From THE INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

LONG-TERM EXPOSURE TO AIR POLLUTION FROM ROAD TRAFFIC AND LUNG FUNCTION IN CHILDREN AND ADOLESCENTS

Erica S. Schultz

Stockholm 2016

Front cover illustration by Alida Bothma.
All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Eprint AB 2016

ISBN 978-91-7676-444-2

Long-term exposure to air pollution from road traffic and lung function in children and adolescents

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Erica S. Schultz

Principal Supervisor:
Erik Melén
Karolinska Institutet
Institute of Environmental Medicine

Co-supervisors:
Jenny Hallberg
Karolinska Institutet
Institute of Environmental Medicine

Göran Pershagen
Karolinska Institutet
Institute of Environmental Medicine

Matteo Bottai
Karolinska Institutet
Institute of Environmental Medicine

Opponent:
Urs Frey
Basel University
Children's Hospital

Examination Board:
Andrei Malinovschi
Uppsala University
Department of Medical Sciences

Anders Blomberg
Umeå University
Department of Public Health and Clinical Medicine

Sofia Carlsson
Karolinska Institutet
Institute of Environmental Medicine

“Breathing and thinking are the two most important processes, one for sustaining life and the other for giving it a purpose.”

Tasneem Hameed

ABSTRACT

Lung function in early life is an important predictor of peak lung function and later decline in adults. However, lung development may also be influenced by factors later in childhood and adolescence. Identification of susceptible periods and modifiable factors affecting lung development already during infancy and childhood could help promote respiratory health later in life. The overall aim of this thesis was to investigate potential environmental determinants of lung function in children and adolescents, with particular focus on exposure to traffic-related air pollution.

The papers in this thesis were based on the BAMSE study, a longitudinal population-based birth cohort of children followed until adolescence. The parents of all children born in predefined urban and suburban areas of Stockholm County between February 1994 and November 1996 were invited to enroll their children. Symptoms of allergy-related disease, life-style factors and major exposures were assessed from questionnaires filled out at the ages of 1, 2, 4, 8, 12 and 16 years. Lung function was measured using spirometry at 8 and 16 years of age, with the addition of impulse oscillometry at age 16 years. The assessment of individual long-term exposure to traffic-related air pollution was based on dispersion modeling, using emission inventories and data on road traffic, meteorological conditions and topography, at relevant geographical locations. Time-weighted annual averages of nitrogen oxides (NO_x) and particulate matter with an aerodynamic diameter of less than 10 μm (PM_{10}) were assessed over the life course.

The influence of long-term exposure to traffic-related air pollution on lung function at 8 and 16 years of age was assessed, including life course analyses. Pollution exposure during the first year of life, but none of the other time periods examined, was significantly associated with reduced lung function at 8 and 16 years of age. No associations were observed for the change in lung function between the two time points for any of the time windows explored. This suggests that exposure in early life influences early lung growth and that lung function thereafter tracks with age. In addition, we observed that air pollution during the first year of life was associated with small but significant increase of small airways resistance. Associations appeared stronger in subjects with asthma at 16 years.

Exposure to air pollution and other environmental factors was investigated in relation to lung function growth between childhood and adolescence, using quantile regression on the 10^{th}, 50^{th} and 90^{th} percentiles, corresponding to low, median, and high lung function growth. Out of 20 examined variables, birth weight, asthma heredity and environmental tobacco smoke exposure in infancy were the only independent predictors of lung function growth.

In summary, exposure to traffic-related air pollution during infancy was associated with decreased lung function, including in the small airways, in childhood and adolescence. Air pollution and other environmental factors assessed after infancy had little impact on lung function growth, supporting the notion of a susceptible period early in life with tracking of lung function thereafter.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following four studies, which will be referred to by their Roman numbers.

I. **Schultz ES**, Gruzieva O, Bellander T, Kull I, Svartengren M, Bottai M, Hallberg J, Melén E, Pershagen G. Traffic-related air pollution and lung function in children at 8 years of age - A Birth Cohort Study. Am J Respir Crit Care Med. 2012; 186(12):1286-91.

II. **Schultz ES**, Hallberg J, Bellander T, Bergstrom A, Bottai M, Chiesa F, Gustafsson PM, Gruzieva O, Thunqvist P, Pershagen G, Melén E. Early-Life Exposure to Traffic-related Air Pollution and Lung Function in Adolescence. Am J Respir Crit Care Med. 2016;193(2):171-7.

III. **Schultz ES**, Hallberg J, Bellander T, Gustafsson PM, Bottai M, Bellander T, Bergstrom A, Kull I, Gruzieva O, Thunqvist P, Pershagen G, Melén E. Early life exposure to traffic-related air pollution and lung function in adolescence assessed with impulse oscillometry. J Allergy Clin Immunol. 2016; 138(3):930-32.e5.

IV. **Schultz ES**, Hallberg J, Thacher J, Pershagen G, Bellander T, Bergstrom A, Kull I, Guerra S, Thunqvist P, Gruzieva O, Gustafsson PM, Bottai M, Melén E. Predictors of lung function change between childhood and adolescence. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

AX	Area of reactance
BAMSE	Children (Barn), Allergy, Milieu, Stockholm, Epidemiology
BC	Black carbon
CI	Confidence interval
CHS	The Children's Health Study
CO	Carbon monoxide
DM	Dispersion model
ETS	Environmental tobacco smoke
ESCAPE	European Study of Cohorts for Air Pollution Effects
FEF	Forced expiratory flow
FEV_1	Forced expiratory volume during 1 second
$FEV_{0.5}$	Forced expiratory volume during 0.5 second
FVC	Forced vital capacity
GALA	Genes-Environments and Admixture in Latino Americans
GINI	German Infant Nutritional Intervention
I	Inertance
IgE	Immunoglobulin E
INMA	INfancia y Medio Ambiente
IQR	Interquartile range
LISA	Lifestyle-related Factors on the Development of Immune System and Allergic disease
LLN	Lower limit of normal
LF	Lung function
LUR	Land use regression
MAAS	Manchester Asthma and Allergy Study
MEF	Maximal expiratory flow
MEFV	Maximal expiratory flow volume
MMEF	Maximal mid-expiratory flow
NO_2	Nitrogen dioxide
NO_x	Nitrogen oxides
OR	Odds ratio
O_3	Ozone
P	Percentile
PEF	Peak expiratory flow
PIAMA	The Prevention and Incidence of Asthma and Mite Allergy
PM_{10}	Particles with an aerodynamic diameter of less than 10 µm
$PM_{2.5}$	Particles with an aerodynamic diameter of less than 2.5 µm
PM_{coarse}	Particles with an aerodynamic diameter between 2.5 and 10 µm
R	Resistance
RSV	Respiratory syncytial virus
SAGE	Study of African Americans, Asthma, Genes, and Environment
SES	Socioeconomic status
SHS	Secondhand smoke (the same as ETS in my studies)
TLC	Total lung capacity
TRAP	Traffic-related air pollution
TSP	Total suspended particles
WHO	World Health Organization
X	Reactance
Z	Impendance

1 INTRODUCTION

Subjects with symptoms suggestive of respiratory diseases, such as asthma or chronic obstructive lung disease (COPD), generally demonstrate various types and degrees of lung function impairments. Reduced lung function per se is associated with increased morbidity and mortality, even among healthy non-smoking individuals with only modestly reduced lung function[1,2]. Lung function in early life has been shown to be an important predictor for peak lung function in adults and later decline[3,4].

From birth and up to late adolescence in women and to the mid-twenties in men, the lung and airways grow and mature with body growth[5], implying that lung development is influenced by factors not just in early life, but also later in childhood and adolescence. Identification of such modifiable factors already during infancy and childhood could help in promoting respiratory health later in life. There is a clear need for a better understanding of the periods when the lung and airways grow and are most vulnerable.

The overriding goal of this thesis was to identify potential environmental predictors of lung function in children and adolescents, with a focus on traffic-related air pollution.

2 BACKGROUND

2.1 LUNG DEVELOPMENT AND GROWTH

A schematic illustration of the airway tree, which has on average 23 generations of branches, is provided in Figure 1. The conducting airways, from generation 0 (trachea) to approximately generation 15 are lined with bronchial epithelial cells. Here, no gas exchange occurs, which contrasts to the more peripheral acinar airways (the acinar lung zone), where alveoli, i.e., gas-exchanging surfaces, appear. As the branching of the intra-acinar airway tree continues, alveoli increase in number, and in the last generation the branching ends with alveolar sacs[6].

The small airways are by convention defined as those with an airway diameter of 2 mm or less in the adult. This corresponds to approximately airway generation 8 and more distally, and comprises the more peripheral conducting airways (up to generation 15), as well as the bronchioles and alveolar regions where gas exchange occurs.

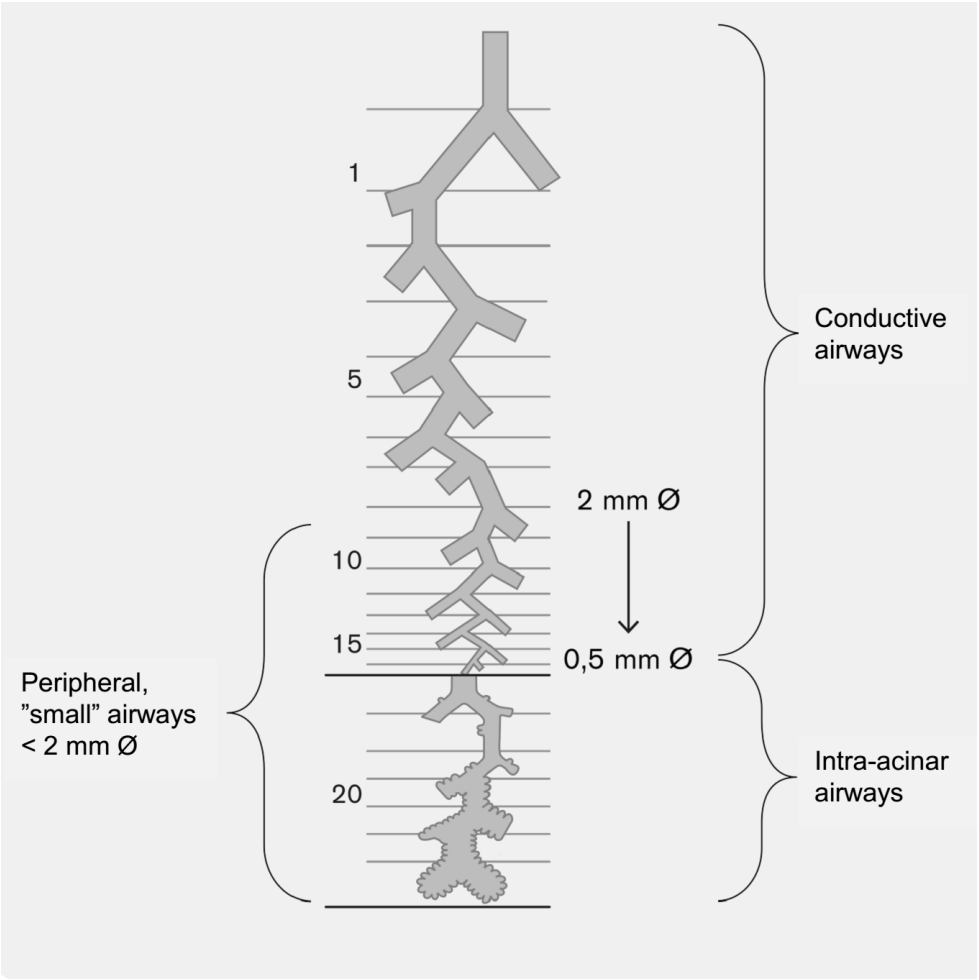

Figure 1. Schematic drawing of the airway generations (modified by courtesy of Per Gustafsson).

Development of the lung starts with the formation of trachea, the major bronchi and buds when the embryo is approximately 4-6 weeks of gestational age. After approximately week

29 the alveoli begin to form, a process that was thought to be completed at about 2 to 4 years of postnatal age[7]. However, the duration of the alveolar development period has been under debate lately, as relatively recent studies suggest that alveolarisation may continue into school age[8], and even up to adulthood[9]. These observations may indicate an increased period of vulnerability and, at the same time, a window of opportunity to recover from previous insults.

The lung and airways continue to expand as the body grows, until late adolescence for women and the mid-twenties for men[7]. However, the lungs and airways do not grow proportionally in all individuals. This concept, which is physiologically normal, explains some of the between-individual variation in flow relative to lung volume and is known as dysanaptic growth[10]. Compared with females, males often start life with small-sized airways relative to their lung volume, and with increasing age, the thorax grows and the airways expand accordingly[11]. Consequently, there may be gender differences in the pathogenesis of airway disease.

Longitudinal studies have revealed that, taking height and gender into consideration, the deviation of an individual's value of lung function from the population average, remains rather constant independent of age. This is known as tracking of lung function, and a person is said to follow his or her own trajectory if crude lung function values are increasing as expected, relative to age, height, and gender[3,7]. In Figure 2, lung function tracking is illustrated by the solid blue (optimal lung function trajectory), green (mid lung function trajectory), and red lines (poor lung function trajectory), while the dashed lines indicate changes in trajectories, due to various causes, some of which will be discussed in the next chapter.

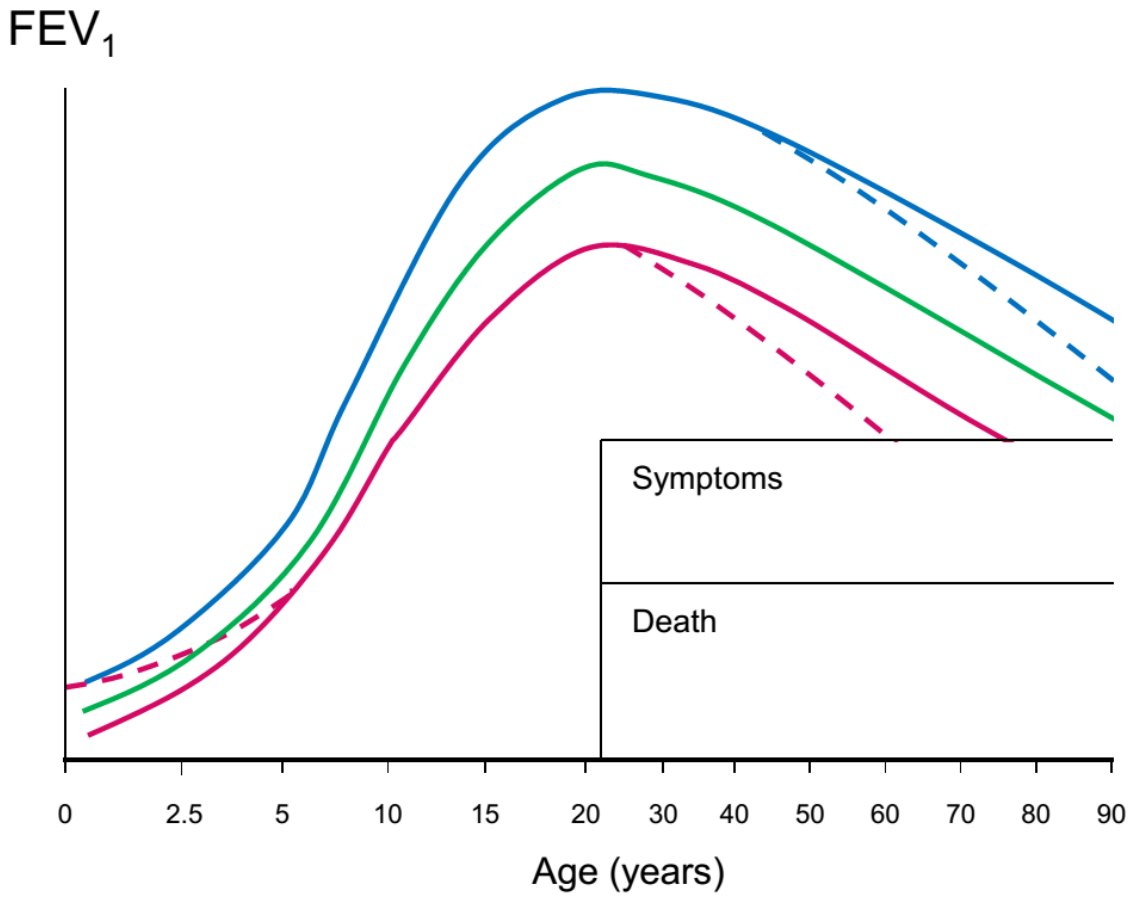

Figure 2. Example of lung function trajectories over the life course (solid lines), and changes after insults to predicted growth and decline (dashed lines).

In this thesis I will use the concept lung function tracking when discussing that someone follows his/her predicted path of lung development (corresponding to the solid lines in Figure 2). In Study IV, the lung function values of a subject are defined to track if they remain within the same percentile cut-offs at both 8 and 16 years: < 25th percentile, 25th to 75th percentile, or > 75th percentile, which correspond to the low, mid and high lung function trajectories. On the other hand, the potential change of trajectory (corresponding to the dashed lines) are investigated and assessed as the 10th and 90th percentile differences between 8 and 16 years, i.e., low and high increase of the delta lung function values.

2.1.1 Factors affecting lung development

Factors known to influence prenatal lung development negatively are congenital abnormalities, such as abnormalities in the chest wall, kidneys and diaphragmatic hernia, in addition to maternal factors, such as oligohydramnion, nutritional factors, maternal exposures to alcohol, nicotine, air pollution etc. Another well-known risk factor for lung function impairment is preterm birth. Subjects born preterm have in general impaired lung function, with varying degrees of severity, depending on type and duration of insult, as well as the developmental stage at which it occurs[7]. Low birth weight and lower respiratory tract illness during infancy and early childhood have also been identified as important determinants for long-term respiratory health[12,13]. Further, postnatal exposure to parental tobacco smoke has shown negative impact on lung function measurements in children[14] and adults[15], although the effect is difficult to separate from exposures during pregnancy. In addition, there are some factors that are suggested to positively affect the development of lung function, such as exposure to farming environment[16], and breastfeeding during infancy[17].

Observations from studies on the prenatal and postnatal period often support the concept of lung function tracking[3,18], with potential consequences for long-term respiratory health. However, many studies only report measurements of lung function from a single moment in time, and therefore a potential continued deterioration of lung function is not captured.

Even though a large proportion of the expected maximal lung function achieved is determined from pre- and postnatal life, the predicted trajectory of lung function may still be affected by factors during later life. Longitudinal studies on lung function have primarily focused on specific respiratory phenotypes or disease severity[19-23]. However, the potential impact from environmental exposures after pregnancy and infancy on altered growth in lung function has scarcely been investigated[24,25]. Identification of such modifiable factors in childhood could help in promoting respiratory health later in life.

2.2 LUNG FUNCTION MEASUREMENTS

Objective assessment of lung function is fundamental in the diagnosis, management and understanding of respiratory diseases. Further, it is widely used in interventional studies, as well as in epidemiological evaluations of risk factors for respiratory health outcomes. There are several components that can affect the measured lung function value, for example stiffness, size, and shape of the thorax, as well as differences in airway wall and lung parenchyma. Therefore, there are several methods available to measure lung function, which often complement each other.

In the articles included in this thesis, lung function has been assessed by dynamic spirometry (Studies I, II, and IV) and by impulse oscillometry (IOS) (Study III). A description of the theory underlying these methods, in addition to brief explanations of the determinants of the respective indices retrieved, is presented in the following sections.

2.2.1 Dynamic spirometry

Dynamic spirometry is the most used lung function method, both in clinical and research settings. There are extensive guidelines for standardized procedures as well as quality criteria, and when correctly performed spirometry gives detailed and reliable information regarding the ventilatory capacity of the lungs and airways[26].

The maximal volumes and flows are recorded from a maneuver when the subject, after a full inspiration, exhales with maximally forced effort (Figure 3). The total volume of air exhaled is called forced vital capacity (FVC), and is a representation of the size of the lungs, although it does not include the volume of air left in the lungs after airway closure. Therefore, a low value of FVC indicates small lungs or air trapping. The amount of air exhaled during the first second (FEV_1), or during half a second ($FEV_{0.5}$) in small children, gives an indication of flow limitations. A low flow indicates higher resistance or smaller caliber of the large and medium-sized airways.

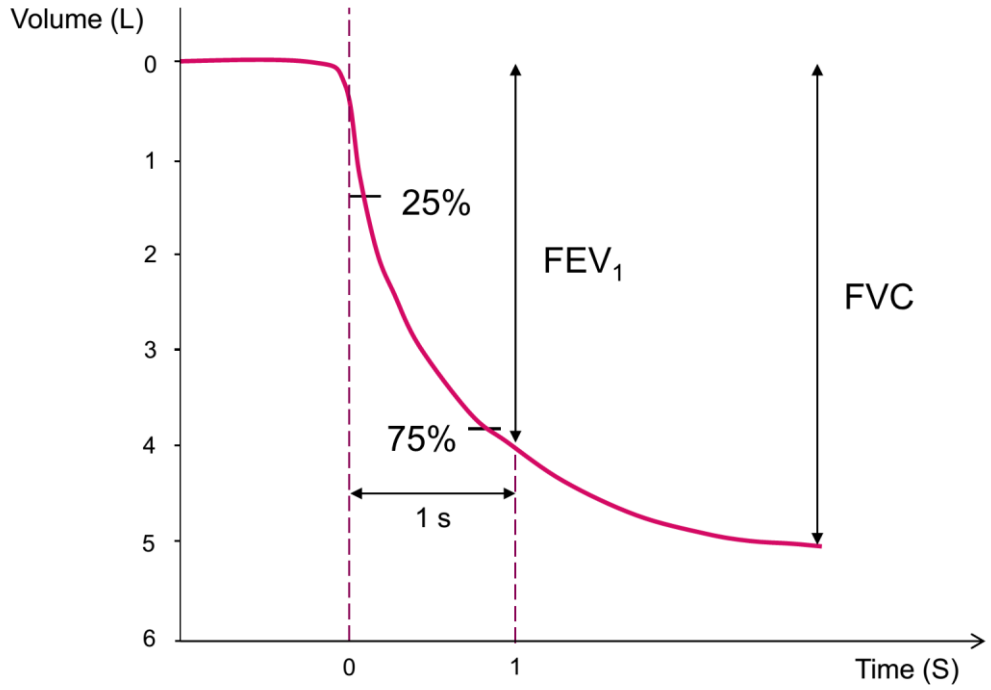

Figure 3. Forced volume/time curve, which illustrates the exhaled volume as a function of time. FEV_1 is the volume expired during the first second, and FVC is the forced expiratory vital capacity (modified by courtesy of Per Gustafsson).

Flows can similarly be measured at other time points during exhalation, and are then usually labeled based on the time at which the flow was measured, or during the interval during with the volume of air was measured. For example maximal expiratory flow at 50 % of FVC is labeled MEF_{50}, and forced expiratory flow between 25-75 % of FVC is called FEF_{25-75} or MMEF (maximal mid-expiratory flow).

FEV_1 has been shown to be highly reproducible, with relatively low variability compared with flow measured at other time points. For this reason, FEV_1 is the most commonly used measure of lung function for clinical decision-making as well as for research[27].

The ratio of FEV_1 over FVC (FEV_1/FVC) gives an indication of the relative size of the airways compared with the lung volumes, and a low ratio is indicative of airway obstruction, while a high ratio may be indicative of a restrictive lung pattern if accompanied by a low FVC.

2.2.2 Impulse oscillometry (IOS)

The forced oscillations technique (FOT), for which IOS is one commercially available system, is based on physiological concepts described already in 1956[28]. The principle of FOT is that mechanically induced pressure impulses are superimposed on tidal breathing through the airway system, producing recordable waveforms. Sophisticated signal processing is then used to extract components of respiratory mechanics from the recorded waveforms, providing information from both the large and small airways.

For many lung diseases, like COPD and asthma, the small airways are a major site of pathology, and the severity of disease is often rather significant before changes in spirometry measurements appear[29]. As the small airways are relatively difficult to study, they are sometimes referred to as "the quiet zone" of the lung[30]. There are now, however, commercially available and easily accessible measurement systems of lung function that can discriminate between large and small airway effects. One such system is impulse oscillometry (IOS)[31]. IOS is easy to use, as it is effort-independent and performed during tidal breathing. There are published guidelines from the European Respiratory Society on recommended procedures for measurement and clinical applications[32].

Using the IOS technique, pressure impulses are applied at the mouth with a fixed frequency of 5 times per second and from which frequencies in expiratory and inspiratory impedance (Z) between 5 and 35 Hz are derived[33]. Higher frequencies (>20 Hz) reach the large or intermediate airways, while lower frequencies (< 15 Hz) are transmitted further into the small airways, as far as the peripheral conductive airways. For this reason, low frequencies represent small and large airways (total airway) and high frequencies represent only the large airways. It is therefore feasible to discriminate between effects in the large and parts of the small airways by subtracting the large airway effect from the total[31].

For simplicity and clarity, I have in this thesis used the simplified term "small airways" for the peripheral conductive airways, as measured using IOS.

Impedance (Z) is a complex function of all forces that hinder air flow into and out of the lung and is further separated into the two components resistance (R) and reactance (X). Resistance is defined as the ratio of the drop in pressure (in Pascal, Pa) over an airway segment and the flow ($L \cdot s^{-1}$) through that segment, and can be viewed as a measure of "friction." Reactance is described simplistically as the amount of recoil generated against the pressure wave and reflects two opposing forces: capacitance (C) and inertance (I). Capacitance represents the elasticity of the airways and chest wall, while inertance reflects the forces required to move the column of air in the airways.

A schematic illustration of the components of Z is seen in Figure 4. At low frequencies, the C forces dominate over the I forces, as the ability of the lungs to stretch is primarily in the small airways. At intermediate frequencies, called resonant frequency (f_{res}), the total reactance is null, because C and I are balanced against each other. When X is plotted against frequency, the area under the curve between 5 Hz and f_{res} is called the area of reactance (AX). This single value of AX therefore includes all frequencies where the C forces (i.e., the elastic properties) dominate over the I forces[32]. In other words, AX reflects the elastance (reduced compliance) of the small airways.

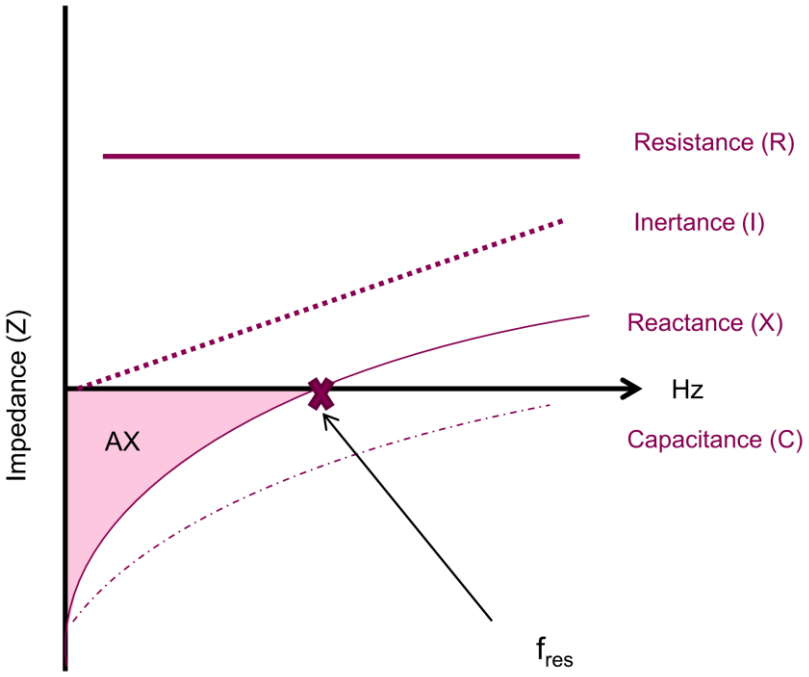

Figure 4. Schematic illustration of the components of impedance (Z) and its relation to frequency of applied oscillations, in a healthy adult subject without frequency dependence of resistance (modified by courtesy of Per Gustafsson).

In healthy adults, resistance is similar across all frequencies, while in young children and in adults and adolescents with small airway obstruction, R is higher at low frequencies than at high frequencies; hence the name frequency dependence of resistance. In practice, the frequency dependence of resistance is expressed as resistance at 5 Hz less the resistance at 20 Hz (R5-R20), and is an index of obstruction in the small airways[31]. As a consequence, the AX and R5-R20 indices are highly correlated[34].

2.3 OUTDOOR AIR POLLUTION

Air pollution (outdoor and indoor) is a global problem and one of the most important environmental risk factors for human health. It carries responsibility for approximately 10 % of annual deaths globally. As much as 85 % of the world's population lives in cities with outdoor air pollution levels exceeding the WHO Air Quality Guidelines for PM_{10}[35]. The trend towards global urbanization implies that more and more people will become highly exposed if further control of emissions is not applied.

Ambient (outdoor) air pollution constitutes a complex mixture of compounds, which vary in concentration depending on sources, geography, topography, wind direction and speed, temperature, ultraviolet radiation, and relative humidity. The concentrations of pollutants may be correlated both in time and space, because they come from the same sources and are distributed similarly. Therefore, in studies of health effects, it may be difficult to discern the importance of one pollutant from the other.

Ambient air pollution consists of organic and inorganic liquid and solid particles suspended in air (particulate matter – PM), as well as different type of gases such as ozone (O_3), nitrogen oxides (NO_x) and carbon monoxide (CO), as well as vapors, as volatile organic carbons (VOCs)[36].

Classification of particles is often according to size, and this provides information about possible health effects. For example, particulate matter with an aerodynamic diameter of less than 10 µm (PM_{10}) is inhalable and reaches the lower airways, and is subdivided into PM_{coarse} (PM with a diameter between 2.5 and 10 µm), which reach the proximal airways, and $PM_{2.5}$ (PM < 2.5 µm), which reach the more peripheral regions of the lungs where gas exchange occurs. Ultrafine particles consist of those with a diameter of less than 0.1 µm, which contribute little to the total mass, but are higher in numbers, have a large surface area, and are suggested to reach past the alveolar wall into the blood circulation[37].

The most important process contributing to levels of ambient air pollution in urban settings relates to the combustion of fuels used in road, aircraft and sea traffic, as well as in residential heating, industry, and power plants. Due to the proximity between people and sources, road traffic is particularly important for population exposure to ambient air pollution. Therefore, in the studies included in this thesis, we focus on road-related air pollution (in the thesis termed "traffic-related"). As indicators for traffic-related air pollution we use traffic-NO_x (sum of NO and NO_2), which is primarily from road-traffic exhaust, and traffic-PM_{10} which is primarily related to road wear by studded tires, sanding and salting of roads in the winter, and wear of vehicle (breaks, clutch, and tires). The smaller fraction of traffic-PM_{10} ($PM_{2.5}$ and ultrafine particles contribute little to the total mass of PM_{10}) is more closely related to combustion of road-traffic fuels[38]. Figure 5 illustrates the relative contribution of road traffic compared with other sources of NO_x and PM_{10} in the Greater Stockholm area[39].

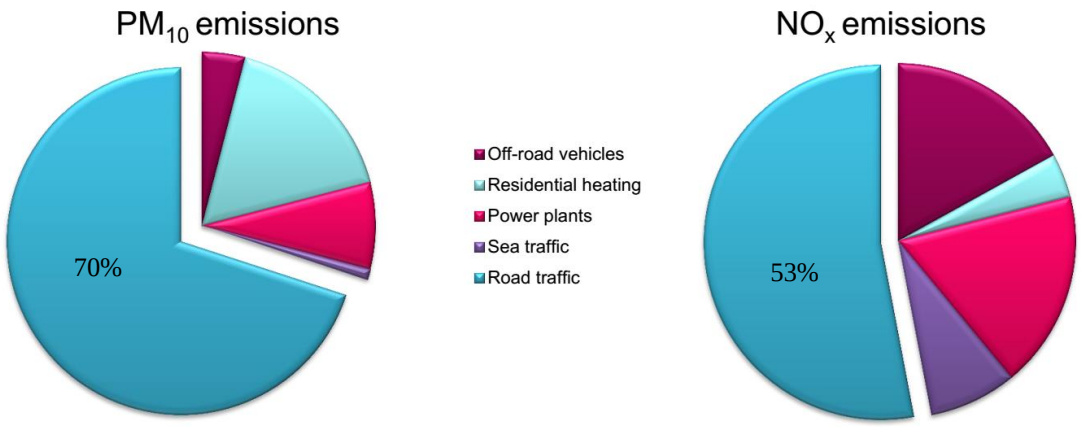

Figure 5. Relative contribution of emissions of PM_{10} and NO_x (in %) from various sources in the Greater Stockholm area during year 2003.

2.4 RESPIRATORY HEALTH EFFECTS OF AIR POLLUTION EXPOSURE IN CHILDREN AND ADOLESCENTS

2.4.1 Mechanisms

The exact mechanisms by which air pollution affects the lungs and airways are not known. It has been hypothesized that oxidative stress and airway inflammation are important processes[40]. It has for example been suggested that inhaled particles provoke the generation of reactive oxygen species. This, as well as direct damage by highly oxidative gases such as ozone and NO_2, induces oxidative stress and inflammatory responses[36].

Variations in genes have been associated with deficits in lung function growth[41], and suggested to be related to the intracellular protective system against the formation of hazardous reactive oxygen species, and oxidative stress, such as the GST enzyme family[42]. Epigenetics has been proposed as one of the links between exposure to air pollution and respiratory health effects, for example through methylation of genes involved in immune-mediated inflammatory response[43].

Studies investigating exhaled nitric oxide in humans support the notion that inflammatory processes may play a role for the observed respiratory health effects by exposure to air pollution[44]. Nitric oxide is an established biomarker of airway inflammation and several studies show a relation between exposure to air pollution and increased levels of the exhaled fraction of nitric oxide (FeNO). This has been observed for short-term exposures, long-term exposures, markers of traffic-related air pollution, and even in children with no history of airway damage[44].

A chronic airway response due to the inflammation processes has been suggested. For example, in studies of human histological lung tissue, correlations have been observed between exposure to high PM levels and small airway remodeling by greater amounts of fibrous tissue and muscle[45].

2.4.2 Long-term air pollution exposure and lung function

To review the published literature within the main focus area of this thesis, i.e., long-term air pollution and lung function in children and adolescents, I searched the PubMed databases for publications from 2006 and onwards, in addition to identifying references from a review by Gotschi and colleagues from 2008. In all, 31 articles with cross-sectional lung function data (Table A1) were included and 12 articles with longitudinal lung function data (Table A2). For details, please see appendix.

Evidence on the negative effects of exposure to air pollution on a child's respiratory health is accumulating. Studies have compared lung function levels in children living in different communities with varying levels of ambient air pollution measured at central monitoring stations or at schools[24,46-60]. Traffic measurements such as traffic density or proximity to high ways have also been used as exposure estimates[58,60-64], as well as modeled individual data using land use regression (LUR) models[65] or dispersion models (DM)[66,67]. Most, but not all studies[54,60,62,68] have observed negative impact from traffic-related air pollution on lung function. Heterogeneity in study designs, exposure assessment, confounder control, as well as lung function measures used across the studies, may have contributed to differences in results[69].

Lung development starts in utero, but a considerable maturation continues after birth, which makes the lungs potentially vulnerable to the effects from exposure to air pollution. Infants are relatively immobile and are often in a pram during outdoor transportation, at the level of motor exhaust emissions. Infants and children may also be more exposed to air pollution relative to their size, due to higher ventilation per minute. In addition, the immune system of infants and young children is not fully developed, which may contribute to an increased vulnerability to the effects of exposure to air pollution[70]. However, few studies have investigated exposure during the infancy period in relation to lung function in children[66,67] and none in adolescents. As a consequence, the long-term effects remain unknown, as does the relative impact of early life exposure vs. exposure later in childhood.

Moreover, there are knowledge gaps concerning potential periods of susceptibility and potential patterns of exposure over the life course. Reports of longitudinal lung function data suggest an attenuated lung function growth in children and/or adolescents exposed to high levels of ambient air pollution during time periods later than the first year of life[24,52,71]. On the other hand, a recovery of negative effects of previous exposure to air pollution has been observed in children moving to less polluted areas[46]. Altogether, these studies indicate that periods of exposure during later life also have importance. The majority of published cross-sectional and longitudinal studies have only investigated later life exposures (usually around school ages, see Table A1 and Table A2 in appendix), and therefore potential dynamic changes of exposure to air pollution during the life-course, in addition to influences on lung function growth, remain largely unknown.

Most investigations linking air pollution exposure to lung function have employed measurements of total airway resistance and large rather than small airway function measured using spirometry[27]. In experimental studies on mice, small aerosol particles of a size range typical of traffic-related air pollution are deposited in the small airways[72] and indices of small airway function correlate with health status and asthmatic symptoms in both children and adults[73,74]. However, it is not known whether exposure to traffic-related air pollution influences small airway function.

2.5 AIMS

The overall aim of this thesis was to investigate potential environmental determinants of lung function in children and adolescents, with particular focus on exposure to traffic-related air pollution.

Specific research questions:

- Does exposure to long-term traffic-related air pollution influence lung function during childhood and adolescence? (Studies I and II)

- Can we identify any particular time window of exposure to traffic-related air pollution during the life course that influences lung function up to adolescence? (Study II)

- Does exposure to air pollution influence small airway function? (Study III)

- Do exposures to air pollution and other environmental factors have an impact on lung function growth between childhood and adolescence? (Studies II and IV)

3 MATERIAL AND METHOD

3.1 AN OVERVIEW

Table 1: Overview of the studies on which the present thesis is based. For details, please see the original articles.

Subpopulation of the BAMSE-cohort	Data	Statistical analyses
Study I. n = 1,924 (47%) with TRAP exposure from all periods, spirometry from 8 years of age and no missing confounders.	*Exposures:* Traffic-related air pollution (TRAP, 0-1 year, 1-4 years, 4-8 years, continuous). *Outcomes:* Spirometric indices from 8 years – raw values and % predicted based on our own cohort[#] (continuous and dichotomized). *Confounders*: Age, gender, height, municipality at birth, and heredity for asthma and/or allergies. *Effect modifiers*: Sensitization, asthma, gender	Linear regression on the mean. Logistic regression.
Study II. n = 2,278 (56%) with TRAP exposure from first year of life, spirometry data from 16 years of age and no missing confounders.	*Exposures:* TRAP (0-1 year, 1-8 years, 8-16 years, continuous and dichotomized) *Outcomes*: Spirometric indices from 16 years (raw values and GLI 2012 z-scores[*]), and change in spirometry between 8 and 16 years. (continuously and dichotomized). *Confounders*: Age, gender, height, weight, and municipality at birth. *Effect modifiers*: Gender, sensitization, asthma, maternal smoking during pregnancy or in infancy, ETS at 16 years, gestational age, and own smoking by the subject.	Chi2 test. T-test, with finite population correction factor. Likelihood ratio test. AIC. Linear regression on the mean. Mixed effect models (random intercepts) with time interaction. Logistic regression. Life-course models (mixed effect models).
Study III. n = 2,415 (59%) with TRAP exposure from first year of life, IOS data from 16 years of age and no missing confounders.	*Exposures*: TRAP (0-1 year, 15-16 years of age, continuous) *Outcomes:* IOS at 16 years (continuous). Above *vs.* below the predicted 95th percentile based on own cohort[#]. *Confounders*: Age, gender, height, weight, municipality at birth. *Effect modifiers:* Gender, sensitization, and asthma.	Likelihood ratio test (confounder analyses). T-test, with finite population correction factor. Linear regression on the median. Logistic regression. Quantile regression on the 95th percentile (to generate internal reference values).
Study IV. n = 1,425 (35%) with FEV_1data from both 8 and 16 years of age	*Exposures*: Individual characteristics; birth weight, preterm birth, season of birth, and asthma heredity. Environmental factors at 0-1 year and/or at 8 years; breastfeeding, lower respiratory tract infections, maternal smoking during pregnancy, older sibling, ETS, level of parental education, NO_x, socioeconomic status, mold and dampness, antioxidant intake. *Outcomes:* Change of FEV_1 between 8 and 16 years. Low *vs.* mid/high FEV_1 at 8 and 16 years (Low < 25th percentile, based on own cohort)[#]. *Confounders*: Gender, increase in height, weight and age between 8 and 16 years, FEV_1 at 8 years.	Chi2-test. T-test. Quantile regression on the 10th, 50th and 90th percentiles. Quantile regression on all percentiles (to generate internal reference values). Logistic regression.

List of abbreviations: TRAP= Traffic-Related Air Pollution, ETS = Environmental tobacco smoke exposure, AIC= Akaike's information criterion, GLI= Global lung function initiative, IOS = Impulse oscillometry, NO_x = Nitrogen oxides. [#] internal reference values. * based on GLI 2012[75].

3.2 THE BAMSE PROJECT

The studies were based on the BAMSE cohort, a longitudinal population-based birth cohort of children followed until adolescence. The Swedish acronym BAMSE stands for "Barn, Allergi, Miljö, Stockholm, Epidemiology," the English translation being "Children, Allergy, Environment, Stockholm, Epidemiology." BAMSE is also a popular bear in a children's cartoon. The original aim was to characterize risk factors for the development of asthma and other allergic diseases. To this end, the recruitment areas included both urban and suburban environments (Figure 6), with a wide range of exposures such as housing, socio-economic factors, and traffic-related air pollution. The parents of all children born in the predefined areas of Stockholm County between February 1994 and November 1996 were invited to enroll their children.

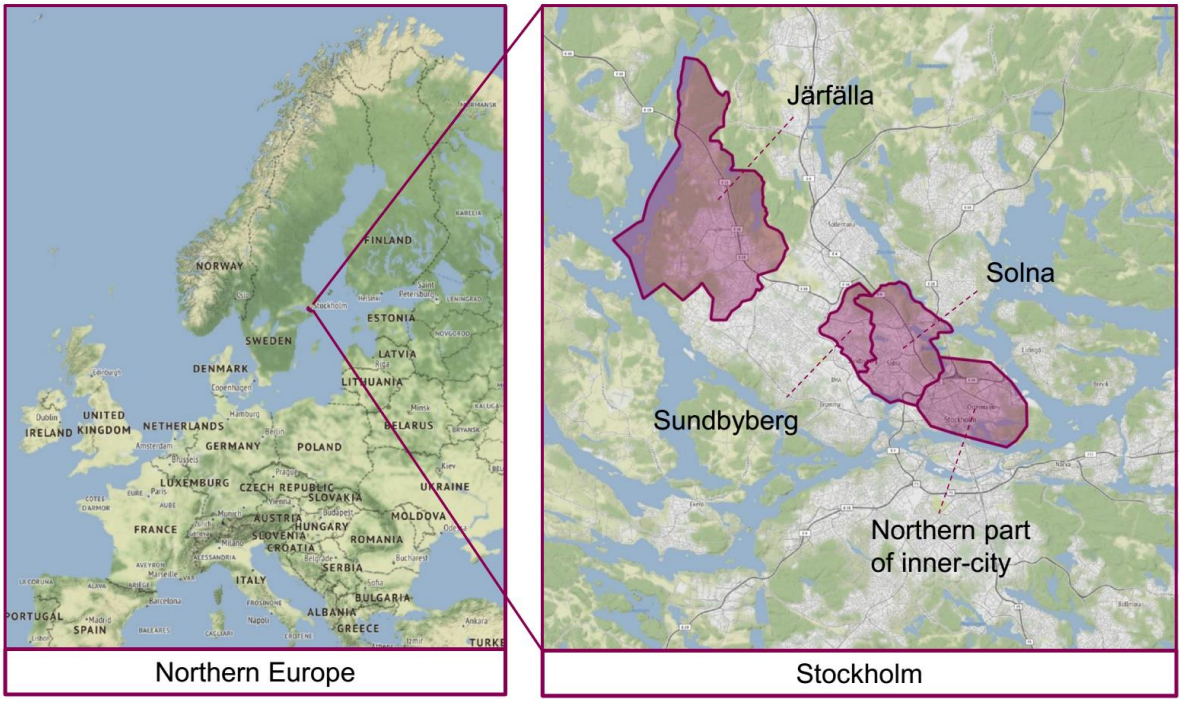

Figure 6. Areas in Stockholm, County, Sweden, from which the BAMSE cohort was recruited. Maps adapted from "© OpenStreetMap contributors"

Figure 7 depicts a flowchart of the recruitment and characterization processes. Of the 7,221children born during the study period, 4,089 were finally included. The baseline questionnaire included detailed questions on current residence, environmental factors and parental allergies. In 1996, a short questionnaire on major exposures and parental allergies was sent both to participants purposely excluded and to non-responders, with response rates of 83 % and 58 %, respectively[76]. The willingness to participate was not influenced by parental asthma or atopy, although parental smoking was more prevalent among non-participants than participants (18 % vs. 9 % for maternal smoking).

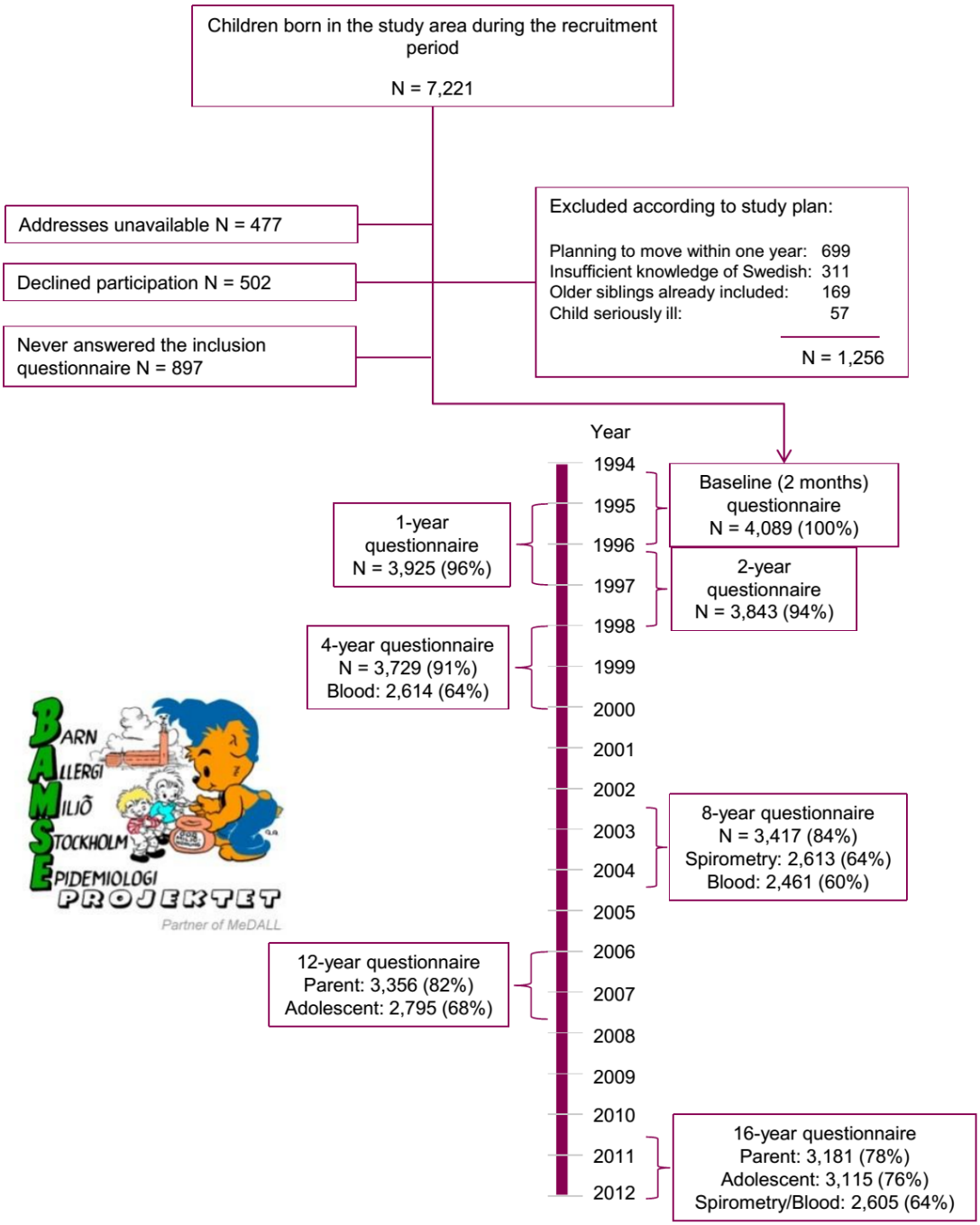

Figure 7. Flowchart illustrating characterization of the BAMSE cohort. The brackets indicate periods of data collection.

3.2.1 Follow-ups

When the children included reached 1, 2, 4, 8, 12 and 16 years of age, their parents received questionnaires focusing mainly on symptoms of allergy, lifestyle factors and major exposures. In addition, the children filled out their own questionnaires at 12 and 16 years of age. Children for whom questionnaires were filled out at 4, 8 and 16 years of age were invited to undergo a clinical examination, including anthropometric measurements, tests of lung function and blood sampling (see Figure 7).

3.3 AIR POLLUTION ASSESSMENT

3.3.1 Long-term traffic-related air pollution assessment

The assessment of individual exposures to long-term air pollution in this thesis was based on calculations using emission inventories and a physical model combining data on road traffic, meteorological conditions and topography, at relevant geographical locations. The calculations are based on assumptions of the atmospheric dispersion of the pollutants, hence the name dispersion model.

The assessment of air pollution exposure in all studies was done by dispersion modeling. Information on residential, daycare, and school addresses were obtained from the postal questionnaires, supplemented with Swedish tax authority records, and transformed into geographic coordinates using a property register developed and managed by the Swedish mapping, cadastral, and land registration authority.

Calculations of emissions were based on the emission source database of the Stockholm and Uppsala Air Quality Management Association (Now East Sweden Air Quality Management association; OSLVF). This database was first constructed in 1993 and covers 1990-2015. It includes geo-referenced information about various sources of air pollution[38]. For the studies in the present thesis, data regarding local traffic in road links were included, such as road type, traffic counts, the share of heavy traffic and speed limits.

Temporally resolved emissions of pollutants were calculated at these road links using the EVA model of the Swedish National Road Administration[77], which describes emissions from tail pipe (NO_x) and road wear (PM_{10}). For the NO_x calculations, we used emission data based on the inventories for the years 1990, 1995, 2000, 2002, 2004, 2006, 2010 and 2015. For the PM_{10} calculations, the inventory from year 2004 was used and applied to all years as road wear was assumed to be stable over the study period[78]. Linear interpolations were made to cover all years during the study period.

The annual mean concentrations of NO_x and PM_{10} over the whole study area were calculated using a Gaussian air quality dispersion model and a wind model, both of which are part of the Airviro Air Quality Management System (SMHI, Norrköping, Sweden; http://airviro.smhi.se). The emissions estimated at the road links, were assumed to spread (disperse) according to local meteorological conditions and topography[66,79]. Air pollution concentrations were estimated 2 m above ground or building level, resulting in an underestimation of ground level concentrations in street canyons with heavy traffic[80]. Therefore, street canyon contributions were calculated for addresses within 30 m of the most polluted street segments in the Stockholm inner city with multistory houses on both sides, using the Airviro street canyon model (http://airviro.smhi.se). PM_{10} levels were additionally adjusted for average road moisture during the winter season (included in assessment update for Studies II-IV).

The calculations were performed in a 25-m grid for the densely populated areas of the inner city, and in a 100-m or 500-m grid for other urban and rural areas, respectively.

Measurements at air quality monitoring stations were used to rescale the air pollution concentration fields estimated from the dispersion modeling. The geocoded addresses were subsequently assigned an annual mean pollution concentration.

Age- and municipality-specific information for the hours that children were at day care and school facilities was used to estimate the annual time-weighted average exposure to NO_x and PM_{10}.

Validation studies

Validation of our 1-year outdoor residential estimates of levels of traffic-NO_x (1995 to 1999) were done by recalculation to annual levels of traffc-NO_2 and comparison of these values with 1-month outdoor measurements of NO_2 (from all sources) for 487 of children in the cohort, showing a correlation of 0.74[66]. Estimated pollutant levels during later years (2008-2011) were validated within the European Study of Cohorts for Air Pollution Effects (ESCAPE). In Stockholm, correlations between estimates from dispersion models and results from monitoring stations were 0.755 for NO_2 and 0.580 for PM_{10}[81].

Measured personal exposure and estimated outdoor levels correlated reasonably well in a study of 250 adults in Stockholm. As an example, 20 % of the 7-day observed inter-individual variation (R^2) in NO_2 was explained by modeled annual levels at the home address and 16 % at the working address[82].

3.3.2 Estimated levels and air quality standards

The estimated time-weighted mean levels of traffic-PM_{10} (in µg/m^3) for the whole cohort were 5.8, 4.7 and 4.2 for the first year of life, ages 1 to 8, and ages 8 to 16, respectively (Table 2). The corresponding levels for traffic-NO_x (in µg/m^3) were 20.9, 13.0, and 7.0.

Table 2: Time weighted mean levels (in µg/m^3) of traffic-NO_x and traffic-PM_{10} for the whole cohort.

	First year of life[#]		1 to 8 years of age*		8 to 16 years of age†	
Air pollutant	Mean	5th to 95th percentile	Mean	5th to 95th percentile	Mean	5th to 95th percentile
PM_{10}	5.8	9.3	4.7	8.1	4.2	8.4
NO_x	20.9	43	13.0	28.5	7.0	17.6

[#]Data includes 4,014 subjects who had complete exposure information for the first year of life.
* Data includes 3,006 subjects who had complete exposure information for ages 1 to 8.
†2,823 subjects who had complete exposure information for ages 8 to 16.

The correlations between estimated mean levels of traffic-PM_{10} and traffic-NO_x for different time periods varied from 0.58 to 0.98 (Table 3). PM_{10} and NO_x show high correlations within time periods, with R_s coefficients above 0.97. Correlations between time periods also showed high coefficients. For exemple, R_s between PM_{10} in the first year of life and ages 1 to 8 years,

and ages 8 to 16 years, were 0.81 and 0.59, respectively. Similar correlations were seen for NO_x between the time periods.

Table 3: Spearman rank correlations (R_s) between levels of NO_x and PM_{10} during different time periods in all subjects.

Air pollutant (time period)	NO_x (0-1)	PM_{10} (0-1)	NO_x (1-8)	PM_{10} (1-8)	NO_x (8-16)	PM_{10} (8-16)
NO_x(0-1)	1.00					
PM_{10} (0-1)	0.97	1.00				
NO_x(1-8)	0.84	0.82	1.00			
PM_{10}(1-8)	0.79	0.81	0.98	1.00		
NO_x(8-16)	0.59	0.59	0.79	0.81	1.00	
PM_{10}(8-16)	0.58	0.59	0.78	0.82	0.98	1.00

According to the air quality standard in Sweden, which corresponds to the current EU regulations, the annual average of ambient PM_{10} should not exceed 40 µg/m^3, which is the same as for NO_2 (There are no standards for NO_x)[83]. However, the WHO air quality guidelines (AQG)[84] are stricter for PM_{10}, with a maximal annual average concentration of 20 µg/m^3. The corresponding Sweden's Environmental Objective for "clean air" is even stricter: 15 µg/m^3 [85].

To facilitate comparison between our estimated traffic-related air pollution concentrations and the current air quality standards in Sweden, a recalculation from estimated traffic-related air pollution levels to estimated total outdoor annual levels was made by adding urban PM_{10} background levels (of 10 µg/m^3)[86] to our PM_{10} estimates of traffic-related air pollution during the first year of life. The estimated total annual average PM_{10} levels in the BAMSE cohort ranged between 10.2 and 32.9 µg/m^3, thus not exceeding the current Swedish standards. When doing the same for NO_x, although recalculated to NO_2 using the formula $NO_2=NO_x$ [(0.66 +34/(NOx +100)); 66] and adding the background levels (3 µg/m^3), 2.8 % of the children were found to live in streets that exceed the Swedish standards.

3.3.3 Short-term assessment used in Studies I and III

For the purpose of sensitivity analyses, we included short-term exposures to PM_{10} (Study I), NO_x (Study III) and ozone (Study I and Study III). Hourly mean values for PM_{10}/NO_x were measured on two streets in the Stockholm city center, on the rooftop of one building and at 3 meters above street level. Measurements were also performed at a rural station located 70 km southwest of Stockholm (Aspvreten). Approximately 1 to 3 % of the observations were missing each year. Missing values for PM_{10}/NO_x at one of the city center measuring stations were imputed using predictions from a linear regression model based on PM_{10}/NO_x for the same point in time from the other two stations. If data from the rural station was lacking it was excluded from the imputation model. After imputation, less than 0.1 % of the values were missing.

Ozone was measured at a rural station situated 70 km northeast of Stockholm (Norr Malma). To predict missing values at this station observations from another rural station located 20 km southwest of Stockholm (Alby) were used. Before imputation the yearly number of missing values was up to 1.6 %. After imputation, no ozone observation was missing.

3.4 LUNG FUNCTION ASSESSMENT

3.4.1 Spirometry used in Studies I,II and IV

Spirometry was performed at 8 years of age using the 2200 Pulmonary Function Laboratory (Sensormedics, Anaheim, CA) and at age 16 using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA). The same spirometry test protocol was used at both time-points, including calibration and quality criteria. Briefly, several curves of maximal expiratory flow volume (MEFV) were recorded and further analyzed if the subject's effort was coded as maximal by the test leader, the MEFV curve passed a visual quality inspection, and the two highest $FEV_{0.5}$ (only at 8 years of age),FEV_1, and FVC were reproducible according to the ATS/ERS criteria[26].

3.4.2 Impulse oscillometry used in Study III

Lung function testing was performed at 16 years of age using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, CA). The subject was sitting upright with the neck in a neutral position, and wearing a nose clip. Measurement was performed during tidal breathing with the lips tightly sealed around the mouthpiece and with the cheeks supported by the hands. Signals free from artefacts (sighs, swallows, coughs and air leaks) and that lasted for at least 20 seconds were saved for analysis. Quality control was performed at the time for examination by visual inspection of the waveforms, and given that coherence, which is a measure of testing reliability, was > 0.80 at 10 Hz. At least two technically accepted recordings were performed per subject. The mean value of the resistance at 5 Hz (R5), the resistance at 20 Hz (R20), the frequency dependence of resistance between 5 and 20 Hz (R5-R20) and the area under the curve of negative resistance values (AX) were extracted. AX may boost the assessed response inappropriately compared with R5-R20, because it multiples two partly independent reactions of the airways (see background – IOS). We therefore "linearized" AX by reporting its square root ($AX^{0.5}$). The IOS system was calibrated each day with a reference resistance of 0.20 kPa L^{-1}·s.

3.5 VARIABLE DEFINITIONS

For variable definitions used in the included studies, please see Table A3 (for effect modifiers) and Table A4 (for confounder variables).

3.6 STATISTICAL ANALYSES

In general, we used standard statistical models for epidemiological research, which are summarized in Table 1 in Chapter 3.1 "An overview." In this chapter, I will further explain the models that are more unusual within this area of research.

3.6.1 Life course analyses

To examine the influence of possible air pollution exposure trajectories between birth and 16 years of age, we performed several life course analyses in Study II. The selected time intervals were 0-1 year of age, 1 to 8 years of age, and 8 to 16 years of age. After categorization to high (= 1) or low (= 0) exposure for each time interval (dichotomized on the NO_x median for the middle time window, age 1 to 8 years corresponding to 10.9 µg/m³), subjects were categorized into one out of eight patterns of air pollution exposure levels, namely: $1 =$ "0,0,0"; $2 =$ "1,0,0"; $3 =$ "0,1,0"; $4 =$ "0,0,1"; $5 =$ "1,1,0"; $6 =$ "1,0,1"; $7 =$ "0,1,1" $8 =$ "1,1,1".

To formally test the importance of different exposure time intervals during a child's life course (0 to 1 year, 1 to 8 years, and 8 to 16 years) on the FEV_1 at 8 and 16 years, we used likelihood ratio tests to compare alternative nested life-course regression mixed models ("critical period," "accumulation," or "sensitive period") with a fully saturated model, comprising all possible NO_x exposure patterns over the time intervals[87,88].

Underlying hypotheses for each model:

- The "saturated "model makes no assumptions about mean FEV_1 across the eight possible patterns of dichotomized NO_x exposure levels over the three time intervals; mean FEV_1 is allowed to take on any value for any given exposure pattern regardless of the other patterns.
- The "critical period" model hypothesizes that it is exposure only during one time interval (0-1, 1-8, or 8-16 years) that is associated with mean FEV_1. In this model, exposure during other intervals is irrelevant. For example, the first critical period model would comprise the case in which it is only exposure in the first year (0-1) that determines mean FEV_1 in the first 16 years of life.
- The "accumulation" model assumes that it is the cumulative number of high exposure intervals across the life course that impacts the trend of mean FEV_1, while the chronical order in which these intervals occur is irrelevant.
- The "sensitive period" model postulates that high exposure at each time interval contributes additively and independently to mean FEV_1.

The alternative models were compared with each other using the Akaike's information criterion (AIC).

3.6.2 Quantile regression

Quantile regression is a method that allows for the possibility that the association between the independent variable (exposure) and the dependent variable (outcome) may differ over the distribution of the dependent variable; thus, the association may be assessed for any selected percentile(s). For example, exposure to traffic-related air pollution and external tobacco smoke may influence those with poor lung function differently from those with optimal lung function. This method is robust to outliers, and may be used on data that is non-normally distributed. The coefficients of quantile regression can be interpreted similarly to those of linear regression, except that they indicate the change in percentile, not mean, of the dependent variable for each unit change of the independent variable[89].

The quantile regression method was used in Study III to assess the association on the median (i.e., the 50^{th} percentile) between exposure to traffic-related air pollution and IOS variables, as these variables are not normally distributed. In the same study, quantile regression was used to estimate the 95^{th} percentile of each IOS index in relation to age, height, weight, separated by gender.

Similarly, in Study IV, the method was used to assess the associations between predictors and FEV_1 increase between 8 and 16 years on the 10^{th}, 50^{th} and 90^{th} percentile (i.e., low, mid, and high lung function growth). In addition, we assessed the estimated 25^{th}, 50^{th}, and 75^{th} percentile of FEV_1 at 8 and 16 years of age in relation to age, height and weight, separated by gender.

3.7 ETHICAL CONSIDERATIONS

The BAMSE study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden. The parents of all participating children received written information about the purpose of the study and provided informed consent. Families were informed about the procedures and were free to withdraw at any stage

4 RESULTS AND DISCUSSION

In this section, the main findings of the thesis are presented and discussed, with reference to the respective study. For further details, please see the individual papers.

4.1 LUNG FUNCTION SUMMARY STATISTICS

Spirometry

A summary of anthropometry and lung function data obtained at the 8- and 16- year clinical investigations is presented in Table 4. The mean FEV_1 increase between 8 and 16 years of age was 1,752 mL and 2,673 mL, for females and males, respectively. Compared with the most widely used reference population today, the Global Lung function Initiative (GLI)[75], this corresponds to a change in z-scores of -0.51 SD in females and -0.40 SD in males, which according to the reference equation would indicate an average decline in lung function trajectory over the period. Results for FVC show a similar pattern.

To have an ideal fit to the GLI, the mean z-score should have a value of 0 with a SD of 1. The main reason for the observed decline between 8 and 16 years of age is that the BAMSE population has values of approximately 0.40 above the reference population at 8 years of age, and that lung function has normalized at 16 years compared with GLI. The SDs are below 1 for all measurements. Within the GLI datasets with n > 1,000 subjects, the mean z-scores ranged from -0.22 to 0.30[90]. Possible underlying reasons for the mean z-score difference between the studies and the GLI reference values may be secular trends, small datasets, ethnicity/biology, and technical differences[75,90].

A population average z-score of slightly below or above 0 does not influence the association between an exposure (such as traffic-related air pollution) and lung function (used continuously). However, it will influence the proportion of individuals having values below a certain threshold. Given a perfect fit to the GLI reference population, the proportion of individuals having lung function values below the lower limit of normal (LLN, defined as < -1.645 SD) should be approximately 5 %. In BAMSE, the corresponding proportion was 1 to 2 % at 8 years of age. Therefore, in Study I, we used our own population to calculate if an individual had lung function values < 80 %, and < 85 % of predicted.

Table 4: Summary statistics of anthropometry and lung function variables from 8 and 16 years of age for males and females separately (in subjects with lung function data available).

	8 year follow-up						16-year follow-up					
	Females			Males			Females			Males		
Variable	No	Mean (SD)	%	No	Mean (SD)	%	No	Mean (SD)	%	No	Mean (SD)	%
Age (yr)	1,016	8.33 (0.49)		1,036	8.45 (0.48)		1,321	16.70 (0.41)		1,233	16.72 (0.41)	
Height (m)	1,016	1.32 (0.06)		1,036	1.33 (0.06)		1,321	1.67 (0.06)		1,233	1.80 (0.07)	
Weight (kg)	1,016	30,13 (5.66)		1,036	30.51 (5.37)		1,321	60.89 (9.46)		1,233	70.45 (11.68)	
Spirometry												
FEV_1 (mL)	971	1,732 (256)		991	1,827 (282)		1,185	3,484 (446)		1,058	4,500 (645)	
FVC (mL)	965	1,987 (295)		957	2,142 (339)		1,150	4,036 (528)		973	5,383 (777)	
FEV_1/FVC (%)	920	87.3 (5.3)		912	85.2 (5.9)		1,122	86.5 (6.1)		933	83.7 (6.6)	
Z-score[#]												
FEV_1	971	0.46 (0.96)		991	0.36 (0.93)		1,185	-0.05 (0.91)		1058	-0.04 (0.96)	
FVC	965	0.62 (0.89)		957	0.56 (0.92)		1,150	0.15 (0.89)		973	0.15 (0.95)	
FEV_1/FVC	920	-0.35 (0.89)		912	-0.35 (0.90)		1,122	-0.36 (0.95)		933	-0.31 (0.98)	
< LLN z - FEV_1	18		1.9	19		1.9	35		3.0	47		4.4
< LLN z - FVC	10		1.0	17		1.8	23		2.0	22		2.3
Impulse oscillometry	No	Median (IQR)	%	No	Median (IQR)	%	No	Median (IQR)	%	No	Median (IQR)	%
R5 ($Pa \cdot L^{-1} \cdot s$)							1,261	395 (105)		1,191	320 (95)	
R20 ($Pa \cdot L^{-1} \cdot s$)							1,261	375 (90)		1,191	305 (75)	
R5-R20 ($Pa \cdot L^{-1} \cdot s$)							1,261	20 (55)		1,191	15 (45)	
$AX^{0.5}$ $(Pa \cdot L^{-1})^{0.5}$							1,261	16 (6)		1,191	13 (5)	
> 95th percentile*: R5-R20							89		7.0	86		7.2
> 95th percentile*: $AX^{0.5}$							83		6.6	85		7.1

Abbreviations: No =number, SD = Standard deviations, yr = years, LLN = Lower limit of normal (< -1.645 SD), [#]z-score calculated using GLI 2012, * > 95th percentile calculated by quantile regression using age, height, and weight as explanatory variables in males and females separately.

IOS

The impulse oscillometry data are not normally distributed and therefore we present the data as the median and the interquartile range (IQR). The median R5 and R20 (in $Pa \cdot L^{-1} \cdot s$) at 16 years of age were 395 and 375 for females, and 320 and 305 for males, respectively. Comparable measurements have been observed in a population from northeast Poland, consisting of children and adolescents up to 18 years and > 150 cm in height[91]. There are, to our knowledge, no other published reference equations for IOS in the adolescent age range.

To discriminate those "worst-off," we used our own "lung healthy" population and calculated a predicted 95th percentile using, age, height, and weight, as explanatory variables in males and females separately. Applying the same equations for all subjects, approximately 7% had values above the 95th percentile of R5-R20 and $AX^{0.5}$, indicating a subpopulation that might be at increased risk of obstruction in the small airways.

Figure 8 illustrates some basic comparisons between the FEV_1 categories using LLN as a cut-off, and the IOS categories using the 95th percentile as a cut-off. For example, only 18 of 78 subjects having below LLN of FEV_1 are also above the 95th percentile of R5-R20, indicating that different subgroups are captured by the different methods.

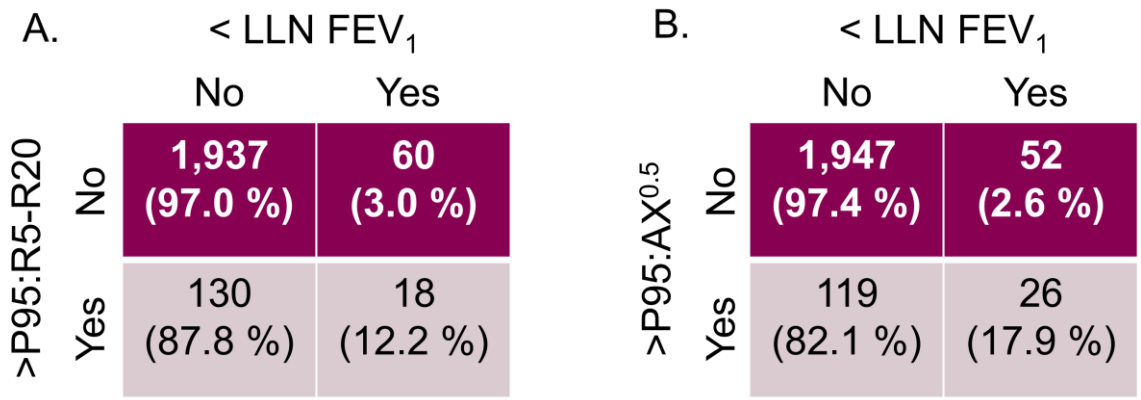

Figure 8. Two-by-two tables comparing the number of individuals having below the lower limit of normal (LLN: < -1.645 SD) for FEV_1 at 16 years of age and those above the 95th percentile (> P95) for the small airway indices R5-R20 (A) and $AX^{0.5}$ (B) at 16 years of age.

4.2 TRAFFIC-RELATED AIR POLLUTION AND LUNG FUNCTION

We found that exposure to traffic-related air pollution during the first year of life, but none of the other time periods examined, was significantly associated with reduced lung function at 8 years of age (Study I) and that this effect remained to some extent at 16 years of age (Study II). We found the strongest associations for FEV_1 and $FEV_{0.5}$ ($FEV_{0.5}$ was only analyzed at 8 years of age, see Study I), representing mainly the mechanical properties of the large and medium size airways, and not as strong associations for FVC, reflecting lung size (Figure 9).

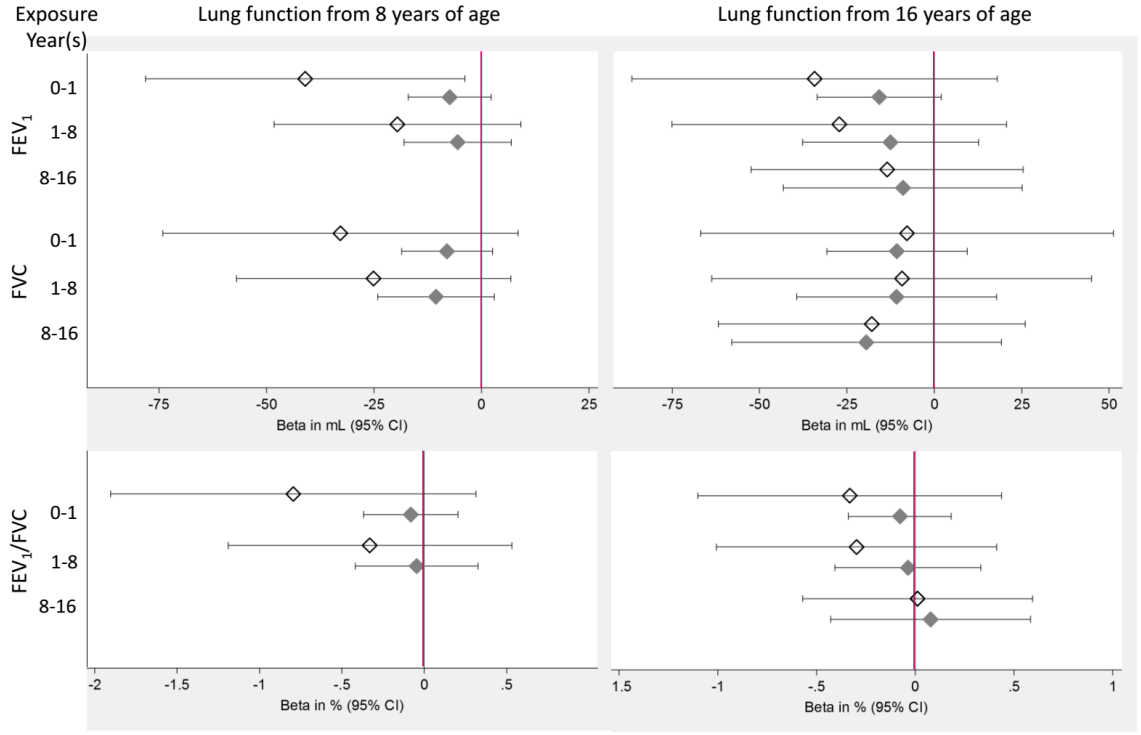

Figure 9. Lung function measurements at 8 and 16 years of age in relation to traffic- PM_{10} exposure (white) per 5 µg/m^3, and traffic-NO_x exposure (grey) per 10 µg/m^3 in different time periods of life. Analyses were made by linear regression on the mean, adjusted for gender, age, height, municipality at birth, heredity for asthma and/or allergies (at 8 years only), and weight (at 16 years only).

The larger effect estimate observed for FEV_1 than for FVC is in agreement with studies from Austria[51], China[92] ,the United States[25 ,47 ,48 ,65 ,93], Puerto Rico[94], and Norway[67], even though many of these studies observed the strongest effect estimate for mid-expiratory flows (FEF_{25-75}), which we did not explore. FVC has in some studies shown stronger associations than FEV_1[52 ,56 ,95-97].

Differences in the effects on the spirometry variables by emissions from traffic may partly be explained by a mixture of components which can differ between the studies. The car fleet, road wear and fuel composition may differ between study areas, as may the use of studded tires. Most epidemiological studies using modelled exposures, including the ones in this thesis, cannot separate the different components of the emissions effectively[98 ,99]. We focused on NO_x and PM_{10} as exposure estimates, which are both derived from traffic, and are

therefore correlated in time and space. In Stockholm, PM_{10} is primarily influenced by coarse particles (> 2.5 µm), even though it also contains smaller particles.

From an individual perspective, the mean estimated effect on lung function at 8 and 16-years of age observed in our studies is rather small (FEV_1; -2.3 % at 8 years and -0.9 % at 16 years of age per 5 µg/m^3 of PM_{10}), although even a minor shift in the population distribution of lung function may increase the prevalence of subjects presenting lung function values below clinical thresholds. In our studies, this was indicated as an increased risk of having less than 80 % and 85 % of the predicted value at 8 years of age (Study I) and less than the lower limit of normal (< -1.645 SD) at 16 years of age (Study II) (Figure 10).

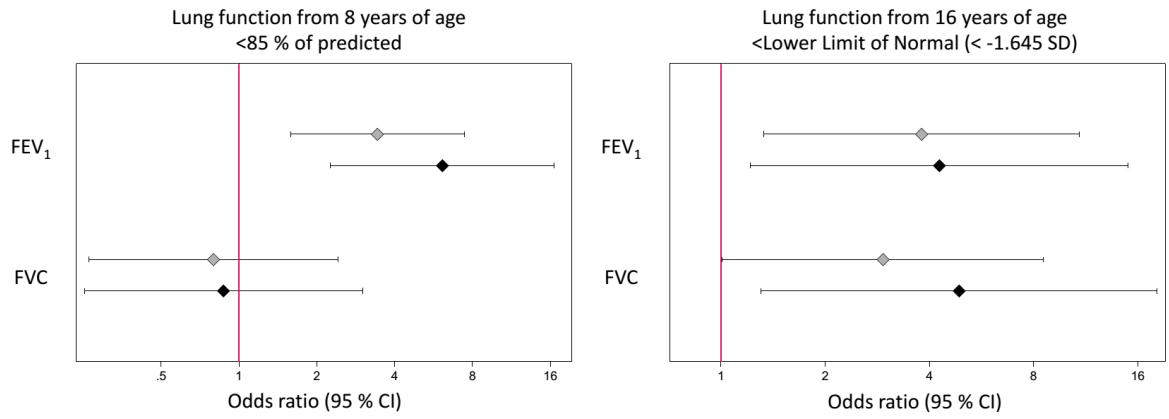

Figure 10: Odds ratios for the association between low FEV_1 and FVC at 8 and 16 years of age, and exposure to traffic-NO_x (grey) and traffic-PM_{10} (black) during the first year of life. Odds ratios were calculated by logistic regression adjusted for municipality at birth (and heredity at 8 years of age). The results are presented at 8 years for a 7 µg/m^3 difference in PM_{10} and for a 47 µg/m^3 difference in NO_x. At 16 years the air pollution levels were dichotomized on the median years 1 to 8 to be considered high- or low-exposed, corresponding to 10.9 µg/m^3 for NO_x and 4.4 µg/m^3 for PM_{10}. Definition of low lung function; At 8 years: < 85 % of predicted value based on age, sex, height, and weight and interaction of sex with age, height, and weight. At 16 years of age: < -1.645 SD of z-scores based on reference equation from the Global Lung Initiative 2012[75].

Our results showing increased risk of having values of lung function below clinical thresholds are supported in one recent publication from the Children's Health Study in California. In this study, Gauderman and colleagues initiated and followed three cohorts during separate calendar periods, at the same time as air pollution levels improved. They observed that the risk of exhibiting FEV_1 values below 80 % of predicted at 15 years of age declined from 7.9 % to 6.3 % to 3.6 % across the three time periods, indicating a substantial improvement in public health following decreased levels of air pollution[25].

In Sweden, we have relatively low levels of air pollution[35]. Adding the background levels from air pollution to the estimated local contribution from traffic during the first year of life, as shown in the air pollution assessment section, revealed that none of the children exceeded the current air quality standard for PM_{10} and the corresponding number for NO_x was only 2.8 %. However, we still observed an increased risk of exhibiting low lung function values, suggesting that current air quality standards are not sufficient to protect infants.

Longitudinal analyses of lung function change

In addition to cross-sectional analyses of lung function data at 8 and 16 years, the longitudinal change of FEV_1 between 8 and 16 years of age, was investigated in Study II. No associations were observed for the change of lung function between 8 and 16 years for any of the time windows explored. This suggests that exposure in early life influences early lung growth and that the level of lung function thereafter tracks with age, as has been reported by others[3].

Even though several studies highlight the importance of exposures prenatally or during infancy for subsequent respiratory health[7,15,100], there are reports showing attenuated lung function growth in relation to exposure during later childhood and adolescence[24,52,71], as well as recovery of previous deleterious effects in subjects moving to less polluted areas[46]. Potential reasons for the discrepancies in the longitudinal results, as well as in the impact on lung function from exposure to air pollution during different periods in life, are further discussed in the next section of this thesis (see Section 4.3: "Timing of air pollution exposure").

4.3 TIMING OF AIR POLLUTION EXPOSURE

In all of the studies in this thesis, we dealt with questions concerning the timing of air pollution exposure. At the time when this project was initiated, only one such study had appeared[67]. Cross-sectional results on lung function from Study I and Study II indicated that the first year of life was a critical time window for exposure to air pollution. These findings are in line with previous findings suggesting that exposures and events during fetal and early years of life have a substantial effect on long-term respiratory health[7].

To examine possible exposure trajectories in greater depth (Study II), we speculated on three different life course models that could potentially explain the associations with FEV_1 at 8 and 16 years of age, namely the "critical period" model, the "accumulation" model, and the "sensitive period" model[87,88]. While exposure during the first year of life was found to be an important determinant of lung function, the difference in the Akaike's information criterion between these models was too small to justify the selection of one model over the other. Using the sensitive period model, exposure during the first year of life was associated with FEV_1 decrements of -71 mL (95 % CI: -152 to 11) up to 16 years, and the corresponding deficit using the critical period model was -80 mL (95 % CI: -160 to 0). Repeatedly being under high exposure (two time intervals) appeared in the accumulation model to exert a negative impact on FEV_1 as well (-89 mL, 95% CI: -175 to -5) (Figure 11).

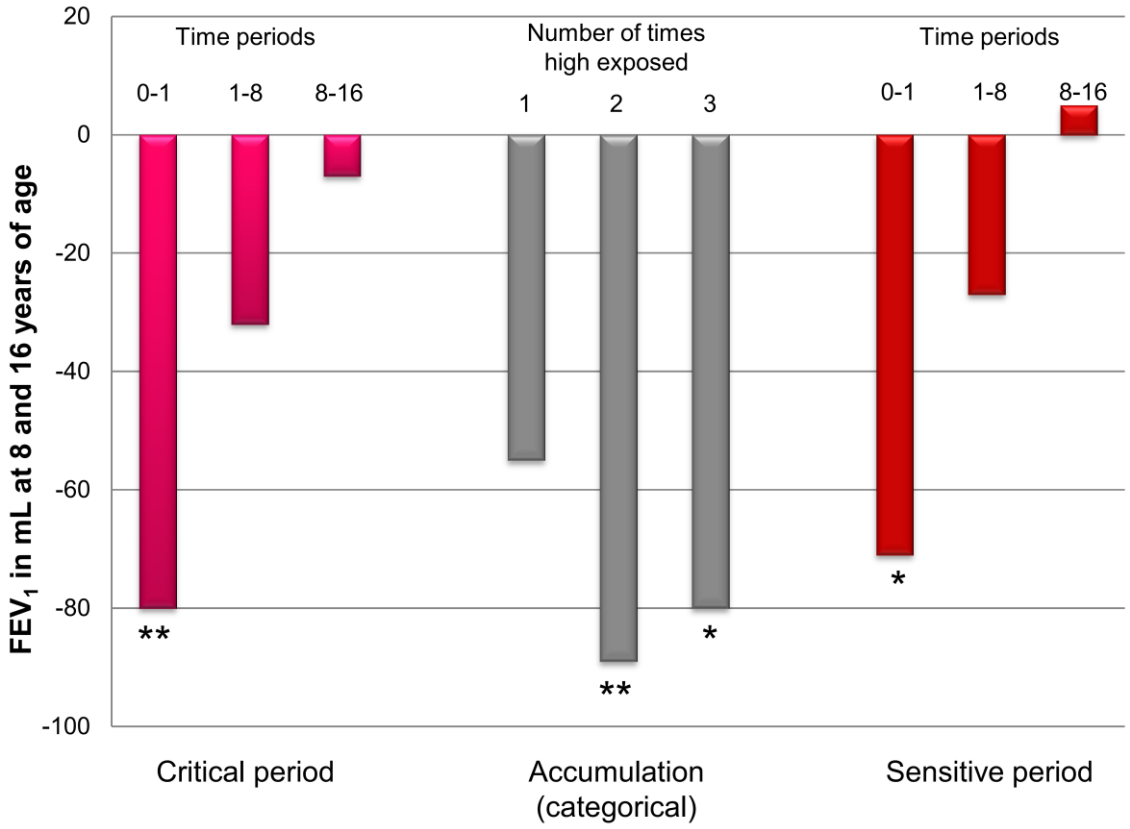

Figure 11. Mixed effects model for FEV_1 at 8 and 16 years of age, for alternative life course NO_x exposure (high or low) models ($n_{all\ subjects}$=1,931). Exposure dichotomized on median exposure for age 1 to 8 years, corresponding to 10.9 µg/m^3, to be considered high- or low-exposed. P values: * ≤ 0.10, ** ≤ 0.05 compared with low-exposed at each time period (critical or sensitive period models) or 0 times high-exposed (accumulation model). Model specifications; Critical period model: Each time period as main effect in three separate models (i.e., each model assumes only one time period is important). Accumulation model: Summed score of number of times of high exposure (i.e., assume all time periods are important, with interchangeable effect sizes). Sensitive period model: All time periods as main effects in a single model (i.e., assumes all time periods are important, with effect sizes that may differ).

It should be noted that the levels of air pollution decreased during the course of our studies, limiting the possibility of discerning the importance of exposure during adolescence. The mean local traffic-related contribution to the regional background air pollution levels was 5.8, 4.7, and 4.2 for traffic-PM_{10}, and 20.9, 13.0, and 7.0 for traffic-NO_x during the first year of life, 1 to 8 years of age, and 8 to 16 years of age, respectively, for the included children. The main explanation for the observed decreasing trend in traffic-related NO_x over the study period is the introduction of catalytic converters in private cars. Particle levels remained stable at the individual addresses over the study period, likely because the uses of studded tires did not change[78]; therefore, the small observed decrease in the time-weighted PM_{10} average results from children moving to less polluted areas.

Our results highlighting the impact of exposure to air pollution in infancy on lung function in childhood and adolescence are not necessarily in disagreement with the reported effect on impairments in lung function levels and growth from later life exposures. Most studies on lung function growth focus only on exposure during later life[24,46-50,52], and the potential impact of exposures during the first year of life in these cohorts is therefore unknown.

To date, there are five studies (aside from the studies included in this thesis) that have reported on the relative impact of early life vs. current exposure to traffic-related air pollution. A Norwegian cohort study of 2,307 nine-and ten-year-olds showed that both early life and lifetime exposure to PM_{10} and NO_2 were negatively associated with lung function[67], even though early life had slightly stronger effects. A meta-analysis of five European birth cohorts within the ESCAPE collaboration showed associations mainly with exposures at the current address and lung function at 6-8 years of age, not with the address at birth[101]. Mölter and colleagues have followed 1,185 children from birth to 11 years of age within the MAAS cohort[71]. They observed strong effects mainly from exposure to PM_{10} during early life and postbronchodilator FEV_1 at 5 and 11 years of age. Fuertes et al report from a German cohort of 2,266 fifteen-year-olds, overall null findings irrespective of exposure time periods[102]. In a recent report, Rice and co-authors investigate 614 children (mean age 7.7 years) and conclude that first year of life, lifetime and past year exposure to $PM_{2.5}$ and black carbon were negatively associated with mainly FVC (not as strongly with FEV_1), but that only the latter two time periods were statistically significant[96]. Taken together, the evidence of the role of exposure timing for effects of air pollution exposure on lung function in children appears inconclusive.

A majority of the BAMSE children lived at the same address during their first year of life as during the pregnancy period. Therefore, we cannot rule out that at least part of the observed effect from first year of life exposure to air pollution is attributable to pregnancy exposure. We acknowledge that other studies have observed an influence on neonatal lung function from pregnancy exposure[103], especially during second and third trimesters[104].

Respiratory health effects related to short-term exposure to air pollution have repeatedly been reported, especially related to asthma exacerbations[105]. In Study I and Study III, sensitivity analyses including additional adjustments for short-term exposure three to seven days before each lung function test showed no effect on the observed estimate for long-term exposure. Our study was, however, not designed to optimize the short-term assessment to air pollution, and the correlation between measured personal exposures and short-term exposures is rather poor[82]. In addition, it should be noted that our short-term air pollution data were derived from monitoring stations, and consequently estimate changes in total ambient pollution concentrations, and not solely the traffic-related compounds.

4.4 SMALL AND LARGE AIRWAYS

The small airways may be vulnerable to the effects of exposure to air pollution because inhaled fine and ultrafine particles (closely linked to NO_x) may be deposited in this region[37,106]. As spirometry measurements are insensitive to changes in the small airways[27], we therefore investigated the relation between air pollution exposure during the first year of life and IOS indices in 2,415 sixteen-years-old adolescents (Study III).

We found a median increase in R5-R20 of 2.0 $Pa \cdot L^{-1} \cdot s$ (95 % CI: 0.3 to 3.6, P value = 0.02) and $AX^{0.5}$ of 0.17 $(Pa \cdot L^{-1})^{0.5}$ (95 % CI: 0.01 to 0.34, P value = 0.04) per 10 $\mu g/m^3$ increase of exposure to NO_x during the first year of life; both measurements reflecting small airway function. Further adjustment for subsequent exposure time windows, short-term exposures, or moving during follow-up did not change the results. No associations were observed for either R5 or R20 (reflecting total and proximal airway resistance, respectively), or for exposure one year prior to the measurements of lung function (data not shown).

Stratified analyses of first year of life exposure to traffic-NO_x suggested stronger associations in subjects with asthma at 16 years (R5-R20 of 6.7 $Pa \cdot L^{-1} \cdot s$, 95 % CI: 0.1 to 13.3), but not in those with allergic sensitization at 16 years. The effect modification in R5-R20 analyses was only significant for asthma (P value = 0.04 for interaction). The results for PM_{10} were in line with those of NO_x, but did not reach significance.

The observed effects on median small airway function were small and likely without clinical implication, at least in healthy subjects and/or in areas with low air pollution levels. To determine whether exposure to air pollution was associated with a more severe small airway obstruction, we analyzed NO_x and PM_{10} exposure in relation to the odds of having R5-R20 and $AX^{0.5}$ above vs. below the estimated 95th percentile (> P95). Associations were indicated between exposure to traffic-NO_x and PM_{10} during the first year of life and > P95 of R5-R20 and $AX^{0.5}$ (Figure 12). In these analyses, PM_{10} exposure appeared most prominently associated with the IOS indices.

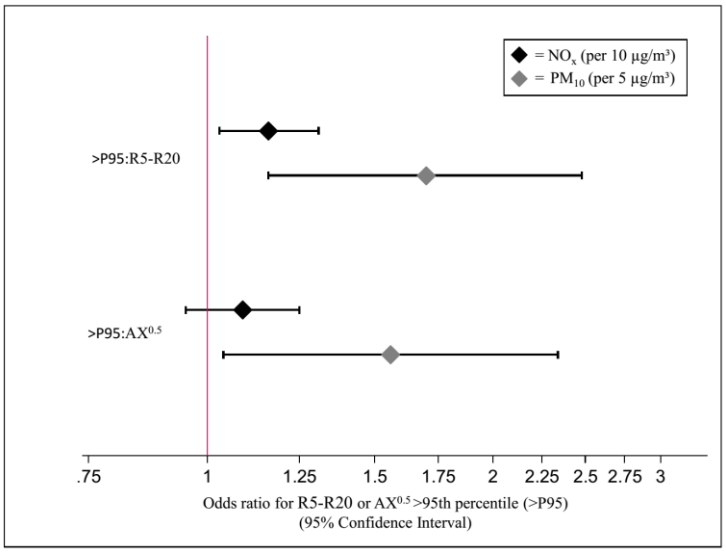

Figure 12. Odds ratios for the association between first year of life exposure to traffic-related air pollution and above vs. below the estimated 95th percentile of R5-R20 or $AX^{0.5}$ at age 16 years. Calculated by logistic regression and adjusted for municipality at birth. P95 based on quantile regression within the BAMSE population using age, height and weight as explanatory variables for males and females separately.

The lack of associations for the large and total airway indices, R20 and R5 respectively, are not in concordance with our previous findings from Study I and Study II, where we observed an effect mainly in FEV_1. FEV_1 in healthy individuals represents primarily the mechanical properties of the large and medium-sized airways, and may therefore also be viewed as a measure of large and total airway resistance. Relying on the assumption that we measure large airway resistance with these two techniques, one would expect a comparable association between all these indices[29]. However, the exact (patho-) physiological concept underlying IOS measures is not entirely clear. The measures are based on principles of physiological information, physical transmission, and mathematical modeling[107], and are supported by literature showing associations between measurements and disease, as well as symptom severity and response to treatment[31,108]. Therefore, it may not be surprising that our results, as well as those of others[29,109], show discrepancies in effects when comparing spirometry and IOS. We observed an association between air pollution exposure and R5-R20 as well as $AX^{0.5}$, particularly in subjects with asthma. On the other hand, the estimate for FEV_1 at 16 years of age was only observed in those without asthma (although there was no significant interaction term). This may be an indication that we do not capture the same underlying physiological mechanism with these different methods. As discussed in Section 4.1, only 18 out of 78 individuals having below the LLN for FEV_1 had abnormal values of R5-R20 (i.e., > 95^{th} percentile), which further illustrates this concept.

Other studies have observed effects on FEV_1 and FVC measured using spirometry[25,47,48,51,65,67,92-94]. In addition, there are reports of associations between exposures to air pollution and forced mid-expiratory flows, like FEF_{25-75}, and suggests that the results represent small airway effects[47,49-51,99]. This interpretation has lately been subject to debate, and the general view is now that the mid-expiratory flows do not contribute with additional information over what is provided by the FEV_1 and FVC[27]. Therefore, our results of air pollution exposure in relation to small airway effects measured with IOS may be considered novel.

To summarize the results regarding the association between air pollution exposure and potential location of airway effects, we observed associations with both the small and the large airways at 16 years of age. The effect estimates are, however, small and with wide confidence intervals; therefore, the results should be interpreted cautiously.

4.5 SUSCEPTIBLE SUBGROUPS

In our modern society, everybody is more or less exposed to air pollution. However, certain people may suffer more or exhibit more harm due to for example: old age[110], existing medical conditions[36], low socioeconomic status[111], and genetic susceptibility[112]. For the studies included in the present thesis we have focused on sub-analyses with respect to asthma, sensitization and gender status (Studies I to III). In Study II, environmental tobacco smoke (ETS), and maternal smoking during pregnancy or infancy were also explored.

4.5.1 Asthma and sensitization

With respect to subgroup analyses, we observed that the association between exposure to air pollution during the first year of life and FEV_1 differed depending on whether the outcome was assessed at 8 or 16 years of age. In Study I, we observed stronger associations between first year of life exposure to PM_{10} (per 7 $\mu g/m^3$) and FEV_1 at 8 years of age in those sensitized, and in those with asthma, with deficits of -136.9 mL (-224.1 to -49.7), and -90.6 mL (-293.4 to 112.3), respectively. However, the effect modification was not statistically significant (P values of 0.13 and 0.69, respectively). At 16 years of age (Study II), the association with FEV_1 did not remain in either sensitized or non-sensitized; instead, an association was primarily suggested among those without asthma (-16.8 mL: 95 % CI, -35.1 to 1.5 vs. 24.5 mL: 95 % CI,-52.8 to 101.8 in those with asthma, per 10 $\mu g/m^3$ of NO_x). However, the effect modification was not statistically significant at this age (P values of 0.26 and 0.82, for sensitization and asthma, respectively).

Contrary to the null results for FEV_1 in subjects with asthma at 16 years of age, we observed stronger effects for the IOS measurements. On the other hand, in line with FEV_1 results at 16 years, no apparent effect modification was present depending on sensitization status.

There is a risk that we are underpowered to detect significant interactions and that our stratified results may show spurious findings. Our mixed results seem to be in concordance with other epidemiological studies on asthma-pollution modification of lung function, as many show no differences[25,47,48,63,67,95,96], while others have shown stronger effects in those with asthma[67,98,102], and sometimes results are only presented for those without asthma[24,93].

Despite the conflicting results regarding effect modification by asthma, our associations observed on the small airway indices (using the IOS method) in adolescents with asthma should be highlighted. It is possible that the peripheral airways in people with asthma are more affected than those of healthy peers, and it has been observed in experimental settings that the deposition of inhaled ultrafine particles (which is closely linked to modeled NO_x) is increased in asthmatics, relative to healthy subjects[106].

Effect modification of air pollution-lung function associations by sensitization has not been well investigated in epidemiological studies. In one study, Morales and colleagues found stronger associations on FEV_1 in allergic (allergic asthma, atopic dermatitis, eczema, or allergic rhinitis) 4.5-year-olds exposed to air pollutants during pregnancy and lifetime, compared with non-allergic children[113]. Janssen and colleagues reported that associations

between truck-traffic counts and respiratory symptoms were only observed among children with bronchial hyper-responsiveness and/or sensitization[62], indicating a sensitive subgroup. However, no associations were observed in the same study regarding spirometry outcomes. As part of the ESCAPE project, stratified analyses based on sensitization status for the relation between air pollution exposure and lung function at 6-8 years of age did not reveal any systematic associations[101]. The studies are rather heterogeneous, therefore direct comparison between studies may be difficult.

Evidence supporting sensitization as an effect modifier comes from a high-risk cohort study, where the authors observed that early exposure to allergens in combination with secondhand smoke exposure increased the risk for incident asthma, compared with having neither of the exposures[114]. Such findings are supported by controlled exposure studies[115,116].

The mechanisms behind how this potential interaction has its effect are not clear, but it has been suggested that genetically susceptible children more often present with impaired epithelial barrier function, which subsequently increases the airway's vulnerability to early life air pollution exposure[117]. Epigenetic effects on DNA have been proposed as a potential link by which sensitization-pollution interaction influences the lung function[118]. Based on the results presented in this thesis, and a summary of available literature, the effect modification by asthma or sensitization status of the relation between air pollution exposure and lung function remains inconclusive.

4.5.2 Maternal smoking during pregnancy and environmental tobacco smoke exposure (ETS)

Based on data from 16 years of age (Study II), further stratified analyses revealed that an association was primarily suggested between air pollution exposure during the first year of life and FEV_1 in those whose mothers did not smoke during pregnancy or in infancy (-20.5 mL, 95 % CI: -39.5 to -1.5) and in those who were not exposed to environmental tobacco smoke at 16 years (-19.7 mL, 95 % CI: -38.5 to -1.0) (Figure 13). P values for interactions were 0.02 and < 0.01, respectively.

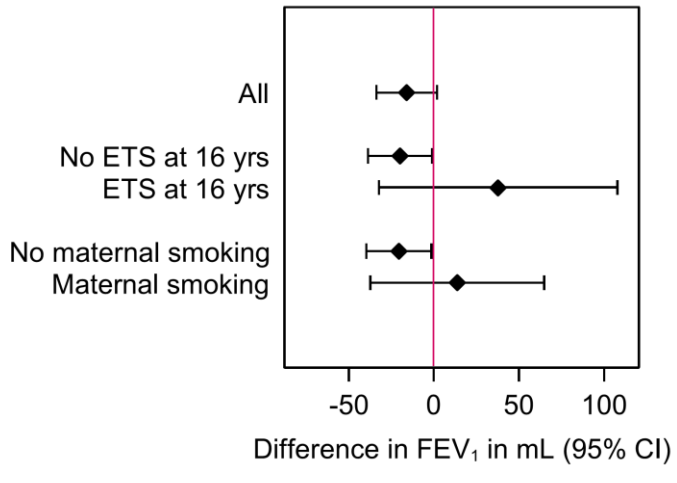

Figure 13. Stratified analyses of NO_x exposure (per 10 µg/m³) during the first year of life and FEV_1 at 16 years. Analyses were made by linear regression on the mean, adjusted for gender, age, height and weight, as well as municipality at birth. Definitions of subgroups: Maternal smoking = the mother smoked at least one cigarette per day at the time of questionnaire 0 and/or smoked at least one cigarette per day at any point of time during the pregnancy. ETS = Environmental tobacco smoke exposure, any of the parents smoked daily at the time of questionnaire 16.

Data regarding the role of smoking as an effect modifier for the association between traffic air pollution and lung function are limited. Svendsen et al report results concordant with ours, from a study in El Paso, Texas, where they observed a stronger association between land use regression modeled NO_x and FVC in those living in houses without exposure to secondhand smoke[95]. This is supported by results from subgroup analyses in the Children's Health Study which point towards an effect in those with no history of smoking[24,25]. However, studies by Rice and colleagues[96], as well as Horak and co-authors[50], revealed no differences between children exposed to household smoking and children not exposed.

Previously it has been suggested that there may be a stronger effect if children are exposed to both parental smoking and own smoking[119]. However, our results do not support a combined effect of being exposed to both parental smoking and exposure to traffic-related air pollution. It may be that the detrimental effects from traffic-related air pollution are less pronounced than the harm already done by passive smoking, and thus difficult to detect in subjects exposed to tobacco smoke.

4.5.3 Gender differences

All of the air pollution studies presented within this thesis, with stratified analyses based on gender, showed that the associations between first year of life exposure to air pollution and lung function were only observed among males. For example, at 8 years of age (in Study I), the association between first year of life exposure to PM_{10} and FEV_1 was -79.6 mL, (95 % CI: -155.7 to -3.5) and -37.1 mL, (95 % CI: -112.7 to 38.4) in males and females respectively, for a 7 µg/m³ difference in exposure, although the apparent effect modification was not significant (P value = 0.35). The effect estimate in males for traffic-NO_x (per 10 µg/m³) was for the IOS index R5-R20 (Study III) 3.2 $Pa \cdot L^{-1} \cdot s$(95 % CI: 1.2 to 5.2) and for FEV_1 at 16 years of age (Study II) the corresponding estimate was -30.4 mL (95 % CI: -59.1 to -1.7). The interaction coefficients were, however, non-significant with P values of 0.56 and 0.68, respectively.

Gender differences on lung function in response to air pollution or other environmental stimuli may be present due to inherited differences in lung and airway development and physiology. For example, surfactant production starts earlier in the lungs of neonatal females than males[120], which may be one reason why infant females have lower airway resistance and higher air-flow rates compared to infant males[121]. In addition, dysanaptic growth may influence gender differences[10]. Dysanaptic growth has an embryological basis and males present already at birth with a higher total number of alveoli and alveolar surface area than females, but at the same time the growth of the airways lags behind, resulting in relatively more narrow airways in infant to adolescent males compared with females[120]. Males may therefore during infancy, childhood and early adolescence have a pulmonary phenotype more susceptible to the deleterious effects of air pollution exposure[122]. During puberty, however, the risk in males compared with females may become reversed as several studies indicate influence by estrogens (female sex hormone) on increased incidence and severity of asthma[123]. Thus a gender-related vulnerability to the detrimental effects from exposure to air pollution may be influenced by age.

Our results of a suggestive stronger effect of air pollution exposure in males compared with females are in concordance with some of the published epidemiological studies within this area[25,55,92,93]. However, there are as many reporting stronger effects in girls[53,56,61,67], and a majority showing no differences in associations[47-49,52,59,95,124]. In addition, the mixed results regarding the role that gender has in relation to air pollution – lung function association is not obviously explained by age, as the observed differences in results cover the entire age range. Based on the published studies to date, there seems to be no conclusive evidence regarding gender-pollution interaction effects on lung function.

4.6 LUNG FUNCTION GROWTH AND TRAJECTORIES

In Study IV, we investigated different environmental exposures and individual characteristics in infancy and school age in relation to lung function growth between the ages 8 and 16 years. This was calculated using quantile regression on the 10^{th}, 50^{th} and 90^{th} percentiles, corresponding to low, median and high lung function growth. Out of 20 examined variables, we found that birth weight, asthma heredity and ETS (referred to as secondhand smoke in the original paper) in infancy were the only independent predictors of lung function. For example, compared with those with a birth weight between 3,000 to 3,998 grams, children with a birth weight < 3,000 grams had reduced growth on the 50^{th} percentile of -95.0 mL (95 % CI: -153.2 to -36.8) and children with a birth weight ≥ 4,000 grams had increased growth on the 90^{th} percentile of 163.8 mL (95 % CI: 85.1 to 242.4), respectively. Children exposed to ETS in infancy had reduced growth on the 90^{th} percentile of -134.0 mL (95 % CI: -236.5 to -31.5). None of the investigated exposure variables were significantly associated with low lung function growth (i.e., on the 10^{th} percentile) (Table 5).

Table 5: Multivariate model of first year of life and 8-year factors on FEV_1 change between 8 and 16 years, results only presented for exposures with significant results.

Adjustment level 1[#]	10^{th} percentile			50^{th} percentile			90^{th} percentile		
	Beta	95 % CI		Beta	95 % CI		Beta	95 % CI	
Birth weight (g)									
531-2,995	-39.7	-140.6	61,2	**-95.0**	**-153.2**	**-36.8**	-86.4	-197.3	24.6
3,000-3,999	Ref			Ref			Ref		
4,000-5,940	60.5	-32.2	153,2	**88.0**	**9.6**	**166.5**	**163.8**	**85.1**	**242.4**
ETS in infancy	11.2	-70.1	92,6	-31.9	-97.0	33.3	**-134.0**	**-236.5**	**-31.5**
Asthma heredity	-10.5	-82.6	61,6	**-72.2**	**-125.6**	**-18.8**	**-90.5**	**-173.6**	**-7.4**

Number of subjects in the analysis: 1,366. Exposure factors included were those that fell out as significant in a stepwise selection procedure, testing all variables that had a P value of ≤ 0.10 in the minimal model analyses (that was only adjusted for gender). Bold numbers indicate P value < 0.05.
Abbreviations: ETS = Environmental tobacco smoke exposure. CI = Confidence interval.
[#] Adjusted for gender, ETS at 8 years, and mold dampness at 2 months of age.

We did not find any clear or consistent influence from the 8 year exposure variables on lung function growth, supporting previous studies by others reporting an influence from intrauterine events and early postnatal life on lung development[18,125]. However, surprisingly few infancy variables were significantly associated with lung function growth considering

what has been reported before regarding the influence of, for example, preterm birth[126,127], maternal smoking during pregnancy[15,128] and early life respiratory infections[129,130]. This motivated us to further investigate whether the infancy variables were associated with having a persistent low lung function trajectory (< 25th percentile of FEV_1 at both age 8 and 16 years).

We observed that predictors for having a persistent low lung function trajectory (compared with mid/high) were premature birth, being born in the spring or autumn compared with the winter, presence of older sibling(s) and having had a lower respiratory tract infection (respiratory syncytial virus infection according to questionnaire) before 19 months of age (Figure 14).

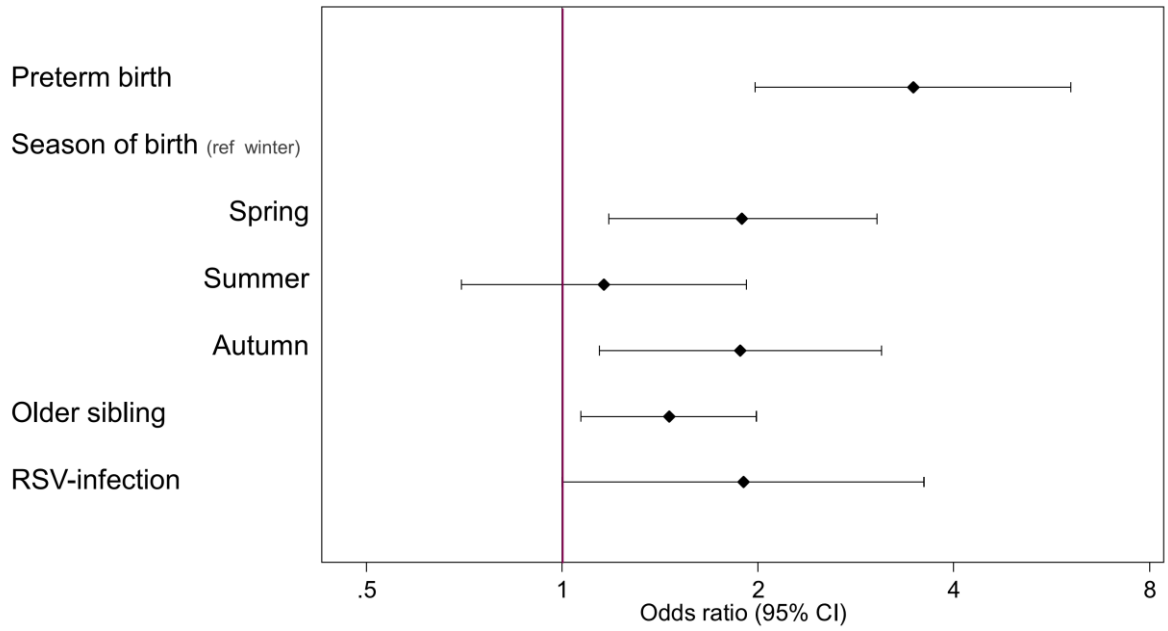

Figure 14. Exposures from infancy and the odds ratio of having a low FEV_1 trajectory (<25th percentile at 8 and 16 years of age) compared with the mid/high trajectory. Odds ratios are calculated in multivariate models including mutual adjustment of all variables in figure in addition to gender, and year of birth. Abbreviations: RSV=Respiratory syncytial virus.

A well-known major risk factor for impaired lung development is maternal smoking during pregnancy[15]. Turner and co-authors observed that those exposed to maternal smoking during pregnancy had initial decrements in lung function at 6 years of age, which resolved up to age 18[18]. We could not observe any associations with maternal smoking during pregnancy and lung function growth between 8 and 16 years of age. However, our data indicated that the effect of *in utero* exposure is set before 8 years of age, with an odds ratio of 1.44 (95 % CI: 0.95 to 2.19) for being below the 25th percentile at both 8 and 16 years of age. Therefore, children exposed during pregnancy may be more likely to retain a low lung function position over the life course, at least up to adolescence. On the other hand, ETS during infancy was shown to be negatively associated with lung function growth (independently of exposure to ETS at 8 years of age). Consequently, children exposed to

ETS in infancy may gradually become worse in their lung function compared with their unexposed peers.

Preterm birth was observed as the main risk factor for having a persistent low lung function trajectory. This association remained, when adjusting for maternal smoking during pregnancy and birth weight, in addition to all the other tested variables. Our results support previous studies showing detrimental impact on lung function at school age in ex-preterm subjects[126,131]. However, the lack of association between preterm birth and lung function growth between 8 and 16 years of age to some extent contradicts reports of catch-up effects in children and young adults born preterm[127,132]. The discrepancy in observed associations for lung function growth, in our study and others, may be explained by differences in study population and time trends, as one study was limited to children with a birth weight <2,000 grams[127] and the other included subjects born approximately 15 years prior to our cohort[132].

In addition to preterm birth, we observed that subjects with a low lung function trajectory, compared with mid/high, were more likely to have had a lower respiratory tract infection in infancy. Moreover, other variables that may be related to lower respiratory tract infections were similarly associated with an increased risk of having a low lung function trajectory, including presence of older sibling(s) and autumn birth[133-135].

We did not observe any associations between exposure to air pollution and growth of lung function in any percentiles. This is in line with our results from Study II. However, in Study I and Study II, we demonstrated that the risk of exhibiting FEV_1 values below clinical thresholds is increased at both 8 and 16 years of age, altogether indicating that exposure to air pollution during infancy influences lung growth in early life and that the level of lung function thereafter tracks with age. The latter results do not match our lack of significant findings between exposure to air pollution and persistent low lung function trajectory (Study IV). The most reasonable explanation may be that the definition used for low lung function trajectory (i.e., below the 25^{th} percentile), does not define an extreme enough phenotype to identify an association with exposure to air pollution.

4.7 STRENGTHS AND LIMITATIONS

4.7.1 Strengths

In general, the studies included in this thesis have several strengths, such as the prospective design, the relatively large number of participants, the individual long-term assessment of air pollution exposure incorporating residential addresses as well as addresses to daycare and schools, the detailed assessment of several potential confounders, the evaluation of the influence of short-term exposure, the objectively measured sensitization based on IgE measurements, and the repeated objectively measured lung function at both 8 and 16 years of age.

4.7.2 Random error

Random errors result from fluctuations around a true value because of sampling variability that cannot be readily explained[136]. It is expressed by the width of the confidence interval and depends on the sample size, but also on the prevalence of exposure and outcome and the quality of exposure and outcome measures. Confidence intervals were computed in all studies included in this thesis to demonstrate the precision of the risk estimates. The sample size varied between 1,425 in Study IV and 2,415 in Study III in the main analyses, which are rather high numbers. However, the confidence intervals can still be quite wide.

The main exposure in this thesis is the long-term traffic-related air pollution. These estimates are modeled and a delicate issue is to incorporate the available and important predictors in the model, but at the same time not introduce too much uncertainty[38]. The correlation between measurements at continuous monitoring stations and modeled exposure were 0.74 for first year of life exposure to traffic-NO_x[66]. Although considered good, this may still explain some of the variability.

There is variability in measured human lung function as well, both related to spirometry[137] and IOS[32]. In brief, we have between-subject variability, mainly due to age, height, weight, and gender, which we adjust for. Within-subject variability that includes day-to-day variability also exists, as does within-day variability, which may depend on time of day (highest values at noon), but remains largely unexplained[137]. The variability may be larger in subjects who smoke or have respiratory diseases[138,139]. Therefore, we may lose some precision in our sub-analyses of, for example, subjects with asthma.

4.7.3 Systematic error

Systematic errors refer to the type of error that remains, independent of study size. Such errors undermine the internal validity of a study, as they can lead to both over- and underestimations of associations[136].

4.7.3.1 Selection of participants

The BAMSE response rates during the follow-ups are comparatively high for questionnaire data, with 78 % of the original cohort still remaining at 16 years of age. Subjects who remained in the study did not differ significantly from the original cohort. For respective studies included in this thesis, the study population varied between 35 % in Study IV and 59 % in Study III, compared with the full cohort. Background data in these individuals differed slightly compared with the original cohort, for example in variables related to low SES, exposure to environmental tobacco smoke, and maternal smoking during pregnancy, with these being more prevalent among nonparticipants. No differences were observed regarding prevalence of asthma and allergies. We find it unlikely that selection influenced our point estimate to any major degree. The precision, however, may be less, especially in Study IV with the lowest participation rate, as our study participants are a more homogenous group than the nonparticipants, which could give less contrast of data.

4.7.3.2 Misclassification

Some misclassification of true individual exposure to air pollution has probably affected our results, especially since we do not have access to individual time-activity data and indoor environment[140]. However, several validation studies have shown good agreement between modeled exposure levels and outdoor measurements[81,141,142], as well as between modeled outdoor levels and personal exposure measurements[82]. The errors in the air pollution assessment are most likely independent from our outcome and such misclassification is expected to weaken any true associations.

Lung function was measured using dynamic spirometry according to ATS/ERS standards by trained, experienced personnel blinded for the exposure levels[26]. For this reason imprecisions are likely due to causes discussed in the random error section. However, we do not have post-bronchodilator measurements in BAMSE, which potentially could have influenced our results, especially for FEV_1, as results from the MAAS cohort indicated stronger associations after bronchodilation[71].

For the longitudinal analyses on lung function, we only have two time points of lung function measurement. This may increase the risk of regression towards the mean. In Study IV, we approached this problem by including sensitivity analyses adjusting for FEV_1 at age 8 years, which did not have an impact on the associations; accordingly, this was unlikely a problem[143]. The use of two different spirometers at 8 and 16 years, although both devices met the ATS/ERS recommendations, could potentially introduce some misclassification. Since all study subjects used the same spirometer at the respective follow-ups, a measurement error is unlikely to contribute to the observed associations between exposures and lung function.

Furthermore, other variables may be influenced by varying degrees of misclassification. For example, in Study IV, information on many of the exposure factors was collected via questionnaire. There may be an underreporting of exposures, such as, maternal smoking during pregnancy, due to awareness of the negative health consequences. Nevertheless, information about all exposures was gathered before lung function measurements were

performed; therefore, any misclassification is likely non-differential and would tend to dilute any true association. However, imprecision in the assessment of covariates may lead to residual confounding.

4.7.3.3 Confounding

In our studies as well as in many other epidemiological studies we do not merely want to establish an association, but also to get some insights on potential causal relationships. Therefore, we do our best to control for confounding. We have addressed confounding by adjusting for known and/or suspected confounding variables (chosen by convention in addition to statistical criteria) by incorporating them in regression models.

One important source of potential bias that deserves consideration when evaluating the effects of long-term traffic-related air pollution is socioeconomic status (SES). Socioeconomic status is a complicated variable, comprising a mixture of a person's income, education, employment, and social status, which may exert an impact on health by various pathways[144]. Therefore, different SES definitions have been used in different epidemiological studies. Interestingly, the relation of SES and air pollution concentrations differs between countries, with low SES and living near roads with heavy traffic often being highly correlated[145,146], while in Stockholm a reverse relation is observed, mainly because high SES families tend to live in the inner city.

In the included studies, we have explored the potential impact of SES by incorporating a variable mainly based on self-reported individual (parental) occupations. The tested SES variable did not influence our associations significantly, and was therefore not included in the models. However, residual confounding by this and other factors cannot be entirely ruled out[65,111].

From a methodological point of view, the assessment of health effects of exposure to air pollution in children should be less influenced by confounding than studies in adults, because many variables may be of importance only in the latter group, such as occupational exposure, own smoking, exposure while commuting, etc[147].

4.7.4 Generalizability

The BAMSE cohort is a population-based study which enables for generalizability of our results to the general population of respective ages. However, the original cohort comprises 75 % of those eligible. Parental smoking was more prevalent among nonparticipants, but the willingness to participate was not influenced by parental asthma or atopy (further explained in Section 3.2 "The BAMSE project")[76].

5 CONCLUSIONS AND IMPLICATIONS

- We observed associations between exposure to air pollution during infancy and lung function decrements in children and adolescents. The observed effects are small in absolute terms and from an individual perspective, but may have important implications on a population level, as a slight decrease in the population mean increases the number of subjects exhibiting lung function levels below clinical thresholds.

- We observed stronger associations between exposure to air pollution during infancy and increased small airways resistance in adolescents with asthma compared with those without asthma. This suggests that children and adolescents with asthma may be a susceptible subgroup.

- We identified a group of children with persistent low lung function throughout childhood, with preterm birth and factors related to lower respiratory tract infections in infancy as the main risk factors. This supports the hypothesis of early life as a sensitive period where negative exposures have a lasting impact on lung function development – hence these children may be at increased risk of developing chronic airflow obstruction in adulthood.

- In Stockholm, the levels of air pollution are generally below the current Swedish air quality standards. Despite this, we observed negative effects on lung function in children and adolescents, indicating that the current air quality standards are not sufficient to protect against detrimental effects in the lungs and airways. In addition to reducing the levels of air pollution from sources like traffic, decisionmakers should take impact from air pollution into consideration in city planning of new residential areas, schools and daycare facilities.

"As the twig is bent, so grows the tree"

Alexander Pope

6 FUTURE PERSPECTIVES

Evidence today suggests an adverse impact of early life exposure to air pollution on lung function, at least up to adolescence. Whether these lung function deficits persist into adulthood, and subsequently result in a reduced maximally attained lung function, or a prolonged growth phase, is unknown. There is therefore a need to follow pregnancy and birth cohorts with air pollution data up to adulthood when the plateau of lung function is reached.

We observed an increased risk of lung function deficits, especially in relation to exposure during the first year of life, and much less in relation to effects during later time periods. The question remains whether first year of life exposure is a proxy for exposure during pregnancy. The relative impact of pregnancy vs. early life exposure is sparsely investigated and when initiating new cohorts, effort should be made to capture both pregnancy and lifetime exposures and measure lung function already in infancy. Starting up a new cohort in the Stockholm area could also shed light on the potential influence of reduced NO_x levels, as an explanation for the lack of associations from exposures during later time periods.

In our studies, we investigated NO_x and PM_{10} in relation to lung function. These air pollutants are modeled and should be viewed as proxies for traffic-related air pollution. Further research, preferably experimental, can give insights into identification of the pathogenic characteristics of air pollution. In addition, caution has to be taken to consider the plausible impact of multiple exposures, interaction effects and possible susceptible subgroups.

We observed an association between air pollution exposure during the first year of life, and increased levels of IOS indices related to small airway function in adolescents, especially in those with asthma. This is a novel finding that needs to be confirmed. If replicated, it could facilitate further research on whether subjects with asthma are more vulnerable in their small airways to the deleterious effects of air pollution, and/or whether subjects born with a susceptibility (for example genetic polymorphism) are predisposed to both the negative effects of air pollution and later development of asthma.

There is a need to publish reference values in a normal population for IOS indices, especially in children. This would facilitate the use of IOS in epidemiological studies. In addition, a suitable cutoff to discriminate normal from suspected abnormal needs to be explored, as well as validation of the R5-R20 index as a detection tool for small airway function.

IOS is suggested to be a more sensitive measurement than spirometry. Changes in short-term exposures, such as air pollution or allergen load (pollen season), can be hypothesized to influence the respiratory mechanics, which may be captured by IOS. This association may be even stronger when investigating inspiratory resistance, as this has shown a greater day-to-day variability than expiratory resistance[148].

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Den maximala lungfunktionen nås i ung vuxen ålder och är till stor del förutbestämd av den lungfunktion individen hade tidigt i livet. Lungfunktionsutvecklingen kan dock till viss del påverkas av faktorer under barndomen och tonåren. Identifiering av viktiga tidsperioder och modifierbara faktorer som påverkar lungutvecklingen tidigt i livet kan bidra till främjad luftvägshälsa senare i livet. Syftet med denna avhandling var att undersöka långtidseffekterna av exponering av olika miljöfaktorer, med särskilt fokus på luftföroreningar från vägtrafiken, på lungfunktionsutvecklingen hos barn och ungdomar.

Delarbetena i denna avhandling baserades på BAMSE-studien, en longitudinell populationsbaserad födelsekohort av 4000 barn som följts tills tonåren. Från upprepade enkäter i åldrarna 1, 2, 4, 8, 12 och 16 år insamlades information om symptom på allergirelaterade sjukdomar, livsstilsfaktorer och miljörelaterade exponeringar. Barnens lungfunktion mättes med spirometri vid 8 och 16 års ålder, samt med impulsoscillometri vid 16 års ålder. Utomhuskoncentrationer av kväveoxider (NO_x) och inandningsbara partiklar (PM_{10}) från vägtrafiken beräknades för alla barnens hem-, förskole- och skoladresser från födseln och framåt med hjälp av spridningsmodeller.

Exponering för luftföroreningar från vägtrafiken under barnens första levnadsår var relaterad till nedsatt lungfunktion vid 8 och 16 års ålder. Inget samband observerades mellan exponering för luftföroreningar någon gång i livet och lungfunktionstillväxt mellan de två tidpunkterna. Detta tyder på att exponering under första levnadsåret påverkar tidig lungfunktionstillväxt, men att individuell lungfunktion därefter följer en given utvecklingskurva. Exponering för luftföroreningar under första levnadsåret var dessutom negativt relaterad till ökning av luftvägsmotståndet i de små luftvägarna. Detta samband var starkare hos ungdomar med astma. Förutom luftföroreningar undersöktes andra miljöfaktorer och individuella födelsekarakteristika i relation till lungfunktionstillväxt mellan barndom och tonåren. Av 20 undersökta faktorer var det endast födelsevikt, ärftlighet för astma, samt exponering för passiv rökning under spädbarnstiden som påverkade lungfunktionstillväxten.

Sammanfattningsvis tyder resultaten i den här avhandlingen på att exponering för luftföroreningar under spädbarnstiden kan påverka barns och tonåringars lungfunktion, inklusive funktionen av de små luftvägarna. Vidare stödjer resultaten konceptet om en viktig period tidigt i livet och att lungfunktionen hos en individ därefter följer den utstakade utvecklingen.

8 ACKNOWLEDGEMENTS

First of all, I would like to express a special thank you to all the children and families of the BAMSE cohort. Without your participation, this thesis could never have been written. In addition, there are many people who have inspired, supported and encouraged me during these years – I would especially like to express my gratitude to:

Erik Melén, my Principle supervisor on this project. I am so grateful that you have been by my side during these years. You have a unique way of creating team spirit, and despite an intense work schedule, there is room for quick feed-back (no matter the time of day), friendship and debate on philosophical matters. I would like to emphasize that your Supervisory style has been the winning concept for me. Never do you tell how or when things are to be done (unless I ask), but you continuously question me, and thereby always push me and the work forward. Brilliant, just like you!

Göran Pershagen, my co-supervisor and head of the department of Environmental Epidemiology. I will always be grateful to you for giving me the opportunity to discover joy and curiosity that research gives. Your leadership skills will be my career inspiration. I admire your effectiveness and ability to convey that I and my skills (and that of other students) are of importance too.

Jenny Hallberg, my co-supervisor, that has become a dear friend over the years. For introducing me to the field of lung function methodology and practice. For shoring me up when needed and giving me faith in my own knowledge.

Matteo Bottai, co-supervisor and professor in Biostatistics, with tutorial skills second to none. One of few that can explain complicated statistical concepts so that I could understand them. I will especially remember, That anything goes, it is just a matter of interpretation.

Tom Bellander, expert within the field of Air Pollution. Your knowledge goes way beyond that. You are wise, your logic, accurate interpretations are impressive, as is your stylistic superiority in wording. However, above all else, I would like to express my appreciation and gratitude for your friendship, laughs and for showing that there is always room to enjoy life.

Magnus Wickman, what a fortunate coincidence that I bumped into you during my pediatric course at medical school. Thanks for making me aware of research as an option after my exam and opening the door to BAMSE. Without you, I would not be here today.

The Skövde team: **Per Gustafsson** for being an enthusiastic cicerone for all matters related to lung physiology, development and testing. It is with heartfelt appreciation I thank you as Tutor and Guide as how I may proceed in the future. Young at heart, you showed me that one can combine clinical work, methodological theory, with engineering, curiosity and subsequently research. **Sanna Kjellberg,** for inviting me to the world of lung function testing beyond spirometry.

All the BAMSE nurses and administrators group: especially **Eva Hallner, Sara Nilsson, and André Lauber**, for keeping the BAMSE-data in such a good shape and responding so nicely each time I in panic could not find a variable or needed support.

The colleagues from CAMM, for enlightening discussions during Monday meetings and "Friday fika", which now, when you are no longer in our corridor, is a sadly missed memory.

My fellow lung function team buddies: **Petra Um Bergström**, for your optimism and fun times during conferences, **Per Thunqvist**, for discussions on lung physiology, methodology and clinical implications. Also for your company working back-to-back at the office, but most of all for being a role model for me with your endless energy and ability to share your time between work and a private life.

Co-authors: I truly enjoyed the moments, when I received your comments on our manuscripts. Your experiences in various fields made the manuscript take great leaps forward every time you edited it. Many of you I have already mentioned, but also **Flaminia Chiesa** for introducing life course modeling and hands on coding tips, **Stefano Guerra** for input on respiratory infections, **Inger Kull** for your encouragement, **Anna Bergström** for being a critical manuscript reader with so many spot on reflections. Your input was invaluable when I was blind to my flaws. Your willingness to share epidemiological methods and thinking has been greatly appreciated. **Jesse Thacher**, for advice on writing on English, but most of all for introducing me to the "Kingkiller Chronicle". **Olena Gruzieva**, for great team-work with our common study. In addition for being such a caring person (handing me pieces of chocolate when working late) and taking the responsibility whenever it was time for celebrations.

Anna-Carin Olin, for being a friendly face outside KI and a true inspiration. I admire your entrepreneurship and hard work. You show that it is possible to be an inventor in a quite traditional field.

For fruitful discussions over the years, I like to thank my **Journal club friends**.

Former and present students and post-docs at the unit of Environmental epidemiology: **Anna Gref, Alva Wallas, Simon Kebede Merid, Petter Ljungman, Marcus Dahlberg, Michal Korek, Jennifer Protudjer, Charlotta Eriksson, Helen Rosenlund , Annika Sääf**, and **Johanna Penell**. Also **Sandra Ekström** and **Jessica Magnusson**, with a room just across the corridor, You often ended up in the firing line of my sometimes too spontaneous questions, suggestions and mood swings. I appreciated having you there, and I hope I haven´t bothered you too much, **Niklas Andersson** for your sharp eyes and mind related to coding **Andrei Pyko** for your patience with my lack of computer skills, and help with automatization scripts etc. Also those that have been here on- and- off: **Marit Westman, Mirja Vetander, Natalia Ballardini, Emma Johansson, Katarina Stenberg-Hammar, Åsa Neuman, Anna Asarnoj**, and **Lilly-Ann Mohlkert**. I would like to thank all of you for your contributions to create such a stimulating and great research atmosphere!

Diogo El Gaudio for being my feeder in times of despair and for fun escapes (coffee & training). Thanks for being such a good listener.

My dream team from medical school: **Mia Johansson** and **Sofia Gyllenswärd**, for all the laughs, cries, brunches, hiking, paddling, spa-days, dinners, drinks, historical excursions, tennis……..not to forget, cocktails.

My extended families: **Charlotte** and **Robert Elvander**, and **Karolina** and **Andreas Detlofsson**, for always being there, for me and my family. You are truly great! A special thanks to you Lotta, as you have been highly intimately involved and supportive during all PhD-student years. You are the only one that really knows the research and private me.

My brother and sister: **Christer** and **Carina**, you are both role models to me; Christer you have a unique combination of inner calmness, but still energetic personality. Carina; efficient as few and incredibly caring and helpful. You both have always been supportive (although no Nintendo as an 7-years old thanks to Carina). Your greatest achievements, however, are that you added more wonderful people to my life: **Magnus, Corinne, Emilia** and **Liv, Eva, Nils**.

My husband **Magnus**, This one is hard to write, as nothing in print can describe my gratitude to you. You don´t care about my research, but as it is important to me, you are my most dedicated supporter. You give me balance in life, and make me a better person. You are my love! Together with our children **Leon** and **Nora**, you make me long for home every day, and repeatedly remind me of what truly matters.

My parents: **Monica** for introducing me as a child to medicine and spreading the humanistic perspective and **Albert** for teaching me to question and be curious. Both of you, for saying that I can become anything and succeed in whatever I decide to do in life and most of all for your unconditional love. Early life matters and you gave me the best start in life.

9 REFERENCES

1. Schunemann HJ, Dorn J, Grant BJ, et al. Pulmonary function is a long-term predictor of mortality in the general population: 29-year follow-up of the Buffalo Health Study. Chest 2000;**118**(3):656-64

2. Young RP, Hopkins R, Eaton TE. Forced expiratory volume in one second: not just a lung function test but a marker of premature death from all causes. Eur Respir J 2007;**30**(4):616-22

3. Stern DA, Morgan WJ, Wright AL, et al. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet 2007;**370**(9589):758-64

4. Lange P, Celli B, Agusti A, et al. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. The New England journal of medicine 2015;**373**(2):111-22

5. Knudson RJ, Slatin RC, Lebowitz MD, et al. The maximal expiratory flow-volume curve. Normal standards, variability, and effects of age. The American review of respiratory disease 1976;**113**(5):587-600

6. Weibel ER. What makes a good lung? Swiss medical weekly 2009;**139**(27-28):375-86

7. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med 2013;**1**(9):728-42

8. Yammine S, Schmidt A, Sutter O, et al. Functional evidence for continued alveolarisation in former preterms at school age? Eur Respir J 2016;**47**(1):147-55

9. Narayanan M, Owers-Bradley J, Beardsmore CS, et al. Alveolarization continues during childhood and adolescence: new evidence from helium-3 magnetic resonance. Am J Respir Crit Care Med 2012;**185**(2):186-91

10. Green M, Mead J, Turner JM. Variability of maximum expiratory flow-volume curves. Journal of Applied Physiology 1974;**37**(1):67-74

11. Merkus PJ, Borsboom GJ, Van Pelt W, et al. Growth of airways and air spaces in teenagers is related to sex but not to symptoms. Journal of applied physiology (Bethesda, Md : 1985) 1993;**75**(5):2045-53

12. Burrows B, Knudson RJ, Lebowitz MD. The relationship of childhood respiratory illness to adult obstructive airway disease. Am Rev Respir Dis 1977;**115**(5):751-60

13. Barker DJ, Godfrey KM, Fall C, et al. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. Bmj 1991;**303**(6804):671-5

14. Cook DG, Strachan DP, Carey IM. Health effects of passive smoking. 9. Parental smoking and spirometric indices in children. Thorax 1998;**53**(10):884-93

15. Moshammer H, Hoek G, Luttmann-Gibson H, et al. Parental smoking and lung function in children: an international study. Am J Respir Crit Care Med 2006;**173**(11):1255-63

16. Campbell B, Raherison C, Lodge CJ, et al. The effects of growing up on a farm on adult lung function and allergic phenotypes: an international population-based study. Thorax 2016

17. Guilbert TW, Wright AL. Does breastfeeding impact lung function and asthma risk? American journal of respiratory and critical care medicine 2012;**185**(8):801-2

18. Turner S, Fielding S, Mullane D, et al. A longitudinal study of lung function from 1 month to 18 years of age. Thorax 2014;**69**(11):1015-20

19. Belgrave DC, Buchan I, Bishop C, et al. Trajectories of lung function during childhood. Am J Respir Crit Care Med 2014;**189**(9):1101-9

20. Lodrup Carlsen KC, Mowinckel P, Hovland V, et al. Lung function trajectories from birth through puberty reflect asthma phenotypes with allergic comorbidity. J Allergy Clin Immunol 2014;**134**(4):917-23.e7

21. Bisgaard H, Jensen SM, Bonnelykke K. Interaction between asthma and lung function growth in early life. Am J Respir Crit Care Med 2012;**185**(11):1183-9

22. Sears MR, Greene JM, Willan AR, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;**349**(15):1414-22

23. Hallberg J, Thunqvist P, Schultz ES, et al. Asthma phenotypes and lung function up to 16 years of age-the BAMSE cohort. Allergy 2015;**20**(10):12598

24. Gauderman WJ, Avol E, Gilliland F, et al. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med 2004;**351**(11):1057-67

25. Gauderman WJ, Urman R, Avol E, et al. Association of improved air quality with lung development in children. N Engl J Med 2015;**372**(10):905-13

26. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;**26**(2):319-38

27. Quanjer PH, Weiner DJ, Pretto JJ, et al. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J 2014;**43**(4):1051-8

28. Dubois AB, Brody AW, Lewis DH, et al. Oscillation mechanics of lungs and chest in man. J Appl Physiol 1956;**8**(6):587-94

29. Shi Y, Aledia AS, Tatavoosian AV, et al. Relating small airways to asthma control by using impulse oscillometry in children. J Allergy Clin Immunol 2012;**129**(3):671-8

30. McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. European clinical respiratory journal 2014;**1**

31. Goldman MD, Saadeh C, Ross D. Clinical applications of forced oscillation to assess peripheral airway function. Respir Physiol Neurobiol 2005;**148**(1-2):179-94

32. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. The European respiratory journal 2003;**22**(6):1026-41

33. Komarow HD, Myles IA, Uzzaman A, et al. Impulse oscillometry in the evaluation of diseases of the airways in children. Ann Allergy Asthma Immunol 2011;**106**(3):191-9

34. Kjellberg S, Houltz BK, Zetterstrom O, et al. Clinical characteristics of adult asthma associated with small airway dysfunction. Respiratory medicine 2016;**117**:92-102

35. WHO. Ambient air pollution: A global assessment of exposure and burden of disease. World Health Organization Report 2016

36. Perez L, Rapp R, Kunzli N. The Year of the Lung: outdoor air pollution and lung health. Swiss medical weekly 2010;**140**:w13129

37. Brunekreef B, Holgate ST. Air pollution and health. Lancet (London, England) 2002;**360**(9341):1233-42

38. Johansson C, Hadenius A, Johansson PÅ, et al. The Stockholm Study on Health effects of Air Pollution and Its Economic Consequences. Part I. NO2 and Particulate Matter in Stockholm.: Stockholm Environment and Health Protection Administration, Box 8136, 117 27 Stockholm, Sweden. Available: http://slb.nu/slb/rapporter/pdf6/lvf1999_Ex1.pdf [accessed 11 September 2010], 1999.

39. Johansson C, Andersson C, Bergström R, et al. Exposure to particles due to local and non-local cources in Stockholm- Estimates based on modelling and measurements 1997-2006. ITM-rapport 175, 2008.

40. Romieu I, Garcia-Esteban R, Sunyer J, et al. The effect of supplementation with omega-3 polyunsaturated fatty acids on markers of oxidative stress in elderly exposed to PM(2.5). Environmental health perspectives 2008;**116**(9):1237-42

41. Gilliland FD, Gauderman WJ, Vora H, et al. Effects of glutathione-S-transferase M1, T1, and P1 on childhood lung function growth. Am J Respir Crit Care Med 2002;**166**(5):710-6

42. Palli D, Saieva C, Gemma S, et al. GSTT1 and GSTM1 gene polymorphisms and gastric cancer in a high-risk italian population. Int J Cancer 2005;**115**(2):284-9

43. Gruzieva O, Merid SK, Melen E. An update on epigenetics and childhood respiratory diseases. Paediatric respiratory reviews 2014;**15**(4):348-54

44. Annesi-Maesano I, Dinh-Xuan AT. Is exhaled nitric oxide a marker of air pollution effect? The European respiratory journal 2016;**47**(5):1304-6

45. Churg A, Brauer M, del Carmen Avila-Casado M, et al. Chronic exposure to high levels of particulate air pollution and small airway remodeling. Environmental health perspectives 2003;**111**(5):714-8

46. Avol EL, Gauderman WJ, Tan SM, et al. Respiratory effects of relocating to areas of differing air pollution levels. Am J Respir Crit Care Med 2001;**164**(11):2067-72

47. Gauderman WJ, McConnell R, Gilliland F, et al. Association between air pollution and lung function growth in southern California children. Am J Respir Crit Care Med 2000;**162**(4 Pt 1):1383-90

48. Gauderman WJ, Gilliland GF, Vora H, et al. Association between air pollution and lung function growth in southern California children: results from a second cohort. Am J Respir Crit Care Med 2002;**166**(1):76-84

49. He QQ, Wong TW, Du L, et al. Effects of ambient air pollution on lung function growth in Chinese schoolchildren. Respir Med 2010;**104**(10):1512-20

50. Horak F, Studnicka M, Gartner C, et al. Particulate matter and lung function growth in children: a 3-yr follow-up study in Austrian schoolchildren. European Respiratory Journal 2002;**19**(5):838-45

51. Neuberger M, Moshammer H, Kundi M. Declining ambient air pollution and lung function improvement in Austrian children. Atmospheric Environment 2002;**36**(11):1733-36

52. Rojas-Martinez R, Perez-Padilla R, Olaiz-Fernandez G, et al. Lung function growth in children with long-term exposure to air pollutants in Mexico City. Am J Respir Crit Care Med 2007;**176**(4):377-84

53. Frye C, Hoelscher B, Cyrys J, et al. Association of lung function with declining ambient air pollution. Environ Health Perspect 2003;**111**(3):383-7

54. Dockery DW, Speizer FE, Stram DO, et al. Effects of inhalable particles on respiratory health of children. Am Rev Respir Dis 1989;**139**(3):587-94

55. Lee YL, Wang WH, Lu CW, et al. Effects of ambient air pollution on pulmonary function among schoolchildren. Int J Hyg Environ Health 2011;**214**(5):369-75

56. Peters JM, Avol E, Gauderman WJ, et al. A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. Am J Respir Crit Care Med 1999;**159**(3):768-75

57. Schwartz J. Lung function and chronic exposure to air pollution: a cross-sectional analysis of NHANES II. Environ Res 1989;**50**(2):309-21

58. Sugiri D, Ranft U, Schikowski T, et al. The influence of large-scale airborne particle decline and traffic-related exposure on children's lung function. Environ Health Perspect 2006;**114**(2):282-8

59. Raizenne M, Neas LM, Damokosh AI, et al. Health effects of acid aerosols on North American children: pulmonary function. Environ Health Perspect 1996;**104**(5):506-14

60. Nicolai T, Carr D, Weiland SK, et al. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. Eur Respir J 2003;**21**(6):956-63

61. Brunekreef B, Janssen NA, de Hartog J, et al. Air pollution from truck traffic and lung function in children living near motorways. Epidemiology 1997;**8**(3):298-303

62. Janssen NA, Brunekreef B, van Vliet P, et al. The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. Environ Health Perspect 2003;**111**(12):1512-8

63. Dales R, Wheeler A, Mahmud M, et al. The influence of living near roadways on spirometry and exhaled nitric oxide in elementary schoolchildren. Environ Health Perspect 2008;**116**(10):1423-7

64. Wjst M, Reitmeir P, Dold S, et al. Road traffic and adverse effects on respiratory health in children. Bmj 1993;**307**(6904):596-600

65. Islam T, Urman R, Gauderman WJ, et al. Parental stress increases the detrimental effect of traffic exposure on children's lung function. Am J Respir Crit Care Med 2011;**184**(7):822-7

66. Nordling E, Berglind N, Melen E, et al. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. Epidemiology 2008;**19**(3):401-8

67. Oftedal B, Brunekreef B, Nystad W, et al. Residential outdoor air pollution and lung function in schoolchildren. Epidemiology 2008;**19**(1):129-37

68. Hirsch T, Weiland SK, von Mutius E, et al. Inner city air pollution and respiratory health and atopy in children. Eur Respir J 1999;**14**(3):669-77

69. Gotschi T, Heinrich J, Sunyer J, et al. Long-term effects of ambient air pollution on lung function: a review. Epidemiology 2008;**19**(5):690-701

70. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy 2000;**55**(8):688-97

71. Molter A, Agius RM, de Vocht F, et al. Long-term exposure to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort. Environ Health Perspect 2013;**121**(10):1232-8

72. Kuehl PJ, Anderson TL, Candelaria G, et al. Regional particle size dependent deposition of inhaled aerosols in rats and mice. Inhal Toxicol 2012;**24**(1):27-35

73. van der Wiel E, ten Hacken NH, Postma DS, et al. Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review. J Allergy Clin Immunol 2013;**131**(3):646-57

74. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. Lancet Respir Med 2014;**2**(6):497-506

75. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;**40**(6):1324-43

76. Wickman M, Kull I, Pershagen G, et al. The BAMSE project: presentation of a prospective longitudinal birth cohort study. Pediatr Allergy Immunol 2002;**13 Suppl 15**:11-3

77. Hammarström U, Karlsson B. Costs and exhaust emissions for road planning (Fordonskostnader och avgasemissioner för vägplanering, EVA). Swedish National Road and Transport research Institute, S-581 95 Linköping, Sweden, Report T 150 (in Swedish), 1994.

78. Johansson C, Burman L. The Stockholm trial-Effects on Air Quality and Health. SLB report 2006;**4**

79. Bellander T, Berglind N, Gustavsson P, et al. Using geographic information systems to assess individual historical exposure to air pollution from traffic and house heating in Stockholm. Environ Health Perspect 2001;**109**(6):633-9

80. Gidhagen L, Johansson C, Langner J, et al. Urban scale modeling of particle number concentration in Stockholm. Atmospheric Environment 2005;**39**(9):1711-25

81. de Hoogh K, Korek M, Vienneau D, et al. Comparing land use regression and dispersion modelling to assess residential exposure to ambient air pollution for epidemiological studies. Environ Int 2014;**73**:382-92

82. Bellander T, Wichmann J, Lind T. Individual exposure to NO2 in relation to spatial and temporal exposure indices in Stockholm, Sweden: the INDEX study. PLoS One 2012;**7**(6):e39536

83. Ordinance on ambient air quality http://www.swedishepa.se/upload/in-english/legislation/the-swedish-environmental-code/environmental-quality-standards/air-quality-ordinance-sfs-2010-477.pdf, 2010.

84. WHO. Air quality guidelines-global update 2005. World Health Organization report 2005

85. Sweden´s environmental objectives- An introduction: Swedish Environmental Protection Agency, 2012.

86. Environment and Health Administration. Luftföroreningar i Stockholm och Uppsala län.LVF 2005:9. 2005

87. Mishra GD, Chiesa F, Goodman A, et al. Socio-economic position over the life course and all-cause, and circulatory diseases mortality at age 50-87 years: results from a Swedish birth cohort. Eur J Epidemiol 2013;**28**(2):139-47

88. Mishra G, Nitsch D, Black S, et al. A structured approach to modelling the effects of binary exposure variables over the life course. Int J Epidemiol 2009;**38**(2):528-37

89. Bottai M, Frongillo EA, Sui X, et al. Use of quantile regression to investigate the longitudinal association between physical activity and body mass index. Obesity (Silver Spring, Md) 2014;**22**(5):E149-56

90. Quanjer PH, Stocks J, Cole TJ, et al. Influence of secular trends and sample size on reference equations for lung function tests. The European respiratory journal 2011;**37**(3):658-64

91. Nowowiejska B, Tomalak W, Radlinski J, et al. Transient reference values for impulse oscillometry for children aged 3-18 years. Pediatric pulmonology 2008;**43**(12):1193-7

92. Gao Y, Chan EYY, Li LP, et al. Chronic effects of ambient air pollution on lung function among Chinese children. Archives of disease in childhood 2013;**98**(2):128-35

93. Gauderman WJ, Vora H, McConnell R, et al. Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study. Lancet 2007;**369**(9561):571-7

94. Neophytou AM, White MJ, Oh SS, et al. Air Pollution and Lung Function in Minority Youth with Asthma in the GALA II (Genes-Environments and Admixture in Latino Americans) and SAGE II (Study of African Americans, Asthma, Genes, and Environments) Studies. Am J Respir Crit Care Med 2016;**193**(11):1271-80

95. Svendsen ER, Gonzales M, Mukerjee S, et al. GIS-Modeled Indicators of Traffic-Related Air Pollutants and Adverse Pulmonary Health Among Children in El Paso, Texas. American Journal of Epidemiology 2012;**176**:S131-S41

96. Rice MB, Rifas-Shiman SL, Litonjua AA, et al. Lifetime Exposure to Ambient Pollution and Lung Function in Children. Am J Respir Crit Care Med 2016;**193**(8):881-8

97. Wang M, Gehring U, Hoek G, et al. Air Pollution and Lung Function in Dutch Children: A Comparison of Exposure Estimates and Associations Based on Land Use Regression and Dispersion Exposure Modeling Approaches. Environmental Health Perspectives 2015;**123**(8):847-51

98. Eeftens M, Hoek G, Gruzieva O, et al. Elemental Composition of Particulate Matter and the Association with Lung Function. Epidemiology 2014;**25**(5):648-57

99. Gehring U, Beelen R, Eeftens M, et al. Particulate Matter Composition and Respiratory Health The PIAMA Birth Cohort Study. Epidemiology 2015;**26**(3):300-09

100. Turnovska TH, Marinov BI. The influence of air pollution during intrauterine development and early childhood on respiratory functions at later age. Int J Hyg Environ Health 2009;**212**(5):519-32

101. Gehring U, Gruzieva O, Agius RM, et al. Air Pollution Exposure and Lung Function in Children: The ESCAPE Project. Environmental Health Perspectives 2013;**121**(11-12):1357-64

102. Fuertes E, Bracher J, Flexeder C, et al. Long-term air pollution exposure and lung function in 15 year-old adolescents living in an urban and rural area in Germany: The GINIplus and LISAplus cohorts. International Journal of Hygiene and Environmental Health 2015;**218**(7):656-65

103. Korten I, Ramsey K, Latzin P. Air pollution during pregnancy and lung development in the child. Paediatric respiratory reviews 2016

104. Latzin P, Roosli M, Huss A, et al. Air pollution during pregnancy and lung function in newborns: a birth cohort study. Eur Respir J 2009;**33**(3):594-603

105. Zheng XY, Ding H, Jiang LN, et al. Association between Air Pollutants and Asthma Emergency Room Visits and Hospital Admissions in Time Series Studies: A Systematic Review and Meta-Analysis. PLoS One 2015;**10**(9):e0138146

106. Chalupa DC, Morrow PE, Oberdorster G, et al. Ultrafine particle deposition in subjects with asthma. Environ Health Perspect 2004;**112**(8):879-82

107. Bates JH, Suki B. Assessment of peripheral lung mechanics. Respir Physiol Neurobiol 2008;**163**(1-3):54-63

108. Bickel S, Popler J, Lesnick B, et al. Impulse oscillometry: interpretation and practical applications. Chest 2014;**146**(3):841-7

109. Oppenheimer BW, Goldring RM, Herberg ME, et al. Distal airway function in symptomatic subjects with normal spirometry following World Trade Center dust exposure. Chest 2007;**132**(4):1275-82

110. Gouveia N, Fletcher T. Time series analysis of air pollution and mortality: effects by cause, age and socioeconomic status. Journal of epidemiology and community health 2000;**54**(10):750-5

111. Cakmak S, Hebbern C, Cakmak JD, et al. The modifying effect of socioeconomic status on the relationship between traffic, air pollution and respiratory health in elementary schoolchildren. Journal of environmental management 2016;**177**:1-8

112. Patel MM, Miller RL. Air pollution and childhood asthma: recent advances and future directions. Current opinion in pediatrics 2009;**21**(2):235-42

113. Morales E, Garcia-Esteban R, Asensio de la Cruz O, et al. Intrauterine and early postnatal exposure to outdoor air pollution and lung function at preschool age. Thorax 2014

114. Carlsten C, Brauer M, Dimich-Ward H, et al. Combined exposure to dog and indoor pollution: incident asthma in a high-risk birth cohort. Eur Respir J 2011;**37**(2):324-30

115. Jorres R, Nowak D, Magnussen H. The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med 1996;**153**(1):56-64

116. Carlsten C, Blomberg A, Pui M, et al. Diesel exhaust augments allergen-induced lower airway inflammation in allergic individuals: a controlled human exposure study. Thorax 2016;**71**(1):35-44

117. Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. Immunological reviews 2011;**242**(1):205-19

118. Clifford RL, Jones MJ, MacIsaac JL, et al. Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation. J Allergy Clin Immunol 2016

119. Guerra S, Stern DA, Zhou M, et al. Combined effects of parental and active smoking on early lung function deficits: a prospective study from birth to age 26 years. Thorax 2013;**68**(11):1021-8

120. Carey MA, Card JW, Voltz JW, et al. It's all about sex: gender, lung development and lung disease. Trends in endocrinology and metabolism: TEM 2007;**18**(8):308-13

121. Doershuk CF, Fisher BJ, Matthews LW. Specific airway resistance from the perinatal period into adulthood. Alterations in childhood pulmonary disease. Am Rev Respir Dis 1974;**109**(4):452-7

122. Hsu HH, Chiu YH, Coull BA, et al. Prenatal Particulate Air Pollution and Asthma Onset in Urban Children. Identifying Sensitive Windows and Sex Differences. Am J Respir Crit Care Med 2015;**192**(9):1052-9

123. Keselman A, Heller N. Estrogen Signaling Modulates Allergic Inflammation and Contributes to Sex Differences in Asthma. Frontiers in immunology 2015;**6**:568

124. Eenhuizen E, Gehring U, Wijga AH, et al. Traffic-related air pollution is related to interrupter resistance in 4-year-old children. Eur Respir J 2013;**41**(6):1257-63

125. Turner S, Zhang G, Young S, et al. Associations between postnatal weight gain, change in postnatal pulmonary function, formula feeding and early asthma. Thorax 2008;**63**(3):234-9

126. Kotecha SJ, Edwards MO, Watkins WJ, et al. Effect of preterm birth on later FEV1: a systematic review and meta-analysis. Thorax 2013;**68**(8):760-6

127. Kotecha SJ, Watkins WJ, Paranjothy S, et al. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. Thorax 2012;**67**(1):54-61

128. McEvoy CT, Spindel ER. Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health. Paediatric respiratory reviews 2016

129. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999;**354**(9178):541-5

130. Edmond K, Scott S, Korczak V, et al. Long term sequelae from childhood pneumonia; systematic review and meta-analysis. PLoS One 2012;**7**(2):e31239

131. den Dekker HT, Sonnenschein-van der Voort AM, de Jongste JC, et al. Early growth characteristics and the risk of reduced lung function and asthma: A meta-analysis of 25,000 children. J Allergy Clin Immunol 2016;**137**(4):1026-35

132. Narang I, Rosenthal M, Cremonesini D, et al. Longitudinal evaluation of airway function 21 years after preterm birth. Am J Respir Crit Care Med 2008;**178**(1):74-80

133. Mack I, Kieninger E, Cangiano G, et al. Rhinovirus Infections and Associated Respiratory Morbidity in Infants: A Prospective Cohort Study. The Pediatric infectious disease journal 2016

134. Copenhaver CC, Gern JE, Li Z, et al. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 2004;**170**(2):175-80

135. Wu K, Gamazon ER, Im HK, et al. Genome-wide interrogation of longitudinal FEV1 in children with asthma. Am J Respir Crit Care Med 2014;**190**(6):619-27

136. Rothman KJ, Greenland S, Lash T. *Modern Epidemiology*. 3 ed: Philadelphia: Lippincott Williams & Wilkings, 2008.

137. Borsboom GJ, van Pelt W, van Houwelingen HC, et al. Diurnal variation in lung function in subgroups from two Dutch populations: consequences for longitudinal analysis. American journal of respiratory and critical care medicine 1999;**159**(4 Pt 1):1163-71

138. Tweeddale PM, Alexander F, McHardy GJ. Short term variability in FEV1 and bronchodilator responsiveness in patients with obstructive ventilatory defects. Thorax 1987;**42**(7):487-90

139. Rozas CJ, Goldman AL. Daily spirometric variability: normal subjects and subjects with chronic bronchitis with and without airflow obstruction. Archives of internal medicine 1982;**142**(7):1287-91

140. Avol EL, Navidi WC, Rappaport EB, et al. Acute effects of ambient ozone on asthmatic, wheezy, and healthy children. Research report (Health Effects Institute) 1998(82):iii, 1-18; discussion 19-30

141. Eneroth K, Johansson C, Bellander T. EXPOSURE - Comparison between Measurements and Calculations Based on Exposure Modelling. Report LVF 2006:12. http://www.slb.nu/slb/rapporter/pdf/lvf2006_12.pdf Accessed 11 June 2011.

142. Johansson C, Andersson C, Bergström R, et al. Exposure to particles due to local and non-local sources in Stockholm - Estimates based on modelling and measurements 1997 - 2006. Department of Applied Environmental Science, Stockholm University. ITM 2008:175. http://www.slb.nu/slb/rapporter/pdf8/itm2008_175.pdf Accessed 10 June 2011.

143. Tantisira KG. In asthma, the apple falls faster than the pear. J Allergy Clin Immunol 2009;**123**(5):1075-6

144. Adler NE, Ostrove JM. Socioeconomic status and health: what we know and what we don't. Annals of the New York Academy of Sciences 1999;**896**:3-15

145. Searing DA, Rabinovitch N. Environmental pollution and lung effects in children. Current opinion in pediatrics 2011;**23**(3):314-8

146. O'Neill MS, Jerrett M, Kawachi I, et al. Health, wealth, and air pollution: advancing theory and methods. Environmental health perspectives 2003;**111**(16):1861-70

147. Heinrich J, Slama R. Fine particles, a major threat to children. International journal of hygiene and environmental health 2007;**210**(5):617-22

148. Goldman MD, Carter R, Klein R, et al. Within- and between-day variability of respiratory impedance, using impulse oscillometry in adolescent asthmatics. Pediatric pulmonology 2002;**34**(4):312-9

149. Hoek G, Pattenden S, Willers S, et al. PM10, and children's respiratory symptoms and lung function in the PATY study. Eur Respir J 2012;**40**(3):538-47

150. Schultz ES, Gruzieva O, Bellander T, et al. Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. American journal of respiratory and critical care medicine 2012;**186**(12):1286-91

151. Schultz ES, Hallberg J, Gustafsson PM, et al. Early life exposure to traffic-related air pollution and lung function in adolescence assessed with impulse oscillometry. The Journal of allergy and clinical immunology 2016;**138**(3):930-32.e5

152. Schultz ES, Hallberg J, Bellander T, et al. Early-Life Exposure to Traffic-related Air Pollution and Lung Function in Adolescence. Am J Respir Crit Care Med 2016;**193**(2):171-7

10 APPENDIX

10.1 SUMMARY TABLES

I searched the PubMed databases for English-language publications from 2006 and onwards, with the terms (TRAP OR (traffic AND(pollut* OR emission* OR PM* OR NO* OR particle*))) AND (“Lung function” OR spirometry OR “pulmonary function” OR “forced expiratory” OR “FEV*” OR “Impulse oscillometry” OR “forced oscillat* techniq*” OR “FOT” OR “lung volume” OR “small airway*” OR “peripheral airway”) AND (Children* OR “school-age*” OR “pre-school*” OR infant* OR adolesce*) ,as well as (TRAP OR traffic OR pollut* OR emission* OR PM* OR NO* OR particle*))) AND (“Impulse oscillometry” OR “small airway*" OR "peripheral airway*”).

I also identified references from the bibliographies of these publications and from a review article by Gotschi et al from 2008[69]. Included articles had longitudinal air pollution data (about 1-year estimates) or proxies for long-term traffic-related air pollution (like traffic counts/density). Articles with ozone as the only exposure estimate were excluded. In summary, 31 articles with cross-sectional lung function data (Table A1) were included, as were 12 articles with longitudinal lung function data (Table A2).

Table A1: Cross-sectional studies of long-term exposure to air pollution and lung function (LF) in children and adolescents

Author, year	Country (cohort name)	N	Ages (years)	Exposure(s)	Outcome(s)	Subgroup(s)	Main findings
Brunekreef, 1997[61]	Netherlands	877	7-12	Distance to motorway, traffic density, school indoor measurements PM_{10}, NO_2	FEV_1, FVC, PEF, FEF_{25-75}	Gender	Negative association of truck traffic on LF, stronger effects in girls
Cakmak, 2016[111]	Canada	1,528	9-11	Traffic-counts and $PM_{2.5}$, NO_2, SO_2 (LUR-annual averages)	FEV_1, FVC, eNO	SES	Effects on FEV_1 and FVC in low income group.
Dales, 2008[63]	Canada	1,613	11	NO_2 , $PM_{2.5}$, PM_{coarse}, $PM_{2.5\ soot}$, SO_2 (LUR annual average at current address), roadway density	FEV_1, FVC, eNO	Asthma	Significant positive effects for roadway density and PM on eNO, stronger in subjects with asthma.
Dockery, 1989[54]	U.S (The 6 cities study)	5,422	10-12	TSP, $PM_{1.5}$, $PM_{2.5}$,SO_4, SO_2, O_3, and NO_2 (central city monitoring stations, daily, monthly, annual mean)	FEV_1, FVC, $FEV_{0.75}$, and FEF_{25-75}	Asthma and wheeze	Null effects for lung function
Eeftens, 2014[98]	Europe (ESCAPE)	4,659	6-8	PM_{10} and $PM_{2.5}$: elemental composition (LUR at current addresses)	FEV_1, $FEV_{0.5}$, FVC, PEF	Asthma status	Small effects related to nickel and sulfur. Heterogeneity across cohorts. Stronger effects in children with asthma.
Eenhuizen, 2013[124]	Netherlands (PIAMA)	880	4	NO_2, $Pm_{2.5,\ soot}$ (LUR annual averages at birth address)	Interrupter resistance (R_{int})	Gender, Parental allergy	Positive association between air pollution at birth address and R_{int}, no effect modification.
Frye, 2003[53]	Germany	1,911	11-12	Total suspended particles (TSP) and SO_2 - daily and annual averages - community monitoring	FEV_1, FVC	Gender	Effects of reduction in TSP and an increase in FVC and possible to a smaller degree in FEV_1, especially in girls.
Fuertes, 2015[102]	Germany (LISA & GINI)	2,266	15	NO_2, $PM_{2.5}$, $PM_{2.5}$absorbance, O_3 (LUR - annual averages at birth, 10-yr and current residential address)	FEV_1, FVC, FEF_{25}, FEF_{50}, FEF_{75}, FEF_{25-75}, PEF, < LLN	Gender, Asthma status	No convincing overall effects, but indications of effects from current exposure in subjects with asthma
Gao, 2013[92]	China	3,168	8-10	PM_{10}, SO_2, NO_2, O_3 (Lifetime and current annual averages from local monitoring stations)	FEV_1, FVC, FEF_{25-75}, FEF_{75}	Gender	FEV_1, FEF_{25-75} and FEF_{75} were significantly lower in boys in high pollution district than in low pollution district.
Gehring, 2013[101]	Europe (ESCAPE)	5,921	6-8	NO_2, NO_x, PM_{10}, $PM_{2.5}$, PM_{coarse}, $PM_{2.5}$ absorbance (LUR- annual averages at birth and current address)	FEV_1, $FEV_{0.5}$, FVC, PEF	Asthma, gender sensitization	Estimated levels of NO_2, NO_x, $PM_{2.5\ absorbance}$, and $PM_{2.5}$ at the current address, but not at the birth address, were associated with small decreases in lung function.
Gehring, 2015 [99]	Netherlands (PIAMA)	3,702	11-12	PM constituents, $PM_{2.5}$, PM_{10} (LUR- annual averages at birth and current address)	FEV_1, FVC, FEF_{25-75}	Allergy, SES	Copper and iron (from $PM_{2.5}$) at current address was negatively associated with FEV_1, also FEF_{25-75} (with copper from PM_{10})
Hirsch, 1999[68]	Germany	1,137	9-11	SO_2, NO_2, CO, benzene, O_3 (Modeled previous year averages based on measurements stations-residential and school addresses)	FEV_1, FEF_{25-75}, BHR	None	Null effects for lung function and BHR
Hoek, 2012[149]	Multicentre	22,809	6-12	PM_{10} , NO_2, and SO_2 from local monitoring stations, approximately one year previous spirometry test.	FEV_1, FVC, FEF_{25-75}, PEF	Gender,wheeze, sensitization	Null effects in combined analyses on lung function.
Islam, 2011[65]	US (CHS)	1,399	11	NO_x, NO_2, NO (LUR at school and resident)	FEV_1, FVC	Parental stress level, No asthma	Sign negative association of NO_x and FEV_1, in high stress households. Effect remained in non-asthmatics.
Janssen, 2003[62]	Netherlands	1,726	7-12	Truck/car traffic counts, $PM_{2.5}$ and NO_2 (Estimated averages during previous year based on measurements at schools)	FEV_1, FVC, FEF_{25-75}, BHR	Sensitized, +BHR	Null effects for lung function and BHR
Lee, 2011[55]	Taiwan (TCHS)	3,957	12-13	CO, NO_x, NO, NO_2, O_3, SO_2, $PM_{2.5}$, PM_{10} (community based monitoring data- annual, and monthly averages)	FEV_1, FVC, FEF_{25-75}, PEF	Gender, asthma	Sign negative association of annual CO, NO_x, NO_2 and NO with FVC and FEV_1, especially in boys.
Morales , 2015[113]	Spain (INMA)	620	4.5	NO_2 and benzene (LUR; Trimester specific, first year of life, previous year, current(1 week))	FVC, FEV_1, PEF, FEF_{25-75}, < 80 % predicted	Gender asthma, SES, allergy	Strongest association on LF after exposure in 2nd trimester of pregnancy, especially among allergic children and those of low SES. (Negative associations, but not significant for the other time periods)

Table A1. (Continued)

Author, year	Country (cohort name)	N	Ages (years)	Exposure(s)	Outcome(s)	Subgroup(s)	Main findings
Neophytou, 2016[94]	U.S and Puerto Rico (GALA II and SAGE II)	1,968	8-21	NO_2, SO_2, O_3, $PM_{2.5}$, PM_{10} (Calculated at residents from 4 monitoring stations. Monthly, annual, and lifetime averages)	FEV_1, FVC FEF_{25-75}	Global genetic ancestry	Lifetime average and first year of life $PM_{2.5}$ was associated with reduced FEF_{25-75} and FEV_1
Nicolai, 2003[60]	Germany	904	9-11	Proximity to traffic and traffic counts, benzene, NO_2, and soot (Estimated residential averages during previous year based on measurements stations)	Spirometry	SHS	Null effects for lung function and BHR
Nordling, 2008[66]	Sweden (BAMSE)	2,599	4	NO_x, PM_{10}, SO_2 (First year of life averages based on dispersion model at residential addresses)	PEF	Gender, wheezing	Effects on PEF, most strong for PM_{10}. No effect modification.
Oftedal, 2008[67]	Norway	2,307	9-10	NO_2, PM_{10}, $PM_{2.5}$ (Early life, lifetime, and current annual averages based on dispersion model at residential addresses)	FEV_1, FVC, FEF_{25}, FEF_{50}, PEF	Gender, asthma, ethnicity	Negative association between all time periods of exposures and PEF, FEF_{25} and FEF_{50}, especially in girls. Slightly stronger effect from first year of life exposures. Stronger PM, and weaker NO_2 effects in asthmatics.
Peters, 1999[56]	U.S	3,293	9-16	NO_2, PM_{10},O_3, acid vapor (12 communities - local monitoring stations during yrs 1986-1990, and year 1994)	FEV_1, FVC, FEF_{25-75}, PEF	Gender	Effects on FEV_1, FVC and FEF_{25-75} in girls. Stronger effects from current exposure.
Raizenne, 1996[59]	U.S and Canada	10,251	8-12	Particles and gaseous pollutants. Community level: Previous year averages based on measurements.	FEV_1, FVC, $FEV_{0.75}$, FEF_{25-75}, PEF, FVC < 85 % predicted	Gender	Effects on FEV_1, FVC and FEF, especially strong in association with particle acidity. No gender difference.
Rice, 2016[96]	U.S (Project Viva)	614	8	Proximity to major road, $PM_{2.5}$ (hybrid model) and BC (LUR):LUR and hybrid model: First year of life, life time, Previous year	FEV_1, FVC, bronchodilator response	Gender, SES, SHS, asthma	Prior year and lifetime PM and BC –significant with FVC (and none significant with FEV_1), also higher OR of <80% predicted. No effect modification by asthma, gender or SHS. Stronger effects in high income households.
Schultz, 2012[150]	Sweden (BAMSE)	1,924	8	PM_{10}, NO_x (first year of life, 1-4 yrs, and 4-8 yrs averages based on DM at addresses)	FEV_1, $FEV_{0.5}$, FVC, < 80 %, < 85% predicted	Gender, sensitization	Associations between first year of life exposure and mainly FEV_1.
Schultz, 2016[151]	Sweden (BAMSE)	2,415	16	PM_{10}, NO_x (first year of life, previous year averages based on DM at addresses)	Impulse oscillometry measurements	Gender, asthma, sensitization,	Associations between first year of life exposure and indices related to function in the small airways, especially in those with asthma.
Schwartz, 1989[57]	U.S	3,922	6-24	TSP, O_3, NO_2, SO_2 (Community level annual averages)	FEV_1, FVC, PEF	No	Effects on lung function from all pollutants (except SO_2). Threshold effects for TSP, and O_3.
Sugiri, 2006[58]	Germany	2,574	5-7	TSP and SO_2 -daily and annual averages - community monitoring (background levels), and residential distance to major road	TLC, airway resistance	No	Better total lung capacity (TLC) when TSP decreased, but not related to distance from traffic
Svendsen, 2012[95]	U.S	1,529	10	NO_2 and diesel related compounds (LUR at school and residential address)	FEV_1, FVC, < 85% predicted	Gender, Asthma, SHS	Negative associations between NO_x and FVC. Increased OR of < 85 % predicted FEV_1 and FVC. Stronger effect in children not exposed to SHS.
Wang, 2015[97]	The Netherlands (PIAMA)	1,058	8	NO_2 , $PM_{2.5}$, PM_{10}, $PM_{2.5\ soot}$ (LUR and DM - birth and current address)	FEV_1, FVC, PEF	No	Negative associations all pollutants and FEV_1 and FVC, not PEF
Wjst, 1993[64]	Germany	4,320	9-11	Traffic-density in school district (as a proxy for long-term exposure - minimum of 5 years at current residence)	FEV_1, PEF, FEF_{25}, FEF_{50}, FEF_{75},	No	Effects on PEF and FEF_{25}, and FEF_{50}.

Table A2: Longitudinal studies of long-term exposure to air pollution and lung function in children and adolescents

Author (year)	Country (cohort)	N	Age at start (follow-up time) [#]	Exposure(s)	Outcome(s)	Subgroup(s)	Main findings
Avol, 2001[46]	U.S (CHS)	110	10 (5)	PM_{10}, NO_2, O_3 (Annual averages from community monitoring stations)	FEV_1, FVC, FEF_{25-75}, PEF	No	LF (not FVC) lowered when subjects moved to high PM_{10}-level areas and increased when moving to low PM_{10} areas.
Gauderman, 2000[47]	U.S (CHS)	3,035	4th, 7th and 10th graders (4)	PM_{10}, $PM_{2.5}$, PM_{coarse}, NO_2, O_3, inorganic acid vapor (Annual averages from community monitoring stations)	FEV_1, FVC, FEF_{25-75}, FEF_{75}	Gender, Asthma	Most effects in fourth graders: deficits in LF growth related to increase in all pollutants (except O_3). No effect modification by asthma or gender.
Gauderman, 2002[48]	U.S (CHS)	1,678	4th graders (4)	PM_{10}, $PM_{2.5}$, PM_{coarse}, NO_2, O_3, inorganic acid vapor, elemental carbon (Annual averages from community monitoring stations)	FEV_1, FVC, FEF_{25-75}, FEF_{75}, PEF	Gender, Asthma	Replication, but less strong effects of results from Gauderman 2000. Effects from O_3. No effect modification by asthma or gender.
Gauderman, 2004[24]	U.S (CHS)	1,759	10 (8)	PM_{10}, $PM_{2.5}$, PM_{coarse}, NO_2, O_3, inorganic acid vapor, elemental carbon (Annual averages from community monitoring stations)	FEV_1, FVC, FEF_{25-75}, FEF_{75}, FEV_1< 80 % predicted	No Asthma , No smoking	Effects of NO_2, acid vapor, EC, and $PM_{2.5}$ on LF. Increased OR of <80% predicted if high exposed. Effect remained in non-asthmatics and non-smokers
Gauderman, 2007[93]	U.S (CHS)	3,677	10 (8)	Proximity to traffic, regional measurements	FEV_1, FVC, FEF_{25-75}, FEF_{75}, FEV_1< 80 % predicted	Gender, No asthma, No smoking	Effects on FEV_1 and FEF_{25-75} growth independently for proximity to traffic and regional levels. Effects mainly in boys. Effect remained in non-asthmatics and non-smokers
Gauderman, 2015[25]	U.S (CHS)	2,120	11 (4)	PM_{10}, $PM_{2.5}$, PM_{coarse}, NO_2, O_3, (Annual averages from community monitoring stations)	FEV_1, FVC, < 90 %, <85 % , and <80 % predicted	Gender, Asthma status	Improvements in FEV_1 and FVC growth related to declining levels of PM_{10}, $PM_{2.5}$, and NO_2. Stronger effects in boys.
He, 2010[49]	China	1,983	8 -10 (0.5)	PM_{10}, SO_2, NO_2 (Lifetime and current annual averages from local monitoring stations)	FEV_1, FVC, FEF_{25}, FEF_{25-75}, FEF_{75}	Gender	Children living in high polluted district showed significant deficits in FEV_1, FEF_{25} and FEF_{25-75} growth. No effect modification by gender.
Horak, 2002[50]	Austria	860	6 (3)	PM_{10}, NO_2, O_3 (6-month averages from community monitoring stations in proximity to school)	FEV_1, FEF_{25-75}	Asthma, ETS	Effects of NO_2 and O_3 on FVC and FEV_1, Summer PM_{10} were negatively associated with growth of FEV_1 and FEF_{25-75}. No clear effect modification.
Mölter, 2013[71]	U.K (MAAS)	1,185	3 (8)	PM_{10} and NO_2 (Different time-windows over the life course and previous year, individual estimates by micro-environmental model based on LUR)	sRAW, FEV_1, bronchodilator	No	Small, but significant deficits in growth of FEV_1. Stronger effects after bronchodilator treatment, especially in relation to early life exposures.
Neuberger, 2002[51]	Austria	3,451	Elementary school age (5)	NO_2, SO_2, TSP (calculated at school addresses from regional monitoring stations)	FEV_1, FVC, FEF_{25}, FEF_{25-75}, FEF_{75}	No	Faster FEF_{25}, and FEF_{25-75} growth in areas with NO_2 level reductions
Rojas-Martinez, 2007[52]	Mexico	3,170	8 (3)	PM_{10}, NO_2, SO_2, O_3 (6-month average from community monitoring stations in proximity to school)	FEV_1, FVC, FEF_{25-75}	Gender	Reduced FEV_1 and FVC growth (also for ratio) in areas with high levels of PM_{10}. NO_2, and O_3. No convincing gender difference.
Schultz, 2016[152]	Sweden (BAMSE)	2,278	8 (8)	PM_{10}, NO_x (first year of life, 1-8yrs, 8-16 yrs averages based on DM at addresses)	FEV_1, FVC,< LLN	Gender, asthma, sensitization, ETS, Maternal smoking during pregnancy	Associations between first year of life exposure and FEV_1 at 8 and 16 years of age, but not with the change between 8 and 16 years. Effect modification by ETS and maternal smoking during pregnancy and/or infancy.

[#]In years if not otherwise stated.

10.2 VARIABLE DEFINITIONS

10.2.1 Table A3: Assessment and definitions of effect modifiers

Table A3: Assessment and definitions of effect modifiers

Variable	Assessment and definitions	Used in Study
Asthma at 8 and 16 years	At least 4 episodes of wheeze in the last 12 months or at least 1 episode in combination with prescription of inhaled corticosteroids.	I, II, III
Sensitization at 8 and 16 years	Any common inhalant and/or food allergens: IgE values for Phadiatop ® [a mix of common inhalant allergens: birch, timothy, mugwort, cat, dog, horse, mold and house dust mite] ≥0.35kU/l and /or IgE-value for fx5® (a mix of common food allergens: cow's milk, egg white, soy bean, peanut, cod fish and wheat) ≥0.35kU/l	I, II, III
Environmental tobacco smoke exposure (ETS):	Any of the parents smoked daily at the time of questionnaires 16 (Independent of the amount of cigarettes).	II
Adolescent smoking at sixteen years:	Smoking regularly or occasionally at the time of questionnaire 16.	II
Maternal smoking during pregnancy or at baseline (2 months):	The mother smoked at least one cigarette per day at the time of questionnaire 0 and/or smoked at least one cigarette per day at any point of time during the pregnancy.	II
Gestational age	Information retrieved from medical birth registry 3 categories: < 37 weeks, 37 to 41 weeks, > 41 weeks	II

10.2.2 Table A4: Assessment and definitions of covariates

Table A4: Assessment and definitions of covariates

Variable	Assessment and definitions	Used as confounder in Study
Municipality at birth	Place of residence at time of birth: Järfälla, Sundbyberg, Solna, Northern part of inner-city	I, II, III, IV[#]
Heredity for asthma/allergy:	Mother and/or father with doctor's diagnosis of asthma and asthma medication and/or doctor's diagnosis of hay fever in combination with furred pets- and/or pollen allergy at the time of questionnaire 0.	I
Short-term air pollution exposure	Hourly mean values from roof top monitoring stations: PM_{10} (Study I), and NO_x (Study III) levels for 1 to 3 days prior LF-test, or 1 to 7 days prior LF- test. Used also in combination with relative humidity, temperature, and O_3	I*, III*
Moving any time during follow up	Change place of residence any time during age 0 to 16 years.	III*

[#]Only used in analyses of traffic-related air pollution exposure
*Only in sensitivity analyses