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Environmental Exposures And Childhood Pulmonary Function

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ENVIRONMENTAL EXPOSURES AND CHILDHOOD PULMONARY FUNCTION

by

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DEDICATION

To my parents, Mom…you have been the main source of motivation and the strongest support system for me. You have inspired me to work hard and achieve greatness. I still remember you saying to me when I graduated from junior college that it does not matter what you do in your life just try to be the best in whatever you do. I could not have been here if it was not for you. It is impossible to thank you adequately for everything that you have done for me. Dad, you are not with us anymore but I know that your love was unconditional and you would have been proud of me today.

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ABSTRACT

Fetal origins of adult disease hypothesis states that adverse influences early in developmental period and particularly during intrauterine life can result in permanent changes in physiology which may lead to increased disease risk of chronic diseases in adulthood. Both fetal and adolescent period are critical time periods for development of lungs. Any adverse environmental exposures during these critical periods of lung growth is a form of programming which can have long term effects on pulmonary function. The purpose of this dissertation was to examine the association between different environmental exposures and pulmonary function in children and late adolescents. The first objective was to investigate the association between sensitization to house dust mites (HDM) allergen on skin prick test (SPT) and pulmonary function during late adolescence (18 years of age) within the birth cohort of Isle of Wight (IOW). The second objective was to assess the combined effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in late adolescence. This association was also tested in the birth cohort of IOW at 18 years of age. The third objective was to examine relationship between body burden of dichlorodiphenyl dichloroethene (DDE) and pulmonary function in pre-adolescent children (8-10 years of age) from federal state of Hesse, Germany.

The results from the first objective suggested that there exists an inverse dose response relationship between wheal diameter (immunological response obtained on SPT

to HDM allergen) and pulmonary function parameters- forced expiratory volume in one second (FEV_1), ratio of forced expiratory volume in one second over forced vital capacity (FEV_1/FVC) and forced expiratory flow at 25-75% ($FEF_{25-75\%}$). Stratified analysis by history of asthmatic wheezing episodes showed reduced FEV_1 and FVC in individuals with no history of asthmatic wheezing attacks.

For the second objective we found that the offspring of mothers who smoked during pregnancy are more likely to smoke during adolescence. Results showed that in girls maternal smoking during pregnancy and adolescent smoking had both independent and joint inverse effects on FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ when compared with no exposure. Path analysis demonstrated that the effects of maternal smoking during pregnancy in girls were mediated through height, weight and adolescent smoking. In boys, adolescent smoking had direct inverse effect on FEV_1/FVC ratio and effects of maternal smoking were mediated through its effects on adolescent smoking.

Results from the third objective demonstrated that DDE had crude inverse associations with height, weight, FEV_1 and FVC in children of age 8-10 years. Further analysis showed that although DDE exposure did not affect pulmonary function directly in children, it had indirect effect on pulmonary function mediated through height and weight at age 8-10 years.

Children are particularly susceptible to environmentally induced lung diseases. All three environmental exposures studied in this dissertation showed adverse effects on pulmonary function during childhood and late adolescence. These findings indicated that the wheal diameter on SPT which is mostly used to determine sensitivity to HDM allergen could also serve has an indicator of underlying abnormal pulmonary function

even in individuals without asthma symptoms. Second objective showed that exposure to gestational and adolescent smoking lead to reduced flow in mid as well as large airways. Adolescent girls were more vulnerable to smoking effects than boys. Finally, the use of path analysis improved the understanding of underlying directional or non-directional relationships between height, weight and DDE exposure on pulmonary function. These findings have implications in the areas of environmental epidemiology, respiratory epidemiology and child health epidemiology.

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μg/L Blood levels of DDE in microgram per liter.

List of Abbreviations

AGFI ..... Adjusted Goodness of Fit Index

ANOVA ..... Analysis of Variance

ATS ..... American Thoracic Society

CFI ..... Comparative Fit Index

CM ..... Centimeter

DDE ..... Dichlorodiphenyl dichloroethene

DDT ..... Dichlorodiphenyl trichloroethane

ETS ..... Environmental Tobacco Smoke

$FEF_{25-75\%}$ ..... Forced Expiratory Flow at 25-75%

FeNO ..... Fractional exhaled Nitric Oxide

FEV_1 ..... Forced Expiratory Volume in One second

FIML ..... Full Information Maximum Likelihood

FVC ..... Forced Vital Capacity

GLI ..... Global Lung Function Initiative

HDM ..... House Dust Mite

IgE ..... Immunoglobin E

IOW ..... Isle of Wight

KG ..... Kilogram

L ..... Liter

M ..... Meter

MCMC ..... Markov Chain Monte

CarloMI ..... Multiple Imputations

MM ..... Millimeter

PPB ..... Parts Per Billion

RMSEA ..... Root Mean Square Error of Approximation

SPT ..... Skin Prick Test

WHO ..... World Health Organization

CHAPTER 1

INTRODUCTION

It is a well-known fact that the environment we live in plays an important role in determining our health. Children in particular are more vulnerable to environmental effects because most of the time they have no control over their prenatal or postnatal environment including the quality of the air they breathe, the water they drink, the food they eat, and their place of residence. The lungs are a common site of environmentally induced diseases. Epidemiological studies in last 40-50 years have shown a worrying increase of respiratory disorders in children. According to European Respiratory Society's report, about one-quarter of primary care consultations among children relate to respiratory complaints and asthma and allergic disorders are the most common form of respiratory disorders in children.[1]

Epidemiological studies have provided evidence that a number of environmental factors contribute in the development of asthma.[2-4] These include family history of asthma,[5,6] indoor allergens,[7-9] air pollutants,[10-12] tobacco smoke,[13-17] prenatal factors[18-20] and exposure to chemical irritants.[21] These environmental factors are considered not only to result in a higher prevalence of asthma in children but are also associated with reduction in pulmonary function in children. Studies have shown that infants who had family history of asthma had lower pulmonary function and increased airway hyperreactivity.[6,22] Evidence from other epidemiological studies also suggest that risk

factors such as a positive reaction to allergy skin test,[23,24] air pollution,[25,26] maternal smoking during pregnancy and personal smoking by adolescents may lead to reduction in pulmonary function in adolescents and adults.[13-17]

The respiratory tract has a resilient immune system which can reduce the effect of potentially harmful inhaled environmental pollutants mentioned above. However, the protective mechanism can overcome depending on type, duration and concentration of pollutant, genetic predisposition, other co-existing conditions, demographic factors such as age, sex, race, socio-economic status, etc. The breakdown of the immune system may set a chain of acute reactions such as inflammation, swelling and acute cellular response characterized by infiltration of neutrophils. If the acute phase remains unresolved this leads to proliferation of immune cells and connective tissue resulting in granulomas and fibrosis and ultimately remodeling of the airways. Chronic cytokine mediated inflammation is also known to alter the physiology of smooth muscles of airways causing smooth muscle cells proliferation along with increased contractility and reduced relaxation.[27] These inflammatory and structural changes may cause remodeling of airways and impairment in lung volumes and airflow which are detected through pulmonary function tests (PFTs) or spirometry. PFTs are a valuable tool for evaluating the respiratory system and play a vital role in screening and assessing the severity of asthma by providing accurate measurements of air volume and airflow. PFTs are widely used in population based studies investigating environmental exposures and pulmonary function in children and adults.[25,26,28-30]

In this dissertation, my aim was to study the associations between three such environmental exposures or reaction to allergen and pulmonary function in children,

namely reactivity on skin prick test (SPT) to house dust mites (HDM) allergen, the combined effect of maternal smoking during pregnancy and current smoking by offspring, and dichlorodiphenyl dichloroethene (DDE).

Literature Review

House Dust Mite

House dust mites (HDM), *Dermatophagoides pteronyssinus* (Der p), is one of the most common indoor allergen.[31] HDM allergy is a significant public health problem affecting 60-130 million of the world's population and HDM are predominantly found in warm and humid climate.[32] A study conducted in New South Wales province of Australia demonstrated that children of age 8-11 years living in coastal region where climate was more hot and humid had higher prevalence of sensitivity to HDM than children living in inland areas.[33] The European Community Respiratory Health Survey- I reported that the mean prevalence of sensitization to HDM was 21.7 % in 15 European countries.[34]

There is strong evidence of a link between HDM sensitization and asthma.[9,33,35-40] A meta-analysis study reported a high overall prevalence of asthma (21 %) with HDM sensitization.[41] Sporik *et al.* have suggested a causal association between HDM exposure during infancy and development of asthma in early childhood.[42] In addition, a randomized controlled trial showed that children who were provided with zippered vinyl cover for pillows, mattresses and box springs and instructions to make bedrooms house dust free showed fewer wheezing episodes and lower peak expiratory flow rate than children who were not provided with intervention.[43]

Custovic *et al.* reported that levels of HDM found in beddings and mattresses were negatively correlated with the achieved percent of the predicted values of forced expiratory volume in one sec (FEV_1) and bronchial hyperreactivity.[44] However, the evidence from large birth cohort studies suggests that mere exposure to HDM is not sufficient to 'cause' asthma but it is the ability to mount immunological response to HDM allergen and genetic predisposition of individual that determines the risk of asthma.[45-47] Langley *et al.* reported that asthmatics who were exposed and sensitized to HDM had a more severe form of asthma in terms of lower percent predicted FEV_1 and higher BHR than those who were exposed but not sensitized.[24] The American Thoracic Society (ATS) recommends use of fractional exhaled nitric oxide (FeNO) which is considered as an indirect marker of airway inflammation in the diagnosis of eosinophilic airway inflammation as well as monitoring of airway inflammation in asthmatics.[48] Studies have shown that there is a strong positive association between HDM sensitization and fractional exhaled nitric oxide (FeNO) levels.[35-37,39]

The immunological responses to HDM are mediated through both innate and adaptive immune system.[49-51] The mechanism of how HDM exposure induces innate response is still unclear. Mechanisms like activation of dendritic cells (DC), C-type lectin pathway and protease signaling along with Toll-like receptors (TLRs) and NOD-like receptors (NLRs) are suggested to induce innate immunity.[49-51] The adaptive immune response is characterized by immunoglobin E (IgE) production along with recruitment of IL-4, IL-5, and IL-13.[49] SPT measures response of HDM specific IgE if an individual is sensitized to HDM. Not only there is a dose-response relationship between increasing

exposure to house dust mites and having a positive SPT to HDM but individuals with positive SPT have higher risk for developing asthma.[9,42,52]

Evidence from animal and human studies suggest that chronic exposure to indoor allergens may lead to mucosal inflammation of the airways which in turn could result in remodeling of both larger and smaller airways.[53-56] HDM has been specifically implicated in the remodeling of airways through its role in activation of epithelial-mesenchymal transition (EMT) mechanism which is central to the structural changes in airways.[49,57,58] Airway remodeling characterized by epithelial changes, thickening of the basement membrane, hyperplasia of smooth muscle along with mucus-secreting and goblet cells, and increased airway vascularity may lead to irreversible loss of pulmonary function.[59]

The bulk of literature on HDM has focused on prevalence of asthmatic symptoms, severity of asthma and bronchial hypersensitivity (BHR). Some studies have demonstrated that FEV_1 is reduced in asthmatic patients exposed and sensitized to HDM.[24,44,60] There is also a positive correlation between size of wheal on SPT and IgE and FeNO levels.[36,61] Additionally, one study found that clinical severity of asthma increased with increased wheal size on SPT to HDM.[62] Nevertheless, it is unknown whether degree of sensitization to HDM i.e. the wheal size on SPT has a quantitative association with pulmonary function. Although FEV_1 is considered as important pulmonary function measurement related to asthma, studies have shown that forced expiratory flow at 25 to 75% ($FEF_{25-75\%}$) could detect slight airway obstruction in asthmatic children with normal FEV_1.[63,64] Another study demonstrated a reduced $FEF_{25-75\%}$ in young adults diagnosed with allergies to HDM, cats and dogs but normal FEV_1, FVC and FEV_1/FVC ratio and no asthma symptoms.[65] Therefore, it is possible that

children sensitized to HDM may not have asthmatic symptoms and reduced FEV_1; but have reduction in $FEF_{25-75\%}$.

Additionally, studies have reported that the dose-response relationship between allergen exposure and sensitization is not linear.[45,66,67] So it is important to assess the effects on pulmonary function at different levels of sensitizations for example –mild, moderate, and severe. To our knowledge no population-based study has explored the quantitative association between degree of sensitivity to HDM and pulmonary function parameters, namely forced vital capacity (FVC), FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$. Our aim is to investigate these associations in hypothesis one of this dissertation.

Maternal smoking during pregnancy and adolescent smoking

The development of the lungs is long and complex process which starts during the 4th week of gestation and continues into late adolescence/early adulthood. While growth of airways is mostly completed *in-utero*, the development of alveoli is completed by 2 years of age and after that growth in lung volumes is mainly due to increase in size of alveoli and not number.[68-70] During adolescence, in boys, lung and thoracic development occurs during and until the end of puberty while in girls, lung development is almost finished following menarche.[71] Therefore, both fetal and adolescent period are critical time periods for development of lungs. Any adverse environmental exposures during these critical periods of lung growth is a form of programming which can have long term effects on pulmonary function. Maternal smoking during pregnancy and adolescent smoking are two such exposures which could have a repeated and/or long term damaging effects on the lungs.

Maternal smoking during pregnancy constitutes a public health problem as the most important *in-utero* harmful exposures. Smedberg *et al.* reported an overall smoking prevalence of 26 % in pregnant women in 15 European countries.[72] Adverse effects of maternal smoking during pregnancy is not just limited to pregnancy outcomes and fetal growth retardation but several studies have shown that maternal smoking could also increase the risk of cardio-pulmonary and behavioral disorders in offspring during childhood.[73,74] Smoking is also a growing public health problem in adolescents as more adolescents take up smoking every day.[75,76] According to the World Health Organization (WHO), the prevalence of tobacco use among adolescents aged 13-15 years is 20 % in boys and 15 % in girls in Europe.[76] Current survey data from United Kingdom indicates that about 207,000 children aged between 11-15 years starts smoking every year.[75]

The underlying patho-physiological mechanisms of adverse effects of tobacco smoke are well documented. Persistent exposure to smoking causes inflammation and epithelial changes in bronchial tissues which leads to secretory congestion and fibrosis causing remodeling of airways and destruction of alveoli resulting in loss of gas exchange.[77] Although no histological study describing the pathological effects of maternal smoking on the airway development during fetal period have been conducted in humans, one can hypothesize that smoking will adversely affect the development of lungs during the gestational period as a result of blood flow restriction to the placenta due to nicotine induced vasoconstriction, poor nutritional status of the mother associated with the anorexigenic effect of nicotine and carbon monoxide exposure.[78] An animal model presented by Rehan *et al*. to study the effects of maternal smoking on fetal lung development showed that *in-utero* exposure to tobacco smoke alters the normal

homeostatic epithelial-mesenchymal interaction in the developing alveolus, resulting in production of myofibroblasts in larger as well as smaller airways, which is a common finding in asthma and chronic lung disease.[79]

Evidence from several sources show that maternal smoking during pregnancy leads to a higher risk of recurrent lower respiratory symptoms[80-83] and increases risk of asthma attacks during infancy and early childhood.[3,18,84] Some studies have also demonstrated a reduced pulmonary function in adolescents exposed to maternal smoking during *in-utero* period.[85-87] Additionally, Jaakkola *et al.* reported that maternal smoking during pregnancy was associated with high prevalence of childhood asthma and these effects were mediated through the negative effects of maternal smoking during pregnancy on fetal growth in seven years old Finnish children.[88] Adolescent smoking on the other hand has been associated with dose-related decline in pulmonary function and also increased risk of new-onset asthma.[13-16] Smoking also has differential effects in adolescent males and females. Even though pulmonary function decreased with increase in smoking in both boys and girls, girls were considered to be more vulnerable to the effects of smoking on pulmonary function growth than boys.[14,89] A study also found that with increased urinary cotinine levels in boys (age 6-8 years) exposed to household smoking there was increased bronchial hyperresponsiveness represented by increased percentage ratio of the amplitude over the mean (AVAM) of the diurnal peak flow rates.[90] However, these results were not seen in girls.

Furthermore, it has been suggested that maternal smoking during pregnancy can influence smoking in offspring.[91,92] Kandel *et al.* found that adolescent daughters of mothers who smoked during pregnancy were more likely to smoke in their adolescence

and that this association was significantly dose related.[92] Additionally, they found that maternal smoking influence on adolescent smoking was stronger than paternal smoking and also stronger in female children than male children.[91]

In line with this research few studies have investigated the combined effects of maternal smoking during pregnancy and personal smoking by adolescents on pulmonary function. Gilliland *et al.* found that children between age 8-15 years who smoked regularly and who were also exposed to maternal smoking during pregnancy had a more than eight fold increased risk of new-onset asthma than non-smokers.[13] Hayatbakhsh *et al*. reported that *in-utero* exposure to maternal smoking was associated with a reduction in FEV_1 and $FEF_{25–75\%}$ in males of 21 years after accounting for maternal smoking after pregnancy and smoking by children at age 14 years.[93] Rizzi *et al*. found a mild airway obstruction in adolescent male smokers and deficit in smaller airway function in passive smokers who were additionally exposed to maternal smoking during pregnancy.[94] However, another study found no effect of either current adolescent smoking or maternal smoking during pregnancy on pulmonary function.[95]

Since maternal smoking during pregnancy and personal smoking by adolescents are closely associated,[91,92] personal smoking by adolescents may act as a mediating variable in the "causal" pathway between maternal smoking during pregnancy and pulmonary function in adolescents (Figure 1.1). Additionally, maternal smoking during pregnancy has also been associated with reduced height and increased obesity in children[96-98] which in turn is associated with pulmonary function[99-102] making height and weight possibly intervening variables. The inconsistent results in association between maternal smoking during pregnancy and pulmonary function in adolescents may be

attributed to the fact that most of the traditional regression analyses methods used to analyze gestational and adolescent smoking are inflexible and cannot take intervening variables into consideration. To elucidate these complex relationships path analysis or structural equation models provides an approach using multiple mathematical equations. Path analysis is very flexible; a variable acting as an independent variable in one equation can act as a dependent variable in another equation, thus allowing to include intervening or mediating variables in the model.[103] Finally, these multiple linear regression equations generates the direct, indirect and total effects of each variable on the outcome which can be used to develop causal path diagram.[103,104] In our second hypothesis we aim to improve the assessment of gestational and adolescent smoking for lung function in children using path analyses.

Figure 1.1: Causal pathway between maternal smoking during pregnancy, adolescent smoking and pulmonary function

Dichlorodiphenyl Dichloroethene (DDE)

Dichlorodiphenyl trichloroethane (DDT) is a synthetic chemical belonging to organochlorine chemicals family and is recognized as persistent organic pollutants. DDT was a widely used insecticide in the world until it was banned in most developed

countries by the end of 1980s. However, it is still largely used in developing countries for malaria control and in agriculture. DDT is toxic and a major public health concern. Dichlorodiphenyl dichloroethene (DDE) is principal metabolite of DDT which exists in environment in all forms of life, humans, plants, animals, water, air and soil.[105,106] Humans are largely exposed to DDE through food chain in food stuff such as fruits, vegetables, fish and meat.[107] DDE is highly lipophilic and has very long half-life. It bioaccumulates in adipose tissue to a higher extent and bloodstream and breast milk to a lesser extent.

DDE is also known to have effect on three important factors related to respiratory health- immune function, growth and respiratory infections/diseases. Firstly, DDE may alter the immune function by acting on both cellular and humoral immunity.[108] There is evidence from both animal and human studies suggesting that DDE exposure is associated with increased production of cytokines and nitric oxide production in macrophages, leading to inflammatory reactions, cytokine imbalance and immune dysregulation.[109-111] DDE seems also associated with changes in T-cell mediated response involving IL-4 which can induce epithelial cell proliferation, fibrosis, and mucus secretion to differing extents.[109,112] These immunological changes due to persistent exposure to DDE may lead to remodeling of both smaller and larger airways. Secondly, studies have shown that DDE adversely affects height and pubertal growth in children. Studies evaluating association between prenatal DDE exposure and postnatal growth have shown decreased height at ages 1, 4 and 7 years.[113] Postnatal exposure to DDE has also been associated with decreased height in girls of age 8 years.[114] Some other studies however, have found no such association between DDE exposure and postnatal

growth.[115-117] DDE's anti-androgenic properties have been known to interfere with puberty in both human and animal studies.[116,118-120] One study has reported that *in-utero* exposure to DDE is associated with reduced age at menarche by one year.[121] High levels of DDE have also been found in girls with precocious puberty.[122] Finally, pre-natal exposure to DDE has been linked with respiratory disorders like lower respiratory tract infections, episodes of wheezing, otitis media and increased risk of asthma in early childhood.[20,123-126]

Nevertheless, there is lack of investigations on the association between DDE exposure and pulmonary function. As height, which is one of the important factors related to pulmonary function,[127] is associated with DDE exposure we can hypothesize that DDE exposure may have an effect on pulmonary function, either direct or via the influence of growth (height). Hence, in the pathway between DDE exposure and pulmonary function height may act as an intervening variable (Figure 1.2).

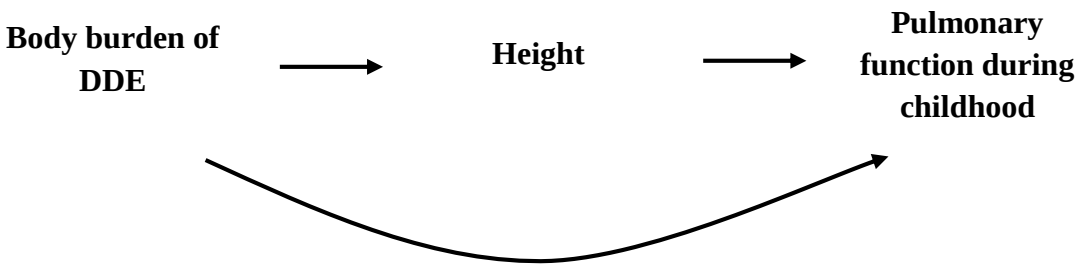


Figure 1.2: Causal pathway between body burden of DDE, growth and pulmonary function

Additionally, average pulmonary function growth varies greatly with height, sex and age in children and adolescents.[128-130] Thus, it is important to take into consideration

relationships between various factors which may be affected by DDE exposure when assessing association between DDE exposure and pulmonary function. As explained earlier, path analyses (structural equation models) are one of the appropriate approaches to accommodate intervening or mediating variables in the analysis. Path analysis takes into account various directional and non-directional associations between a set of variables on outcome while estimating direct, indirect and total effects of each variable on the outcome. Hence, in hypothesis three, we will assess the direct and indirect of DDE on pulmonary function markers.

Scope of Study

The overall objectives of this research are to examine the association between three different environmental exposures, namely (1) allergic sensitization to house dust mite allergens (SPT to HDM), (2) maternal smoking during pregnancy (gestational smoking) and adolescent smoking, and (3) dichlorodiphenyl dichloroethene (DDE) and pulmonary function in children and adolescents. The first step was to investigate the association between immunological response to HDM allergen on SPT and pulmonary function during late adolescence within the birth cohort of Isle of Wight (IOW). The next step was to assess the combined effects of gestational smoking and adolescent smoking on pulmonary function in late adolescence. This association was also tested in the birth cohort of IOW. The third step was to examine relationship between body burden of DDE and pulmonary function in pre-adolescent children in a study conducted in the federal state of Hesse, Germany. In both step two and step three, we used path analysis methods to address the possibility of intervening or mediating variables.

Hypotheses and Specific Aims

Hypothesis 1 (H-1): With an increase in wheal size (severity of sensitization) to HDM allergen on SPT pulmonary function decrease in adolescents of age 18 years

- Specific Aims (SA) 1.1: To compare FEV_1, FVC, FEV_1/FVC, $FEF_{25-75\%}$ and FeNO between those children who tested positive on HDM SPT and those who tested negative.
- SA 1.2: To determine the association between increase in wheal size on HDM SPT and above mentioned pulmonary function parameters
- SA 1.3: To categorize the degree of sensitization based on the wheal size and compare pulmonary function in these categories
- SA 1.4: To compare pulmonary function among various groups depending on the duration of positive SPT

Hypothesis 2 (H-2): Maternal smoking during pregnancy and current smoking by the offspring have negative effect on pulmonary function in adolescents after accounting for the intervening effect of current smoking by offspring

- SA 2.1**:** To determine the joint and independent effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function parameters in adolescents of age 18 years separately in boys and girls using linear regression
- SA 2.2: To investigate if current smoking by adolescents act as a mediating variable in the "causal" pathway between maternal smoking during pregnancy and pulmonary function in adolescents using path-analytical models.

Hypothesis 3 (H-3): Persistent body burden of DDE in children affect pulmonary function in childhood, directly or indirectly via its effect on height and weight in children

- SA 3.1: To determine the association between blood levels of DDE with pulmonary function parameters namely FEV_1, FVC and FEV_1/FVC
- SA 3.2: To explore the relationship between blood levels of DDE, height, weight and pulmonary function using path analyses

Significance of the study

Hypothesis 1:

As mentioned earlier HDM allergy is a significant public health problem in Western countries. Although many previous studies have shown an association between HDM exposure and asthma, to our knowledge, this study is one of the first to explore the quantitative association between wheal diameter on SPT and pulmonary function. It is important to extend the research on HDM beyond its association with asthma because any negative effects that HDM sensitization may have on pulmonary function especially during the growth phase of the lungs may have long term respiratory health consequences.

Hypothesis 2:

While maternal smoking during pregnancy and adolescent have been individually linked with lower pulmonary function during adolescence, little is known about the combined effects of both these factors on pulmonary function. After birth pulmonary function undergoes a phase of growth, reaching its peak in early adulthood. Therefore, both the *in- utero* and adolescent periods are important for the growth of the respiratory system. Additionally, behavioral studies have also shown an association between

maternal smoking during pregnancy and smoking by offspring. Path analysis would be appropriate to disentangle these complex relationships between maternal smoking during pregnancy, adolescent smoking and pulmonary function in adolescents. Our study is the first to examine these relationships using path analysis.

Hypothesis 3:

Given that both prenatal and postnatal DDE exposure is associated with asthma, an additional investigation of the association between DDE and pulmonary function will show whether the above association is due to immune response or can also be explained by pulmonary effects. DDE is known to have negative effect on height during childhood which may act as an intervening variable on the 'causal' pathway between DDE and pulmonary function. This association was investigated using path analysis. This study is the first to use robust analytical methods like repeated measures analysis and path analysis to assess the association between DDE and pulmonary function.

Study Outline

The dissertation is divided into six chapters. The first chapter provides the hypotheses of the research, including specific aims, as well as the scope and significance of the research. The second chapter provides the methodology for each specific aim. The third, fourth, and fifth chapters contain hypotheses 1, 2 and 3, respectively. The final chapter contains a discussion of the findings, public health implications, limitations, and summary of the entire dissertation.

CHAPTER 2

METHODS

Addressing three different exposures of interest, we used data sets from two different population. Since the first two hypotheses were tested using the Isle of Wight (IOW) birth cohort the methods related to these two hypotheses are described together with the exception of the exposures and statistical analyses methods which are provided separately for H-1 and H-2. The third hypothesis was tested using a longitudinal study conducted in Germany; its methods are presented separately.

SAS Version 9.3 (SAS Institute, Cary, NC, USA) was used for all statistical analysis.

Hypotheses 1 and 2:

Study Population

The IOW birth cohort was established with the aim to study the natural history of asthma and allergic conditions. The island is close to the British mainland, semi-rural, without heavy industry and largely composed of Caucasians (99 %). Children born between January 1989 and February 1990 on the IOW were recruited to participate in this study. In total, 1,536 mothers/child pairs born in the study period were contacted. After exclusion of perinatal deaths, adoptions, and refusals of informed consent was obtained

from 1,456 participants. Children were followed at the ages of 1 (n=1,167), 2 (n=1,174), 4 (n=1,218), 10 (n=1,373) and 18 years (n=1,313). Detailed questionnaires were completed for each child at each follow-up regarding asthma and allergic conditions. When a visit was not possible, a telephone questionnaire was completed or a postal questionnaire sent for completion and return. Ethics approval was obtained from the Isle of Wight Local Research Ethics Committee at recruitment of this birth cohort born on the Isle of Wight, United Kingdom, between January 1989 and February 1990. Additional approval was acquired for year 1 and 2 follow-ups (No 05/89; dated 08/22/1988) and the Ethics Committee approved an extension for this study to allow follow-up at 4 years (dated 01/17/93). Subsequently, at 10 years follow-up, we obtained permission from the Isle of Wight Local Research Ethics Committee for the follow-up as well as collection of blood for genetic studies into asthma and allergy (No. 18/98, dated 07/20/1998). For the 18-year follow-up, ethics approval was given by the Isle of Wight, Portsmouth and SE Hampshire Local Research Ethics Committee (No. 06/Q1701/34, dated 06/16/2006).[130-132]

Exposures

Hypothesis 1: Skin Prick Test (SPT) for HDM

To determine allergic sensitization status, SPT at ages 1 and 2 years were performed on children with any symptoms of eczema, asthma or rhinitis. SPT were also performed at ages 4, 10 and 18, regardless of symptoms on most children enrolled in the study by a standardized method to a panel of common indoor and outdoor allergens: house dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*, milk, hens' egg, wheat,

soya, cod and peanut and, in addition, histamine and physiological saline acted as the positive and negative controls respectively (Alk-Abello, Horsholm, Denmark). Single-headed lancets were used and the skin pricked at an angle of 90°. The wheal diameter was recorded at 15 minutes.[133] Primarily, we used results from HDM SPT performed at 18 years of age. We used sensitization to HDM as both continuous and categorical variable of exposure. For SA 1.1, we categorized individuals into two groups based on their wheal size on SPT- positive (≥ 3 mm) and negative (< 3 mm). For SA 1.2, the actual size of wheal diameter (mm) on SPT was used as exposure. For SA 1.3, in order to determine degree of sensitization we categorized wheal size into 3 categories: >5 mm diameter (moderate/severe), 3-5 mm diameter (mild) and < 3 mm diameter (no sensitization). For SA 1.4, to test whether longer duration of having a positive SPT affects pulmonary function we divided individuals into 3 groups- SPT positive at age 18 years only, SPT positive since age of 10 years, and SPT positive since age of either 4 or 2 or 1 years. For the simplicity, we labeled these groups as diagnosed with positive SPT during late-adolescence, pre-adolescence and early childhood, respectively.

Hypothesis 2: Maternal smoking during pregnancy and current smoking by adolescents of age 18 years

The main exposures of interests were maternal smoking during pregnancy and current personal smoking by adolescents of age 18 years. Birth and obstetric records were used to obtain information on maternal smoking during pregnancy. To determine the current smoking status children were asked "Do you currently smoke?" at age 18 years. Information was also collected on number of cigarettes smoked per day, past smoking and duration of smoking. Pack years were calculated from cigarettes smoked per day and

duration of smoking and used as main indicator of current smoking at 18 years of age. Adolescent smoking was defined as a history of ever smoking more than 100 cigarettes. To investigate the independent and joint effects of maternal smoking during pregnancy and adolescent smoking we categorized exposure into the following mutually exclusive categories: none, exposure to maternal smoking only, adolescent smoking only and exposure to both maternal smoking and adolescent smoking. This is comparable to a prior categorization used by Sadegnejad *et al.* to combine exposure to maternal smoking during pregnancy and environmental tobacco smoke in the cohort of IOW at age 10 years.[134]

Outcomes

Pulmonary function parameters

Pulmonary function tests were performed at 10 and 18 years of age. FVC, FEV_1, $FEF_{25-75\%}$ and PEFR were measured using a Koko Spirometer and software with a portable desktop device (both PDS Instrumentation, Louisville, KY, USA). Spirometry was performed and evaluated according to the American Thoracic Society (ATS) criteria. Children were required to be free of respiratory infection for 2 weeks and not to be taking any oral steroids and were advised to abstain from any β-agonist medication for 6 hours and from caffeine intake for at least 4 hours.[130-132]

Fractional exhaled nitric oxide (FeNO) measurements

FeNO was measured (Niox mino, Aerocrine AB, Solna, Sweden) according to ATS guidelines.[48] A biofeedback mechanism was used to maintain the expiratory flow rate at 50 ml/s and subjects exhaled against resistance to prevent upper airway contamination. Measurements were made in a standardized manner with the subject

standing without a nose clip; FeNO was measured before spirometric testing. Participants were rescheduled if they had clinical symptoms consistent with either current infection or a recent (within 2 weeks) asthma exacerbation, or had required antibiotics or oral steroids in the preceding 2 weeks.[133]

Covariates

Birth weight, sex, height, weight, body mass index (BMI), use of oral or inhaled steroids, parental history of asthma and allergic conditions, active smoking at 18 years, exposure to environmental tobacco smoke (ETS) at 18 years, and socio-economic status (SES) were considered as potential confounders and were adjusted in explanatory models. Information on birth weight was obtained from the hospital records. Height and weight were measured before the spirometric tests at age 18; BMI was calculated. Information on use of oral or inhaled steroids, parental history of asthma and allergic conditions were inquired from the study participants at age 18 Exposure to ETS at age 18 was inquired from questions of "any smoking in the household". Information on the SES was based on the following three variables: (a) the British socioeconomic classes (1–6) derived from parental occupation reported at birth; (b) the number of children in the index child's bedroom (collected at age 4 years); and (c) family income at age 10 years.[131] Additionally, adjustment for sensitization to other indoor and outdoor allergens, namely cats, dogs, grass pollen, tree pollen, *Alternaria alternata*, *Cladosporium herbarum*, milk, hens' egg, wheat, soya, cod and peanut was also done. A participant was considered atopic if he/she had a positive SPT to any of these allergens.

Statistical Analysis

Of total 1,456 participants 838 went through spirometry tests at 18 years of age. To address the issue of missing data on one or more confounders we used multiple imputations procedure PROC MI with multivariate normal distribution to generate ten new data sets. SAS uses Markov Chain Monte Carlo (MCMC) which assumes that all the variables in the imputation model have a joint multivariate normal distribution. For the continuous variables we generated the missing values which ranged between the minimum and maximum observed values.

Hypothesis 1:

For specific aims related to H-1linear regression techniques were used to determine association between exposure to HDM and pulmonary function parameters- FEV_1, FVC, FEV_1/FVC ratio, $FEF_{25-75\%}$ and FeNO at 18 years of age. The analysis for imputed data sets were performed in two steps when the exposure was continuous (SA 1.1) and in three steps when exposure was categorical (SA 1.2, 1.3 and 1.4). The first step was to obtain data sets containing parameter estimates by PROC MIXED on each imputed data set. We did not use general linear model analysis (PROC GLM) because PROC GLM does not support imputation of categorical variables without monotone missing pattern. Using PROC MIXED did not add any additional concerns and was more flexible. Additionally, when the exposure variable was categorical (SA 1.2, 1.3 and 1.4) we also obtained estimates for least square means and standard errors in step one. Next we used PROC MIANALYZE to combine the parameter estimates from each imputed data set and generate valid statistical inferences about the parameters. In the third step (only for categorical exposure) we again used PROC MIANALYZE to compare least

square means for categorical exposure variable based on least square means estimates and standard errors obtained from each imputed data set in step one.

We adjusted for the effects of sex, height, weight, BMI, sensitization to other indoor or outdoor allergens, use of oral or inhaled steroids, parental history of asthma and other allergic conditions, active smoking, exposure to ETS and SES. We also stratified analysis according to history of asthma wheezing attacks. We selected those confounders/effect modifiers which changed the estimates by 10 %.

Hypothesis 2:

To test H-2 we again used imputed data sets and followed the 3 step analysis plan as in H-1. For SA 2.1, in the first step we used PROC MIXED model to obtain the parameter estimates for each imputed data set separately in boys and girls. To investigate the independent and joint effects of both maternal smoking during pregnancy and adolescent smoking, we used exposure with mutually exclusive categories: none, exposure to maternal smoking only, adolescent smoking only and exposure to both maternal smoking and adolescent.

In the next two steps we used PROC MIANALYZE to generate valid parameter and least square means estimates. The models were adjusted for birth weight, current weight, atopy, parental history of asthma, exposure to ETS and SES. We used backward elimination method and selected the confounders/effect modifiers that changed the estimates by 10 %. To account for the differential effects of lung growth with respect to sex and height we used interaction term between sex and height. As explained earlier current smoking by adolescents, height and weight may act as mediating variables in the

"causal" pathway between maternal smoking during pregnancy and pulmonary function in adolescents. To explore this further in SA 2.2 we used path analysis and specifically the Covariance Analysis of Linear Structural Equations (PROC CALIS). Path analysis provides an approach based on conceptual model and multiple mathematical equations. Path analysis is very flexible; a variable acting as a cause in one equation can act as an effect in another equation, thus allowing to include intervening or mediating variables in the model. These multiple linear regression equations generates the direct, indirect and total effects of each variable on the outcome which can be used to develop causal path diagram. While direct effects represent the association between exposure and outcome without any intervening/mediating variables, indirect effects represent the association between exposure and outcome via intervening/mediating variables. The total effects of exposure on outcome is obtained by adding direct and indirect effects. We used Full Information Maximum Likelihood (FIML) method to determine parameter estimates which addresses the issue of missing values for covariates. Pack years was used as an indicator of adolescent smoking in all path analysis models. The adequacy of model fit was determined by several statistics: a chi-square p-value > 0.05 if chi-square test statistic is close to 0, comparative fit index (CFI) > 0.9, adjusted goodness of fit index (AGFI) > 0.9 and root mean square error of approximation (RMSEA) < 0.06..

Hypothesis 3:

Study Population

We used data from a longitudinal study conducted in the south of the federal state of Hesse, in central Germany, between 1994 and1997. Children were recruited from 3 different regions. One is in the Rhine Valley, with low mountains on both sides, within a

15-km radius around an industrial waste incinerator and is also used intensively for vegetable production. A second region, also industrial and agricultural, is 20 km north of the incinerator area. The third region is located in low mountains (about 400 m above sea level).[114] After obtaining permits from the Data Protection Agency of the State of Hamburg, Germany, from the Ministry of Cultural Affairs of Hesse, Germany, and from the local school committees, parents of 1091 second grade school children in 18 townships were asked to participate in this study. Informed consent, according to the requirements of the Ethical Committee of the Board of Physicians and the Data Protection Agency of the State of Hamburg, was obtained from all participating parents. Parents were asked to let their children participate in phlebotomy only when passive smoking in the private household had not exceeded 10 cigarettes per day in the previous 12 months. Children and their parents participated in the 3 repeated surveys (December 1994–April 1995, January to May1996, January to June 1997).[114]

Exposure

Organochlorines in Blood

A blood sample was taken as part of the first visit. DDE and 7 PCB congeners (101, 118, 138, 153, 170, 180, 183, and 187) were analyzed at the Institute of Toxicology, University of Kiel, Germany. DDE and PCB concentrations were determined from 5-mL samples of whole blood by performing high-resolution gas chromatography (HRGC, Model 3400, Varian, Gloucester, Mass) with a 63Ni electron capture detector. The detection limit (the signal/low-noise ratio) was 0.02 g/L for DDE and each PCB congener. For extraction and clean-up procedures, magnesium-silica gel and n-hexane were used (9 g of silica gel was deactivated with 3 % water and given in a

chromatography column 22 mm in diameter and 48 mm in length for elution). The capillary column amounted to 30 m in length and 0.25 mm in diameter, containing nitrogen as a carrier gas. The congeners were determined by retention times on the chromatograms and were identified by comparison with known standards. In addition, reliability was tested with gas chromatography– mass spectometry. The laboratory successfully participated in nationwide interlaboratory quality assessments in Germany for DDE and PCB determinations.[114]

Outcomes

Pulmonary function parameters

Pulmonary function tests were conducted at 8, 9 and 10 years of age using a Masterscope (Software Release 4.0; Erich Jaeger, Würzburg, Germany). The instrument was calibrated daily and each child performed two forced expiratory maneuvers according to the ATS guidelines in standing position wearing a nose clip. Two flow/volume curves were accepted as reproducible if the difference between FVC measurements was ≤ 5 %. The highest FVC and FEV_1 values were then selected for statistical analysis.

Covariates

Self-administered questionnaires were used in the survey. Information was collected on child's age, birth weight, birth order, maternal and paternal education, maternal and paternal height and smoking during pregnancy, and breast-feeding duration. Height and weight were measured for each child in 3 consecutive examinations 1 year apart (ages of 8, 9, and 10 years). Environmental tobacco smoke (ETS) was assessed as

smoking in the child's home in the previous 12 months (per day: no cigarettes, 1-10 cigarettes, 11-20 cigarettes, 20-30 cigarettes, >30 cigarettes).[114]

Statistical Analysis

Out of 691 enrolled children, 632 performed PFTs in year 1995, 598 in year 1996 and 558 in year 1997. However, DDE was measured only in years 1995 and 1997. About 328 (52 %) children in 1995 and 214 (61.7 %) children in 1997 who had PFT also had information on DDE. From year 1996, we selected children who also participated in PFT (n=328) either in 1995 or 1997. Since, DDE exposure was not measured in 1996 we imputed values for those children based on their respective values in years 1995 and 1997 using multiple imputation methods. Multiple imputations were also used for missing data on one or more confounders. As a first step, correlations between DDE exposure and height, weight, FEV_1, FVC and FEV_1/FVC were determined using PROC CORR method.

For SA 3.1, we used linear mixed models (PROC MIXED) to assess the association between blood levels of DDE and repeated pulmonary function measurements. Compound symmetry covariance structure matrix was selected based on lowest Akaike information criteria and the Bayesian Schwarz information criterion after considering unstructured, compound symmetry and autoregressive covariance structure matrices. All models were adjusted for sex, birth weight, breast feeding duration, height, weight, smoking during pregnancy, parental history of asthma and ETS. We used backward elimination method to select and keep the confounders in the model that changed the estimates by 10 %. Since relationship between pulmonary function and height varies by sex we included interaction between height and sex in explanatory models.

The possibility that effect of DDE exposure on pulmonary function in childhood is mediated through its effect on height cannot be tested using repeated measurement analysis since these models do not take into consideration intervening variables. Therefore, we explored the relationship between DDE exposure, height, weight and pulmonary function in childhood by path analysis using the Covariance Analysis of Linear Structural Equations procedure (SA 3.2). However, since body burden of DDE, height and weight are correlated we were not able to generate positive convergence estimates for maximum likelihood optimization method. Therefore, we performed two separate path analysis model on cross-sectional data for years 1995 and 1997 using Full Information Maximum Likelihood (FIML) method which takes into consideration the missingness. The adequacy of model fit was determined by the following statistics: a chi-square p-value > 0.05 if the chi-square value is close to zero which indicates little difference between the expected and observed covariance matrices comparative fit index (CFI) > 90, adjusted goodness of fit index (GFI) > 90 and root mean square error of approximation (RMSEA) < 0.06 (SUGI).

CHAPTER 3

RELATIONSHIP BETWEEN IMMUNOLOGICAL RESPONSE TO HOUSE DUST MITE SENSITIZATION AND PULMONARY FUNCTION IN LATE ADOLESCENCE

Introduction

House dust mites (HDM) allergy is a significant public health problem in the Western countries.[32,34] A survey conducted in four European countries showed that 15 % of the survey population presenting with allergy symptoms like repetitive sneezing, nasal discharge, stuffy nose, etc. had physician diagnosed allergy to HDM.[135] The role of HDM in the pathogenesis of asthma has been extensively studied and results have shown a positive association between exposure and sensitization to HDM and prevalence of asthma symptoms, severity of asthma and bronchial hyperreactivity (BHR).[9,35-40,60] Some studies have even suggested a causal association between HDM exposure and development of asthma in early childhood.[42,43] Additionally, There is also a strong positive relationship between HDM exposure and fractional exhaled nitric oxide (FeNO) levels which is an indirect marker of airway inflammation and used for monitoring treatment in asthma.[35,36]

Custovic *et al.* reported that levels of HDM found in beddings and mattresses were negatively correlated with percent predicted values of forced expiratory volume in one

sec (FEV_1) and bronchial hyperreactivity.[44] However, the evidence from large birth cohort studies suggests that mere exposure to HDM is not sufficient to 'cause' asthma but it is the ability to mount immunological response to HDM allergen and/or the genetic predisposition that determines the risk of asthma.[24,45-47] This ability to mount immunological response to HDM allergen can be measured in terms of wheal size on skin prick test (SPT) and is mediated through immunoglobulin E (IgE). There seems to be a dose-response relationship between increasing exposure to house dust mites and having a positive SPT to HDM.[9,42,52] It has also been reported that total serum IgE and allergen specific IgE are inversely associated with pulmonary function in both children and adults.[136-138] Additionally, a study also observed an increased severity of asthma with increased wheal size on SPT for HDM.[62]

Evidence from animal and human studies suggest that chronic exposure to indoor allergens leads to mucosal inflammation and remodeling of both larger and smaller airways and ultimately may alter pulmonary function.[53-56] Although FEV_1 is the most important parameter considered when diagnosing asthma there is growing body of evidence suggesting role of smaller airways in pathology of asthma.[139-142] $FEF_{25-75\%}$ is a spirometric variable used to detect small airway obstruction. $FEF_{25-75\%}$ has a marked physiological variability, but epidemiological studies suggest that $FEF_{25-75\%}$ is an important pulmonary function parameter which could detect slight airway obstruction in non-asthmatic children with normal FEV_1.[63,64] Another study demonstrated a reduced $FEF_{25-75\%}$ in young adults diagnosed with allergies to HDM, cats and dogs but with normal FEV_1 and no asthmatic symptoms.[65] Thus, $FEF_{25-75\%}$ is a significant parameter

and a marker for early inflammatory involvement of the small airways in individuals with allergic disease and no asthma.

Any exposure leading to adverse effects on pulmonary function especially during phase of growth might have long term consequences. Although the role of HDM in asthma has been well-established, it is unknown whether wheal size on SPT has a quantitative association with pulmonary function. Our main aim in this study was to assess the association of degree of sensitization to HDM and duration of positive SPT to HDM allergen with pulmonary function parameters namely- FEV_1, FVC, FEV_1/FVC ratio $FEF_{25-75\%}$ including average FeNO levels in the birth cohort of Isle of Wight (IOW) during late adolescence.

Methods

Study Population

We used data from the IOW birth cohort. Between January 1989 and February 1990, 1,536 mothers/child pairs were contacted to be enrolled in the IOW birth cohort after obtaining approval from the Isle of Wight Local Research Ethics Committee at recruitment. After exclusion of perinatal deaths, adoptions, and refusals and obtaining approval from the local research ethics committee 1,456 participants were available for follow-up at 1, 2, 4, 10 and 18 years of age.[130,131]

Skin Prick Test for HDM

To determine allergic sensitization status, SPT at ages 1 and 2 years were performed on children with symptoms of eczema, asthma or rhinitis. At ages 4, 10 and 18 years SPT were performed regardless of symptoms on most children enrolled in the study

by a standardized method to a panel of common indoor and outdoor allergens: HDM, grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*, milk, hens' egg, wheat, soya, cod and peanut. Histamine and physiological saline acted as the positive and negative controls respectively (Alk-Abello, Horsholm, Denmark). Single-headed lancets were used and the skin pricked at an angle of 90°. The wheal diameter was recorded at 15 minutes.[133,143]

Pulmonary Function

Pulmonary function tests were conducted at 10 and 18 years of age. FVC, FEV_1, $FEF_{25-75\%}$ and PEFR were measured using a Koko Spirometer and software with a portable desktop device (both PDS Instrumentation, Louisville, KY, USA). Spirometry was performed and evaluated according to the American Thoracic Society (ATS) criteria. Children were required to be free of respiratory infection for 2 weeks and not to be taking any oral steroids and were advised to abstain from any β-agonist medication for 6 hours and from caffeine intake for at least 4 hours.[130-132]

Fractional Exhaled Nitric Oxide Measurements

FeNO was measured (Niox mino, Aerocrine AB, Solna, Sweden) according to ATS guidelines.[48] A biofeedback mechanism was used to maintain the expiratory flow rate at 50 ml/s and subjects exhaled against resistance to prevent upper airway contamination. Measurements were made in a standardized manner with the subject standing without a nose clip; FeNO was measured before spirometric testing. Participants were rescheduled if they had clinical symptoms consistent with either current infection or

a recent (within 2 weeks) asthma exacerbation, or had required antibiotics or oral steroids in the preceding 2 weeks.[133]

Potential Confounders

We considered sex, height, weight, body mass index (BMI), use of oral or inhaled steroids, history of asthmatic wheezing attacks, parental history of allergy, active smoking at 18 years, exposure to environmental tobacco smoke (ETS) at 18 years, and socio-economic status (SES) as potential confounders. We also adjusted for sensitization to other indoor and outdoor allergens, namely cats, dogs, grass pollen, tree pollen, *Alternaria alternata*, *Cladosporium herbarum*, milk, hens' egg, wheat, soya, cod and peanut. A participant was considered atopic if he/she had a positive SPT to any of these allergens. Height and weight were measured before the spirometric tests at age 18; BMI was calculated using formula- (weight in kg)/ (height in meters). Information on use of oral or inhaled steroids, parental history of asthma and other allergic conditions and active smoking was inquired from the study participants at age 18. Exposure to ETS at age 18 was inquired from questions of "any smoking in the household". Information on the SES was based on the following three variables: (a) the British socioeconomic classes (1–6) derived from parental occupation reported at birth; (b) the number of children in the index child's bedroom (collected at age 4 years); and (c) family income at age 10 years.[131]

Statistical Analysis

Of total 1,456 participants 838 went through spirometry tests at 18 years of age. As a first step we used t-test and analysis of variance (ANOVA).to compare pulmonary

function across sex, degree of sensitization and duration of sensitization categories. We used linear regression techniques to determine all the associations. Our outcomes of interest were pulmonary function parameters FEV_1, FVC, FEV_1/FVC ratio, $FEF_{25-75\%}$ and FeNO at 18 years of age. The allergic sensitization to HDM on SPT was used as exposure in four different ways. First we used the wheal size (diameter in mm) obtained on SPT at 18 years of age as a continuous variable. If SPT was not performed at 18 years of age then we used results from SPTs performed at 10 years of age. Next we classified SPT as positive and negative based on wheal diameter; mean diameter of 3 mm or more than the negative control was considered as positive SPT. For these analysis we also included interaction terms between wheal diameter/ positive or negative SPT and history of wheezing attacks. If the interaction terms were significant (p-value: < .0001) then we stratified the analysis by history of wheezing. We categorized wheal size into 3 categories in order to determine degree of sensitization: >5 mm (moderate/severe), 3-5 mm (mild) and < 3 mm (no sensitization). Finally, to test whether longer duration of having a positive SPT affects pulmonary function we divided individuals into 3 groups- SPT positive at age 18 years only, SPT positive since age of 10 years, and SPT positive since age of either 4 or 2 or 1 years. For the simplicity, we labeled these groups as diagnosed with positive SPT during late-adolescence, pre-adolescence and early childhood, respectively. All explanatory models were adjusted for potential confounders mentioned earlier. As relationship of pulmonary function with height varies with sex we also included the interaction term between height and sex in the explanatory models. We selected those confounders which changed the estimates by 10 %. To address the issue of missing data on one or more confounders we used multiple imputations to generate ten

new data sets. All data sets were analyzed separately for linear regressions. Finally all results were combined and valid statistical inferences were generated using MIANALYZE procedure.

Results

Out of 838 participants who had spirometry at 18 years of age 443 (52.9 %) were girls. Majority of participants belonged to middle social status (78.3 %) based on parental occupation, number of children in one bedroom and annual family income. About 28.5 % had history of asthma wheezing episodes, 31.7 % had history of other atopic conditions and 13.6 % had used steroids (nasal or oral) for asthma attacks prevention. The prevalence of smoking for this age group was 33 % and about 39.1 % were exposed to some form of ETS. About 29 % were tested positive for HDM allergy on SPT; 12.4 % had wheal diameter of > 5 mm, 16.5 % had wheal diameter of 3-5 mm and 71.1 % had wheal diameter of < 3 mm. Among those who tested positive 30.6 % had positive SPT since early childhood, 26.9 % had since pre-adolescence and 42.6 % were diagnosed during late adolescence. (Table 3.1). The average diameter of the wheal was 1.7 ± 2.6 mm (Table 3.2). Boys had overall higher FEV_1, FVC, $FEF_{25-75\%}$ and FeNO measures than girls, except for FEV_1/FVC ratio which was higher in girls (Table 3.3). The participants with positive SPT had higher FVC and FeNO levels while lower FEV_1/FVC ratio (Table 3.4). Among the group with wheal diameter > 5 mm and group with wheal diameter of < 3 mm differences were observed for FVC and $FEF_{25-75\%}$; for FEV_1/FVC ratio and FeNO levels differences were seen within all groups (Table 3.5). FEV_1/FVC ratio and $FEF_{25-75\%}$ were lower in the group with positive SPT since early childhood when compared to the groups who had positive SPT since either pre-adolescence or late adolescence. FeNO

levels were higher in the group with longer duration of positive SPT when compared to any group with lower duration (Table 3.6).

HDM as continuous variable

Results from the linear regression showed significant interaction between wheal diameter (mm) and history of wheezing for FVC, FEV_1/FVC, $FEF_{25-75\%}$ and FeNO levels (Table 3.7). These results were adjusted for height, weight, sex, current smoking, history of asthma wheezing episodes and other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS and SES. Stratified analysis demonstrated reduction in FVC and FEV_1 and increase in FeNO levels in participants who did not report any history of asthma wheezing episodes. On the other hand, in the group of participants with history of asthma wheezing episodes there was statistically significant reduction in FEV_1/FVC ratio and $FEF_{25-75\%}$ and increase in FeNO levels (Table 3.8).

HDM as categorical variable

The interaction between SPT (positive or negative) and history of wheezing episodes was also significant after adjusting for height, weight, sex, current smoking, history of asthma wheezing episodes and other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS and SES (Table 3.9). The differences in least square means between positive and negative SPT showed significantly lower FEV_1/FVC ratio and $FEF_{25-75\%}$ in participants with positive SPT and history of wheezing episodes. No such differences were seen in other group with no history of wheezing. The average FeNO levels were higher in participants with positive SPT irrespective of the history of asthma (Table 3.10).

Degree of sensitization

There were significant differences in all pulmonary function parameters except FVC when comparing group with wheal diameter of > 5 mm to group with wheal diameter of < 3 mm. Additionally, $FEF_{25-75\%}$ was also lower in group with wheal diameter of > 5 mm when compared to group with 3-5 mm wheal diameter. FeNO levels were higher in groups with higher degree of sensitization when compared to groups with lower degree of sensitization (Table 3.11).

Duration of sensitization

The group of participants who had positive SPT since early childhood had lower FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ and higher FeNO when compared to group who tested positive in late adolescence. FEV_1/FVC ratio and $FEF_{25-75\%}$ were also lower in early childhood diagnosed group when compared to pre-adolescence diagnosed group. Lower FEV_1/FVC ratio and higher FeNO were observed in pre-adolescence diagnosed group when compared to late-adolescence diagnosed group (Table 3.12).

We were not able to test the differences within groups of degree of sensitization and duration of sensitization stratified by asthmatic wheezing status because of small size.

Discussion

The results of the present study show significant reductions in FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ and increase in average FeNO with every 1 mm increase in wheal diameter on SPT at age 18 years. Stratified analysis demonstrated decline in FEV_1/FVC ratio and $FEF_{25-75\%}$ were limited to participants with asthma wheezing episodes only.

Other significant findings were reductions in FEV_1 and FVC with increase in wheal size in the group with no history of asthmatic wheezing.

Previous studies have demonstrated that the HDM exposure is associated with increased risk of asthma, increased bronchial airway responsiveness, and increased in both exhaled and nasal fractional nitric oxide. A study found a negative association between concentration to Der p group 1 of HDM found in beddings and percent predicted FEV_1.[44] However, Langley *et al.* demonstrated that participants who were exposed and sensitized to HDM had a more severe form of asthma in terms of lower percent predicted FEV_1, higher BHR and FeNO levels than the participants who were only exposed but not sensitized.[24] Few other studies have reported that increased degree of sensitization was associated with severity of asthma.[60,62] However, there is no literature on association between the wheal size on SPT and pulmonary function parameters.

Decreased FEV_1/FVC and $FEF_{25-75\%}$ along with increased FeNO levels in this group suggests a possibility of ongoing inflammation of larger as well as smaller airways. Asthma can often pose a diagnostic challenge because of inaccurate history of clinical symptoms and lack of evidence of airway obstruction on physical examination. Current guidelines by National Asthma Education and Prevention Program (NAEPP) to diagnose asthma requires at least a partial reversibility in airflow obstruction determined by an increase in FEV_1 of $\geq$ 200 mL and $\geq$ 12 % from baseline measures after bronchodilator administration.[144] However, Rao *et al*. have reported that a low $FEF_{25-75\%}$ in a group of asthmatic children with normal FEV_1 could predict severity of asthma.[64] Few other studies have also shown reductions in $FEF_{25-75\%}$ in individuals allergic specifically to HDM but with no wheezing episodes in both asthmatic[145] and non-asthmatic children.[65]

We did not find any significant reductions in $FEF_{25-75\%}$ in group with no asthmatic symptoms, however we did find reductions in FEV_1 and FVC. Our findings indicate that participants reactive to HDM on SPT but with no history of asthma wheezing attacks may still have underlying abnormal pulmonary function.

Continuous exposure to perennial allergens has been associated with persistence of the inflammatory response that may lead to a worsening of respiratory function. Our findings related to FeNO were consistent with Moody *et al.* who reported positive correlation between wheal size on HDM SPT and FeNO and nasal nitric oxide levels in asymptomatic Pacific Islanders.[36]

The subgroup analyses for degree of sensitization showed significant reduction in FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ in the group with > 5 mm of wheal diameter compared to group with < 3 mm wheal diameter (no sensitization). There were no differences in FEV_1, FVC and FEV_1/FVC ratio for comparisons between 3-5 mm vs < 3 mm or > 5 mm vs 3-5 mm. However, $FEF_{25-75\%}$ was reduced in group with wheal diameter 3-5 mm when compared to < 3mm wheal diameter. These results could possibly suggest that the effects of HDM sensitivity on larger airways or lung capacity may not become evident until the response on SPT reaches 5 mm wheal size but effects on small airways can be observed at mild sensitization of 3-5 mm wheal diameter. Although Koshak *et al.* reported increase in severity of asthma with increased degree of sensitization to indoor allergens including HDM, there was no information on pulmonary function or FeNO levels.[62]

There are some limitations to this study. We did not use predicted values for pulmonary function parameters and this may limit the generalizability of our results.

Although there is plethora of published prediction/ reference equations for spirometric indices for European population, the generalizability of these equations is questionable as many are based on data collected decades ago on small numbers of subjects specific to a set of population and so may affect their applicability to this cohort. However, the new reference equations for wider age range and multi-ethnic groups developed as a part of Global Lung Function Initiative (GLI) could be used in future studies.[127] $FEF_{25-75\%}$ is known to have highest variability among all pulmonary function measurements. The spirometery data used in this study is cross-sectional and therefore could not address the variability in $FEF_{25-75\%}$. Finally, sensitivity to HDM is often related with sensitivity to other indoor allergens especially cats and dogs. Although we adjusted for sensitivity to any allergen other than HDM in our analyses, background correlations could have biased the final estimates.

In conclusion, this study was one of the first to explore the quantitative association between immunological response to HDM on SPT and pulmonary function parameters. The wheal size on SPT should be considered as a potentially important indicator of underlying abnormal pulmonary function even in individuals without asthma symptoms. Future research should be focused on longitudinal studies to examine whether abnormal pulmonary function related to sensitivity to HDM in non-symptomatic individuals have any long term consequences.

Table 3.1: Baseline characteristics for adolescents with pulmonary function data at age 18 years in the Isle of Wight data

Characteristic		N	%	Missing
Sex	Boys	395	47.1	0
	Girls	443	52.9	
HDM result on SPT	Positive (≥ 3 mm)	242	29.0	2
	Negative (< 3 mm)	594	71.1	
Degree of sensitization	> 5 mm	104	12.4	2
	3-5 mm	138	16.5	
	< 3 mm	594	71.1	
Duration of positive SPT to HDM	Since age 4 years	74	30.6	1
	Since age 10 years	65	26.9	
	Only at age 18 years	103	42.6	
Asthma wheezing episodes	Yes	239	28.5	0
	No	599	71.5	
Current smoking	Yes (≥ 100 cigarettes)	264	33.0	38
	No (< 100 cigarettes)	536	67.0	
Atopic sensitization to allergens other than HDM	Yes	262	31.7	11
	No	565	68.3	
Maternal history of allergy	Yes	317	38.6	17
	No	504	61.4	
Paternal history of allergy	Yes	205	25.2	23
	No	610	74.9	
Socio-economic status (SES)	Low	109	13.3	21
	Mid	640	78.3	
	High	68	8.3	
Exposure to environmental tobacco smoke (ETS)	Yes	300	39.1	71
	No	467	60.9	
Use of steroids	Yes	114	13.6	0
	No	724	86.4	

Table 3.2: Average wheal diameter on SPT, anthropometric and pulmonary function parameters at age 18 years

Variable	N	Median	Mean	SD	Missing
HDM wheal diameter (mm)	836	0	1.7	2.6	2
Height (cm)	838	170.7	170.9	9.2	0
Weight (kg)	834	65.5	67.8	13.6	4
Body Mass Index	834	23.2	23.2	4.3	4
FEV_1 (L)	838	3.9	4.0	0.8	0
FVC (L)	838	4.5	4.6	0.9	0
FEV_1/FVC Ratio (%)	838	88	87.3	7.2	0
$FEF_{25-75\%}$ (L)	838	4.3	4.4	1.1	0
FeNO (ppb)	812	22.2	26.4	29.4	26

SD= Standard deviation, FEV_1= Forced expiratory volume in one second,
FVC= Forced vital capacity, $FEF_{25-75\%}$= Forced expiratory flow at 25-75%,
FeNO= Fractional exhaled nitric oxide, ppb= parts per billion

Table 3.3: Unadjusted average pulmonary function among boys and girls (t-test)

Pulmonary function parameter	Boys (n=395)			Girls (n=443)			
	N	Mean	SE	N	Mean	SE	P-value
FEV_1 (L)	395	4.62	0.03	443	3.47	0.02	<.0001
FVC (L)	395	5.35	0.04	443	3.96	0.03	<.0001
FEV_1/FVC (%)	395	86.55	0.37	443	98.92	0.33	0.0058
$FEF_{25-75\%}$ (L)	395	4.99	0.06	443	3.95	0.04	<.0001
FeNO (ppb)	381	22.38	1.04	431	15.79	1.03	<.0001

SE= Standard error
Mean and SE estimates are not adjusted for any covariates

Table 3.4: Unadjusted average pulmonary function among groups of positive and negative SPT for HDM (t-test)

Pulmonary function parameter	HDM SPT=Positive (n=242)			HDM SPT=Negative (n=594)			
	N	Mean	SE	N	Mean	SE	P-value
Wheal Diameter (mm)	242	5.37	0.12	594	0.20	0.03	<.0001
FEV_1 (L)	242	4.04	0.05	594	3.99	0.03	0.4529
FVC (L)	242	4.74	0.06	594	4.56	0.04	0.0103
FEV_1/FVC (%)	242	85.67	0.53	594	87.93	0.27	0.0002
$FEF_{25-75\%}$ (L)	242	4.36	0.08	594	4.48	0.04	0.2228
FeNO (ppb)	235	34.33	0.02	575	14.52	0.01	<.0001

Mean and SE estimates are not adjusted for any covariates

Table 3.5: Unadjusted average pulmonary function among groups of degree of sensitization to HDM (ANOVA)

Pulmonary function parameter	≥ 5 mm (n=104)		3-5 mm (n=138)		< 3 mm (n=594)	
	Mean	SE	Mean	SE	Mean	SE
FEV_1 (L)	4.04	0.80	4.04	0.07	4.00	0.03
FVC (L) [a]	4.81	0.09	4.69	0.08	4..56	0.04
FEV_1/FVC (%) [a,b,c]	84.46	0.69	85.58	0.60	87.93	0.29
$FEF_{25-75\%}$ (L) [a]	4.23	0.11	4.46	0.01	4.48	0.05
FeNO (ppb) [a,b,c]	42.03	1.07	29.41	1.06	14.52	1.03

P-value <0.05: a= ≥ 5 mm vs < 3 mm, b= ≥ 5 mm vs 3-5 mm, c= 3-5 mm vs < 3 mm
ANOVA= Analysis of variance
Mean and SE estimates are not adjusted for any covariates

Table 3.6: Unadjusted average pulmonary function among groups with different duration of positive SPT (ANOVA)

Pulmonary function parameter	Since 4 years (Early childhood) (n=74)		Since 10 years (Pre-adolescence) (n=65		Only at 18 Years (Late adolescence) (n=103)	
	Mean	SE	Mean	SE	Mean	SE
FEV_1 (L)	3.97	0.09	3.97	0.01	4.14	0.08
FVC (L) [a]	4.83	0.11	4.63	0.12	4.75	0.09
FEV_1/FVC (%) [a,b,c,d]	82.55	0.82	85.84	0.88	87.88	0.69
$FEF_{25-75\%}$ (L) [a,b,c]	3.99	0.13	4.40	0.14	4.62	0.11
FeNO (ppb) [a,b,d,e,f]	44.16	1.08	38.58	1.08	26.70	1.07

P-value <0.05: a= since 4 years vs negative, b= since 4 years vs only at 10 years, c= since 4 years vs since 10 years, d= since 10 years vs negative, e = since 10 years vs only at 18 years, f = only at 18 years vs negative

Mean and SE estimates are not adjusted for any covariates

Table 3.7: Adjusted linear regression models for pulmonary function and wheal diameter on SPT for HDM (n=838)

	FEV_1 (L)			FVC (L)		
	Estimate	SE	P-value	Estimate	SE	P-value
Wheal diameter (mm)	-0.02	0.01	0.0226	-0.02	0.01	0.0245
History of wheezing =yes	-0.12	0.05	0.0114	-0.13	0.05	0.0135
Wheal diameter*history of wheezing	-0.001	0.01	0.9711	0.04	0.01	0.0024
	FEV_1/FVC (%)[$]			$FEF_{25-75\%}$ (L)		
	Estimate	SE	P-value	Estimate	SE	P-value
Wheal diameter (mm)	-0.02	0.14	0.8977	-0.02	0.02	0.2145
History of wheezing =yes	-0.13	0.74	0.8645	-0.14	0.11	0.1805
Wheal diameter*history of wheezing	-0.75	0.19	0.0001	-0.06	0.03	0.0350
	FeNO (ppb)					
	Estimate	SE	P-value			
Wheal diameter (mm)	1.10	1.01	<.0001			
History of wheezing =yes	1.09	1.07	0.1932			
Wheal diameter*history of wheezing	1.04	1.02	0.0134			

FeNO: N=812

All models adjusted for height, weight, sex, current smoking, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS, SES, and height*sex interaction

height*sex interaction not significant

Table 3.8: Adjusted linear regression models for pulmonary function and wheal diameter on SPT for HDM stratified by wheezing history

Pulmonary function parameter	History of wheezing (n=239)			No history of wheezing (n=599)		
	Estimate	SE	P-value	Estimate	SE	P-value
FEV_1(L)	-0.02	0.01	0.1177	-0.02	0.01	0.0142
FVC (L)	0.02	0.01	0.1890	-0.02	0.01	0.0264
FEV_1/FVC (%) [$]	-0.78	0.19	<.0001	-0.03	0.14	0.8122
$FEF_{25-75\%}$ (L)	-0.08	0.03	0.0030	-0.03	0.02	0.1825
FeNO (ppb)	1.14	1.02	<.0001	1.10	1.01	<.0001

All models adjusted for height, weight, sex, current smoking, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS, SES, and height*sex interaction

[$]height*sex interaction not significant

Table 3.9: Adjusted linear regression models for pulmonary function for positive and negative SPT for HDM (n=838)

	FEV_1 (L)			FVC (L)		
	Estimate	SE	P-value	Estimate	SE	P-value
Wheal diameter (mm)	-0.06	0.05	0.2058	-0.08	0.06	0.1395
History of wheezing =yes	-0.13	0.05	0.0116	-0.14	0.06	0.0146
Wheal diameter*history of wheezing	-0.01	0.07	0.8554	0.24	0.08	0.0041
	FEV_1/FVC (%)[$]			$FEF_{25-75\%}$ (L)		
	Estimate	SE	P-value	Estimate	SE	P-value
Wheal diameter (mm)	0.37	0.75	0.622	-0.03	0.11	0.7489
History of wheezing =yes	-0.13	0.76	0.8679	-0.16	0.11	0.1277
Wheal diameter*history of wheezing	-4.54	1.14	<.0001	-0.32	0.16	0.0491
	FeNO (ppb)					
	Estimate	SE	P-value			
Wheal diameter (mm)	1.67	1.07	<.0001			
History of wheezing =yes	1.07	1.07	0.3266			
Wheal diameter*history of wheezing	1.37	1.11	0.0021			

FeNO: N=812

All models adjusted for height, weight, sex, current smoking, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS, SES, and height*sex interaction

height*sex interaction not significant

Table 3.10: Differences in least square means between positive and negative SPT for HDM from adjusted linear regression models for pulmonary function stratified by wheezing history

Pulmonary function parameter	History of wheezing (n=239)			No history of wheezing (n=599)		
	Difference	SE	P-value	Difference	SE	P-value
FEV_1(L)	-0.08	0.08	0.3288	-0.07	0.05	0.1588
FVC (L)	0.14	0.09	0.1018	-0.08	0.06	0.1551
FEV_1/FVC (%) [$]	-4.42	1.18	0.0002	0.29	0.75	0.6962
$FEF_{25-75\%}$ (L)	-0.37	0.17	0.0298	-0.05	0.11	0.6535
FeNO (ppb)	2.19	1.13	<.0001	1.69	1.07	<.0001

All models adjusted for height, weight, sex, current smoking, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS, SES, and height*sex interaction

[$]height*sex interaction not significant

Table 3.11: Differences in least square means between groups of different degree of sensitizations for HDM from adjusted linear regression models for pulmonary function (n=838)

Pulmonary function parameters	> 5 mm (n=104) **vs** < 3 mm (n=594)			3-5 mm (n=138) **vs** < 3 mm (n=594)			> 5 mm (n=104) **vs** < 3-5 mm(n=138)		
	Difference	SE	P-value	Difference	SE	P-value	Difference	SE	P-value
FEV_1 (L)	-0.13	0.05	0.0118	-0.02	0.05	0.6232	-0.11	0.06	0.0549
FVC (L)	-0.01	0.06	0.8442	0.02	0.05	0.6903	-0.30	0.07	0.6181
FEV_1/FVC (%)	-2.30	0.82	0.0053	-0.67	0.72	0.3508	-1.63	0.90	0.069
$FEF_{25-75\%}$ (L)	-0.35	0.17	0.0027	-0.02	0.10	0.8293	-0.33	0.13	0.0098
FeNO (ppb)	2.25	1.08	<.0001	1.68	1.07	<.0001	1.34	1.08	0.0003

FeNO: N=812

All models adjusted for height, weight, sex, current smoking, history of asthma wheezing episodes, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS and SES

Table 3.12: Differences in least square means between groups of different degree of sensitizations for HDM from adjusted linear regression models for pulmonary function (n=838)

Pulmonary function parameters	Since age 4 years (n=74) **vs** only at age 18 years (n=103)			Since age 4 years (n=74) **vs** since age 10 years (n=65)			Since age 10 years (n=65) **vs** only at age 18 years (n=103)		
	Difference	SE	P-value	Difference	SE	P-value	Difference	SE	P-value
FEV_1 (L)	-0.25	0.07	0.0003	-0.15	0.08	0.0554	-0.15	0.08	0.0554
FVC (L)	-0.04	0.08	0.5937	-0.02	0.09	0.7755	-0.02	0.09	0.7755
FEV_1/FVC (%)	-4.75	1.08	<.0001	-2.44	1.17	0.0379	-2.44	1.17	0.0379
$FEF_{25-75\%}$ (L)	-0.66	0.15	<.0001	-0.49	0.17	0.0032	-0.49	0.17	0.0032
FeNO (ppb)	1.36	1.10	0.0013	1.08	1.11	0.4647	1.08	1.11	0.4647

FeNO: N=812

All models adjusted for height, weight, sex, current smoking, history of asthma wheezing episodes, other atopic conditions, maternal and paternal history of allergy, use of steroids, ETS and SES

CHAPTER 4

MATERNAL SMOKING DURING PREGNANCY, ADOLESCENT SMOKING AND PULMONARY FUNCTION IN LATE ADOLESCENCE

Introduction

Gestational smoking and adolescent smoking are an important public health problems. Smedberg *et al.* reported an overall smoking prevalence of 26 % in pregnant women in 15 European countries.[72] According to the World Health Organization (WHO), the prevalence of tobacco use among adolescents aged 13-15 years is 20 % in boys and 15 % in girls in Europe.[146] Current survey data from United Kingdom indicates that about 207,000 children aged between 11-15 years starts smoking every year.[75] The independent effects of both maternal smoking during pregnancy and adolescent smoking on children's respiratory health have been extensively investigated. Evidence from several sources show that maternal smoking during pregnancy increased risk of recurrent lower respiratory symptoms[80-83] and asthma attacks during infancy and early childhood years.[3,18,84] Many epidemiological studies conducted in adolescents have reported reduction in pulmonary function and increased risk of new-onset asthma with increased personal smoking by adolescents.[13-17] However, the joint effects of both maternal smoking during pregnancy and adolescent smoking are less extensively studied. The limited information available suggests that adolescent smoking may exacerbate the damage already caused to lungs by maternal smoking during pregnancy and may

eventually cause greater risk of asthma and pulmonary function deficit.[13,93,94] Smoking also has differential effects in adolescent males and females. Even though pulmonary function decreased with increase in smoking in both boys and girls, girls were found to be more vulnerable to the effects of smoking on pulmonary function growth than boys.[14,89] Additionally, a link between maternal smoking during pregnancy and personal smoking by adolescents has also been reported. Intergenerational studies by Kandel *et al.* suggested a significant dose related association between both gestational and current smoking by mothers and smoking by female offspring during adolescence.[91,92]

Both fetal and adolescent period are critical time periods for development of lungs. Therefore, any exposure to tobacco smoke during these periods is a form of programming which can have long term effects on pulmonary function. Because maternal smoking during pregnancy and smoking during adolescence are highly correlated, both exposures need to be considered in assessing the adverse effects of tobacco smoke on pulmonary function. Adolescent smoking may act as an intervening factor in the pathway between maternal smoking during pregnancy and reduction in pulmonary function in adolescents. Additionally, maternal smoking during pregnancy has also been associated with reduced height and increased obesity in children[96-98] which in turn is associated with pulmonary function[99-102] making height and weight possibly intervening variables.

The IOW birth cohort offers an opportunity to investigate the independent and joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in late adolescence. We used pulmonary function data collected in participants at 18 years of age to assess the complex relationships between maternal smoking during pregnancy and pulmonary function mediated through adolescent

smoking, height and weight (both also collected at age 18 years) using path analysis (structural equation models).

Methods

Study Population

Between January 1989 and February 1990 1,536 mothers/child pairs were contacted to be enrolled in the IOW birth cohort after obtaining approval from the Isle of Wight Local Research Ethics Committee at recruitment. After exclusion of perinatal deaths, adoptions, and refusals and obtaining approval from the local research ethics committee 1,456 participants were available for follow-up at 1, 2, 4, 10 and 18 years of age.[130-132]

Exposure

The main exposures of interests were maternal smoking during pregnancy and current personal smoking by adolescents of age 18 years. Birth and obstetric records were used to obtain information on maternal smoking during pregnancy. To determine the current smoking status children were asked "Do you currently smoke?" at age 18 years. Information was also collected on number of cigarettes smoked per day, past smoking and duration of smoking. Adolescent smoking was defined as a history of ever smoking more than 100 cigarettes. To investigate the independent and joint effects of maternal smoking during pregnancy and adolescent smoking we categorized exposure into the following mutually exclusive categories: none, exposure to maternal smoking only, adolescent smoking only and exposure to both maternal smoking and adolescent smoking. This is comparable to a prior categorization used by Sadegnejad *et al.* to

combine exposure to maternal smoking during pregnancy and environmental tobacco smoke in the cohort of IOW at age 10 years.[134] Pack years were calculated from cigarettes smoked per day and duration of smoking and used as main indicator of current smoking for path analysis at 18 years of age.

Outcomes

Pulmonary function tests were performed using a Koko Spirometer and software with a portable desktop device (both PDS Instrumentation, Louisville, KY, USA) to measure FVC, FEV_1, $FEF_{25-75\%}$ and PEFR. Spirometry was performed and evaluated according to the American Thoracic Society (ATS) criteria. Children were required to be free of respiratory infection for two weeks and not to be taking any oral steroids and were advised to abstain from any β-agonist medication for six hours and from caffeine intake for at least four hours.[132] FeNO was measured (Niox mino, Aerocrine AB, Solna, Sweden) according to ATS guidelines.[48] Measurements were made in a standardized manner with the subject standing without a nose clip; FeNO was measured before spirometric testing. Participants were rescheduled if they had clinical symptoms consistent with either current infection or a recent (within 2 weeks) asthma exacerbation, or had required antibiotics or oral steroids in the preceding 2 weeks.[133] We log transformed the FeNO values to get a normal distribution. The final estimates were back transformed.

Potential Confounders

Birth weight, sex, height and weight measured at 18 years, BMI (18 years), history of allergic conditions at 18 years (atopy), and socio-economic status (SES)

determined at 10 years of age were considered as potential confounders and were adjusted in explanatory models. Information on birth weight was obtained from the hospital records. Height and weight were measured before the spirometric tests at age 18; BMI was calculated. Information on the SES was based on the following three variables: (a) the British socioeconomic classes (1–6) derived from parental occupation reported at birth; (b) the number of children in the index child's bedroom (collected at age 4 years); and (c) family income at age 10 years.[131]

Statistical Analysis

Of total 1,456 participants 838 participated in spirometric testing at 18 years of age. In the initial analyses we used t-tests and analysis of variance (ANOVA) to test the differences in pulmonary function among different groups of smoking. Linear regression analyses were then used to determine the independent and joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function. To address the issue of missing data on one or more confounders we used multiple imputations procedure PROC MI with multivariate normal distribution to generate ten new data sets. We used PROC MIXED model to obtain the parameter estimates for each imputed data set. Next, we used PROC MIANALYZE to generate valid parameter. The models were adjusted for height, weight, birth weight, atopy, ETS and SES. We used backward elimination method and selected the confounders that changed the estimates by 10 %. To account for the differential effects of lung growth with respect to sex and height we introduced interaction term between sex and height in the models.

As explained earlier, current smoking by adolescents, height and weight possibly act as intervening variables in the pathway between maternal smoking during pregnancy

and pulmonary function in adolescents. Adjusting on intervening variables in regression analyses can disrupt the underlying path. Therefore, we explored these relationships by path analysis using Covariance Analysis of Linear Structural Equations. We used Full Information Maximum Likelihood (FIML) method to determine parameter estimates which addresses the issue of missing values for covariates. Pack years was used as an indicator of adolescent smoking in all path analysis models. The adequacy of model fit was determined by several statistics: a chi-square p-value > 0.05 if chi-square test statistic is close to 0, comparative fit index (CFI) > 0.9, adjusted goodness of fit index (GFI) > 0.9 and root mean square error of approximation (RMSEA) < 0.06. The data were analyzed using the SAS statistical package (version 9.3; SAS Institute, Cary, NC, USA).

Results

Out of 838 participants who had spirometry at 18 years of age 443 (52.9 %) were girls. Majority of participants belonged to middle social status (78.3 %) based on parental occupation, number of children in one bedroom and annual family income. About 41.4 % had history of other atopic conditions. The prevalence of adolescent smoking among this age group was 33 % and about 20.1 % were exposed to maternal smoking during fetal period. About 9.2 % of participants had both smoking exposures (Table 4.1). Average anthropometric and pulmonary function measures in the cohort selected for this study are presented in Table 4.2.

On average boys had higher birth weight, height, weight, FEV_1, FVC and $FEF_{25-75\%}$ and lower BMI and FEV_1/FVC ratio when compared to girls (Table 4.3). These estimates were not adjusted for any covariates. The unadjusted estimates for FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ were lower in adolescent smokers (> 100 cigarettes) when

compared to non-smokers (Table 4.4). Adolescents who were exposed to *in-utero* maternal smoking also showed lower FEV_1/FVC ratio and $FEF_{25-75\%}$ (Table 4.5). Additionally, lower FEV_1/FVC ratio and $FEF_{25-75\%}$ were observed among adolescents who were exposed to both forms of tobacco exposure compared to those who were not exposed at all (Table 4.6). Interestingly, we found higher FeNO levels in non-exposed adolescents than adolescents exposed to either one or both forms of smoking (Table 4.4 and 4.6).

To determine the independent and joint effects of maternal smoking during pregnancy and adolescent smoking with pulmonary function, we conducted linear regression using four mutually exclusive categories of smoking exposure- maternal smoking during pregnancy only, adolescent smoking only, both and none (reference category). All the final models were adjusted for height, weight, birth weight, atopy and ETS (Table 4.7). Models for FVC and FEV_1 were additionally adjusted for interaction term sex*height. The results demonstrated individuals who were exposed to both maternal smoking and adolescent smoking had lower FEV_1/FVC ratio (-1.99 % ± 0.93, p-value: 0.0328) and $FEF_{25-75\%}$ (-0.25 L ± 0.13, p-value: 0.0522) than those who were not exposed at all. Group exposed to adolescent smoking only and group exposed to both forms of smoking showed higher FeNO levels when compared to reference category (no smoking exposure) (Table 4.7). Additionally, FeNO levels were also found to be higher in group with both smoking exposures (0.76 ± 1.11 ppb, p-value: 0.0088) when compared to the group exposed to maternal smoking only (data not shown in tables). No independent effects of either maternal smoking during pregnancy or adolescent smoking were observed on any spirometric measures in the total cohort.

In stratified analysis, boys did not show reduction in spirometric measures in relation to any form of smoking. However there was increase in FEV_1 in the group exposed to maternal smoking during fetal period when compared to the reference category (Table 4.8). In girls we found that both maternal smoking during pregnancy and adolescent smoking had independent inverse effects on pulmonary function. While girls who were exposed only to maternal smoking during fetal period had reductions in both FEV_1/FVC ratio and $FEF_{25-75\%}$; girls with exposure only to adolescent smoking showed reduction in FEV_1 when compared to the group with no exposure. Additionally, there were also joint inverse effects of both maternal smoking and adolescent smoking on FEV_1/FVC ratio and $FEF_{25-75\%}$ (Table 4.9). Both boys (except those exposed to maternal smoking only) and girls exposed to any form of smoking had higher FeNO levels compared to reference category (Table 4.8 and 4.9).

All path analysis models fulfilled the criteria of model fit. Table 4.10 A presents the path coefficients for total, direct and indirect effects of maternal smoking during pregnancy on adolescent smoking, birth weight, height and weight at age 18 years in boys. In boys maternal smoking during pregnancy had inverse effects on birth weight and height at age 18 years and positive effect on adolescent smoking (Table 4.10 A). The path analysis assessing the effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function was conducted separately in boys and girls and all models were adjusted for birth weight, height and weight at 18 years of age and history of any atopic conditions, ETS and SES. ETS and SES did not contribute significantly to the variance of final path analysis models and their removal from model did not change the path coefficients. Path analysis in boys showed that neither maternal smoking nor

adolescent smoking had any effects on FEV_1, FVC or $FEF_{25-75\%}$, but adolescent smoking did have a direct inverse effect on FEV_1/FVC ratio (Table 4.11 B). Since maternal smoking during pregnancy had positive effect on adolescent smoking possibly effect of maternal smoking was carried forward to FEV_1/FVC through adolescent smoking (Figure 4.1).

On the other hand, in girls maternal smoking during pregnancy had positive effects on adolescent smoking and weight at 18 years of age while no effect on either birth weight or height at age 18 years (Table 4.10 B). As linear regression models, path analysis results also showed that adolescent smoking had direct inverse effect on FEV_1 in girls and although the maternal smoking did not have any direct effects its effects on FEV_1 were mediated through adolescent smoking and weight (Table 4.12 A, Figure 4.2). Additionally, maternal smoking had both direct and indirect effects mediated through weight at age 18 years of age on FEV_1/FVC ratio and $FEF_{25-75\%}$ but no effects of adolescent smoking (Tables 4.12 B, Figure 5.3 and 5.4).

Discussion

We studied the IOW birth cohort to assess the independent and joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function during late adolescence. The results of linear regression showed that in boys there were no adverse independent or joint effects of maternal smoking during pregnancy and adolescent smoking. However, the path analysis suggested that adolescent smoking had inverse effects on FEV_1/FVC ratio in boys. The path analysis also demonstrated that maternal smoking during pregnancy had direct effect on adolescent smoking in both boys and girls, thus confirming that offspring of mothers who smoked during pregnancy are

more likely to smoke during adolescence. Therefore, even though maternal smoking during pregnancy did not have any direct effect on FEV_1/FVC ratio in boys, it is possible that its adverse effects were mediated through its effects on adolescent smoking.

In girls both adolescent smoking and maternal smoking had independent as well as joint adverse effects on FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$, respectively. Our path analysis findings corroborated the results from linear regression. It demonstrated that adolescent smoking had direct inverse effect on FEV_1 and the effects of maternal smoking during pregnancy were mediated through its effects on adolescent smoking and weight. Additionally, we also found direct inverse effects of maternal smoking during pregnancy on FEV_1/FVC and $FEF_{25-75\%}$ but no effects of adolescent smoking. Thus, although linear regression results suggested that maternal smoking during pregnancy and adolescent smoking had joint adverse effects on FEV_1/FVC and $FEF_{25-75\%}$ in girls, path analysis demonstrated these effects were mainly due to maternal smoking during pregnancy and adolescent smoking did not contribute significantly to the reduction in FEV_1/FVC and $FEF_{25-75\%}$.

Not many studies have investigated the joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in adolescents. A study conducted by Rizzi *et al.* in healthy adolescent boys in Milan showed a mild airway obstruction in current smokers not exposed to maternal smoking during fetal period and deficit in smaller airway function in current passive smokers exposed to maternal smoking during fetal period.[94] There was however no information on pulmonary function among current smokers who were exposed to *in-utero* maternal smoking. Sherrill *et al.* examined the effects of smoking on lung function in children of age 9 to 15 years in a

longitudinal cohort study conducted in New Zealand.[95] They reported pulmonary function deficit in 9-15 years old children exposed to current parental smoke but no effect of either current adolescent smoking or maternal smoking during pregnancy. One possible explanation for the discrepancy between our results and results by Sherrill *et al.* may be the differences in the ages of participants. The participants in our study (18 years old) were older than Sherrill *et al.* (9-15 years old) by 3-9 years; the prevalence of smoking increases with age. Additionally, only a small number of participants were exposed to maternal smoking during pregnancy (n=10); in our study the prevalence of maternal smoking during pregnancy was 20.1 % (n=168). Another study by Hayatbakhsh *et al.* conducted in young adults reported that *in-utero* exposure to maternal smoking was associated with a reduction in FEV_1 and $FEF_{25–75\%}$ in males of 21 years after accounting for maternal smoking after pregnancy and smoking by children at age 14 years.[93] However, this study did not consider information on current smoking status by participants. Our findings related to differences in boys and girls were comparable to those by Holmen *et al.* and Gold *et al.*[14,89] Both these studies investigated the effects of adolescent smoking on pulmonary function after adjusting for passive smoking. Holmen *et al.* in a cohort of 13-18 years old from Norway found that adolescent smoking was associated with reduction in pulmonary function only in girls.[89] Although Gold *et al.* found lower FEV_1/FVC ratio and $FEF_{25-75\%}$ in both boys and girls of age 10 -18 years in association with adolescent smoking, they found that girls were more vulnerable to adverse effects of smoking and had lower growth of pulmonary function than boys.[14] Our findings related to maternal smoking during pregnancy were partially comparable to a study by Hollams *et al.* who reported reduction in FEV_1/FVC ratio and $FEF_{25-75\%}$ in

association with maternal smoking during pregnancy in current adolescent non-smokers.[147]

Although the unadjusted estimates of FeNO were lower in those exposed to either form of smoking (Table 4.4, 4.5 and 4.6) the adjusted linear regression estimates demonstrated higher FeNO levels in both boys and girls who were exposed either to maternal smoking during fetal period or current smoking or both (Tables 4.8 and 4.9). Previous studies have reported increased FeNO levels in infants and young children who were exposed to maternal smoking during pregnancy.[148,149] To our knowledge no study has examined this relationship in adolescents.

The underlying pathophysiological mechanisms of adverse effects of personal smoking are well documented. Persistent exposure to smoking causes inflammation and epithelial changes in bronchial tissues which leads to secretory congestion and fibrosis causing remodeling of airways and destruction of alveoli resulting in loss of gas exchange.[77] Although there have been no histological study describing the patho-physiological effects of maternal smoking on the airway development during fetal period in humans; one can hypothesize that smoking will adversely affect the development of lungs during the gestational period as a result of blood flow restriction to the placenta due to nicotine induced vasoconstriction, poor nutritional status of the mother associated with the anorexigenic effect of nicotine and carbon monoxide exposure.[78] An animal model presented by Rehan *et al.* to study the effects of maternal smoking on fetal lung development showed that *in-utero* exposure to tobacco smoke alters the normal homeostatic epithelial-mesenchymal interaction in the developing alveolus, resulting in production of myofibroblasts.[79] Myofibroblasts contribute to the formation of fibrosis in

larger as well as smaller airways and is a common finding in asthma and chronic lung disease.[79] Previous studies have also shown an association between maternal smoking during pregnancy and higher FVC in children.[150-152] Studies have suggested that maternal smoking during pregnancy or exposure to parental smoking during early childhood may cause disproportional growth of lung parenchyma and airways known as dysnaptic growth of lungs in children.[69,151,153,154] Our findings were different from previous studies, we found increased FEV_1 in boys in relation to maternal smoking during pregnancy. It is possible that maternal smoking during pregnancy disrupted the process of normal lung growth and altered the pattern of growth of the airways in relation to lung size.

There are some limitations to our study. Because of negative attitude towards smoking during pregnancy the self-reported information on smoking during pregnancy was probably underestimated and it was not validated by objective measurements. Under-reporting of smoking prevalence may bias our estimates for maternal smoking during pregnancy towards the null. We also did not use predicted values for pulmonary function parameters and this may limit the interpretation of our results in clinical settings. Our modeling strategy with linear regression for pulmonary function may have introduced some errors in our estimates. Maternal smoking during pregnancy affects birth weight which in turn has independent association with pulmonary function.[155,156] Therefore, adjusting on birth weight in linear regression may have introduced bias. However, use of path analysis allowed us to include birth weight as mediating factor in the explanatory models which helped disentangle the complex relationship between numerous determinants of pulmonary function. Additionally, in linear regression we had small sample size in four smoking exposure categories stratified by sex but this was not a

problem in path analysis as maternal smoking during pregnancy and adolescent smoking were considered as two independent factors.

In conclusion our findings suggest that maternal smoking during pregnancy has an adverse association with adolescent smoking in offspring. The joint effects of maternal smoking during pregnancy and adolescent smoking may be responsible for larger deficit in pulmonary function than the independent effect of either exposure. However, these finding were significant only in girls. The results of the path analysis deepened our understanding of relationships between various determinants of pulmonary function. The increased FeNO levels and reduction in FEV_1/FVC and $FEF_{25-75\%}$ values found in girls suggest that tobacco smoke leads to chronic inflammation and remodeling in small as well as larger airways. These data demonstrate that not only tobacco exposure has differential inverse effects on pulmonary function among adolescent boys and girls, but maternal smoking during pregnancy has a measurable adverse effect on the pulmonary function which is independent of any direct use of cigarettes by adolescents. These findings are especially important because lower pulmonary function during adolescence may possibly lead to chronic obstructive pulmonary disease and small airway disease in adulthood. Our results confirm the need of intervention strategies of tobacco cessation in adolescents and women of reproductive age. Furthermore, reduction in maternal smoking during pregnancy may not only reduce the burden of chronic respiratory diseases in the long term but may also reduce the burden of smoking in the adolescents.

Table 4.1: Baseline characteristics for adolescents with pulmonary function data at age 18 years in the Isle of Wight data

Characteristic		N	%	Missing
Sex	Boys	395	47.1	0
	Girls	443	52.9	
Maternal smoking during pregnancy	Yes	168	20.1	4
	No	666	79.9	
Adolescent smoking	Yes (≥ 100 cigarettes)	264	33.0	38
	No (< 100 cigarettes)	536	67.0	
Maternal smoking during pregnancy + adolescent smoking	Both	73	9.2	42
	Maternal smoking during pregnancy only	86	10.8	
	Adolescent smoking only	189	23.7	
	None	448	56.3	
Environmental tobacco smoke (ETS)	Yes	300	39.1	71
	No	467	60.9	
History of any atopic condition	Yes	343	41.4	10
	No	485	58.6	
Socio-economic status (SES)	Lower	109	13.3	21
	Middle	640	78.3	
	Higher	68	8.3	

Table 4.2: Average anthropometric and pulmonary function parameters at age 18 years

	N	Mean	Median	SD	Missing
Birth weight (kg)	825	3.41	3.42	0.51	13
Height (cm)	838	170.88	170.70	9.17	0
Weight (kg)	834	67.79	65.50	13.60	4
BMI	834	23.21	22.15	4.33	4
FEV_1 (L)	838	4.01	3.91	0.78	0
FVC (L)	838	4.61	4.47	0.93	0
FEV_1/FVC (%)	838	87.28	88.00	7.16	0
$FEF_{25-75\%}$ (L)	838	4.44	4.32	1.14	0
FeNO (ppb)	822	26.27	16.00	29.28	0

SD= Standard deviation, FEV_1= Forced expiratory volume in one second, FVC= Forced vital capacity, $FEF_{25-75\%}$= Forced expiratory flow at 25-75%, FeNO= Fractional exhaled nitric oxide, ppb= parts per billion

Table 4.3: Comparison of smoking exposure, anthropometric and pulmonary function measures among boys and girls at age 18 years (t-test)

Variable		Boys		Girls	
		N	%	N	%
Maternal smoking during pregnancy	Yes	75	19.1	93	21.0
	No	317	80.9	349	79.0
Adolescent smoking	Yes (≥ 100 cigarettes)	113	30.1	151	35.6
	No (< 100 cigarettes)	263	69.9	273	64.4
Maternal smoking during pregnancy + adolescent smoking	Both	32	8.6	41	9.7
	Maternal smoking during pregnancy only	42	11.3	44	10.4
	Adolescent smoking only	80	21.4	109	25.8
	None	219	58.7	229	54.1

Variable	Boys			Girls			
	N	Mean	SE	N	Mean	SE	P-value
Birth weight (kg)	389	3.48	0.03	436	3.36	0.02	0.0009
Height (cm)	395	177.8	0.33	443	164.7	0.29	<.0001
Weight (kg)	394	71.22	0.64	440	64.71	0.65	<.0002
BMI	394	22.51	0.19	440	23.84	0.23	<.0003
FEV_1 (L)	395	4.62	0.03	443	3.47	3.47	<.0001
FVC (L)	395	5.35	0.04	443	3.96	0.03	<.0001
FEV_1/FVC (%)	395	86.55	0.37	443	87.92	0.33	0.0058
$FEF_{25-75\%}$ (L)	395	4.99	0.06	443	3.95	0.04	<.0001
FeNO (ppb)	387	22.26	1.04	435	15.74	1.03	<.0001

SE= Standard error

Mean and SE estimates are not adjusted for any covariates

Table 4.4: Unadjusted average pulmonary function stratified by adolescent smoking status (t-test)

Pulmonary function parameter	Smoker (>100 cigarettes) (n=264)		Non-smoker (≤ 100 cigarettes) n=(536)		
	Mean	SE	Mean	SE	P-value
FEV_1 (L)	3.91	0.05	4.06	0.03	0.015
FVC (L)	4.55	0.06	4.65	0.04	0.1571
FEV_1/FVC (%)	86.49	0.45	87.68	0.31	0.0275
$FEF_{25-75\%}$ (L)	4.29	0.07	4.51	0.05	0.0083
FeNO (ppb)	15.59	11.05	19.97	1.03	<0.001

FeNO: smoker=260, non-smoker=524
Mean and SE estimates are not adjusted for any covariates

Table 4.5: Unadjusted average pulmonary function stratified by maternal smoking during pregnancy (t-test)

Pulmonary function parameter	Maternal smoking during pregnancy				
	Yes (n=168)		No (n=666)		
	Mean	SE	Mean	SE	P-value
FEV_1 (L)	3.96	0.06	4.02	0.03	0.3399
FVC (L)	4.61	0.07	4.61	0.04	0.9642
FEV_1/FVC (%)	86.15	0.56	87.55	0.28	0.0236
$FEF_{25-75\%}$ (L)	4.28	0.09	4.48	0.04	0.0424
FeNO (ppb)	22.93	2.20	27.15	1.16	0.0988

FeNO: Maternal smoking during pregnancy - yes=168, no=653
Mean and SE estimates are not adjusted for any covariates

Table 4.6: Unadjusted average pulmonary function stratified by combined smoking exposure status (ANOVA)

Pulmonary function parameter	Maternal + adolescent smoking (n=73)		Only maternal smoking during pregnancy (n=86)		Only adolescent smoking (n=189)		None (n=448)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
FEV_1 (L)	3.87	0.09	4.07	0.08	3.94	0.06	4.05	0.04
FVC (L)	4.54	0.11	4.72	0.10	4.56	0.07	4.63	0.04
FEV_1/FVC (%)*	85.73	0.84	86.57	0.78	86.74	0.52	87.90	0.34
$FEF_{25-75\%}$ (L)*	4.14	0.13	4.44	0.12	4.35	0.08	4.52	0.05
FeNO (ppb)*	19.40	3.46	26.22	3.16	24.04	2.13	27.88	1.39

FeNO: Maternal + adolescent smoking=71, only maternal smoking during pregnancy=85, only adolescent smoking=187, none=437
*= significant differences in Maternal + adolescent smoking vs none
ANOVA= Analysis of variance

Table 4.7: Joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in total cohort (n=838)

Pulmonary function parameter	Difference †	SE	P-value
FEV_1 (L) $			
Maternal + adolescent smoking	-0.05	0.06	0.4379
Maternal smoking only	0.05	0.06	0.3724
Adolescent smoking only	-0.04	0.04	0.2662
FVC (L) $			
Maternal + adolescent smoking	0.05	0.07	0.4873
Maternal smoking only	0.11	0.06	0.0903
Adolescent smoking only	0.01	0.04	0.8666
FEV_1/FVC (%)			
Maternal + adolescent smoking	-1.99	0.93	0.0328
Maternal smoking only	-1.06	0.87	0.2237
Adolescent smoking only	-1.05	0.60	0.0822
$FEF_{25-75\%}$ (L)			
Maternal + adolescent smoking	-0.25	0.13	0.0522
Maternal smoking only	-0.05	0.12	0.689
Adolescent smoking only	-0.10	0.09	0.247
FeNO (ppb)*			
Maternal + adolescent smoking	0.68	1.09	<.0001
Maternal smoking only	0.91	1.09	0.2296
Adolescent smoking only	0.81	1.06	0.0002

FeNO: n=822
Reference category= none
All models are adjusted for birth weight, sex, height, weight, history of any atopic condition, environmental tobacco smoke (ETS)
† Differences in least squares means from linear regression models
$ Models for FVC and FEV_1 are additionally adjusted for interaction term sex*height
* Difference was also significant between both vs maternal smoking only 0.76 ± 1.11 (p-value 0.0088)

Table 4.8: Joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in boys (n=395)

Pulmonary function parameter	Difference †	SE	P-value
FEV_1 (L)			
Maternal + adolescent smoking	0.04	0.10	0.6722
Maternal smoking only	0.21	0.09	0.0245
Adolescent smoking only	0.03	0.07	0.6687
FVC (L)			
Maternal + adolescent smoking	0.08	0.12	0.4952
Maternal smoking only	0.18	0.10	0.0897
Adolescent smoking only	0.12	0.07	0.1062
FEV_1/FVC (%)			
Maternal + adolescent smoking	-0.60	1.47	0.6835
Maternal smoking only	1.28	1.32	0.3323
Adolescent smoking only	-1.41	0.94	0.1327
$FEF_{25-75\%}$ (L)			
Maternal + adolescent smoking	-0.05	0.23	0.8175
Maternal smoking only	0.29	0.20	0.1545
Adolescent smoking only	-0.08	0.15	0.5666
FeNO (ppb)*			
Maternal + adolescent smoking	0.66	1.15	0.0029
Maternal smoking only	1.04	1.14	0.7693
Adolescent smoking only	0.74	1.09	0.001

FeNO: n=387
Reference category= none
† Differences in least squares means from linear regression models
All models are adjusted for birth weight, height, weight, history of any atopic condition, ETS
* Differences were also significant between both vs maternal smoking only 0.63 ± 1.18 (p-value 0.0061) and maternal smoking only vs adolescent smoking only 1.38 ± 1.14 (p-value=0.0156)

Table 4.9: Joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in girls (n=443)

Pulmonary function parameter	Difference †	SE	P-value
FEV_1 (L)			
Maternal + adolescent smoking	-0.11	0.07	0.1052
Maternal smoking only	-0.08	0.07	0.1983
Adolescent smoking only	-0.10	0.05	0.0221
FVC (L)			
Maternal + adolescent smoking	0.02	0.07	0.8159
Maternal smoking only	0.03	0.07	0.6453
Adolescent smoking only	-0.09	0.05	0.0892
FEV_1/FVC (%)			
Maternal + adolescent smoking	-2.84	1.20	0.0182
Maternal smoking only	-2.88	1.16	0.0131
Adolescent smoking only	-0.79	0.79	0.313
$FEF_{25-75\%}$ (L)			
Maternal + adolescent smoking	-0.37	0.15	0.0127
Maternal smoking only	-0.32	0.14	0.0289
Adolescent smoking only	-0.12	0.10	0.239
FeNO (ppb)*			
Maternal + adolescent smoking	0.72	1.12	0.0031
Maternal smoking only	0.82	1.11	0.0554
Adolescent smoking only	0.86	1.08	0.0442

FeNO: n=435
Reference category= none
† Differences in least squares means from linear regression models
All models are adjusted for birth weight, height, weight, history of any atopic condition, ETS

Table 4.10 A: The standardized direct, indirect and total effects of maternal smoking during pregnancy on birth weight, height, weight and adolescent smoking in boys (n=395)

Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Birth weight	-0.23	0.05	<.0001	-0.23	0.05	<.0001			
Height at 18 years	-0.13	0.05	0.0071	-0.09	0.05	0.0459	-0.04	0.02	0.0977
Weight at 18 years	0.02	0.05	0.6587	0.04	0.05	0.3933	-0.02	0.01	0.0921
Adolescent smoking (pack years)	0.21	0.05	<.0001	0.21	0.05	<.0001			

Effect of maternal smoking during pregnancy on each variable is adjusted by other covariates explanatory models.
Goodness of Fit Criteria: Chi-squared test statistic=1.85, p-value 0.9326; AGFI=0.9999; CFI=1; RMSEA=0

Table 4.10 B: The standardized direct, indirect and total effects of maternal smoking during pregnancy on birth weight, height, weight and adolescent smoking in girls (n=443)

Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Birth weight	-0.05	0.05	0.2925	-0.05	0.05	0.2925			
Height at 18 years	-0.03	0.05	0.5115	-0.08	0.04	0.0861	0.05	0.02	0.0222
Weight at 18 years	0.18	0.05	0.0001	0.18	0.05	<.0001			
Adolescent smoking (pack years)	0.26	0.05	<.0001	0.26	0.05	<.0001	-0.01	0.01	0.3099

Effect of maternal smoking during pregnancy on each variable is adjusted by other covariates explanatory models.
Goodness of Fit Criteria: Chi-squared test statistic=9.24, p-value 0.1601; AGFI=0.9997; CFI=0.9877; RMSEA=0.035

Table 4.11 A: The standardized direct, indirect and total effects of maternal smoking during pregnancy and adolescent smoking along with other covariates on FEV_1 and FVC in boys (n=395)

FEV_1									
Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Maternal smoking during pregnancy	0.003	0.05	0.9536	0.10	0.04	0.0274	-0.09	0.03	0.0034
Adolescent smoking (pack years)	-0.04	0.04	0.3854	-0.04	0.04	0.3854			
Birth weight	0.23	0.05	<.0001	0.10	0.04	0.0204	0.13	0.03	<.0001
Height	0.51	0.04	<.0001	0.51	0.04	<.0001			
Weight	0.25	0.05	<.0001	0.06	0.04	0.1512	0.19	0.03	<.0001
Atopy	-0.07	0.04	0.0748	-0.07	0.04	0.0748			
FVC									
Maternal smoking during pregnancy	0.003	0.05	0.9585	0.06	0.04	0.1398	-0.06	0.03	0.0794
Adolescent smoking (pack years)	0.05	0.04	0.2142	0.05	0.04	0.2142			
Birth weight	0.17	0.05	0.0010	0.02	0.04	0.5935	0.14	0.03	<.0001
Height	0.53	0.04	<.0001	0.53	0.04	<.0001			
Weight	0.36	0.04	<.0001	0.16	0.04	0.0002	0.19	0.02	<.0001
Atopy	-0.03	0.04	0.4194	-0.03	0.04	0.4194			

Model Fit Criteria: FEV_1- Chi-squared test statistic=1.85, p-value 0.9326; AGFI=0.9999; CFI=1; RMSEA=0
FVC- Chi-squared test statistic=1.81, p-value 0.9364; AGFI=0.9999; CFI=1; RMSEA=0

Table 4.11 B: The standardized direct, indirect and total effects of maternal smoking during pregnancy and adolescent smoking along with other covariates on FEV_1/FVC and $FEF_{25-75\%}$ in boys (n=395)

FEV_1/FVC									
Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Maternal smoking during pregnancy	0.01	0.05	0.7996	0.06	0.05	0.2611	-0.05	0.02	0.0281
Adolescent smoking (pack years)	-0.12	0.05	0.0176	-0.12	0.05	0.0176			
Birth weight	0.08	0.05	0.1070	0.11	0.05	0.0396	-0.02	0.02	0.1100
Height	-0.04	0.05	0.4531	-0.04	0.05	0.4531			
Weight	-0.17	0.05	0.0006	-0.15	0.05	0.0037	-0.02	0.02	0.4546
Atopy	-0.06	0.05	0.2399	-0.06	0.05	0.2399			
$FEF_{25-75\%}$									
Maternal smoking during pregnancy	-0.01	0.05	0.8627	0.07	0.05	0.1972	-0.07	0.02	0.0007
Adolescent smoking (pack years)	-0.09	0.05	0.0877	-0.09	0.05	0.0877			
Birth weight	0.18	0.05	0.0004	0.13	0.05	0.0136	0.05	0.02	0.0012
Height	0.22	0.05	<.0001	0.22	0.05	<.0001			
Weight	0.06	0.05	0.1926	-0.02	0.05	0.7494	0.08	0.02	0.0002
Atopy	-0.05	0.05	0.3169	-0.05	0.05	0.3169			

Model Fit Criteria: FEV_1/FVC- Chi-squared test statistic=1.86, p-value 0.9324; AGFI=0.9999; CFI=1; RMSEA=0
$FEF_{25-75\%}$- Chi-squared test statistic=1.85, p-value 0.9327; AGFI=0.9999; CFI=1; RMSEA=0

Table 4.12 A: The standardized direct, indirect and total effects of maternal smoking during pregnancy and adolescent smoking along with other covariates on FEV_1 and FVC in girls (n=443)

FEV_1									
Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Maternal smoking during pregnancy	-0.06	0.05	0.2296	-0.03	0.04	0.5035	-0.03	0.03	0.3237
Adolescent smoking (pack years)	-0.10	0.04	0.0139	-0.10	0.04	0.0139			
Birth weight	0.16	0.05	0.0007	0.03	0.04	0.4623	0.13	0.03	<.0001
Height	0.48	0.04	<.0001	0.48	0.04	<.0001			
Weight	0.23	0.05	<.0001	0.08	0.04	0.0636	0.15	0.02	<.0001
Atopy	-0.04	0.04	0.3751	-0.04	0.04	0.3751			
FVC									
Maternal smoking during pregnancy	0.05	0.05	0.3280	0.07	0.04	0.0685	-0.03	0.03	0.3985
Adolescent smoking (pack years)	-0.12	0.04	0.0021	-0.12	0.04	0.0021			
Birth weight	0.21	0.05	<.0001	0.07	0.04	0.0920	0.14	0.03	<.0001
Height	0.51	0.04	<.0001	0.51	0.04	<.0001			
Weight	0.29	0.04	<.0001	0.14	0.04	0.0010	0.16	0.02	<.0001
Atopy	-0.003	0.04	0.9458	-0.003	0.04	0.9458			

Model Fit Criteria: FEV_1- Chi-squared test statistic=9.25, p-value 0.1601; AGFI=0.9997; CFI=0.9877; RMSEA=0.035
FVC- Chi-squared test statistic=9.16, p-value 0.165; AGFI=0.9997; CFI=0.99; RMSEA=0.03545

Table 4.12 B: The standardized direct, indirect and total effects of maternal smoking during pregnancy and adolescent smoking along with other covariates on FEV_1/FVC and $FEF_{25-75\%}$ in girls (n=443)

FEV_1/FVC									
Variables	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Maternal smoking during pregnancy	-0.17	0.05	0.0003	-0.17	0.05	0.0006	0.00	0.02	0.9572
Adolescent smoking (pack years)	0.03	0.05	0.4864	0.03	0.05	0.4864			
Birth weight	-0.09	0.05	0.0536	-0.06	0.05	0.2492	-0.03	0.01	0.0125
Height	-0.08	0.05	0.1158	-0.08	0.05	0.1158			
Weight	-0.11	0.05	0.0181	-0.09	0.05	0.0768	-0.02	0.02	0.1245
Atopy	-0.07	0.05	0.1448	-0.07	0.05	0.1448			
$FEF_{25-75\%}$									
Maternal smoking during pregnancy	-0.14	0.05	0.0032	-0.14	0.05	0.0037	0.00	0.02	0.8554
Adolescent smoking (pack years)	-0.01	0.05	0.7750	-0.01	0.05	0.7750			
Birth weight	0.04	0.05	0.4166	-0.01	0.05	0.8468	0.05	0.02	0.0015
Height	0.16	0.05	0.0016	0.16	0.05	0.0016			
Weight	0.11	0.05	0.0251	0.06	0.05	0.2340	0.05	0.02	0.0038
Atopy	-0.06	0.05	0.2314	-0.06	0.05	0.2314			

Model Fit Criteria: FEV_1/FVC- Chi-squared test statistic=9.12, p-value 0.1657; AGFI=0.9998; CFI=0.978; RMSEA=0.0343
$FEF_{25-75\%}$- Chi-squared test statistic=9.23, p-value 0.1612; AGFI=0.9997; CFI=0.9773; RMSEA=0.0348

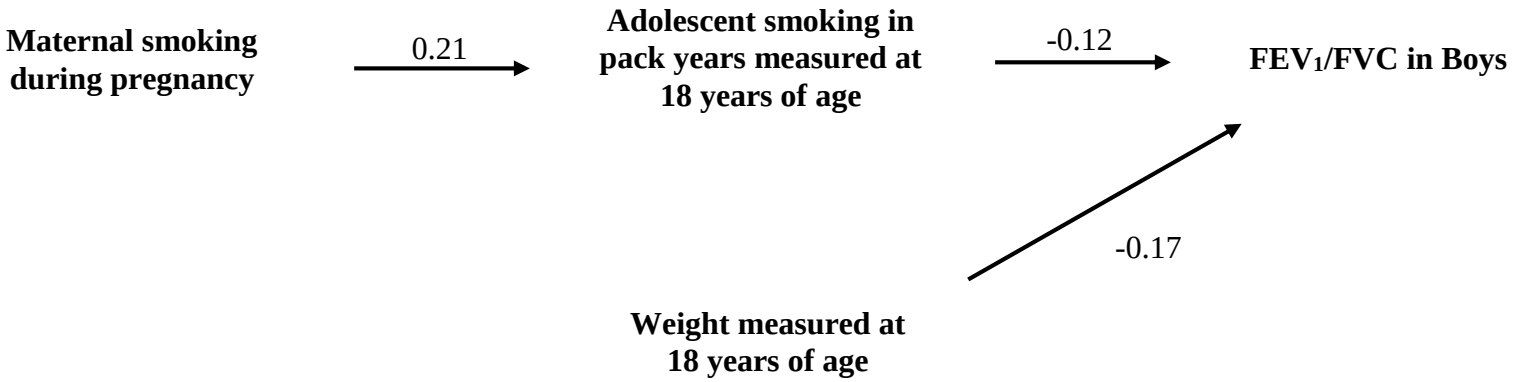


Figure 4.1: Analytical path model exploring the standardized direct effects of maternal smoking during pregnancy and adolescent smoking on FEV_1/FVC in boys (path coefficients from Table 4.10 A and 4.11 B)

The path coefficients represented by solid arrows are direct effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

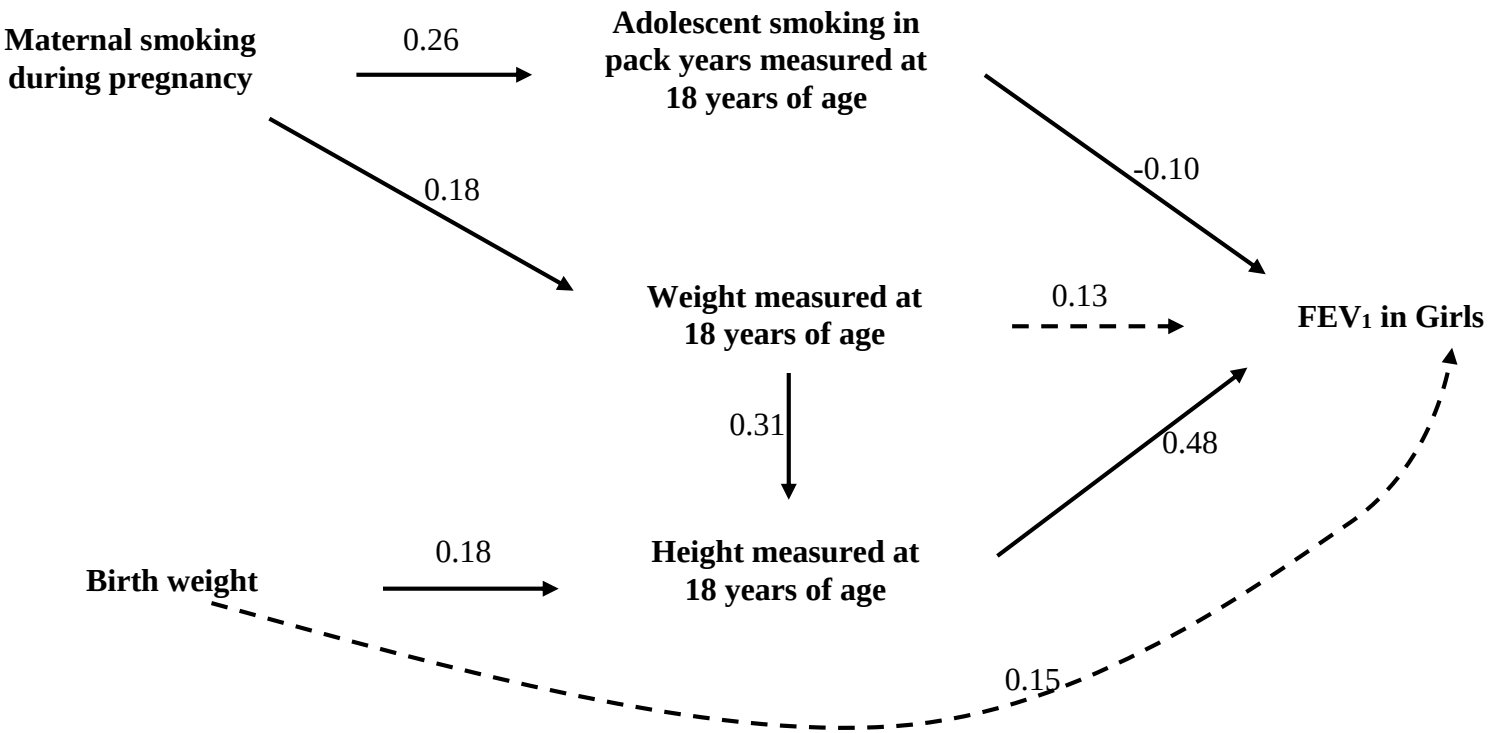


Figure 4.2: Analytical path model exploring the standardized direct and indirect effects of maternal smoking during pregnancy and adolescent smoking on FEV_1 in girls (path coefficients from Table 4.10 B and 4.12 A)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

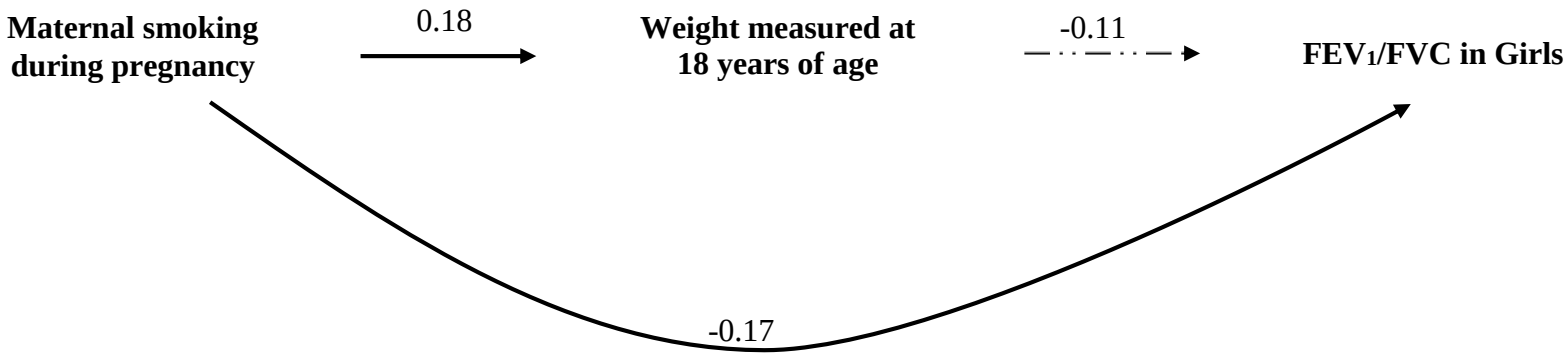


Figure 4.3: Analytical path model exploring the standardized direct and total effects of maternal smoking during pregnancy and adolescent smoking on FEV_1/FVC in girls (path coefficients from Table 4.10 B and 4.12 B)

The path coefficients represented by solid arrows are direct effects while those represented by dashed and dotted arrows (— · · — ▶) are total effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

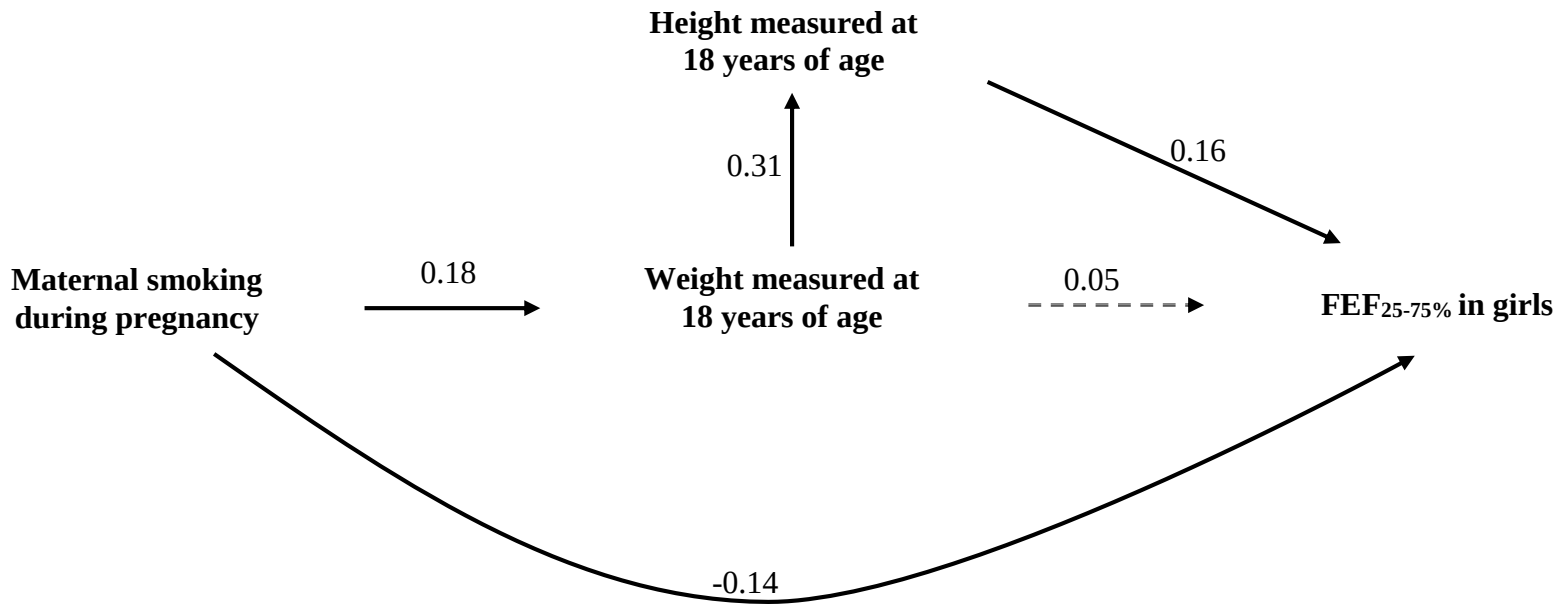


Figure4.4: Analytical path model exploring the standardized direct and indirect effects of maternal smoking during pregnancy and adolescent smoking on $FEF_{25-75\%}$ in girls (path coefficients from Table 4.10 B and 4.12 B)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

CHAPTER 5

EXPOSURE TO DICHLORODIPHENYL DICHLOROETHENE (DDE) AND CHILDHOOD PULMONARY FUNCTION

Introduction

Dichlorodiphenyl dichloroethene (DDE) is principal metabolite of dichlorodiphenyl trichloroethane (DDT) which is a synthetic chemical belonging to organochlorine chemicals family and is recognized as persistent organic pollutants.[106] DDT was a widely used insecticide until it was banned in most developed countries by the end of 1980s.[105,106] However, it is still largely used in developing countries for malaria control and in agriculture. Because of its lipophilic properties and very long half-life DDE persists in environment in all forms of life. [105,106] Humans are exposed to DDE through food chain which accumulates in adipose tissue, bloodstream and breast.[105,106]

Evidence from longitudinal studies has shown that both prenatal and postnatal exposure to DDE leads to reduction in growth of height and increase in BMI during childhood.[113,114,157] Not only the anti-androgenic properties of DDE are known to interfere with puberty reducing the age of menarche,[121] but high levels of DDE have also been found in girls with precocious puberty.[157] DDE is also considered to alter both cellular and humoral immunity with increased production of cytokines and nitric oxide.[108-111] Prenatal exposure to DDE has also been linked with respiratory disorders

like lower respiratory tract infections, episodes of wheezing, otitis media and increased risk of asthma in early childhood.[20,123-126]

In children and adolescents, average pulmonary function growth varies greatly with height, weight, sex and age of pubertal growth spurt.[128-130] Exposure to DDE may not only disrupts growth of height and weight in children, but immunological responses to persistent exposure to DDE may also alter physiology of airways through remodeling during this critical period of lung growth. To date, no study has ever investigated relationship between burden of DDE and pulmonary function in children. We hypothesized that DDE exposure affect pulmonary function in childhood, directly or indirectly via height and weight in addition to its immunological role. To assess these complex relationships we used path analysis approach (structural equation models). Path analysis is an extension of multiple regression and it offers flexibility to generate estimates for hypothesized causal associations between sets of variables. Path analysis also allows the use of intervening or mediating variables in the explanatory models which ultimately provide the direct, indirect and total effects of each variable on the outcome which can be used to develop causal path diagram.

An environmental epidemiologic study was conducted in central Germany, to investigate the effects of persistent body burden of polychlorinated biphenyls (PCBs) and DDE on health of children.[114] In addition to showing a small reduction in height in girls,[114] other results from this study showed that DDE was related with increased total IgE and asthma.[125,158] In this research paper, our aim is to investigate the effects of persistent DDE exposure on childhood pulmonary function in this study. We provide results from repeated measurement analysis followed by results from path analysis.

Methods

Study Population

We used data from a longitudinal study conducted in the south of the federal state of Hesse, in central Germany, between 1994 and1997. Children were recruited from 3 different regions which were located around industrial waste incinerator among other industries (e.g., chemical plants). One is in the Rhine Valley, with low mountains on both sides, within a 15-km radius around an industrial waste incinerator and is also used intensively for vegetable production. A second region, also industrial and agricultural, is 20 km north of the incinerator area. The third region is located in low mountains (about 400 m above sea level).[114] After obtaining permits from the Data Protection Agency of the State of Hamburg, Germany, from the Ministry of Cultural Affairs of Hesse, Germany, and from the local school committees, parents of 1091 second grade school children in 18 townships were asked to participate in this study. Informed consent, according to the requirements of the Ethical Committee of the Board of Physicians and the Data Protection Agency of the State of Hamburg, was obtained from all participating parents. Parents were asked to let their children participate in phlebotomy only when passive smoking in the private household had not exceeded 10 cigarettes per day in the previous 12 months. Children and their parents participated in the 3 repeated surveys (December 1994–April 1995, January to May1996, January to June 1997).[114]

Organochlorines in Blood

A blood sample was taken as part of the first visit. DDE concentrations were determined from 5-mL samples of whole blood by performing high-resolution gas

chromatography (HRGC, Model 3400, Varian, Gloucester, Mass) with a 63Ni electron capture detector at the Institute of Toxicology, University of Kiel, Germany. The detection limit was 0.02 µg/L for DDE. In addition, reliability was tested with gas chromatography– mass spectometry. The laboratory successfully participated in nationwide interlaboratory quality assessments for DDE and PCB determinations.[114]

Pulmonary Function

Pulmonary function tests were conducted at 8, 9 and 10 years of age using a Masterscope (Software Release 4.0; Erich Jaeger, Würzburg, Germany). The instrument was calibrated daily and each child performed two forced expiratory maneuvers according to the American Thoracic Society (ATS) guidelines in standing position wearing a nose clip. Two flow/volume curves were accepted as reproducible if the difference between FVC measurements was ≤ 5%. The highest FVC and FEV_1 values were then selected for statistical analysis.

Covariates

Self-administered questionnaires were used in the survey. Information was collected on child's age, birth weight, birth order, maternal and paternal education, maternal and paternal height, smoking during pregnancy, and breast-feeding duration. Height and weight were measured for each child in 3 consecutive examinations 1 year apart (ages of 8, 9, and 10 years) before each spirometric examination. Environmental tobacco smoke (ETS) was assessed as smoking in the child's home in the previous 12 months (per day: no cigarettes, 1-10 cigarettes, 11-20 cigarettes, 20-30 cigarettes, >30 cigarettes).[114]

Statistical Analysis

For the purpose of this research paper, we used pulmonary function data only from those children who had information on blood levels of DDE either in 1995 or 1997. As DDE exposure was not measured in 1996, for this year we selected children who had PFT done either in 1995 or 1997 with information on DDE exposure. The final data set contained 1000 observations with 344 participants. We imputed values for DDE exposure in 1996 based on the values in years 1995 and 1997 using multiple imputation methods. Multiple imputations were also used for missing data on one or more confounders. As a first step, linear correlations between DDE exposure and height, weight, FEV_1, FVC and FEV_1/FVC were determined using PROC CORR for years 1995 and1997. Next we used linear mixed models (PROC MIXED) on imputed data sets to assess the association between blood levels of DDE and repeated pulmonary function measurements. Compound symmetry covariance structure matrix was selected based on lowest Akaike information criteria and the Bayesian Schwarz information criterion after considering unstructured, compound symmetry and autoregressive covariance structure matrices. All models were adjusted for age, sex, birth weight, breast feeding duration, height, weight, smoking during pregnancy, parental history of asthma and ETS. We selected those covariates for the final model which changed the pulmonary function estimates for DDE exposure by 10 %. To account for differential association between height and pulmonary function in boys and girls we included interaction terms of height with sex. Finally all results were combined and valid statistical inferences were generated using MIANALYZE procedure.

Further, we explored the relationship between DDE exposure, height, weight and pulmonary function in childhood by path analysis using the Covariance Analysis of Linear Structural Equations procedure. However, since DDE exposure was not measured in 1996 even after using Full Information Maximum Likelihood (FIML) method which takes into consideration the missingness the final path models did not converge for repeated measures data. Therefore, we used two separate path analysis model for cross-sectional data from years 1995 and 1997. However, we included measurements on the body burden of DDE, height and weight obtained in year 1995 in the linear equations for path analysis models in year 1997. The adequacy of model fit was determined by several statistics: a chi-square p-value > 0.05 if chi-square test statistic is close to 0, comparative fit index (CFI) > 90, adjusted goodness of fit index (GFI) > 90 and root mean square error of approximation (RMSEA) < 0.06.

Results

Out of 691 enrolled children, 632 performed PFTs in year 1995, 598 in year 1996 and 558 in year 1997. However, DDE was measured only in years 1995 and 1997. A total of 328 (52 %) children in 1995 and 214 (61.7 %) children in 1997 had information on DDE. Table 5.1 shows the average anthropometric, pulmonary function and DDE exposure measures for each year. About 13 % had history of asthma, while prevalence of maternal and paternal asthma ranged from 3.4 % - 4.3 % and 5.3 % - 5.8 %, respectively over a period of 3 years. The prevalence of *in-utero* exposure to at least some amount of maternal smoking ranged from 29.4 % - 30 %. Similarly, the prevalence for ETS was 32.3 % - 35.5 % (Table 5.2)

Boys had significantly higher FEV_1 and FVC than girls in all years unadjusted for any covariate. However, FEV_1/FVC ratio was higher in girls than boys suggesting much lower FVC in girls than boys (Table 5.3). Table 5.4 shows the Spearman's correlations between DDE blood levels, anthropometric measures and pulmonary function. There was weak negative correlation between DDE levels and height, weight, FEV_1 and FVC.

Figure 5.1 demonstrates that height has a positive relationship with both FVC and FEV_1 but higher level of DDE slightly blunts this relationship at higher levels of height in year 1995 (median age of participants 8 years). However, the effect of DDE on relationship of height with FVC and FEV_1 is diminished in year 1997 (median age of participants 10 years).

The final data set with repeated measurements contained 344 participants with 1000 observations. All the linear mixed models were adjusted for age, height, weight, sex, breastfeeding status, exposure to *in-utero* maternal smoking and ETS. There were no significant associations between body burden of DDE and any pulmonary function parameter. As expected, height and weight had positive association with FVC and FEV_1 and no effect on FEV_1/FVC ratio. The interaction term between sex and height was significant for FVC and FEV_1/FVC ratio models but not for FEV_1 model (Table 5.5).

Path analysis at 1995 showed that DDE exposure had negative total effects on both height (direct: 1.3 cm per μg/L of DDE, indirect: -0.16 cm per μg/L of DDE, total: -0.28 cm per μg/L of DDE) and weight (total: -0.24 kg per μg/L of DDE) after controlling for effects of age, sex, breast feeding and maternal smoking during pregnancy (Table 5.6). Further, path analysis for the year 1995 demonstrated that DDE exposure did not

have any direct effects on any pulmonary function but had significant inverse indirect and total effects on both FVC (indirect: -0.20 L per μg/L of DDE; total: -0.17 L per μg/L of DDE) and FEV_1 (indirect: -0.20 L per μg/L of DDE; total: -0.15 L per μg/L of DDE) (Tables 5.7 A and B). For year 1997, we included measurements on height, weight and DDE levels obtained in 1995. There were no total effects of body burden of DDE in 1997 on either height or weight in 1997 after adjusting for height and weight in 1995 (Table 5.8). However, there were significant inverse indirect effects of body burden of DDE measured in 1995 on both height and weight measured in 1997 mediated through its inverse effects on height and weight measured in 1995 (Figure 5.5 and 5.6). Similarly, DDE measured in year 1997 had no total effects on either FVC or FEV_1 or FEV_1/FVC but DDE measured in year 1995 had indirect inverse effects on FVC and FEV_1 measured in year 1997 mediated through its effects on height and weight measured in both years (Figure 5.9 A-C).

Figures 5.3 to 5.6 shows significant standardized direct and indirect effects of DDE exposure, height and weight on FVC and FEV_1 at both years 1995 and 1997. The solid arrows represent the direct effects while the dotted arrows represent the indirect effects. The covariates with non-significant p-value for total effects were not depicted in these path diagrams but are included in Tables 5.7 A-C and 5.9 A-C. The path coefficients suggested that there was a direct effect of DDE exposure on height and weight, but no direct effect on either FVC or FEV_1. However, as height and weight explains FVC and FEV_1, the diminishing effects of DDE exposure on height and weight were carried forward towards FVC and FEV_1 (indirect effect) with height and weight acting as intervening variables.

Discussion

A study conducted in South-Hesse, Germany from 1995-1997 in children of age 8 to 10 years suggest that dichlorodiphenyl dichloroethene (DDE) blood level may indirectly reduce lung function. Path analysis show negative correlations of DDE with height, weight and pulmonary function suggesting that height and weight may act as intervening/mediating factors in the association between DDE exposure and pulmonary function. The results from path analysis indicate that DDE exposure had direct inverse effects on reduced height and weight but not on pulmonary function. However, the inverse effect of DDE exposure was carried forward to FVC and FEV_1 indirectly through its effect on height and weight in children of age 8 years. Although height and weight continued to have positive direct effects on both FVC and FEV_1 these effects were attenuated by the inverse effects of DDE exposure on height and weight. Further analysis at age 10 years (1997) did not show any direct or indirect effect of DDE measured in 1997 on height, weight or pulmonary function after adjusting for height and weight at age 8 years. This shows that that with increase in age the direct effect of DDE on height and weight diminishes. Nevertheless, the inverse effect of DDE measured at age 8 years (1995) on height and weight persisted at age 10 years.

DDE is known to affect endocrine system through its estrogenic and anti-androgenic properties.[105] Karmaus *et al*. reported that *in-utero* exposure to DDE is associated with reduced age at menarche by one year in a Michigan angler cohort.[121] High levels of DDE have also been found in girls with precocious puberty who migrated to Belgium from developing countries.[122] Early age of menarche is associated with decrease in growth of height post-menarche, thus affecting the final adult height.[130,159] A

prospective study by Ribas-Fito *et al*. found that increased prenatal DDE concentrations were associated with decreased height at 1, 4, and 7 years of age in both boys and girls.[113] On the other hand, a study by Gladen *et al*. suggested that prenatal exposure to DDE was associated with increased height at puberty in boys.[116] In the same cohort from Hesse, Karmaus *et al*. demonstrated that growth during childhood was significantly reduced in girls with high DDE concentrations measured at 8 years of age but no effect was seen in boys.[114] One possible explanation for the conflicting findings in previous studies may be the adjustment of intervening factors in explanatory models. The effects of DDE on height seem to vary by sex and DDE is directly associated with weight which in turn is directly associated with height. Therefore adjusting on weight may distort the true relationship between DDE exposure and height. Similarly when assessing the association between DDE and pulmonary function in children; adjusting on mediating variables weight and height may introduce mediator-outcome confounding.[160] Additionally, introducing interaction term between height and DDE in the explanatory models may generate biased estimates because of mediator-exposure interaction.[160] Another methodological problem arises from the fact that while analyzing pulmonary function in a population based study adjustment has to be made on height and weight. Our path analysis results may partially address these issues. The results suggested that DDE exposure had overall (total) inverse effect on height at age 8 years; but breakdown of this total effect showed a positive direct effect and inverse indirect effect. Our path diagram demonstrated that the indirect negative effect may have been mediated through weight (Figure 5.3 and 5.4). Thus, by using path analysis we were able to separate the confounding aspects of height and weight while assessing the association between DDE

and pulmonary function at the same time taking measurement errors into consideration. We were however not able to corroborate these finding separately in boys and girls because of small sample size.

There are some other important limitations to our study. We did not use predicted values for pulmonary function parameters and this may limit the interpretation of our results in the clinical settings. We did not have DDE exposure measured at age 9 years (1996). The average DDE level for 1996 generated through multiple imputation technique was slightly higher than years 1995 and 1997; having actual values for DDE would have been more reliable. Furthermore, it would have been more prudent to show the relationships between DDE, height, weight and pulmonary function through path analysis using repeated measurements. However, the structure of data did not allow the final model to converge and hence we conducted path analysis at a cross-sectional level.

In addition to its effects on endocrine system DDE also alters immune function. There is evidence from both animal and human studies suggesting that DDE exposure is associated with increased production of cytokines and nitric oxide production in macrophages, leading to inflammatory reactions, cytokine imbalance and immune dysregulation.[109-111] It also changes T-cell mediated response involving IL-4 which can induce epithelial cell proliferation, fibrosis, and mucus secretion to differing extents.[109,112] A study investigating the effect of organochlorine compounds and lead on immune biomarkers in the present cohort demonstrated an increased levels of IgE, IgE count on basophils, and the reduction of eosinophilic granula at higher DDE concentrations.[108] Such immunological changes due to persistent exposure to DDE may add to remodeling of both smaller and larger airways which is evident from

epidemiological studies reporting higher risk of developing asthma in children exposed to DDE.[20,124-126]

Pulmonary function in children is determined by complex relationships between many factors like age, sex, race, height, weight and onset of puberty. These relationships are further complicated by exposure to DDE which is known to affect one or more of these determinants of pulmonary function. Although DDE exposure does not seem to directly influence pulmonary function, it does inversely affect height and weight, which in turn are related to pulmonary function. Therefore, poor growth in height and weight at an early age contributes to lower FVC and FEV_1. Whether DDE exposure modifies physiology of airways leading to lower pulmonary function is unknown. However, our path analysis demonstrated that reduction in pulmonary function parameters are at least partially due to its effects on height and weight. Additionally, other research conducted in this cohort of children from federal state of Hesse, Germany has demonstrated reduction in childhood growth,[114] increased IgE levels[108] and increased risk of asthma and otitis media[158] which would explain reduction in FVC and FEV_1.

To date, there has been no study investigating the association between DDE exposure and pulmonary function in children. In this study data on DDE exposure and PFT were assessed under standard conditions reducing the information bias. Use of path analysis allowed us to include the intervening factors in the explanatory models which helped disentangle the complex relationship between DDE exposure and pulmonary function. Finally, as in every longitudinal study, missing data was an issue which we overcame by multiple imputations.

In conclusion, we suggest that the relationship between height and pulmonary function is modified by DDE exposure during childhood. The use of path analysis improved the understanding of underlying directional or non-directional relationships, allowing the estimation of direct, indirect and total effects of height, weight and DDE exposure on pulmonary function. Further studies are needed to test this association in another sample, preferable from a region with continuous high DDT application.

Table 5.1: Baseline characteristics for children of age 8-10 years with pulmonary function data in the years 1995, 1996 and 1997

Characteristic		Year 1995			Year 1996			Year 1997		
		N	%	Missing	N	%	Missing	N	%	Missing
Sex	Female	127	45.4	0	144	43.9	0	153	44.5	0
	Male	153	54.6		184	56.1		191	55.5	
Doctor diagnosed asthma	Yes	37	13.3	1	44	13.5	1	46	13.4	2
	No	242	86.7		283	86.5		296	86.6	
Maternal asthma	Yes	12	4.3	3	11	3.4	1	12	3.5	2
	No	265	95.7		316	96.6		330	96.5	
Paternal asthma	Yes	16	5.8	5	17	5.3	4	18	5.3	5
	No	259	94.2		307	94.8		321	94.7	
Breast feeding	Yes	244	87.5	1	279	85.3	1	293	85.4	1
	No	35	12.5		48	14.7		50	14.6	
Maternal smoking during pregnancy (per day)	>30 cigarettes	3	1.1	3	4	1.2	5	4	1.2	7
	20-30 cigarettes	3	1.1		4	1.2		4	1.2	
	11-20 cigarettes	11	4.0		13	4.0		14	4.3	
	1-10 cigarettes	66	23.8		74	22.9		78	23.2	
	None	194	70.0		228	70.6		237	70.3	
Environmental tobacco smoke (ETS) (per day)	>30 cigarettes	2	0.7	4	5	1.6	6	5	1.5	6
	20-30 cigarettes	5	1.8		5	1.6		5	1.5	
	11-20 cigarettes	19	6.9		19	5.9		21	6.2	
	1-10 cigarettes	72	26.1		75	23.3		79	23.4	
	None	178	64.5		218	67.7		228	67.5	

Table 5.2: Average anthropometric measures, pulmonary function parameters and DDE exposure

Variable	Year 1995 (n=280)			Year=1996 (n=328)			Year=1997 (n=344)		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Age (years)	8.28	8	0.54	9.25	9	0.48	10.30	10	0.54
Height (cm)	132.51	133	5.79	137.22	137	6.03	143.31	143.5	6.55
Weight (kg)	30.02	29	6.48	32.96	32	6.81	38.63	37	8.86
FEV_1 (L)	1.80	1.78	0.28	1.88	1.88	0.29	2.13	2.12	0.32
FVC (L)	2.05	2.01	0.33	2.25	2.25	0.33	2.50	2.47	0.39
FEV_1/FVC ratio (%)	87.93	88.49	6.16	83.81	84.96	7.18	85.39	85.77	5.44
DDE (µg/L)*	0.41	0.29	0.43	0.55	0.51	0.30	0.42	0.32	0.39

SD= Standard deviation, FEV_1= Forced expiratory volume in one second, FVC=Forced expiratory capacity,
DDE= Dichlorodiphenyl dichloroethylene
*DDE levels were not measured in 1996

Table 5.3: Unadjusted average pulmonary function among boys and girls (t-test)

Year=1995					
Pulmonary function parameters	Boys (n=153)		Girls (n=127)		
	Mean	SE	Mean	SE	P-value
FVC (L)	2.13	0.03	1.96	0.03	<.0001
FEV_1 (L)	1.85	0.02	1.73	0.02	0.0004
FEV_1/FVC (%)	87.20	0.54	88.81	0.47	0.0263
Year=1996					
Pulmonary function parameters	Boys (n=184)		Girls (n=144)		
	Mean	SE	Mean	SE	P-value
FVC (L)	2.34	0.02	2.12	0.02	<.0001
FEV_1 (L)	1.93	0.02	1.81	0.02	<.0002
FEV_1/FVC (%)	82.50	0.56	85.48	0.52	0.0001
Year=1997					
Pulmonary function parameters	Boys (n=191)		Girls (n=153)		
	Mean	SE	Mean	SE	P-value
FVC (L)	2.59	0.03	2.40	0.03	<.0001
FEV_1 (L)	2.17	0.02	2.09	0.03	0.0231
FEV_1/FVC (%)	83.99	0.40	87.13	0.40	<.0001

SE= Standard error

Table 5.4: Spearman's correlations for DDE with anthropometric and pulmonary function measures for years 1995 and 1997

Variables	Year=1995 (n=280)		Year=1997 (n=344)	
	Spearman's correlation	P-value	Spearman's correlation	P-value
DDE (µg/L)-Height (cm)	-0.29	<.0001	-0.21	<.0001
DDE (µg/L)-Weight (kg)	-0.28	<.0001	-0.29	<.0001
DDE (µg/L)-FEV_1 (L)	-0.15	0.0107	-0.15	0.007
DDE (µg/L)-FVC (L)	-0.20	0.001	-0.18	0.0006
DDE (µg/L)-FEV_1/FVC (%)	0.08	0.1572	0.09	0.1004

Table 5.5: Adjusted linear mixed models for pulmonary function, DDE exposure, height, weight and sex

Covariates	FVC (L)			FEV_1 (L)			FEV_1/FVC (%)		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE (µg/L)	-0.004	0.02	0.8172	0.002	0.02	0.8975	0.40	0.55	0.4755
Height (cm)	0.03	0.00	<.0001	0.02	0.00	<.0001	0.01	0.06	0.826
Weight (kg)	0.01	0.00	<.0001	0.01	0.00	<.0001	-0.08	0.05	0.096
Sex: Female	-0.36	0.20	0.0682	0.08	0.02	<.0001	12.7	6.71	0.0588
Height*Sex	0.004	0.00	0.0066				-0.11	0.05	0.0212

All models were additionally adjusted for breast feeding duration, history of maternal smoking during, current environmental tobacco smoke exposure

Compound symmetry covariance matrix was selected based on lowest Akaike Information Criterion (AIC)

Height*sex interaction had p-value >0.05 in explanatory model for FEV_1; therefore not included in the final model

Table 5.6: The standardized direct, indirect and total effects of DDE along with other factors on height and weight from path analysis model at years 1995

Covariates		Height			Weight
		Direct	Indirect	Total	Direct/Total
DDE (µg/L)	Estimate	1.3	-1.59	-0.28	-0.24
	SE	0.35	0.38	0.05	0.05
	P-value	0.0002	<.0001	<.0001	<.0001
Age (years)	Estimate	-1.14	1.36	0.2	0.20
	SE	0.33	0.36	0.05	0.05
	P-value	0.0005	0.0002	<.0001	<.0001
Weight (kg)	Estimate	6.72		6.72	
	SE	0.23		0.23	
	P-value	<.0001		<.0001	
Sex	Estimate	-0.14		-0.14	
	SE	0.04		0.04	
	P-value	0.0003		0.0003	
Breast feeding (weeks)	Estimate	-0.60	0.63	0.03	0.09
	SE	0.41	0.45	0.07	0.07
	P-value	0.1474	0.1650	0.6573	0.1613
Maternal smoking during pregnancy	Estimate	0.05	-0.09	-0.04	-0.01
	SE	0.33	0.36	0.05	0.05
	P-value	0.8684	0.7963	0.4586	0.7963

Goodness of Fit Criteria: Chi-squared test statistic=5.7, p-value < 0.05; AGFI=0.9991; CFI=0.9936; RMSEA=0.0749

Weight is the first endogenous variable determined by exogenous variables- DDE, age, breast feeding and maternal smoking during pregnancy and hence only has direct effects and no indirect effects.

Table 5.7 A: The standardized direct, indirect and total effects of DDE along with other factors on FVC from path analysis model at years 1995

Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE (μg/L)	-0.17	0.05	0.0015	0.03	0.04	0.4702	-0.20	0.04	<.0001
Age (years)	0.05	0.05	0.3034	-0.10	0.04	0.0079	0.16	0.04	<.0001
Height (cm)	0.50	0.05	<.0001	0.50	0.05	<.0001			
Weight (kg)	3.60	0.30	<.0001	0.26	0.05	<.0001	3.34	0.34	<.0001
Sex	-0.27	0.04	<.0001	-0.20	0.04	<.0001	-0.07	0.02	0.0007
Breast feeding (weeks)	0.02	0.07	0.7238	0.02	0.05	0.7349	0.04	0.05	0.3775
Maternal smoking during pregnancy	-0.05	0.06	0.3412	-0.03	0.04	0.4705	-0.02	0.04	0.5365
Environmental tobacco smoke	0.02	0.04	0.5858	0.02	0.04	0.5858			

Goodness of Fit Criteria: Chi-squared test statistic=5.7, p-value < 0.05; AGFI=0.9991; CFI=0.9936; RMSEA=0.0749

Table 5.7 B: The standardized direct, indirect and total effects of DDE along with other factors on FEV_1 from path analysis model at years 1995

Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE (μg/L)	-0.15	0.06	0.0053	0.04	0.04	0.3555	-0.20	0.04	<.0001
Age (years)	0.07	0.05	0.1935	-0.08	0.04	0.0424	0.15	0.04	<.0001
Height (cm)	0.47	0.05	<.0001	0.47	0.05	<.0001			
Weight (kg)	3.41	0.32	<.0001	0.27	0.05	<.0001	3.14	0.4	<.0001
Sex	-0.22	0.04	<.0001	-0.16	0.04	0.0001	-0.07	0.02	0.0008
Breast feeding (weeks)	0.06	0.07	0.3948	0.02	0.05	0.7088	0.04	0.04	0.3857
Maternal smoking during pregnancy	-0.07	0.06	0.2469	-0.05	0.05	0.3247	-0.02	0.04	0.538
Environmental tobacco smoke	0.03	0.05	0.5652	0.03	0.05	0.5652			

Goodness of Fit Criteria: Chi-squared test statistic=5.7, p-value < 0.05; AGFI=0.9991; CFI=0.9931; RMSEA=0.0753

Table 5.7 C: The standardized direct, indirect and total effects of DDE along with other factors on FEV_1/FVC from path analysis model at years 1995

Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE (μg/L)	0.07	0.06	0.2412	0.03	0.06	0.6079	0.04	0.03	0.0440
Age (years)	0.002	0.06	0.9688	0.03	0.06	0.5904	-0.03	0.03	0.0569
Height (cm)	0.12	0.08	0.1303	-0.12	0.08	0.1303			
Weight (kg)	-0.79	0.47	0.0897	-0.02	0.07	0.8185	-0.78	0.51	0.1298
Sex	0.13	0.05	0.0157	0.12	0.06	0.0378	0.02	0.01	0.1630
Breast feeding (weeks)	0.06	0.07	0.3664	0.07	0.07	0.3287	-0.01	0.01	0.6122
Maternal smoking during pregnancy	-0.02	0.06	0.7895	-0.02	0.06	0.7317	0.01	0.01	0.5111
Environmental tobacco smoke	-0.001	0.06	0.9836	-0.001	0.06	0.9836			

Goodness of Fit Criteria: Chi-squared test statistic=5.7, p-value < 0.05; AGFI=0.9992; CFI=0.9894; RMSEA=0.0749

Table 5.8: The standardized direct, indirect and total effects of DDE along with other factors on height and weight from path analysis model at years 1997

Height in year 1997									
Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE in year 1997 (μg/L)	0.02	0.02	0.4313	0.02	0.02	0.3948	-0.001	0.00	0.365
DDE in year 1995 (μg/L)	-0.24	0.05	<.0001				-0.24	0.05	<.0001
Height in year 1995 (cm)	0.92	0.02	<.0001	0.92	0.02	<.0001			
Weight in year 1995 (kg)	1.44	0.54	0.008				1.44	0.54	0.008
Weight in year 1997 (kg)	0.04	0.03	0.1594	0.04	0.03	0.1594			
Age (years)	0.02	0.02	0.2757	0.02	0.02	0.2961	0.001	0.00	0.3043
Sex	-0.03	0.04	0.3937	0.11	0.02	<.0001	-0.14	0.04	0.0001
Breast feeding (weeks)	0.04	0.07	0.5174	0.04	0.03	0.1324	0.003	0.06	0.9628
Maternal smoking during pregnancy	0.01	0.05	0.8168	0.03	0.02	0.2229	-0.02	0.05	0.7756
Weight in year 1997									
Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE in year 1997 (μg/L)	-0.03	0.02	0.265	-0.03	0.02	0.265			
DDE in year 1995 (μg/L)	-0.19	0.05	<.0001				-0.19	0.05	<.0001
Weight in year 1995 (kg)	0.92	0.01	<.0001	0.92	0.01	<.0001			
Age (years)	0.03	0.02	0.16	0.03	0.02	0.16			
Breast feeding (weeks)	0.08	0.07	0.2039	0.03	0.03	0.246	0.05	0.06	0.4054
Maternal smoking during pregnancy	0.01	0.05	0.7914	0.01	0.02	0.6888	0.01	0.05	0.9225

Goodness of Fit Criteria: Chi-squared test statistic=33.1, p-value < 0.05; AGFI=0.898; CFI=0.994; RMSEA=0.048

Table 5.9 A: The standardized direct, indirect and total effects of DDE along with other factors on FVC from path analysis model at years 1997

Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE in year 1997 (μg/L)	0.01	0.04	0.836	0.01	0.03	0.8675	0.002	0.02	0.8945
DDE in year 1995 (μg/L)	-0.18	0.04	<.0001				-0.18	0.04	<.0001
Age (years)	0.05	0.04	0.1421	0.03	0.03	0.3179	0.02	0.01	0.1759
Height in year 1997 (cm)	0.48	0.10	<.0001	0.48	0.10	<.0001			
Weight in year 1997 (kg)	0.278	0.05	<.0001	0.26	0.04	<.0001	0.02	0.02	0.1771
Height in year 1995 (cm)	0.52	0.04	<.0001	0.08	0.11	0.4726	0.44	0.10	<.0001
Weight in year 1995 (kg)	1.04	0.31	0.0007				1.04	0.31	0.0007
Sex	-0.25	0.04	<.0001	-0.23	0.04	<.0001	-0.03	0.02	0.2686
Breast feeding (weeks)	0.0004	0.06	0.9956	-0.04	0.04	0.3166	0.04	0.05	0.397
Maternal smoking during pregnancy	-0.06	0.05	0.252	-0.07	0.04	0.0612	0.01	0.04	0.8344
Environmental tobacco smoke	0.08	0.04	0.0407	0.08	0.04	0.0407			

Goodness of Fit Criteria: Chi-squared test statistic=33.1, p-value < 0.05; AGFI=0.898; CFI=0.994; RMSEA=0.048

Table 5.9 B: The standardized direct, indirect and total effects of DDE along with other factors on FEV_1 from path analysis model at years 1997

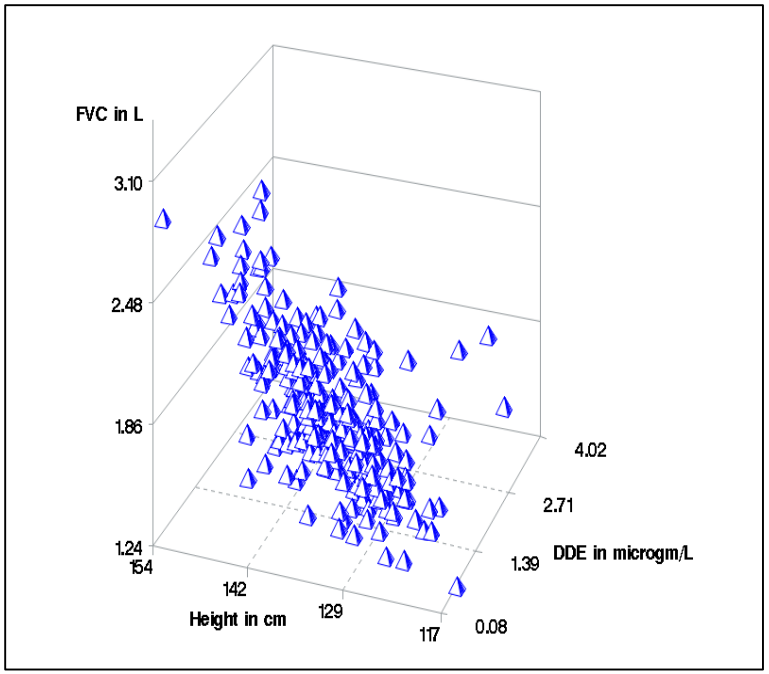
Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE in year 1997 (μg/L)	0.02	0.04	0.6785	0.02	0.04	0.6909	0.002	0.02	0.9072
DDE in year 1995 (μg/L)	-0.17	0.04	<.0001				-0.17	0.04	<.0001
Age (years)	0.08	0.04	0.0536	0.06	0.04	0.1345	0.02	0.02	0.1596
Height in year 1997 (cm)	0.53	0.12	<.0001	0.53	0.12	<.0001			
Weight in year 1997 (kg)	0.23	0.05	<.0001	0.23	0.05	<.0001			
Height in year 1995 (cm)	0.51	0.05	<.0001	0.01	0.12	0.9419	0.51	0.11	<.0001
Weight in year 1995 (kg)	0.21	0.05	<.0001				0.21	0.05	<.0001
Sex	-0.13	0.04	0.0021	-0.11	0.04	0.0055	-0.02	0.03	0.467
Breast feeding (weeks)	0.04	0.07	0.5065	0.001	0.05	0.9879	0.04	0.05	0.3583
Maternal smoking during pregnancy	-0.09	0.06	0.1203	-0.1	0.04	0.0213	0.01	0.04	0.7723
Environmental tobacco smoke	0.07	0.04	0.1091	0.07	0.04	0.1091			

Goodness of Fit Criteria: Chi-squared test statistic=33.7, p-value < 0.05; AGFI=0.909; CFI=0.994; RMSEA=0.043

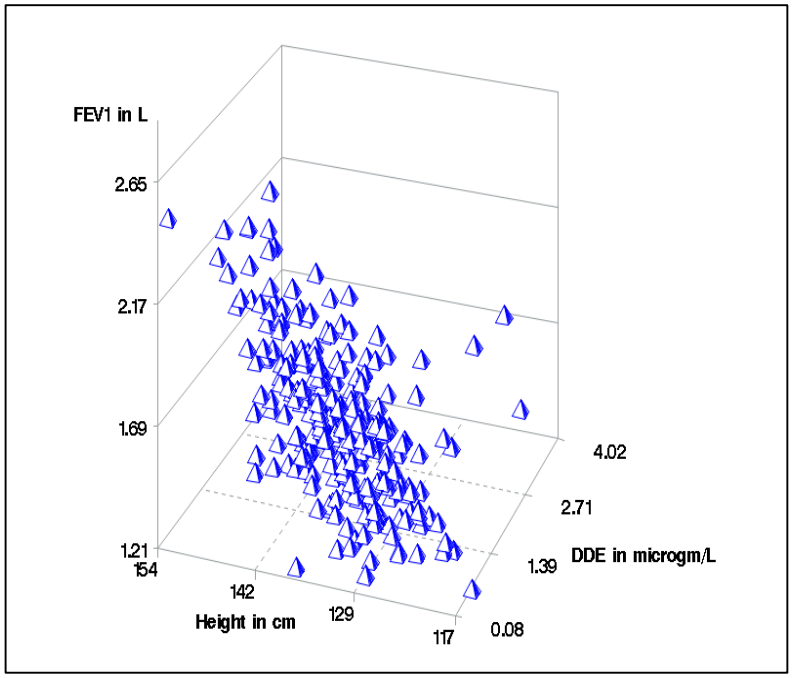
Table 5.9 C: The standardized direct, indirect and total effects of DDE along with other factors on FEV_1/FVC from path analysis model at years 1997

Covariates	Total			Direct			Indirect		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
DDE in year 1997 (μg/L)	0.06	0.18	0.7405	0.06	0.18	0.7558	0.004	0.00	0.4165
DDE in year 1995 (μg/L)	0.02	0.19	0.9107	-0.01	0.19	0.9574	0.03	0.02	0.0441
Age (years)	0.03	0.05	0.6091				-0.001	0.00	0.8015
Height in year 1997 (cm)	0.07	0.17	0.6749	0.07	0.17	0.6749			
Weight in year 1997 (kg)	-0.09	0.07	0.209	-0.09	0.07	0.2035	0.003	0.01	0.6893
Height in year 1995 (cm)	-0.06	0.07	0.3977	-0.13	0.17	0.4582	0.07	0.15	0.6749
Weight in year 1995 (kg)	-0.17	0.09	0.0607				-0.17	0.09	0.0607
Sex	0.3	0.05	<.0001	0.28	0.05	<.0001	0.02	0.02	0.4248
Breast feeding (weeks)	0.09	0.07	0.1836	0.09	0.07	0.1684	-0.004	0.01	0.7591
Maternal smoking during pregnancy	-0.06	0.06	0.3292	-0.06	0.06	0.316	0.001	0.01	0.8656
Environmental tobacco smoke	-0.02	0.06	0.765	-0.02	0.06	0.765			

Goodness of Fit Criteria: Chi-squared test statistic=28.6, p-value < 0.05; AGFI=0.906; CFI=0.995; RMSEA=0.044

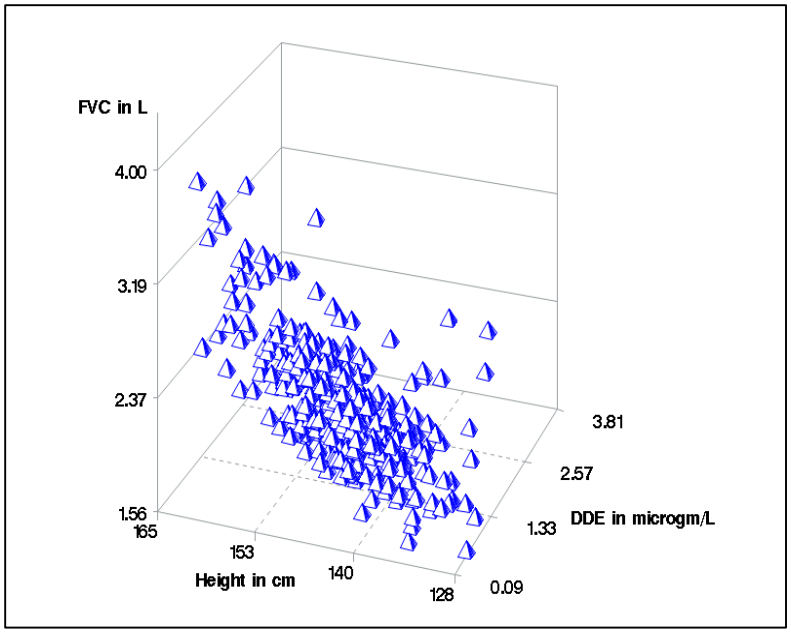


I. Relationship between DDE (µg/L) and height (cm) with FVC (L)

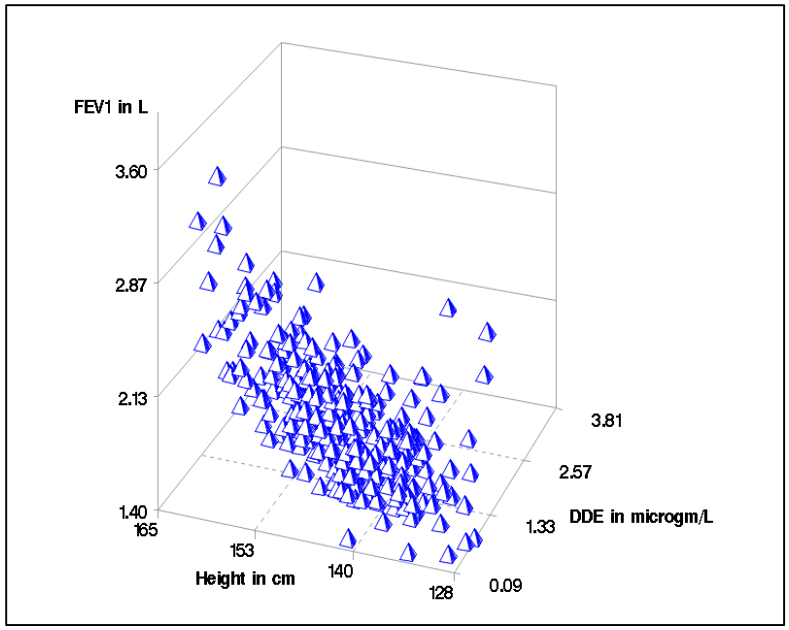


II. Relationship between DDE (µg/L) and height (cm) with FEV_1 (L)

Figure 5.1: Three dimensional scatter plot showing relationship between DDE and height with FVC and FEV_1 in year 1995 (median age: 8 years)



I. Relationship between DDE (µg/L) and height (cm) with FVC (L)



II. Relationship between DDE (µg/L) and height (cm) with FEV_1 (L)

Figure 5.2: Three dimensional scatter plot showing relationship between DDE and height with FVC and FEV_1 in year 1997 (median age: 10 years)

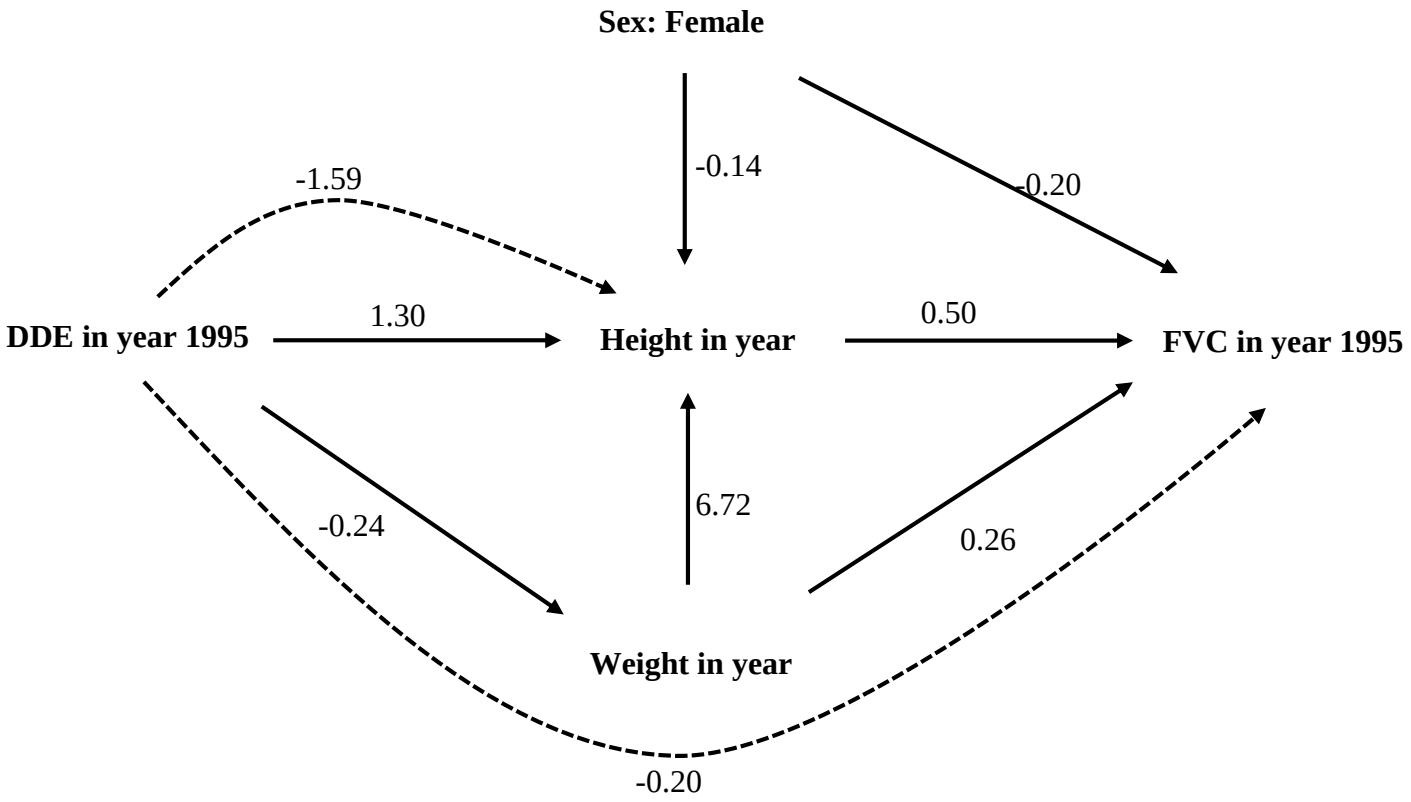


Figure 5.3: Analytical path model exploring the standardized direct and indirect effects of DDE exposure, height, weight and FVC at year 1995 (path coefficients from Table 5.6, and 5.7 A)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

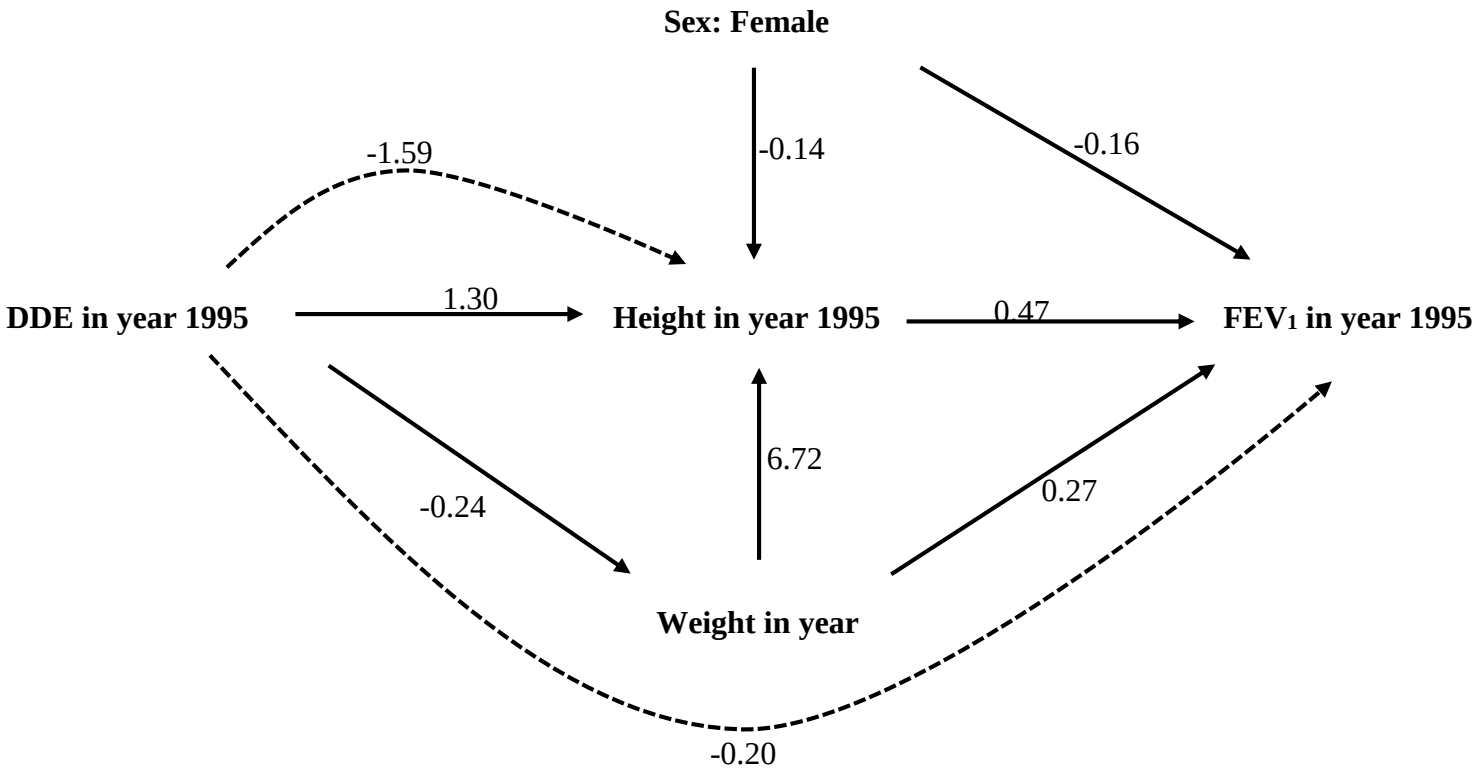


Figure 5.4: Analytical path model exploring the standardized direct and indirect effects of DDE exposure, height, weight and FEV_1 at year 1995 (path coefficients from Table 5.6 and 5.7 B)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

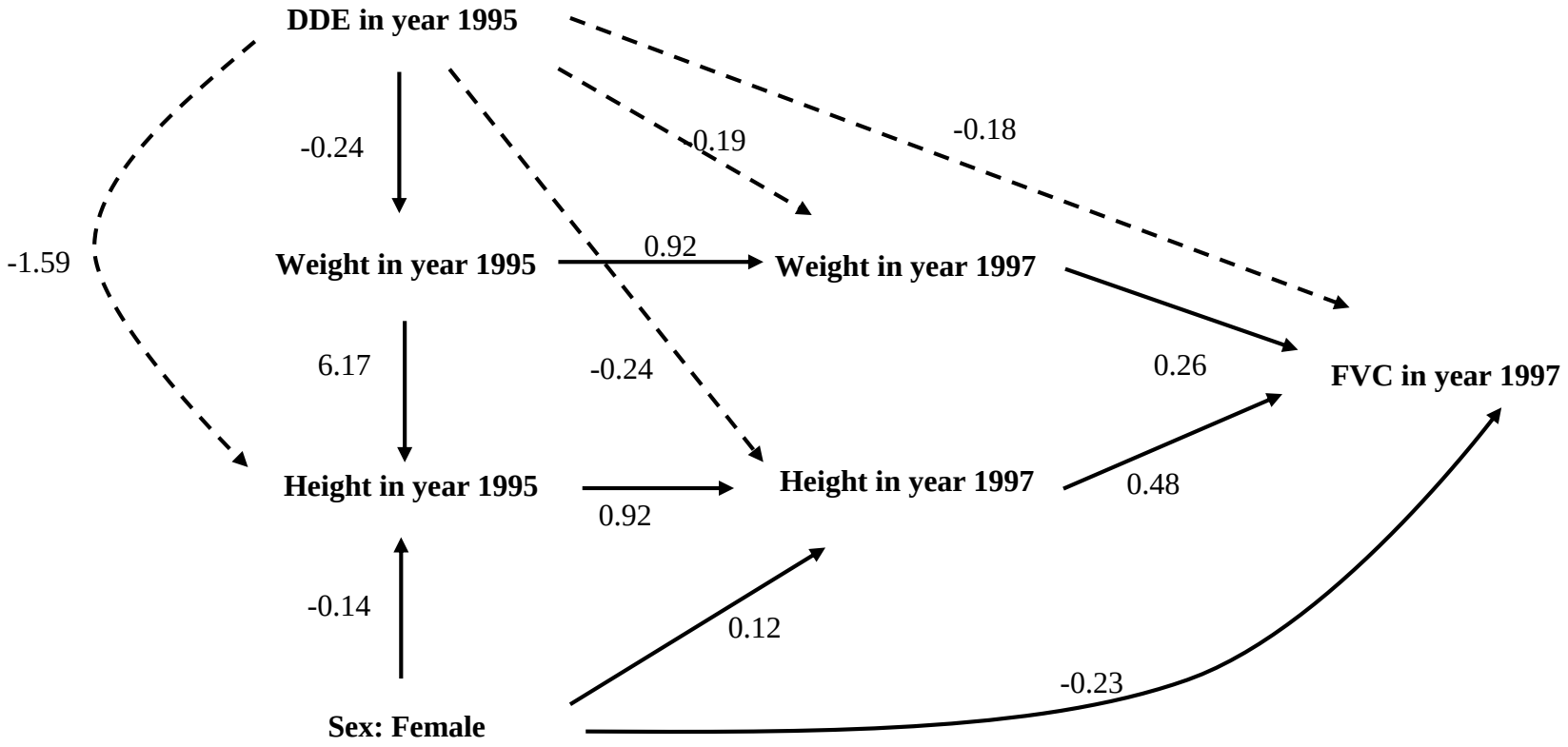


Figure 5.5: Analytical path model exploring the standardized direct and indirect effects of DDE exposure, height, weight and FVC at year 1997 (path coefficients from Table 5.6, 5.8 and 5.9 A)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

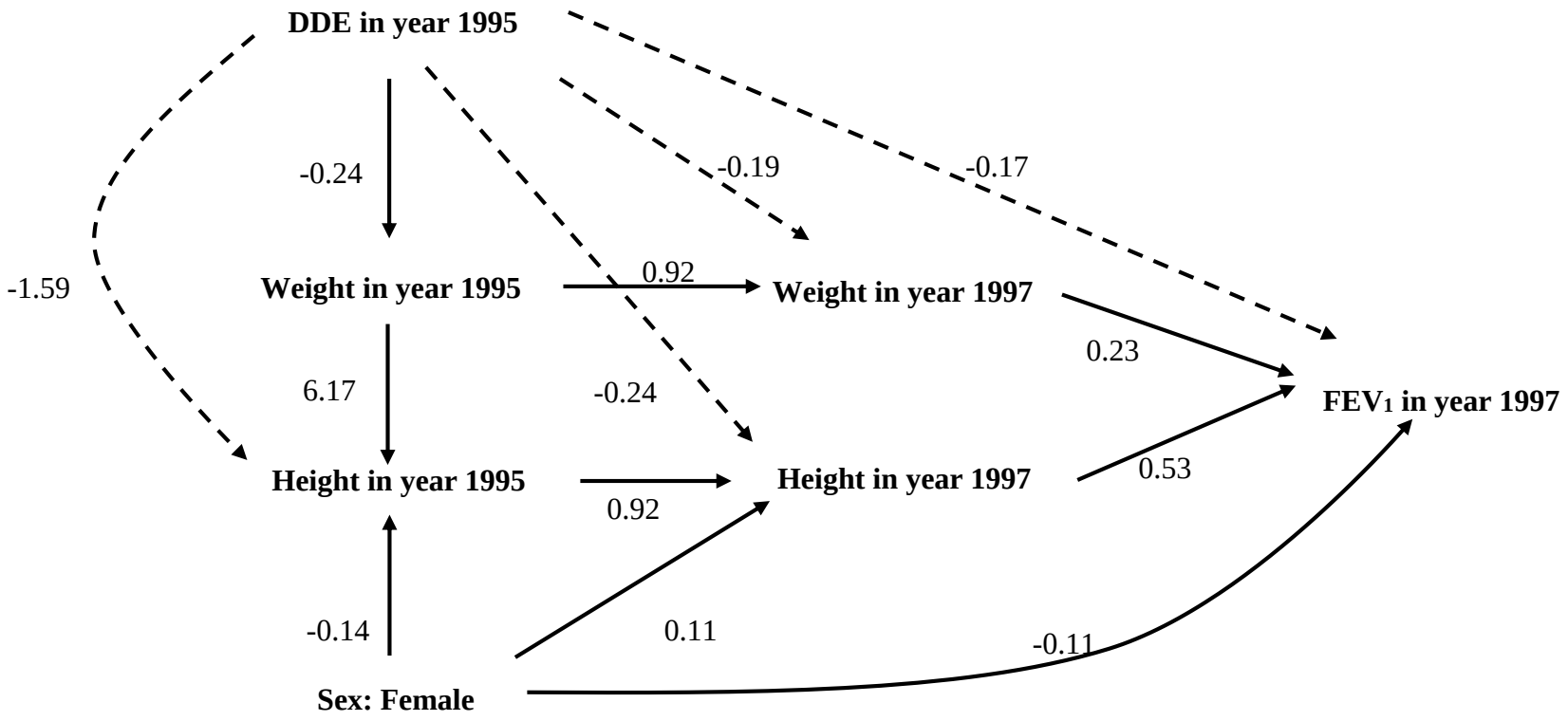


Figure 5.6: Analytical path model exploring the standardized direct and indirect effects of DDE exposure, height, weight and FEV_1 at year 1997 (path coefficients from Table 5.6, 5.8 and 5.9 B)

The path coefficients represented by solid arrows are direct effects while those represented by dashed arrows are indirect effects. The coefficients for covariates with non-significant total effects are not depicted in this diagram.

CHAPTER 6

DISCUSSION

According to the World Health Organization's report on environmental burden of diseases 20 % of lower respiratory tract diseases in developed countries are because of environmental causes.[1] Most commonly investigated exposures associated with respiratory diseases and pulmonary function are air pollution, indoor and outdoor allergens, tobacco smoke and exposure to chemical irritants. In children research related to associations between these exposures are often focused on risk of asthma and lower respiratory tract infections and rarely provide information on pulmonary function. However, since the development of pulmonary function stretches from newborns to young adulthood it is important to study these exposures in relation to pulmonary function as it may have long term consequences. In this dissertation, we studied three such environmental exposures- sensitization to house dust mite, joint effects of maternal smoking during pregnancy and adolescent smoking and dichlorodiphenyl dichloroethene (DDE) in relation to pulmonary function in children and late adolescents.

The first hypothesis investigated whether there is an association between immunological response to HDM and pulmonary function during late adolescence (18 years of age) after controlling for other atopic conditions. Immunological responses to HDM allergen were measured in terms of wheal size on SPT. To investigate this aim we

used data from the Isle of Wight (IOW) birth cohort, which was established in 1989 with the aim to study the natural history of asthma and allergic conditions. Our study is one of the first to explore the direct association between wheal size and pulmonary function. In second hypothesis we investigated the independent and joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in adolescents of age 18 years in IOW birth cohort. Although there is an abundance of literature on independent effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function, little information is available on the joint effects of gestational and adolescent smoking, their underlying pathways, and relationships between these exposure and other determinants of pulmonary function. In third hypothesis our aim was to investigate the effects of persistent body burden of DDE on pulmonary function in a cohort of children aged 8 to 10 years in Germany between 1994 and 1997. This study is one of first to investigate this association although previous studies have shown link between DDE exposure, asthma and atopic conditions.

Interpretation of Findings

Hypothesis 1

In this study we found that increase in wheal diameter on SPT was associated with reduced FEV_1, FEV_1/FVC ratio and $FEF_{25-75\%}$ and increased FeNO levels. Further we also found that although FEV_1 and FVC were reduced in adolescents with history of asthma wheezing episodes, adolescents without history of wheezing also demonstrated greater reduction in FEV_1/FVC and $FEF_{25-75\%}$ with every 1 mm increase in wheal diameter.

Previous studies have explored HDM exposure mostly in relation to risk of asthma and atopic diseases, bronchial hyperresponsiveness, FeNO and percent predicted FEV_1 levels.[24,42,44,45] Although one study by Koshak *et al.* reported increased severity of asthma with increased wheal diameter on SPT to HDM, no other studies have investigated the association between wheal size and pulmonary function parameters.[62]

In the present study, every 1 mm increase in wheal diameter, we found a reduction of pulmonary function and an increase in exhaled nitric oxide (FeNO) levels, suggesting that participants reactive to HDM on SPT but with no history of asthma or wheezing attacks still experience reduction pulmonary function. This may be explained by a possible inflammation of the airways or a reduced airway growth. Although in clinical settings an individual is usually considered as sensitive to HDM allergen if the wheal diameter is ≥ 3 mm, our results related to degree of sensitization suggested that reduction in pulmonary function only becomes evident at wheal diameter of > 5 mm.

Hypothesis 2

The findings of this investigation supported the hypothesis that maternal smoking during pregnancy and current adolescent smoking have inverse effect on pulmonary function in adolescents after accounting for the intervening effect of active smoking in adolescence. We tested this hypothesis first by using standard linear regression techniques. Our results indicated that smoking during pregnancy and adolescence had differential effects on pulmonary function among boys and girls. We found that girls are more vulnerable to effects of smoking than boys. Exposure to maternal smoking during fetal period is associated with increased adolescent smoking and decreased height and

weight in the offspring. Additionally, adolescent smoking, height, and weight are also important determinants of pulmonary function making them intervening variables on pathway between gestational smoking and pulmonary function at 18 years of age. Hence, conditioning on these intervening variables may generate biased estimates in linear regression. In order to address the issue of intervening variables, we conducted path analysis separately in boys and girls. Path analysis first demonstrated that adolescent smoking had direct inverse effect on FEV_1/FVC ratio in boys. Gestational smoking did not have any direct effects on FEV_1/FVC but its effects were mediated through adolescent smoking. Similarly in girls, adolescent smoking had direct inverse effect on FEV_1 and effects of maternal smoking during pregnancy were mediated through adolescent smoking. However, path analysis also demonstrated that the adverse effects on FEV_1/FVC and $FEF_{25-75\%}$ in girls were mainly due to maternal smoking during pregnancy and adolescent smoking did not contribute significantly to the reduction in these pulmonary function measures.

Not many studies have investigated the joint effects of maternal smoking during pregnancy and adolescent smoking on pulmonary function in adolescents. Few studies who have investigated this association were limited either by lack of generalizability[94] or small sample size of participants with exposure to maternal smoking[95] or lack of information on current smoking status.[93] However, our findings related to differences in boys and girls were comparable to those by Holmen *et al.* and Gold *et al.*[14,89] Both these studies investigated the independent effects of adolescent smoking on pulmonary function after adjusting for passive smoking. Holmen *et al.* in a cohort of 13-18 years old participants from Norway found that adolescent smoking was associated with reduction

in pulmonary function only in girls. Although Gold *et al.* found that a lower FEV_1/FVC ratio and a lower $FEF_{25-75\%}$ in both boys and girls of age 10 -18 years were associated with adolescent smoking, they found that girls were more vulnerable to adverse effects of smoking and had lower growth of pulmonary function than boys.[14] However in our study we found that girls were more affected by gestational smoking than personal smoking. Additionally, previous studies have reported increased FeNO levels in infants and young children who were exposed to maternal smoking during pregnancy.[148,149] To our knowledge no study has examined the relationship between gestational smoking and FeNO in adolescents. In our study we found that both maternal smoking during pregnancy and adolescent smoking had independent as well as joint positive effects on FeNO.

One of the advantage of our study is that we used path analysis to disentangle the relationships between determinants of pulmonary function in different periods of child development i.e. gestation to adolescence. Standard regression analysis does not take into consideration the temporal sequence and thus is ill-suited for modeling relationships which are composed of effects mediated through intervening variables. Path analysis on the other hand accommodates intervening variables in the model since it is based on both conceptual theories and mathematical equations. Pulmonary function during childhood and adolescent period is determined by complex relationships between many factors like age, sex, height, weight, race and smoking status. Failure to understand the underlying 'causal' pathways and relationships between these factors can lead to unintentional adjustment on intervening or mediating variables. This may not only distort the causal

pathway but may also lead to an over-adjustment bias affecting the precision of the relationship between exposure and outcome .[161]

Hypothesis 3:

Our third hypothesis was – persistent body burden of dichlorodiphenyl dichloroethene (DDE), a by-product of the pesticide dichlorodiphenyl trichloroethane (DDT), affects pulmonary function in childhood, directly or indirectly via its effect on height and weight in children. In our first step, we found that there was a weak correlation between DDE exposure and - height, weight, FEV_1 and FVC. Next we used, linear mixed models to explore the association between DDE exposure and pulmonary function pattern. Although our repeated measurement analysis showed an association of height, weight and sex with pulmonary function there was no significant association between DDE exposure and pulmonary function. However, our path analysis showed that DDE exposure measured at age 8 years (1995) had direct inverse effects on height and weight measured at age 8 years and indirect inverse effects on height and weight measured at age 10 years (1997). Further, it also suggested that DDE exposure had indirect effects on FEV_1 and FVC mediated through its effects on height and weight in children of age 8 years. We also observed indirect inverse association between DDE exposure measured at age 8 years and FEV_1 and FVC measured at age 10 years mediated through height and weight measured at both 8 and 10 years. However, there was no association between DDE exposure measured at age 10 years and FEV_1 and FVC measured also at age 10 years.

DDE is an exposure which is known to affect three important determinant of pulmonary function: height, weight and immune function. There are conflicting reports on effects of DDE on height. Some studies have suggested that DDE reduced height in both boys and girls;[113] while other have reported that it reduced height only in girls[114] while increased height in boys during pubertal period.[116] Literature also shows that DDE exposure leads to early puberty in girls through its estrogenic effects.[121] Our results showed that DDE exposure was directly related to reduction in height and weight at age 8 years and indirectly at age 10 years, although we were not able to test this hypothesis separately in boys and girls. Both height and weight are related to an increase in lung capacity and therefore any exposure that adversely affects height and weight should affect FVC. Our results corroborated this hypothesis. Additionally, role of DDE exposure in dysregulation of immune function may also lead to remodeling of airways. Previous study conducted in the same cohort as the present study have also shown that higher DDE concentrations increased levels of IgE, IgE count on basophils, and the reduction of eosinophilic granula.

Study Limitations

The major limitation of this dissertation is related to lack of use of predicted values for pulmonary function parameters and this may limit the interpretation of our results in clinical settings. The percent of predicted values are recommended by American Thoracic Society for diagnosing asthma. Although there is plethora of published prediction/reference equations for spirometric indices for European population, the generalizability of these equations is questionable as many are based on data collected decades ago on small numbers of subjects specific to a set of population and so may

affect their applicability to this cohort. However, the new reference equations for wider age range and multi-ethnic groups developed as a part of Global Lung Function Initiative (GLI) could be used in future studies.[127] In addition to this limitation there some other limitations related to each hypotheses. Regarding hypothesis 1, while exploring association between HDM sensitivity we adjusted for sensitivity to other indoor and outdoor allergens. Often sensitivity to HDM is related with sensitivity to other indoor allergens especially cats and dogs. This correlation could have biased the final estimates in linear regression models; however, it provided the net effect of house dust mite sensitization. In hypothesis 2, information on smoking during pregnancy was self-reported. Because of negative attitude towards smoking during pregnancy this information may have been underestimated and it was not validated by objective measurements. Under-reporting of smoking may have biased our estimates for maternal smoking during pregnancy towards the null. Another important limitation is small sample sizes in four smoking exposure categories in boys and girls in linear regression models which results in a lower statistical power to detect significant differences. In the third hypothesis the major limitation was that we were not able to test our hypothesis separately in boys and girls using path analysis. DDE is known to affect height and weight differently in boys and girls and thus, may have differential effects on pulmonary function in boys and girls. However, because of small sample size our path analysis models were not able to generate positive convergence estimates for maximum likelihood optimization method.

Public Health Implications

Hypothesis 1:

Allergy is a major health problem in the Western countries. The prevalence of asthma is high in the populations with a high prevalence of atopy. In general, asthma diagnoses are based on clinical presentation of symptoms and reduction in FEV_1. But often the history of asthmatic symptoms like wheezing and nocturnal cough provided by children or their parents is unreliable and it is not possible to diagnose airway obstruction on physical exam. Additionally, diagnosis of slight airway obstruction is difficult as patients may not exhibit clinical symptoms associated with it. In such situations the reduction in pulmonary function may go undetected unless the physician orders for pulmonary function tests. In these settings, wheal diameter may serve as a potential indicator of underlying abnormal pulmonary function. Monitoring of individuals reactive to HDM but with no history of asthma may be beneficial as it may alert physicians to abnormality in pulmonary function and in the long term may reduce the burden of chronic respiratory diseases in adults.

Hypothesis 2:

The findings from hypothesis two have both clinical and public health significance. The increased FeNO levels and reduction in FEV_1/FVC and $FEF_{25-75\%}$ values found in girls suggest that gestational smoking leads to chronic inflammation and remodeling in small airways along with larger airways. These findings are especially important because lower pulmonary function during adolescence may possibly lead to chronic obstructive pulmonary disease and small airway disease in adulthood. The

findings demonstrate that not only tobacco exposure has differential inverse effects on pulmonary function among adolescent boys and girls, but maternal smoking during pregnancy has a measurable adverse effect on the pulmonary function which is independent of any direct use of cigarettes by adolescents. Our results corroborated the need of intervention strategies of tobacco cessation in adolescents and women of reproductive age. Furthermore, reduction in maternal smoking during pregnancy may also lead to reduction in adolescent smoking which in turn may not only lower the burden of chronic respiratory diseases but also other chronic diseases like cardiovascular diseases and cancers.

Hypothesis 3:

Although the pesticide dichlorodiphenyl trichloroethane (DDT) has been banned for more than three decades now, its principal metabolite DDE remains a global public health issue. Its adverse effects on more than one determinant of pulmonary function namely height and weight makes it difficult to determine its true effect on pulmonary function. In this study we used a more sophisticated approach of path analysis to study the interrelationships between DDE exposure, height, weight and pulmonary function. Although our results suggested that the adverse effects of DDE on pulmonary function were mediated through height and weight, results from previous studies in the same cohort in Hesse, Germany suggest DDE exposure may possibly lead to structural damage to airways through its effects of immune system. However, this hypothesis can only be corroborated by histo-pathological studies. We must point out that although our study population belonged to a developed country, the investigation of this hypothesis in a

population with high use of DDT for agricultural or malaria prevention purposes would give a clearer picture of the impact of DDE on pulmonary function.

Summary

The ultimate goal of this dissertation was to enhance our knowledge and understanding of how environmental exposures, namely sensitization to HDM, gestational smoking and adolescent smoking, and DDE, impact pulmonary function in children and adolescents. We started by exploring the association between wheal size on SPT for HDM and pulmonary function in adolescents of age 18 years in hypothesis one.We found that increased wheal diameter on SPT was associated with reduced pulmonary function in adolescents of age 18 years irrespective of history of wheezing attacks. Our results also demonstrated that wheal diameter on SPT could serve as a potential indicator of abnormal pulmonary function in asymptomatic individuals sensitized to HDM. With hypothesis two we assessed the joint effects of gestational and adolescent smoking on pulmonary function. The results suggest that girls are more vulnerable to smoking effects than boys. Path analysis further demonstrated that not only adolescent tobacco exposure has differential inverse effects on pulmonary function among adolescent boys and girls, but also gestational smoking has a measurable adverse effect on the pulmonary function, independent of any direct use of cigarettes by adolescents. Finally, in our third hypothesis we investigated associations between body burden of DDE exposure and pulmonary function parameters in children of age 8-10 years. Previous research in the field of DDE and respiratory health has been limited to investigations of upper and lower respiratory illnesses and risk of asthma. We were able

to demonstrate an indirect association of DDE with pulmonary function mediated through height and weight.

These results emphasize the importance of the adverse effects of environmental exposures on pulmonary function in children and adolescents. The reduction in pulmonary function in children and adolescents is associated with early onset of chronic respiratory diseases in adulthood leading to increased burden of respiratory diseases and impaired quality of life. Therefore, early detection of reduction in pulmonary function may not only reduce the burden of respiratory diseases on healthcare system but may also improve the health and quality of life at individual level. Our first two hypotheses were investigated in a population with high prevalence of allergy and smoking and the third hypothesis related to DDE exposure was investigated in a cohort from a developed country with lower levels of DDE than detected in children from developing and underdeveloped countries. Future research should be focused on investigating these associations in a more generalizable population of children in longitudinal studies..

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