

**Childhood asthma and reduced lung function:**

**What comes first?**

**by**

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## 1. PREFACE

### 1.1 Acknowledgement

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## 1.2 Summary of thesis

### *Introduction*

The increasing prevalence of asthma seen worldwide during the last decades has in some recent reports shown a levelling off. Asthma has been associated with reduced lung function both in childhood, adolescence and adulthood and reduced lung function in infancy has been identified as a risk factor for lower respiratory illness in the first years of life. However, the association of impaired lung function early in life to obstructive airway disease later in life is still unsettled. Likewise is our knowledge of which factors influence lung function development from birth into childhood limited. The main aim of the present thesis was to explore the overall association between lung function and asthma through childhood and the specific research questions were:

- I. What is the prevalence of asthma among school children in Oslo?
- II. Is lung function reduced in children with asthma?
- III. What factors are associated with lung function development through childhood?
- IV. Is lung function at birth associated with lung function later in childhood?
- V. Is lung function at birth associated with asthma later in childhood?

### *Methods*

The present thesis reports results from the prospective birth cohort “the Environment and Childhood Asthma (ECA) Study” in Oslo established in 1992. At birth 3754 term infants were enrolled and a representative sub sample of newborns had lung function measurements by tidal flow volume loops (n=802) and passive respiratory mechanics (n=664) at mean 2.7 days ( $\pm$  S.D. 0.9). Starting at the maternity ward, extensive questionnaires were completed by the parents every six months the first two years of life. Cohort children with recurrent

bronchial obstruction (rBO) the first two years of life (n=306) together with age matched controls without any episodes of bronchial obstruction were recruited to a nested case-control study with a clinical examination, including lung function measurements by tidal flow volume loops, at two years of age. The two years assessment was attended by 516 children, of whom 140 children had lung function measured at birth.

Of the 1019 children attending a 10 year follow-up study including lung function measurements, bronchial provocation tests, skin prick tests and a structured interview, 616 children had lung function measurements at birth. The latter group of children was representative for the general cohort at birth, and was suitable for prevalence studies.

### *Results*

At 10 years of age the life time prevalence of asthma was 20.2% and 11.1% had current asthma. Children with current asthma had reduced  $t_{PTEF}/t_E$  at birth and reduced FEV<sub>1</sub> and FEF<sub>50</sub> at 10 years. Asthma ever was associated with reduced  $t_{PTEF}/t_E$  and Crs at birth.

Lung function development from birth to two years of age was not influenced by the presence of atopic eczema or rBO by two years whereas current asthma and maternal smoking during pregnancy were significant predictors for FEF<sub>50</sub> even after adjusting for lung function at birth. Lower respiratory tract infections during the first two years of life did not significantly influence lung function development from birth to 10 years of age.

$t_{PTEF}/t_E$  at birth correlated reasonably well with  $t_{PTEF}/t_E$  at two years of age and tracking of lung function the first two years of life was found to be present, independent of the expression of allergic diseases. Crs at birth was significantly associated with FEV<sub>1</sub> at ten years and  $t_{PTEF}/t_E$  at birth was a significant predictor of FEF<sub>50</sub> at 10 years.

Children with both rBO and AE by two years, a high-risk phenotype for atopic diseases in school age, had significantly reduced  $t_{PTEF}/t_E$  both at birth and at two years of age.



Reduced lung function at birth was found to be a risk factor for asthma ever by 10 years as well as current asthma at 10 years with an adjusted odds ratio of about 2. In a high-risk group of children with  $\text{CrS} < \text{median}$  and  $t_{\text{PTEF}}/t_{\text{E}} < 0.20$  the prevalence of asthma ever was 45.5% and current asthma 28.1%, respectively.

### *Discussion*

The reported prevalence of asthma is the highest ever in Norway and indicates a substantial increase compared to surveys in the mid nineties. The findings of reduced lung function in children with current asthma at the age of 10 years are in line with several previous studies, but this is the first report of reduced lung function at birth in children with asthma in school age.

Lung function at two years of age was associated with lung function at birth, but not with concurrent allergic disease expression. Lung function at 10 years was associated with lung function at birth, maternal smoking during pregnancy and current asthma at ten years, but not with lower respiratory tract infections the first two years of age. These results suggest that, although lung function development through childhood may be modulated by lower respiratory tract disease and environmental factors, tracking is present from the very beginning of extra uterine life.

The novel finding of reduced lung function at birth as a risk factor for asthma in childhood differs from the few previous studies addressing this issue. The Perth study found an association to wheeze, but not to asthma in school aged children. This discrepancy may be due to methodological differences based in type of lung function measurements at birth as well as the sample size in the ECA study being several times larger than all other studies to date with this focus. Our results point to factors that have already in utero exerted an effect that is implicated in asthma development in a subpopulation of children. These children at

risk may be identified by the reduced lung function present at birth, although such measurements are unlikely to be valuable for individual subject prediction.

### *Conclusions*

In conclusion the results presented in the present thesis indicate that the prevalence of childhood asthma has increased substantially since the mid nineteen-nineties. Asthma is associated with reduced lung function both at birth and at 10 years of age and although some factors may modulate lung function development during childhood, tracking of lung function is dominant through childhood and starts very early in life. Reduced lung function at birth is a risk factor for asthma through childhood pointing to pre natal factors being implicated in the development of asthma in some children.

### 1.3 Abbreviations

AE	Atopic eczema
AHR	Airway hyper responsiveness
BO	Bronchial obstruction
Crs	Compliance of the respiratory system
CV	Coefficient of variation
$\Delta t_{PTEF}/t_E$	Change in $t_{PTEF}/t_E$ from birth to two year of age
DDA	Doctors diagnosed asthma
ECA-1	The Environment and Childhood Asthma Study part 1
ECA-2	The Environment and Childhood Asthma Study part 2
FEF <sub>50</sub>	Forced expiratory flow at 50% of forced expiratory volume
FEF <sub>25-75</sub>	Mean forced expiratory flow between 25%-75% of forced expiratory volume
FEV <sub>1</sub>	Forced expiratory volume in 1 second
FEV <sub>1</sub> /FVC	Ratio of forced expiratory volume in 1 second to forced vital capacity
FEV <sub>0.5</sub>	Forced expiratory volume in 0.5 second
FEV <sub>0.75</sub>	Forced expiratory volume in 0.75 seconds
FRC	Functional residual capacity
FVC	Forced vital capacity
ISAAC	International Study of Asthma and Allergy in Childhood
LRIs	Lower respiratory tract infections
OR	Odds ratio
$P_j$	Mean jacket inflation pressure
PW	Persistent wheeze
$\rho$ (rho)	Spearman's two tailed correlation coefficient

rBO	Recurrent bronchial obstruction
Rrs	Resistance of the respiratory system
RTC technique	Rapid thoracoabdominal compression technique
RV/TLC	Ratio of residual volume on total lung capacity
sGaw	Specific airway conductance
SPT	Skin prick test
$t_{\text{PTEF}}/t_{\text{E}}$	Ratio of time to peak tidal expiratory flow to total expiratory time
$V'_{\text{max,FRC}}$	Maximal expiratory flow at functional residual capacity
$V_{\text{PTEF}}/V_{\text{E}}$	Volume expired at peak tidal expiratory flow on total expired volume

## 1.4 List of papers

### *Paper I*

Lødrup Carlsen KC, Håland G, Devulapalli CS, Munthe-Kaas M, Pettersen M, Granum B, Løvik M, Carlsen KH. Asthma among every fifth child in Oslo, Norway. A 10-year follow-up of a birth cohort study. *Allergy* 2006; 61:454-460.

### *Paper II*

Håland G, Lødrup Carlsen KC, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M, Carlsen KH. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; 355:1682-1689.

### *Paper III*

Håland G, Carlsen K-H, Devulapalli CS, Pettersen M, Mowinckel P, Lødrup Carlsen KC. Lung function development in the first 2 yr of life is independent of allergic diseases by 2 yr. *Pediatr Allergy Immunol* 2007; 18: 528-534.

### *Paper IV*

Håland G, Lødrup Carlsen KC, Mowinckel P, Munthe-Kaas MC, Devulapalli CS, Berntsen S, Carlsen K-H. Lung function at 10 years is not impaired by early childhood lower respiratory tract infections. In press.

## 2. GENERAL INTRODUCTION

### 2.1. Asthma in childhood

#### 2.1.1 Definitions and phenotypes

Our understanding of bronchial asthma has evolved tremendously since the first description in the Hippocratic (460-370 B.C.) writings (1). The term asthma, derived from the ancient Greek word for panting, was for more than 2000 years mainly used as description of symptoms: episodes of laboured, rapid breathing with wheeze, chest tightness and coughing. The attempts to define the disease were likewise largely based upon symptoms. However, as our understanding of the physiology of the airways and the pathophysiology of asthma has improved new features of the disease have been added to the definition. However, no strict definition of asthma has been agreed upon and the Global Initiative for Asthma (GINA) has changed to the term “operative description” when describing asthma:

*“...a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment” (2).*

This “operative description” describes the pathology (chronic inflammation), physiological features (airway hyperresponsiveness (AHR) and variable airflow obstruction) and symptoms (wheezing, breathlessness, chest tightness and coughing) associated with

asthma. From a paediatric and epidemiological point of view this description has two major limitations.

First, this description may in many ways be useful in diagnosing and monitoring asthma in school age children, adolescent and adults as both airway inflammation, AHR and airflow obstruction can be measured. However, measurement of these objective features in preschool children is more difficult and partly unreliable, and the underlying pathological and physiological features of asthma are less explored in this age group. Attempts have therefore been made to approach this issue in a more pragmatic manner. The “Nordic consensus report on asthma management” states that *“the diagnosis of asthma can be strongly considered in the following circumstances:”*

- “1. From the third attack of airway obstruction during the last year is current asthma.*
- 2. One attack of asthmatic symptoms occurring after the age of 2 years.*
- 3. Irrespective of age in an attack in children with eczema, food allergy or other allergy.*
- 4. If the child does not become free of symptoms when infection has ceased, or has persistent symptoms for more than a month”(3).*

In the *“Third International Pediatric Consensus Statement on the Management of Childhood Asthma”*, asthma among children younger than three years is defined as:

*“.....recurrent wheezing and/or persistent coughing in a setting where asthma is likely and other rarer conditions have been excluded” (4).*

These two latter definitions have in common that the diagnosis of asthma is based upon clinical judgement and not on objective measures.

The second and most important limitation of the GINA “operative description” is that the described features do not apply to all subjects with asthma. No single feature is universal or unique to asthma. The dominant cells of inflammation vary as bronchial biopsies from patients with allergic asthma showed no correlation between AHR and the number of different inflammatory cells (5), but an eosinophilic inflammation was associated with reduced lung function (5). Others have found patients with severe asthma to have a predominance of neutrophilic inflammation in their bronchial mucosa (6). Population studies have shown AHR to be present in schoolchildren not reporting respiratory symptoms or having diagnosed asthma (7-9) and in patients with allergic rhinitis, but who do not have asthma (9-11). On the other hand, studies examining AHR in wheezing preschool children did not find the same response to airway challenge tests as in older children and adults (12;13). It is well known that asthmatic patients often have normal lung function between attacks, even among patients with severe asthma reduced lung function is not a consistent finding. (14-16). The symptoms associated with asthma are likewise not unique to patients with asthma but may be caused by several other conditions especially in children (17).

This heterogeneity in the “asthma population” and the overlap in airway characteristics between those with and those without asthma have increased the uncertainty of epidemiological studies of asthma, making it more difficult to interpret result of prevalence studies and especially to analyze time trends and compare results from different geographical areas.

In order to explain the heterogeneity among asthmatic patients, attempts have been made to define phenotypes with different characteristics, but who fit under the “asthma umbrella”. Among children the phenotypes have been created to explain the different natural history of- (18;19) and to predict the prognosis of early life wheezing (20). In the Tucson



birth cohort study the children were “phenotyped” at six years as no wheezing (never wheezed), transient early wheezing (wheezing before the age of three, but not at six years), late onset wheezing (wheezing at six years, but not before three years of age) or persistent wheezing (wheezing both before the age of three years and at six years) (18). In this retrospective perspective cord serum IgE levels were unrelated to wheezing phenotype, but compared to children who had never wheezed the children with persistent wheezing had significantly higher IgE level at nine months of age and at six years. They also had a significantly higher prevalence of positive skin test reactivity as was the case among the late onset wheezers at six years of age (18). Lung function measurements showed the transient early wheezers to have reduced maximal expiratory flow at functional residual capacity ( $V_{\max, \text{FRC}}$ ) at birth and the transient early wheezers as well as the persistent wheezers to have reduced  $V_{\max, \text{FRC}}$  at six years of age (18).

At 11 years of age, after a re-examination including measurements of peak flow variability and bronchial challenge testing, the Tucson investigators defined three wheezing phenotypes in children (19): Transient early wheezers associated with reduced lung function at birth that persists through childhood; Non-atopic wheezers associated with increased peak flow variability long after the wheezing symptoms had ceased. The third group was the IgE-associated wheeze/asthma that may exist at any age during childhood and was related to the combination of atopy, increased responsiveness to metacholin and increased peak flow variability. This was in line with the phenotypes previously described by Wilson (21) with a hypothetical prevalence as shown in figure 1.

In the German Multicenter Atopy Study (MAS study), a prospective birth cohort study, four phenotypes were defined based upon the presence or not of wheeze and atopic eczema (AE) by two years of age (20). Children with both wheeze and AE by two years were characterized by a specific sensitization pattern (sensitization to less prevalent allergens) at

two years, a high risk of current wheeze at seven years and reduced lung function at seven years compared to children without wheeze and AE by two years thereby representing a high risk group for asthma in school age (20).

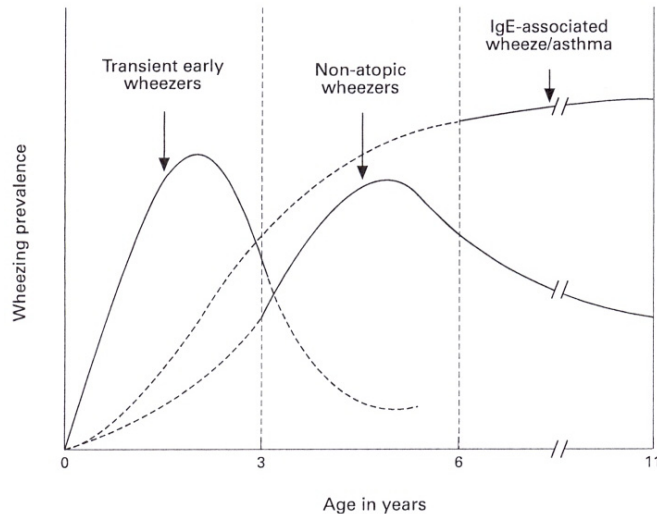


Figure 1. Hypothetical yearly peak prevalence of wheezing for the three different wheezing phenotypes in childhood. Reprinted with permission from Stein et al. *Thorax* 1997; 52(11): 946-952.

However, none of the described phenotypes represent distinct entities, and there is a substantial degree of overlap in the clinical expression as the severity of the symptoms as well as the underlying pathology and physiological features between the phenotypes.

### 2.1.2. Epidemiology

#### *Global trends*

During the last decades the prevalence of asthma has shown an almost epidemic increase in the western world (22). The increase, which was first reported among children (23), has been

associated with western life style and urbanization (24-28). The prevalence rates show a wide variation world wide as shown by the International Study of Asthma and Allergy in Childhood (ISAAC) (29). This study, using a validated and translated standardized questionnaire at different study centres in different parts of the world, found an approximately 20-fold difference in prevalence of asthma symptom between the countries with the highest figures (Great Britain, Australia and New Zealand) and those with the lowest figures (Albania, Indonesia), figure 2 (29). Earlier studies have shown an east-west gradient within Europe in prevalence of asthma symptoms (30;31) and this gradient was also apparent in the ISAAC study (29).

However, several more recent studies report a levelling off or even a decrease in asthma prevalence (32-35), all from westernized countries where previously high prevalence rates have been reported. Other studies from western-Europe (36-39), eastern-Germany (40) and Australia (41) report no change in the trends of asthma prevalence. The results are conflicting and even studies from the same country show discrepancies (32;41). This divergent trend in asthma prevalence is also reflected in the phase three of the ISAAC study (42), a follow up at mean seven years after the initial phase. Although the mean prevalence of asthma symptoms in young children (six-seven years of age) increased, there was a wide variety with several centres, especially in the western world, showing a levelling off and even a decrease in the prevalence of asthma. Among older children (13-14 years of age) there was no increase the mean asthma prevalence and the divergence was even more pronounced (42).

There is however one major limitation to much of the epidemiological research among asthma in children: The definition of asthma. Most studies use the symptom of wheeze as a surrogate for asthma, but this symptom may represent other airway abnormalities than asthma. In a study assessing the prevalence of wheeze, asthma and other airway diseases in Sweden, Finland and Estonia the prevalence of wheeze was highest in Estonia, but the

reported prevalence of asthma far lower than in the Scandinavian countries (43). Furthermore, in most countries outside the English speaking ones the term wheeze does not exist as such and a standardization of translation has thus been challenging.



Figure 2. 12-month prevalence of self-reported asthma symptoms among 13-14 years old children from written questionnaires in the International Study of asthma and Allergy in Childhood (ISAAC) Phase I study. Reprinted with permission from the ISAAC Steering Committee, Lancet 1998; 351 (9111): 1225-1232.

### *National trends*

The first report of asthma prevalence among school age children in Norway was by Claussen in 1948 (44), who reported a prevalence of 0.4%. Later studies have reported steadily higher prevalences, figure 3. Although direct comparison between the first (1948, 1954 and 1978) (44-46) and the latest studies (47-52) is difficult as different questionnaires were used, the studies in 1981, 1993 and 1994 (50-52) included the same questionnaires and were all conducted in Oslo. In the latest study conducted in 1994 a prevalence of 9.3% was reported (52), the highest reported thus far in Oslo. Contemporary studies from Northern Norway (12.3%) (47) and Mid-Norway (10.2%) (49) report comparable prevalence, while a study from West-Norway (53) reported a lower prevalence (5.4%). However, all these studies report lower prevalences of asthma than studies from many other western European countries (29).

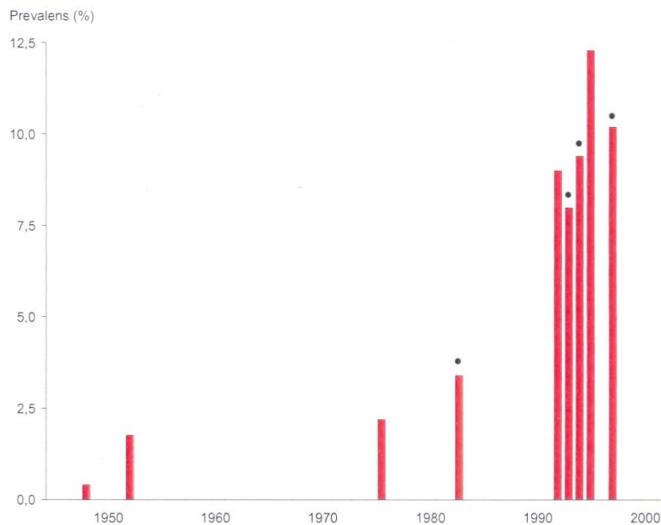


Figure 3. The prevalence of asthma in Norway 1948-1994. Studies marked with a dot has applied identical questionnaires. Reprinted with permission from Carlsen KH, Tidsskr Nor Laegeforen 2001; 121(7):836-840.

## **2.2. Lung function during childhood**

### *2.2.1. Historical aspects*

The works by Erasistratus (c. 280 BC) and Galen (129-201) demonstrating the role of the diaphragm as a muscle of respiration, the origin and function of the phrenic nerve and the function of the intercostal and the accessory muscles has been regarded as the earliest studies on lung function (54). The knowledge of the respiratory system, including anatomical aspects, the role of ventilation in maintaining life and gas exchange evolved slowly thereafter for 1500 years. The birth of respiratory physiology as such took place in the seventeenth century (54). Borelli measured in 1679 the maximal volume of a single inspiration (54) and in 1846 Hutchinson defined vital capacity as “the greatest voluntary expiration following the deepest inspiration”, designed the first spirometer for its estimation and reported it to be related to the height of the person (54). Measurement of residual volume by gas dilution technique was first introduced by Davy in 1800 (55). Although the gas dilution technique has been modified several times thereafter, the basic principle of equilibrium between the air in the lung and a known volume of an insoluble inert gas is still used in the assessment of lung volumes.

In 1950 “Standardization of Definitions and Symbols in Respiratory Physiology” was published by leading American respiratory physiologists defining the various volumes and its abbreviations (56) still used today as described in figure 4. The use of whole body plethysmography for the measurement of lung volumes and airway resistance was introduced by DuBois and colleagues in 1956 (57). About a century after Hutchinson developed the spirometry, a rotating drum was added, enabling measurement of absolute volume expired or inspired in a given time (e.g. FEV<sub>1</sub>) and the rate of change of volume with time (i.e. flow rates) (55).

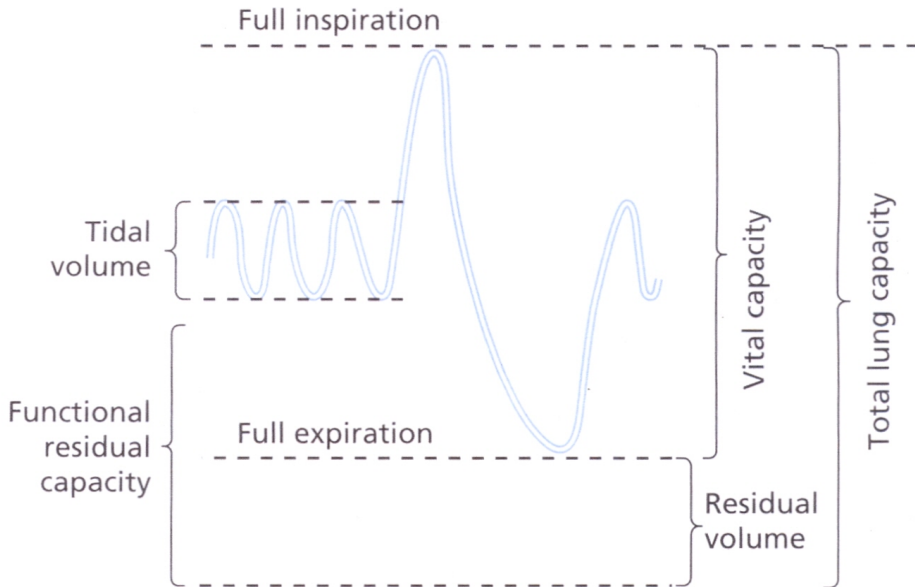


Figure 4. Total lung capacity and its subdivisions. Reprinted with permission from SJ Bourke. Lecture Notes in Respiratory Medicine, 7<sup>th</sup> edition 2007. Blackwell Publishing

Knowledge of the control of respiration evolved after the observation that a lesion in medulla oblongata interfered with normal breathing reported in the first part of the nineteenth century (54). Based upon the findings by Hering and Breuer 50 years later that this region received information about distension of the lung via the vagus nerve, the understanding of the self-regulation mechanism evolved. The exploration of the role of hypoxemia and hypercapnia, as well as the exploration and understanding of the muscle spindles in respiratory muscles and pulmonary receptors contributed to increased understanding of this regulatory mechanism (54).

### 2.2.2. Methods of lung function measurement in infants and children

The progress in our understanding of respiratory physiology has been followed by a concurrent advance in methods and equipment for lung function measurements both for

research and clinical purposes. However, most of these methods have been developed for use in adults and later been modified for use in children mature enough for the cooperation necessary to perform most of these tests.

For lung function measurement in infants and young children who are unable to cooperate adequately, several methods have been developed. Both measurement by tidal flow volume loops, passive and dynamic respiratory mechanics, forced expiration by the forced deflation technique or the rapid thoracoabdominal compression (RTC) technique, whole body plethysmography and gas dilution techniques have been applied in the assessment of infant lung function (58). However, due to insufficient standardization of most of these techniques, they have mainly been used for research purposes, and no “gold standard” for infant lung function has been agreed upon.

Lung function measurement by tidal flow volume loops was first described in 1957 (59). In 1981 Morris and Lane published a study comparing the shape of the flow to time and flow to volume curves in obstructed and healthy subjects during tidal breathing (60). They reported a significant correlation between the ratios of time to peak tidal expiratory flow to total expiratory time ( $t_{PTEF}/t_E$ ) and volume expired at peak tidal expiratory flow to total expired volume ( $V_{PTEF}/V_E$ ) and  $FEV_1$ ,  $FEV_1/FVC$  and airway resistance among adults (60).

In infants measurements by tidal flow volume loops are made with the infant lying supine, awake or asleep, quietly breathing (61;62). Noise, tactile stimuli and other factors that may influence the breathing pattern of the infant must be reduced to a minimum as it is of great importance that the infant has a natural, reproducible and regular breathing pattern. A flow meter, usually a pneumotachograph, is attached to a face mask with a “putty” kept tight with a minimal pressure over the mouth on the infant to ensure no air leak (61). As for other techniques of lung function measurements in infants the dead space and the resistance of the



equipment must be reduced to a minimum. A sampling rate of  $\geq 200$  Hz is recommended when measuring  $t_{PTEF}/t_E$ , for other parameters a lower sampling rate may be sufficient (61). Although a minimum number of 10 consecutive stable cycles ( $\geq 30$  seconds recording) are recommended for analysis (63) previous studies have reported 4-50 cycles with various standardization criteria (64-66). The recently published recommendations for tidal flow measurements in infants can also be applied in older children with a few modifications (63): The children should be awake and sitting and a mouthpiece may be applied instead of a face mask.

Although several parameters have been calculated from tidal breathing (67) most studies report  $t_{PTEF}/t_E$  or  $V_{PTEF}/V_E$  which have shown to correlate well with each other (60). These ratios are usually calculated by the computer software based upon a time flow plot as shown in figure 5 for  $t_{PTEF}/t_E$  (62). Although it is still unsettled what the tidal flow volume ratios exactly represent, it is assumed that *“timing of PTEF is due to an interaction between the mechanical properties of the lungs and airways on one hand and central control of breathing on the other”* (63).  $t_{PTEF}/t_E$  has been used to discriminate between infants and children with and without obstructive airway disease in several studies (60;64;66;68) and documented the effect of  $\beta$ -2 agonist inhalation in young children (64;66;69) although there has been a considerable overlap. Measurements of  $t_{PTEF}/t_E$  has been shown to be reproducible (65;70) and the coefficient of variation (CV) has in most studies but one (65) been about 25% (60;66;70;71). In infants parameters of tidal flow volume measurement have correlated poorly with lung resistance (72), respiratory resistance (73) and specific airway conductance (68), but reasonably well with  $V_{max,FRC}$  (68;74). However, in older children significant correlation was found between  $t_{PTEF}/t_E$  and  $FEV_1$  and forced expiratory flow at 50% of forced expiratory volume ( $FEF_{50}$ ) with a correlation coefficient of about 0.5 (60;66;75).

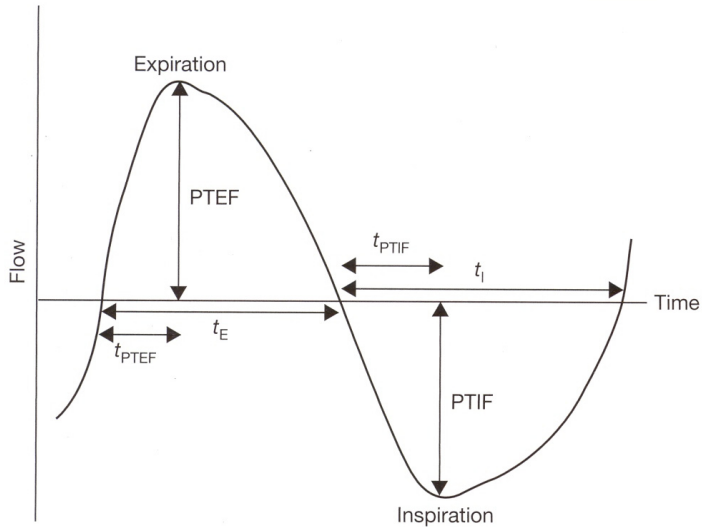


Figure 5. Flow time plot for tidal breathing parameters. PTEF/PTIF: Peak tidal expiratory/inspiratory flow.  $t_E$ : expiratory time.  $t_{PTEF}$ : time to reach PTEF.  $t_I$ : inspiratory time  $t_{PTIF}$ : time to reach PTIF. Reprinted with permission from Carlsen KCL, Carlsen KH: Paediatric Pulmonary Function Testing. Prog Respir Res. Basel, Karger, 2005, vol 33, pp 10-19.

Indices of respiratory mechanics may be assessed by passive or dynamic measurement in infants (76). Dynamic measurements are performed during spontaneous breathing (with the respiratory muscles actively contracting) and allow assessment of the different components of the respiratory system, but will not be discussed in detail here. In contrast to dynamic respiratory mechanics measurements, passive respiratory mechanics measurements give information about compliance and resistance of the entire respiratory system by the single or multiple occlusion technique, with the single occlusion technique being the most applied method and will be discussed in more detail (76). Measurements are performed with the infant lying quietly breathing with a slight extension of the neck. A face mask, held tightly over the infant's mouth and nose, is connected to a flow meter. (77). Between the face mask

and the flow meter a shutter/valve with a pressure port connected to a pressure transducer with a range of  $\pm 5$  kPa is included. Data acquisition starts after a stable tidal respiration has been achieved, verified on a real time display. At end inspiration the shutter is closed and due to the Hering Breuer Inflation reflex the respiratory muscles will relax and pressure equilibrium in the respiratory system (from the alveoli to the mouth) will be reached as there is no flow (77). The occlusion is sustained until pressure equilibration has occurred (a pressure plateau with change of pressure  $< 20$  Pa for 100 ms). Pressure is measured at the airway opening at equilibrium and flow is measured at a sampling rate of 200 Hz. The flow volume curve obtained upon opening the shutter, fig 6, is used for calculation of compliance of the respiratory system ( $C_{rs}$ ) and resistance of the respiratory system ( $R_{rs}$ ) as well as time constant of the respiratory system ( $\tau_{rs}$ ).

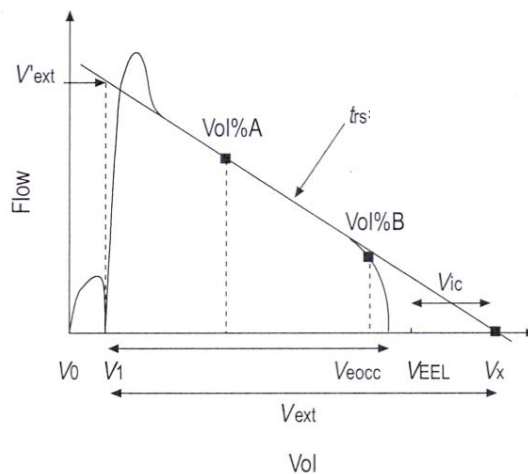


Figure 6. Flow volume curve obtained following end inspiratory occlusion for the single occlusion technique.  $V'_{ext}$ : extrapolated flow.  $V_{ext}$ : extrapolated volume.  $V_1$ : Volume at time of occlusion.  $\tau_{rs}$ : time constant.  $V_x$ : intersection of  $\tau_{rs}$  with volume axis.  $VEEL$ : End expiratory lung volume.  $Veocc$ : volume at end expiration.  $V_{ic}$ : difference between  $V_x$  and  $Veoc$ .

Reprinted with permission from Gappa et al. Eur Respir J 2001; 17: 141-148.

Crs is calculated as change in (extrapolated) volume to change in pressure from opening of shutter to end of expiration ( $Crs = \Delta V_{ext} \cdot \Delta P_{oa}^{-1}$ ), Rrs as change in pressure to change in flow (extrapolated) ( $Rrs = \Delta P_{oa} \cdot \Delta V'_{ext}^{-1}$ ) and  $\tau rs$  is given by the product of Crs and Rrs ( $\tau rs = Crs \cdot Rrs$ ) (77). Criteria for acceptable measurements are in addition to the criteria for pressure plateau, smooth expiration to within 10% of previous expiration and linearity of at least 40% of the flow volume curve. A mean of three to five acceptable manoeuvres should be reported (77).

Measurement of infant lung function by the RTC technique was first introduced by Adler and Wohl in 1978 (78). This technique, which allows measurements of forced expiration in uncooperative infants and children, has later been improved by Taussig and coworkers (79). The infants must be sedated, lying in a supine position with a tight face mask, connected to a flow meter, covering the mouth and nose (80). The dead space of the equipment should be kept to a minimum and measures must be taken to ensure no air leak in the system (81). The recommended sampling rate for data acquisition for RTC measurement is 200 Hz as for tidal breathing measurements (81). The infant to be assessed wears a “squeeze jacket” with an inflatable inner layer wrapped tightly around the thorax, abdomen and upper thighs (80;81). After a stable end expiratory functional residual capacity (FRC) level has been achieved through 5-10 stable tidal loops the “squeeze jacket” is inflated at end tidal inspiration and the pressure is maintained through the entire forced expiration (81). Each curve is accepted or rejected according to given criteria (81), assessed by means of a real time display of inspiratory and expiratory volumes, mean jacket inflation pressure ( $P_j$ ), airway pressure and flow volume curve. The procedure is repeated until the minimal  $P_j$  (optimal pressure) necessary to obtain maximal flow at FRC is discovered. The optimal pressure is then used to perform 3-5 partial expiratory flow volume curves for calculations of mean  $V'_{max,FRC}$  ( $\text{mL} \cdot \text{s}^{-1}$ ) (81) as shown in figure 7.

$V'_{\max, \text{FRC}}$  is thought to represent the function of the small peripheral airways (79) and RTC measurement have been applied in a number of epidemiological, clinical and physiological studies (80), but no widely accepted reference material is available. The high variability in reported  $V'_{\max, \text{FRC}}$  from different centres is probably due to the lack of reliable volume landmark for measurement of  $V'_{\max, \text{FRC}}$  as FRC varies according to respiratory rate, degree of sedation and airway calibre (80).

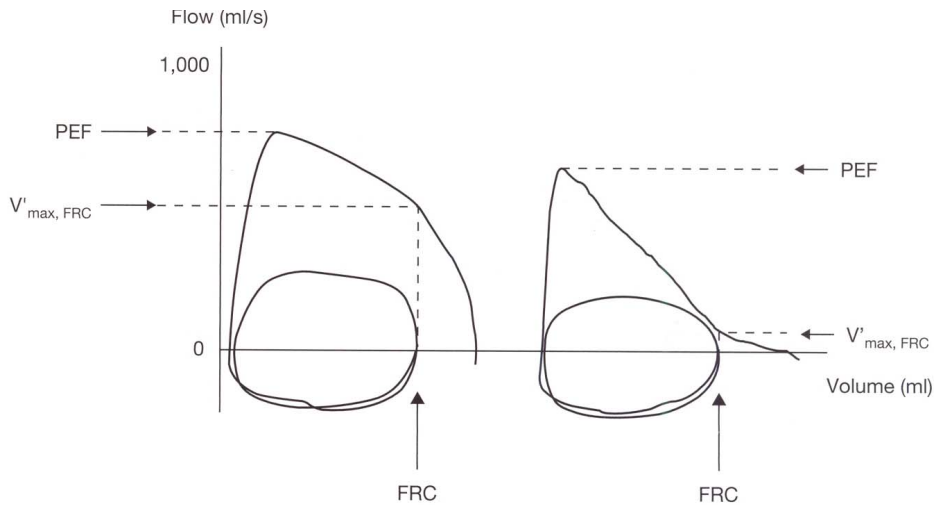


Figure 7. Partial expiratory flow volume curve obtained by rapid thoracoabdominal compression technique for calculation of maximal expiratory flow at functional residual capacity ( $V'_{\max, \text{FRC}}$ ) in a healthy infant and an infant with bronchiolitis. PEF: Peak expiratory flow. FRC: Functional residual capacity. Reprinted with permission from Modl M, Eber E: Paediatric Pulmonary Function Testing. Prog Respir Res. Basel, Karger, 2005, vol 33, pp 34-43.

Forced expiratory flow volume measurement (spirometry) is the most commonly used lung function test in children able to cooperate. It is widely used both in clinical practice and

for research purposes and internationally updated guidelines for preschool children (63) as well as older children and adults (82) are available. The child should be sitting and the procedure carefully described. A successful measurement depends on total cooperation from the child, and a trained and dedicated technician (83). A mouthpiece is connected to a flowmeter, usually a pneumotachometer. The procedure starts by full inhalation to total lung capacity followed by forceful expiration to residual volume for a minimum of 3 seconds in children younger than 10 years of age and minimum 6 seconds in children 10 years or older (82). Specific criteria for successful start and completion of the manoeuvre have been agreed upon (82) and in children an interactive computer animated system may be helpful as many children have problem fulfilling these criteria (63). According to guidelines, three acceptable manoeuvres should be achieved (82), and both flow volume and volume time curves should be displayed for evaluation. The most common parameters derived from forced expiratory flow volume measurement are FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, FEF<sub>50</sub> and forced expiratory flow at 25%-75% of FVC (FEF<sub>25-75</sub>). Spirometry is reproducible in well cooperating children. Several studies have addressed acceptability and reproducibility of spirometry among preschool children (84-90) and the latest ERS/ATS guidelines (63) conclude that criteria for successful measurements in adults were not appropriate in preschool children. Only two acceptable manoeuvres are sufficient, and forced expiratory volume in 0.5 (FEV<sub>0.5</sub>) as well as 0.75 (FEV<sub>0.75</sub>) seconds should be reported.

### *2.2.3. Lung function development*

In the embryo the lung bud develops during the fourth week of development. The lung bud soon divides into the primary bronchial buds which enlarge to form the two main bronchi. By 24 weeks approximately 17 generations of subdivisions have been formed, and a further 7 orders of airway development form during postnatal life (91). At 24 weeks of gestation the

terminal sacs are present making gas exchange possible. Primordial alveoli are present from about 32 weeks, but characteristic mature alveoli develop mainly after birth (91).

From birth until three years of age lung volume increases mainly due to an increasing number of alveoli, however alveoli may still develop up to age eight years (91;92). Thereafter increase in lung volume is mainly due to expansion of the alveoli (92) with a 13 fold increase in total lung volume from the prenatal period until seven years of age (93). The increase continues thereafter until early adult age with varying speed. Several studies have shown that the growth spurt of the lungs is out of phase with that of the body (94-97) and the lung growth continues after the height growth has ceased (97). The relative distribution between the different “sub volumes of the lung” changes also by ages as the ratio of residual volume to total lung capacity (RV/TLC) increases by age (98).

In contrast to the alveoli, the number of conducting airways is complete at birth and thereafter increase only in size (92), with the peripheral airways increasing relatively more than the proximal airways (99). After multiplication of alveoli is complete, lung growth is assumed to be isotropic, meaning that lung size and airway size develop symmetrically (92;97;100).

Gender related airway size changes significantly during puberty. In infancy (101) and during childhood (102-105) girls have larger airways than boys for a given volume although the total height adjusted lung volume is larger in boys than girls already from early life (106). During puberty boys have a larger increase in airway size compared to lung size than girls (98) resulting in a shift with males having higher maximal flows than females in adulthood (107;108).

The phenomenon of tracking, meaning the predictability of future values by early measurements (109), has been shown to be a characteristic of lung development. Dockery and coworkers reported in 1983 that in children 6-11 years tracking in lung function was nearly as

great as tracking in height (tracking index,  $\tau$ ,  $>0.90$ ) when evaluating  $FEV_1$  and FVC (110). This finding has later been confirmed for several indices of lung function measurement both in childhood and in adolescent (111). In the Dunedin study, tracking was found to be present from childhood into adulthood and to be independent of symptoms of lower respiratory disease (112). However, the question of how early tracking begins has been unsettled. Tracking has been reported in the neonatal period measured by tidal flow volume loops (70), in the first nine months of age in low birth weight infants as well as in infants with normal birth weight measured by the raised volume RTC technique (113), and in the first year of life in preterm children without neonatal respiratory disease (114). Furthermore, significant correlation was found for  $t_{PTEF}/t_E$  (Spearman's two tailed correlation coefficient ( $\rho$ ) 0.423) and specific airway conductance ( $\rho=0.374$ ) measured at eight weeks and one year of age (115). On the other hand an Australian study found that tracking was not consistent through the first year of life neither when  $V'_{max,FRC}$ ,  $t_{PTEF}/t_E$  or  $Cr_s$  were evaluated (116). Studies reporting longitudinal results of lung function from early childhood into school age are divergent. In the Perth study children with persistent wheezing (PW) until 11 years of age had reduced lung function shortly after birth using the RTC technique, at six years and at eleven years, but for other wheezing phenotypes there was no significant trend of tracking (117). This is in contrast to the Tucson study, using the same technique for lung function measurement, reporting children with PW to have reduced lung function at six years only while transient early wheezers had reduced lung function both at six years and shortly after birth (18). Follow up studies at 11 and 16 years confirmed that tracking was evident from school age into adolescence independent of wheezing phenotype (118). However, further support to the hypothesis that tracking starts early in life comes from a longitudinal study in children with bronchopulmonary dysplasia reporting a high correlation between  $V'_{max,FRC}$  at two years and  $FEV_1$  and  $FEF_{25-75}$  in school age (119).



#### *2.2.4 Factors associated with lung function growth*

Although tracking of lung function has been demonstrated during most of the “lung growth period”, several factors have been associated with lung function abnormalities. Maternal smoking during pregnancy has been associated with reduced lung function at, or shortly after birth (120-122) as has a family history of asthma, maternal hypertension during pregnancy (120) and maternal respiratory tract infections during pregnancy (123). The effect of antenatal maternal smoking on lung function has been shown to be sustained through the first year of life in a study with repeated lung function measures (124) as well into later childhood in several studies (125;126). Cross-sectional (125-128) as well as in longitudinal studies (129;130) of exposure to second hand smoke during pre school- as well school age have demonstrated a significant negative effect on lung function.

Active asthma in childhood is associated with reduced values of forced flow ( $FEV_1$ ,  $FEF_{25-75}$ , and  $FEV_1/FVC$ ) both in males and females (128;131-134) on a group level, but most studies fail to show an impact on lung volume (FVC) (128;131;133). This is in line with the results of longitudinal studies including children with asthma (133;134) and/or repeated measurements of AHR (97;133;135). A reduced growth of airway size was found as indicated by less increase of indices of airway flow in children with asthma and/or AHR. Lung volumes were either not affected (97;134;135) or slightly increased (133) indicating that asthmatics have a non-isotropic growth of lung function. However, none of the studies included preschool children and only one study (134) included children younger than 8 years of age.

Lower respiratory tract infections (LRIs) early in life have in several studies been associated with reduced forced flow values, but not lung volume, both in school age (136-140) and in adulthood (141-143). Pneumonias (139;141;142), no-pneumonia LRIs (139), bronchitis (137) as well as undefined LRIs (136;138;140;143) have been reported to give impaired lung function later in life. However, most of these studies have a retrospective

design and do not control for lung function early in life prior to the potential insult by the airway pathogen. Neither has the microbial airway pathogen causing the LRIs been identified in most of these studies. Other studies have found an association between Respiratory syncytial virus (144-147) as well as *Mycoplasma pneumonia* (148;149) infections in early life and subsequent reduced lung function .

During recent years several longitudinal studies (150-155) have confirmed the negative impact of air pollutants on lung growth previously reported from cross sectional studies (156-158). The effect has been on both forced flow values as well as on lung volumes. In two large longitudinal studies the magnitude of the effect of air pollution exposure on lung growth was found to be similar (150) or slightly greater (151) than the effect of exposure to maternal smoking. A recently published study reported a reduced growth in lung function from school age into early adult age in children living within 500 meters from a motorway (159). However, although the studies are consistent in their finding of a detrimental effect on lung function development there is still controversy on which pollutant (ozone, particulate matter with a diameter less than 10  $\mu\text{m}$  and 2.5  $\mu\text{m}$ , nitrogen dioxide acid vapour, organic carbon and elemental carbon) is most harmful as varying effects have been found in different studies. However, air pollution will not be further discussed in any detail in the present thesis.

#### *2.2.5. Early lung function as a predictor of obstructive airway diseases later in life*

The first report of an association between reduced lung function early in life, before any symptoms of respiratory illness, and subsequent increased risk of lower respiratory diseases came from the Tucson pediatric study in 1988 (160). Infants with wheezing during the first year of life had reduced  $t_{\text{PTEF}}/t_{\text{E}}$  and respiratory conductance (sGaw) at about two months of age prior to any symptoms of lower respiratory disease. At a follow up study by six years of age it was found that children with transient early wheezing (see section 2.1.1 for definition)

had reduced  $V'_{\max, \text{FRC}}$  at birth and at six years of age whereas children with persistent wheezing had reduced  $V'_{\max, \text{FRC}}$  at six years only (18). The findings from Tucson have later been confirmed in several studies: Children reporting wheeze the first year of life had reduced  $t_{\text{PTEF}}/t_{\text{E}}$ , sGaw and increased airway resistance in the first week of life (161). Reduced  $V'_{\max, \text{FRC}}$  at one month of age was found in children who wheezed during the first year(s) of life in unselected populations (162;163) as well as in an atopic high risk population (164). In one study stratifying into “wheeze groups” (first year only, second year only, both first and second year) showed all “wheeze groups” to have reduced  $V'_{\max, \text{FRC}}$  at one month, but only children who wheezed both the first and second year to have reduced Crs compared to never wheezers (163). Reduced  $t_{\text{PTEF}}/t_{\text{E}}$  early in life has similarly been associated with increased risk of recurrent bronchial obstruction (rBO) (165) and doctors diagnosed asthma (116) by two years of age.

Although most studies find an association between impaired early life lung function and symptoms of obstructive airway disease in the first years of life the associations to obstructive lower airway diseases later in life is less clear. In the Perth cohort reduced  $V'_{\max, \text{FRC}}$  at one month of age was associated with persistent wheeze (PW) until 11 years of age and recent wheeze at 11 years but not doctors diagnosed asthma by 11 years of age (117). This is in contrast to the results from the Tucson cohort reporting children with PW to have reduced lung function at six, 11 and 16 years of age but not at 2 months of age (118). However, due to differences in definition of PW, the studies are not entirely comparable.

### **3. AIMS OF THE PRESENT STUDY**

The overall objective of the studies included in the present thesis was to determine the overall association between lung function and asthma through childhood.

The specific research questions were:

- I. What is the prevalence of asthma among school children in Oslo?
- II. Is lung function reduced in children with asthma?
- III. What factors are associated with lung function development through childhood?
- IV. Is lung function at birth associated with lung function later in childhood?
- V. Is lung function at birth associated with asthma later in childhood?

## 4. METHODS AND SUBJECTS

### 4.1. Design of study

The present thesis reports results from the prospective birth cohort study *the Environment and Childhood Asthma (ECA) Study part 1 (ECA-1) and 2(ECA-2)*, figure 8. The ECA study was established January the 1<sup>st</sup> 1992 as a collaborative study between Department of Pediatrics at Ullevål University Hospital and Section for Epidemiology at National Institute of Public Health. At the maternity wards of the two municipal hospitals in Oslo and 3.754 (75% of all eligible) infants were recruited at birth during the 15 months inclusion period according to the following criteria.

Inclusion criteria:

- Birth weight more than 2000 grams
- Absence of any illness likely to impair respiration (severe respiratory, cardiovascular, neuromuscular or metabolic disease)
- No requirement for assisted ventilation or oxygen therapy after beyond 6 hours after birth

Exclusion criteria:

- Plans to move out of Oslo the next six months
- Insufficient language comprehension by the parents to be able to complete questionnaires

Before leaving the maternity ward (mean age ( $\pm$  SD) 2.7 (0.9) days) lung function assessments were performed in 802 infants at the largest maternity ward at Ullevål University Hospital by tidal flow volume loops and passive respiratory mechanics .

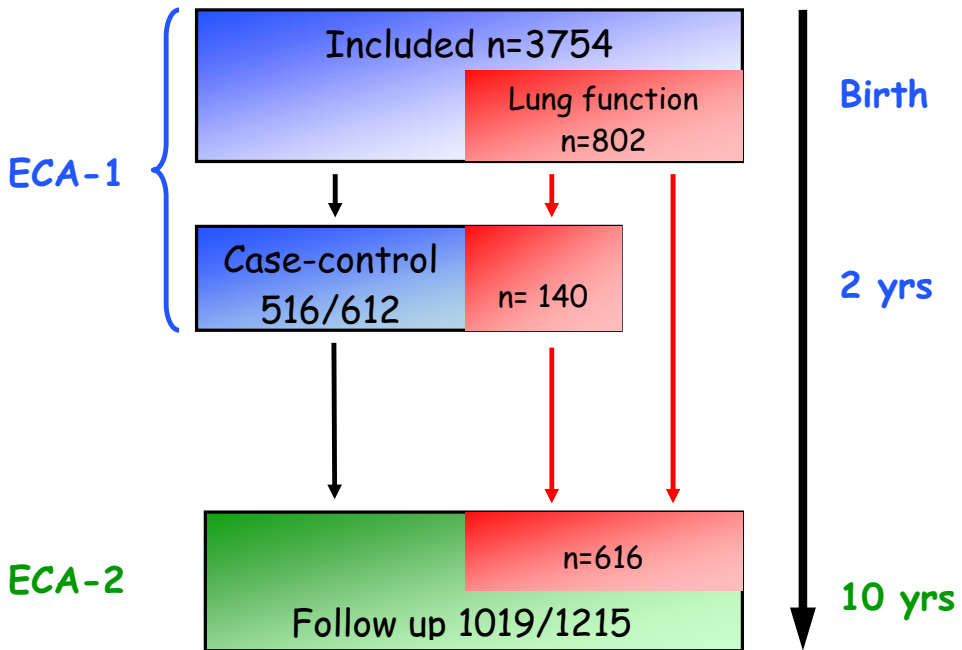


Figure 8. Simplified study design of the Environment and Childhood Asthma Study. ECA-1/ECA-2: Environmental and Childhood Asthma Study part 1/part 2. Blue boxes: ECA-1. Green Box: ECA-2. Red boxes: Children with lung function measurements at birth.

Starting at the maternity ward, parents of the children completed extensive half yearly questionnaires until two years of age. A nested case-control study was established with cases identified by these questionnaires and clinical registration cards with doctors documenting signs of bronchial obstruction (BO) as well as direct referrals from well baby clinics, hospital admissions and family doctors. A case was a child with rBO defined by at least two episodes of BO or BO lasting more than 4 months before two years of age. At least one episode of BO had to be confirmed by a doctor. The controls were the children without BO born closest in time to the case. The defined 306 cases and 306 controls were invited to a clinical assessment at enrolment if younger than 20 months of age and all cases and controls were invited to clinical assessment at two years (mean age 25 months) including lung function measurement

by tidal flow volume loops before and after inhalation of salbutamol, skin prick test, blood samples and a structured interview. A total of 562 children attended at least one of the assessments of whom 516 children (265 cases, 251 controls) attended the two year visit.

At ten years of age all traceable children who had attended at least one of the clinical visits the first two years of life and/or had lung function measurements at birth were invited to a follow up study (ECA-2). One thousand and nineteen of the 1215 eligible children (84%) attended the two days of examination (within a week) including forced expiratory flow volume measurements on both days, measurements of bronchial hyperresponsiveness by metacholine challenge test and exercise test by a treadmill run, skin prick test, measurement of fractionated exhaled nitric oxide, collection of blood samples, urine samples and dust from the child's mattress and an extensive interview of the parent(s) to be filled out by the study doctor. All personnel involved in the data collection in ECA-2 were blinded to the results of the assessments in ECA-1.

## 4.2. Subjects

### *Paper 1, 2 and 4:*

The study population of these three papers include the 616 children (332 boys, 284 girls) with lung function measurements at birth who attended the 10 year follow up study (mean age ( $\pm$ SD), 10.9 ( $\pm$  0.9) years). Sufficient information was available to classify 614 children for asthma ever/history of asthma and 606 for current asthma, for definitions see section 4.4.

These 614 children (614 with lung function measurement by tidal flow volume loops and 500 by passive respiratory mechanics) are included in paper 1 and 2. They did not differ in baseline characteristics compared to the remainder of the entire cohort of 3.754 infants, but were slightly longer and heavier at birth compared to the 188 children with lung function at birth who did not participate in the 10 year follow up, table 1.

Table 1. Baseline characteristics of subjects included in paper 1 and 2 compared to the remainder of the ECA study population.

Characteristics	Children included in	Children not included in the	
	the follow up study	follow up study	
	n=614	n=3140	
		Lung function at birth	
		yes (n=194)	no (n=2946)
Gender (% boys)	53.7	51.6	51.0
Birth weight (kg)	3.59 (0.49)	3.50 (0.48)*	3.55 (0.51)
Birth length (cm)	50.5 (2.2)	50.2 (2.2)**	49.7 (3.6)
$t_{\text{PEF}}/t_{\text{E}}$	0.32 (0.11)	0.31 (0.11)	NA
Compliance of the respiratory system (ml.cmH <sub>2</sub> O <sup>-1</sup> .kg)	4.16 (1.19)	4.08 (1.17)	NA
Parental asthma (%)	12.0	12.3	12.2
Parental rhinoconjunctivitis (%)	29.7	27.4	27.2
Maternal smoking in pregnancy			
daily or occasionally (%)	24.8	30.6	24.4

Values are given as mean  $\pm$  S.D or per cent when stated. NA denotes not applicable. Parental refers to mother, father or both. \*p=0.03, \*\*p= 0.04 compared to children included in the follow up. Reproduced with permission from Håland et al, N Engl J Med 2006; 355: 1682-1689.



Paper 4 includes 607/616 children attending the 10 year follow up with lung function measurements at birth, information of lower respiratory tract infections the first two years of life and successful lung function measurement at 10 years. Mean age was 10.9 ( $\pm$  0.8) years, and among these there was no significant differences in baseline characteristics compared to the 195 children with lung function measurements at birth not included in the study.

### *Paper 3*

The study population of paper 3 comprises the 135 children included in the nested case control study with lung function measurements at birth (135 by tidal flow volume loops and 117 by respiratory mechanics), information about rBO and AE by two years and attendance in the two year assessment. They had more often parents with rhinoconjunctivitis (37.8% vs. 27.4%,  $p=0.016$ ) but did otherwise not differ in baseline characteristics compared to the 667 children with lung function measurements at birth not included in the study. Ninety children had successful lung function measurements at two years.

## **4.3. Methods**

### *Parental questionnaires/interviews*

The half yearly questionnaires and the parental standardized interview at the two year assessment included questions about symptoms of lower respiratory disease and atopic diseases, use of anti-asthmatic medication, lower and upper respiratory infections, exposure of the child to tobacco smoke during intrauterine life and the first two years of life and respiratory and atopic diseases in parents and siblings.

At the ten year follow up the parents responded to an extensive interview completed by the study doctor, including core ISAAC questions about symptoms of lower respiratory diseases validated in Norwegian (166), having a doctors diagnosis of asthma, use of anti

asthmatic medication, atopic diseases in 1<sup>st</sup> degree relatives, infections, socioeconomic and home environmental issues, physical activity and diet. Environmental factors were to some extent assessed in detail, but will not be further discussed in the present thesis.

### *Lung function measurements*

At birth 802 awake infants had lung function measurements by tidal flow volume loops and 664 by passive respiratory mechanics lying on the investigators lap (167). At two years of age lung function was measured by tidal flow volume loops in children free of lower respiratory tract symptoms sitting on the lap of a parent. Short acting  $\beta$ -2 agonists were withheld since the day before (none used long acting  $\beta$ -2 agonists) and theophyllamins and antihistamins for 72 hours.

Flow was measured using a pneumotachograph with a flow range of 0-10  $l \cdot \text{min}^{-1}$  at birth (8311 series, Hans Rudolph, Missouri, USA) and 0-30  $l \cdot \text{min}^{-1}$  at 2 years (4500 series, Hans Rudolph, Missouri, USA) attached to an appropriate face mask with an air inflated cuff to make it closely fitting to the face of the child, ensuring no air leak. The dead space of the system was 2.4 ml (8311 series) and 3.2 ml (4500 series) respectively, and that of the face masks was 8.5-11ml (167). Volume was calculated by the digital integration of the flow signal with a frequency of 256 samples $\cdot\text{sec}^{-1}$ .  $t_{\text{PTEF}}/t_{\text{E}}$  was calculated by separate measurements of the time to peak expiratory flow and total expiratory time by the software programme of the computer of the SensorMedics 2600 System (SensorMedics Corp., Anaheim, CA, USA). The flow and volume signal were calibrated daily by a 100 ml precision syringe (Hans Rudolph, Missouri, USA).

The reported result for each child is the mean of four representative curves chosen from of eight loops stored on the computer. These eight curves were obtained from a series of

breaths with as stable volume and shape of the loops as possible and as low respiratory rate possible.

Respiratory system mechanics were measured in the newborn babies using the single breath, passive occlusion technique (167). A stable baseline level was established through several tidal cycles followed by an airway occlusion created by an automatic slide valve. The occlusion was sustained until equilibrium between alveolar and airway pressure was obtained i.e. a pressure plateau with variation  $\leq \pm 0.125$  cm H<sub>2</sub>O for 100 ms was recorded by the computer. The passive expiratory flow-volume loop obtained upon opening the slide valve was registered and used for measurement Crs (ml·cmH<sub>2</sub>O<sup>-1</sup>) and calculation of resistance of the respiratory system (Rrs) (cmH<sub>2</sub>O·ml<sup>-1</sup>·s). The reported results are the mean of the accepted curves (range 2-14) obtained for each infant.

At ten years lung function was measured by forced expiratory flow volume loops according to the European standard (168). Long acting  $\beta$ -2 agonists, theophyllamines and leukotrien antagonists were withheld for 72 hours, antihistamins for 5 days, short acting  $\beta$ -2 agonists for 8 hours and inhaled corticosteroids the same day, respectively. Baseline lung function was measured on two occasions before challenge testing within a week at least four weeks after any symptoms of LRIs by trained and experienced technicians using a Sensor Medics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA). FEV<sub>1</sub>, FEF<sub>50</sub> and FVC are reported as percent predicted based upon the reference values of Zapletal (169) and FEV<sub>1</sub>/FVC as absolute value from the best of the two baseline measurement.

#### *Assessment of airway hyperresponsiveness*

At the ten year follow up study AHR was assessed by a direct method (metacholin challenge test) and indirect method (exercise challenge) within a week, both at least four weeks after any symptoms of lower respiratory tract disease. Long acting  $\beta$ -2 agonists, theophyllamines

and leukotrienantagonists were withheld for 72 hours, antihistamins for 5 days, short acting  $\beta$ -2 agonists for 8 hours and inhaled cortocosteroids the same day, respectively.

Metacholin challenge test was performed according to ATS Guidelines (170) by inhaling doubling doses of metacholin nebulized by the inhalation triggered Spira Dosimeter (Spira Respiratory Care Center Ltd, Hämeenlinna, Finland) until a fall in FEV<sub>1</sub> of 20 percent (metacholin provocation dose) or the maximal dose of metacholin (22.4  $\mu$ mol) was reached.

Exercise challenge test was performed by treadmill running 6-8 minutes without preceding warm-up according to a standardized protocol (171). Effort was made to reach a heart rate of 95% of maximum (220-age) the last 4 minutes of the running. Lung function was measured before and three, six, 10, 15 and 20 minutes after the test. A test was regarded positive with a 10% or more decrease in FEV<sub>1</sub> 3-20 minutes after the running.

#### *Skin Prick Tests*

Skin prick test (SPT) was performed according to the Nordic standard (172) at the two year assessment to the following standardized allergens (Greer laboratories INC, Lenoir, NC, USA): egg white, cows milk, *dermatophagoides pteronyssinus*, timothy grass, cat dander, dog dander, silver birch, mugwort and *Cladosporium herbarum*. Histamine 10mg/ml was used as positive control and saline as negative control. A test was considered positive with a wheal of half the size of the positive control.

At ten year SPT was performed to the following 15 common allergens (Soluprick<sup>®</sup>, ALK, Abello, Denmark): House dust mites (*Dermatophagoides* (D.) *pteronnyssinus* and *D. farinae*), german cockroach, dog, cat, and rabbit dander, birch, timothy (grass) and mugwort pollens, moulds (*Cladosporium herbarium* and *Alternaria alternata*), egg white, milk, peanut and codfish. The test was performed according to international standard (173) with a positive

test to a specific allergen defined as a mean wheal diameter  $\geq 3$ mm larger than the negative control (0.9%NaCl).

#### 4.4. Definitions and outcomes

*Asthma ever* (paper I) and *history of asthma* (paper II) was defined by fulfilling at least two of the following three criteria:

1. Dyspnoea, chest tightness and/or wheezing 0-3 years and/or 4-10 years
2. Doctor's diagnosis of asthma ever
3. Use of asthma medication ( $\beta$ -2 agonist, sodium chromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) 0-3 years and/or 4-10 years

*Current asthma* (paper I, II and IV) was defined as asthma ever/history of asthma plus at least one of the following criteria:

1. Dyspnoea, chest tightness and/or wheezing during the last 12 months
2. Use of asthma medication ( $\beta$ -2 agonist, sodium chromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) during the last 12 months
3. Positive exercise test at the ten year follow up

*Wheeze ever* (paper I) was defined by a positive response to the questions "Has your child experienced dyspnoea, chest tightness and/or wheezing during the age periods 0-3 years and/or 4-10 years?"

In paper III the four clinical phenotypes were defined by the presence or not of AE and rBO the first two years of life: no rBO or AE, AE only, rBO only and both AE and rBO.

Severe AHR in paper II was defined as a metacholin provocation dose of less than 1  $\mu$ mol.

## 4.5. Statistical analyses

Baseline characteristics are given as mean values with standard deviation (SD) or per cent when stated. Results are given as mean with 95% confidence interval unless otherwise stated. For continuous variables differences between unpaired groups were assessed using the two sample t-test assuming normal distribution and a one-sample t-test was used to compare paired variables. Categorical variables were compared using the Pearson's Chi-Square test. Multiple groups were compared using one-way analysis of variance (ANOVA), or the non parametric Kruskal-Wallis tests when appropriate. Tukey's Post Hoc test was used when the ANOVA analyses indicated a significant difference between groups. Pearson's coefficient of correlation was used to assess possible associations.

Level of agreement between  $t_{PTEF}/t_E$  at birth and two years of age was assessed by Weighted Kappa ( $\kappa$ ) given by lung function categorized at the two time points (paper III). A two sided p-value of 0.05 was considered statistical significant. Analysis were performed with Statistical Package for Social Sciences version 11.0 (paper II) version 12.0 (paper I) and version 14.0 (paper III and IV) for Windows (SPSS, Chicago, IL, USA) and Statistical Analysis System version 9.1.13 (SAS, Cary, NC USA) in paper III.

### *Categorization of lung function*

Based upon lung function measurements at birth for all 802 children according to  $t_{PTEF}/t_E$  and 664 according to Crs the children were categorized into quartiles to evaluate lung function development from birth to two years of age (paper III). The middle two quartiles were merged since possible differences are more likely to occur between extreme values. Change in lung function from birth to two years of age ( $\Delta t_{PTEF}/t_E$ ) was calculated as  $t_{PTEF}/t_E$  at two years minus  $t_{PTEF}/t_E$  at birth.

To assess changes in lung function from birth to ten years of age (paper IV) z-scores were calculated based upon mean and SD calculated at birth for all children (as they were healthy at the time) and at 10 years for all children without any LRIs the first two years of life and without a history of asthma. Changes in lung function were calculated as z scores at ten years minus z-scores at birth.

Cut off values of lung function at birth for calculation of risk of a history of asthma and current asthma at ten years in paper II were based upon commonly used statistical approaches of median values, as well as the previously identified clinical cut-off of  $t_{PTEF}/t_E < 0.20$  (165).

### *Regression analysis*

Logistic regression analyses were performed to estimate odds ratio (OR) for asthma ever and current asthma in paper I including gender, allergic sensitisation and parental history of allergic disease (asthma and/or rhinoconjunctivitis) as covariates. In paper II the logistic regression analyses assessing OR for a history of asthma and current asthma included gender, maternal smoking in pregnancy, birth length, birth weight and parental asthma and parental rhinoconjunctivitis reported at birth of the index child. In addition, OR for Crs was also adjusted for  $t_{PTEF}/t_E \leq 0.20$ , and ORs for  $t_{PTEF}/t_E$  were adjusted for Crs <median.

To assess possible predictors of lung function ( $t_{PTEF}/t_E$ ) at two years and change in lung function from birth to two years of age ( $\Delta t_{PTEF}/t_E$ ) in paper III a multiple linear regression model was created including maternal smoking during pregnancy, gender, clinical phenotype at two years, age in months at two years assessment, birth weight and birth length and parental asthma and parental rhinoconjunctivitis reported at birth of the index child as covariates. For  $\Delta t_{PTEF}/t_E$  as outcome  $t_{PTEF}/t_E$  at birth was included in the analysis as well. Similarly was lung function at ten years of age (i.e. FEV<sub>1</sub>, FEF<sub>50</sub>, FVC, FEV<sub>1</sub>/FVC) evaluated

(paper IV) using multiple linear regression analyses including current asthma at ten years, positive SPT to any allergen at ten years, parental asthma, parental rhinoconjunctivitis, maternal smoking during pregnancy, gender,  $t_{PTEF}/t_E$ , Crs, Rrs, parental asthma and parental rhinoconjunctivitis reported at birth of the index child and LRIs the first two years of life either categorized as pneumonia yes/no, bronchiolitis yes/no and bronchitis yes/no or summarized in total number of LRIs as independent variables. The final multivariate model was build as described by Hosmer and Lemeshow (174). For the significant predictors results are given as regression coefficient (B) with 95% CI and p-value and adjusted  $R^2$  are given for the final model. The influence of co-linearity was assessed using the variance inflation factor (VIF) and the Studentized deleted residuals were used to assess the normality of the model. Cook's d was used to assess the influence of the single observations.

#### *Regression to the mean*

Controlling for “regression towards the mean” was performed for  $\Delta t_{PTEF}/t_E$  in paper III by taking the average of the initial and final measurement and calculating the correlation between this value and the observed change, as described by Altman (175)

## **4.6. Ethical issues**

Written informed consent was obtained from parent(s) of all the included infants at the maternity ward before inclusion in the ECA study. Renewal of the consent was obtained at enrolment into the 10 follow up study after written information to the parents as well as the child. The participants were informed that they could withdraw from the study at any time. The study was approved by the Norwegian Data Inspectorate and the local Committee for Medical Ethics made no objections to the study. The study was registered in the Norwegian



Biobank Registry, Ullevål University Hospital in Oslo.

## 5. RESULTS OF THE STUDIES

### 5.1. Prevalence of asthma among school children in Oslo

In the ten year follow up study 20.2% of the children fulfilled the criteria of asthma ever and 11.1% the criteria of current asthma (paper I). Doctors diagnosis of asthma was reported in 16.1% of the children and 30.6% reported wheeze ever. Current asthma and wheeze ever were more frequent among boys than girls (14.4% vs 7.1%,  $p=0.008$  and 36.9% vs 22.5%,  $p=0.002$  respectively) but boys and girls did not differ significantly with respect to asthma ever (22.9% vs 17.0%,  $p=0.086$ ) and doctors diagnosed asthma. Similar numbers of children reported wheeze before four years of age (8.8%) and at age 4-10 years of age (9.1%) whereas 12.6% reported wheeze both before and after the age of four years.

Compared to no asthma children and asthmatic children without current symptoms, children with current asthma were characterized by a higher prevalence of positive SPT (56.1% vs 26.0% in no asthma children and 26.8% in asthma children without current symptoms,  $p<0.001$ ) and parental asthma and/or rhinoconjunctivitis (67.2% vs 49.1% and 49.6% respectively,  $p<0.024$ ) reported at ten years (paper I).

### 5.2. Lung function in children with asthma

Lung function was reduced both at birth as well as at 10 years in children with asthma. Children with current asthma at 10 years had significantly lower values of  $FEV_1$  and  $FEF_{50}$  compared to children with no asthma and asthmatic children without current symptoms, table 2 (paper I). Children fulfilling the criteria of a history of asthma as well as current asthma had reduced  $t_{P_{TEF}/t_E}$  and  $Crs$  (although not statistical significant for the current asthma group) at birth compared to the no asthma group, table 2 (paper II).

Table 2. Lung function at birth and 10 years of age in children with and without asthma.

	No asthma	Asthma, no current symptoms	Asthma ever	P value	Current asthma	P value
$t_{PTEF}/t_E$	0.322 (0.312-0.332)	—	0.298 0.278-0.317	0.03	0.293 0.266-0.321	0.05
Crs	4.241 (4.125-4.356)	—	3.876 (3.639-4.114)	0.005	3.942 (3.614-4.270)	0.08
FEV <sub>1</sub>	99.1 98.3-100.0	100.3 (97.7-102.8)	—		95.8 (93.3-98.3)	0.021
FEF <sub>50</sub>	91.0 (89.4-92.7)	86.7 (82.1-92.1)	—		79.0 (73.8-84.0)	<0.001

Values are given as mean with 95% confidence interval for  $t_{PTEF}/t_E$  and Crs measured at birth and FEV<sub>1</sub> and FEF<sub>50</sub> measured at ten years. For  $t_{PTEF}/t_E$  and Crs p values are given for comparison with no asthma group, for FEV<sub>1</sub> and FEF<sub>50</sub> p values are given for trend.

### 5.3. Factors associated with lung function development through childhood

Lung function development from birth to two years of age measured by  $t_{PTEF}/t_E$  was not influenced by the presence of AE and/or rBO (paper III), figure 9. In a multiple linear regression model including maternal smoking during pregnancy, gender, parental asthma, parental rhinoconjunctivitis, clinical phenotype at two years (presence of AE and/or rBO), age in months at two years assessment, birth weight, birth length and  $t_{PTEF}/t_E$  at birth as covariates only  $t_{PTEF}/t_E$  at birth was significantly associated with  $\Delta t_{PTEF}/t_E$  from birth to two years of age

when this covariate was introduced. In the final model including 90 subjects, the explained variability of  $\Delta t_{PTEF}/t_E$  ( $R^2=0.353$ ,  $p<0.001$ ) was then mainly explained by  $t_{PTEF}/t_E$  at birth.

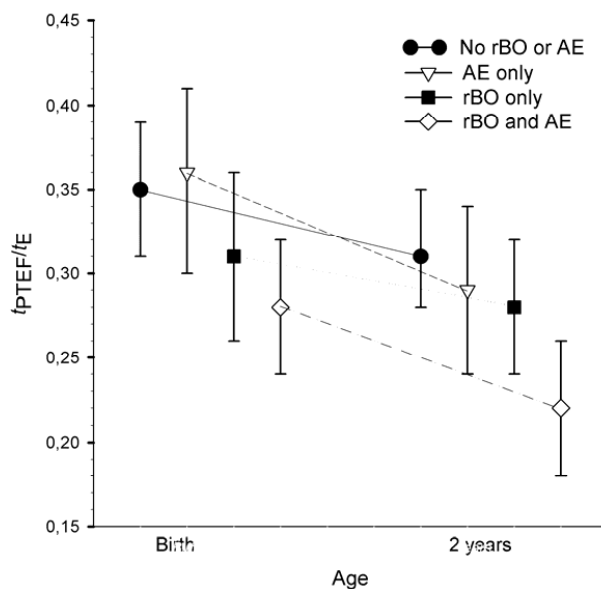


Figure 9. Lung function measured by tidal flow volume loops (mean  $\pm$  95% confidence interval) at birth and two years of age according to clinical phenotype by 2 years. rBO: recurrent bronchial obstruction. AE: atopic eczema. Reprinted with permission from Håland et al *Pediatr Allergy Immunol* 2007; 18: 528-534.

Reported bronchiolitis, bronchitis and three or more LRIs the first two years of life were associated with reduced  $FEF_{50}$  at ten years of age. Likewise was bronchiolitis associated with reduced  $Crs$  and  $t_{PTEF}/t_E$  at birth. However, after stratifying for gender these associations were found in girls only (paper IV).

LRIs the first two years of life were not significantly associated with impaired lung function development from birth to 10 years of life, figure 10. Neither pneumonias, bronchitis, bronchiolitis or the total number of LRIs were predictors of any lung function indices at ten years of age when included in a linear regression model together with parental

rhinoconjunctivitis, parental asthma, maternal smoking during pregnancy, gender, current asthma at ten years, positive SPT to any allergen at ten years, and lung function at birth ( $t_{PTEF}/t_E$ , Crs and Rrs). For  $FEF_{50}$  the significant predictors were current asthma ( $B=9.47$ , 4.70-14.24,  $p<0.001$ ), male gender ( $B= 6.72$ , 3.74-9.70,  $p<0.001$ ), maternal smoking during pregnancy ( $B=3.51$ , 0.073-6.95,  $p=0.045$ ) and reduced  $t_{PTEF}/t_E$  at birth ( $B= 14.86$ , 1.43-28.30,  $p=0.03$ ), but the adjusted  $R^2$  was poor (0.075).

Controlling for the same factors for each gender separately left only current asthma at ten years as significant predictor of reduced  $FEF_{50}$  at ten years when including total numbers of LRI the first two years of life (Girls  $B=14.22$ , 5.87-22.56,  $p=0.001$ . Boys  $B=7.93$ , 2.05-13.82,  $p=0.008$ ).

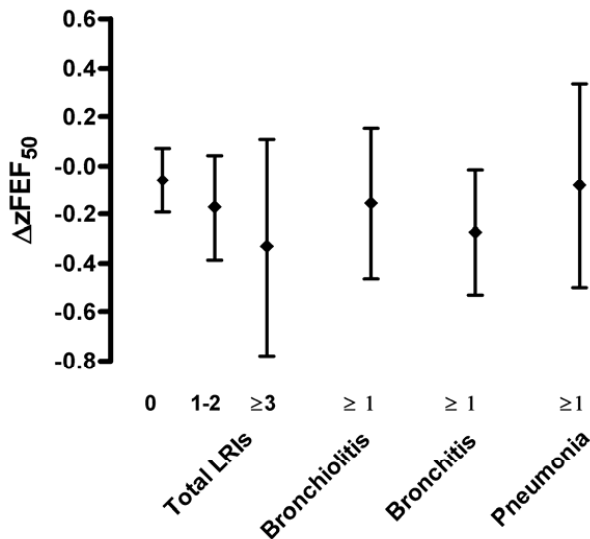


Figure 10. Change in lung function (mean with 95% confidence interval) by z-scores from birth (by  $t_{PTEF}/t_E$ ) to 10 years of age (by  $FEF_{50}$ ) for reported bronchiolitis, bronchitis, pneumonia and total number of reported lower respiratory tract infections (LRIs) the first two years of age.  $p > 0.1$  for all groups compared to no LRIs. Reprinted with permission Håland et al. *Pediatr Allergy Immunol*. In press.

### 5.4. Lung function from birth to ten years

Children with  $t_{PTEF}/t_E$  in the lowest quartile at birth had significantly lower  $t_{PTEF}/t_E$  at two years of age compared to the upper quartiles (0.22 (0.18-27) vs. middles 0.27 (0.24-0.30), and upper 0.35 (0.31-0.38) respectively, overall  $p<0.001$ ). The  $t_{PTEF}/t_E$  at birth correlated significantly with  $t_{PTEF}/t_E$  at two years, ( $r=0.475$ ,  $p<0.001$ ), and the measure of agreement was  $\kappa=0.42$  (0.26-0.58,  $p<0.001$ ). Rrs and Crs at birth were not significantly associated with  $t_{PTEF}/t_E$  at two years, but a weak correlation ( $r=0.11$ ,  $p=0.003$ ) was found between  $t_{PTEF}/t_E$  at birth and Rrs at birth, paper III. Only  $t_{PTEF}/t_E$  at birth (adjusted  $R^2=0.23$ ,  $p=0.001$ ) was a significant predictor of  $t_{PTEF}/t_E$  at two year in a regression analysis including maternal smoking during pregnancy, gender, parental asthma, parental rhinoconjunctivitis, clinical phenotype at two years (presence of AE and/or rBO), age in months at two years assessment, birth weight, birth length and  $t_{PTEF}/t_E$  at birth as covariates, paper III.

Although there was a significant difference in  $\Delta t_{PTEF}/t_E$  from birth to two years of age between the upper and lower  $t_{PTEF}/t_E$  birth quartile, this difference was no longer significant after controlling for regression to the mean, paper III.

At ten years children with  $t_{PTEF}/t_E$  below mean at birth had significantly lower  $FEF_{50}$  compared to children with  $t_{PTEF}/t_E$  above mean, and children with Crs below compared to above median at birth had significantly lower  $FEV_1$  compared to children with Crs above median, table 3 (paper II).

$t_{PTEF}/t_E$  at birth was a significant predictor for  $FEF_{50}$  at 10 years, as described in section 5.3., (paper IV).

### 5.5. Lung function at birth and asthma later in childhood

Children with clinical phenotype of rBO and AE at two years had lower  $t_{PTEF}/t_E$  at birth compared to children with no rBO or AE (0.28 (0.23-33) vs 0.34 (0.31-0.37),  $p=0.027$ ), figure

9. Although not statistically significant, the children with this phenotype had the highest prevalence of positive SPT and use of inhaled corticosteroids, a marker of severity of obstructive airway disease, by two years, paper III.

Compared to children with no asthma, children fulfilling the criteria of asthma ever/history of asthma had lower  $t_{PTEF}/t_E$  (0.30 (0.28-0.32) vs 0.32 (0.31-0.33),  $p=0.03$ ) and Crs at birth (3.88 (3.64-4.11) vs 4.24 (4.13-4.36),  $p=0.005$ ) as well as children with current asthma had lower  $t_{PTEF}/t_E$  (0.29 (0.27-0.32),  $p=0.05$ ) and a trend towards lower Crs (3.94 (3.61-4.27),  $p=0.08$ ), paper II.

Children with  $t_{PTEF}/t_E$  at birth below median were significantly more likely to have asthma ever as well as current asthma by ten years, table 3.  $t_{PTEF}/t_E$  below 0.20 at birth was significantly associated with increased prevalence of asthma ever, and children with Crs below median at birth had more often a history of asthma ever and current asthma by ten years, table 3. Severe airway hyperresponsiveness (metacholine provocation dose  $<1 \mu\text{mol}$ ) at ten years was associated with  $t_{PTEF}/t_E < 0.20$  as well as below median at birth, table 3 (paper II).

Children with both  $t_{PTEF}/t_E < 0.20$  and Crs below median represented a high risk group for a history of asthma (45.5% vs 19.1%,  $p < 0.001$ ) and current asthma (28.1% vs 10.0%,  $p=0.002$ ), figure 11 (paper II).

After adjustment for gender, parental asthma, parental rhinoconjunctivitis and maternal smoking in pregnancy (ORs for Crs was also adjusted for  $t_{PTEF}/t_E \leq 0.20$ , and ORs for  $t_{PTEF}/t_E$  were adjusted for Crs below median)  $t_{PTEF}/t_E < 0.20$  remained a significant risk factor for a history of asthma (OR 1.90 (1.06-3.42)) and a value of  $t_{PTEF}/t_E$  below median a risk factor for current asthma (2.10 (1.12-3.93)). Crs below median was a predictor for both asthma ever (OR 2.18 (1.39-3.38)) and current asthma (2.01 (1.10-3.66)).

Table 3. Outcomes at ten years of age according to lung function measured at birth.

Outcomes at 10 years	$t_{PTEF}/t_E$		$t_{PTEF}/t_E$		Crs	
	<0.20	>0.20	<median	>median	< median	>median
Asthma ever (%)	<b>31.3</b>	18.5	<b>24.3</b>	16.2	<b>27.4</b>	14.8
Current asthma (%)	14.6	10.5	<b>14.6</b>	7.5	<b>15.0</b>	7.7
Severe AHR (%)	<b>12.7</b>	5.1	<b>9.1</b>	4.9	5.2	8.9
Current use of ICS (%)	5.3	3.9	<b>5.9</b>	2.4	5.2	2.7
FEV1 % predicted	98.9	98.9	98.2	99.6	<b>97.8</b>	99.7
mean, 95% c.i.	96.6-101.2	98.1-99.8	97.1-99.4	98.5-100.7	<b>96.4-99.1</b>	98.5-100.9
FEF50 % predicted	88.2	88.6	<b>87.8</b>	91.0	88.3	90.5
(mean, 95% c.i.)	83.8-92.7	88.0-91.2	<b>85.6-90.0</b>	88.9-93.1	85.8-90.8	88.1-92.8

Severe airway hyperresponsiveness (AHR): metacholin challenge dose <1  $\mu$ mol that leads to a 20% reduction in FEV<sub>1</sub>. Reproduced with permission from Håland et al, N Engl J Med 2006; 355: 1682-1689.



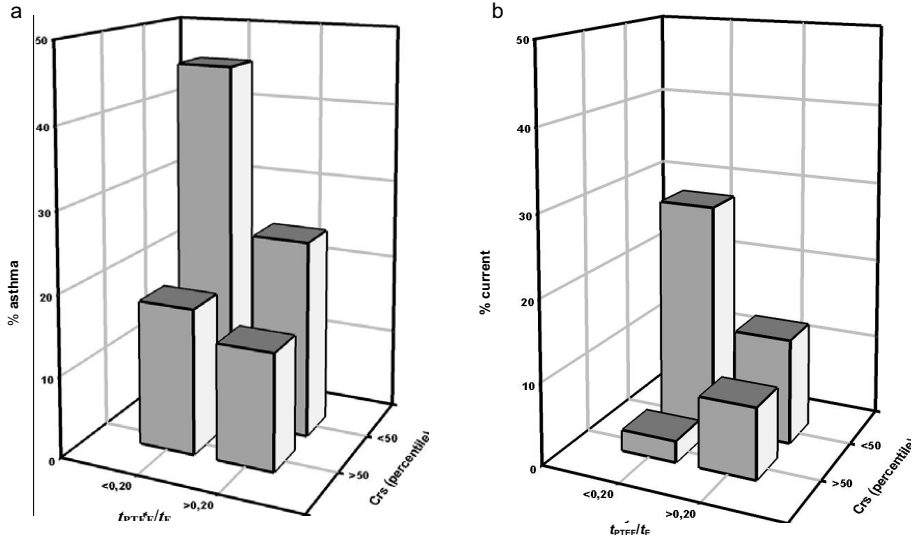


Figure 11. Prevalence of asthma ever (figure a) and current asthma (figure b) at ten years by  $t_{PTEF}/t_E$  below 0.20 and Crs below median at birth.

## 6. GENERAL DISCUSSION

### 6.1. Prevalence of asthma among school children in Oslo

The results of paper I of a lifetime prevalence of asthma of 20.2% and current asthma in 11.1% of the children are the highest ever reported in Norway. The study indicate a substantial increase in prevalence of asthma among school children in Oslo compared to the 1994 survey reporting a lifetime prevalence of asthma of 9.3% and current asthma in 5.5% of the children (52). These two studies are however not directly comparable due to methodological differences as lifetime prevalence of asthma was defined as doctors diagnosed asthma (DDA) in 1994. However, in our study DDA was reported in 16.1% which further support an increased asthma prevalence from the mid nineties.

The findings in the ECA study are supported by a Danish study conducted in 2001 reporting current asthma among 11.7% of school children living in Copenhagen, an urban population comparable to ECA population (36). In the Danish study current asthma had increased from 5.3% in a survey in 1986 applying the same questionnaire and a positive SPT to at least one out of 10 allergens among 46 % of the current asthmatics (36) compared to 56.1% in the ECA study including 15 allergens.

The results from the Norwegian Young-HUNT study, conducted in central Norway 1995-97, differed from ours reporting current asthma, by the employed definition (DDA and wheeze last 12 months), in 8.5% of the girls and 7.1% of the boys (176). Current wheeze was reported among 29.0% of girls and 20.4% of boys. In another report from the same cohort, asthma prevalence was estimated to 14.7% (girls 18%, boys 11%) when defined as current wheeze and AHR (177), emphasizing the challenge of asthma labelling as previously discussed. However, these studies are not directly comparable to the ECA study due to methodological differences and the study population being older (13-16 years) meaning that

many of the included adolescents had gone through puberty, a period known to influence the prevalence of respiratory symptoms and asthma (178).

In contrast to our study, several other studies have reported a levelling off or even a reduction in asthma prevalence (32-35). Most of these studies have however come from areas with a higher prevalence than reported in the previous Norwegian studies. This may lead to speculations that a “saturation level” of asthma prevalence has been reached in these areas, with their given genetic background and the present environment. However, for most of these studies the observation time has been rather short and they are based on questionnaires and do not include objective measures like assessment of AHR (33-35). These limitations are also valid for a northern Norway survey conducted in 2000 finding the life time prevalence of asthma (13.8%) to be unchanged from a similar study in 1995 in 9-11 year old children (179). Whether we are approaching a “saturation level” in Norway can only be determined by future surveys employing methods identical to previous studies.

## **6.2. Lung function in children with asthma**

The presented result from paper I that school children with asthma have reduced mean forced flow values ( $FEV_1$  and  $FEF_{50}$ ) is in line with previous studies (128;131;132) including children from six years of age (131). The results from the two years assessment (paper III) of reduced lung function measured by tidal flow volume loops among children with both rBO and AE by two years of age, the more “severe” clinical phenotype with increased risk of wheeze and allergies in school age (20), adds further evidence to the association between asthma and reduced lung function. These findings raise the question whether the reduced lung function is *due to* impairment of lung function development in children with asthma or if it was present prior to disease expression; i.e. is reduced lung function a marker of increased susceptibility of asthma?

*Lung function development in children with asthma*

In paper IV we report current asthma to be a significant predictor of lung function at ten years even when including lung function at birth in the analyses. Previous longitudinal studies assessing the impact of lower respiratory tract illness on lung function development from childhood into adolescent provide conflicting results. Decreased growth in airway calibre represented by reduced airflow values ( $FEV_1$ ,  $FEV_1/FVC$ ,  $FEF_{25-75}$ ), but not in lung volume (FVC) have been reported in children with AHR (97;135) or persistent asthma (180). Other studies find reduced lung function in children with lower respiratory tract illness already in early school age, but their further lung function development to be independent of lower respiratory tract illness (112;118;134;181). However, the latter studies did not include measurement of AHR and some studies stratified the children according to wheezing phenotype (112;118) as may represent, as previously discussed, other airway abnormalities than asthma. Furthermore, wheezing was relatively infrequent among many children, thus likely to have a mild disease only. Disease severity has previously been associated with level of AHR (182).

The apparent paradox that current asthma at ten years predicted lung function at ten years (paper IV), but clinical phenotype by two years was not associated with lung function at two years (paper III) may be explained by the factors discussed above. At two years the children were categorized according to symptoms only and not by a diagnosis of asthma or the presence of AHR. Another possible explanation is that the duration of the disease had been too short to cause permanent impairment of lung function at two years of age, indicating that airway remodelling was not present. Assessment of airway remodelling is not the scope of the present thesis and objective measures of airway remodelling were not included in the ECA study. The issue will therefore not be further discussed.

However, only 90 children were included in the two years analyses which might have been too few to detect small, but real effects on lung function development the first two years of life by factors assessed.

### **6.3. Other factors associated with lung function development**

The reduced lung function in school age in children reporting LRIs the first two years of life (paper IV) is in line with several previous studies (136-140). However, the novelty of the ECA study is the possibility to adjust for lung function at birth in a large scale study. We could thereby conclude that lung function development from birth to ten years of age was not influenced by LRIs reported during the first two years of life (paper IV). Results from the 11 year follow up of the Tucson birth cohort (139) support our conclusion as children with radiologically ascertained pneumonia as well as LRIs other than pneumonia in the first three years of life had reduced FEV<sub>1</sub> and FEF<sub>25-75</sub> at 11 years as well as reduced  $V'_{\max, \text{FRC}}$  at two months of age. However, the number of children with lung function measurements shortly after birth included was low and the finding of reduced lung function prior to any LRIs was not significant among the eight children with measurement of  $V'_{\max, \text{FRC}}$  and X-ray confirmed pneumonia (139). In an Australian prospective study, children with bronchiolitis the first two years of life were found to have significant reduced  $V'_{\max, \text{FRC}}$  at one month of age as well as FEF<sub>25-75</sub> at 11 years of age compared to children without bronchiolitis (183). The level of reduction in lung function was comparable at the two time points, but also in this study the sample size was small including only 16 children with bronchiolitis. Our results are further supported by several reports identifying reduced lung function during infancy as a risk factor for wheezing lower respiratory illness during the first years of life (160-163;184) as wheezing in this age group is closely associated with LRIs.

In paper IV LRIs were classified as pneumonias, bronchitis or bronchiolitis based upon doctors diagnosed disease reported by the parents in the questionnaires. In these

diagnostic entities there is a risk of misclassification due to the overlap in symptoms (185). However, in the study by Castro-Rodriguez and coworkers including chest X-rays to differentiate LRIs, both radiologically ascertained pneumonias as well as other LRIs the first years of life were associated with reduced lung function both in infancy and at 11 years of life (139). Thus our study, and others, do not support the hypothesis of a causal association between LRIs early in life and reduced lung function in school age, but rather points to initial reduced lung function as a risk factor or a marker of increased susceptibility to LRIs the first two years of life. However, we were not able to control for specific microbial airway pathogens involved in the LRIs as collecting microbial samples was not a part of our cohort study. Previous publications have found LRIs caused by Respiratory syncytial virus (139;144-147) and *Mycoplasma pneumonia* (148;149) in early life to be associated with impaired lung function later in life. One may then speculate that some airway pathogens, independent of the clinical presentation, cause a small impairment of lung function not identified by our study. However, none of these previous studies report lung function measurements prior to the potential insult by the LRI.

Maternal smoking during pregnancy has been associated with reduced lung function at birth (121) in our cohort, and in paper IV found to be a significant predictor of FEF<sub>50</sub> at ten years of age even when lung function at birth was included in the regression analyses. Although the association just reached statistical significance ( $p=0.045$ ) and was not evident when stratifying for gender, it is in line with previous studies (125;126). We did not control for exposure to second hand smoke in the first years or at ten years, but other studies have reported the effect of post natal exposure to be non significant when adjusted for in utero exposure (125;186). Although maternal smoking during pregnancy was not a significant predictor of lung function (by  $t_{PTEF}/t_E$ ) at two years or  $\Delta t_{PTEF}/t_E$ , this may be due to the number

of children with lung function measurement both at birth and two years being too small to detect possible differences.

#### 6.4. Lung function from birth to ten years

Although there is evidence that several factors influence lung function development through childhood, significant tracking of lung function was demonstrated by tidal flow volume parameters from birth to two years of age. This adds further evidence to the hypothesis that tracking is present from the very beginning of extra uterine life. Several other studies have found tracking to be evident during the first year of life (113-115), but there is to our knowledge no other reports including lung function measurement both at birth and at two years. The first years of life have been considered the most vulnerable period for insults affecting growth and development of the airways (187). In our study lung function development from birth was independent of clinical phenotype defined by rBO and AE indicating a strong tracking even in the first years of life.

However, as previously discussed, we can not rule out that some environmental factors may induce small, but real changes in lung function since the correlation between  $t_{PTEF}/t_E$  at birth and two years was 0.475. However, sample sizes far larger than our 90 may be required to identify such.

$t_{PTEF}/t_E$  below median and Crs below median at birth were associated with reduced FEF<sub>50</sub> and FEV<sub>1</sub> respectively at ten years of age. Furthermore,  $t_{PTEF}/t_E$  at birth was a significant predictor of FEF<sub>50</sub> at ten years. These findings indicate that tracking is evident from birth into school age, but the association may appear to be weaker than the tracking during the first two years of life and the previously reported tracking during school age (110) and into adolescence (111) and adulthood (112). There are several possible explanations for this discrepancy. First, at birth lung function measurements were performed by tidal flow

volume loops and passive respiratory mechanics. It is not known exactly what the tidal flow volume loop parameter  $t_{PTEF}/t_E$  represents, but it is assumed that “*timing of PTEF is due to an interaction between the mechanical properties of the lungs and airways on one hand and central control of breathing on the other*” (63). Crs and Rrs represent mechanical properties of the respiratory system. At ten years, lung function was measured by forced expiratory flow volume loops and the parameters derived represent airway calibre of the large ( $FEV_1$ ) and smaller ( $FEF_{50}$ ) airways as well as lung volume (FVC). Although  $t_{PTEF}/t_E$  has been found to correlate with  $FEV_1$  and  $FEF_{50}$  (60;66;75) we are actually comparing indices that at least partly represent different qualities of the airways. In addition, although measurement of lung function in infancy and school age is reproducible, it does show variation. Most studies report a CV of about 25% for  $t_{PTEF}/t_E$  (60;66;70;71). The normal variation of  $FEV_1$  has been reported to be up to 5% within a day and up to 12% from week to week in healthy adults (188). This illustrates the problems of comparing results obtained by different methods at different time points. This obstacle can partly be overcome by calculating z-scores (118), or group the children according to quartiles (189) as has been done in the ECA study.

A second possible explanation for discrepancy in tracking the first years of life and from birth to school age is that lung function is modulated from birth to school age by factors not identified. Previous studies exploring the association between lung function in infants and in school age have provided conflicting results when the children were categorized according to wheezing phenotype (18;117). However, in a recently published study children with  $V'_{max,FRC}$  in the lowest quartile at two months of age had significantly reduced  $FEV_1$ ,  $FEF_{25-75}$ , and  $FEV_1/FVC$  at both 11, 16 and 22 years of age (189), and a significant correlation coefficient (Pearson's  $r$ ) of about 0.35 was found between infant  $V'_{max,FRC}$  and  $FEF_{25-75}$  and  $FEV_1/FVC$  at the three time points.



## 6.5. Lung function at birth and asthma later in childhood

Our finding of significant reduced lung function at birth in children with asthma ever as well as current asthma (paper II) at ten years extends previous findings of an association between reduced lung function at birth and respiratory illness during the first years of life. Several studies, including one from our own cohort, have demonstrated reduced  $t_{PTEF}/t_E$  to increase the risk of wheeze associated lower respiratory tract illness in the first years of life (160;161;165;184) as well as doctors diagnosed asthma by two years (116). Furthermore, reduced Crs at one month of age has been associated with persistent wheezing the first two years of life (163) and reduced  $V'_{max,FRC}$  have been found to precede wheeze during the first years of life (162-164;184). These studies support our finding of reduced  $t_{PTEF}/t_E$  at birth in children with the both rBO and AE by two years of age, a sub sample of children with the highest prevalence of positive SPT and use of inhaled corticosteroids by two years (paper III), and increased risk of wheeze as well as atopic diseases in school age (20).

Few studies that have explored the association of early infant lung function and lower respiratory tract illness further on to school age (18;117). Both included measurement of infant lung function by the RTC technique and the results have been conflicting with only one study (117) finding an association between reduced  $V'_{max,FRC}$  and wheeze, but not asthma, at six and 11 years of age.

The inconsistency of our results with previous findings may be explained by methodological differences. Whereas  $V'_{max,FRC}$  is thought to reflect airway calibre,  $t_{PTEF}/t_E$  is believed to be a complex measure of lung function including airway calibre, mechanical properties of the airway system (60;73;75) as well as respiratory control (72). Direct comparison between these two parameters of lung function (as well as Crs) is therefore not feasible. Although small airway calibre shortly after birth may be a risk factor for obstructive airway disease in the first years of life, the findings in paper II suggest that other

characteristics of the respiratory system at birth identify infants at increased risk of asthma in school age. The results further suggest that markers of increased risk of asthma in school age are detectable already at birth.

Another methodological difference that may explain the discrepancy is the sample size: our cohort included 614 children with lung function measures at birth as well as 10 years, a population sample of more than three times the Perth cohort (117) and almost five times the Tucson cohort (18). Thus, the power to detect associations was improved compared to the previous studies. The adjusted ORs for current asthma and asthma ever are comparable with those previously reported for a family history of asthma (190). However, the clinical implication of reduced lung function at birth on an individual level is still unsettled. As previously discussed, the inter- and intra individual variation in  $t_{PTEF}/t_E$  is well known (167) and the positive predictive value for later asthma was low (14-31%) for all cut off values for  $t_{PTEF}/t_E$  as well as Crs. Thus, our data do not support the use of lung function measurements by tidal flow volume loops or respiratory mechanics as screening tests at birth for the risk of subsequent asthma. This conclusion is further supported by the fact that few measures have proven effective in preventing the development of asthma in children at risk and the measurement of lung function by these techniques are time consuming. However, lung function measurements in infancy provide a unique opportunity to assess possible modifying environmental effects upon, not only immunological or clinical disease expression, but also physiological development through childhood.

## 6.6. Strengths and limitations

### *Strengths*

The ECA study is a prospective birth cohort study including detailed questionnaires every six months during the first two years of life and follow up at 10 years of age. It was designed to

investigate asthma and factors associated with asthma development during childhood, with an emphasis on environmental exposure. It contains a well characterized population of mainly, but not exclusively, Norwegian ethnicity. The longitudinal design with inclusion early in life and later follow up reduces the risk of recall bias and misclassification thereby increasing the level of evidence (191). The follow up rate of 77% and the children included being representative for the total ECA cohort at birth makes inclusion bias unlikely.

The ECA cohort includes the largest population of children with lung function measurements in infancy known to date. The sample size, and the fact that our findings are supported by other studies make a type I error in paper II and a type II error in paper IV unlikely.

At ten years the definition of asthma ever was based on a combination of symptoms, medication and clinical diagnosis and not only on one of these criteria as has been customary. For the definition of current asthma an objective measure of AHR was included as well. We believe that these more comprehensive definitions reduce the risk of misclassifications thereby increasing the validity of our findings. However, we also included the core questions from the standardized ISAAC questionnaires, making it possible to compare our results with other studies.

All the study doctors and technicians involved in the ten years assessment were blinded to the results of the lung function measurements at birth and two years of age as well as other results from ECA-1.

### *Limitations*

AHR has been found to be associated with reduced growth of lung function during childhood and adolescence (97;133;135). In one prospective study AHR at one month of age was associated with reduced levels of FEV<sub>1</sub> and FVC as well as doctors diagnosed asthma at six

years of age (192). The ECA study did not include measurement of AHR early in life or at two years of age and it was therefore not possible to adjust for this factor in our analyses. However, there is currently no standardised method for AHR measurements in early childhood, and in particular not for the TFV or passive respiratory mechanics measurements employed in early childhood in the present study.

As previously discussed the study sample in paper III may have been too small to identify small, but real effects of different factors on lung function development by two years. Due to the previously described intra- and inter individual variation of tidal flow volume measurements one would expect the somewhat small study sample to further reduce our power to detect tracking from birth to two years of age. However, despite these limitations we found significant tracking of lung function the first two years of life.

## **6.7. Future perspectives**

The results of the papers included in the present thesis raises two main questions. First: Why do some children have reduced lung function at birth making them more susceptible to lower respiratory tract illness during childhood? Tobacco smoke during pregnancy, parental atopy and maternal lower respiratory tract infections during pregnancy have, as previously discussed, been associated with reduced lung function early in life, but these factors can only partly explain the impaired lung function observed among some infants. Whether the factors decisive for impaired intra uterine lung function evolution are primary genetic, environmental or a combination of these can not be decided upon by the present knowledge and needs further investigation. New studies addressing this question should ideally recruit mothers ahead of pregnancy and include close follow up of the mothers during pregnancy and of the children after birth. These studies are challenging to conduct, but identifying these risk factors

may make preventive measures possible either on a population level or in children at risk of developing asthma.

The second question that the present thesis raises is whether lung function at birth is a predictor for obstructive airway disease even in adult life? The association between lower respiratory tract illness in childhood and reduced lung function and increased risk of obstructive pulmonary disease in adult life has been known for many years (193). The findings presented in the present thesis give evidence to the hypothesis that children with lower respiratory tract illness during childhood have reduced lung function already at birth and, although smoking has been identified as the major risk factor for decline in lung function and adult chronic obstructive pulmonary disease, the question of how much of adult obstructive airway disease can actually be attributed to reduced lung function at birth (and thereby prenatal factors) is still to be answered. To answer this question infants included in birth cohorts with lung function measurement shortly after birth, like the ECA study, have to be followed into adult life.

## 7. CONCLUSION

Based upon the results of the included papers and previous knowledge in the research areas explored in the present thesis the following conclusions can be drawn on the specific research questions:

1. Among schoolchildren in Oslo the lifetime prevalence of asthma and current asthma was 20.2% and 11.1% respectively. The findings indicate a substantial increase in prevalence when compared with surveys conducted 10 years earlier.
2. Children with current asthma at ten years of age had reduced lung function by FEV<sub>1</sub> and FEF<sub>50</sub> at ten years as well as at birth and children with asthma ever have reduced lung function at birth.
3. Lung function development from birth to ten years of age is negatively associated with current asthma at ten years and maternal smoking during pregnancy. LRIs the first two years of life had no significant impact on lung function development in contrast to previous hypotheses.

Lung function development by  $t_{PTEF}/t_E$  from birth to two years of age was not influenced by clinical phenotype by two years (presence of rBO and/or AE) or maternal smoking during pregnancy in our relatively small population size.

4. Lung function at birth was associated with lung function at two years as well as at ten years of age.  $t_{PTEF}/t_E$  at birth correlated reasonably well with  $t_{PTEF}/t_E$  at two years of age and lung function tracking was present the first two years of life independent of clinical phenotype by two years.  $t_{PTEF}/t_E$  at birth was likewise a significant predictor of lung function at ten years of age and reduced levels of  $t_{PTEF}/t_E$  and Crs at birth was

associated with impaired lung function at ten years of age. The findings add further evidence to the hypothesis that tracking of lung function starts very early in life

5. Reduced lung function at birth was a risk factor for asthma by 10 years and associated with the clinical phenotype by two years including both rBO and AE. The value of lung function measurements at birth on an individual level was limited due to the low predictive value, whereas the value in a prospective study is much greater providing a basis for understanding natural development and environmental influences in disease development.

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**ERRATA**

The following spelling errors have been corrected since submission to the doctoral committee:

Page 9 line 13 "cocurrent" has been substituted with "concurrent"

Page 9 line 20 "is" has been deleted

Page 10 line 6 "mid" has been substituted with "since"

Page 58 line 21 "an" has been deleted

Page 63 line 24 "adolescent" has been substituted with "adolescence"

Page 64 line 4 and 6 "represents" has been substituted with "represent"

Page 66 line 15 "facts" has been substituted with "fact"

Page 68 line 1 "included" has been substituted with "include"

Page 70 line 20 "reasonable" has been substituted with "reasonably"

Since submission to the doctoral committee reference 16 in the reference list has been published:

Lang AM, Carlsen KH, Haaland G, Devulapalli CS, Munthe-Kaas MC, Mowinckel P, Lødrup Carlsen KC. Severe asthma in childhood: assessed in 10 year olds in a birth cohort study. *Allergy* 2008; 63: 1054-1060.