups in question. Age (years) was calculated as the difference between examination date and birth date using the automated procedure of SPSS, with ages recorded to two decimal places.

4.6 Statistical methods

All statistical calculations were performed using SPSS version 18.0 (Chicago, USA) or versions 20-22 (IBM Corp., Armonk, NY). The two-sided level of significance was set at $p \leq 0.05$, pertaining to the probability of a group difference with no *a priori* assumption about which group would show superior performance. Data were reported as means with 95% confidence intervals (CIs) or ranges for continuous, normally

distributed data; medians with ranges or geometric means with 95% CIs for continuous, skewed data; or counts with percentages of total for categorical data, as appropriate. The distribution of continuous variables was assessed by descriptive statistics, histograms, and Q-Q plots. Variables that appeared markedly skewed were log10 transformed prior to analysis (DRS and $FE_{NO\ sa}$) to meet the assumption of normally distributed data. Non-paired group comparisons were performed with Student's t-test or analysis of variance (ANOVA) for normally distributed continuous variables, Mann-Whitney U test or Kruskal-Wallis test for non-normally distributed, continuous variables, and exact chi-square tests or Fishers' exact 2-sided mid-p values[225] for categorical variables, as appropriate.

Associations between spirometry measures obtained in 2001-2002 and 2008-2009 were assessed with simple linear regression analyses, and prediction equations and coefficients of determination (R^2) were provided for z-FEV_1 and z-FEF_{25-75}.

Backward, stepwise linear regression analysis was used to assess associations between neonatal variables (e.g., GA, BW, BW z-scores, BPD, number of days on the ventilator, number of days with O_2, treatment with surfactant, and antenatal and postnatal steroids, active management of PDA) in EPB subjects, and background variables (maternal smoking, current self-reported smoking, wheeze last 12 months, use of asthma medication last 12 months, current asthma, asthma ever, familial asthma, and atopy) in EPB and TB subjects versus spirometry indices as the dependent variables. The independent variables were selected based on clinical and empirical understanding of factors known to or assumed to be associated with the outcome variables. These were included in the regression equations, provided they showed a relationship with the dependent variable of at least 0.3 in correlation analyses, and provided the total number of variables entered into the final equations did not exceed 1/10 of the population sample size. As relatively little power might be required in order for regressions to exhibit statistical significance, we also reported coefficients of determination (R^2) in order to relate the results to some means of practical importance[226].

According to the design, (i.e., individually matched TB subject for each EPB subject) we used statistical analyses appropriate for a paired design whenever possible. Paired group comparisons were performed with the mixed linear model (MLM) (EPB subgroups vs. TB groups). Interaction models were constructed to address divergent trends in PF for TB subjects in relation to EPB with increasing severity of BPD, in relation to increasing GA (GA category), or in relation to the different EPB birth cohorts.

For longitudinal data, the MLM has the advantage of allowing paired analyses to be performed for matched data where one subject of the pair declined to participate at one assessment during the study period (and hence were examined only once)[227]. The method of MLM was also used to explore development of PF variables that were measured repeatedly over time, exploring both between- and within-subject changes (EPB subgroups vs. TB vs. development from first to second follow-up)[227]. The model included the continuous PF variable, subject group (TB, EPB with and without BPD) and time, and the interaction for time×group. A significant interaction would imply a different development of PF between subject groups over time.

We used the general linear model in SPSS to test potential different effects from background variables in EPB and TB (interactions).

The mean represents a simple statistical model of the centre of a score distribution. The results from MLM analyses are given as *estimated means,* which account also for within-subject variability; thus, they can be slightly different from the *observed means*.

4.6.1 Power calculations

The original cross-sectional set-up from 2001-2002 was designed to have 90% power for detecting differences (significance level $p \leq 0.05$) exceeding 7.5% in predicted FEV_1 between EPB and TB subjects. However, because of the different design and also subject attrition, the full longitudinal study (Paper #I; EP_{1982} and EP_{1991} cohorts) eventually achieved 85% power to detect changes for z-FEV_1 of 0.6 from visit 1 to visit 2 (significance level $p \leq 0.05$). The smaller study (Paper #II; EP_{1982} cohort) achieved 79% power to detect a difference in z-FEV_1 of 0.6 between EPB and TB. All

power calculations at second follow-up were done *post hoc*, performed after the original description and inclusion of study subjects, as the number of participants at that stage was given by the design. For the study of 11-year-old subjects born in 1999 (Paper #III; EP_{1999} cohort), provided 60 cases were included, the study had 80% power to detect a difference in z-FEV_1 of 0.5 between EPB and TB (significance level $p \leq 0.05$).

4.7 Ethics

The Regional Committee on Medical Research Ethics of the Western Norway Health Authority approved all studies. All participating subjects and/or their parents or guardians gave informed written consent. All participants were informed of the opportunity to withdraw at any time during the studies.

The PF tests we used are employed on a daily basis in pulmonary clinics throughout the Western world and none are considered to represent any risk or particular discomfort to the participants. Children were offered local anaesthetics (Emla®) applied to the skin before blood sample collection in order to minimize pain.

Participants were individually informed of all findings, such as tests for allergy, lung function and exercise capacity. Subjects were referred for further investigations or for subsequent treatment in cases where the assessment revealed suspicion of medical conditions.

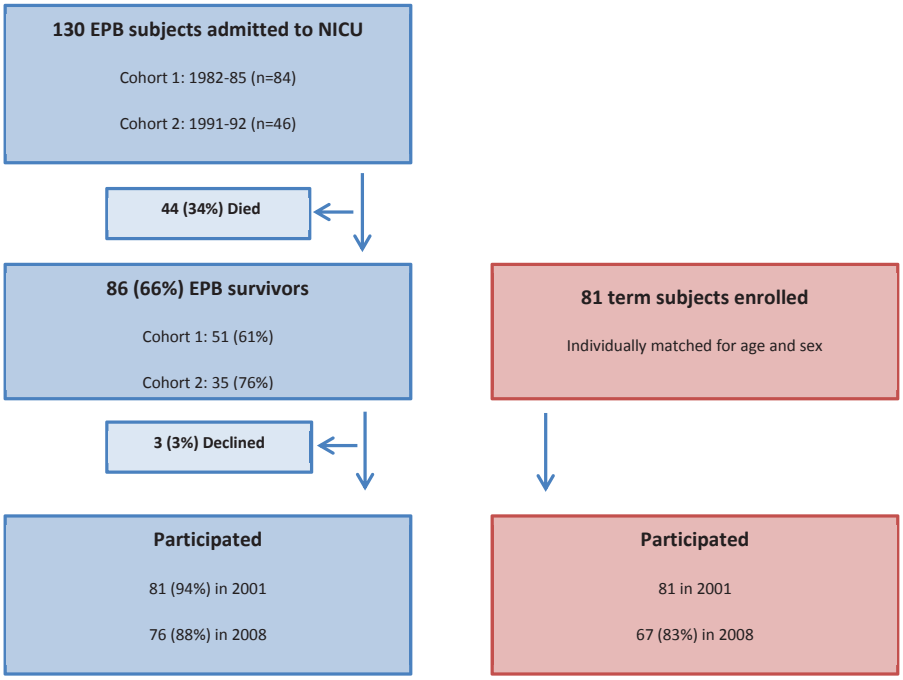
5. RESULTS

This section presents results from the three papers, as well as supplementary results, referred to herein as unpublished data, intended to clarify the reported results.

5.1. Subjects, Paper #I and Paper #II

The recruitment of participants born in the two inclusion periods 1982-85 and 1991-92 is depicted in Figure 5. The most immature survivors were born at GA 23 weeks, and the lowest BW was 580 g in EP_{1991} and 570 g in EP_{1982}. Seven participants were included solely by using the BW criterion, two in EP_{1991} (GA 30 and 31 weeks), and five in EP_{1982} (mean GA 30 weeks). Eight (17%) and five (14%) subjects were SGA in the EP_{1982} and EP_{1991} cohorts, respectively[228]. NICU mortality was 39% in the EP_{1982} cohort (mean GA 27.3) and 26% in the EP_{1991} cohort (mean GA 26.7) ($p = 0.157$).

Figure 5: The recruitment scheme of extremely preterm (EPB) and term-born (TB) subjects for assessment in 2001 and 2008.



5.2 Paper #I: Longitudinal course of lung function (Research area 1)

Compared to TB, EPB had lower z-FEV_1 and z-FEF_{25-75} at all assessments in both cohorts (11 to 18 years old and 18 to 25 years old). Z-scores were lower for EPB with a neonatal history of BPD than for EPB with no such history. Flow-volume parameters tracked in parallel for the TB and subgroups of EPB, with non-significant interaction terms (EPB vs. TB vs. time). Airway obstruction was present from mid-childhood to adulthood in EPB, with no relative improvement or deterioration, neither over time nor versus the control groups. R^2 values for data obtained at the first vs. the second assessment ranged from 0.64 to 0.82. Some decline in small airway dimensions (FEF_{25-75}) was noted for most groups at 25 years of age.

The reference equation (Global Lung Function Initiative (GLI) 2008)[209] fitted the control population reasonably well, with two exceptions, FEF_{25-75} and FEV_1/FVC measured at 18 years of age in EP_{1982} (mean z-scores = 0.47 and 0.53, respectively; 95% CIs not including zero). Four variables (FEV_1, FVC, FEV_1/FVC, and FEF_{25-75}) were tested twice in two control populations. The likelihood that 95% CIs should not include zero for 2 out of 16 variables was estimated to be approximately 19%.

5.2.1 Pulmonary function related to perinatal and background variables

In regression models, postnatal treatment with corticosteroids remained the only perinatal (negative) predictor of z-FEV_1 at 18 years in EP_{1991} ($R^2 = 0.39$), no variable remained significant at 25 years in EP_{1982}. For background variables, use of asthma medication in the 12 preceding months at 11 years ($R^2 = 0.39$) and current asthma at 18 years ($R^2 = 0.28$) both remained negative predictors for z-FEV_1 at 18 years in EPB in the 1991-92 inclusion period; no variable remained significant in TB. In the 1982-85 inclusion period, maternal smoking ($R^2 = 0.35$, with other variables) negatively predicted z-FEV_1 in TB at 25 years, no predictor remained significant in EPB.
We tested potential different effects from background variables in EPB and TB (interactions) by the general linear model. The only significant effect ($p = 0.021$) was use of asthma medication before the visit at 11 years for those born in 1991-92, where z-FEV_1 was negatively associated in EPB but positively associated in TB. Due to small numbers, this cannot be interpreted confidently.

The findings of similar tracking of spirometry parameters were robust to adjustments, with neither perinatal nor background variables influencing the interaction terms used to compare the developmental trajectories of z-FEV_1 in the various subgroups.

5.2.2 Differences in pulmonary function between cohorts

Subjects from both cohorts were assessed at age 18 years (Table 2); respectively at the first examination (2001-02) of EP_{1982} and the second examination (2008-09) of EP_{1991}. Spirometry parameters were similar for the BPD subgroups, except for EPB with moderate-to-severe (m/s) BPD, where z-FVC was lower in the EP_{1982} cohort (difference: –1.01; p = 0.020), and z-FEV_1/FVC was higher (difference: 1.02; p = 0.049).

Table 2: Z-score differences for spirometry parameters obtained at 18 years of age for two cohorts of EP and matched TB subjects born 1982-1985 (EP_{1982}) and 1991-1992 (EP_{1991}).

z-score	Subject	Mean difference	95% CI	p-value
FEV_1	Control	0.28	(-0.18, 0.75)	0.233
	EP no BPD	-0.11	(-1.15, 0.92)	0.816
	EP mild BPD	-0.60	(-1.76, 0.56)	0.299
	EP m/s BPD	-0.17	(-0.95, 0.61)	0.656
FVC	Control	-0.07	(-0.54, 0.39)	0.754
	EP no BPD	0.15	(-1.14, 1.44)	0.806
	EP mild BPD	-0.71	(-2.05, 0.63)	0.288
	EP m/s BPD	-1.01	(-1.84, -0.17)	**0.020**
FEV_1/FVC	Control	0.53	(0.01, 1.05)	**0.046**
	EP no BPD	-0.30	(-1.29, 0.67)	0.511
	EP mild BPD	0.05	(-0.80, 0.90)	0.910
	EP m/s BPD	1.02	(0.01, 2.03)	**0.049**
FEF_{25-75}	Control	0.62	(0.15, 1.09)	**0.010**
	EP no BPD	-0.28	(-0.97, 0.42)	0.407
	EP mild BPD	-0.34	(-1.18, 0.51)	0.421
	EP m/s BPD	0.45	(-0.50, 1.40)	0.335

Values are observed mean differences with 95% CIs between the EP_{1982} and EP_{1991} cohorts and their respective matched TB control groups, all examined at 18 years of age. A positive difference translates to higher z-scores in the EP_{1982} cohort. P-values denote cohort differences, as assessed by independent samples t-tests/ANOVA. Unpublished data.

5.3 Paper #II: Lung function in adulthood (Research area 2)

Lung function abnormalities persisted into adulthood. Assessed at 25 years of age**,** z-scores for FEV_1, FEF_{25-75}, and FEV_1/FVC were reduced and airway resistance increased in EPB compared to TB. RV/TLC increased with the severity of BPD. BHR to methacholine and the bronchial lability index were increased, whereas bronchial responsiveness to exercise and salbutamol and exhaled NO were similar to that observed in TB. Deficits were relatively modest for most variables, and for z-FEV_1**,** partly reversed by β-agonist. Respiratory symptoms were uncommon and similar in both groups. PF progressed in parallel from 18 to 25 years in EPB and TB.

5.3.1 Effect of different reference equations

An ERS Task Force (GLI) published updated reference equations for spirometry across all ages in 2008 and 2012[208,209]. Therefore, different equations were used in Papers #I and #II. Spirometry parameters at 25 years of age were compared, revealing no differences in results or statistical conclusions using the different sets of reference equations (Appendix III).

5.3.2 Gender effects

In relation to changes from 18 to 25 years of age, there were no interaction effects with gender for any PF variable in EPB. In TB, significant effects were observed for z-FEV_1/FVC, with a greater decline in males than females (-0.69 vs. -0.20; $p = 0.024$), and for FRC% predicted (males decreased -3.8 vs. females increased 4.3; $p = 0.041$).

5.3.3 Bronchial lability

The question whether airway obstruction in EPB is a fixed phenomenon rather than a reversible one, was explored as follows:

a) Instead of reporting salbutamol responses in percentages of baseline values, we reported post-salbutamol responses as z-scores[83]. Numerically and statistically, the response to β-agonist was similar in EPB and TB, as well as in BPD subgroups. Thus, the group differences remained unchanged. Compared to levels predicted by the reference equations, there was barely any residual airway obstruction after

salbutamol administration, except that z-FEV_1 was moderately reduced in one BPD subgroup.

b) Linear regression models were constructed, with the dependent variables z-FEV_1 and z-FEV_1/FVC vs. the independent variables responsiveness to methacholine (DRS), exercise, and salbutamol, and the latter two variables combined (bronchial lability). Models with interaction terms were also constructed to test whether potential effects differed between EPB and TB. DRS was associated with lower z-FEV_1, if both groups were tested together (B = -0.51; $p \leq 0.001$) and separately (EPB: B = -0.34; p = 0.035 vs. TB: B = -0.52; p = 0.019), with no group interaction (p=0.489). Findings were similar for z-FEV_1/FVC, if both groups were tested together (B = -0.27; p = 0.039). However, if split by EPB and TB, the associations were not significant, and no interaction was found (p = 0.807). Adding bronchial lability to the model increased the effect of DRS on z-FEV_1 (B = -0.66; $p \leq 0.001$), when both groups were tested together and independently (EPB: B = -0.46; p = 0.028 vs. TB: B = -0.82; p = 0.005); similar results were found in EPB and TB (p = 0.983). Bronchial lability was not associated with z-FEV_1, but with z-FEV_1/FVC in both groups (p = 0.001). No group interaction was found (p = 0.269), suggesting similar effects in EPB and TB.

c) Adjusting z-FEV_1 and z-FEV_1/FVC for DRS and/or bronchial lability index did not influence the statistical differences between EPB and TB. Similarly, adjustment for the exercise or the salbutamol response did not change the differences.

d) The number of subjects with z-scores < -1.64 (lower limit of normal [LNN]) according to ERS, ATS, and GLI[83,205,208], was calculated. Nine of 45 EPB and 2 of 39 TB had z-FEV_1 less than lower limit of normal (LLN), with an OR = 4.6 (95% CI: 0.9 to 22.9; p = 0.061). Ten EPB and 1 TB had z-FEV_{25-75} less than LLN, with an OR = 11.2 (95% CI: 1.4 to 91.9; p = 0.025). One of 32 EPB and 0 TB had z-FEV_1 and z-FEF_{25-75} less than LLN following administration of salbutamol. Clinically, the post-salbutamol results thus indicate a change of the baseline group differences, that is, the differences between EPB and TB disappeared.

5.4 Subjects, Paper #III

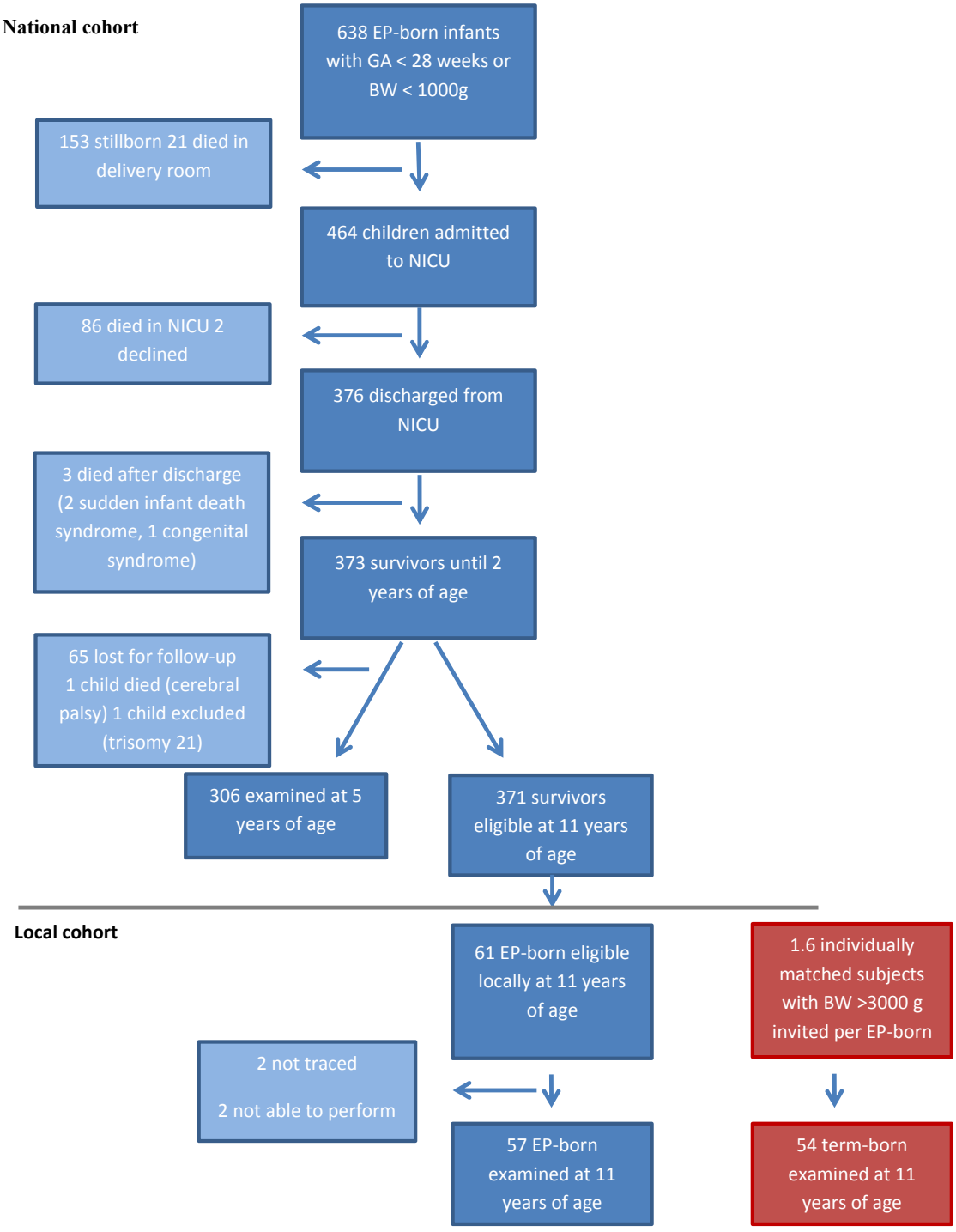
The recruitment of participants born in the inclusion period 1999-2000 is depicted in Figure 6. Sixty-one of 87 of the local cohort (70%) were eligible (26 died before admittance or in the NICU). Two children were not found, 2 were excluded because they were not able to perform (cerebral palsy (CP)), and 57 EPB were examined. All but 3 were Caucasian (1 Arab, 1 African, 1 mixed ethnic origin).

The most immature survivors were born at a GA of 24 weeks, and the lowest BW was 450 g. Eleven (19%) subjects were included solely by the BW criterion, at mean GA 29.2 weeks (range: 28-31weeks). Overall, 20 (35%) participating EPB were SGA[228], and 100% of those in the GA category ≥ 28 weeks. SGA infants had a 200 gram lower mean BW ($p < 0.001$) despite a 2 weeks higher GA ($p < 0.001$), compared to AGA infants. Compared to the EP_{1991} cohort, the incidence of SGA was higher in the EP_{1999} cohort ($p = 0.033$).

5.5 Paper #III: Lung function in recent EPB (Research area 3)

In the group born preterm in 1999-2000, we observed deficits in z-FEV_1, z-FEV_1/FVC, and z-FEF_{25-75} and higher BHR to methacholine in EPB compared to TB. Fifteen subjects had z-FEV_1 below LLN, 4 TB, 4 EP no BPD and 7 EP BPD. Other PF outcomes did not differ significantly between the EPB and TB groups. For EPB with BPD, improvements were observed for important PF variables compared to those born similarly EP in 1991-1992; i.e. nearly one decade before. In regression models, improvements related to use of antenatal corticosteroids and surfactant, both administered more frequently in EP_{1999} than in EP_{1991}. PF was unrelated to GA. The findings suggest that better neonatal management might improve long-term pulmonary outcome.

Figure 6: Subject recruitment of Project Extreme Prematurity. Candidate subjects for the local study came from Western Norway Health Authority. Included subjects were born extremely premature or at extremely low birthweight in 1999-2000.



The only perinatal variable predicting z-FEV_1 at 11 years of age in EP_{1999} was a negative impact from BW z-scores. The same result was found for EP_{1991} in a final model that also included antenatal steroids and days with supplemental O_2. No background variables were significant predictors of z-FEV_1 at 11 years in either EPB or TB in any of the two birth cohorts.

For the NO variables, regressions were performed with independent variables proposed to influence NO parameters (FEV_1, FVC, age, height, and atopy)[215] with results basically as expected (Appendix IV).

5.5.1 Differences between centres

PF data were collected from two centres. Differences in z-RV and RV/TLC ratios were found, the values being higher in TB and EPB without BPD from SUS (Table 3).

Table 3: Lung function data at 11 years of age from EP- and term born subjects born in 1999-2000 in either Bergen or Stavanger.

		Control N=54	**EP no BPD** N=26	**EP BPD** N=31
Variable		Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
FEV_1	z	0.26 (-0.31, 0.81)	0.15 (-0.66, 0.95)	-0.18 (-0.91, 0.57)
FVC	z	0.36 (-0.19, 0.90)	0.30 (-0.39, 0.99)	-0.20 (-0.95, 0.55)
FEV_1/FVC	z	-0.13 (-0.65, 0.40)	-0.08 (-0.94, 0.78)	-0.05 (-0.83, 0.74)
FEF_{25-75}	z	0.13 (-0.42, 0.69)	0.02 (-0.80, 0.84)	-0.10 (-0.82, 0.63)
DRS	G. mean	2.2 (0.2, 1.2)	3.0 (0.1, 1.5)	1.3 (0.3, 5.2)
TLC	z	-0.21 (-0.89, 0.47)	-0.52 (-1.13, 0.08)	-0.00 (-0.74, 0.84)
FRC	z	-0.55 (-1.40, 0.31)	-0.41 (-1.33, 0.51)	-0.39 (-1.44, 0.67)
RV	z	**-0.91 (-1.65, -0.18)**	**-1.13 (-2.06, -0.21)**	0.40 (-0.45, 1.25)
RV/TLC	%	**-4.9 (-8.4, -1.3)**	**-6.7 (-12.7, -0.7)**	3.2 (-2.2, 8.7)
R_{aw}	z	-0.33 (-0.68, 0.02)	-0.13 (-0.69, 0.43)	-0.31 (-2.0, 1.4)
DL_{CO}	Mmol/kPa min	-0.21 (-0.82, 0.41)	-0.55 (-1.58, 0.47)	-0.23 (-1.72, 1.26)
VA	l	0.32 (-0.70, 0.06)	**-0.76 (-1.32, -0.21)**	-0.26 (-0.91, 0.39)
K_{CO}	Mmol/kPa min l	0.09 (-0.07, 0.26)	0.20 (-0.02, 0.42)	0.0 -0.26, 0.26)

Values are observed mean (95% CI) differences between measurements taken at Bergen vs. Stavanger facilities. Positive differences translate to higher values in those examined in Stavanger compared to those examined in Bergen. DRS values are geometric means (G.mean). Unpublished data.

5.6 Changes over time spans, from 1982 to 2000

5.6.1 Preterm born subjects

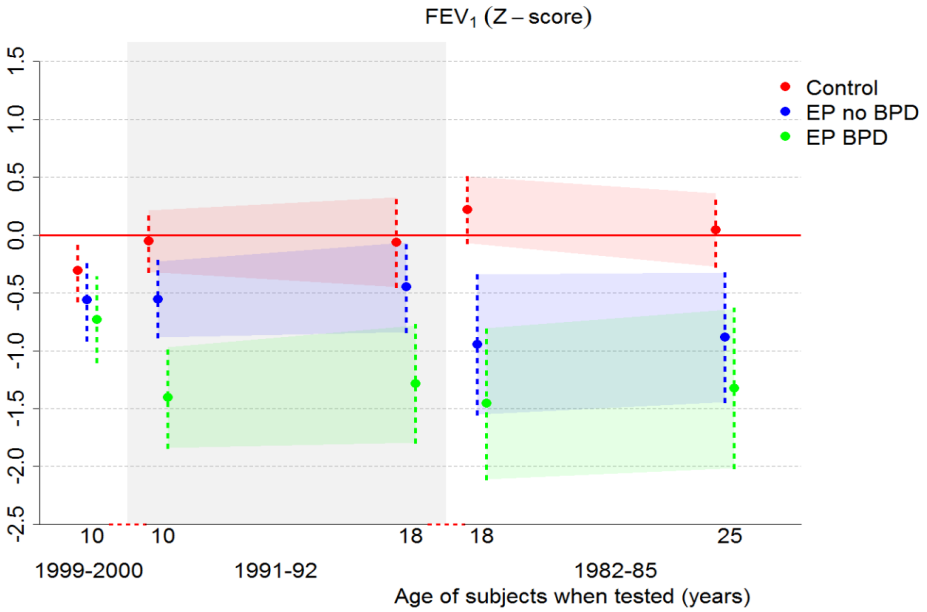


Figure 7: Development of z-FEV_1 in three consecutive cohorts of EPB and TB subjects born in Western Norway in 1982-1985 (EP: n = 48; TB: n = 46); 1991-1992 (EP: n = 35; TB: n = 35); and 1999-2000 (EP: n = 57; TB: n = 54). EPB subjects were grouped by the presence or absence of bronchopulmonary dysplasia (BPD), defined as O_2 supplementation given at GA 36 weeks. Dots indicate mean values, and shaded areas are 95% confidence intervals.

Plotting the data obtained for z-FEV_1 as mean values with 95% confidence intervals versus a time scale that reflects the decades into which the three participating EPB cohorts were born, the following picture emerges (Figure 7):

For subjects born EP in the 1980s, there was extensive overlap of confidence intervals between EPB with and without neonatal BPD regarding z-FEV_1, but no such overlap between the two EPB subgroups and the control group. For subjects born EP in the 1990s, there was no overlap of z-FEV_1 confidence intervals between the two BPD subgroups; however, there was overlap between the EPB subgroup with no history of BPD and the control group, possibly indicating that those born EP without developing

BPD in that decade had better outcome. For the cohort born EP at the turn of the millennium, also the subgroup that *did* develop BPD had improved, with z-FEV_1 confidence intervals overlapping with the subgroup with no history of BPD as well as with the TB control group, possibly indicating improvements also for EPB subjects with neonatal BPD and presumably the most turbulent neonatal history.

5.6.2 Term-born controls.

There were no significant differences in z-FEV_1 between 11-year-old TB subjects in the 1999 versus 1991 cohorts (p = 0.199), or between 18-year-old TB subjects in the 1991 versus 1982 cohorts (p = 0.233) (Table 4).

Table 4: FEV_1, expressed as z-scores, for term-born subjects in Western Norway over three decades.

	1999-2000		**1991-1992**		**1982-1985**
N	54	35	28	46	39
Age, Years	11	11	18	18	25
Male n (%)	29 (54)	13 (37)	7/27 (26)	25 (54)	21/39 (54)
FEV_1; z	-0.31	-0.05	-0.06	0.28	0.042
	(-0.57, -0.04)	(-0.35, 0.25)	(-0.45, 0.32)	(-0.04, 0.61)	(-0.36, 0.45)

Values are counts (%) and means (95% CI). Unpublished data.

The proportion of male subjects was similar in the three TB cohorts, with 25 of 46 (54%) TB in 1982, 14 of 35 (37%) TB in 1991, and 29 of 54 (54%) TB in 1999 (p = 0.236). At 18 years of age, the proportion was dissimilar (p = 0.042). The corresponding male proportion in EPB was 27 of 48 (56%), in 1982, 13 of 35 (37%) in 1991 and 29 of 57 (51%) in 1999 (p = 0.219).

6. DISCUSSION

Our findings indicate presence of widespread pulmonary abnormalities in EPB subjects born in three different decades and tested at ages spanning from 10 to 25 years. Spirometry parameters tracked in parallel from childhood to adulthood in subjects born EP and at term, with no signs of pubertal catch-up growth or early adult deterioration in any subgroups. Respiratory symptoms decreased with age in the EPB groups. Impaired pulmonary function persisting to adulthood might herald future early onset COPD in subgroups. Addressing cohort effects; i.e. changes over time in comparable EPB groups born in different decades, revealed a picture of possible improvements, with less impairments of PF in EPB children born at the turn of the millennium compared to comparable children born in the early 1990s, possibly related to changes in neonatal treatment. Airway obstruction in EPB was unrelated to exhaled NO, and the question if airway obstruction was a fixed or reversible characteristic remained unresolved.

6.1 Methodological considerations

6.1.1 Statistical considerations

Study design

The purpose of biomedical research is to generate knowledge that is applicable to other groups than those studied. The population sample under study should be an unbiased sample of the larger population that the study aims to describe. A bias is a factor that influences the measure of interest, without being precisely measured or accounted for. Valid generalizations cannot be made from unrepresentative or biased samples.

In studies of outcomes following preterm birth, sample selection bias arises when the study population differs from the general population in relation to the exposure or the outcome. Studies of EPB should preferably be population based. In centre-based studies, not all preterm babies are born in the centre, and as those born in the centre might be selected from high-risk pregnancies, the occurrence of later disabilities might be higher. Our studies were population based with minor loss to follow-up (minimum

93% follow-up rate), and despite relatively few participants, the risk of selection bias was small. Thus, the natural history of respiratory symptoms and findings in EPB participants of the present study are likely to apply also to other EPB populations in high-income countries.

Survival bias arises when the study population (due to the exposure) has an increased mortality risk, and only survivors are included. This issue is particularly relevant to the present study, as only EPB survivors could be studied. Data for total survival and NICU survival are provided for the EP_{1999} cohort. We have no data on those not admitted to the NICU for the EP_{1982} and EP_{1991} cohorts. Changes in survival rates over time, or survival rates at variance with other centres, could potentially have biased our results. In the EP_{1982} cohort, overall survival was 52 of 84 (62%); in the EP_{1991} cohort, it was 35 of 47 (74%). For the national EP_{1999} cohort, overall survival rate to discharge was 376 of 462 of infants (81%) admitted to NICU. These survival rates are all comparable to other studies[26,36,126,191,229-231].

Recall bias is particularly relevant, and difficult to avoid, as families of EPB more easily might recall important factors occurring around pregnancy and birth, and be more aware of health problems that could be due to preterm birth.

A cohort is a population group defined by a certain characteristic. The EP_{1982} and EP_{1991} cohorts were defined retrospectively, after (preterm) birth. The prime objective was to include all EPB (GA < 29 weeks). Early ultrasound scans were not performed in mothers giving birth in the early 1980s, leading to our inclusion of the BW criterion, to counter the possible uncertainty of establishing delivery dates by LMP alone. The organization of neonatal care, in which all EPB were cared for in one NICU, ensured the inclusion would have been the same had they been defined prospectively[168]. The EP_{1999} cohort was defined prospectively and all stillborn and live births registered in both NICUs were included for study, provided written consent[12].

Ideally, the physician and technician should be blinded to the patient status in studies aiming to assess differences in outcomes between exposed and unexposed individuals. In our studies, the physician and researcher were the same, being responsible for

collecting data, which involved finding patients from medical records and registries, inviting them to the study, fitting them into the lab schedule, examining them, plotting and analysing the data, and discussing the findings – and was thus not blinded. This might have biased the results.

Our frames of a longitudinal observational study design preclude the attainment of solid data that could explain cause and effect in terms of long-term health outcomes. However, the studies might point at potential associations between preterm birth, neonatal treatments and later outcome that could be evaluated in other studies.

Population sample

A control group of subjects was used in order to compare the outcome between those exposed (EPB) and not exposed (TB) to the factor (preterm birth) under study. Our control subjects were matched to individual cases by age and gender. The main purpose of matching was to control for known and unknown confounding variables that might influence the comparison. As matching was used in the design, we used paired statistical analyses. Careful consideration was taken to identify and measure important baseline prognostic variables that could possibly differ between the exposure groups - and these were attempted adjusted for in the final analysis.

Most EPB consented to participate, whereas some TB declined the first invitation. For recruitment of the EP_{1982} and EP_{1991} cohorts, approximately 1.3 TB was invited to recruit a full 1:1 match. For the EP_{1999} cohort, 60 controls were invited in order to obtain 38 participants in Bergen (1.6 controls per EPB). We were unable to recruit a full one-to-one match. There was only one TB excluded for medical reasons (in the EP_{1982} cohort), who later received a lung transplant to treat a severe lung condition. The strict recruitment system and the low exclusion rate in TB might have increased the generalizability of the findings in the control population.

Studies that invite friends, family or classmates of the index subject, or hospital staff or medical students[126,186,232] to participate, are at risk of creating a biased control group, some with a personal interest of being tested, e.g. due to a perceived health problem, factors that may impact on the results and statistical conclusions. Sampling

selection bias may also have been present in this thesis, in that those most fit and well performing, and possibly living in homes with higher-educated parents, better realize the importance of participating in research. Theoretically, this situation could elevate the average group performance and thus bias the results. Studies have indicated that mothers of EPB in general attain a lower level of higher education than mothers of TB babies[11], and children born to higher-educated parents usually fare better in terms of health and physical fitness. We did not attempt to match for socioeconomic status in the control populations, partly because of the rather 'flat' socioeconomic structure in Norway and complexity of recruitment. Assessed in retrospect the educational level did not differ significantly between the mothers of the EPB and TB groups in the EP_{1982} and EP_{1991} cohorts[168], or for the mothers of the EP_{1999} cohort[233], leading us to conclude that the risk of such bias was low.

For the inclusion of participants, we conclude that the presence of sample bias and known presence of confounders were low for the EPB and TB participants, and we therefore consider the results valid. Thus, the data can reliably indicate respiratory outcomes for comparable groups born EP in high-income countries.

Statistical power

Due to the relatively small number of participants, group differences that may have been truly present could have gone undetected due to lack of statistical power (type II errors). Thus, the null hypothesis, that there were no differences in PF between EPB and TB, might have been falsely retained.

The issue of power was discussed when the original study on the EP_{1982} and EP_{1991} cohorts was planned in 2000. Given relevant published distribution data for FEV_1 at the time and our own pilot data collections, we designed a study that could detect what were assumed relevant group differences between EPB and TB. In the tables, the full study results have been broken down to even smaller subgroups, one of which was the group comprising BPD subjects, thereby further limiting statistical power. Instead of reporting p values, we have generally used 95% CIs to address uncertainties regarding estimated mean values for subgroups, except for the interaction terms introduced to assess developmental differences.

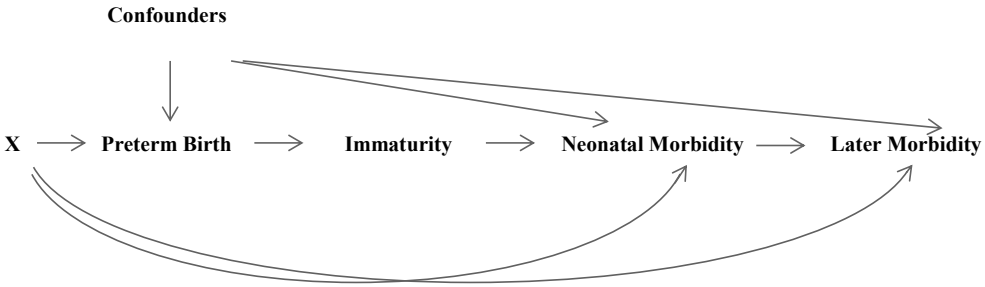
Ideally, more participants should have been included in order to reduce the risks of particularly type II errors. However, a "near significant" p-value does not necessarily become smaller (and hence 'more' significant), as extra data are added[234]. Therefore, we have consciously avoided making any claims of a trend towards statistical significance in the presentation of the results. In addition, p-values around 0.05 - whichever side they lie on - represent only modest degrees of evidence. Studies such as ours should be repeated to gain more evidence-based knowledge.

External validity is the extent of generalizability of the results from a study sample to a larger unstudied but presumably similar population. We conclude on this issue that ideally more participants should have been included, though our results should be valid due to the cited characteristics of the study groups and the design and performance of the studies[235]. The most important findings were the pattern of group differences between those born EP and at term, which we interpret as being reasonably robust for all the three cohorts, whereas subgroup differences should be cautiously interpreted.

Regression variables and assumptions

The foundation of this thesis rests on studies of children born at a low GA. GA at birth is an important predictor of morbidity in the short[12,15] and long term[4], being a proxy index for immaturity but also a measure of the background pathophysiology that leads to preterm labour and birth[24] (Figure 7). GA at birth might be an intermediate variable, occurring in between (because of, or leading to) other possible prenatal and perinatal variables (e.g., inflammation, growth restriction, infection, positive pressure ventilation, PDA). Those born most immature will also be most prone to receive most intensive care treatment[12,26]. Our study inclusion criteria employed restricted ranges for GA and BW for EPB participants, thus reducing – but not eliminating - potential confounding effects of GA on outcomes[24].

Figure 7: Possible associations between preterm birth and later morbidity (modified from Wilcox et al.)[24].



The study design did not allow for further exploration of the antenatal history of the EPB participants, as we had limited access to prenatal data, such as intrauterine growth curves, inflammatory changes in the placenta, or vaginal swabs. An infant born at 24 weeks GA might be exposed to many extra weeks of supplemental O_2 compared to an infant born at 27 weeks GA, simply because of immaturity and poor or insufficient respiratory efforts, not because of more RDS. We found that the number of days with supplemental O_2 correlated negatively with GA (B = -7.07; p = 0.38; R^2 = 0.09) in the EP_{1999} cohort. Thus, the variable days with O_2 could in fact be a simple function of GA and not as much of poor respiratory function. FEV_1 in childhood has been associated with the duration of O_2 therapy in several studies[13,180,236,237], though this issue seems not to be settled in preterm populations[60,144].

Considerations relating to collinearity also affect other parts of this thesis. The study highlights important issues that relate to respiratory abnormalities after EP birth:

(1) What are basic causal mechanisms? And
(2) Are these inevitably linked to living extra-uterine throughout the third trimester - or manageable - given optimal postnatal care?

As for causal mechanisms, we are faced with the statistical challenge of collinearity between possible risk factors and limited options for organizing randomized controlled trials that could distinguish confounding or intermediate variables from factors truly

involved. Complications and negative events in these vulnerable infants tend to aggravate each other mutually. Observational statistical models struggle with this scenario. Examples are neonatal use of corticosteroids or O_2 treatment to overcome problems related to pulmonary immaturity, and the observed links with poor PF in later life[179,238]. It remains debatable whether it is the remedies themselves, or the conditions they are used to treat, that are to be blamed. The present study faces these same challenges, and can only report what remain significant associations from carefully constructed regression models.

6.1.2 Subjects' ability to cooperate with lung function testing

Obtaining valid test results is for most tests dependent upon subject cooperation. Values can be underestimated, but seldom overestimated; only if the patient used drugs or stimulating agents like caffeine, or in specific situations, like the estimation of a high FEF_{25-75} with a falsely low FVC. Another possibility for obtaining underestimated or overestimated values is that the equipment lacks proper calibration.

Obtaining a valid result requires that the test subject is given appropriate procedural explanation and demonstration, verbal encouragement and proper feedback on how to perform maximally and adjust the performance. Testing of children and adolescents is by nature demanding. In our studies, the youngest participants were 10 years old. A major challenge in working with EPB is that neurodevelopmental delay is common. The most immature subjects are most prone to neonatal disease that affects both their brain and lungs, and subjects with neurodevelopmental delays may therefore have more severe lung disease[95]. At the same time, they might be unable to complete PF tests, because of an inability to cooperate, thereby constituting bias in all studies of EPB. The true nature of impaired PF is therefore difficult to estimate. We included all disabled subjects that were able to perform spirometry, thereby increasing the generalizability and external validity of the findings.

The following specific findings for disabilities in the cohorts were:

1) EP_{1982} cohort: Cerebral ultrasound was introduced in our NICU around 1980 and used systematically and routinely from 1984 onward (Prof. T. Markestad, personal

communication). Thus, complete data are unavailable, especially for conditions such as intraventricular haemorrhage and periventricular leukomalacia. In EP_{1982} assessed at 18 years of age, 8 (17%) had major disabilities (defined as disabilities preventing attendance at regular school; blindness, deafness, quadriplegia, and psychiatric disabilities); four (9%) had disabling CP, three (7%) were blind, three (7%) were deaf, and two (4%) had psychiatric disorders; i.e. overlapping disorders in several. Six (13%) subjects had minor disabilities.

2) EP_{1991} cohort: No EPB had major disabilities; seven (20%) had minor disabilities.
3) EP_{1999} cohort: Two (3%) EPB were excluded; one (2%) with severe CP accompanied by tetraplegia and a ventriculo-peritoneal shunt; one (2%) with minor CP. Both subjects were unable to cooperate in reproducing flow-volume curves; a third subject with CP accompanied by deafness was able to perform simple spirometry, which was included; no other PF tests could be completed.

6.1.3 Data preparation

For the EP_{1982} and EP_{1991} cohorts, antenatal and neonatal data were based on hard copies of obstetric and paediatric records, respectively. Medical records were available for all but one EPB in the EP_{1982} cohort, for whom the discharge summary and maternal recall data were used. For the EP_{1999} cohort, data were prospectively collected and entered into data files; however, data on days on O_2 supplementation were collected retrospectively. Medical records were unavailable for two, and for four of 57 EPB, there was no available information on additional days of O_2 supplementation after discharge from the hospital. Three of these patients used O_2 supplementation after discharge during the nights for some months, one was transferred to a hospital in another part of the country.

Raw data for PF tests, clinical examination, anthropometric measures, patient information, blood and urine samples, questionnaires, and other information for each subject were manually entered directly into a data file with assigned variables determined by the operator. To avoid mistakes, all data were entered twice.

We conclude that the acquisition and preparation of data was adequate.

6.1.4 Definition of intrauterine growth restriction

BW is an easily available measure of the wellbeing of the newborn, reflecting both duration of gestation and fetal growth. SGA was defined as BW below the 10th percentile for GA[228]. Being SGA may be due both to genetic factors defining a constitutional small size (normally small; due to maternal height, weight, ethnicity and parity) or to foetal growth restriction (abnormally small), the latter describing a foetus that has not reached its growth potential and is smaller than expected due to environmental factors that cause compromised nutrient supply. SGA infants have higher GA than infants of similar BW and thus are more mature, but if growth retarded, they have higher rates of neonatal morbidity when compared to infants at the same GA with appropriate BW[239,240].

According to the DOHaD concepts, being born SGA might implicate adverse consequences for later respiratory health. Data on intrauterine growth were not available, so we have no data on "true" intrauterine growth restriction. This is a limitation. However, in very preterm infants, as in our study populations, SGA status is likely to represent IUGR[241].

The slightly different inclusion criteria for the cohorts might have had implications, with GA < 29 weeks and BW < 1001 g in the EP_{1982} and EP_{1991} cohorts vs. GA < 28 weeks and BW < 1000 g in the EP_{1999} cohort. The inclusion of infants that were one week earlier in GA at birth but with no changes in the BW criterion leads to a higher proportion of SGA infants in the EP_{1999} cohort. We found that SGA subjects in that cohort had the most impaired PF when assessed at 11 years, and that BW z-score was the only neonatal predictor of z-FEV_1.

6.1.5 BPD definition

BPD is currently defined by the need for supplemental O_2 at certain ages[40]. A standardized assessment of a ´need for O_2 supplement´ has not yet been implemented into most NICU routines. Some studies indicate that BPD incidence would be lower if the diagnosis was set by specific indications, like the room air challenge[242,243], and that the incidence varied with varying definitions (28 days vs. 36 weeks)[26]. Ever-changing definitions are a challenge when comparing studies of long-term effects.

The evolution in NICU care implies that hypoxaemia in room air and thereby inefficient pulmonary gas exchange has been evaluated by different physiologic assessments in different eras. In our centre, transcutaneous O_2 tension (target TcO_2 > 6.5-7 kPa) was used in the early 1980s; O_2 saturation (oximetry) in the 1990s (target SaO_2: 91-93%); later on, arterial tension of O_2 (PaO_2) was used in addition to the previous methods (Prof. T. Markestad, personal communication). BPD has also been defined by radiological findings (often assessed at 28 postnatal days), although it shows a poor correlation with PF[133,157,187,244].

BPD in the present studies was defined as mild if O_2 supplementation was required at 28 postnatal days and moderate/severe (m/s) if still required at 36 weeks GA[40]. However, O_2 is not always given continually to EPB. In the case of ROP treatment by cryotherapy - which is rather frequent in this population - an infant might require O_2 during sedation; however, this was only counted as ´requiring O_2 supplementation´ if it was given for more than 12 hours, according to the definition. The same was applied to children undergoing any other procedure requiring sedation. Some infants had intermittent episodes of O_2 requirement after the O_2 supplement was originally discontinued; these days were counted only if such an episode was due to respiratory symptoms like tachypnoea, dyspnoea or grunting, and consisted of two or more days of supplement. These days were subsequently added to the subject's total number of days with O_2. The diagnosis of BPD was made if O_2 supplementation was administered at the cut-offs of 28 postnatal days or 36 weeks GA, also according to the definition[40].

The BPD definition just considered is simple and useful for studies such as this thesis. However, the presence of BPD contains no additional information on anatomy, mechanics, inflammation, or pathology in the respiratory organs, a fact that is important to consider when interpreting the results[125,135].

6.1.6 Pulmonary function (PF) tests

Laboratory conditions and logistics

The standardized conditions for the test laboratories and the equipment ensured that testing commenced with minimal procedural variation.

Spirometry

For the EP_{1982} and EP_{1991} cohorts, the reported values were selected according to standardized recommendations. Obtaining reproducible spirometry curves according to ERS and ATS recommendations[202,205] might be difficult to achieve in children[245]. A learning effect in performance of spirometry is present, as children with asthma (tested earlier) perform better, with more reproducible parameters[246]. For the EP_{1999} cohort, spirometry values from the second day were chosen. For those four who only had one test (declined further participation after the first examination), this first test was used.

FEV_1 and FVC are relatively robust indices, with within-subject reproducibility (i.e., CV) in the range 1-11%, and reasonable reproducibility of measurements made on different days in adults[79]. The forced expiratory flows (e.g. FEF_{25-75}) have a larger CV[247]. FEF_{25-75} might be artefactual high unless the technician is alert and pushes the child to avoid finishing the exhalation manoeuvre too early[205].

An important note is that spirometry represents function, not structure, specifically not of the distal airways ('silent' parts of the lungs), and is only indirectly related to respiratory mechanics or lung size. This is particularly relevant to our studies. Potentially damaged terminal airway structures are impossible to assess by spirometry alone. Due to EP birth, these structures, meant to develop (partly) intrauterine, have developed within the frames of the NICU, and might as such be abnormal. The mechanisms behind the observed airway obstruction are poorly understood. Questions such as whether developmental insults to the peripheral airways also affect the more proximal conducting airways[248], or whether premature birth and/or hyperoxic insults resulting from NICU care somehow alter the responses of the airways to later environmental influences, remain unanswered. Drawing firm conclusions on lung growth and development by spirometry only is therefore questionable.

Lung volumes

Measuring absolute lung volumes is technically challenging. Different techniques are used: body plethysmography, nitrogen washout, helium dilution method, or varying radiographic imaging methods[206]. Plethysmography is most widely used, with the determination of FRC as the key component[206]. Assuming that the intrathoracic gas volume is equal to FRC at airflow occlusion is only true if the tested subject has airways that communicate with the airway opening (i.e., the mouth). The gas dilution methods may underestimate volumes in subjects with obstructive airways (seemingly present in most EPB), as segments of the lungs that are collapsed or filled with secretions do not contribute to gas exchange and dilution (trapped gas). The plethysmograph may overestimate lung volumes, as it includes also non-ventilated lung compartments, and results may be elevated by gas in the mouth or the abdomen.

In a study conducted by our group on the EP_{1982} and EP_{1991} cohorts, the 1.96 standard deviations (SDs) for the differences between repeated measurements of FRC, TLC, and RV were 20%, 8%, and 40% of the mean values of the two measurements, respectively, obtained by using the panting technique. That is, for a difference to be considered unrelated to procedural variations, a relatively large difference should be observed[249]. Achieving reliable and reproducible results is demanding on the operator and the patient. Thus, fewer participants were able to produce satisfactory plethysmograph results compared to spirometry testing.

Plethysmograph specific airways resistance (R_{aw}) is defined as pressure loss in the respiratory system per unit of flow, and is widely used to determine airways resistance, although no formal standardization of test conditions and performance exist[250]. The measurement of R_{aw} at rest may allow assessment of obstruction in patients who are unable to perform satisfactory spirometry manoeuvres. A narrow airway has larger resistance than a wide airway. We opted to present R_{aw}, and the increased resistance somehow confirmed the airway obstruction by spirometry in our studies.

Volume measurements and effects of changing equipment over time

Patients were tested in the same laboratory during 2001 to 2012. We used the same type of equipment from the same manufacturer (SensorMedics®) for all assessments,

although the software was upgraded at HUS and SUS (2005) and the external cabinet changed (HUS). Analysing the raw-data using the inbuilt software algorithm provided by the manufacturer at the times the examinations were performed versus using the software algorithm that was in use after 2005 on all data, revealed systematic and significant differences. Thus, we opted to re-analyse and report all data using the software from 2005 (Appendix V, Tables V1 and V2). These findings were reported back to the manufacturer, so far with no response.

Lung diffusing capacity DL_{CO}

DL_{CO} represents the total uptake of CO (mmol) by the lung per unit of time (min) and driving pressure (kPa). The diffusion of CO over the alveolar membrane is complex, and is influenced by a range of processes. The test conditions need to be standardized to BTPS, body position, exercise and Hb affinity for CO, which is influenced by alveolar O_2 partial pressure, and carboxyhaemoglobin as in smoking[203,207].

Intersession CVs of 10-15% have been reported[251]. In our populations, CVs < 10% were found for all subgroups and a reproducibility of measurements in the same range in EPB and TB[212].

In Paper #II, for the EP_{1982} cohort at 25 years of age, small differences in unadjusted DL_{CO} disappeared with correction for volumes and Hb (g/dl), weakening the idea that DL_{CO} in adults was influenced by EP birth. This might be due to weaknesses pertaining to the method per se, or due to catch-up growth in acinar structures, although the studies were not designed to explore such theories. However, correcting for these factors also implies the introduction of new variables with their own distributions and varying repeatability. We therefore opted to report DL_{CO} both as raw data, normalized for volume and corrected for Hb.

One may speculate whether the higher blood Hb observed in 25 year-old EPB adults somehow may reflect an adjustment or a compensation for a life-long history with lower than average lung diffusion capacity. The study design did not allow further penetration of this interesting issue. Clinically, differences were minor. These may

represent a random finding. Few studies have reported Hb values in EPB[156], and further studies are needed.

Nitric oxide (NO)

In the human respiratory system, NO is synthesized in small amounts in normal physiological conditions (constitutive synthases), and in higher amounts in inflammatory processes (inducible synthases). Expired NO concentration (FE_{NO}) is considered a biomarker of certain pulmonary inflammatory conditions, used in both diagnostics and follow-up of inflammatory lung diseases, such as asthma. As it is a non-invasive method, it is useful in both adults and children. In asthma patients, $FE_{NO\ sa}$ has correlated with sputum eosinophils[252]. Extended NO analysis is a promising tool in monitoring diseases in which NO metabolism might be altered[215].

As direct measurement of NO in the peripheral airways is not possible, mathematical models have been constructed to differentiate between the various sites of NO production[216]. When exhaled NO is measured at multiple flow rates, airway NO flux ($J_{aw}NO$) and alveolar NO (C_ANO) can be calculated. The slope and intercept value of the linear regression line between NO output and exhalation flow rate correspond to C_ANO and $J_{aw}NO$, respectively[215]. Alveolar NO output has been measured in healthy children, and reference values have been collected and published[253].

In studies of children, two-compartment NO parameters are correlated with FEV_1 and FVC; height is found to be the strongest predictor of the variation[253]. The test requires subjects to maintain a constant expiratory flow of 300 ml/sec for a certain time, and e.g. 4 seconds corresponds to 1.2 litres of expired air[214]. As this is not far from the predicted FVC in some 11-year-olds, this was challenging, and four EPB and five TB failed.

We assessed of alveolar NO in order to determine if reduced PF in EPB could be related to peripheral inflammatory processes in the acinar structures. The question is relevant, as these subjects were born before the completion of these structures. We could not find that any of the parameters for exhaled NO in the EPB group differed

from those of the TB group, supporting previous studies that conclude that eosinophilic airway inflammation is not a typical feature of EPB children.

Bronchial hyperesponsiveness (BHR)

BHR refers to the process of abnormal airway narrowing in response to specific (allergens) or non-specific stimuli (e.g. histamine, methacholine, cold air, exercise)[79]. These stimuli induce smooth muscle contraction, probably both by direct agonistic properties (e.g. methacholine is a muscarinic [M3] agonist) and by indirect stimulation of non-muscular components of the airway wall, which somehow affect the extent of smooth muscle contraction[254].

BHR correlates with low pre-test FEV_1 in adults[220,255,256]. BHR is a typical characteristic of asthma, but there is significant overlap between the extent of BHR in asthmatic and non-asthmatic subjects, and it is challenging to set the cut-off between a normal and abnormal response[79,255]. The test has a high negative predictive value in relation to asthma. Positive results are more difficult to interpret[257].

The extent of BHR can be expressed as the provocative methacholine dose that induces a given percentage decrease in FEV_1, often 20% (PD20). By doing so, a provocative dose can only be estimated if the decrease in FEV_1 is greater than 20% within the dose-frame prescribed by the protocol, as it will otherwise be censored at the maximum dose allowed. This is a limitation to epidemiological studies. The majority of healthy subjects never cross the critical threshold level, and their degree of response cannot be quantified. Recent work suggests 7.8-8 µmol methacholine as cut-off for identifying subjects with moderate BHR[204,219,258].

BHR can also be given as a continuous non-censored indicator, such as the slope of the curve that describes the percentage decrease in FEV_1 per given dose of methacholine (dose-response slope, DRS)[218]. A slope is not censored, and all participating subjects will thereby contribute data to statistical comparisons[259]. The cut-off threshold of DRS has been suggested at 2.39%/µmol, corresponding to more than 20% decline in FEV_1 after a cumulative dose of less than 8.37 µmol[219]. BHR is a non-specific term to

designate any deviations in the DRS, which is related to sensitivity (the position of onset), reactivity (the slope), or the maximal response (the plateau) in the slope[79].

We calculated DRS as suggested by O'Connnor et al.[218]. In Paper #II we used DRS ≥ 2.39%/µmol as a cut-off for a positive response. DRS is a skewed variable that was log-transformed to achieve a normally distributed set of values. Results were reported as geometric means, prompting mathematical considerations (Figure 8).

Figure 8: Mathematical considerations regarding differences in log-transformed parameters.

Mean log-transformed values are *x1* and *x2*
Mean geometric values are *g1* and *g2*

$x1 = \log g1$ and $x2 = \log g2$ and $g1 = 10^{x1}$ and $g2 = 10^{x2}$
$x1 - x2 = \log g1 - g2 = \log (g1/g2)$ and $g1 - g2 = 10^{x1} - 10^{x2}$

$g1/g2 = 10^{(x1-x2)}$ = antilog of the difference $x1-x2$, which is not equal to the difference between the geometric means, but the ratio between them, which is always positive.

Ratio < 1 means a negative difference (*x1* is lower than *x2*)
Ratio > 1 means a positive difference (*x1* is higher than *x2*)

We found higher BHR to methacholine in EPB compared to TB at all assessments in all cohorts. The mechanisms are unknown. BHR might be due to altered airways because of ongoing inflammatory processes, structural defects, changes in the receptors for methacholine or other altered systemic immunologic responses induced by preterm birth and subsequent early life inflammatory insults [260]. Such changes may hamper proper layout of collagen and elastic fibres in the lung parenchyma, with adverse consequences for acinar structure and function, peri-bronchial airway support and elastic recoil pressures. We aim to explore this further.

Airway reversibility to salbutamol

Bronchodilators act on β2 receptors in the airways, mediating smooth muscle relaxation and bronchial dilation, facilitating airflow. The bronchial dilation is measured by spirometry as increased FEV_1. Bronchodilator drugs are less likely to be effective in subjects with a presumed normal smooth muscle tone, like in subjects with 'normal' FEV_1. The responses tend to be higher in those with a lower baseline FEV_1[83].

Tests for BHR, EIB, and reversibility to salbutamol should be performed on different days, as they might interfere with one another. The present study involved all these tests, and it was discussed whether to schedule participants for two or three examination days in order to obtain a 'clean' test of salbutamol reversibility. With the EP_{1982} and EP_{1991} cohorts, we piloted the three-day approach for a short period. However, in the spirit of not letting 'the perfect becomes the enemy of the good', we decided to proceed with a two-day-schedule, testing salbutamol response after the exercise test. Testing over three days carried a risk of subjects loosing motivation. The EP_{1999} participants were not EIB-tested, and lung function was assessed over two days; applying the BHR test and the salbutamol reversibility test on separate days.

Assessment of salbutamol reversibility subsequent to the exercise test carries a risk of bias in the direction of underestimating a positive response to the β2 agonist in subjects with EIB. In the event of a reduced FEV_1 after exercise, this might mask an increase in the same parameter following the administration of the β_2 agonist. We used the 'bronchial lability index' as an outcome parameter from the present study. This index represents the sum of changes in FEV_1 after exercise and salbutamol. Therefore, and given the test set-up, we may have obtained a lower bronchial lability index for those who in fact did have a bronchial lability. Only one subject did have a significant EIB response. Moreover, the controlled design of the study to some extent 'protected' the study from missing potential group differences, as all groups and subgroups were exposed to the same schedule of testing. Finally, the authors of the reference that was cited for this bronchial lability index in fact used a test set-up similar to ours, and provided their test subjects with a β_2 agonist after the end of a free running test[223].

The ERS and ATS guidelines describe the assessment of reversibility to salbutamol, and they do not suggest that it is performed in conjunction with an EIB test.[83,205]. As the test set-up was similar in all participating groups and subgroups, and as the responses to exercise as well as to salbutamol were similar in the EPB and TB groups, we argue that these issues did probably not influence the group differences.

6.1.7 Reference equations

In principle, reference equations compare the patient's test results to those of a healthy person of the same ethnicity, gender, body height, and age[83]. For PF tests, reference equations are generally available for preschool children *or* children up to 18-19 years of age *or* adults older than 18 years of age. Only very few of these references cover the complete age span from 10 to 25 years[261].

The raw data obtained from the test situation may be expressed as the percentages of the values predicted or as the number of SDs that the raw data deviate from the expected mean values given by the reference equation (z-scores). A workshop at the ERS 2012 meeting discussed how to report PF-data (personal reference notes). The conclusion from the workshop was that both percentages of predicted and z-scores could be acceptable. Using percentages of predicted is scientifically strictly not correct unless the data scatter is proportional to the predicted value, which is often not what is observed[79]. Z-scores are calculated as the difference between the observed value and the mean expected value for the population, divided by the standard deviation of the reference population. By design, z-scores are normally distributed, with mean = 0 and SD = 1. In the present study, PF data were mainly presented as z-scores if proper reference equations were available, with the LLN defined by the fifth percentile of the distribution, corresponding to a z-score of -1.64[83,262]. Otherwise, data were presented as percentages of predicted or ratios or simply as raw data, as appropriate.

The present study applied a paired design, with one individually matched term-born subject included as comparison for each preterm born participant. In order to use the test results of these controls as the expected mean in our studies, instead of published reference equations, the control groups should ideally have consisted of at least 150 subjects of each gender[235]. However, the control groups of our study contributed to safer evaluation of the data obtained from the EPB groups than would have been possible had the assessments relied solely on published reference data[156,186,209].

Spirometry

For spirometry variables we used the all-age reference equations published by the GLI in 2008 (Paper #I) and 2012 (Papers #II and #III) [208,209]. One set of equations covered

the entire age span of the subjects, making it easy for others to compare their results with those of our study (www.lungfunction.org). The question of what change in z-score over the age span studied we consider relevant or important in a broader context is difficult to answer. The GLI provides LLNs, although we are uncertain as to what functional consequences these limits might have in early adulthood. Aerobic capacity is largely unrelated to FEV_1 at this age[156,221], unless FEV_1 is severely reduced or aerobic capacity is extraordinary high. The answer must involve our reflections about the process of age-related decline in FEV_1 and prospects of early onset COPD in later life[94]. In 2007, Baraldi and Fillipone published a review speculating that relatively modest deficits at a younger age may have clinical and functional consequences at an older age. They also stated: 'It is not known whether the decline in respiratory function in adults, who had bronchopulmonary dysplasia as children will be normal, or whether it will be early or accelerated'[95]. Rather small differences could potentially be of interest in a lifetime perspective[93], particularly for those with BPD, as they have the most pronounced PF deficits.

Interestingly, z-FEV_1 in TB seemed to be numerically lower for each successive decade (Figure 5, Table 4), although differences were non-significant. Thus, we conclude that this most likely represent random findings in small samples[235].

We studied the effect of gender in Paper #II, revealing no effects in EPB, neither on change in PF from 18 to 25 years nor in PF at 25 years of age. TB males on the other hand, appeared to decline more for some flow-volume parameters than females.

Lung volumes

Lung volumes relate to body size; standing height is the most important correlative variable. During puberty, lung growth not always synchronizes with somatic growth, such that lung growth lags behind the increase in height during the pubertal growth spurt, followed by a shift during adolescence[83,85,263,264].

We used the equation provided by Stocks et al. (Dr. Stocks and Dr. Quanjer, personal communication) for lung volume calculation in Paper #II [210]. Our youngest participant was 15.5 years, although he had a height of 155.6 cm, within the height range used for

the equations (155-195 cm). The equation had limitations in fitting our population, as it assumes a plateau phase during this age span. In our study, volume raw data increased from 18 to 25 years in all subgroups of patients.

In the EP_{1999} cohort, the equation from Rosenthal et al. for Caucasian children 4-19 years, was chosen[211]. This covered the age span, and enabled us to compare the volumes of our cohort with relevant cohorts from other Caucasian populations, e.g., the EPICure study[149]. Two non-Caucasians were analysed using this same equation.

DL_{CO}

It is problematic to find appropriate reference equations for diffusion capacity. As it seems, this is due to inter-laboratory differences that are larger than for spirometry; probably related to numerous factors that might influence the measurements, such as the method used to calculate DL_{CO} and inaccuracies introduced by adjusting DL_{CO} values to alveolar volume, Hb concentration, carboxyhaemoglobin concentration and altitude[203]. We chose to report diffusion capacity as raw data, as the prediction equations[211] used in comparable populations did not seem to fit the control population particularly well[149].

6.1.8 CLD versus asthma

Asthma is defined (modified) by The Global Initiative for Asthma (GINA)[265] as:

(a) A history of variable respiratory symptoms

- Wheezing, shortness of breath, chest tightness, cough

(b) A history of variable expiratory airflow limitation

- Low FEV_1 and preferably also low FEV_1/FVC

In a recently published international consensus statement on paediatric asthma, the diagnosis rests on recurrent episodes of wheezing triggered by various stimulants such as viral infections, irritants and exercise; symptoms include cough, wheezing, and tightness of the chest[266]. Asthma is associated with inflammatory changes in the airway mucosa, suggested to cause symptoms and PF abnormalities.

Studies of EPB show that airway obstruction[124,179,192], BHR, and reversibility to β2 agonists[126,149,156,237]—also findings of this thesis—are characteristic features, like in

patients with asthma. Wheezing in early childhood is a trait found in most EPB children[267], but the aetiology is unknown.

The term ‘asthma' is frequently used as a label for respiratory symptoms after EP birth, although these conditions most likely represent different disease entities[147,268]. There is growing evidence that lung disease subsequent to preterm birth and “classical childhood asthma” represent different disease entities[268]. The eosinophilic inflammatory pattern characteristic of “classical asthma” has not been demonstrated in lung disease after EP birth[148,149]; also not in this thesis. Asthma treatment is often prescribed to children with lung disease after preterm birth, although the evidence for effect is weak. To our knowledge, no studies have shown an effect on basic PF after administering corticosteroids to EPB with airway obstruction[269,270], although it might decrease bronchial lability.

There are disagreements in the literature as to what extent lung disease after preterm birth is in fact a ‘dead’ structural sequela or an active disease. Recent data suggest the latter, based on findings compatible with airway oxidative stress[150-152]. Obviously, ‘classical asthma’ may develop also in EPB, complicating research and statistical models. Our group has published data relevant for this discussion[147].

Although by nature CLD and asthma seem to differ, the two conditions tend to exhibit symptoms that are difficult to distinguish and tend to be similarly described by patients[95,147]. Information on respiratory diagnoses and symptoms in the study populations were therefore assessed with the ISAAC questionnaire, as this is considered a rigorously validated instrument[200]. The ISAAC questionnaire obtains data on several symptoms found in asthmatic subjects, although no single variable distinguishes ‘true current asthma’. We therefore combined variables in order to increase the validity of such a diagnosis. As there is no consensus, we selected combinations of answers. The varying definitions, although not completely different, reflect to some degree the uncertainty of the asthma diagnosis, and are a weakness.

6.1.9 Verification of smoking

We measured urinary cotinine, a major nicotine metabolite and a biomarker for nicotine use, at 18 years of age to provide validation of the uncertain nature of self-reported smoking data. Cotinine is detectable in the urine at least 36 hours after smoking exposure[271]. The self-reported responses were reasonably similar to the cotinine data, with three positive tests for detectable cotinine levels in 57 self-reported non-smokers. We assumed the data from age 18 years had some bearing on the character of the responses also at 25 years; and included the data as background in Paper #2.

6.2 Discussion of the main results of the study

6.2.1 Longitudinal course of PF after preterm birth (Research area 1)

The 1982-1985 and 1991-1992 cohorts

The study indicates that pulmonary function is persistently reduced from mid-childhood to early adulthood after EP birth, and that the rate of growth and development during this period does not differ from that of TB peers. Our findings contradict what have been reported from some studies[186] but are consistent with others[155]. The observed impairments might represent potential precursors for later COPD, at least in EPB subgroups[93]. These dreary but still unresolved prospects have received growing attention. So far, neither we nor other research groups have been in position to unravel this challenging issue, simply as large groups of EPB survivors have not yet reached late adulthood. In this context, it seems relevant to bring up the relatively recent dispute regarding the 'London cohort', one of few preterm groups that have been followed longitudinally beyond 20 years of age. It was argued in a letter to the American Journal of Respiratory and Critical Care Medicine that a PF decline was evident in the data set from mid-childhood to adulthood, whereas the authors (A. Bush, M. Rosenthal and I. Narang) argued the opposite, though acknowledged that the lack of a longitudinal control group precluded giving definitive answers[186,188].

The normal human ageing process implies a gradual decline of lung function from the age of 25-30 years. This situation has led to various hypotheses regarding future pulmonary health prospects of EPB individuals, as most of them seem to fail to reach their optimal PF levels in early adulthood. Concerns remain whether EPB individuals will commence on this decline at an earlier stage and if the decline during adulthood will follow the same or steeper trajectories than in TB individuals [272]. Concerns also remain to what extent external factors (like smoking) will add impact to this risk scenario, and lead to an earlier onset and/or a steeper decline in EPB compared to TB individuals[95]. Studies of unselected background populations have shown tracking of obstructive airway diseases from relatively early in life and into adult life[91,93]. Age-related decline in PF varies between sub-groups [96,273] and the process may be accelerated by cigarette smoking[93], childhood pneumonias[274], asthma[275], and

BHR[276,277]. These traits are highly relevant to EPB individuals[175,278] and may contribute to an increased risk of COPD[97,131,190,279].

Our study design has limitations. Data from two assessments on two cohorts were used to draw trajectories from mid-childhood to early adulthood. Each participant was examined twice at most, and none was studied throughout the complete period from 10-25 years of age. Neonatal lung function data was not available.

Interestingly, there was a downward trend for z-scores (and raw data) for FEV_1/FVC and $FEF_{25–75}$ from 18 to 25 years of age, which affected all subgroups. This suggests that age-related development of small airway obstruction possibly had commenced at that stage, and hence that the PF plateau might have ended by this age[95] (Table 9).

Apart from the effects imposed by BPD, the only perinatal variable with significant long-term influence on FEV_1 in the EPB group was postnatal treatment with corticosteroids (EP_{1991}), whereas maternal smoking contributed negatively in TB (EP_{1982}). The remaining assessed neonatal and background variables had no detectable effect on the longitudinal course of PF.

We found that spirometry data obtained at first examination strongly predicted spirometry data obtained at the next. Data from the two measurements were acquired in the same subjects and are by nature related. Narang et al. reported only weak associations in their studies, following children from mid-childhood to the age of 21 using a reference equation validated from 4-19 years[186], findings that provoked vigorous debate. In view of the severe airway obstruction that has been reported in subgroups of EPB children and adolescents, the issue of longitudinal development may have an important long-term bearing and appears to be too little explored.

Table 9: Maximal flows and volumes (absolute mean values with 95% CIs) in two cohorts of EPB and TB subjects.

		EP_{1991} cohort		**EP_{1982} cohort**	
Age, years (SD)		**10.5 (0.4)**	**17.8 (0.4)**	**17.7 (1.2)**	**24.9 (1.2)**
FEV_1, litre	Control	2.14 (2.05, 2.24)	3.65 (3.40, 3.89)	4.07 (3.81, 4.31)	4.10 (3.85, 4.35)
	All EP	1.80* (1.68, 1.92)	3.30** (3.10, 3.49)	3.30* (3.02, 3.58)	3.50* (3.22, 3.78)
	EP non BPD	1.82 (1.66, 1.99)	3.31 (3.08, 3.54)	3.39 (3.04, 3.75)	3.59 (3.24, 3.93)
	EP BPD	1.68 (1.47, 1.89)	3.27 (2.87, 3.67)	3.08 (2.66, 3.50)	3.24 (2.78, 3.69)
FVC, litre	Control	2.44 (2.35, 2.54)	4.22 (3.95, 4.50)	4.59 (4.30, 4.88)	4.92 (4.61, 5.24)
	All EP	2.15* (2.00, 2.29)	4.09 (3,80, 4,38)	3.93** (3.58, 4.30)	4.47 (4.10, 4.84)
	EP non BPD	2.10 (1.89, 2.30)	3.87 (3,53, 4,22)	4.04 (3.58, 4.51)	4.57 (4.11, 5.03)
	EP BPD	2.17 (1.92, 2.41	4.43 (3.91, 4.94)	3.74 (3.20, 4.28)	4.18 (3.58, 4.78)
FEF_{25-75}, litre/s	Control	2.51 (2.26, 2.77)	4.01 (3.60, 4.42)	4.78 (4.37, 5.18)	4.31 (3.97,4.64)
	All EP	1.86* (1.65, 2.07)	3.30** (2.96, 3.64)	3.41* (3.09, 3.73)	3.18* (2.87, 3.49)
	EP non BPD	2.08 (1.83,2.33)	3.63 (3.27, 3.98)	3.53 (3.13, 3.91)	3.23 (2.87, 3.58)
	EP BPD	1.52 (1.19, 1.84)	2.78 (2.15, 3.42)	3.09 (2.51, 3.67)	3.04 (2.31, 3.77)
FEV_1/FVC (%)	Control	87.9 (86.0, 89.7)	86.6 (83.7, 89.6)	88.9 (86.9, 90.9)	83.7 (81.7, 85.8)
	All EP	84.3** (81.3, 87.2)	81.6** (78.0, 85.3)	84.9* (82.6, 87.3)	79.0* (76.7, 81.3)
	EP non BPD	87.6 (83.8, 91.3)	86.3 (82.7, 89.9)	85.1 (82.8, 87.5)	79.2 (77.0, 81.4)
	EP BPD	77.7 (73.2, 82.1)	74.3 (68.4, 80.3)	83.3 (76.6, 90.0)	78.4 (71.1, 85.6)

* $p<0.005$, ** $p<0.05$, both for differences between EPB and TB subjects.

The study contained data from two regional birth-cohorts, overlapping at the age of 18 years, and therefore allowed for assessment of cohort effects; i.e. assessments of 18-year-old adolescents born in the early 1980s and the early 1990s. The results are given in section 5.2.2 of this thesis. Spirometry parameters were similar, except that those born EP in 1982-85 with a neonatal history of m/s BPD had lower z-FVC and a higher z-FEV1/FVC. Standard NICU care improved dramatically from 1980 to 1990[29], and EPB were exposed to different treatment techniques and policies. There was a trend towards decreased mortality, particularly for the smallest infants[26], a trend modestly reflected in our study, although not statistically significant. Mortality was addressed only for live-born EPB infants admitted to the NICU, a possible bias in this context. Improved access to better care and consequently different attitudes towards the limit of viability in the two inclusion periods might have influenced the data. A shift in the neonatal clinical course may have occurred, with less traumatic survival for infants who would have been exposed to tougher interventions in the 1980s, paralleled by recruitment of more immature survivors in the 1990s that would have died in earlier eras. Changes in outcomes may be difficult to predict due to such cohort effects[95], and direct comparisons of EPB from these periods should be cautiously interpreted. One may speculate that the observed difference in FVC may represent changes that reflect a more restrictive PF pattern of the 'old BPD' of the 1980s due to stronger neonatal trauma during that era[71].

Having said this; similar findings with parallel PF trajectories for all subgroups in both inclusion periods strengthens the overall notion that PF tracks through childhood and puberty also for EPB groups, seemingly irrespective of the 'level of the path'.

6.2.2 Adult respiratory outcomes of EP birth (Research area 2)

The 1982-1985 cohort

Adult EPB participants of this study had relatively mild airway obstruction and increased levels of BHR, results that are compatible with most other relevant studies[133,141,155,157,186,187]. Nevertheless, the issue of adult respiratory outcomes in this group has not been settled[188], and may further be subject to changes over time due to

changes in perinatal and neonatal practices and treatment schemes. A recent review in the journal *Chest* stated: 'It is uncertain if lung function impairment extends into adulthood or whether "catch up" lung growth occurs'[280]. Studies of adult lung health in EPB adults are scarce, and tend to have few participants, control groups of varying quality, and/or designs that are not population based[141,186,187]; factors that all may contribute to biased conclusions.

Data obtained at 25 years is important, as most PF variables are likely to peak approximately at this stage, and thus be predictive of what may occur in the future[90,93]. It is of interest that most of the PF variables did not change much between 18 and 25 years of age, and that lung growth and development thus appeared to be essentially completed by the age of 18. We also acknowledge that the z-scores for some spirometry variables tended to increase during the period, in fact for some variables more in the EPB than in the TB group (Paper #II). This might imply that some lung growth continued in EPB, and some degree of 'catch-up growth' cannot be excluded.

Contrary to the 'London group', we found BHR in a large proportion of EPB adults, whereas EIB and reversibility to salbutamol was modest. However, when the latter variables were combined into the concept of bronchial lability, there were minor but significant group differences. As pointed out in epidemiological studies[255,256], there is an association between FEV_1 and BHR in the general population, a feature we reproduced in our study. Ulrik et al. suggested adjustment for pre-challenge FEV_1 when performing histamine challenge tests, a strategy not generally adopted[204]. Increased BHR is independently associated with a more rapid decline in FEV_1 with ageing, and is a predictor of later asthma and COPD[273,276,281,282]. Recently, increased BHR predicted new-onset asthma and COPD in a large unselected population covering the age span of 20-44 years of age, findings highly relevant also to our populations[259].

While there is relatively solid evidence for persistent airway obstruction in EPB children and adolescents, the question if and to what extent this obstruction is permanent or a reversible phenomenon related to bronchospasm, remains unanswered. Airway obstruction may be caused by airway injuries that lead to scarring and impaired airway growth and development, or by increased airway smooth muscle tone

due to some active process. Conceivably, the latter scenario could clinically be easier to treat[269,270,283]. In the present study, BHR and bronchial lability were negatively and similarly associated with baseline FEV_1 in the EPB and the TB groups, reproducing reports from population studies[255,256,284]. After salbutamol administration, the number of subjects with z-FEV_1 below LLN was similar in both groups, and the mean value approached zero in the EPB group. Thus, the 'signals' on this issue were equivocal, and we were unable to estimate what fractions of airway obstruction were attributable to airway reactivity and bronchospasm versus irreversible changes. We conclude that the data support the assumption that the fixed and irreversible part of airway obstruction was mild in most EPB adults.

6.2.3 Change of respiratory outcomes over time (Research area 3)

The 1991-92 versus the 1999-2000 cohorts

Some authors have expressed optimism regarding long-term lung health in subjects born EP during the recent years; i.e. in the so-called post surfactant era of NICU care of the late 1990s and thereafter[196,237]. However, two large studies showed that airway obstruction persists, particularly after BPD, with both studies revealing lower z-FEV_1 and z-FEF_{25-75} in EPB compared to TB[126,179]. The EPICure study examined 10 year-olds born EP in 1995 (GA < 26 weeks)[126], and the Victorian Infant Collaborative Study Group examined 8 year-olds born EP in 1997 (mean GA = 26.5 weeks)[179]. Data from the latter study were also compared to those of children born similarly preterm in the same region in 1991-1992 (mean GA = 26.7 weeks). Although survival rates were higher in the most recent birth-cohort, data were relatively similar, in fact showing some improvement in z-FEV_1 and z-FVC in EPB without BPD, but with reduced z-FEV_1/FVC. In light of the possibility that the most recent birth-cohort potentially recruited more children at risk of unfavourable outcomes, this might be interpreted as positive development. This situation highlights an important challenge when dealing with time-related cohort effects: Better treatments potentially benefit all NICU graduates, but increased survival of more immature and vulnerable individuals may adversely affect outcomes[95,125].

In accordance with the Australian study, our data revealed better respiratory outcomes for the most recent EP_{1999} cohort compared to the EP_{1991} cohort, despite slightly more extreme inclusion criteria and marginally better survival rates in the EP_{1999} cohort. Adjustment for differences in administration of antenatal corticosteroids and surfactant, removed the advantage of the EP_{1999} cohort regarding z-FEV_1; a finding that particularly applied to those with BPD. Better neonatal treatment might as such be involved in a gradual process of improving the respiratory outcomes of these children.

However, associations do not necessarily reflect causation. By design, there are important limitations to observational studies, including ours, as factors included in the regression models might have been outnumbered by influences (known and unknown) not accounted for that could have modulated outcome. Although a matched design makes the study more robust, the study was vulnerable to potential population differences, environmental changes, or other possible external alterations that may have occurred over the study period. Examples are lack of data on maternal smoking in the TB groups, precluding analyses of effects from this potentially important factor. Potential bias introduced by the two-centre design was mitigated by using paired statistical analyses according to the matched design.

BPD was <u>not</u> a negative predictor of future FEV_1 in the EP_{1999} cohort, consistent with some[237] and contradicting other and particularly older studies[126,179,285]. The same was true for duration of oxygen supplementation, with findings contradicting some studies[13,237] and consistent with others[126,179]. Thus, the study challenged the notion that neonatal BPD and prolonged O_2 requirement are inevitably linked to poor long-term respiratory outcome. Particularly BHR, but also low z-FEV_1, were linked to the group that was included based on BW < 1000 gram irrespective of GA; i.e. a group solely consisting of infants born SGA. Thus, the data points to other potential explanatory factors, such as developmental issues potentially of intrauterine origin[24]. The question if low BW z-scores worked differently over different GA categories was tested in interaction models, and no effect could be revealed (p=0.299). As few EPB had low BW z-scores in the lower GA categories, such associations would have been difficult to substantiate.

Low BW for gestation and IUGR have been associated with poor health outcomes in the general population[286] and with increased mortality as well as morbidity and poor future health in preterm born populations [287,288] . With better treatment, such factors may be more apparent than earlier, when prenatal factors might have been masked by postnatal factors; that is, consequences from harsh but life-saving interventions administered to immature infants in the neonatal period might have overshadowed "weaker signals" from intrauterine influences. The lack of associations between O_2 supplementation and BPD on future PF might indicate that contemporary NICU medicine is capable of preserving health and development of the immature lung through the necessary life-saving interventions without inflicting as severe injuries as in the earlier eras of NICU care. Alternatively, these findings might merely reflect the inaccuracy of the BPD diagnosis[40,125,135,242].

We have advocated caution when interpreting comparisons of outcome data from EPB treated under different eras of NICU care. The same cautions should of course be applied to the associations and findings in Paper #III. Nevertheless, the data-set can be seen as indications of a possible positive future development, characterized by recruitment of ever more healthy graduates from our NICUs. In this context, it seems pertinent to put some relevant factors on the agenda. Firstly, the great improvements in survival reported for EPB neonates in the 1980s and early 1990s have not occurred to the same extent in later eras[15]; specifically, this development seems to be less evident in the most recent data sets. As it seems, approximately 23 weeks GA may represent an absolute biological lower limit of viability, given the treatment principles currently available. Thus, previous development characterized by recruitment of ever more immature infants may gradually subside and eventually come to a halt. Secondly, the great advances of NICU medicine that happened during the 1980s and early 1990s, such as the introduction of surfactant treatment[289], has not occurred to the same extent after. Relatively small changes characterized the 1990s more than single big leaps of development. Most areas of NICU medicine went through continuous refinements, such as better standardization of antenatal and perinatal care, a higher level of competence among neonatologists and nurses regarding the special needs of these infants, and a better exploitation of technological advances, such as patient

coordinated assisted ventilation and various forms of oscillation. Thirdly, these continuous refinements on a multitude of levels, combined with constant small shifts of attitudes towards limits of viability, will require systematic studies to evaluate outcomes and thereby provide the necessary feedback to improve treatment schemes.

A recent report from the US Food and Drug Administration focused particularly on scientific progress in the medicinal therapeutic areas[290]. Future research in this area needs to deal with a multitude of minor changes of perinatal and neonatal management, each with its own impact on survival, and all with potential for a variety of effects that may support or oppose better overall outcomes. Large-scale multicentre or even multi-national treatment protocols with strong and widespread adherence and with integrated and coordinated procedures for long-term follow-up are imperative, or else effects will pass undetected in statistical modelling, due to factors such as power issues or complex co-linearity among variables of potential explanatory nature.

The study covers the decade from the early to the late 1990s, during which a nearly complete use of antenatal corticosteroids and surfactant treatment was achieved, whereas use of positive pressure ventilation and active treatment of PDA was tuned down. For the EP_{1991} cohort, surfactant was administered as rescue treatment only, as prescribed by the Osiris® trial of exogenous synthetic surfactant (Exosurf®). For the EP_{1999} cohort, the natural surfactants Survanta® (bovine derived) or Curosurf® (porcine derived) were administered prophylactically, or as a rescue treatment. Prophylactic surfactants have later proved benificial[127], and there have been indications of better early respiratory status after administration of natural rather than synthetic surfactants, factors possibly contributing to the more beneficial outcomes of the EP_{1999} cohort [291]. These later studies support our finding that the advantage of EP_{1999} versus EP_{1999} disappeared for z-FEV_1 when adjusted for disproportionate use of this treatment.

Alveolar NO

Few studies have performed tests of alveolar NO in EPB[292]. No differences between EPB and TB were evident in our study, confirming also findings from other studies of one-compartment airway NO[148,149]. This supports the theory advancing the idea that eosinophilic inflammation is absent in central as well as peripheral parts of the airways

of these children. It does not, however, exclude the theory of some other means of active inflammation or metabolic process being involved, perhaps of neutrophilic origin[150].

6.2.5 Changes over time in western Norway

Consecutive population-based cohorts of subjects born EP in different eras of neonatal intensive care medicine have been followed at our centre for decades. As reported in Chapter 5.6.1 and illustrated in Figure 7, a picture emerges that might indicate a gradual improvement in pulmonary outcomes since the early 1980s. Thus, the findings reported in Paper #III of possibly better outcomes for EPB with BPD in the EP_{1999} cohort, might herald a positive trend with still better future respiratory prospects for recent EPB survivors.

7. CLINICAL IMPLICATIONS

COPD, the world's third leading cause of death[293], is a heterogeneous disease characterized by partly irreversible and progressive airflow obstruction[294]. It is most often associated with cigarette smoking[78], though may also develop in non-smokers[295], and increasing evidence suggest early life factors are involved[94,96].

The first large cohorts of 'pre-surfactant' EPB survivors are today in their early 30s, whereas 'post-surfactant' survivors are in their early 20s. Lung-health prospects through middle age and beyond must therefore remain speculative[95,131,133,175]. Many start adult life with low FEV_1 and high BHR. The clinical relevance of these findings made at the threshold of adulthood is yet unknown. The rate of future decline in FEV_1 will determine if or when EPB might cross the limits of PF associated with symptoms and disease.

Our findings suggest that EPB young adults can have impaired PF with little or no symptoms, and therefore may fail to seek advice in the health care system[63,97]. This underscores the importance of asking patients about early life events. Thus, preterm birth is not of interest to only paediatricians, but should also be in the focus of primary care physicians and adult pulmonologists. Adult pulmonologists are often unaware of the birth history and early life risk factors of their patients[65].

Around 300 children are born EP annually in Norway (MBRN). They are seen regularly in outpatient clinics until 5 years of age in order to identify impairments or shortcomings. Thereafter, providing no disabilities or major problems emerge, their care is left to the regular health facilities[296].

Scheduled health care visits until adulthood might be beneficial for EPB, but the effect would have to be evaluated before introducing such a large measure. Although demanding in terms of immediate costs, it could avert future costs related to chronic adult disease[62]. The lack of effective interventions poses a problem. Early detection of respiratory impairment might enable early implementation of measures to optimize lung health, preferably in advance of the individuals crossing the critical threshold for symptoms. This could include non-smoking strategies; measures to achieve a healthy

lifestyle, such as dietary, physical fitness and vocational guidance, and influenza immunisations[91,297].

7.1 Future prospects for better lung health in EPB subjects?

Wilcox stated 'Preterm babies carry the burden of whatever pathology triggered their early birth'[24]. Infants born preterm after disadvantageous early intrauterine exposures most probably cannot be 'cured' by postnatal care in a NICU. However, one may easily envision the opposite; i.e. that 'poor' but necessary neonatal handling, such as hyperoxia and various ventilatory aids, can potentiate already existing injuries in such unfortunate infants, possibly to an extent exceeding the effects in those born more 'healthy'.

More fortunate infants that enter postnatal life after more advantageous intrauterine existence might be more robust to postnatal exposures. These 'healthy' preterms might be harmed by poor postnatal handling, but may have the potential to develop normal lungs – given the theoretical scenario of an 'optimal NICU environment'. Irrespective of various scenarios and speculations, it seems that the continuous strive for better handling of preterm born babies is worthwhile.

The data presented in the last paper of this thesis are highly encouraging. Individuals born at extremely low GA at appropriate birth weight in Western Norway in 1999-2000 had pulmonary function that did not differ much from normal at 11 years of age. In the group of children under study, poor PF was largely a phenomenon observed in those with a history of low gestation and being light for age at birth. One way of interpreting this, is that our NICUs were in fact capable of delivering a standard of care that facilitated 'close to normal' lung development in infants born after shortened but otherwise normal pregnancies. Thus, one might postulate that such infants can in fact develop relatively unharmed, given optimal postnatal handling in a well-functioning NICU. Of course - such speculations need confirmation.

Regarding future prospects for growth and repair after the neonatal period, our study gave no indication that the bronchial tree is likely to exhibit catch-up growth throughout childhood and adolescence. The situation might be somewhat different

regarding acinar growth, and recent data indicate that catch-up alveolarization might occur[73,298]. These studies provide optimism that repair mechanisms might come into play to mend the pessimistic picture conveyed from lung autopsies of infants who have died from BPD, portraying severe alveolar dysplasia. Thus, post-neonatal development of acinar structures might facilitate an increase of the gas-exchanging surface and thereby aid future gas exchange. These findings may explain the relatively minor differences in diffusion capacity shown in this and other studies[149,156,186], and also the close to normal peak exercise capacity that has been reported by several research groups, including our own[156,299].

In conclusion, the continued strive to optimize the standard of care that we offer to extremely preterm born infants is worthwhile, and is likely to further improve their pulmonary health.

7.2 Future studies

To provide unbiased and firm estimates of lifetime respiratory outcomes after EP birth, properly powered population-based studies should prospectively include consecutive premature births, and provide comprehensive follow-up, ideally from birth through adulthood and beyond. Well-matched TB control subjects should be included. These may seem ambitious but nevertheless reasonable objectives, considering the human and financial costs involved in neonatal intensive care medicine.

Although the respiratory impairments of these individuals are well described, the aetiology is poorly understood, and thus, evidence based treatments become difficult to institute. We should aim at tackling this in the future. There have been few prospective studies on interventions to prevent BPD in preterm born infants[300,301] or on post-neonatal treatments of preterm born children with lung disease[269,270]. Researchers should design prospective intervention studies, and healthcare workers should focus on how to prevent preterm birth, and on how to prevent BPD from developing in preterm born infants.

8. CONCLUSIONS

The overall 0-hypothesis of this study stated:

H_0: There is no difference in PF or PF trajectories between children, adolescents, and young adults born EP and at term during the period 1982-2000.

The study rejected the first part of the statement, as significant deficits was observed for PF over the complete study period for most EPB subgroups, whereas the second part was sustained, as no differences could be found for PF tracking.

Research area 1

We observed tracking of PF in all study groups. PF in EPB survivors born before and after the introduction of surfactants changed in parallel with TB control subjects through puberty and early adulthood, with no apparent differences between subgroups of EPB. There were no signs of catch-up growth or early onset / accelerated impairments of PF during the study period.

Research area 2

For subjects born EP in the early 1980s, impairments in PF persisted into adulthood, most pronounced in EPB with a neonatal history of BPD. Most impairment was modest, except BHR. Respiratory symptoms seemed to diminish in adulthood, despite the presence of persistent and reduced PF.

Research area 3

Children born EP in 1999-2000 had poorer PF than matched term-controls, but respiratory outcomes were better than of comparable children born preterm 8 years earlier, particularly for subjects with neonatal BPD. BPD and low GA did not predict poor respiratory outcome; instead intrauterine growth restriction appeared as an important factor. We found no differences in alveolar NO between EPB and TB. Inhaled salbutamol improved airflow and reduced deficits relative to TB controls, but the response did not differ between the EPB and TB groups. Whether PF abnormalities after EP birth represents structural sequelae or ongoing processes, should be further evaluated.

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10. ERRATA

Paper #I

Table 2 Perinatal data: Gestational age (weeks), mean (range) for all EPB in 1991-1992 should be 26.7, not 26.9.

Paper #II

Paper #III

11. APPENDIX

APPENDIX I

Til de som har astma og / eller bruker medisiner og skal delta i lunge-forskning ved Barneklinikken i Bergen

I den studien du skal være med på, vil vi teste lungefunksjonen. For at testene skal bli riktige, bør kroppen ikke være påvirket av astma-medisiner, og heller ikke av kaffe, te, cola. Derfor er det fint om du stopper å bruke dette en periode før timeavtalen hos oss. Hvor lenge før timeavtalen du bør stoppe, varierer (se under).

Dersom du har astma og tror at du vil få ubehag av å utsette astma-medisinene, er det fint om du ringer oss. Dersom du slutter med astma-medisiner og blir tett eller tungpustet, begynner du med medisiner igjen slik du pleier når du er tett eller tungpustet. Ring oss i så fall ved første anledning.

Vi vil helst undersøke lungene dine når du er så god som mulig.

Derfor: Hvis du har hatt en luftveisinfeksjon eller en merkbar forverring av astma de siste to ukene før avtalt time på Barneklinikken, ber vi deg ta kontakt med oss.

Følgende tidsintervall gjelder mellom siste dose medisiner og timen på Barneklinikken:

Hurtigvirkende anfallsmedisin (åpnere) som Bricanyl, Ventoline eller Airomir	8 timer før
Langtidsvirkende medisin som Oxis, Serevent, Seretide eller Symbicort	48 timer før
Cortison til inhalasjon, som Pulmicort, Flutide, Becotide eller Aerobec	24 timer før
Singulair	72 timer før
Lomudal	24 timer før
Allergimedisiner som Ceterizine, Clarityn, Zyrtec, Polaramin eller Phenamin	1 uke før

I tillegg ber vi deg om ikke bruke:

Kaffe, te eller cola 4 timer før

Maria Vollsæter

Lege, Barneklinikken, Stipendiat Prosjekt Ekstrem Prematuritet

Tlf 48068282

APPENDIX II

Prosjekt Ekstrem Prematuritet – PEP

Registrering av antall dager med oksygentilskudd etter fødsel

Som en grei regel er det lurt å lese epikrisen fra nyfødtoppholdet først, mange leger har her regnet sammen antallet dager med oksygenbehandling allerede!

Dersom ikke tallet finnes her, finnes det på følgende måte, ved å bruke (journal) og hovedsakelig kurvene fra nyfødtavdelingen i papirjournalene

I summen av antall dager inngår:

Antall dager med respirator/SIMV

+ Antall dager med nasal/tube CPAP – også på romluft!

+ Antall dager med oksygentilskudd på trakt/nesemaske

= Antall dager med oksygentilskudd

Man teller med den dagen barnet er født, og med den dagen barnet slutter, så lenge sistnevnte er på dagtid og barnet altså har hatt tilskudd om natten denne datoen.

Det er fint om det også registreres om barnet brukte oksygen ved 28 dagers alder (ja/nei).

Generelt gjelder at den oksygenbeh som registreres, skal være uttrykk for den *daglige beh* av nyfødte barn, ikke den som blir gitt ved spesielle anledninger.

Dette presiseres som følgende:

Dersom barnet har vært av oksygen/CPAP/respirator i noen dager, og så blir satt på igjen, adderer man disse dagene til det totale antallet, såfremt oksygenbeh blir gitt grunnet kronisk lungesykdom/immaturitet/persisterende ductus i *to eller flere dager*.

Oksygenbeh gitt på grunnlag av annen tilkommet sykdom som ikke har med lungene å gjøre, eks sepsis, sentrale apnoer, intracerebrale blødninger, diafragmaparese, teller ikke med dersom dette tilkommer etter seponering.

Oksygenbeh gitt i forbindelse med div inngrep (kryobeh for ROP, kirurgi for div tilstander) teller med bare dersom det er gitt i mer enn 12 timer.

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APPENDIX III **Table III** Comparison of using two different GLI all-age equations for spirometry parameters expressing pulmonary function of 45 EPB divided by respective BPD-subgroups and 39 matched TB controls examined at 25 years of age

		Controls N=39	Non BPD N=11	Mild BPD N=22	M/S BPD N=12	Interaction p	EP vs. control p
FEV_1	Z_{2008}	0.07 (-0.36, 0.50)	-0.52 (-1.29, 0.24)	-1.06 (-1.60, -0.52)	-1.33 (-2.06, -0.60)	0.776	**0.001**
	Z_{2012}	0.042 (-0.36, 0.45)	-0.54 (-1.26, 0.17)	-1.06 (-1.56, -0.55)	-1.33 (-2.02, -0.65)	0.757	**<0.0005**
	$\%_{2008}$	100.8 (95.9, 105.8)	94.1 (85.4, 102.7)	87.9 (81.8, 94.1)	84.9 (76.6, 93.2)		
	$\%_{2012}$	100.4 (95.5, 105.3)	93.6 (85.0, 102.3)	87.2 (81.1, 93.3)	84.1 (75.8, 92.3)		
FVC	Z_{2008}	0.05 (-0.43, 0.53)	0.29 (-0.56, 1.13)	-0.71 (-1.31, -0.11)	-0.73 (-1.54, 0.08)	0.419	0.209
	Z_{2012}	0.07 (-0.37, 0.50)	0.28 (-0.49, 1.04)	-0.64 (-1.18, -0.10)	-0.63 (-1.37, 0.10)	0.395	0.086
	$\%_{2008}$	100.5 (95.5, 105.6)	103.4 (94.1, 112.0)	92.5 (86.2, 98.8)	92.3 (87.7, 100.8)		
	$\%_{2012}$	100.9 (95.8, 105.9)	103.4 (94.4, 112.3)	92.7 (86.4, 99.1)	92.4 (83.9, 101.0)		
FEV_1/FVC	Z_{2008}	0.03 (-0.29, 0.35)	-1.13 (-1.70, -0.57)	-0.60 (-1.00, -0.20	-0.70 (-1.24, -0.16)	0.129	**0.001**
	Z_{2012}	-0.08 (-0.41, 0.25)	-1.20 (-1.79, -0.61)	-0.76 (-1.18, -0.35)	-0.92 (-1.49, -0.36)	0.194	**0.002**
	$\%_{2008}$	99.4 (96.8, 102.1)	90.2 (85.5, 94.9)	94.5 (91.1, 97.8)	92.4 (87.9, 96.9)		
	$\%_{2012}$	-	-	-	-		
FEF_{25-75}	Z_{2008}	0.06 (-0.31, 0.43)	-1.21 (-1.90, -0.53)	-1.10 (-1.56, -0.64)	-1.28 (-1.90, -0.65)	0.525	**<0.0005**
	Z_{2012}	-0.06 (-0.42, 0.29)	-1.33 (-1.99, -0.66)	-1.23 (-1.68, -0.78)	-1.41 (-2.01, -0.80)	0.468	**<0.0005**
	$\%_{2008}$	102.7 (95.1, 110.4)	74.0 (59.7, 88.3)	78.3 (68.7, 87.9)	74.0 (61.0, 87.1)		
	$\%_{2012}$	99.4 (92.0, 106.8)	71.4 (57.6, 85.2)	74.9 (65.6, 84.2)	70.8 (58.2, 83.5)		

Values are observed means (95% CI). Unpublished data.

APPENDIX IV:

Exhaled NO regression analyses

FE_{NO} was associated with FEV_1 (β 13.1; p=0.006), FVC (β 11.1; P=0.001), and atopy (β 4.3; p<0.001) (R^2 =0.42 for all models) in TB. For EPB without BPD, FE_{NO} was associated with height (β 0.49; p=0.004), atopy (beta 3.7; p=0.012), and FVC (β 9.1; p=0.008) (R^2 = 0.56 for all models), whereas in EPB with BPD FE_{NO} was associated only with atopy (β 4.1;p=0.031;R^2 = 0.18).

Alveolar concentration of NO (C_ANO) was not associated with any variable in TB and EPB with BPD. In EPB without BPD, C_ANO was associated with age (beta 0.38; p=0.010), FEV_1 (β 1.42; p=0.037), and FVC (β 1.28;p=0.022) (R^2 = 0.51 for all models).

APPENDIX V

Table V1: Differences in plethysmographic volumes when using old (2001) and new software (2005). Data from 18-year-old subjects (N=86) participating in 2001 (EP1982)

	Control N=46	**EP no BPD** N=29	**EP BPD** N=11
TLC, *l*	0.12 (0.07, 0.17)	0.15 (0.00, 0.29)	0.12 (0.07, 0.16)
FRC, *l*	0.00 (-0.01, 0.01)	0.05 (-0.10, 0.00)	0.01 (-0.01, 0.02)
RV, *l*	0.13 (0.09, 0.17)	0.09 (0.01, 0.18)	0.12 (0.07, 0.16)
RV/TLC, *ratio*	1.83 (1.23, 2.42)	1.00 (0.13, 1.87)	1.73 (1.05, 2.41)

Values are means with 95 % CI for absolute measured lung volumes. Volumes measured in litres and analyzed by paired samples t-tests. A positive difference indicates that the volumes are higher when analyzed by the new software (new 2005 algorithm). Unpublished data.

Table V2: Differences in plethysmographic volumes from11-year-old subjects (N=69) participating in 2001 (EP1991)

	Control N=35	**EP no BPD** N=22	**EP BPD** N=12
TLC, *l*	0.05 (0.02, 0.08)	0.01 (-0.06, 0.07)	0.05 (-0.02, 0.13)
FRC, *l*	-0.00 (-0.03, 0.03)	-0.05 (-0.13, 0.02)	0.00 (-0.04, 0.05)
RV, *l*	0.10 (0.07, 0.13)	0.06 (0.01, 0.10)	0.07 (-0.03, 0.17)
RV/TLC, *ratio*	2.60 (1.93, 3.27)	2.23 (0.77, 3.68)	1.96 (-0.52, 4.44)

Values are means with 95 % CI for absolute measured lung volumes. Volumes measured in litres and analyzed by paired samples t-tests. A positive difference indicates that the volumes are higher when analyzed by the new software (new 2005 algorithm). Unpublished data.

12. Papers I, II and III

III

PLOS | ONE

RESEARCH ARTICLE

Children Born Preterm at the Turn of the Millennium Had Better Lung Function Than Children Born Similarly Preterm in the Early 1990s

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Abbreviations: BW, birthweight; CA_{NO}, alveolar NO; COPD, chronic obstructive pulmonary disease;

Abstract

Objective

Compare respiratory health in children born extremely preterm (EP) or with extremely low birthweight (ELBW) nearly one decade apart, hypothesizing that better perinatal management has led to better outcome.

Design

Fifty-seven (93%) of 61 eligible 11-year old children born in Western Norway in 1999–2000 with gestational age (GA) <28 weeks or birthweight <1000 gram ($EP_{1999–2000}$) and matched term-controls were assessed with comprehensive lung function tests and standardized questionnaires. Outcome was compared with data obtained at 10 years of age from all (n = 35) subjects born at GA <29 weeks or birthweight <1001 gram within a part of the same region in 1991–92 ($EP_{1991–1992}$) and their matched term-controls.

Results

$EP_{1999–2000}$ had significantly reduced forced expiratory flow in 1 second (FEV_1), FEV_1 to forced vital capacity (FEV_1/FVC) and forced expiratory flow between 25–75% of FVC ($FEF_{25–75}$), with z-scores respectively -0.34, -0.50 and -0.61 below those of the term-control group, and more bronchial hyperresponsiveness to methacholine (dose-response-slope 13.2 vs. 3.5; p<0.001), whereas other outcomes did not differ. Low birthweight z-scores, but not neonatal bronchopulmonary dysplasia (BPD) or low GA, predicted poor outcome. For children with neonatal BPD, important lung-function variables were better in $EP_{1999–2000}$ compared to $EP_{1991–1992}$. In regression models, improvements were related to more use of antenatal corticosteroids and surfactant treatment in the $EP_{1999–2000}$.

CPAP, continuous positive airway pressure; DLCO, lung diffusion capacity for carbon monoxide; DRS, dose-response-slope; EP, preterm born participants of the present study; ERS, European Respiratory Society; FE_{NO}, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; $FEF_{25–75}$, forced expiratory flow between 25 and 75% of vital capacity; FRC, functional residual capacity; FVC, forced vital capacity; GA, gestational age; Hb, haemoglobin; Jaw_{NO}, bronchial flux of NO; KCO, diffusion capacity corrected for alveolar volume; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PDA, persistent ductus arteriosus; PD20, the dose methacholine required to lower FEV1 $\geq$ 20% of baseline level; R_{aw}, airway resistance; ROP, retinopathy of prematurity; RV, residual volume; SGA, small for gestational age; SPT, skin prick test; TLC, total lung capacity; VA, alveolar volume.

Conclusions

Small airway obstruction and bronchial hyperresponsiveness were still present in children born preterm in 1999–2000, but outcome was better than for children born similarly preterm in 1991–92, particularly after neonatal BPD. The findings suggest that better neonatal management not only improves survival, but also long-term pulmonary outcome.

Introduction

Since the 1990s most infants born extremely preterm or with extremely low birthweight (hereafter referred to as EP-born) in high-income countries have survived to discharge [1, 2]. Birth at this stage of pregnancy implies that gas exchange must take place in fetal lungs, often leading to the syndrome of bronchopulmonary dysplasia (BPD) [3]. Life-long pulmonary prospects after EP birth and BPD are unknown, and concerns have been expressed for future functional deficits, such as early onset respiratory insufficiency [4, 5].

Neonatal pulmonary autopsy data suggest that EP birth may lead to severe bronchoalveolar abnormalities, but structural data from later life are scarce [6]. However, a range of functional abnormalities have repeatedly been described in EP-born survivors, such as airway obstruction, bronchial hyperresponsiveness, pulmonary hyperinflation and impaired gas diffusing capacity. Generally, those with neonatal BPD do worse. This scenario applies to EP-born adults from the early era of neonatal intensive care units (NICUs) as well as to children exposed to the far more advanced treatments introduced in the 1990s [7–12]. As lung function seems to track from childhood to adulthood [9, 13, 14], early onset chronic obstructive pulmonary disease (COPD) is a feared scenario in high-risk subgroups [4, 5].

Better NICU management benefits all infants born preterm, but it also facilitates survival of more immature and vulnerable individuals who may be at particular risk of severe long-term morbidity [2, 15]. Therefore, respiratory health and lung function after EP birth have been closely monitored in population-based controlled longitudinal studies in Western Norway since the 1980s [13, 16]. With the present study, we aimed to address respiratory outcomes at 11 years of age in our most recent cohort born EP in 1999–2000 ($EP_{1999–2000}$) and to compare the findings with those of children born similarly preterm in 1991–1992 ($EP_{1991–1992}$). We hypothesized that changes in perinatal care during the 1990s were associated with respiratory improvements [16, 17].

Materials and Methods

Detailed descriptions are provided in S1 File, and the inclusion process is visualized in Fig 1.

Subjects, definitions and neonatal background data

The $EP_{1999–2000}$ included all NICU admitted infants born in 1999–2000 with gestational age (GA) <28 weeks or birth weight (BW) <1000 gram within Western Norway Health Authority, which serves a population of approximately 1.1 million. It was a regional selection of a national cohort [2]. The infants were treated at one of the two regional NICUs (Bergen and Stavanger). The $EP_{1999–2000}$ data were compared with data obtained at 10 years of age from all (n = 35) subjects born with GA <29 weeks or BW <1001 gram within a part of the same region (population 615 000) in 1991–92 and treated at the NICU in Bergen ($EP_{1991–1992}$). $EP_{1991–1992}$ data have been published previously in a different context [13, 16] and relevant data are included in

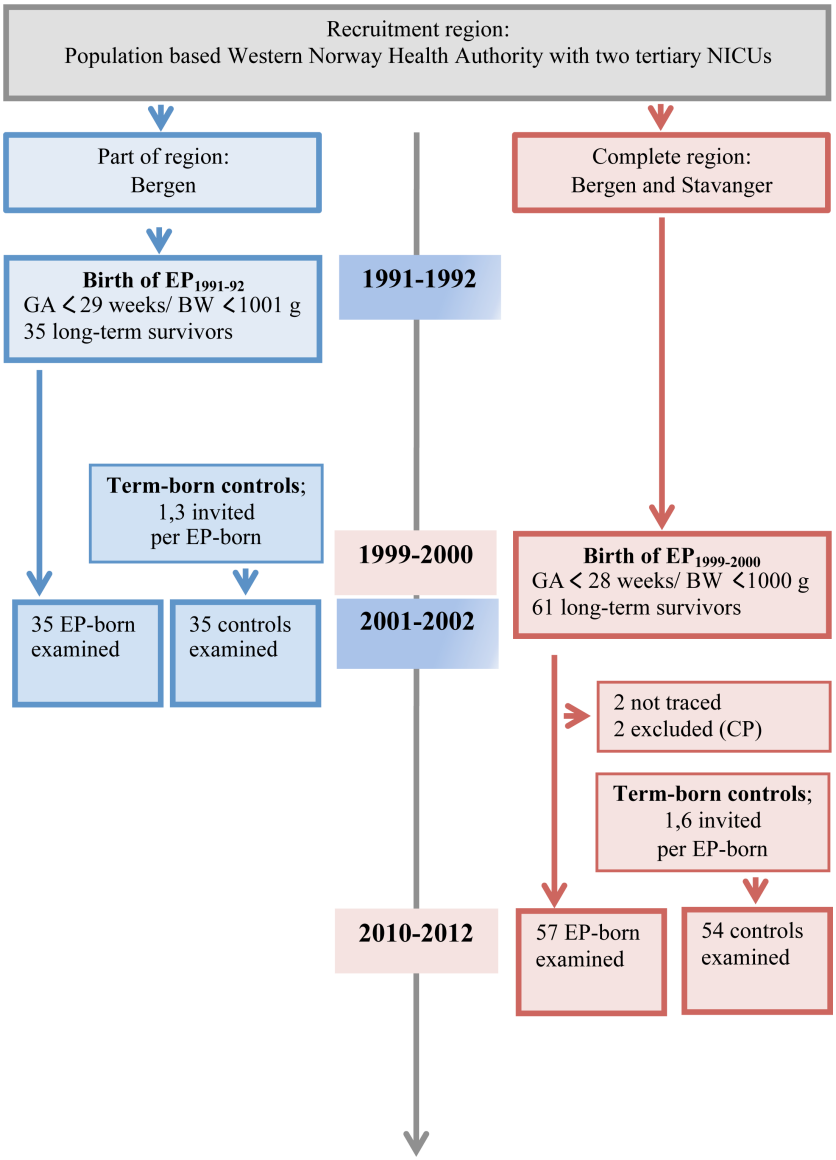

Fig 1. The recruitment of subjects in two cohorts of premature (EP) and matched term-born subjects, one cohort born in 1991–92 and examined in 2001–2002, and one cohort born in 1999–2000 and examined in 2010–2012.

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the present paper. The acronym 'EP-born' is used in this article to represent participants born preterm in both inclusion periods.

For each EP-born participant of both birth-cohorts, the next-born child in the same maternity ward of the same gender with GA >37 weeks and BW >3000 grams were identified from birth protocols and invited as control. If that individual declined, the next-born eligible child was invited until a match was obtained. Background data were extracted from compulsory notifications to the Medical Birth Registry of Norway, registration-forms developed for the study and questionnaires completed by the parents, including the International Study on Asthma and Allergy in Childhood (ISAAC) questionnaire [18]. Small for gestational age (SGA) was defined as BW $<10^{th}$ percentile for GA according to Norwegian growth curves [19]. Z-scores for BW and later anthropometric measures were calculated with reference to Norwegian growth curves [19, 20]. BPD was defined as still dependent on oxygen supplementation at 36 weeks' postmenstrual age [3]. Oxygen supplementation was given according to similar department policy in the two inclusion periods in the two participating NICUs, and at 36 weeks' GA it was generally provided through low flow nasal cannulas guided by pulse oximetry targeting 90–95% saturation. Important changes of treatment between the two inclusion periods were more extensive use of surfactant and antenatal corticosteroids and a change from synthetic (Exosurf®) (31) to natural surfactant (Curosurf®) (Table 1) (32, 33). Most areas of neonatal intensive care medicine had gone through refinements, such as better standardization of antenatal and perinatal care, a higher level of competence among neonatologists and nurses regarding the special needs of these vulnerable infants, and better exploitation of technological advances, such as patient coordinated assisted ventilation and various forms of oscillation.

The studies were approved by the regional committee on medical research ethics in Western Norway Health Authority (REC West), and parents of all participants gave written consent.

Measurements

The two birth-cohorts were examined at 10–11 years of age in 2001–2002 and 2010–2012, respectively, using the same type of equipment and the same examination program, except that nitric oxide was not studied in $EP_{1991-1992}$. The children were seen on two separate days at the University Hospitals in Bergen or Stavanger according to place of birth. Spirometry, static lung volumes and pulmonary diffusing capacity for carbon monoxide (*DLCO/KCO*) were measured with Vmax equipment (*SensorMedics Inc*, *Anaheim*, *USA*), applying standard quality criteria [21, 22] with data standardized for age, height and gender [23, 24], except *KCO* reported as raw data. Fractional exhaled nitric oxide (Fe_{NO}) was measured with Exhalyzer CLD-88 (*EcoMedics*, *Switzerland*), according to ATS/ERS recommendations [25]. Alveolar NO (ppb) (CA_{NO}) and bronchial flux of NO (nl/sec) (Jaw_{NO}) were calculated using three different flows (30, 100 and 300 ml/sec) [26]. Methacholine provocation was performed with an inhalation-synchronised dosimetric nebulizer (*Spira Electra*, *Finland*), providing baseline FEV_1 ≥65% predicted [27, 28]. The test continued until a fall of 20% or more compared to baseline FEV_1, or until the maximum dose of 11.5 μmol methacholine. Dose-response slope (DRS) was calculated as the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose of methacholine (%/μmol) [29]. Reversibility to salbutamol was assessed by measuring FEV_1 before (baseline) and 10–15 minutes after administering 0.1 mg/10 kg salbutamol (*Ventoline*) from a metered dose inhaler via a spacer (*Volumatic*); an increase ≥12% was considered positive response [30]. Methacholine provocation and salbutamol reversibility tests were done on separate days.

Table 1. Perinatal data comparing children born preterm in 1991–92 ($EP_{1991–92}$) and in 1999–2000 ($EP_{1999–2000}$).

		$EP_{1991–92}$ cohort	$EP_{1999–2000}$ cohort	p-value[a]
Subjects; n (%)	Control	35	54	
	All EP	35	57	
	- non BPD	23 (66)	26 (46)	0.067
	- BPD	12 (34)	31 (54)	
Female gender;n (%)	Control	22 (63)	25 (46)	0.135
	All EP	22 (63)	28 (49)	0.209
	- non BPD	17 (74)	16 (62)	0.379
	- BPD	5 (42)	12 (39)	0.860
Birthweight, gram; mean (SD)	Control	3564 (275)	3701 (434)	0.073
	All EP	933 (204)	850 (175)	0.039
	- non BPD	976 (195)	873 (200)	0.073
	- BPD	851 (203)	831 (151)	0.722
Birthweight; sds- score	All EP	-0.36 (0.9)	-0.80 (1.3)	0.054
	- non BPD	-0.37 (0.9)	-0.97 (1.4)	0.076
	- BPD	-0.32 (0.9)	-0.66 (1.2)	0.386
Gestational age, weeks; mean (SD)	All EP	26.7 (1.7)	26.8 (1.6)	0.974
	- non BPD	27.2 (1.7)	27.3 (1.6)	0.788
	- BPD	25.8 (1.5)*	26.3 (1.4)**	0.381
Small for gestational age (SGA); n (%)	All EP	5 (14)	20 (35)	0.030
	- non BPD	3 (13)	12 (46)	0.015
	- BPD	2 (17)	8 (26)	0.570
Postnatal days with oxygen treatment; median (range)	All EP	49 (2–180)	65 (0–250)	0.109
	- non BPD	34 (2–70)	44 (0–78)	0.254
	- BPD	92 (61–180)	79 (44–250)	0.080
Ventilator days; median (range)	All EP	4.0 (0–55)	5.0 (0–24)	0.618
	- non BPD	1.3 (0–40)	2.5 (0–21)	0.212
	- BPD	12.7 (2–55)***	8.0 (0–24)***	0.011
Antenatal corticosteroids; n (%)	All EP	15/34 (44)	46 (81)	<0.001
	- non BPD	11 (48)	21 (81)	0.005
	- BPD	4/11 (36)	25 (81)	0.012
Surfactant; n (%)	All EP	17 (49)	49/56 (88)	<0.001
	- non BPD	7 (30)	20/25 (80)	0.001
	- BPD	10 (83)	29 (94)	0.367
Postnatal corticosteroids; n (%)	All EP	10 (29)	18 (32)	0.772
	- non BPD	2 (9)	1 (4)	0.549
	- BPD	8 (67)	17 (55)***	0.510
Closing of PDA; n (%)	All EP	17 (49)	13 (23)	0.013
	- non BPD	7 (30)	3 (12)	0.122
	- BPD	10 (83)	10 (32)	0.004
Maternal smoking in pregnancy; n (%)	Control	9 (26)	-	-
	All EP	13 (37)	13/52 (25)	0.205
	- non BPD	10 (43)	5/24 (21)	0.111
	- BPD	3 (25)	8/28 (29)	0.957

Figures are means (SD), medians (ranges) or counts (%).

[a] The p-value denotes differences between those born in 1991–92 and in 1999–2000.

* P-values for group differences between EP non BPD vs. EP BPD within each cohort, * p<0.05,

**p<0.01,

***p<0.001.

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Statistical analysis

Non-paired groups were compared using independent sample t-tests, Mann-Whitney U-test, Fishers exact 2-sided mid-p value [31] or odds ratios (OR), and paired data with the mixed linear model (MLM) of SPSS, as appropriate. For $EP_{1999–2000}$, multiple backward regression models were constructed in order to address potential associations between neonatal data and selected background data (listed in the Results chapter) vs. current FEV_1. Independent variables were included if the correlation with the dependent variable was > 0.3 and the bivariate correlation with other independent variables was < 0.7. Interaction terms were used to test if effects (differences in lung function between EP-born and matched term-controls) differed between EP-born subgroups; i.e. BPD vs. non BPD, GA-categories (GA $\leq$25 vs. 26–27 vs. $\geq$28 weeks) and birth-cohort (1991–1992 vs. 1999–2000). The interaction terms were tested for influence from selected neonatal factors that varied between the two preterm born cohorts (see Results). Providing 60 cases were included, the study had 80% power to detect a difference in z-FEV_1 of 0.5 between $EP_{1999–2000}$ and matched term-controls, given a two-sided significance level of 0.05. SPSS (version 21.0) was used for computations.

Results

Subjects (Fig 1)

In $EP_{1999–2000}$, 61 eligible EP-born were discharged alive, two could not be traced and two were excluded due to cerebral palsy, leaving 57 (93%) participants, all but three Caucasians. In $EP_{1991–1992}$, all 35 eligible EP-born survivors participated, all Caucasians. On average, respectively 1.6 and 1.3 term-born subjects were approached to recruit a full 1:1 control group for the two inclusion periods. In $EP_{1999–2000}$, uneven drop-outs eventually caused a numeric gender difference; i.e., 49% EP-born vs. 46% controls were female ($p = 0.85$).

Perinatal data (Table 1)

For subjects admitted to the NICU, survival rates were 81% ($EP_{1999–2000}$) vs. 74% ($EP_{1991–92}$) ($p = 0.57$). Due to slight differences in the inclusion criteria, mean BW was lower and the proportion born SGA was higher in $EP_{1999–2000}$. Eleven subjects (19%) were included solely by BW (i.e. GA $\geq$28 weeks) in $EP_{1999–2000}$ (mean GA (range) 29.2 (28–31) weeks), and two solely by BW (i.e. GA $\geq$29 weeks) in $EP_{1991–92}$ (GA 30 and 31 weeks). The number of participants born at GA $\leq$27 weeks was 46/57 (81%) and 21/35 (60%), respectively. For subjects with BPD in $EP_{1999–2000}$ compared to $EP_{1991–1992}$, mean GA and BW did not differ, but days on ventilator were fewer, a higher proportion had received prenatal corticosteroids and surfactant treatment and fewer had artificial closure of persistent ductus arteriosus (surgery or indomethacin).

Anthropometric data and respiratory symptoms (Table 2)

At follow-up, $EP_{1999–2000}$ was one year older than $EP_{1991–1992}$. Anthropometric measures were similar except that the control children for $EP_{1999–2000}$ had slightly lower BMI. The prevalence of ever having been diagnosed with asthma was similar for the two preterm-born cohorts, and the proportion was significantly higher than for their respective term-born groups. Current respiratory symptoms (wheezing) and use of asthma medication tended to be rarer in $EP_{1999–2000}$ than in $EP_{1991–92}$ and not significantly different from term-controls, contrasting $EP_{1991–92}$. Wheeze was unrelated to airflow-limitation, with one report of current wheeze in the overall 15 subjects with z-FEV1 below -1.64, which is the lower limit of normal [23].

Table 2. Anthropometric data and respiratory symptoms at age 11 comparing children born preterm in 1991–92 ($EP_{1991–92}$) and in 1999–2000 ($EP_{1999–2000}$).

		$EP_{1991–92}$ cohort n = 35	$EP_{1999–2000}$ cohort n = 57	p-value[a]
Age; years	Control	10.6 (0.4)	11.7 (0.7)	<0.005
	All EP	10.4 (0.4)	11.4 (0.6)	<0.005
	- non BPD	10.4 (0.5)	11.4 (0.6)	<0.005
	- BPD	10.4 (0.4)	11.5 (0.6)	<0.005
Height; z-score	Control	0.02 (0.9)	0.00 (1.1)	0.925
	All EP	-0.51 (1.2)*	-0.41 (1.0)*	0.654
	- non BPD	-0.52 (1.3)	-0.38 (1.0)	0.658
	- BPD	-0.49 (1.1)	-0.43 (1.1))	0.868
Weight; z-score	Control	0.17 (1.0)	-0.14 (1.1)	0.153
	All EP	-0.48 (1.4)*	-0.33 (1.05)	0.570
	- non BPD	-0.40 (1.7)	-0.43 (1.0)	0.956
	- BPD	-0.61 (0.8)	-0.25 (1.1))	0.303
BMI; z-score	Control	0.25 (0.9)	-0.22 (1.0)	0.033
	All EP	-0.28 (1.4)	-0.16 (1.0)	0.644
	- non BPD	-0.18 (1.6)	-0.35 (1.1)	0.660
	- BPD	-0.46 (0.9)	0.00 (1.0)	0.178
Asthma ever	Control	3 (9)	5 (9)	0.933
	All EP	12 (34)*	15 (26)*	0.427
	- non BPD	6 (26)	5 (19)	0.587
	- BPD	6 (50)	10 (32)	0.309
Asthma medication last 12 months	Control	1 (3)	3 (6)	0.838
	All EP	5 (14)	4 (7)	0.075
	- non BPD	1 (4)	3 (12)	0.775
	- BPD	4 (33)	1 (3)	0.006
Wheeze last 12 months	Control	2 (6)	5 (9)	0.587
	All EP	11 (31)*	8 (14)	0.055
	- non BPD	6 (26)	4 (15)	0.383
	- BPD	5 (42)	4 (13)	0.060
Atopy	Control	8 (23)	20/50 (40)	0.105
	All EP	9 (26)	12/55 (22)	0.675
	- non BPD	8 (35)	6/25 (24)	0.435
	- BPD	1 (8)	6/30 (20)	0.415

Figures are means (SD), medians (ranges) or counts (%).

[a] The p-value denotes differences between those born on 1991–92 and in 1999–2000.

* P-values for group differences between term controls vs. all EP within each cohort, * p<0.05. Atopy registered as minimum one positive SPT (skin prick test) or IgE test in the $EP_{1991–92}$ cohort and as minimum one positive SPT in the $EP_{1999–2000}$ cohort.

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Lung function for $EP_{1999–2000}$

Data on $EP_{1991–1992}$ have been published previously [13, 16], and relevant data are presented in Table C in S1 File. For $EP_{1999–2000}$, data are presented in Tables 3 and 4 and Table D in S1 File. Flow-volume loops were satisfactorily obtained from all participants. Failure rates were minor also for most other lung function tests, except measures of nitric oxide and lung diffusion (12–14 of 57). The failure rates were similar for EP-born and term-controls on the individual tests; details are presented in S1 File. EP-born had lower z-scores for FEV_1, FEV_1/FVC and $FEF_{25–75}$, higher airway resistance (Raw), and higher DRS from the methacholine challenge than term-

Table 3. Lung function variables in 11 year old children born preterm (EP) in 1999–2000 and matched term-born controls, split by neonatal bronchopulmonary dysplasia (BPD).

		Controls N = 54	All EP N = 57	EP non BPD N = 26	EP BPD N = 31	Mean difference (95% CI) All EP vs. Control	* P values EP vs. Control
FEV_1	*Z*	-0.31(-0.57, -0.04)	-0.65 (-0.90, -0.41)	-0.56 (-0.91, -0.21)	-0.73 (-1.10, -0.37)	-0.35 (-0.70, 0.01)	0.04
FVC	*Z*	-0.16 (-0.42, 0.09)	-0.17 (-0.41, 0.07)	-0.17 (-0.48, 0.13)	-0.17 (-0.54, 0.20)	-0.01 (-0.36, 0.33)	0.96
FEV_1/FVC	*Z*	-0.30 (-0.54, -0.05)	-0.80 (-1.07, -0.54)	-0.69 (-1.06, -0.31)	-0.90 (-1.29, -0.52)	-0.52 (-0.89, -0.16)	0.005
$FEF_{25–75}$	*Z*	-0.53 (-0.79, -0.27)	-1.14 (-1.39, -0.89)	-1.04 (-1.40, -0.68)	-1.22 (-1.58, -0.87)	-0.63 (-0.98, -0.27)	0.001
Raw	*Z*	0.68 (0.51, 0.85)	1.23 (0.77, 1.68)	0.96 (0.72, 1.21)	1.45 (0.61, 2.29)	0.54 (0.05, 1.04)	0.58
Reversibility[a]	*% change (FEV_1)*	5.0 (3.9, 6.1)	6.6 (4.3, 8.8)	5.2 (2.9, 7.4)	7.8 (4.0, 11.5)	1.6 (-0.9, 4.1)	0.18
DRS[b]	*Geometric mean*	3.47 (2.19, 5.50)	13.18 (8.16, 21.37)	11.48 (5.75, 23.44)	14.79 (7.24, 29.51)	3.80 (2.00, 7.24)	<0.001
TLC	*Z*	0.45 (0.13, 0.76)	0.30 (0.05, 0.55)	0.17 (-0.11, 0.46)	0.41 (0.00, 0.82)	-0.15 (-0.55, 0.25)	0.32
FRC	*Z*	-0.34 (-0.74, 0.06)	0.07 (-0.27, 0.41)	-0.26 (-0.67, 0.15)	0.36 (-0.16, 0.89)	0.41 (-0.11, 0.92)	0.06
RV	*Z*	0.27 (-0.09, 0.63)	0.003 (-0.30, 0.30)	0.05 (-0.40, 0.51)	-0.04 (-0.47, 0.38)	-0.27 (-0.73, 0.20)	0.17
RV/TLC	*Ratio*	26.2 (24.4, 27.9)	25.9 (24.0, 27.9)	26.9 (24.0, 29.8)	25.2 (22.4, 27.9)	-0.2 (-2.8, 2.4)	0.75
DLCO	*% predicted*	88.2 (84.3, 91.7)	86.5 (80.6, 92.4)	87.4 (81.4, 93.3)	85.7 (75.0, 96.4)	-1.6 (-8.5, 5.2)	0.68
VA	*% predicted*	97.1 (91.4, 102.9)	97.8 (91.8, 103.7)	92.6 (83.7, 101.4)	102.7 (94.7, 110.8)	0.6 (-7.5, 8.7)	0.65
KCO	*Mmol/kPa. min*	1.74 (1.66, 1.82)	1.69 (1.61, 1.77)	1.77 (1.67, 1.87)	1.61 (1.48, 1.74)	-0.05 (-1.6, 0.06)	0.32
	% predicted	80.0 (76.7, 83.3)	76.3 (72.5, 80.2)	80.0 (75.3, 84.8)	72.8 (66.9, 78.8)	-3.6 (-8.6, 1.3)	0.13
$Fe_{NO\ 0.05}$	*G. mean*	11.77 (9.53, 14.54)	9.65 (7.97, 11.69)	10.46 (7.59, 14.43)	9.08 (7.08, 11.65)	-1.22 (-1.62, 1.09)	0.18
C_ANO	*ppb*	1.48 (1.22, 1.74)	1.25 (0.97, 1.54)	1.30 (0.85, 1.74)	1.22 (0.82, 1.61)	-0.16 (-0.56, 0.25)	0.24
$J_{aw}NO$	*nl/sec*	0.77 (0.57, 0.96)	0.86 (0.54, 1.17)	0.83 (0.47, 1.19)	0.88 (0.36, 1.41)	0.09 (-0.28, 0.46)	0.62
$Fe_{NO\ nasal}$	*ppb*	805 (696, 915)	825 (745, 905)	901 (758, 1044)	764 (675, 854)	20 (-115, 155)	0.73

Figures are observed means (95% confidence intervals), unless otherwise stated. For abbreviations, please see list. Lung diffusion data reported as % predicted and as raw-data, and nitric oxide (NO) data only as raw-data, due to suboptimal reference equations for children.

* Interaction terms testing differences between EP and matched term-born controls in the group with BPD vs. without BPD were non-significant for all variables, and therefore only the p-values for all EP vs. term-born controls were reported.

[a] Reversibility is given as percentage change in FEV_1 after vs. before administration of beta agonist, assuming the pre-value is baseline.

[b] DRS is the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (μmol) of methacholine (%/μmol), reported as geometric means due to a highly skewed distribution.

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Table 4. Lung function variables reflecting airway flow before and after administration of salbutamol, and bronchial responsiveness to methacholine, in 11 year old children born preterm (EP) in 1999–2000, split by gestational age (GA) at birth.

		Controls N = 54	GA ≤ 25 weeks N = 10	GA 26–27 weeks N = 36	GA ≥ 28 weeks N = 11
Gestational age (SD)	*weeks*	-	24.4 (0.5)	26.7 (0.5)	29.2 (1.0)
Birth weight (SD)	*grams*	-	741.2 (107)	904.1 (177)	770.1 (146)
BW z-scores (SD)		-	0.09 (0.9)	-0.54 (1.0)	-2.46 (0.7) ***
No. with SGA[a]	*(% of group)*		1 (10)	8 (22)	11 (100)
No. with BPD	*(% of group)*	-	8 (80)	20 (56)	3 (27) ***
FEV_1	*z*	-0.27 (-0.52, -0.01)	-0.46 (-1.28, 0.37)	-0.54 (-0.82, -0.27)	-1.22 ** (-1.84, -0.59)
	z-Post-β_2	-0.07 (-0.34, 0.21)	0.05 (-1.04, 1.13)	-0.41 (-0.82, 0.01)	-0.67 (-1.29, -0.04)
FEV_1/FVC	*z*	-0.30 (-0.54, -0.05)	-0.13 (-0.98, 0.73)	-0.82 * (-1.12, -0.52)	-1.38 ** (-1.93, -0.83)
	z- Post-β_2	0.22 (0.00, 0.44)	0.39 (-0.39, 1.18)	-0.23 (-0.58, 0.13)	-0.31 (-0.89, 0.28)
$FEF_{25–75}$	*z*	-0.52 (-0.78, -0.27)	-0.92 (-1.59, -0.25)	-1.07 * (-1.37, -0.77)	-1.78 ** (-2.34, -1.22)
	z- Post-β_2	0.06 (-0.20, 0.31)	-0.07 (-0.88, 0.74)	-0.58 (-0.91, 0.24)	-0.50 (-1.17, 0.17)
DRS	*geometric mean*	3.48 (2.21, 5.47)	3.58 (1.11, 14.19)	12.74 ** (6.95, 23.33)	38.54 ** (1.74, 85.31)

Figures are observed means (95% confidence intervals) split by GA categories for those lung function variables that differed between the EP and term-born groups. For abbreviations, please see list. Interaction terms, testing if differences between EP and matched term-born controls were different over the three GA categories, were non-significant for all variables. * p-values testing differences between EP-born subgroups, or (when possible) EP-born subgroups vs. control subjects,

* p<0.05,

** p<0.01,

*** p<0.001.

[a] SGA = small for gestational age.

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controls. DRS was negatively and similarly associated with z-FEV_1 ($p<0.001$) in the EP- and term-born groups (test of interaction, p = 0.21). Static lung volumes (z-TLC, z-FRC, z-RV and RV/TLC), Fe_{NO} and DLCO did not differ significantly between the EP-born and term-born groups. Nine subjects (7 EP-born; 5 BPD and 2 non-BPD, and 2 term-born) had ≥12% increase in FEV_1 on the salbutamol reversibility test (EP vs. term-born, p = 0.18). Mean FEV_1 change before vs. after salbutamol did not differ between those born EP and at term, and responses were negatively associated with z-FEV_1 ($p<0.001$) in both groups (test of interaction, p = 0.24).

There was no effect of neonatal BPD vs. no BPD on any of the assessed lung function variables (non-significant interaction-terms). Lung function variables that differed between the EP- and term-born groups were analyzed also by GA-category, and the deficits were numerically most pronounced for the GA-category ≥28 weeks, although interaction terms were non-significant (Table 4).

Lung function related to perinatal and background variables

For $EP_{1999–2000}$, BW z-score was the only significant predictor of z-FEV_1 at 11 years (B = 0.24; p = 0.04; $R^2 = 0.10$) in a regression model including the following perinatal variables: GA, BW z-scores, days on ventilator, days on oxygen treatment, use of antenatal and postnatal corticosteroids, surfactant treatment and BPD vs. no-BPD. Applying the same model on the $EP_{1991–1992}$, BW z-scores similarly predicted z-FEV_1 (B = 0.35; p = 0.02) in a final model; however, including also days of oxygen (B = -0.011; $p<0.001$) and antenatal corticosteroids (B = 0.59; p = 0.03) ($R^2 = 0.48$, all three variables). Maternal smoking in pregnancy was not associated with z-FEV_1 at age 11. Regressing the background variables asthma ever, asthma medication

last 12 months, wheeze last 12 months, atopy and maternal smoking in pregnancy on z-FEV_1 at age 11, revealed no significant associations for either EP or term-born in any birth-cohort.

Differences between $EP_{1991–1992}$ and $EP_{1999–2000}$

Paired differences between EP-born and matched term-controls for the inclusion periods 1991–1992 and 1999–2000 are given in Table 5 and illustrated in Figs 2 and 3. The differences were significantly smaller for the variables z-FEV_1, z-FVC and z-$FEF_{25–75}$ and RV/TLC for the group *with BPD* born in 1999–2000 compared to the group *with BPD* born in 1991–1992 (tests of interaction). Adjusting these interaction terms for differences between the study periods regarding GA, BW, days on ventilator and PDA management did not influence conclusions, whereas the effect disappeared for z-FEV_1 when surfactant and/or antenatal corticosteroids were included in the model. Differences between EP-born *without BPD* and their matched term-controls did not differ between two inclusion periods.

Discussion

This study showed that respiratory outcomes in mid-childhood were encouraging in children born at extremely low GAs or BWs in 1999–2000. Still, the preterm born participants had more small-airway obstruction and bronchial hyperresponsiveness than matched term-controls, but static lung volumes, diffusing capacity and exhaled nitric oxide did not differ. Outcome data were in most respects unrelated to BPD, and those born most immature did surprisingly well. Compared to children born similarly preterm in the same region in 1991–1992, lung function data were generally better, and particularly for those with neonatal BPD, where significant improvements had occurred for important variables.

Strengths and limitations

The major strengths of this study were the population-based design with almost complete attendance, and recruitment of matched control groups that followed a strict algorithm based on the 'next-born-subject' principle, minimizing risks of selection bias. An age difference of approximately one year at follow-up between the two birth-cohorts was adjusted for by reference equations or by statistical models. Potential bias introduced by a two-center design was limited by statistical analyses performed according to the matched preterm versus term-born structure. The algorithm for inclusion was based on a combined use of GA and BW, initially preferred in 1991–1992 to ensure inclusion of all children perceived to be extremely immature at birth. We continued this approach in 1999–2000, although changing the GA criterion from $<$29 weeks to $<$28 weeks. Due to the inclusion criteria the data may not be generalizable to subjects born extremely preterm in general. A diagnosis of BPD must reflect oxygen treatment algorithms, which to some extent is subject to department policy. In this study, the same senior neonatologists operated the same algorithms for oxygen supplementation in both inclusion periods, making systematic changes unlikely. The study could not discriminate moderate from severe BPD since oxygen treatment at 36 weeks GA was provided through low flow nasal cannulas without recording the exact fraction of inspired oxygen (FiO_2) [3]. By nature, unfavourable events or conditions tend to be linked in neonatal intensive care medicine, often setting up vicious circles that require treatments with long-term side effects. Observational statistical models struggle with these scenarios due to collinearity between variables. To limit spurious associations we therefore excluded the independent neonatal variable considered least meaningful if a correlation coefficient was ≥ 0.7 in the same analysis. This situation calls for cautious interpretation of regression models that aim to address statistical associations between such variables and outcome. The study may be criticized for having few participants. Longitudinal

Table 5. Comparison of lung function indices in two cohorts of children born preterm in 1991–92 ($EP_{1991–92}$) and in 1999–2000 ($EP_{1999–2000}$).

	$EP_{1991–1992}$ cohort			$EP_{1999–2000}$ cohort			
	Control vs. all-EP	Control vs. EP no-BPD	Control vs. EP BPD	Control vs. all-EP	Control vs. EP no-BPD	Control vs. EP BPD	* p-value, control vs. EP BPD
FEV_1; z-score	0.86 (0.44, 1.26)	0.53 (-0.00, 1.07)	1.46 (0.80, 2.13)	0.35 (-0.01, 0.70)	0.25 (-0.30, 0.79)	0.43 (-0.08, 0.94)	0.02
FVC; z-score	0.44 (0.07, 0.82)	0.41 (-0.11, 0.93)	0.51 (-0.14, 1.16)	0.01 (-0.33, 0.36)	0.01 (-0.53, 0.54)	0.01 (-0.49, 0.52)	< 0.001
FEV_1/FVC; z-score	0.57 (-0.03, 1.18)	0.08 (-0.60, 0.77)	1.51 (0.65, 2.36)	0.52 (-0.16, 0.89)	0.41 (-0.15, 0.97)	0.62 (0.09, 1.15)	0.03
$FEF_{25–75}$; z-score	0.94 (0.47, 1.42)	0.54 (-0.07, 1.14)	1.72 (0.96, 2.47)	0.63 (0.27, 0.98)	0.52 (-0.02, 1.06)	0.72 (0.20, 1.23)	0.04
TLC; z-score	0.10 (-0.26, 0.45)	0.24 (-0.24, 0.72)	-0.18 (-0.76, 0.43)	0.15 (-0.25, 0.55)	0.28 (-0.33, 0.88)	0.04 (-0.54, 0.62)	0.74
RV; z-score	-0.17 (-0.56, 0.21)	0.16 (-0.33, 0.65)	-0.79 (-1.39, -0.19)	0.27 (-0.20, 0.73)	0.21 (-0.49, 0.92)	0.31 (-0.37, 0.99)	0.02
RV/TLC	-1.27 (-4.94, 0.11)	-0.59 (-3.93, 2.74)	-5.77 (-9.87, -1.66)	0.21 (-2.40, 2.82)	-0.69 (-4.67, 3.28)	1.01 (-2.82, 4.84)	0.03
DRS; geometric mean	5.01 (2.19, 11.22)	3.47 (1.17, 10.47)	11.22 (2.69, 47.86)	3.80 (2.00, 7.24)	3.31 (1.20, 8.71)	4.27 (1.62, 10.96)	0.36

Figures are mean differences (95% confidence intervals) between EP born subgroups and their respective matched term-born control subjects. The units are z-scores, except the ratio RV/TLC and the variable DRS where figures are differences in the dose response slope to methacholine. Positive figures indicate higher z-scores for the control group or higher DRS for the EP-born group.

* Interaction terms, testing if differences between EP and matched term-born controls were different in the two study periods (1991–92 vs. 1999–2000) were non-significant, except for the groups with neonatal BPD, for which p-values are given.

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studies over decades are by nature difficult to perform as demonstrated by the paucity of similar data. The power calculations indicate reasonable detection limits.

Lung function in the children born preterm in 1999–2000

The lung function abnormalities in the 11 year old children born preterm in 1999–2000 were minor. FEV_1 was mostly within normal limits and they had no signs of abnormal volume distribution or diffusing capacity for carbon monoxide. However, forced mid-expiratory flow ($FEF_{25–75}$) in the range of 75% predicted and significant methacholine hyperresponsiveness indicate that small airway airflow limitation and bronchial abnormalities were still present. Those born most immature did surprisingly well, in that lung function variables did not differ significantly from those of the term-controls. Moreover, the presence of neonatal BPD was not significantly associated with outcome, contrasting some [12, 32], and consistent with one previous study [11]. Most of the differences between preterm and term-born groups seemed to be explained by the *highest* GA-category; i.e. basically SGA infants with GA $\geq$28 weeks who were included on the basis of BW $<$1000 gram, in whom significant airway obstruction and remarkably high bronchial hyperresponsiveness were observed. In adjusted regression models, BW z-score was the only remaining neonatal variable associated with FEV_1 at age 11. Thus, within the frames of todays advanced NICU management, the paradigm that neonatal BPD or extreme immaturity is inevitably linked to poor long-term pulmonary outcome may need to be revised. However, statements regarding causal pathways for novel findings are bound to be speculations within the frames of a study of this kind.

Interestingly, administration of salbutamol increased all airflow variables to within 0.5 z-score of zero; i.e. clearly within normal limits. Salbutamol responses were negatively and

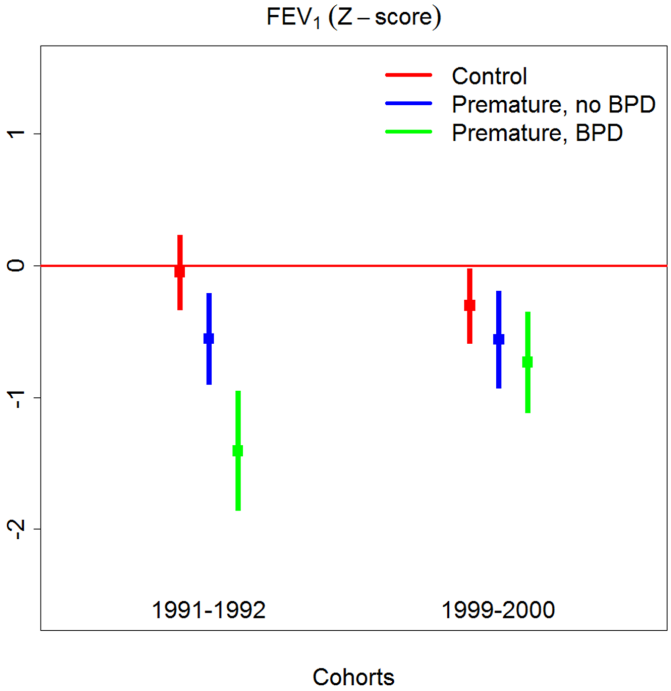

Fig 2. Z-FEV$_1$ in 11 year old term and preterm-born (EP) subjects born in 1991–92 and 1999–2000, EP-born split by the presence of BPD.

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similarly associated with FEV$_1$ in the EP-born and term-control group, further reducing the group differences. Few subjects had salbutamol responses considered clinically significant (≥12% from baseline) [30], and on average the responses did not differ between the EP- and term-born group. The question to what extent airway obstruction after EP birth is a fixed or reversible phenomenon therefore remains unanswered.

Pulmonary abnormalities after EP birth may clinically mimic pediatric asthma, and studies have reported increased respiratory symptoms and more hospital admissions, particularly during the first few years of life [33–35]. In this group of 11 year old children born EP in 1999–2000, current respiratory symptoms and use of asthma medication were reassuringly rare, also in those who were most immature at birth, contrasting some previous studies (8, 12, 36). Childhood asthma is generally characterized by eosinophilic airway inflammation (17), which may be assessed by fractional exhaled nitric oxide (FeNO) (25), with extended FeNO analyses as a new promising tool (26). We found that neither FeNO nor alveolar NO differed between the preterm and term-born group, providing support for the notion that eosinophilic airway inflammation is not involved in lung disease after EP birth (10, 37). However, active inflammatory mechanisms may still be involved, and recent studies have indicated increased oxidative stress in the respiratory system after EP birth, pathways this study was not set up to explore (38, 39).

Some authors have expressed optimism regarding long-term pulmonary outcomes in children who were born EP more recently [11, 36]. Kotecha et al. found in their review milder

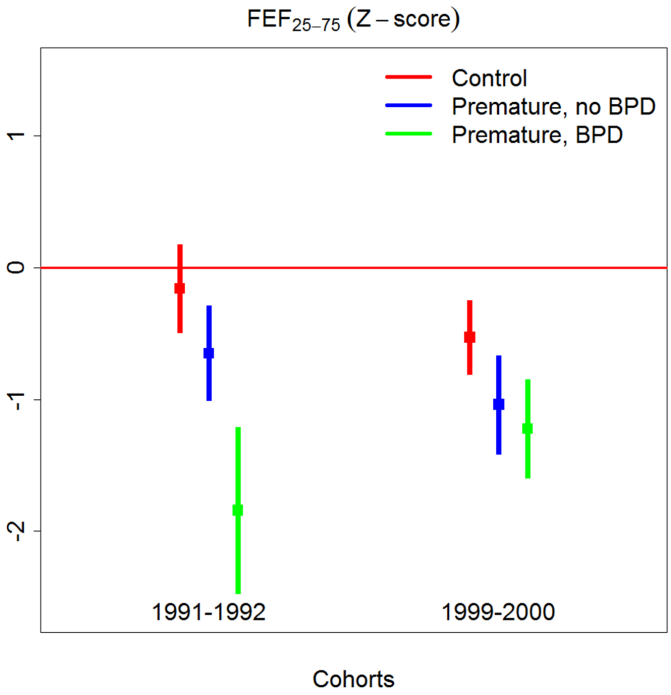

Fig 3. Z-FEF$_{25–75}$ in 11 year old term and preterm-born (EP) subjects born in 1991–92 and 1999–2000, EP-born split by the presence of BPD.

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impairments in FEV$_1$ for preterm born children with neonatal BPD (defined by need of oxygen treatment at 28 days of age) in recent compared to earlier studies [36]. However, two research groups reported data indicating that respiratory abnormalities are still present after EP birth; one regional study of children born at GA < 28 weeks or BW < 1000 grams in 1997 in the state of Victoria, Australia [12, 32], and another based on the EPICure study of children born atGA < 26 weeks in 1995 in the UK or Ireland [10, 32]. Both studies reported significantly lower z-FEV$_1$ and z-FEF$_{25–75}$ in preterm compared to term-born children. The Australian study [12] compared children born in 1997 with a group born in 1991–92, and found overall airflow limitation in both groups, but for those with no history of neonatal BPD, the children born in 1997 had better z-FEV$_1$ and z-FVC than those born in 1991–92. In light of the possibility that more children at risk of unfavorable outcome may have survived in the most recent cohort, this may be interpreted as a positive development. These findings are in line with ours, although we found this positive development particularly evident in the group *with* neonatal BPD. The EPICure study had more extreme inclusion criteria, with mean GA at birth almost two weeks lower (24.9) and BW approximately 100 gram lower (750 gram) than the EP$_{1999–2000}$ cohort of our study, and also a higher rate of neonatal BPD; i.e. 70% compared to 50%. They found no differences between preterm and term-born participants regarding FRC, TLC and alveolar volume, which is comparable to our findings, but elevated RV/TLC and some impairments in DLCO and DLCO/VA; differences that we did not see as clearly in our EP$_{1999–2000}$ cohort but resembling our findings at ten years of age in the EP$_{1991–1992}$ cohort [16]. One

may speculate that more infants born at the limits of viability in the EPICure study may have contributed to these differences in findings.

Comparing lung function of children born preterm in 1991–1992 and 1999–2000

There were significant improvements for z-FEV_1 and other important lung function variables from 1991–1992 to 1999–2000. This was particularly evident for the group of children with neonatal BPD, who presumably had the most turbulent neonatal history. The interaction terms used to assess these improvements were robust for adjustment for most perinatal variables, except that use of antenatal corticosteroids and surfactant eliminated the improvements in z-FEV_1. Thus, statistical modeling of the dataset suggested that more extensive use of these modalities may partly explain the observed improvement with time. However, a limitation that applies to studies of this kind is that a variety of known and unknown influences of possible significance for outcome cannot be accounted for in the applied regression models. Thus, more and larger studies are required to resolve this issue.

The inclusion algorithms varied slightly between the two preterm-born cohorts. Despite this, the two groups had fairly similar BWs and GAs, although with more SGA children included in 1999–2000. More SGA children and more extreme criteria as regards GA in 1999–2000 should theoretically lead to worse outcomes, whereas the opposite was in fact observed. Thus, one may argue that this *strengthens* the notion that outcome did in fact improve for the average infant born preterm during the 1990s.

The field of neonatal intensive care is in constant change, and it has been suggested that pushing the limits of viability might have masked improvements in outcome [4], although some recent studies have indicated otherwise [12, 37]. We have previously reported respiratory health and lung function data for subjects born EP in the early 1980s and 1990s and suggested improvements with time in groups *without* neonatal BPD [13]. The present study which also included children born in this millennium, indicates that improvements has also occurred in children *with* BPD, i.e. the infants with the most turbulent neonatal history.

Conclusion

Eleven year old children born at extremely low GAs or BWs in 1999–2000 had more small-airway obstruction and bronchial hyperresponsiveness than matched term-controls, but lung volumes and diffusing capacity were similar. Outcome was mostly unrelated to BPD, and those born most immature did surprisingly well. Compared to a group born similarly preterm in the same region nearly one decade earlier, lung function was generally better, particularly after neonatal BPD. The findings indicate that infants born preterm in this millennium may have better pulmonary prognosis than previously assumed, and that BPD or extreme immaturity may not necessarily be linked to poor lung function in mid-childhood.

Supporting Information

S1 File.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: MV HC OR KØ TM TH. Performed the experiments: MV HC OR KØ TM TH. Analyzed the data: MV TM TH. Contributed reagents/materials/analysis tools: KS ES. Wrote the paper: MV TM TH.

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Children born preterm at the turn of the millennium had better lung function than children born similarly preterm in the early 1990s

S1 Supporting information

Materials and Methods

Background information

During the period from the early to the late 1990s, there was an increasing use of surfactant and a change from synthetic (Exosurf) to the presumably better natural surfactant (Curosurf), and also more extensive use of antenatal corticosteroids This was noted in the main text of this article. Furthermore, the 1990s were characterized by a large number of relatively small changes in NICU care, more than by single big leaps in development. Most areas of neonatal intensive care medicine had gone through refinements, such as better standardization of antenatal and perinatal care, a higher level of competence among neonatologists and nurses regarding the special needs of these vulnerable infants, and better exploitation of technological advances, such as patient coordinated assisted ventilation and various forms of oscillation. In 1991-1992 oscillation had recently been introduced with ventilators capable of exploiting this technique, in our department Infant Star and SensorMedics. Seven years later these techniques were still in use, although with even more advanced ventilators, and better techniques for patient coordinated ventilation had been developed. Moreover, the skills with which these techniques were used had improved, based on the improved knowledge that had accumulated during the 1990s.

Subjects, data collection and definitions

Asthma medication included inhaled corticosteroids and short or long acting beta 2 agonists (in separate or combined device), anticholinergics and oral leukotriene modifiers (Singulair®). Atopy was defined as minimum one positive skin prick test (SPT) in a panel of relevant airway allergens. Maternal smoking in pregnancy was defined by self-reported daily or occasional smoking during pregnancy with the index subject.

Current height (cm) was measured with a stadiometer and weight (kg) with an electronic weight, and standardized for gender and age [1]. The age was calculated as the difference between the examination date and the birth date using the automated method provided by SPSS, with values entered into the reference equations using two decimals.

Lung function measurements

Subjects born in 1999-2000 ($EP_{1999-2000}$) were seen twice in 2010-2012 at the University Hospitals in Bergen or Stavanger, according to place of birth, with pediatric examination, comprehensive lung function tests, skin prick tests for allergy and anthropometric measurements. Subjects born in 1991-1992 ($EP_{1991-1992}$) went through similar examinations (except nitric oxide measurements) using similar equipment and testing procedures in 2001-2002. Subjects were rescheduled if respiratory symptoms suggestive of an obstructive exacerbation or a viral infection were suspected during the past two weeks. Inhaled corticosteroids and short-acting beta 2 agonists were stopped 1 day prior to testing, and long-acting beta 2 agonists and leukotriene modifiers 2 days prior to testing.

Spirometry and pulmonary diffusing capacity for carbon monoxide (*D*LCO) (single breath method) were measured with Vmax 22 equipment (*SensorMedics Inc, Anaheim, USA*), static

lung volumes with V6200 Autobox Body Plethysmograph (*SensorMedics Inc, Anaheim, USA*), all in sitting position, wearing a nose clip, applying standard quality criteria [2-6]. The spirometry values from the second test day were used, for those subjects (2 EP and 3 term-born) that declined (or were unable to perform) after the first day, values from the first test day were used. Measurements were standardized for age, height and gender [7, 8], and KCO reported as raw-data. Variables recorded were forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}), total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV), airway resistance (Raw), diffusion capacity for CO (DLCO), alveolar volume (VA), and diffusion capacity adjusted for alveolar volume (KCO).

Fractional exhaled nitric oxide (Fe_{NO}) at an exhalation rate of 50 ml/sec was measured with Exhalyzer CLD-88 (*EcoMedics, Switzerland*), according to ATS/ERS recommendations [9]. The test was commenced before spirometry and the other lung function tests. NO-free air was inhaled to near total lung capacity, followed immediately by full exhalation through a dynamic flow restrictor with a target flow of 50 ml/second for at least 6 seconds, in standing position, without wearing a nose clip. Fe_{NO} (ppb) was calculated as the mean value from 3 measurements with coefficient of variation (CV) within 10% acceptability, or if this was not applicable, from the curve with the most stable and horizontal plateau phase of expiration. If the subjects had measures from both test days, mean values from the two days were used for statistical analysis. Fe_{NO} values were in statistical analyses considered log-normally distributed and reported as geometric means. Mathematical models can be used to differentiate the sites of NO production in the respiratory system, specifically bronchial vs. alveolar NO [10, 11]. Alveolar NO (ppb) (CA_{NO}) and bronchial flux of NO (nl/sec) (Jaw_{NO})

were calculated using three different flows (30, 100 and 300 ml/sec), and nasal Fe_{NO} was measured, by exhalation through a nasal prong.

Methacholine provocation (PD20) was performed with an inhalation-synchronised dosimetric nebulizer (*SPIRA Electra, Finland*), providing baseline FEV_1 ≥65% of predicted [12, 13]. Baseline lung function measurements were obtained in sitting position after saline inhalation, followed by inhalation of doubling doses of methacholine via the *SPIRA* dosimeter with controlled tidal breathing according to ATS guidelines. The children inhaled at a flow 0.5 l/s, the aerosolisation started when 100 ml air was inhaled from functional respiratory capacity and the aerosol delivery time was set to 0.5 seconds. The first dose was 0.05 µmol methacholine and the test continued until a fall ≥20 % compared to post-saline (baseline) FEV_1, or until the maximum dose of 11.5 µmol methacholine was reached. Dose-response slope (DRS) was calculated as the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (µmol) of methacholine (%/µmol); in statistical analyses considered log-normally distributed and reported as geometric means [14]. Reversibility to salbutamol was given as percentage change in FEV_1 after vs. before administration of 0.1 mg/10 kg salbutamol (*Ventoline*) from a metered dose inhaler via a spacer (*Volumatic*), assuming the pre-value as baseline. The test was commenced on a separate day from the PD20 test.

Reversibility to salbutamol was assessed by measuring FEV_1 before (baseline) and 10-15 minutes after administering 0.1 mg/10 kg salbutamol (*Ventoline*) from a metered dose inhaler via a spacer (*Volumatic*), an increase ≥12% ((FEV_1 value after - FEV_1 value before) x 100/ FEV_1 value before) was considered positive response [15].

Skin prick tests for house dust mite (*D. Farinae* and *D. Pteronyssinus*), animal dander (cat, dog, horse), pollens (timothy, birch and mugwort) and moulds (*Alternaria* and *Cladesporium*)

were done with standard extracts (Soluprick®SQ, ALK-Abello AS, Hørsholm, Denmark) in accordance with European guidelines[16]. Histamine (10 mg/mL) and the allergen diluent were used as positive and negative controls. A reaction was judged positive if mean of the two perpendicular weal diameters was at least 3.0 mm.

Statistical methods

Means with standard deviations (SD) or 95% confidence intervals (95% CI), medians with ranges, counts with group percentages were calculated. Groups were compared by independent sample t-test, Mann-Whitney U-test, Fisher's exact 2-sided mid-p value or odds ratios (OR), as appropriate. Paired data were compared with the mixed linear model of SPSS, allowing for contributions also from pairs with missing data, using interaction terms to test if effects or differences differed between the various subgroups of the study, that is neonatal BPD vs. no BPD, GA categories (GA ≤25 vs. 26-27 vs. ≥28 weeks) and birth-cohorts ($EP_{1999-2000}$ vs. $EP_{1991-1992}$), a positive interaction would mean that the difference between term-born and EP-born would differ between either BPD groups, GA groups or birth cohorts. The study had 80 % power to detect a difference in FEV_1 z-scores of 0.50 if 60 cases were included in the $EP_{1999-2000}$ cohort, providing a two-sided significance level of 0.05.

Multiple backward regression models were constructed to address potential associations between perinatal data or background data vs. lung function data at age 11, primarily with z-FEV_1 as outcome as it is considered the most robust index of airway obstruction. Variables were entered into the regressions if their individual association with the dependent had a p-value <0.1.

SPSS (version 21.0) was used for computations.

Results

Subjects

Two of the 61 children born EP in 1999-2000 could not be traced, two were excluded due to severe cerebral palsy, none of whom were able to cooperate when doing lung function testing, and a third child was unable to cooperate to other than spirometry. All but three participating subjects were Caucasian.

120 **Table A**: Perinatal data comparing children born preterm in 1991-92 ($EP_{1991-92}$) and in 1999-2000 ($EP_{1999-2000}$).

		$EP_{1991-92}$ cohort	**$EP_{1999-2000}$ cohort**	**Cohort difference**	**p-value[a]**
Subjects; n (%)	Control	35	54		
	All EP	35	57		
	- non BPD	23 (66)	26 (46)	20 (-4, 44)	0.067
	- BPD	12 (34)	31 (54)	20 (-13, 53)	
Female gender; n (%)	Control	22 (63)	25 (46)	17 (-4, 38)	0.135
	All EP	22 (63)	28 (49)	14 (-7, 35)	0.209
	- non BPD	17 (74)	16 (62)	12 (-14, 38)	0.379
	- BPD	5 (42)	12 (39)	3 (-30, 36)	0.860
Birthweight, gram; mean (SD)	Control	3564 (275)	3701 (434)	137 (-28, 301)	0.073
	All EP	933 (204)	850 (175)	-84 (-163, -4)	0.039
	- non BPD	976 (195)	873 (200)	-104 (-218, 10)	0.073
	- BPD	851 (203)	831 (151)	-20 (-134, 94)	0.722
Birthweight; sds-score	All EP	-0.36 (0.9)	-0.80 (1.3)	-0.44 (-0.93, 0.04)	0.054
	- non BPD	-0.37 (0.9)	-0.97 (1.4)	-0.60 (-1.27, 0.08)	0.076
	- BPD	-0.32 (0.9)	-0.66 (1.2)	-0.33 (-1.10, 0.44)	0.386
Gestational age, weeks; mean (SD)	All EP	26.7 (1.7)	26.8 (1.6)	0.01 (-0.7, 0.7)	0.974
	- non BPD	27.2 (1.7)	27.3 (1.6)	0.1 (-0.8, 1.1)	0.788
	- BPD	25.8 (1.5)*	26.3 (1.4)**	0.4 (-0.5, 1.4)	0.381
Small for gestational age (SGA); n (%)	All EP	5 (14)	20 (35)	21 (2, 40)	0.030
	- non BPD	3 (13)	12 (46)	33 (7, 58)	0.015
	- BPD	2 (17)	8 (26)	9 (-19, 37)	0.570
Postnatal days with oxygen treatment; median (range)	All EP	49 (2-180)	65 (0-250)	1.8 (1.0, 3.0)	0.109
	- non BPD	34 (2-70)	44 (0-78)	1.9 (-1.1, 3.4)	0.254
	- BPD	92 (61-180)	79 (44-250)	-1.3 (-1.6, -1.2)	0.080
Ventilator days; median (range)	All EP	4.0 (0-55)	5.0 (0-24)	1.2 (-1.4, 2.2)	0.618
	- non BPD	1.3 (0-40)	2.5 (0-21)	1.7 (-1.2, 3.2)	0.212
	- BPD	12.7 (2-55)***	8.0 (0-24)***	-1.8 (-3.1, -1.1)	0.011
Antenatal corticosteroids; n (%)	All EP	15/34 (44)	46 (81)	37 (17, 57)	<0.001
	- non BPD	11 (48)	21 (81)	33 (6, 60)	0.005
	- BPD	4/11 (36)	25 (81)	45 (17, 73)	0.012
Surfactant; n (%)	All EP	17 (49)	49/56 (88)	39 (20, 58)	<0.001
	- non BPD	7 (30)	20/25 (80)	50 (22, 78)	0.001
	- BPD	10 (83)	29 (94)	11 (-8, 30)	0.367
Postnatal corticosteroids; n (%)	All EP	10 (29)	18 (32)	3 (-16, 22)	0.772

	- non BPD	2 (9)	1 (4)	5 (-9, 19)	0.549
	- BPD	8 (67)	17 (55)***	12 (-21, 45)	0.510
Closing of PDA; n (%)	All EP	17 (49)	13 (23)	26 (6, 46)	0.013
	- non BPD	7 (30)	3 (12)	18 (-5, 41)	0.122
	- BPD	10 (83)	10 (32)	51 (18, 84)	0.004
Maternal smoking in pregnancy; n (%)	Control	9 (26)	-	-	-
	All EP	13 (37)	13/52 (25)	12 (-8, 32)	0.205
	- non BPD	10 (43)	5/24 (21)	22 (-5, 49)	0.111
	- BPD	3 (25)	8/28 (29)	4 (-26, 34)	0.957

Figures are means (SD), medians (ranges) or counts (%). [a] The p-value denotes differences between those born in 1991-92 and in 1999-2000. * P-values for group differences between EP non BPD vs. EP BPD within each cohort, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table B: Anthropometric data and respiratory symptoms at age 11 comparing children born preterm in 1991-92 ($EP_{1991-92}$) and in 1999-2000 ($EP_{1999-2000}$).

		$EP_{1991-92}$ cohort n=35	$EP_{1999-2000}$ cohort n=57	Cohort Difference	p-value[a]
Age; years	Control	10.6 (0.4)	11.7 (0.7)	1.1 (0.8, 1.4)	<0.005
	All EP	10.4 (0.4)	11.4 (0.6)	1.0 (0.8, 1.3)	<0.005
	- non BPD	10.4 (0.5)	11.4 (0.6)	1.0 (0.7, 1.3)	<0.005
	- BPD	10.4 (0.4)	11.5 (0.6)	1.1 (0.8, 1.4)	<0.005
Height;z-score	Control	0.02 (0.9)	0.00 (1.1)	-0.02 (-0.46, 0.42)	0.925
	All EP	-0.51 (1.2)*	-0.41 (1.0)*	0.10 (-0.36, 0.57)	0.654
	- non BPD	-0.52 (1.3)	-0.38 (1.0)	0.14 (-0.51, 0.79)	0.658
	- BPD	-0.49 (1.1)	-0.43 (1.1))	0.06 (-0.67, 0.79)	0.868
Weight; z-score	Control	0.17 (1.0)	-0.14 (1.1)	-0.31 (-0.73, 0.12)	0.153
	All EP	-0.48 (1.4)*	-0.33 (1.05)	0.15 (-0.37, 0.66)	0.570
	- non BPD	-0.40 (1.7)	-0.43 (1.0)	-0.02 (-0.80, 0.76)	0.956
	- BPD	-0.61 (0.8)	-0.25 (1.1))	0.37 (-0.34, 1.08)	0.303
BMI; z-score	Control	0.25 (0.9)	-0.22 (1.0)	-0.46 (-0.89, 0.04)	0.033
	All EP	-0.28 (1.4)	-0.16 (1.0)	0.12 (-0.39, 0.62)	0.644
	- non BPD	-0.18 (1.6)	-0.35 (1.1)	-0.17 (-0.93, 0.60)	0.660
	- BPD	-0.46 (0.9)	0.00 (1.0)	0.46 (-0.19, 1.11)	0.178
Asthma ever	Control	3 (9)	5 (9)	0 (-12, 12)	0.933
	All EP	12 (34)*	15 (26)*	8 (-11, 27)	0.427
	- non BPD	6 (26)	5 (19)	7 (-16, 30)	0.587
	- BPD	6 (50)	10 (32)	18 (-14, 50)	0.309
Asthma medication last 12 months	Control	1 (3)	3 (6)	3 (-6, 12)	0.838
	All EP	5 (14)	4 (7)	56 (35, 77)	0.075
	- non BPD	1 (4)	3 (12)	8 (-7, 23)	0.775
	- BPD	4 (33)	1 (3)	3 (13, 47)	0.006
Wheeze last 12 months	Control	2 (6)	5 (9)	3 (-8, 14)	0.587
	All EP	11 (31)*	8 (14)	17 (0-34)	0.055
	- non BPD	6 (26)	4 (15)	11 (-12, 34)	0.383
	- BPD	5 (42)	4 (13)	29 (18, 56)	0.060
Atopy	Control	8 (23)	20/50 (40)	17 (-14, 47)	0.105
	All EP	9 (26)	12/55 (22)	4 (-14, 22)	0.675
	- non BPD	8 (35)	6/25 (24)	11 (-15, 37)	0.435
	- BPD	1 (8)	6/30 (20)	12 (-13, 37)	0.415

Figures are means (SD), medians (ranges) or counts (%). [a] The p-value denotes differences between those born on 1991-92 and in 1999-2000. * P-values for group differences between term controls vs. all EP within each cohort, * $p<0.05$. Atopy registered as minimum one positive SPT (skin prick test) or IgE test in the $EP_{1991-92}$ cohort and as minimum one positive SPT in the $EP_{1999-2000}$ cohort.

Hospital admissions and respiratory symptoms in $EP_{1999-2000}$

Hospital admissions during the 5 years prior to inclusion were more common in the EP than the term-born group (16/55 vs. 7/53; p=0.044), more so for those at GA ≤25 weeks than GA 26-27 weeks (5/9 vs. 6/35, p=0.030), with no influence from BPD. Respiratory causes were given as reason for admittance in 2 EP and 1 term-born subject. More EP than term-born subjects had ever been diagnosed with asthma (15 vs. 5; OR 3.5; 95%CI 1.2, 10.4), but current asthma did not differ (6 vs. 4; OR 1.5, 95%CI 0.4, 5.5). Current asthma was present in 3/10, 2/36 and 1/11 of those born at GA ≤ 25 weeks, GA 26-27 weeks and GA ≥ 28 weeks, respectively; i.e. significantly more in the most immature group vs. the GA 26-27 weeks group (OR 7.29; 95%CI 1.02, 52.01). Respiratory symptoms were rare and similarly distributed between EP and term-born; i.e. respectively 8 vs. 5 (OR 1.60, 95%CI 0.49, 5.24) had wheeze the last 12 months, 10 vs. 3 (OR 3.79; 95%CI 0.98, 14.65) had used asthma medication after 5 years of age, and 5 vs. 2 (OR 2.50; 95%CI 0.46, 13.47) had lower respiratory tract infections treated by antibiotics after 5 years of age.

Lung function

Flow-volume loops were satisfactorily obtained from all participants, static lung volumes were successfully measured in 53/57 EP and 47/54 term-born subjects, bronchial hyperresponsiveness to methacholine (DRS) was successfully assessed in 55/57 EP and 50/54 term-born subjects, reversibility to salbutamol was successfully assessed in 56/57 EP and 53/54 term-born subjects, Fe_{NO} was successfully measured in 49/57 EP and 48/54 term-born subjects, with alveolar NO obtained from 45/57 and 43/54, respectively, missing data mainly due to equipment failure the first months of the study, and DLCO was successfully measured in in 43/57 EP and 45/54 term-born subjects.

Table C: Comparison of two cohorts of subjects born preterm eight years apart, in 1991-92 ($EP_{1991-92}$) and in 1999-2000 ($EP_{1999-2000}$).

		$EP_{1991-92}$			$EP_{1999-2000}$		
		Control	EP non BPD	EP BPD	Control	EP non BPD	EP BPD
FEV_1	z	-0.05 (-0.35, 0.25)	-0.56 (-0.91, -0.20)	-1.52 (-2.06, -0.97)	-0.31 (-0.57, -0.04)	-0.55 (-0.88, -0.22)	-0.73 (-1.10, -0.37)
FVC	z	-0.05 (-0.35, 0.25)	-0.57 (-0.90, -0.23)	-1.54 (-2.08, -1.00)	-0.16 (-0.42, 0.09)	-0.17 (-0.48, 0.13)	-0.17 (-0.54, 0.20)
FEV_1/FVC	z	0.10 (-0.21, 0.42)	0.02 (-0.42, 0.46)	-1.40 (-2.01, -0.80)	-0.30 (-0.54, -0.05)	-0.69 (-1.06, -0.31)	-0.90 (-1.29, -0.52)
FEF_{25-75}	z	-0.22 (-0.54, 0.10)	-0.74 (-1.10, -0.39)	-1.92 (-2.49, -1.36)	-0.53 (-0.79, -0.27)	-1.04 (-1.40, -0.68)	-1.22 (-1.58, -0.87)
DRS	G.mean*	3.0 (1.7, 5.2)	10.8 (5.3, 22.0)	33.4 (10.6, 105.0)	3.47 (2.19, 5.50)	11.48 (5.75, 23.44)	14.79 (7.24, 29.51)
TLC	z	0.18 (-0.15, 0.52)	-0.06 (-0.37, 0.26)	0.37 (-0.12, 0.86)	0.45 (0.13, 0.76)	0.17 (-0.11, 0.46)	0.41 (0.00, 0.82)
FRC	z	-0.20 (-0.61, 0.20)	-0.71 (-1.18, -0.25)	-0.24 (-0.50, 0.98)	-0.34 (-0.74, 0.06)	-0.26 (-0.67, 0.15)	0.36 (-0.16, 0.89)
RV	z	-0.31 (-0.66, 0.04)	-0.48 (-0.90, -0.05)	0.49 (-0.11, 1.08)	0.27 (-0.09, 0.63)	0.05 (-0,40, 0.51)	-0.04 (-0.47, 0.38)
RV/TLC	%	24.3 (22.5, 26.1)	24.8 (22.0, 27.7)	30.1 (26.2, 33.9)	26.2 (24.4, 27.9)	26.9 (24.0, 29.8)	25.2 (22.4, 27.9)
Raw	z	0.55 (0.31, 0.79)	0.73 (0.46, 1.01)	1.31 (-0.54, 3.15)	0.68 (0.51, 0.85)	0.96 (0.72, 1.21)	1.45 (0.61, 2.29)

Figures are observed means (95 % confidence intervals) for two cohorts of EP born subjects. * For DRS (geometric means), a difference between two values is negative if less than 1 and positive if larger than 1.

Table D: Perinatal data for 57 preterm (EP) born participants born in 1999-2000, split by the presence or absence of bronchopulmonary dysplasia (BPD) and by gestational age (GA) categories.

	EP non BPD N=26	**EP BPD** N=31	**GA ≤25 weeks** N=10	**GA 26-27 weeks** N=36	**GA ≥28 weeks** N=11
Gestational age; weeks	27.3 (1,6)	26.3 (1,4)**	24.4 (0.5)	26.7 (0.5)	29.2 (1.0)
Birthweight; gram	872.5 (200)	830.6 (151)	741.2 (107)	904.1 (177)	770.1 (146)
Birthweight; z	-0.97 (1.4)	-0.67 (1.2)	0.09 (0.9)	-0.54 (1.0)	-2.46 (0.7)***
SGA; n	12 (46)	8 (26)	1 (10)	8 (22)	11 (100)***
BPD, n	-	-	8 (80)	20 (56)	3 (27)***
Antenatal steroids; n	21 (81)	25 (81)	8 (80)	28/34 (82)	10 (91)
Surfactant; n	20/25 (80)	29 (94)	10 (100)	32/35 (91)	7 (64)*
Days on ventilator (range)	3 (0-21)	8 (0-24)***	12 (4-24)	4 (0-17)	3 (0-9)**
Days with CPAP (range)	20 (0-53)	36 (4-81)***	46 (28-72)	28 (2-81)	4 (0-50)***
Days with oxygen	44 (22)	79 (35)***	80 (26)	66 (38)	30 (36)*
Systemic steroids; n	1 (4)	17 (55)***	8 (80)	10 (28)	0***
PDA; n	3 (12)	10 (32)	4 (40)	8 (22)	1 (9)
Intracerebral pathology; n	6 (23)	8 (26)	5 (50)	7 (19)	2 (18)
ROP; n	1/21 (5)	4/28 (14)	4 (40)	0	1/10 (10)***
NEC; n	0	1/26 (4)	0	1 (3)	0

Maternal smoking; n	5/24 (21)	8/28 (29)	1/9 (11)	7/35 (20)	2/8 (25)

Figures are means (SD), medians (range) or counts (%). * refers to p-values; *=p<0.05, **=p<0.01, ***=p<0.001 for the differences between groups based on BPD or GA. Abbreviations: SGA = small for gestational age, i.e. birthweight below 10th percentile; antenatal corticosteroids registered if administered in the last week pre labor, surfactant registered if administered in the rescue room or in the neonatal intensive care unit (NICU); CPAP = continuous positive airway pressure; PDA = persistent ductus arteriosus, here registered if treated by medical (indometacine) or surgical intervention; intracerebral pathology registered if intracerebral bleeding, distended side ventricles or periventricular leukomalacia noted on ultrasound scan assessments in the NICU; ROP = retinopathy of prematurity, here registered if treated by cryosurgery; NEC = necrotizing enterocolitis, here registered if treated surgically; maternal smoking registered as daily or occasional smoking during pregnancy.

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