Lung sequelae after premature birth

A population based, controlled, long-term cohort study

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Acknowledgements

The first fumbling steps leading to this dissertation were taken after a dispute with a paediatric neurologist over pulmonary outcome in low birth weight children. A medical student (Guri Andersen Gott) appeared in my office just after, asking for help with an assignment. She ended up piloting the present study, and the results incited this thesis.

Since the mid 1990's, a well equipped clinical laboratory for lung and exercise physiology has been established at the Pediatric Department. This achievement was the result of a local "joint venture" between paediatric pulmonology and cardiology, and particularly the efforts from a very enthusiastic Professor Gottfried Greve. Since 1997, Ola Røksund has been responsible for implementation of high quality routines and methods. He has developed into a friend and co-worker, and is responsible for all lung function testing in this study. Professor Amund Gulsvik soon became an important mentor in the field of academic pulmonology.

Within this context – a clinical question and an emerging clinical laboratory - a protocol for this particular study was written together with my principal supervisor, Dr. Britt Skadberg. The encouragement and support from her and from the head of the Pediatric Department, Professor Robert Bjerknes, have been vital for the realization of this project. The University of Bergen kindly provided the necessary financial support, and in the winter 2001, identification of eligible preterms began. Ms. Berit Båtsvik soon entered the study group to collect data for her master thesis in nursing. Excellent secretarial support was provided by Ms. Heidi Habbestad, and numerous patient records were provided by the staff at the patient file archive. Statistician Tore Wentzel-Larsen at the Centre for Clinical Research patiently introduced me to the world of statistics, and later, associate Professor Geir Egil Eide became an important co-worker.

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Bergen, October 4th 2005,

Thomas Halvorsen

1. Abbreviations

FEV_1	Forced expiratory volume in first second
PEF	Peak expiratory flow
FVC	Forced vital capacity
FEF_{50}	Forced expiratory flow at 50% of FVC expired
FEF_{75}	Forced expiratory flow at 75% of FVC expired
TLC	Total lung capacity
FRC	Functional residual capacity
RV	Residual lung volume
Raw	Airway resistance
PD_{20}	Provocative dose of methacholine causing a 20% reduction in FEV_1
VLBW	Very low birth weight, i.e. < 1500 grams
ELBW	Extremely low birth weight, i.e. < 1000 grams
PMA	Post menstrual age, i.e. the age in weeks since conception
GA	Gestational age, i.e. the age in weeks since conception
BW	Birth weight
RDS	Neonatal respiratory distress syndrome
BPD	Bronchopulmonary dysplasia
CLD	Chronic lung disease of prematurity
PDA	Persistent ductus arteriosus
A preterm	A subject born at a PMA ≤ 28 weeks or with a BW ≤ 1000 grams
NICU	Neonatal intensive care unit
AHR	Airway hyperresponsiveness
mSv	Milli Sievert

2. Abstract

Background: Survival after extremely premature birth has increased over the past decades, parallel to advances in perinatal and neonatal care. As more vulnerable infants survive, the incidence of bronchopulmonary dysplasia (BPD) has increased. Currently, BPD has been proposed the most common chronic lung disease in infancy. Subsequent to premature birth and neonatal BPD, there are reports of clinical asthma, airway obstruction, airway hyperresponsiveness (AHR), pulmonary hyperinflation and radiological findings in schoolchildren, adolescents and selected samples of adults. There is little population-based knowledge on these issues in young adults who were born prematurely in the early era of neonatal intensive care medicine. Furthermore, outcome in these pioneer subjects has not been compared to outcome in comparable survivors of more recent neonatal intensive care. While inflammation is important in neonatal BPD, there is limited knowledge on the mechanisms underlying later respiratory manifestations. Principal determinants for long-term pulmonary sequelae are not well established. Most knowledge relating to lung sequelae from *premature* birth is based on samples of subjects defined by their *birthweight* and born at tertiary level teaching hospitals. Finally, the feasibility of lung function testing has not been examined in this population.

Aims: In population-based cohorts of long-term survivors from extremely premature birth:

- To assess the feasibility and precision of lung function testing.
- To assess pulmonary outcome in young adults born in the early 1980's.
- To compare pulmonary outcome in subjects born in the early 1980's and in the early 1990's.
- To assess the relevance and importance of the following variables for asthma, airway obstruction and AHR: Inheritance, allergy, selected markers of airway inflammation, exposures to cigarette smoking and various conditions in the neonatal period.
- To establish knowledge regarding pulmonary radiological findings.

Design: Population-based, controlled, historical, prospective cohort study.

Subjects: Preterms consisted of two population-based cohorts of subjects with gestational age ≤ 28 weeks or birthweight ≤ 1000 grams, born consecutively within the study region in western Norway in the years 1982-85 (first birth-cohort) and 1991-1992 (second birth-cohort). For each preterm, the temporally closest term-born and willing subject of the same gender with birthweight between 3.0 and 4.0 kilograms was recruited as control.

Study setting and methods: Subjects were examined twice within two weeks in 2001/2002 at the paediatric Cardio-Pulmonary Laboratory at Haukeland University Hospital with standard equipment and in accordance with international guidelines. Airflow obstruction was assessed from maximum flow volume loops, lung volumes with whole body plethysmography, airway hyperresponsiveness (AHR) with methacholine provocation and bronchial lability with tests for exercise induced asthma and reversibility to salbutamol. Allergy was assessed with skin prick tests. Airway inflammation was assessed by measuring urinary leukotriene E_4, urinary eosinophilic protein X, serum eosinophilic cationic protein and whole blood counts of eosinophilic granulocytes. Radiological imaging of the lungs was done with high resolution CT technique (HRCT). Respiratory symptoms and relevant background information was established with questionnaires and a standardised interview by a paediatrician.

Results: Spirometry and whole body plethysmography are precise and feasible methods for assessment of airway function and lung volumes in subjects born preterm. Young adults born preterm in the early 1980's had more asthma and significant airway obstruction, AHR and pulmonary hyperinflation, compared to control subjects born at term. The incidences of BPD among preterms born in the early 1980's and in the early 1990's were similar. Compared to matched controls, current airway obstruction, AHR and pulmonary hyperinflation were similarly increased in the two preterm cohorts. Furthermore, current deficits in important lung function variables were similarly associated with the severity of neonatal respiratory disease in both cohorts. Compared to matched controls, current FEV_1 was reduced with respectively 18.6% and 18.7% of predicted in the two birth-cohorts in preterms who required supplemental oxygen at 36 weeks postmenstrual age. One-hundred days with neonatal oxygen treatment predicted decreases in current FEV_1 of 12% and in current FEF_{50} of 25%, and these figures were similar in both birth-cohorts. With respect to subsequent small airway obstruction, the influence from a neonatal diagnosis of BPD seemed to have *increased* in the most recent birth-cohort. In preterms, the occurrence of asthma and AHR was unrelated to inheritance, allergy, markers of eosinophilic airway inflammation and cigarette exposures. AHR was instead strongly related to prolonged neonatal requirement for oxygen treatment. Radiological findings were observed in 63 (88%) of the subjects undergoing CT scanning of the lungs. Most of the described pathology seemed relatively minor, such as discrete linear or triangular opacities of unknown prognostic significance. There was significantly less findings in the second birth cohort.

Concluding remarks and implications: Pulmonary sequelae after extremely premature birth were demonstrated throughout childhood and into early adult life. In the two birth-cohorts, adverse outcome was strongly and similarly related to neonatal respiratory morbidity, and to prolonged requirements for oxygen treatment. Asthma and AHR in preterms were unrelated to the assessed markers of airway inflammation and to features usually observed with asthma. These findings suggest irreversible, structural damage to the airways and/or to the pulmonary interstitium as causal factors, rather than an ongoing, active inflammatory disease. Less radiological findings in the second birth-cohort may reflect less structural damage in preterms born in the 1990's. Survival in infants born after short gestation is still associated with long requirements for oxygen treatment. Providing that adverse pulmonary outcome is still linked to prolonged neonatal oxygen exposure, current BPD-survivors may be developing similar lung injury. Relatively minor pulmonary insults in early childhood have been associated with chronic obstructive pulmonary disease (COPD) in adults. There is now evidence to *indicate* that subsets of young people with a neonatal history of BPD may become future COPD patients. Furthermore, there is reason to *speculate* that contemporary BPD-survivors may develop similarly.

3. List of papers

1. Assessment of lung volumes in children and adolescents: comparison of two plethysmographic techniques.

Halvorsen T, Skadberg BT, Eide GE, Røksund OD, Bakke P, Thorsen E.

Clin Physiol Funct Imaging 2005; 25: 62-8.

2. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study.

Halvorsen T, Skadberg BT, Eide GE, Røksund OD, Carlsen KH, Bakke P.

Acta Paediatr. 2004; 93: 1294-300.

3. Better care of immature infants, has it influenced long-term pulmonary outcome?

Halvorsen, T, Skadberg BT, Eide GE, Røksund OD, Markestad T.

Acta Paediatr. 2005, Accepted for publication.

4. Characteristics of asthma and airway hyper-responsiveness after premature birth.

Halvorsen T, Skadberg BT, Eide GE, Røksund OD, Aksnes L, Øymar K.

Pediatr Allergy Immunol. 2005; 16: 487-494.

5. High-resolution CT in ex-premature children.

Aukland SM, Halvorsen T, Fosse KR, Daltveit AK, Rosendahl K.

Am J Roentgenol. 2005, Accepted for publication.

4. Background information

4.1 The epidemiology of premature birth

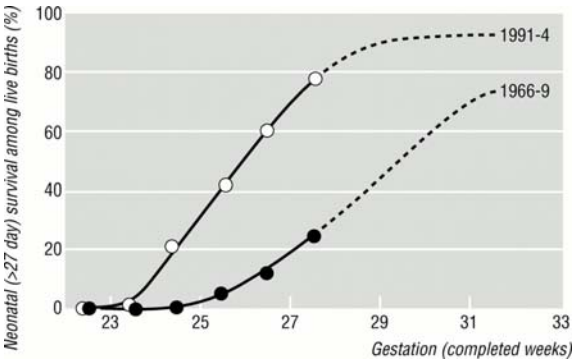
Premature birth is related to low *birthweight* and vice versa. *Birthweight* is a readily available and reliable figure, and therefore often used as a substitute for *gestational age* to define samples and incidences. In research aiming to describe consequences of premature birth, this strategy may introduce inaccuracies due to inclusion of infants that are small for gestational age and therefore more mature than expected from their birthweight. Antenatal organ development is an orderly process governed by genetic information and complex cellular interactions that are related to the fetal stage of development. Impact from premature birth on the normal schedule of pulmonary development thus seems better described through assessment of subjects included on the basis of postmenstrual age (PMA) at birth, rather than birthweight. Few long-term follow-up studies have taken this position in the process of subject inclusion. Therefore, current understanding of long-term consequences from premature birth is to a large extent derived from knowledge based on very low birthweight (VLBW) infants. Since gestational age at birth is critical for lung development and also important for the immediate decision to initiate life support at birth, this is not an optimal situation.

4.1.1 Survival data

The overall *incidence* of premature delivery has been fairly constant in Norway over the last decades, in the range of 1.3-1.5 VLBW infant per 100 births (1). However, premature infants born today face a considerably better chance of *survival* than comparable infants born in the second half of the last century. Perinatal and neonatal mortality rates have declined for all weight classes since the 1960's (1;2). The reasons for this have been manifold, but for VLBW infants, changes have coincided with the introduction of assisted ventilation and the development of neonatal intensive care medicine. From the 1960's to the 1980's, the chances of survival for live born VLBW infants trebled, and for the smallest infants weighing between 751 and 1000 grams, survival improved from nearly zero to 42-75% (3). In the 1990's, development in perinatology and neonatal intensive care medicine was paralleled by further improvements in the survival rates, particularly for the most immature infants (4;5). The *Neonatal Research Network* reported that survival to discharge was 74%, 80% and 84% for VLBW infants born in the years 1988, 1991 and 1995-96, respectively (6). In the second half

of the 1990's, further improvements were noted (7). In Norway in the two years 1999 and 2000, 636 infants were born at PMA < 28 weeks or with birthweight < 1000 g. Of these, 462 (73%) were considered viable and admitted to a NICU (8). Altogether 376 infants survived to discharge, which is 59% of all included infants or 81% of those who were admitted to a NICU.

There are wide variations as to reported outcomes (9). Allen et al. (10) reported survival rates of up to 80% for infants born at 25 weeks' gestation in the early 1990's, while others reported considerably lower survival rates (5). For infants with birthweight less than 750 grams, survival ranged from 32% to 46% (11).



Neonatal survival according to gestational age at birth in two periods.
From Tin, Waryiar and Hey,
BMJ 1997.

These variations reflect several factors. First, there may be real differences in treatment qualities and policies. Second, the study populations are not necessarily comparable, i.e. some are recruited from single tertiary centres and others from geographically defined areas (5). The denominator may be the total number of deliveries or only those infants considered viable and admitted to NICU care. When survival is estimated according to gestational age at birth, the quality of the pregnancy data is crucial. Furthermore, perinatal practice and mortality are influenced by the attitude and the ability of the neonatologists to salvage immature infants at the edge of viability. These matters may also induce changes with respect to classification of perinatal mortality: Extremely vulnerable infants may increasingly have been classified as live births rather than spontaneous abortions or stillbirths (12).

4.1.2 Development of neonatal intensive care

Modern intensive care neonatology commenced with the introduction of mechanical ventilation in the 1960's. In Bergen, mechanical ventilation of a premature infant was first done in 1969 (Gjermund Fluge, personal communication). In the following 35 years, there

have been vast improvements in antenatal, perinatal and neonatal care of premature babies. Better technologies and treatment procedures relating to assisted ventilation were fundamental elements in this development. Better techniques for non-invasive surveillance of blood gases and oxygen tension (13) facilitated more exact administration of ventilatory support and oxygen therapy. Continuous arterial blood pressure surveillance and arterial blood sampling became a safe procedure through better equipment for access to peripheral arteries. The importance of adequate nutrition was gradually recognized (14-17), and intravenous nutrition was facilitated by techniques for central venous access through peripheral veins (18). Treatment of persistent ductus arteriosus (PDA) with surgery or indomethacin became more aggressive as the impact on respiratory distress syndrome (RDS) from over-hydration (19;20) and over-perfusion of the lung (21;22) became evident. More aggressive use of antibiotics (23) and a better understanding of aspects relating to the prevention of neonatal septicaemia have been important, as have better nursing procedures (24). Antenatal corticosteroids was gradually introduced in clinical medicine in the 1980's and early 1990's, reducing perinatal mortality, respiratory distress syndrome and intraventricular haemorrhage (25;26). Beneficial effects from postnatal corticosteroids on RDS was demonstrated in the early 1980's (27;28). In the late 1990's, however, awareness of long-term negative neurological effects (29) limited its use, despite documented positive effects on respiratory morbidity (30-33). Surfactant replacement therapy was introduced in clinical routines in the late 1980's and early 1990's, and had significant impact on acute respiratory morbidity and death rates from RDS (34;35). The importance of standardisation of complex neonatal intensive care schemes together with coordinated and multidisciplinary programs for research, education and quality improvements on all levels, was expressed internationally through the Vermont Oxford Network, established in 1989 in the USA (36;37).

Collectively, these changes have increased perinatal survival, reduced acute neonatal morbidity and increased neonatal survival after premature birth, particularly in the most immature. Thus, more premature infants have been exposed to neonatal intensive care, and more of them have survived. The first large birth-cohorts of these young individuals have only recently started to reach adulthood. Population based knowledge on their long-term pulmonary outcome is virtually non-existent. Since the total number of low-birthweight deliveries remains relatively stable and the survival rates increase, the number of preterms discharged alive is increasing. Chronic neonatal pulmonary morbidity - bronchopulmonary dysplasia (BPD) - has not decreased and may in fact be increasing (4;35;38;39). Measures that

are beneficial for survival and acute neonatal morbidity may not necessarily be beneficial in relation to long-term morbidity. Effects from these evolving changes with respect to long-term respiratory health are poorly described in the literature.

4.2 Chronic neonatal pulmonary morbidity

Pulmonary sequelae after neonatal respiratory distress syndrome were initially described by Shepard and Robertson in the 1960's. In 1967, Northway et al. (40) coined the term "Bronchopulmonary Dysplasia" (BPD) to describe a constellation of clinical, radiological and pathologic findings in oxygen treated, ventilated premature infants. Since then, the characteristics of infants requiring long-term ventilatory support have changed, as have definitions of BPD. In the literature, the term has come to signify a need for supplementary oxygen at various ages, sometimes in combination with abnormal radiological findings. In 1979, Tooley et al. (20) suggested a cut off point of 30 days of oxygen supplementation to define BPD. In 1988, Shennan et al. (41) reported that oxygen requirements at 36 weeks PMA was a better predictor of abnormal pulmonary outcome the first two years of life and introduced the term Chronic Lung Disease of Prematurity (CLD). In 2001, an attempt to categorize the severity of BPD was put forward in a NICHD/NHLBI/ORD work shop summary in Bethesda, USA, by Jobe and Bancalari (39). Infants born before 32 weeks PMA requiring supplemental oxygen for at least 28 days were classified as mild BPD if they were breathing room air at 36 weeks PMA. Requirement for supplemental oxygen < 30% at 36 weeks PMA was defined as moderate BPD whereas requirements ≥ 30% was defined as severe BPD.

Different definitions of BPD and CLD have been used in different studies. As discussed by Bancalari (42), BPD has been defined by the total *number of postnatal days* (28 days) with requirement for supplemental oxygen, by the *postnatal age* (28 days) at which weaning from supplemental oxygen is possible or by the *post menstrual age* (36 weeks) at which weaning from supplemental oxygen is possible. Defining BPD by oxygen treatment at 36 weeks PMA tends to equalize its incidence across gestational ages, as an immature infant of 24 weeks will require much longer duration of oxygen supplementation than a more mature infant to reach that criterion. As oxygen is used both to define and to treat neonatal lung disease, further methodological problems appear. There are few generally agreed upon criteria for discontinuation of oxygen therapy. In a single institution, one can assume that infants in oxygen at a particular postnatal or postmenstrual age have worse lung disease than those in

room air. However, it does not follow that infants in oxygen at one institution are similar to infants in oxygen at another. Conclusions from multi-centre studies may therefore be blurred.

4.3 The epidemiology of neonatal Bronchopulmonary Dysplasia

The reported incidences of BPD vary widely, cross-sectionally as well as longitudinally, and several factors contribute to this variation. Firstly, real differences with respect to patient management (43) and strategies for weaning from oxygen treatment (44) are important. Secondly, the nature of the studied preterm populations varies. With increasing survival of immature infants, the population at risk of developing BPD increases (38). The rates of BPD will therefore vary according to the constitution of the population studied. Thus, results will be influenced by the time period of the study. For the same reason, hospital based studies may produce results that differ from area based studies. Thirdly, the base population from which incidence is calculated varies. The denominator may be *all* infants born prematurely, only *surviving* premature infants, only infants *admitted* to a NICU, only infants within certain categories of *birthweight* or *gestational age*, only *ventilated* infants or only so called "*at-risk infants"* etc. Lastly, differences relating to which infants are labelled and defined as suffering from BPD obviously must influence its incidence (42).

These inconsistencies regarding fundamental medical and epidemiological criteria used to identify BPD may disguise causal factors and seriously hamper the search for causal pathways as well as the understanding of its long-term outcome. Standard diagnostic criteria are critical for rational interpretations of clinical studies using BPD as an outcome or explanatory variable, and for comparisons between them.

In the late 1970's, the reported incidence of BPD in infants mechanically ventilated for RDS ranged from 10-20% (19;45;46). Besides differences with respect to patient populations or management, differences were explained by inconsistent definitions (22). For infants with birthweight between 700-1500 g treated at eight major NICU's in the USA in 1982-1984, significant and substantial differences in the incidence of infants requiring prolonged oxygen supplementation were observed (43). These differences incited discussions still unresolved, relating to strategies for assisted ventilation (47). In the mid 1990's the incidence of BPD defined as oxygen dependency at 36 weeks PMA in infants with birth weight 501-1500g, ranged from 3% to 43% in the 14 different participating centres of the National Institute of Child Health and Human Development (NICHD) Neonatal Network (6). Overall incidence

increased from 19% in 1991 to 23 % in 1996. Bancalari et al. have reported incidences of BPD from the same institution in 1986 and in 2003 (22;42): In 1986, in infants who had been mechanically ventilated and survived to 28 days, incidence ranged from 85% for those weighing below 700 g, to 16% in the 1000-1300 g group and 5% in those weighing more than 1300. In 2003, incidence ranged from 67% in the 500-750 g group to less than 1% in the 1251-1500 g group. Young et al. (4) compared rates of chronic lung disease in VLBW infants in North Carolina in 1984 (48) and 1994. An "at risk-group" was defined as infants ventilated > 48 hours. In 1984, the proportion requiring ventilation or supplemental oxygen at 30 days was 54% and in 1994 it was 68%, a significant increase. Data from Palta et al., suggest an increase in CLD from the pre-surfactant to the post-surfactant era, and survival without CLD morbidity did not change (49). In the EPICure study, focusing on infants born before 26 completed weeks PMA, 74% were still receiving supplemental oxygen at 36 weeks PMA, as were 51% at term (50). In a Swedish study of infants with birth weight < 1000g, 28% were dependent on oxygen supplementation at 36 weeks PMA (51). Among infants born at PMA < 28 weeks or with birthweight < 1000 grams in Norway in 1999 and 2000, 123 (33%) of 376 survivors still required treatment with supplemental oxygen at 36 weeks PMA (8).

In conclusion, the epidemiology of BPD is difficult to interpret for reasons relating to inconsistent definitions and varying population characteristics. Clinically, the severity of the condition seems to decrease while long periods of oxygen supplementation continue to be required, particularly to salvage the smaller infants. Increased survival in the lowest weight classes and among the most immature infants thus seems to occur at a cost of increased *chronic neonatal* pulmonary morbidity. The long-term consequences are largely unknown.

4.4 The aetiology of Bronchopulmonary Dysplasia

The aetiology of a clinical condition characterized by "*prolonged need for oxygen supplementation*" must be multifactorial, i.e. BPD is not a clearly defined disease-entity with a static set of explanatory and prognostic variables, but rather a syndrome with a common denominator being prolonged requirement for supplemental oxygen. Why BPD develops in some premature infants and not in others, is still not well understood.

In a preterm infant, lung development that normally should take place in utero, instead must occur after birth while the lungs function as a gas exchanging organ. Instead of quiet "breathing" of amniotic fluid, the immature lung tissue is mechanically inflated by a gas

mixture with considerably higher oxygen tension, paralleled by stretch and relaxation inflicted by mechanical ventilation (52). Furthermore, at birth the fetal lung is exposed to a series of fundamental physiological changes, such as perfusion with a full cardiac output with considerable higher oxygen tension than during fetal life (42), nutritional alterations (15), and exposure to infections (53), inflammation (54), oxidative stress (55) and proteolytic enzymes (56).

Clearly, the main risk factor for both RDS and BPD is the extent of prematurity (39). Lung development evolves through genetically scheduled phases, starting with an embryonic phase (the first 7 weeks) which is followed by a pseudoglandular (7 to 16 weeks), canalicular (16 to 26-28 weeks) and saccular (26-28 to 32-36 weeks) phase and ends with the alveolar phase, starting at 32-36 weeks and ending at approximately 18 months of postnatal age (57;58). In an infant born prematurely, this developmental process is disturbed and probably interrupted by mechanical ventilation, hyperoxia, left to right shunting through a PDA, altered nutrition, oxidative stress, inflammation, infections and proteolytic enzymes (39). The ability of an immature infant to handle factors such as oxidative, inflammatory and proteolytic stress, may be reduced and may also conceivably vary between individuals (59). It is important to bear in mind that the mere exposure to room air represents hyperoxia relative to intrauterine conditions. The relative importance of the different factors involved, remains to be defined, and multiple pathways to injury seem plausible. Better treatment of RDS and acute neonatal morbidity have facilitated survival of more infants born in the canalicular stage and before formation of terminal acini. The pathology and clinical expression of BPD therefore have changed (58), as have probably also its dominating causes.

The factors causing premature delivery vary, and at birth each neonate already has an individual medical history. The effects from postnatal events, conditions and therapeutic interventions are modulated by the previous antenatal history of the babies (60-62), their genetic constitution (63), possibly by gender (64-66) and by the PMA at which they are born (39). The subsequent treatment modalities and policies these infants are exposed to, have changed over time, and also vary cross-sectionally between countries and hospitals. As survival rates increase, the number infants at risk of developing BPD increase. Due to this heterogeneity of the preterm population and the imprecise definition of BPD, the search for one particular causal factor or sequence of events, seems a difficult exercise.

A fundamental question is whether prematurity *per se* is compatible with normal lung development and growth, and what benefits can be expected from further improvements in treatment schemes and modalities (67). Hjalmarson et al. studied healthy preterm and full-term infants at the same PMA of 40 weeks, and concluded that preterm birth *per se* changed the normal development of lung function (68). In a follow up study, they concluded that ventilatory impairments were also influenced by the extent of neonatal BPD (69). Impairments following BPD were of the same nature as in healthy preterm infants, but the magnitude was related to the clinical severity of BPD. Loss of bronchial function in the first year of life has been reported in premature infants, irrespective of the treatment modalities used, and also in children without apparent neonatal respiratory disease (70-72). Hofhuis et al. (70) found a possible beneficial effect from high-frequency oscillation, whereas a recent report from The United Kingdom Oscillation Study, questioned this (73). These data suggest that reduced lung and airway function following preterm birth may be related to developmental changes caused by the premature birth *per se* as much as to the initial disease severity or treatment effects (72). Because airway development precedes development of the acini and the pulmonary microvasculature, parenchymal development may be *more* disturbed by premature birth than airway abnormalities. However, assessment of potential parenchymal injury and of acinar structure and function is hampered by the lack of readily available non-invasive techniques.

In conclusion, in the development of BPD, the relative contributions from prematurity *per se* versus potentially harmful exposures, events or treatments are difficult to discern, and may vary between individuals according to their genetic predispositions, antenatal history and PMA at birth. Insight in theses matters may only be gained through long-term physiological follow-up studies of population based cohorts of preterms handled according to standardised schemes and definitions.

4.5 The pathology of neonatal Bronchopulmonary Dysplasia

As exogenous surfactant and advances in neonatal critical care medicine have led to less barotrauma and oxygen injury, the pathology of BPD has changed. These changes have been expressed through the concepts "*old*" and "*new*" BPD (58).

4.5.1 "Old" Bronchopulmonary Dysplasia

As reviewed by Coalson (74), pathological changes primarily consisted of severe airway injury and alternating sites of pulmonary overinflation and fibrosis/atelectasis. There were squamous metaplasia and hyperplasia of the airway epithelium, bronchial and bronchiolar mucosal metaplasia, peribronchiolar smooth muscle hypertrophy and interstitial fibrosis. Cardio-vascular lesions consisted of periarteriolar thickening, right ventricular hypertrophy and cardiomegaly with cor pulmonale in the severe cases. Pathological changes were evoked by hyperoxia and ventilator induced injury on a relative immature and surfactant deficient lung.

4.5.2 "New" Bronchopulmonary Dysplasia

During the 1980's, autopsy studies described a "tubular alveolar structure", reduced alveolar numbers and reduced internal surface area measurements (75), suggesting reduced postnatal alveolar growth. Alveolar hypoplasia with enlarged, simplified airspaces, variable amounts of fibrosis of the alveolar interstitial septae and dysmorphic vascular changes have later been consistent findings in BPD autopsies. Impaired alveolar growth and developmental arrest are hallmarks of "new BPD". A reduced surface area for gas exchange and a decrease in the size and complexity of the pulmonary capillary bed are physiological consequences. At 24 weeks PMA, the lung is in its canalicular stage of development and at 30 weeks still not in the alveolar stage. Thus, premature birth and the initiation of pulmonary gas exchange interrupts normal septation and acinar development. During fetal development, the lung exists in a relatively hypoxic environment, and therefore air itself represents hyperoxia. Studies have shown that exogenous surfactant alone does not enhance alveolarisation in BPD-survivors (76;77). In premature baboons, alveolar hypoplasia and vascular developmental arrest were present, despite antenatal steroids and postnatal surfactant replacement (58). The dominant lesions in animal models (77;78) and in humans after modern treatment regimens, are delayed development of the acinus of the lung with abnormal alveolarisation, altered elastic and collagen tissue networks and disturbed vascularisation (52;58;76;79). Lung mechanics may be affected by the described abnormalities. Enlarged airspaces can result in hyperinflation. Furthermore, alveolar wall and parenchymal attachments are structures through which lung recoil exerts its force on small airways to prevent airway closure during exhalation (80). Altered elastic recoil will lead to abnormally low expiratory flow rates (81). Concluding her chapter on the pathology of BPD in a recently published book (74), Coalson expresses her worries that BPD in current extremely low birth weight survivors, may have as serious long-

term consequences in adulthood as in those infants who acquired BPD in the 1960's. Similar concerns are expressed by Hack and Fanaroff (11). To gain knowledge on these issues, premature infants should continually be followed up into adulthood and results compared to previous data.

4.5.3 Pathophysiology of Bronchopulmonary Dysplasia

The early stages of BPD are almost invariably characterized by an intense acute inflammatory response followed by chronic inflammation and airway remodelling (39;82). There is furthermore evidence that antenatal inflammatory mechanisms may influence the occurrence of neonatal BPD (60;62;83). Beyond infancy, the pathophysiology of BPD is poorly described. While an eosinophil-driven inflammatory process is central in asthma (84), we know little about the nature and relevance of airway inflammation in long-term BPD survivors. Airway pathology of BPD beyond infancy has not been properly investigated, and to my knowledge, no studies exist on bronchoalveolar lavage fluid or bronchial biopsies. Recently, Baraldi et al. (85) described low levels of exhaled nitric oxide (eNO) in school aged BPD survivors compared to FEV_1-matched asthmatic children. This underscores the differences between these two types of paediatric obstructive lung disease. However, due to a similar clinical presentation, asthma medication is frequently prescribed (86-88), despite few studies documenting effect (89). A better understanding of the causal mechanisms for airway pathology in BPD survivors is fundamental for adequate treatment. Due to a clinical resemblance with classical asthma, it is particularly critical to examine the importance of ongoing inflammation. One of the aims of this study was therefore to study markers of airway inflammation in relation to airway pathology in preterms and term-born control subjects.

4.6 Long-term pulmonary outcome from premature birth

Assessments of pulmonary function in premature children and adolescents will have to be an assessment of the therapeutic strategies of the past. Therefore, continually long-term surveillance and follow-up studies are required to identify positive trends as well as potentially untoward and iatrogenic consequences of neonatal intensive care medicine. To provide unbiased estimates of long-term outcome, complete cohorts of consecutive premature births, preferably representing entire geographic regions, should be followed into adulthood. Such optimal long-term follow-up studies are, however, demanding and challenging to carry

out. Consequently, the majority of published literature focuses on younger children (65;87;90-95), studies tend to be hospital-based (96-98) rather than population-based (90;91;99) or they focus on particular subsets of preterms with prolonged oxygen dependency (96;100) or requirement for assisted ventilation (101). Birthweight as the sole criterion for inclusion (87;90;97;99;102;103) is practical, but assumptions regarding effects of prematurity *per se* or its treatment might be confused by small-for-date infants. Consequences of premature birth should be assessed in subjects who are included primarily on the basis of gestational age at birth and not on the basis of low birthweight. Thus, one of the aims of the present study was to provide descriptive data for respiratory health and pulmonary function in a complete regional cohort of survivors of extreme prematurity, approaching adulthood.

Advances in perinatal care and neonatal intensive care medicine have altered the surviving preterm population through recruitment of more immature survivors at risk of long-term sequelae - but also reduced the potential for acute injuries. The combined long-term effect of this scenario is poorly described. To elucidate such effects, comparable regional birth cohorts from different eras of intensive care neonatology should be assessed by identical methods, preferably at the same age. However, the time span required makes this optimal strategy demanding. Concurrent assessment at different ages facilitates identical evaluation, but precipitates a discussion on growth effects. Summarising available knowledge on this issue, Eber and Zach (104) stated that hyperinflation may improve with growth whereas airway obstruction improve only slowly or not at all. Thus, with this knowledge at hand, another aim of the present study was to assess possible different patterns in lung function in children who were born extremely premature in the early 1980's and in the early 1990's.

4.6.1 Measurements of pulmonary function in preterms

Objective surveillance of long-term pulmonary outcome from premature birth through lung function testing rests on the notion that the data gathered are valid, i.e. accurate and precise measurements of the current status of the lung. Lung function testing requires extensive cooperation between the tested subject and the pulmonary technician. The tested subject must be able to hear and comprehend a complex series of instructions, and subsequently have the neuromuscular ability to comply with these instructions. Neurological and cognitive impairment is more common in preterms than in term-born children (105). In no published studies have the repeatability of complex lung function tests been assessed in preterms. There are thus reasons to examine to what extent preterms are capable of producing technically

adequate and repeatable lung function tests. Lung volume measurement in a whole body plethysmograph is a complex and a well suited model system, i.e., if successful, one may assume adequate performance also in simpler test situations, such as spirometry.

4.6.2 Bias in sample selection

Follow-up studies of premature children are influenced by the sampling process. Typically, hospital based samples from single, tertiary level, teaching hospitals have been studied instead of area based cohorts. Hospital based studies may include samples that are skewed with respect to adverse neonatal outcomes as well as risk factors for subsequent development of obstructive airway disease. These factors may bias any outcome measure. The recommended approach is to include consecutively born subjects from complete geographical regions (106). However, Escobar et al. found that only 13% of 111 studies on morbidity among surviving VLBW infants were population based (107).

4.6.3 Infancy

Respiratory morbidity is common in infancy, particularly in BPD survivors. Symptoms present as wheezing episodes, and readmission to hospital is common (108-115). Several studies of lung physiology in infants with a history of BPD have demonstrated decreases in forced expiratory flows and hyperinflation (68-72). Recurrent wheezing in preterms has been associated with greater expiratory flow limitation, (116;117) and ventilatory impairments in BPD survivors was related to the clinical severity of the BPD (69).

4.6.4 Childhood, adolescence and adulthood

In later childhood and adolescents, there may be a gradual decline to normal of wheezing episodes (92;97;118;119), although several studies report continual symptoms, particularly after BPD (87;91;120). Later in life, symptoms seem to improve further, even after severe classical BPD, while lung function abnormalities persist (96). Airway obstruction and hyperresponsiveness have been reported in a number of studies of schoolchildren and in a few studies of adolescents. In most studies, inclusion is based on birthweight, samples tend to be recruited from single tertiary centres and drop-out rates tend to be high. Many studies include only selected subgroups of preterms, such as only those with BPD (100) or only those who were ventilated in the neonatal period (101). In the few published population based studies, age tend to be low (91) and subjects with serious neonatal morbidity few (99). Some studies aspiring to describe outcome of premature birth include subjects born close to term (64;65).

4.6.5 Radiographic findings of the chest

The initial chest radiographic appearances of most infants who are born extremely prematurely, are highly abnormal (121), particularly in those infants who go on to develop BPD (122;123). The definition of BPD has been linked to abnormal chest x-rays since the condition was first described by Northway in 1967 (22;40;124). Attempts have also been made to relate the extent and the qualities of the pathology described on early chest x-rays to the subsequent risk of lung function abnormalities years later (125). In recent reports, however, the repeatability of chest radiographic interpretations have been questioned (122), and in a consensus conference in 2000, x-ray findings were not included in the definition of BPD (39).

Subsequent to BPD in infancy, there is a gradual and slow improvement in the radiographic appearance (126-128). Several authors have noted that chest radiographs in later childhood may show only minor abnormalities, despite significant ventilatory dysfunction (96;126). The sensitivity of plain x-ray in diagnosing subtle lung pathology is limited, and indications for high-resolution computer tomography (HRCT) in children have increased (129). With its relatively low radiation dose, HRCT has been suggested as a useful method for follow-up of lung sequelae after premature birth (130). Few studies have presented such follow-up data. Oppenheim et al. (131) described radiological findings in all the 23 examined BPD survivors, born between 1974 and 1992. Their population was highly selected, however, as they were all requiring medical follow-up for pulmonary morbidity. Furthermore they were all fairly young (mean age 4 years) and the population was heterogeneous, with age ranging from two months to 13 years. In another study (132), the 26 subjects (aged 5-18 years) were all recruited due to status as pulmonary patients, as were the five participating subjects in a study of young adults (133). There is no information available in the literature, regarding findings from pulmonary HRCT performed in population-based, unselected cohorts of preterms. Thus, we have no knowledge on expected or typical findings in long-time survivors from premature birth who do not appear as pulmonary patients.

Survival after extremely premature birth has increased over the past decades, parallel to profound advances in perinatal and neonatal care. The radiographic pulmonary findings in premature infants and in BPD have also changed during this time course, both in the neonatal period and in later childhood. The prominent findings of classic BPD have been replaced by

less impressive changes that apparently normalise during later childhood (127). The impression of radiological improvement, has been based on plain chest x-rays. A better understanding of these matters would require population based follow-up data, based on pulmonary HRCT in preterms born and treated in different eras of neonatology. Such assessments would require a reliable system for classification of pathological findings in a population of this kind. There is no available literature discussing relevant HRCT results in this kind of time perspective, and there is no published and validated classification system for relevant radiological findings.

4.7 Summary of background information

Over the past decades, the number of live-born *extremely* premature infants seems to have increased, and there has certainly been a marked increase in survival to discharge after neonatal intensive care treatment. These improved survival rates have been paralleled by profound advances in perinatal and neonatal care. As more vulnerable infants survive, the incidence of bronchopulmonary dysplasia (BPD) has probably also increased. The majority of extremely premature infants require extensive medical intervention, often paralleled by long periods of treatment with supplemental oxygen. Currently, BPD has been proposed the most common chronic lung disease in infancy. Thus, more immature infants are admitted alive into neonatal intensive care, and more of them survive after exposure to better – but also prolonged and more aggressive – treatment. Subsequent to premature birth and neonatal BPD, there are numerous reports of clinical asthma, airway obstruction, airway hyperresponsiveness, pulmonary hyperinflation and radiological findings in schoolchildren, adolescents and selected samples of adults. There is, however, little population-based information on these issues in current young adults born in the early era of neonatal intensive care medicine. Furthermore, no comparisons have been published between these pioneer subjects and comparable preterms exposed to more recent intensive care medicine. While inflammation is important in neonatal BPD, there is limited knowledge on the mechanisms underlying later respiratory manifestations. The importance and relevance of the family history of asthma and atopy, cigarette exposures, allergy and inflammation for long-term lung sequelae subsequent to BPD remains to be established. Principal determinants for airway obstruction and AHR in long-term BPD-survivors are not well described. Nevertheless, asthma-like symptoms in BPD-survivors are often treated with anti-asthma medication with potential for side effects. Most of the knowledge relating to lung sequelae from *premature*

birth is based on samples of subjects defined by their *birthweight* and born at tertiary level teaching hospitals. Objective surveillance of lung-function rests on the notion that the data gathered are precise and accurate measurements of the current status of the lung. Since premature birth is associated with various disabilities, there are reasons to question the feasibility and the precision of lung function testing in preterms. Published information on these issues is scanty.

5. Aims of the present study

5.1 To assess the feasibility and precision of lung function testing in young people who were born extremely preterm, and in comparable subjects, born at term.

5.2 To assess long-term pulmonary outcome after extremely premature birth in a population based cohort of young adults, born in the early 1980's.

5.3 To compare long-term pulmonary outcome subsequent to extremely premature birth in the early 1980's and the early 1990's.

5.4 To characterise the lung disorder that occurs in young people who were born extremely preterm, and compare with subjects born at term.

5.5 To establish knowledge regarding radiological findings on high resolution computer tomography (HRCT) of the lungs in young people born extremely preterm.

6. Study design, subjects and methodology

6.1 Study design

The present thesis reports results from an observational follow-up study of two cohorts of subjects who were respectively exposed (preterms) and not exposed (term-born controls) to extreme premature birth. Preterms were matched individually 1:1 to term-born controls with respect to gender and time of birth, and otherwise unselected except that their birthweight was within 1SD of Norwegian mean. The preterms were defined *after* the event of interest had occurred, implying a historical design. However, antenatal, perinatal and neonatal data were recorded in the patient's charts *before* outcome was assessed, and in this respect the design was historical and prospective.

All subjects were seen twice within a period of approximately two weeks in 2001/2002 at the paediatric Cardio-Respiratory Laboratory at Haukeland University Hospital. On the first test-day, the questionnaire from the *International Study of Asthma and Allergy in Childhood* (ISAAC) (134) and a specifically designed questionnaire were filled in by all subjects and their guardians. Subsequently, a full medical examination was done by one of two consultant pediatricians (TH or BTS). Spirometry with flow volume loops and whole body plethysmography were done on both test-days. Exercise testing and salbutamol responsiveness were assessed on the first test-day, while a methacholine provocation test was

done on the second. HRCT imaging of the lungs was done approximately within the two weeks used for lung function testing. To obtain adequate and comparable conditions for bronchial provocation, reversibility and allergy testing, subjects on medication were instructed to stop prior to testing as appropriate. Antihistamines were stopped one week before testing, long acting β_2 agonists two days before while short acting β_2 agonists and inhaled corticosteroids were stopped from at least 24 hours before testing. No subjects were on leukotriene blockers. No subjects were examined within two weeks of a respiratory tract infection or an asthma event.

6.2 Ethics

The Regional Ethics Committee approved the study. Informed written consent was obtained from participating subjects and their guardians. The study met the criteria listed by Emanuel et al. in their paper "What Makes Clinical Research Ethical" (135). Except venous punctures for blood sampling, no procedures were associated with discomfort or pain. All subjects were given the option to use local anaesthetic plasters (EMLA cream, AstraZeneca, Stockholm, Sweden). Except HRCT imaging, no procedures were considered to have potential for untoward negative consequences. The radiation dose was kept low by using modern equipment and limiting the number of slices. The radiation dose equivalent was estimated to 0.5-1.0 milli Sievert (mSv) per examination. (For comparison, a standard antero-posterior chest x-ray is estimated to 0.02 mSv). The risk of developing cancers due to exposure to low level radiation was discussed by Mossman (136). Exposures below 100 mSv have not been associated with increased risks.

6.3 Subjects

6.3.1 The preterms

The primary criterion for inclusion was gestational age (GA) less than or equal to 28 weeks at birth. In addition, children with birthweight (BW) equal to or below 1000 grams were included, irrespective of GA. All such infants who were born in the two periods January 1982 through December 1985 (first birth-cohort) and February 1991 through June 1992 (second birth-cohort) were included, providing their mothers were residents of one of the two counties *Hordaland* or *Sogn og Fjordane* in Western Norway at the time of delivery. The first inclusion period was set to include survivors from an early era of intensive care neonatology, with access to good quality neonatal data, documented in standardised forms. The second

inclusion period was set to include pre-pubertal children at the age of ten (scheduled to be examined January 2002-June 2002), who were born in an era with access to modern equipment and treatment modalities, such as exogenous surfactant and perinatal use of corticosteroids.

As the NICU at Haukeland University Hospital is the only unit in the region caring for sick premature neonates, the inclusion to the study was truly regional and population based. The regional population was approximately 500.000 and annual birth rates approximately 6700. Five preterms were outborn; the remaining 76 were inborn at the maternity unit at Haukeland University Hospital. NICU admission protocols were used to identify candidates for inclusion. To avoid dropouts due to misclassifications, charts were reviewed from all infants registered with BW below 1750 grams or GA below 32 weeks and in cases were information in the admission protocols were missing or unclear. A total of 346 charts were reviewed by two independent people: A registered nurse who had been working in the department for 10 years and this author.

Eligible infants were considered included if admitted alive into the NICU. That is, changes in attitudes between the two periods, with respect to delivery suite resuscitation of infants at the threshold of viability, may have influenced the constitution of the included populations: In the 1990's, more immature infants may have been considered viable and live-born in the delivery suite, and thus admitted to the neonatal department, compared to the 1980's (12). A possible sample bias of this kind may have increased the vulnerability of the preterms in the 1990 birth-cohort.

The GA set at admittance was reassessed with information from the obstetric and paediatric charts, i.e. copies of the pregnancy reports, ultrasound scans and paediatric assessment. An algorithm in accordance with Yudkin et al. (137) was employed when the neonates were originally admitted, and was also used in this retrospective reassessment. The number of completed weeks since the last menstrual period was compared to gestational age predicted by ultrasound scan performed before 21 weeks gestation and with postnatal assessment by paediatric examination according to Ballard (138). Scan assessment was preferred only if it differed more than two weeks from the estimate based on dates, whereas paediatric assessment was preferred if it differed four weeks or more from that based on dates or ultrasound scan. With nine of the 346 reviewed charts, the determination of gestational age at

birth was difficult, and the decision was left to an external expert on foetal medicine and intrauterine development.

The restricted ranges for GA and BW applied in the process of inclusion were expected to restrict the statistical potential for these factors to predict variability in outcome measures. Effects from GA and BW on lung function outcome are apparent from other studies with wider inclusion criteria. In this study, however, the likelihood for these variables to predict outcome was expected to be low.

Before the preterms and their guardians were asked to participate, the National Registry was consulted to ensure that they were alive. In order to achieve relevant knowledge on their medical status, local hospital registries and charts, and in special situations, also paediatric neurologists were consulted. Severely disabled preterms were initially carefully approached through a telephone call from this author, orally requesting their willingness to participate. All others were approached through a standardised letter of invitation. There were 51 eligible subjects from the first cohort and 35 from the second. From the first cohort, two were inaccessible (one had moved abroad and one never responded), two refused to participate whereas one was excluded from the study due to severe Eisenmenger syndrome. All subjects from the second cohort responded positively.

6.3.2 The term-born controls

One individual control subject was selected for each included preterm. Birth protocols in the obstetric department were used to identify the temporally nearest term-born subjects of the same gender with birth weight between 3.0 and 4.0 kilograms, corresponding approximately to the cut offs for the Norwegian 10^{th} and 90^{th} centiles (139). If this subject refused to participate, the next was approached, and so on. As the control population should reflect the preterm population with respect to all relevant attributes except for gestational age at birth, a bias in its constitution would be a serious source of error. To ensure objectivity in the selection process, this was largely left to an uninvolved secretary with precise instructions. For financial and practical reasons, subjects with a home address implicating more than one hour transportation by car to the Haukeland University Hospital, were excluded. The socio-demographics and the physical nature of the Bergen area, make it unlikely that this geographical constraint should introduce bias with respect to risk factors for obstructive airway disease. The only further criterion for exclusion was a registered diagnosis of a mental

or physical disability, likely to interfere with the test situations. Particularly, asthma was not a criterion for exclusion. There was only one exclusion due to these medical criteria; a young woman suffering from a serious lung condition for which she subsequently received a lung transplant one year later.

The higher the number of subjects that had to be approached to recruit a voluntary control, the higher the likelihood of including subjects with a personal interest in being tested, introducing another possible bias. In this study, 61 (75%) of the primarily 81 invited control subjects responded positively. In cases of refusals, the next eligible subject was asked. To complete a full 1:1 matched control group, an average of 1.3 term-born subjects were approached for each included preterm.

Socioeconomic disadvantage is associated with preterm delivery (140) and probably also with asthma (141). We chose *not* to match the control population for indicators of socioeconomic status. The educational level of the parents of the preterms and controls eventually participating in this study, revealed no significant differences. There was a non significant tendency for mothers of preterms to have stopped education after primary school compared to mothers of term-borns (22% versus 11%, $p = 0.086$), but no such trend for fathers. As socioeconomic status is more related to the educational level of the father (142), the likelihood for this type of selection bias in the control population was therefore considered to be low.

6.4 Lung function tests

Lung function was the main output measure of the study. The focus was on three aspects: Airway dimensions, airway reactivity and lung volumes. Standard, commercially available spirometric equipment was used, produced by SensorMedics inc., Yorba Linda, California, USA. The same experienced and certified (143) pulmonary technician performed all tests, blinded to the status of the test-person (preterm or control) and to any previous results.

6.4.1 Maximum flow volume spirometry

Gas flow was measured with a mass flow sensor incorporated in a Vmax 22 spirometer (SensorMedics inc., Yorba Linda, USA). Two probes, located in the centre of this device, are exposed to the gas stream. An electrical current cause them to heat-up. When particle velocity is present, it asymmetrically alters the temperature distribution around the two probes.

According to Kings Law, the cooling is proportional to the square root of the velocity. The temperature difference between the two sensors quantifies the particle velocity. A change of temperature of the probes, also leads to a change in resistance. The electrical current required to maintain a constant ratio of resistance in these differentially heated censors, is proportional to flow. When flow is measured directly, volume must be derived by integration of the flow information. The accuracy of the flow sensor is verified by the application of a precise and known volume of air over various flow rates. The American Thoracic Society (ATS) requires three different flow rates to cover the flows of interest. The flow sensor was thus calibrated with a 3 litre syringe at slow, medium and fast flow rates before each test. According to the specifications of the manufacturer, the equipment meets the ATS requirements for a diagnostic spirometer, i.e. an accuracy of at least ± 3% of readings or 50 ml, whichever is greater (144).

Subjects were tested in the sitting position, wearing a nose clip. Two measurements were performed, one on each test day. For each measurement, recordings were repeated until at least three technically acceptable curves were obtained. Criteria for acceptability were that exhalation should start instantly and without hesitation, the peak expiratory flow rate should be clearly visible as a peak in the flow-volume loop and there should be no fluctuations. Expiration was considered terminated when a plateau in the volume-time curve was apparent. Criteria for reproducibility were in accordance with the ATS 1994 recommendations, i.e. the largest and second largest FVC and FEV_1 should not differ more than 0.2 litres (144). In the final measurement report, the largest FVC and FEV_1 from acceptable curves were recorded. The other maximal expiratory flow values were obtained from the single curve with the largest sum of FVC and FEV_1. Most subjects were able to provide FVC and FEV_1 with reproducibility well below 0.2 litres. The following spirometric variables were recorded: Forced vital capacity (FVC), peak expiratory flow (PEF), forced expiratory volume in first second (FEV_1) and forced expiratory flow at 50% and 75% of FVC (FEF_{50} and FEF_{75}). The mean values of the two final measurement reports from each of the two days were used in statistical analyses.

6.4.2 Whole body plethysmography

Lung volumes were determined with a combined pressure-flow plethysmograph, Vmax Autobox 6200 (SensorMedics inc., Yorba Linda, USA). This equipment function as a variable-pressure, constant-volume device while measuring the thoracic gas volume (Vtg) and

during quiet breathing, and as a flow plethysmograph during forced expiration. As biological controls, two reference subjects in the laboratory had their lung volumes measured monthly and the results logged and compared. Measurements were done in accordance with the guidelines of the European Respiratory Society (145). Calibration of the flow sensor and the plethysmograph (50 ml, frequency 2 Hz) was performed prior to each test in accordance with the automated procedures and the instructions given by the manufacturer (146). Test subjects were left outside during calibration and individual volume corrections of the plethysmograph were subsequently performed, based on body weight. A bacterial filter (MicroGard, SensorMedics inc., Yorba Linda, USA) with an attached mouthpiece was used. The added deadspace was included in the applied algorithms and dealt with by the software. Deadspace may have some influence on the measurements, as the temperature response to pressure changes within the deadspace and the lung may differ. Therefore, this volume was kept as low as possible. Subjects were tested seated in an upright position with the face pointing straight forward, supporting the cheeks in their hands, wearing a nose-clip. After closing the door and a period of relaxed tidal breathing to allow for thermal stabilisation and familiarisation with the situation, a baseline level of functional residual capacity (FRC) was established from at least four stable tidal breaths. Two techniques were used, the panting and the single inspiratory effort technique (147;148). With the panting technique, test subjects were coached to pant gently with a frequency of about 1 Hz (149). The shutter closed at the end of a tidal expiration (no flow) whereupon a series of at least four panting movements were done against it, recording the corresponding pressure-volume loops. After opening of the shutter, the test subjects made 2-3 tidal breaths before performing a full inspiratory manoeuvre to total lung capacity (TLC) followed by a full expiratory manoeuvre to the residual lung volume (RV). From each acceptable pressure-volume loop, a thoracic gas volume (Vtg) was estimated by applying the mathematical algorithms installed by the manufacturer (146). Subsequently, a mean Vtg was calculated. Pressure-volume loops were considered technically inadequate if the pressure gradient exceeded 1.5 kilopascal (kPa) or if there were obvious “looping” or open ends, nonlinear segments or other major irregularities. Cursor lines for slope calculations were adjusted only in cases of blunt mistakes (150;151). With the single inspiration technique, the breathing frequency was about 20 per minute. The shutter closed at the end of a tidal expiration (no flow), data collection was triggered to start at 0.25 kPa of inspiratory mouth pressure and the shutter was subsequently released when the pressure gradient had increased by another 0.25 kPa. Calculation of Vtg was based on pressure-volume plots recorded in the interval between 0.25 and 0.5 kPa. If the shutter closed at a lung volume that differed from the

established baseline level of FRC, the calculated Vtg was adjusted according to this volume discrepancy to obtain the correct volume of FRC. TLC was subsequently calculated by adding inspiratory capacity (IC) to FRC, and RV by subtracting the expiratory vital capacity (VC) from TLC (146;152). At least three test-manoeuvres were done and each final test-report was based on their average. The following lung volumes were recorded: FRC, TLC, RV and RV/TLC. In addition, airway resistance (Raw) was measured with the panting technique.

The physical principle underlying the mathematical calculation of thoracic gas volume in a body plethysmograph is expressed in Boyle-Mariotte's law. However, the algorithms finally installed in most commercially available equipment, have been subject to modifications based on certain assumptions that have been criticised (153;154). This applies particularly to the single inspiration technique if high pressures are generated against the shutter or if the manoeuvres are prolonged (154). With the equipment used in this study, influence from high pressure gradients and prolonged manoeuvres were avoided by calculating Vtg from pressure-volume plots recorded in a narrow pressure interval between approximately 0.25 and 0.5 kPa.

6.4.3 Test of airway hyperresponsiveness with methacholine chloride

Airway responsiveness to methacholine was determined and expressed by the cumulative dose in µmol which caused a 20% decrease from baseline in FEV_1 (PD_{20}) (155). An automated, inhalation synchronized, dosimetric jet nebulizer, Spira Elektra 2 (Respiratory Care Centre, Hameenlinna, Finland) (156) with 2.0 bar driving pressure and an integrated turbine flow sensor, was used to nebulize methacholine. Nebulization-time was 0.5 seconds, set to begin after 100 ml of air had been inspired. Inspiratory flow was measured and should not exceed 0.5 litres/second. Two concentrations of methacholine were administered from separate dosimeters (2.5 and 25 mg/ml), calibrated to deliver 3.6 µl per 0.5 seconds. This set-up ensured early to mid-inspiratory administration of a known volume of nebulized solution, inhaled with a controlled inspiratory flow rate. Methacholine was administered in doubling amounts, beginning with 0.045 µmol and – unless a positive response - ending at 22.3 µmol. Prior to administration of methacholine, a baseline FEV_1 was determined after inhalation of saline 0.9 mg/ml. Subsequently FEV_1 was measured one minute after each dose of methacholine. PD_{20} was calculated by interpolation between the doses immediately preceding and following the 20% fall in FEV_1.

Interpretation: The diagnostic sensitivity and specificity of the PD_{20} test is influenced by the different methods and equipment used, by the constitution of the population sample that is

tested as well as by the cut-off level set to define a positive response (157). A comparable control population is therefore imperative when AHR is to be assessed in a population subgroup with a particular exposure. In our laboratory, the cut-off value suggested by Godfrey et al. (158) work reasonably well in the routine diagnostic work, and was also employed in paper #1 and paper #2 of this study. The ratio between the maximum percent decline obtained in FEV_1 over the maximum cumulative dose of methacholine (in μmol), was used as an index of the dose-response slope (DRS) (159). Thereby, a continuous index of AHR was obtained also in non-responders. DRS was used as an index of AHR in paper #3 and paper #4.

6.4.4 Test of exercise induced asthma (EIA)

EIA tests were performed within a setting of simultaneous measurement of gas exchange, oxygen consumption and exercise flow volume loops. The cardiopulmonary exercise data are not assessed as a part of this thesis. After establishment of a baseline FEV_1, subjects were running to exhaustion on a treadmill (Woodway ELG 70, Weil am Rhein, Germany), wearing a face mask and connected to a Vmax 29 spirometer and cardiopulmonary exercise unit. To facilitate familiarization with the treadmill in children with potential for various disabilities (86), and to optimize the conditions for cardiopulmonary exercise testing, a modified Bruce protocol was used (160). The primary criterion for test-termination was exhaustion, with support from either a respiratory quotient (RQ) > 1.05, a heart rate > 95% of predicted maximum level (220-age in years), or a plateau in oxygen consumption. FEV_1 was measured 1, 3, 6 and 10 minutes after exercise was stopped (161;162).

Interpretation: The EIA test was considered positive if the largest decline in FEV_1 relative to baseline exceeded 12%.

6.4.5 Test of reversibility to salbutamol

After the last spirometry of the EIA test, 100 μgram salbutamol per 10 kg bodyweight was administered with a metered dose inhaler (Ventoline®) via a plastic spacer (Volumatic®). FEV_1 was measured 10 minutes later.

Interpretation: Reversibility to salbutamol was considered significant if the increase in FEV_1 relative to the baseline measurement obtained before the EIA test, exceeded 12%.

6.5 High-resolution computed tomography (HRCT) of the lungs

HRCT imaging of the lungs was performed with a General Electric Hi Speed Advantage Single-Slice Helical - CT scanner with 1.25 mm slice thickness; 0.5 second scan time, 120

kV, 50-100 mA, lung algorithm and 512-512 matrix. Approximately 10-12 scans in inspiration with 10 mm intervals were followed by 4-5 scans in expiration with 20 mm intervals. Total radiation exposure per examination was estimated to be 0.5 –1.0 mSv. All images were read at a PACS workstation with a window width of +1540 and window level of –400 Hounsfield units. Based on Bhalla scoring system for cystic fibrosis (163) and prior studies in BPD survivors, a scoring system consisting of 14 parameters was constructed. Four parameters of special interest were introduced in addition to those included by Bhalla: (1) small peripheral linear opacities, (2) triangular subpleural opacities, (3) mosaic perfusion, and (4) air trapping in expiration. The evaluation of disease extent was based on a geographic lung map with lung segment borders, in which all radiological findings were recorded.

The examinations were read independently by two experienced paediatric radiologists. Observer one (SMA) read the HRCT images twice separated by a period of at least six months, while observer two (KRF) read the images once during May 2004. None of the observers had knowledge of previous results, clinical findings or lung function tests. Prior to the study, in order to standardise the scoring system, both observers analysed and discussed the findings of four different HRCT examinations (three patients with cystic fibrosis and one premature child who did not participate in this study).

6.6 Assessment of allergic sensitization

Skin prick tests against 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 (164). Histamine (10 mg/mL) and the allergen diluent were used as positive and negative control, respectively. A reaction was judged positive if mean of the two perpendicular weal diameters was at least 3.0 mm.

6.7 Biochemical markers of airway inflammation

Urinary and blood biochemical substances were measured to assess airway inflammation. Blood was drawn at the first test-day, whereas early morning urinary samples were brought on the second day. The urine was aliquoted and put to -80^{O}C. To test stability of the substances, selected samples were left on the bench at room temperature and in the refrigerator for 1-5 days, without significant impact on subsequent measurement results. Urinary creatinine was measured with Jaffes reaction, and urinary leukotriene E4 (U-LTE$_4$) with ACE™ competitive

enzyme immunoassay (Cayman Chemical, Ann Arbor, USA) at the research laboratory in the Paediatric Department in Bergen. All U-LTE_4 analyses were done in duplicate and expressed in ng/mmol creatinine. To measure urinary eosinophilic protein X (U-EPX), frozen urine was shipped on dry ice to the Pharmacia laboratory in Uppsala and analysed with a specific radioimmunoassay (Pharmacia, Uppsala, Sweden). U-EPX was expressed in µg/mmol creatinine. Serum eosinophilic cationic protein (S-ECP) was measured with Pharmacia CAP System ECP™ (Pharmacia Diagnostics AB, Uppsala, Sweden) at the routine laboratory for clinical biochemistry at Haukeland University Hospital in accordance with their routine protocols. S-ECP was expressed in µg/litre. Whole blood eosinophilic leukocytes (B-Eos) were counted ($x10^9$/litre) in a Bayer Technicon H3™ (Bayer, Leverkusen, Germany).

In paper #4, we wished to examine if subjects in the preterm and the term-born population with "high levels" of these markers had increased methacholine responsiveness (High U-EPX, U-LTE_4, S-ECP and B-Eos), and furthermore, if a potential association between "high levels" of these markers and methacholine responsiveness was different in the population born preterm compared to the population born at term. We defined "high levels" as values above the $75^{th.}$ centile. The respective upper quartiles were defined by the values in the preterm and the term-born populations, *separately*. We could alternatively, have defined "high levels" from the complete data set or from control subjects only. Our control population was, however, not an optimal reference population. Furthermore, we had no way to predict in advance if the values of these markers would be similar in the preterm and the term-born population. Theoretically, increased or decreased levels of these substances could be related *also* to features other than airway inflammation. If quartiles were defined from the complete data set and a significant difference existed between the preterm and term-born population, this would influence sample selection differently in the two populations, and thereby potentially influence the results. Therefore, to us it seemed most correct to select subjects belonging to the upper quartiles based on cut-off levels established in the preterm and term-born population, separately.

6.8 Questionnaires

Parents and participating subjects filled in three sets of questionnaires: A validated translated version of the Child Health Questionnaire (CHQ) (165) to assess quality of life, the questionnaire from the International Study on Asthma and Allergy in Childhood (ISAAC) to

assess airway symptoms, and a separate questionnaire designed to map remaining relevant demographic variables and health related information.

6.9 Neonatal data

Patient records were available for all but one preterm. For this single subject, relevant information was collected from discharge summaries and maternal recall. For all other subjects, information on neonatal variables was collected from the original paediatric records, mostly available in standardised forms that were filled in by the nurse on call with *in situ* responsibility for the care of the infant. Information on antenatal variables (maternal corticosteroids) was collected from the obstetric and paediatric records.

6.10 Definitions of BPD, CLD and Asthma

BPD was defined and classified in accordance with suggestions from the workshop on BPD in Bethesda, USA, arranged in 2000 by the National Institute of Child Health and Human Development/National Heart, Lung and Blood Institute/Office of Rare Diseases (39). A diagnosis of BPD was made in infants treated with oxygen for more than 28 days. BPD was defined as mild in infants breathing room air at 36 weeks PMA. The workshop suggests that infants requiring < 30% oxygen at 36 weeks PMA are classified as moderate BPD while those requiring ≥ 30% are classified as severe BPD. In order not to create subgroups that were too small, we chose to analyse all infants receiving oxygen treatment (any concentration) at 36 weeks PMA together as moderate/severe BPD.

CLD. The concept of CLD is often used to classify premature infants with requirement for oxygen treatment at 36 weeks PMA (41). In this study, CLD will correspond to moderate/severe BPD.

Asthma. The main criterion for a diagnosis of asthma was at least one reported episode of wheeze within the preceding 12 months, reported in the ISAAC questionnaire. Not all subjects who report current wheeze have asthma. Therefore, we wished to confirm that reported symptoms were in fact expressions of a clinically relevant and current asthma.

- In paper #1 and #2, asthma was defined by at least one episode of wheeze within the previous 12 months, and additionally, a confirmed airway hyperresponsiveness to methacholine (166). AHR was defined by a decline in FEV_1 exceeding 20% relative to baseline before a cumulative dose of 6.7µmol methacholine had been administered (158).

- In paper #4, asthma was defined by at least one episode of wheeze within the previous 12 months, and additionally, clinical support from *either* a current doctor's diagnosis of asthma when the child was included to the study, *or* from a positive test for exercise induced asthma (EIA) or a significant reversibility to salbutamol.

6.11 Statistical analyses

The study was designed as a 1:1 matched controlled cohort study of two samples of subjects which differed with respect to gestational age at birth, but were otherwise similar. Tests devised to compare paired observations were therefore used to assess data that were available in a paired fashion. In univariate analyses, paired t-tests were used to compare group means, and McNemar's test in cases of categorical data. A paired multivariate two way analyses of covariance was used to assess relationships between multiple explanatory variables and continuous numerical output-variables [the linear mixed model procedure of SPSS (167)]. Interaction terms (168) were constructed to assess potential effect-modifying influence in relation to outcome from a number of factors, e.g. being born in the first versus the second inclusion period, or having a history of non-BPD versus mild BPD versus moderate/severe BPD. Paired odds ratios (OR) and confidence intervals (CI) were estimated according to Breslow & Day with special programming in Maple (169).

In non-paired univariate group comparisons, two sample t-tests were used to compare group means and Pearson's χ^2- test in cases of categorical data. The homogeneity of the variances within compared groups was assessed with Levene's test. Analysis of variance was applied to assess differences between non-paired groups, such as the BPD subgroups of the preterm population. Non-paired multivariate analyses were done with stepwise multiple linear regressions in order to explore relationships between potential explanatory variables and measures of outcome, such as lung function variables. Univariate trend analyses in continuous data were performed with linear regression if normally distributed and Kruskal-Wallis' test if not, and with Pearson's χ^2- test for trend if data were categorical.

Several outcome variables were asymmetrically distributed. Methacholine responsiveness represents a statistical challenge, as the variance in population-based studies is high and its distribution far from Gaussian. However, most researchers assume a lognormal distribution of the dose-response slope (159), as employed in the present study. Variables used to describe airway inflammation (B-Eos, S-ECP, U-LTE4 and U-EPX) were also asymmetrically

distributed, and we applied non-parametric statistical methods (Wilcoxon-Mann-Whitney) for group comparisons, in accordance with previous literature in this field (82).

6.11.1 Statistical power

The size of the study populations was defined in order to achieve a 90% power to detect a difference in FEV_1 of at least 7.5% between preterms and matched control subjects in each of the two birth-cohorts and also between the two preterm birth-cohorts, providing a significance level (α) of 0.05 and 1 SD = 10% predicted.

6.11.2 Particular statistical aspects relating to particular papers

A. The study comparing two methods for lung volume assessment (paper #1)

The approach suggested by Bland and Altman for method comparisons were used (170;171). The repeatability (precision) of each technique was determined by calculating the standard deviations (SD) of the differences between two replicate measurements. Agreement between the two techniques was assessed by calculating the SDs of the differences between paired measurements obtained with the two techniques. The SDs were used to calculate 95% limits of agreement between replicate measurements obtained with the same technique as well as 95% limits of agreement between the two techniques. Repeatability of the techniques and the agreement between them were visualised by plotting the differences between paired measurements against their average values. Concordance between paired measurements was visualised by means of Kaplan-Meier plots (172).

B. The study of HRCT findings in the lungs (paper #5)

Agreement within and between observers regarding scores of radiological findings was visualised with Bland-Altman plots and examined with kappa (κ) statistic (170;171). Differences according to grading were tested with McNemar's test of symmetry. Differences in score between subjects with and without BPD were assed the Mann-Whitney test.

7. Results and summary of papers

7.1 Paper # 1

Whole body plethysmography can by used to assess lung volumes in preterms. Despite somewhat inferior repeatability, preterms did not perform principally different from term-born control subjects (Table 1). By inference, this suggests that spirometry and flow volume loops can be used to assess airway function in preterms, since body plethysmography is a more complex procedure that partly entails adequately performed spirometry. When comparing repeatability for the two applied methods, the fraction of replicate measurements with discordance in the medium range was smaller with the panting than the single inspiratory effort technique. The fraction with a high discordance was comparable for both methods (Figure 1). The likelihood of making large mistakes thus seemed to be similar with both techniques. Therefore, if the standard panting method becomes technically too difficult, the single inspiratory effort method can be used in a clinical setting, but with more caution in research.

Table 1

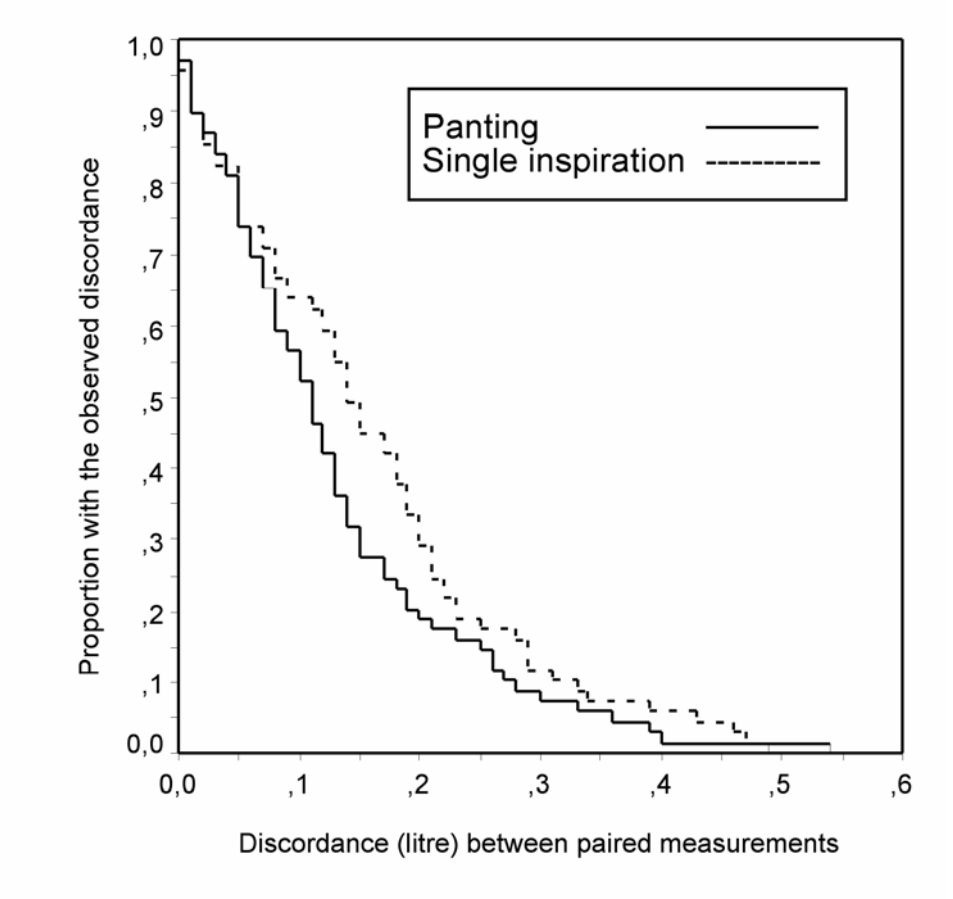
Repeatability of the panting and single inspiratory effort technique

		Panting		Single inspiratory effort	
		1SD *	**95% limits of agreement †**	**1SD ***	**95% limits of agreement †**
FRC	Non-asthmatic controls	0.16	20.3	0.18	23.1
	Non-asthmatic preterms	0.17	23.8	0.18	26.3
	All preterms	0.18	23.3	0.19	28.8
TLC	Non-asthmatic controls	0.13	8.0	0.18	11.5
	Non-asthmatic preterms	0.19	12.9	0.18	12.2
	All preterms	0.21	12.5	0.18	12.4
RV	Non-asthmatic controls	0.14	39.5	0.23	66.7
	Non asthmatic preterms	0.20	60.0	0.23	72.2
	All preterms	0.22	59.9	0.22	72.3

***** 1SD (litres) of the differences between two replicate measurements of the same lung volume, measured with the respective techniques. † 1.96 SDs of the differences between the two replicate measurements, reported as percentage of the means of the two measurements. Data are given for the ten year olds only, non-asthmatic controls (n = 33), non-asthmatic preterms (n = 25) and all preterm (n = 34).

Figure 1

Assessment of lung volumes, method comparison



Discordance between replicate measurements of FRC obtained with the panting and the single inspiratory effort technique in ten-year-old children. The x-axis represents the absolute difference between replicate measurements, and the y-axis represents the proportion of pairs with at least the observed difference. All the 69 tested ten-year-old subjects are included, i.e. preterms and term-born controls.

7.2 Paper # 2

In 18 year old preterms born in the first inclusion period (1982-85), the prevalence of a doctor's diagnosed asthma, regular use of asthma inhalers and recurrent wheeze was increased compared to term-born controls. Prematurity was associated with increased airway obstruction, resistance and hyperresponsiveness, as well as with pulmonary hyperinflation. Differences between preterms and term-born controls were mostly accounted for by subjects with a history of neonatal BPD, although small airway obstruction was markedly increased also in non-BPD subjects. Prolonged neonatal oxygen treatment significantly predicted current FEV_1. Adjusted for height, gender and age, FEV_1 was reduced with a mean of 580 ml/second in subjects with a history of BPD, representing 1-2 decades of normal age related decline.

7.3 Paper # 3

Comparing preterms born in the 1980's and in the 1990's, the incidences of BPD and chronic lung disease (CLD) were similar. Compared to matched controls, current airway obstruction, AHR and pulmonary hyperinflation were similarly increased in the two preterm cohorts. Furthermore, current deficits in important lung function variables were similarly associated with the severity of neonatal respiratory disease in both cohorts (Table 2). Compared to matched controls, current FEV_1 was reduced with respectively 18.6% and 18.7% of predicted in the two birth-cohorts in preterms who required supplemental oxygen at 36 weeks postmenstrual age. One-hundred days with neonatal oxygen supplementation predicted decreases in current FEV_1 of 12%, and in current FEF_{50} of 25%, and these figures were similar in both birth-cohorts. With respect to subsequent small airway obstruction, the influence from a neonatal diagnosis of BPD seemed to have *increased* in the most recent birth-cohort (Table 2, test of interaction, $p = 0.020$). The impact on current FEF_{75} from the number of neonatal days with oxygen supplementation was non-significantly *increased* in the second birth-cohort compared to the first birth-cohort (test of interaction: $p = 0.091$), explaining 37% of the variability in FEF_{75} in the ten-year-olds ($p < 0.001$). Neither gestational age at birth nor birthweight was significant predictors of current lung function.

Table 2

Paired decreases in lung function, by birth cohort and the extent of neonatal bronchopulmonary dysplasia (BPD)

		Extent of BPD †		p- values ‡	
	Birth cohort	**None or mild (n = 55)**	**M/S (n = 24)**	**Differences between birth-cohorts**	**Influence from BPD**
FEV_1	91-92	5.5	18.7	0.901	0.762
	82-85	7.2	18.6		
FEF_{50}	91-92	13.8	36.8	0.698	0.107
	82-85	18.7	40.3		
FEF_{75}	91-92	12.1	41.6	0.566	0.020
	82-85	24.2	36.2		
FVC	91-92	4.9	8.0	0.650	0.388
	82-85	2.6	8.9		
TLC	91-92	1.7	- 1.8	0.818	0.184
	82-85	0.8	1.6		
RV/TLC	91-92	- 0.5	- 7.0	0.810	0.158
	82-85	- 0.8	- 6.4		
DRS *	91-92	-2.5	-28.9	0.134	0.996
	82-85	- 1.7	- 14.8		

The figures are group mean differences between preterms and individually matched controls in percent of predicted, except for the variable * DRS (dose-response slope to methacholine) where differences between preterms and controls are given as median values. † The criteria for classification of BPD are given in the text. ‡ The p-values test "differences between the birth-

cohorts" with respect to lung function outcome, and if the "influence from BPD" on subsequent lung function outcome differed between the birth-cohorts (interaction analyses).

7.4 Paper # 4

Asthma after premature birth was not significantly related to allergy or increased markers of airway inflammation. Increased AHR in preterms was not confined to those with asthma (Table 3). Furthermore, the substantially increased AHR in preterms was unrelated to inheritance, cigarette exposures, allergy and markers of airway inflammation. Instead, AHR was strongly and significantly related to a neonatal history of BPD and prolonged requirement for oxygen treatment (Figure 2). The urinary metabolite leukotriene E4 was increased in preterms compared to term-born controls.

Table 3

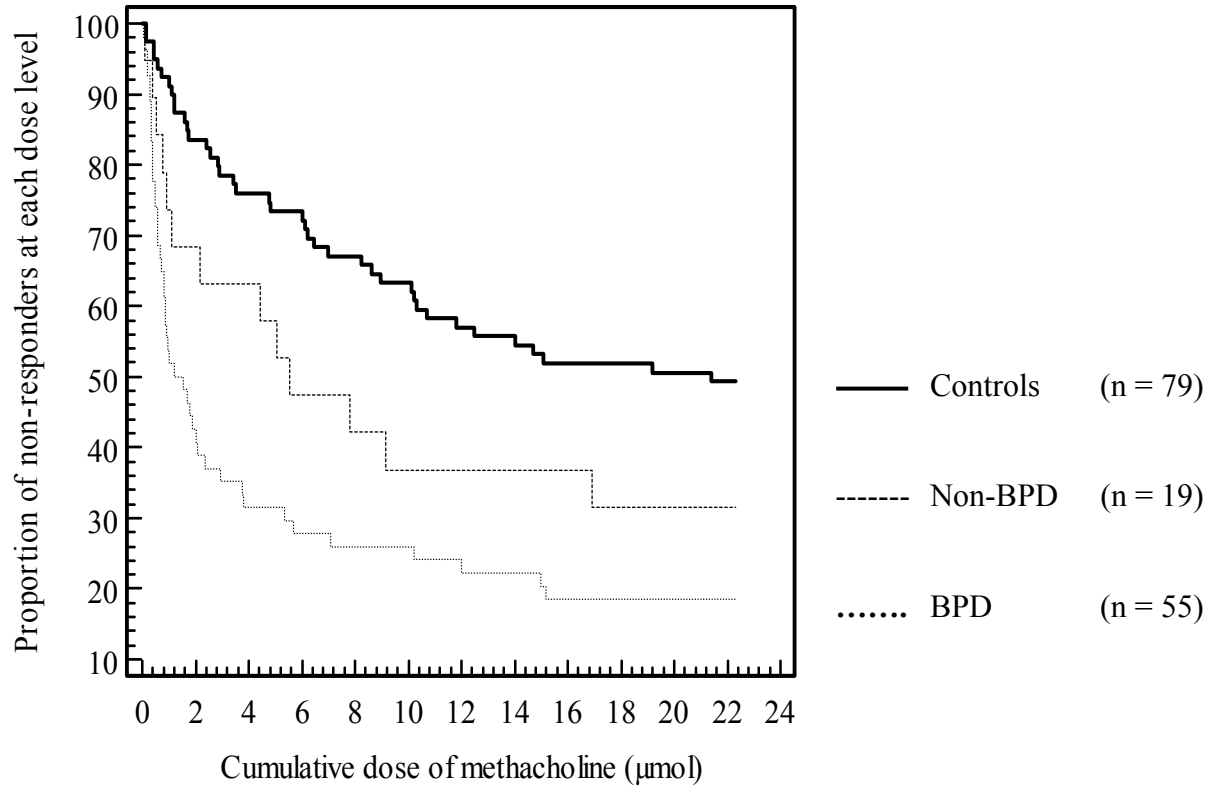
Characteristics of current asthma in preterms and term-born control subjects

	Preterms (n = 81)			Controls (n = 81)		
	Asthma (n = 22)	**Non Asthma** (n = 59)	p-value †	**Asthma** (n = 10)	**Non Asthma** (n = 71)	p-value †
DRS ‡	9.8 (3.6, 26.6)	6.2 (3.7, 10.3)	0.368	12.6 (4.7, 34.3)	1.2 (0.9, 1.7)	< 0.001
Allergy *	5 (22.7%)	5 (8.5%)	0.125	6 (60.0%)	6 (8.5%)	< 0.001
B-Eos	0.130 (0.064, 0.221)	0.100 (0.065, 0.164)	0.259	0.348 (0.229, 0.501)	0.121 (0.084, 0.184)	< 0.001
S-ECP	12.2 (8.8, 18.1)	10.7 (6.9, 18.8)	0.482	17.1 (12.8, 29.0)	10.7 (7.5, 19.6)	0.071
U-EPX	34.4 (27.6, 50.9)	47.5 (31.5, 86.5)	0.131	77.4 (42.3, 95.6)	37.1 (24.7, 52.8)	0.032
U-LTE_4	40.2 (23.0, 60.1)	27.9 (20.5, 48.2)	0.322	39.4 (22.5, 51.2)	23.2 (15.3, 33.3)	0.068

Current asthma was principally defined by recent wheeze and a doctor's diagnosis at study inclusion. * Number (%) in the respective groups with perennial allergies. † Relations between asthmatics and non-asthmatics in the preterm and term-born population, tested with χ^2-test (Allergy), t-test (log_{10} DRS) or Mann-Whitney test. ‡ Dose response slop to methacholine, geometric means (95% CI). B-Eos = eosinophilic granulocytes ($x10^9$/litre), S-ECP = serum eosinophilic cationic protein (µg/l), U-EPX = urinary eosinophilic protein X (µg/mmol creatinine) and U-LTE_4 = urinary leukotriene E4 (ng/mmol creatinine), all reported as median values (25-75 centiles).

Figure 2

Methacholine responsiveness in the preterm and term-born populations



Methacholine responsiveness in young people born extremely preterm, classified by neonatal bronchopulmonary dysplasia, and in matched term-born control subjects. The x-axis represents the total cumulative dose of methacholine given to each subject, censored at the maximum administered dose of 22.3 µmol. The y-axis depicts the proportion of non-responders at any given dose. In this graph, BPD was defined as neonatal requirement for supplemental oxygen ≥ 28 days.

7.5 Paper # 5

HRCT imaging of the lungs was successfully performed in 74 (91%) preterms. One subject was medically unable, and six did not show up. In two cases, images were printed on hard copies but accidentally not stored in the PACS system. Thus, images from 40 subjects of the first birth-cohort and 32 subjects of the second birth-cohort were assessed. The agreement within and between observers of a novel scoring system for radiological findings was moderate, but varied between different parameters.

Radiological findings were revealed in 63 (88%) of the examined preterms. Total score was significantly reduced (i.e. less findings) in subjects of the second inclusion period (1991-92) compared to the first (1982-85). There was a tendency for higher scores in preterms with a history of BPD compared to those without, however, the differences did not reach statistical significance. There were no significant differences in scores between males and females, but maybe a weak tendency for lower total score in females ($p = 0.10$). Most of the described pathology was discrete linear or triangular opacities of unknown prognostic significance. Mosaic perfusion and air trapping observed in respectively 13% and 26% may, however, represent prognostic information. The significance of these findings as putative x-ray precursors of chronic lung damage can only be established through further follow-up of these young people, with concurrent lung function measurements and clinical surveillance.

8. Discussion

Pulmonary sequelae after extremely premature birth were demonstrated in childhood and in early adult life. Adverse outcome at follow-up was strongly and significantly associated with neonatal respiratory morbidity. Subjects in both birth-cohorts with a neonatal history of BPD had similar, significant and substantial decreases in important lung function variables. In 18 year old subjects with a history of BPD, the decrease in FEV_1 corresponded to 1-2 decades of normal age related loss of lung function. Prolonged neonatal oxygen supplementation predicted current decreases in lung function, and for important lung function variables, this association was similar in the two birth-cohorts. Asthma and AHR in preterms were unrelated to markers of airway inflammation and to features usually observed with asthma, such as inheritance and cigarette exposures, and AHR was not confined to subjects with asthma. These findings suggest irreversible, structural damage to the airways and/or to the pulmonary interstitium as causal factors, rather than an ongoing, eosinophilic inflammatory disease. Less radiological findings in the second birth-cohort *may* reflect less structural damage in preterms born in the 1990's.

8.1 Methodological aspects

8.1.1 The population sample

The fundamental endpoint of clinical research is to develop generalizable knowledge. In order to achieve this, the population sample selected for a study should be *representative* and *unbiased*.

Most studies that address the issue of long-term sequelae from *premature birth*, enrol subjects with low (102), very low (87;90;98;99;119) or extremely low (97;173) *birthweight*. Results from studies with this design may be influenced by subjects inappropriately grown for gestational age. Therefore, the principal inclusion criterion in the present study was *gestational age*. In order to include all preterms perceived as extreme, subjects with birthweight ≤ 1000 grams were also included, irrespective of gestational age. There were three of these growth retarded subjects in the first birth-cohort and four in the second.

Population based studies are less likely to be influenced by known or unknown confounding factors compared to studies recruiting from tertiary level teaching hospitals, which is a

commonly employed design. Inclusion to the present study was truly population based since all mothers of the participating preterms were residents within a defined geographical area and, to the best of our knowledge, no preterm neonates born by mothers who were residents of this area, were referred to hospitals outside this area.

In a number of published follow-up studies, the ratio between subjects actually examined versus the number of subjects eligible for inclusion, have been rather low (91;98;99;102;119;120;174;175) and in some studies, the tested fraction was in fact less than 60% (98;99;119). High drop-out rates raise questions as to the representativeness of the sample that was eventually examined. In the present study, the drop-out rate was 6%. This is unusually low in population-based long-term follow-up, and comparable only to an Australian study (97).

Bias due to selective or differential survival is difficult to exclude. The survival rate in the present study was 67%, similar to results reported for comparable populations in comparable institutions at the time (11). We have no reason to suspect that mortality in our institution was related to other factors or conditions than those operating in other comparable institutions. As was pointed out in a review (176), however, this issue can be answered only through full assessment of family background, antenatal exposures and neonatal data also for subjects not surviving.

To conclude on this point, we hold the population sample examined in the present study to be *representative* and *unbiased* for long-term survivors after extremely preterm birth.

8.1.2 Sample size, detection limits and statistical power

The present study entailed assessment of a number of variables with different and partly unknown distributions, some of them far from Gaussian. Subgroups of various sizes were analysed. By nature, the issue of *a priori* sample size calculation becomes complex in a context of this kind. The study was powered to detect clinically relevant decreases in the preterm populations for the main outcome measure, which was FEV_1. In order to directly address uncertainties relating to conclusions, confidence intervals were presented for most calculated mean values (177). The total number of subjects included was 162, of whom 81 were born prematurely and 81 at term. As for a long-term clinical follow-up study in this field, this number is comparable to most previous reports.

In paper #3, we were not able to reject the null-hypotheses, as the two preterm birth-cohorts appeared strikingly similar with respect to FEV_1%. This calls for a discussion over statistical power and detection limits. We had 90% power to detect a difference exceeding 7.5% for FEV_1% between the two complete preterm birth-cohorts. Overlapping confidence intervals supported the notion of equality (177). Subgroup analyses according to neonatal BPD-status necessarily implied fewer subjects in each group, and therefore higher detection limits with respect to differences between them. We had, however, reasons to treat the BPD subgroups as ordinal groups. Therefore, the observed and striking similar trends in the two birth-cohorts with respect to outcome over increasing severity of neonatal BPD, supported the null-hypothesis. Tests of interaction (168) gave further support, as a p-value close to 1.0 with respect to effect from birth cohort on subsequent FEV_1%, made an effect unlikely.

In paper #4, differences between relatively small subgroups were assessed (asthmatics versus non-asthmatics, smokers versus non-smokers, exposed versus unexposed to intrauterine cigarette smoking, subjects with a family history of asthma versus subjects without). In these circumstances, negative conclusions should be treated with caution. Again, confidence intervals were presented for all mean values, to address the issue of uncertainties (177).

A larger population sample may have described these issues with greater certainty. However, large-scale, long-term, clinical studies of samples that are representative for young people with this particular neonatal background, are demanding to carry out. The issues of sample selection and sample size are complex and involves several difficult aspects (135;178-181). We regard the conclusions presented to be statistically adequately documented – even if the sample size by some may be perceived as marginal.

8.1.3 Comparing pulmonary function in subjects of unequal age, height and gender

In order to compare lung function in subjects of unequal gender, age and height, raw-data were transposed to percentages of predicted values, calculated by using recognised prediction equations. FEV_1 was considered the main outcome variable, and "percent predicted" was calculated from a set of regression equations presented by Quanjer et al. (182). FEV_1 was predicted rather well by these equations in control subjects, i.e. the FEV_1 predicted was close to the FEV_1 actually measured in subjects assumed to be healthy and normal, irrespective of

age, height and gender. Subsequently, most statistical comparisons were done within the paired framework of the study, i.e. by assessing paired differences between preterms and matched controls. In paper #3, we assessed outcome in 10-year-olds and 18 year-olds by comparison of paired differences between preterms and matched controls in the two birth-cohorts. These differences were found to be basically similar for important lung function variables. Since the younger age group had approximately seven years of growth to reach the age of the older, this design precipitates a discussion of possible effects of growth: Lung function may develop differently with adolescent growth in preterms compared to subjects born at term. Summarising available knowledge on this issue, Eber and Zach (104) stated that hyperinflation may improve with growth whereas airway obstruction improve only slowly or not at all. We therefore consider the presented comparisons as valid despite the age difference, and we reckon the presented information on long-term trends as important.

8.1.4 Classification of BPD in different time eras

Oxygen is used both to diagnose and to treat BPD. Therefore, the strategies employed for weaning neonates from supplemental oxygen are important factors in the process of classifying neonatal respiratory morbidity. If oxygen supplementation is considered harmful, strategies for weaning from oxygen may also influence the extent of lung injury. One of the strengths of this study was that the senior medical staff was principally unchanged in the two inclusion periods. Despite new technology (pulse-oximetry), decisions regarding weaning from oxygen treatment were made with reference to the same clinical judgement (44).

8.1.5 Test conditions

The assessment of lung function at baseline conditions combined with two different tests for airway responsiveness and a reversibility test for salbutamol as well as measurement of gas exchange during exercise, created logistical challenges. The modified Bruce protocol employed in the exercise set-up, may underestimate the occurrence of exercise induced bronchoconstriction. The protocol was used for two reasons: We wished to combine assessment of exercise induced bronchoconstriction with measurement of gas exchange during exercise, for which purpose a ramp protocol with a rather careful start seemed better suited in this population of children. A standardized protocol with a careful start was anticipated to enhance the success rate in children who were unfamiliar with the test-situation and had a potential for various disabilities.

All subjects accepted the test situation and in no cases did we stop due to anxiety. All subjects were pushed rather hard for the last 4-5 minutes, and most subjects achieved a maximum heart rate above 95% expected. Mean (95% CI) RQ levels were 1.09 (1.08, 1.10). Mean maximum minute ventilation (95% CI) was 77% (74%, 79%) of the expected maximum minute ventilation that was calculated from $FEV_1 \times 35$. In accordance with previous literature (183), mean ventilatory reserves at maximum exercise tended to be less in preterms than in controls (21% versus 25%, respectively, $p = 0.060$). We are relatively confident, that the test conditions were acceptable also in the context of releasing a positive EIA response (161;162).

8.2 Predictors of pulmonary outcome

Neither BW nor GA at birth was significantly related to lung-function outcome. This was a consistent finding in univariate models as well as in multivariate models in combination with other neonatal potential explanatory variables. Clearly, pulmonary outcome from premature birth is related to these two variables (63;102;120). The mere fact that the preterms of the present study differed from term-born controls, supports this notion. In the present study, however, the distribution of these two variables - as defined by the inclusion criteria - was relatively narrow. Therefore, by nature, the design of the present study reduced the statistical potential for BW and GA to influence *variability* in outcome measures. Effects from short gestation and low BW on subsequent lung function, are more likely to be disclosed in studies with wider inclusion criteria (64;102;120). Much of the controversies (104) relating to which are the principal predictors for lung function outcome in subjects of premature birth (i.e. birthweight and gestational age versus neonatal conditions and treatment exposures), may be explained within the context of sample selection. Within the ranges given for BW and GA in the present study, neonatal respiratory morbidity (BPD and CLD) and prolonged oxygen supplementation were strongly and significantly associated with current lung function outcome. Prolonged neonatal requirements for oxygen treatment remained the principal predictive factor for current lung function outcome in multivariate models including other relevant neonatal variables. Inclusion of factors considered to be associated with asthma and airway hyperresponsiveness in unselected populations did not alter this picture. A trend seems to appear from the literature, indicating that BW and GA may be less associated with lung function outcome in studies with the more immature population samples. Our study fits well into this trend: The smaller the babies, the more susceptible they are to postnatal noxious exposures or conditions. The more mature the babies, the more robust they are to neonatal traumas, increasing the importance of factors such as BW and GA for outcome. This concept

is supported by a recent study on exercise capacity in ELBW children by Kilbride et al. (173). They examined a sample of children (9-15 years) born in the late 1980's, selected by their capacity to complete an exercise test. There were no BW or GA differences between subjects with and without CLD (oxygen treatment ≥ 36 weeks PMA). This is certainly unusual. Nevertheless, lung function in ELBW subjects without CLD was close to normal, while ELBW subjects with CLD had highly significant airway obstruction. Hjalmarson & Sandberg (69) studied contemporary BPD infants at an age corresponding to term. There were no differences in BW (approximately 800 g) or GA (approximately 26 weeks) between infants classified as mild, moderate or severe BPD. Although abnormalities were found also in apparently healthy preterms and in mild BPD, those infants with the more severe BPD had the most pronounced functional abnormalities. GA was not related to outcome. This further supports the notion that neonatal respiratory morbidity is the principal predictor of long-term outcome in immature infants. It furthermore indicates that the relationship between prolonged requirements for oxygen and adverse lung function outcome is present also in survivors from contemporary neonatal intensive care. These data may represent an extension into this decade of the results presented in paper #3, showing that an extra day with oxygen treatment was related to similar decreases in lung function in preterms born the 1980's and in 1990's. There is evidence that airway obstruction in premature children track throughout childhood (104). If Hjalmarsons cohort follows the same pulmonary growth pattern, BPD survivors from this millennium, may also develop dysfunctional airways as described in paper #2, #3 and #4. Whether or not this is a noxious effect of oxygen *per se* is unknown and cannot be discerned from our study. Certainly, prolonged requirements for supplemental oxygen may represent the "final common output" of a series of events. A sequence of prenatal and/or postnatal predispositions, events or conditions may trigger lung injuries that bring about requirement for oxygen treatment that *statistically* becomes related to outcome, but with an uncertain *causal* relation. However, an independent noxious effect from oxygen is not biologically implausible. Both scenarios may be true, i.e. prolonged requirement for treatment with oxygen may be a marker of lung injury inflicted by other factors as well as an independent causal factor for *additional* lung injury. Whatever role oxygen supplementation may play in the lungs of immature infants, prolonged neonatal requirements seem to be a continued and well-suited prognostic indicator for subsequent long-term airway obstruction in this group of subjects.

8.3 Biological aspects of airway obstruction and hyperresponsiveness after BPD

Limitation of maximal expiratory flow depends on the resistance of the airways and on the elastic recoil of the lung tissue (81;184). Therefore, one or both of these factors must differ between those who were born prematurely and those who were born at term. The major determinant for airway resistance is the cross sectional dimension of medium sized bronchi. The elastic recoil of the lungs depends to a large extent on the formation and adequate disposition of elastic fibres (185). Adequately formed three dimensional network of elastic fibres is also required for a normal alveolarization (186). The acute phases of RDS and BPD are characterized by a significant inflammatory response in the airway mucosa and in the interstitium of the lungs (54). Activated inflammatory cells release proinflammatory cytokines and various mediators as well as cytotoxic oxygen radicals and proteases, including elastase. Increased concentrations of free elastase and low α1-protease inhibitor activities have been detected in the airway secretion of some infants with BPD, and this imbalance has been suggested as a hallmark of lung injury of preterm infants (54). Altered elastic and fibrous networks is described in lungs after exposure to oxygen supplementation and positive pressure ventilation in preterm animals (77;187) and humans (52). Taken together, there is good evidence to suggest that the structure of the pulmonary interstitium may be affected by premature birth and/or associated oxygen toxicity, baro-traumas, infections and inflammatory responses. This mal-development of the pulmonary interstitium and of the acinar structures may alter peri-bronchiolar support with consequent alterations of elastic recoil and small airway resistance. The decreases in maximal expiratory flows observed in subjects of premature birth in the present study may be explained within this conceptual framework. Jacob et al. (100) studied pulmonary pressure-volume curves of 11 year old children who were born at 24-31 weeks PMA and required oxygen treatment to at least one month after term. They reported reduced elastic recoil pressure at 90% TLC (78% predicted ± 26%), however with a relatively wide scatter. Their overall findings were mixed and seemingly contradictory, but consistent with coexistence of emphysematous changes as well as with fibrosis. No studies have supplied evidence that inhaled steroids improve the airway obstruction occurring after premature birth (89;188). Thus, there is a relatively consistent line of arguments from basic science to clinical studies supporting the notion that airway obstruction subsequent to premature birth represents structural pulmonary sequelae, probably related to altered interstitial elastic and/or fibrous networks. The observations of the present

study fits well into this picture: Airway obstruction in preterms was strongly related to the neonatal respiratory morbidity and we were unable to demonstrate any association between airway obstruction and the examined markers of airway inflammation (paper #4). The association between neonatal respiratory morbidity and outcome seemed to be quantitatively similar in preterms born in the early 1980's and the early 1990's (paper #3).

AHR is observed in most BPD-survivors (104). In asthma, AHR is often taken as evidence of poor inflammatory control (189;190), leading to suggestions of increased treatment intensity. In preterms of the present study, no relation was observed between AHR and markers of airway inflammation, nor was there any association between a diagnosis of asthma and markers of airway inflammation (paper #4). AHR was, however strongly related to neonatal respiratory morbidity and prolonged requirement for supplemental oxygen. Some, but not all, of this effect was mediated through an association between AHR and FEV_1%.

The capacity of isolated airway smooth muscle to shorten in response to a maximal cholinergic stimulus is greater than its shortening capacity in vivo in response to a corresponding stimulus (191). In vivo, the maximal response to methacholine is attenuated by increasing the lung volume at which bronchoconstriction is measured (192). On the bases of these experiments, it has been suggested that the limit to airway smooth muscle shortening in vivo is related to the elastic load that the lung parenchyma imposes on the airway smooth muscle when it contracts (192). In line with this, loss of elastic recoil should induce increased responsiveness to methacholine. Bellofiore et al. (80) treated rat lungs with elastase and found an increase in lung volumes, reduced lung elastic recoil, diffuse parenchymal lung destruction and loss of alveolar attachments to the airway walls. Concurrent with these changes there was an increase in the maximal degree of airway narrowing to inhaled methacholine. These experiments provide support to the notion that AHR after premature birth is not an expression of airway mucosal inflammation, but of pulmonary parenchymal structural sequelae and possibly of distorted elastic and/or fibrous architecture of the interstitium. Structural factors influencing airway narrowing during testing for AHR was recently reviewed by Brusasco & Pellegrino (193). The data provided through the present thesis fits nicely into this theory: Airway obstruction as well as AHR was strongly related to neonatal respiratory morbidity and not associated with current markers of inflammation or features generally associated with childhood asthma (paper #4).

8.4 Future prospects for pulmonary function in children of premature birth

FEV_1 follows a well-described track throughout a lifespan. Subsequent to childhood growth, a stable plateau phase is followed by a decline throughout adulthood (194). The maximum level of FEV_1 obtained, as well as the timing of onset and subsequent rate of decline in adulthood, determine when and if lung function impairment will occur. Thus, the deficit in FEV_1 described for subjects with BPD in paper #2 of this study, represents more than a decade of normal age related decline. Furthermore, the rate of decline in lung function in adulthood differs between population subgroups (194). Cigarette smoking, childhood pneumonias (195;195), asthma (196) and AHR (197) may accelerate the normal aging process of the lung. Our preterm population smoked cigarettes at the same rate as their controls, had experienced more childhood pneumonias, used more asthma inhalers and had a distinctly increased prevalence of AHR. A prospect of an accelerated decline in lung function in adulthood is therefore possible.

Shaheen et al. (195) showed us that relatively minor insults to the lungs of small children may be associated with chronic obstructive airway disease (COPD) at the other end of life. The lungs of extremely premature children are definitely not exposed to minor insults but to major traumas. There is furthermore a coherent line of evidence that these traumas may lead to structural injuries, leaving little prospects of improvement. Already in 1990, in an editorial in the New England Journal of Medicine, Mary Ellen Wohl (198) expressed her worries for the pulmonary future of BPD patients. Hack and Fanaroff (11) expressed similar worries one decade later. Today, in 2005, these concerns still seem relevant.

8.5 Implications for contemporary BPD survivors

We found a similar relation between the extent of neonatal BPD and current lung function in two comparable cohorts of preterms, born in the 1980's and the 1990's. Hjalmarson and Sandberg showed us that infants born in this decade, requiring oxygen treatment at 36 weeks PMA, had lung function abnormalities that partly were related to the extent of neonatal BPD. One may therefore speculate that BPD survivors of contemporary neonatal intensive care medicine will also grow up with lung sequelae, particularly those with prolonged requirements for oxygen treatment. Development of bronchi precedes development of acini, starting with primary septation approximately at 25-26 weeks PMA. Thus, in the most

immature BPD-survivors of today, acinar development takes place solely after birth. These infants therefore, are at risk of suffering from more acinar and interstitial injury than previous BPD-survivors. These facts call for careful follow-up studies.

9. Concluding remarks and implications

The studies of this thesis showed that:

- Lung function testing could be conducted by preterms with a precision that did not differ significantly from term-born control subjects.
- Pulmonary sequelae from extremely preterm birth persisted into early adulthood.
- Pulmonary sequelae in subjects born extremely preterm in the early 1980's and in the early 1990's were of similar magnitudes, and also similarly related to prolonged requirements for supplemental oxygen.
- Asthma and AHR subsequent to extremely preterm birth differed from typical childhood asthma with respect to important features. Current AHR was best explained by neonatal variables.
- High-resolution CT revealed radiological findings in 81% of preterms born in the 1990's and in 93% of preterms born in the 1980's. Relatively minor abnormalities, such as linear, triangular, and subpleural opacities, were the most prevalent findings.

It is only recently that the first large cohorts of survivors from extremely premature birth have reached adulthood. In a life-time perspective, their pulmonary prognosis is unknown. In this context, therefore, extreme neonatal intensive care medicine is experimental medicine. Certainly, survival has increased and morbidity is reduced also in infants born at later gestation. A shift in the neonatal clinical course may have occurred, with relatively eventfree survival of infants previously exposed to harsh interventions, paralleled by recruitment of a new category immature survivors that previously died. The proportion of these infants requiring supplemental oxygen at 36 weeks PMA seem to have settled at approximately $^{1}/_{3}$. Particularly for these subjects, there is reason for concern regarding life-long pulmonary prospects. As survival of these infants has become the rule rather than the exception, their absolute number is rising and their public health importance is increasing. Without medical interference, most of them would have died. Appreciating this scenario, an obligation for proper follow up, treatment and guidance fall upon the health profession that facilitated their survival.

10. References

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