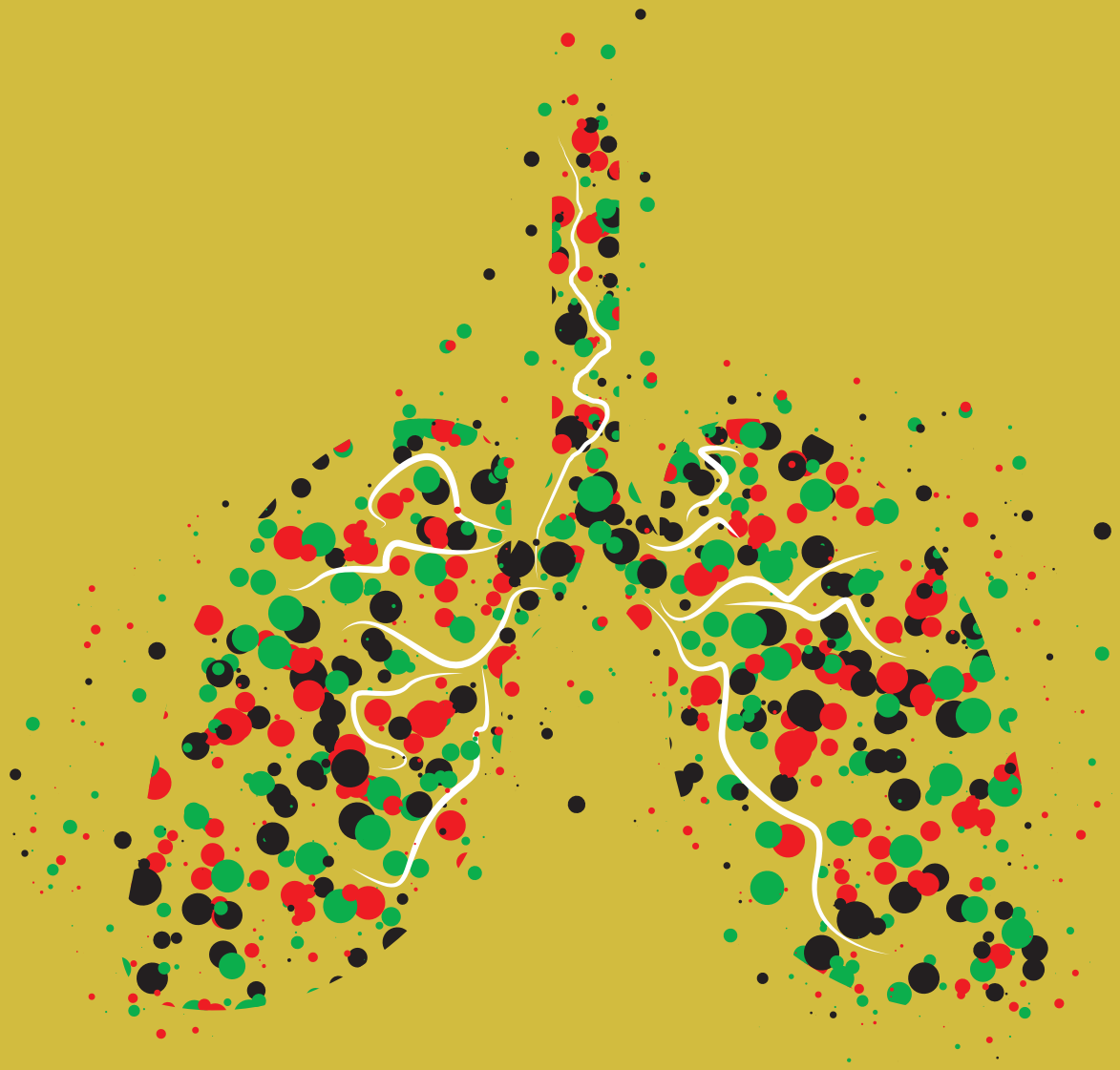

A WINDOW OF OPPORTUNITY

THE IMPORTANCE OF THE TIMING OF ENVIRONMENTAL EXPOSURES OVER THE LIFE COURSE FOR ASTHMA AND LUNG FUNCTION IN ADOLESCENCE

EDITH B. MILANZI

A window of opportunity:
The importance of the timing of environmental exposures over the life course for asthma and lung function in adolescence

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The research leading to the results presented in this thesis has received funding from the Dutch Lung Foundation (*Longfonds*) (Grant number 4.1.14.001)

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Cover design: Ron Zijlmans
Lay-out: RON Graphic Power || www.ron.nu
Printed by: ProefschriftMaken || www.proefschriftmaken.nl

ISBN: 978-94-6380-804-0

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Wat is de invloed van milieublootstellingen gedurende verschillende levensfasen op astma en longfunctie van adolescenten

(met een samenvantting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de grad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
dinsdag 2nd Juni 2020 avonds te 14:30 uur.

door

Edith Bridget Milanzi

Geboren op 11th July 1990 te Lilongwe, Malawi

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CHAPTER 1

1

General Introduction

BACKGROUND

The World Health Organization (WHO) has recognized the role of environmental exposures on human health and further estimates that 24% of the global disease burden and 23% of all deaths could be attributed to environmental exposures as of 2012.[1] Epidemiological studies have elucidated the potential associations between environmental exposures and health. These have provided an understanding of how interventions can be structured and policies implemented in order to reduce disease burden attributed to environmental exposures.

This thesis presents epidemiological research that explores methods that can be used to characterize longitudinal time-varying environmental exposures to determine the relevance of the timing of exposure to secondhand smoke (SHS), pets, dampness or mould, and air pollution during the life course in the associations with asthma and lung function in adolescence.

Asthma and Lung function

Asthma

The Global Initiative for Asthma (GINA) defines asthma as a heterogeneous disease characterized by chronic airway inflammation, recurring periods of wheezing, chest tightness, shortness of breath and coughing that vary over time.[2] Increasing evidence has shown that the prevalence of asthma has increased especially in children. In 2016, the Global Burden of Disease study estimated that 339.4 million people worldwide were affected by asthma corresponding to an increase in age-standardized global prevalence of asthma by 3.6% since 2006.[3,4] In addition, the GINA estimates that there will be an additional 100 million people with asthma by the year 2025.[3] Consequently, asthma has become a significant global health concern. The specific causes of the disease are not clear. Various potentially modifiable environmental exposures and behavioural patterns during prenatal, perinatal, and postnatal periods have been suggested to either prevent or boost asthma development.[5] The independent contribution of genetics, as well as the interaction of environmental exposures with genetic factors in the development of asthma, has also been acknowledged.[5-8]

Lung function

The main function of lungs is the process of gas exchange and a reduced/impaired lung function means that the ability of the lungs to carry out this process is reduced leading to respiratory complications.[9] Objective and quantitative pulmonary function tests (PFTs) are considered early predictors of respiratory morbidity and mortality.[10] The most studied PFTs are forced expiratory flow in one second (FEV_1), forced vital capacity (FVC), and the ratio of FEV_1 and FVC (FEV_1/FVC). They are used to assess airway obstruction and restriction and also reflect lung capacity.[11] The development of the lung starts in the embryo and continues

throughout childhood and into adolescence and exposure to a variety of toxicants and conditions during lung development can potentially affect the overall growth and function of the respiratory system.[12,13] The maximum achieved level of lung function during early adult life has important implications for later life respiratory health and this level can be ascertained by measuring the course of lung function and lung function growth in childhood.[14,15]

Epidemiological evidence on associations of environmental exposures with asthma and lung function

Secondhand tobacco smoke (SHS)

SHS remains a health hazard worldwide.[16] Involuntary exposure to SHS has been shown to be associated with multiple adverse respiratory health effects including asthma. Extensive evidence pointing to these associations has been established in cross-sectional and longitudinal studies.[17-19] Both maternal smoking during pregnancy and postnatal SHS exposure have been established as strong risk factors for childhood asthma and according to the *Surgeon General,* the evidence is sufficient to infer a causal relationship between parental smoking and ever having asthma among children of school age.[19]

In addition to asthma, SHS exposure is also strongly linked to lower lung function, and slower lung function growth in children[20-22] and consequently chronic obstructive pulmonary diseases (COPD) in later life.[23] Existing evidence is deemed suggestive, but not sufficient to infer a causal relationship between long term SHS exposure and decrements in lung function in the general population.[19]

Pet exposure

Current epidemiological evidence on the associations of pet exposure on asthma development and allergic disease is contradictory and the debate is ongoing. While some studies have reported a higher risk of asthma in relation to pet exposure, [24-28] substantial evidence also exists for a lower asthma risk in children that are exposed to pets.[29-32] Similarly, higher risk of sensitization to common allergens has been reported by some studies[33] while other studies have also reported a lower risk of sensitization.[34] However, this relationship has been explored mainly in children and not in adolescents.

The relationship between pet exposure and level of attained lung function and lung function growth during childhood and adolescence has not been extensively investigated and is therefore not well understood. Of the few studies that have studied this relationship in children, inconsistent findings have been reported with some studies reporting lower function[35] and other studies reporting no association[36] or higher lung function in asthmatic girls exposed to pets.[37]

Dampness or mould exposure

Dampness or mould in homes may promote microbial proliferation resulting in production of microbial agents that may contain inflammatory substances and allergens.[38] As such, dampness or mould are also suggested to increase the risk of asthma as well as allergic disease.[39] A number of studies and reviews have consistently found associations between dampness or mould exposure and increased asthma risk and other respiratory outcomes in children and adults.[38-42] Associations between exposure to dampness or mould and lung function have not been extensively explored. Of the few studies that have investigated these associations, weak negative associations were observed in children,[43,44] evidence for these associations beyond childhood is even more limited.

Air pollution

The role of air pollution in a range of adverse health effects, particularly mortality and morbidity due to respiratory diseases has been established.[45] Extensive literature points to lower levels of attained childhood lung function and reduced lung function growth in childhood and adolescence in relation to both short term and long term outdoor air pollution exposure especially to particulate matter (PM) and nitrogen dioxide (NO_2).[46-49] Consequently, it has also been demonstrated that reduced air pollution exposure may be associated with lung function improvement. This has been suggested in children[50-53] and adults.[54]

Timing of residential environmental exposures and asthma and lung function into adolescence

Existing evidence on associations of environmental exposures with asthma and lung function is limited to children or exposure in either early-life, at a specific age or later-life only such that evidence on life course longitudinal exposures and therefore the relevance of timing of exposure is scarce. Apart from lack of life course exposure studies, most studies are also unable to investigate health outcomes in adolescence. This is attributable to the lack of longer follow-ups in most prospective studies. Investigating the relevance of the timing of exposure is essential as exposure during different time periods in the life course may differentially affect associations of exposure with asthma and lung function in later life. Understanding the role of the timing of the different exposures will guide targeted interventions towards reducing the burden of respiratory disease in childhood and, perhaps, beyond.

The PIAMA study

The research presented in this thesis used data from the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort initiated in 1996/97. Pregnant mothers were recruited from antenatal clinics in the north, west and central parts of the Netherlands (Figure 1) and the study started with 3963 newborns. The study was initially set up as an intervention

(Figure courtesy of Brunekreef *et al*,2002[56])

Figure 1. Map of the Netherlands showing original PIAMA participants locations. Some of the participants have since moved to other locations.

study to investigate the effect of mite-allergen avoidance on the incidence of asthma and allergy in childhood and simultaneously, as a study on lifestyle and environmental (indoor and outdoor) risk factors for childhood asthma and allergy. [55,56] The study has proceeded to investigate different epidemiological relationships with outcomes other than allergy and asthma such, as but not limited to, respiratory symptoms, cardiometabolic markers, genetic and lifestyle outcomes. Questionnaires used to obtain data on environmental and lifestyle exposures and on asthma and other allergic and respiratory outcomes were completed by parents during pregnancy, when the child was 3 months old, then annually from 1 up to 8 years and by parents as well as children at 11, 14, 16 and 17 years. Medical examinations were conducted at ages 8, 12 and 16 years to obtain anthropometric measures, measurements of lung function, measurements of IgE levels and blood pressure (Figure 2).

The prospective nature of the PIAMA study and detailed data collection on environmental exposures and health outcomes from birth till adolescence makes this study uniquely positioned to address and provide insight into the relevance of timing of different exposures over the life course in the development of asthma and lung function into

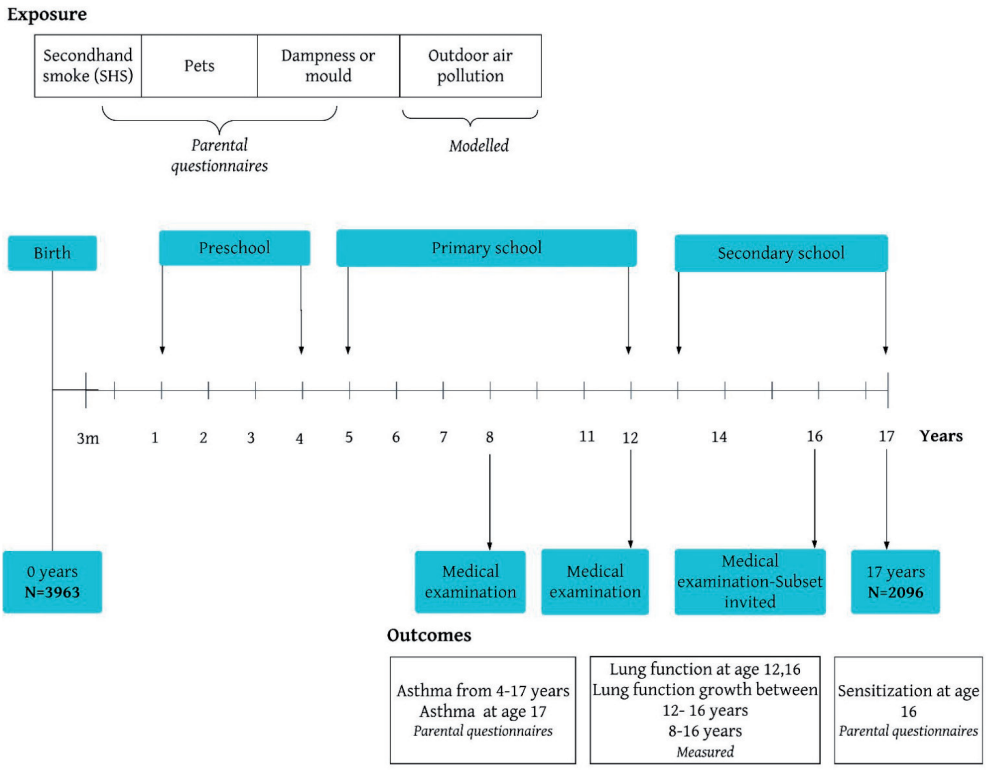

Figure 2. Exposures, outcomes and approaches to investigate the timing of exposure

adolescence. Three approaches were used to investigate the relevance of timing of different exposures over the life course; a) Time windows approach; distinguished as preschool (birth – 4 years), primary school (5-12 years) and secondary school (13-17 years), b) Cumulative exposure scores and c) Longitudinal patterns of exposure (Figure 2). Currently, the study is ongoing and the participants are now approximately 23 years old. By the 17-year follow-up, 2096 participants were still in the study. Authorized institutional ethical review boards from participating institutions (Utrecht, Rotterdam, and Groningen medical centres) approved the PIAMA study.

Aim and thesis outline

The hypothesis of this thesis was that different timing of SHS, air pollution, pet, and dampness or mould exposure throughout the life course might differentially affect asthma and lung function in adolescence. Associations of exposure to SHS, air pollution, pet and dampness or mould exposure during different time windows with the development and prevalence of asthma up to 17 years, lung function (growth) until 16 years were investigated. Additionally, associations with sensitization at age 16 were also investigated because few studies have studied the relationships of pet and dampness or mould exposure with sensitization in adolescence and because sensitization is considered a risk factor for asthma.

The outline of this thesis is as follows:

Chapter 2 describes a study on associations of lifetime SHS exposure with asthma up to 17 years.

Chapter 3 is a study on associations of the timing of pet and dampness or mould exposure with the prevalence of asthma at age 17 and sensitization at age 16.

Chapter 4 describes a short study conducted to assess systematic differences between lung function measurements of two different spirometers that were used to measure lung function at the 16-year PIAMA medical examination, the Jaeger Masterscreen pneumotachograph and the EasyOne spirometers.

Chapter 5 assesses associations of timing of exposure to SHS, pets and dampness or mould with lung function growth between ages 12 and 16, and level of lung function attained at age 12 and at age 16.

Chapter 6 elucidates on the relationship between air pollution exposure during different time windows since birth until adolescence and lung function growth between ages 8 and 16 years, and level of attained lung function at age 16.

Chapter 7 is an invited editorial on associations of air pollution on lung function in adults conducted in the UK Biobank cohort.

In **Chapter 8,** the findings in preceding chapters are summarized and discussed in a broader context of the timing of life course environmental exposure and its role on asthma prevalence and lung function in adolescence, potential implications and steps for further research are also discussed.

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CHAPTER 2

2

Lifetime secondhand smoke exposure and childhood and adolescent asthma: findings from the PIAMA cohort

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Environmental Health (2017) 16:14

ABSTRACT

Background: Secondhand smoke (SHS) exposure is a modifiable risk factor associated with childhood asthma. Associations with adolescent asthma and the relevance of the timing and patterns of exposure are unclear. Knowledge of critical windows of exposure is important for targeted interventions.

Methods: We used data until age 17 from 1454 children of the Dutch population-based PIAMA birth cohort. Residential SHS exposure was assessed through parental questionnaires completed at ages 3 months, 1-8 (yearly), 11, 14, and 17 years. Lifetime exposure was determined as; a) time window-specific exposure (prenatal, infancy, preschool, primary school, and secondary school); b) lifetime cumulative exposure; c) longitudinal exposure patterns using latent class growth modelling (LCGM). Generalized estimation equations and logistic regression were used to analyse associations between exposure and asthma at ages 4 to 17 years, adjusting for potential confounders.

Results: With all three methods, we consistently found no association between SHS exposure and asthma at ages 4 to 17 years e.g. adjusted overall odds ratio (95% confidence interval) 0.67 (0.41 to 1.12), 1.00 (0.66 to 1.51) and 0.67 (0.41 to 1.11) for prenatal maternal active smoking, infancy, and preschool school time window exposures, respectively.

Conclusion: We assessed lifetime SHS exposure using different methods. Different timing and patterns of SHS exposure were not associated with an increased risk of asthma in childhood and adolescence in our study. More longitudinal studies could investigate the effects of lifetime SHS exposure on asthma in adolescence and later life.

BACKGROUND

Exposure to secondhand smoke (SHS) along with exposure to other environmental, lifestyle and genetic factors has been found to be associated with asthma. [1-3] Since asthma is one of the most common chronic respiratory diseases and most asthma begins in childhood and adolescence, exposure to SHS during these periods is of particular interest.[4]

Prenatal and early-life postnatal exposure to SHS has been found to be associated with an increased risk of asthma during the first 10 years of life.[2,5-8] Studies of the association of asthma with SHS exposure later in life and studies of the association between lifetime SHS exposure and adolescent asthma are scarce. Consequently, the relevance of SHS exposure later in life and for adolescent asthma is largely unknown. To date, four prospective studies [6,9-11] have assessed the association of pre and postnatal SHS exposure on asthma in adolescence but results are inconsistent. Only one study out of these considers SHS exposure throughout childhood.

Longitudinal cohort studies seldom pay attention to patterns of exposure over time. Exposure to SHS during different time windows may also differentially affect the presence of asthma, therefore knowledge of critical time windows of exposure is important in implementing targeted interventions. Therefore, in this study, we aim to use three methods to determine the role of timing of SHS exposure and investigate if there is a critical time window of SHS exposure that contributes to asthma up to adolescence and to examine cumulative exposure and detailed longitudinal patterns of exposure from repeated measures and investigate their associations with asthma prevalence at age 17.

METHODS

Study population and design

We obtained data from the Dutch population-based PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort that started with 3963 children born in 1996/1997.[12] Questionnaires were completed by parents during pregnancy, 3 months after birth, then yearly from age 1 to 8; and at ages 11, 14 and 17 by both, parents and children. Questionnaires comprised questions on SHS exposure and asthma symptoms, diagnoses and medication as well as socio-demographic characteristics, parental atopy, and other environmental exposures and lifestyle characteristics.

Our primary study population consists of all adolescent children with complete data on both asthma at age 17 and SHS exposure from pregnancy until age 17 (N=1454, Figure E1).

Ethics statement

Ethical approval was obtained from authorized institutional review boards. Children's parents or legal guardians and children themselves provided written informed consent.

SHS exposure assessment

We assessed SHS exposure through the repeated parental questionnaires from pregnancy till age 17. Exposure at age 17 was assessed using the adolescent questionnaires if the participant had moved out of his/her parents' home (N=50). Postnatal residential SHS exposure was assessed by reports of anybody smoking inside the home (yes; yes, but less than once a week; never). In addition, the number of cigarettes smoked per day for those who answered 'yes' was obtained, and at ages 1-4, information on smoke exposure outside child's home if children regularly spent at least half a day outside their home.

Lifetime SHS exposure was determined using three methods: First, we distinguished five time windows. To optimize the implementation of potential preventive measures during a specific time window we chose time windows for ages that match appropriate settings for prevention in the Netherlands: prenatal period (pregnancy), infancy time window (3 months after birth) corresponding to prevention in well-baby clinics, preschool time window (1-4 years) for prevention through infant health care, and primary school (5-11 years) and secondary school (> 12 years) time windows for prevention in primary and secondary school, respectively. Time window-specific exposures were assessed as detailed in Figure E2. In brief, for the prenatal period, four categories were defined: maternal active smoking, maternal sometimes passive smoking (>4 hours per day), maternal rare passive smoking (1-4 hours per day), and never (no active or passive smoking). Exposure during infancy was defined by the 3-month questionnaire using the original categories. Exposure during preschool, primary school and secondary school time windows was determined through categories created from two to five questionnaires: if the response to the question on anyone smoking in the house was 'yes' for the whole time window, children were classified as 'always exposed'; if the response was 'yes' at least once during the time window, children were classified as 'sometimes exposed'; if the response was 'yes, but less than once a week' at least once and 'never' otherwise, children were classified as 'rarely exposed'; if the response was 'never' during whole time window, children were classified as 'never exposed'

Second, lifetime cumulative SHS exposure was defined based on the well-documented dose-response relationship of SHS and asthma [13] by assigning points to questionnaire responses on SHS exposure (prenatal exposure: maternal active smoking=2 points, any maternal passive smoking=1 point; neither active nor passive smoking=0 points; postnatal exposure for each questionnaire: yes=2 points; yes, but less than once a week=1 point; never=0 points) and summing score points for all questionnaires from pregnancy to age 17. The score ranged from 0 (no SHS exposure) to 26 points (highest exposure at all 13 follow-ups). Children with SHS exposure were divided into three categories of equal size defined as passive low (scores 1-3), medium (scores 4-12) and high (scores 13-26).

Third, in order to combine exposure during different time windows and account for cumulative exposure, we used a data-driven approach known as latent class growth modelling [14] (LCGM, TRAJ procedure in SAS 9.4, Cary, USA) to define lifetime longitudinal patterns of exposure from pregnancy to age 17. These patterns reflect the different underlying subpopulations existent within the whole population based on the probability of being exposed to SHS over time. The procedure allocates individuals based on the probability of belonging to a particular pattern taking into account the status of exposure at each time point and translates it into a cumulative pattern over time. The higher the probability, the higher the likelihood of being allocated to that particular pattern. SHS exposure was dichotomized at each time point for this procedure (any exposure, yes/no). To determine the number and shape of patterns of SHS exposure in the population, we first assumed that there is one homogeneous non-changing pattern of SHS exposure from pregnancy till age 17, by specifying the intercept only. We then further investigated if there was more than one pattern and different pattern shapes by including more groups and higher-order polynomials. We repeated this procedure for models assuming up to five groups of patterns for polynomials up to the order of three (models with six or more patterns did not converge). All models were compared and the best model was defined as one with the smallest BIC (Bayesian Information Criterion).

Asthma definition

Asthma at ages 4 to 17 years was defined by the presence of two out of the following three criteria based on parental questionnaires: wheezing in the past 12 months, doctor-diagnosed asthma ever, and prescription of asthma medication in the past 12 months according to the MeDALL protocol.[15] We also defined asthma phenotypes based on age of onset and persistence as follows: "early transient" defined as any asthma according to the definition described above between 4-6 years but not later, "persistent" defined as asthma between 4-6 and between 14-17 years; "intermediate onset" defined as first asthma between 7-11 years, "adolescent-onset" defined as first asthma between 14-17 years; and never defined as no report of asthma at ages 4-17 years.

Statistical analyses

We used Generalized Estimating Equations (GEE) to analyse associations of asthma at ages 4 to 17 years with SHS exposure during the prenatal, infancy and preschool time windows; multiple logistic regression models to analyse associations of asthma at age 17 with SHS exposure during the primary and secondary school time windows, as well as cumulative scores and the longitudinal patterns of exposure; and polytomous logistic regression to analyse associations of asthma phenotypes with exposure during the prenatal, infancy and preschool time windows. Age-specific estimates of associations with exposure during prenatal, infancy and preschool time windows were obtained from GEE models with

exposure-age interaction terms. In analyses of exposure patterns, the pattern variables were included as exposure variables in the model. Observations were weighted according to posterior probabilities of belonging to a particular pattern of exposure to account for uncertainty in the allocation of individuals to patterns.

Adjusted and unadjusted analyses were performed adjusting for the following potential confounders identified from literature and prior knowledge: parental education (defined as maximum of either mother's or father's education; low, medium, high), sex, parental atopy, breastfeeding (>12 weeks: yes/no), having older siblings (yes/no), maternal age at birth (continuous), active smoking (smoking at least once a week at 14/17 years), resident region at birth (north, middle, west). Time-varying confounders such as presence of pets (yes/no), gas cooking (yes/no), presence of dampness and mould (yes/no), and overweight (yes/no/unknown based on BMI using the International Obesity Task Force sex-specific cut-off points [16]) were selected from the earliest available questionnaire. Exposure to ambient air pollution was estimated by land-use regression modelling [17] and defined as annual average nitrogen dioxide (NO_2) concentration at the home address at birth.

We performed a number of sensitivity analyses. In order to investigate the possible effect of selecting children with complete SHS data, we repeated analyses with extended populations; i.e. children with complete exposure information for a specific time window instead of from birth till age 17 for time window-specific exposures and individuals with asthma data, but incomplete SHS exposure data (N=1871) for the longitudinal patterns. We repeated adjusted analyses with time-varying confounders defined based on the latest available questionnaire (14/17 years). We also excluded active smokers from all analyses and additionally adjusted for low birth weight, which could be on the causal pathway between asthma and SHS exposure. Modifications of the association between SHS and asthma by parental atopy, presence of pets, sex and parental education have been suggested [18-23] and were explored in stratified analyses.

All analyses were performed with SAS version 9.4. Statistical significance was defined by a two-sided alpha of 0.05.

Table 1. Characteristics of study population (N=1454)

Characteristics	**n/N**	**(%)**
Parental atopy (Yes)	749/1454	51.5
Sex (Boys)	721/1,454	49.6
Presence of pets at 3 months (Yes)	674/1454	46.3
Presence of mould at 1 year (Yes)	386/1454	26.5
Breastfeeding > 12 weeks (Yes)	772/1449	53.3
Overweight at 3 years		
Yes	95/1454	6.5
No	1122/1454	77.1
Unknown	237/1454	16.3
Gas cooking (Yes)	1204/1448	83.1
Older siblings (Yes)	727/1454	50.0
Parental education		
Low	125/1454	8.6
Intermediate	464/1454	31.9
High	864/1454	59.5
Region		
North	454/1454	31.1
Middle	633/1454	43.6
Western	367/1454	25.2
Ethnicity (Dutch)	1322/1435	92.1
Active smokers (14/17 years)	193/1454	13.2
	N (Mean (Range))	
Maternal age at birth (years)	1438 (31.1 (18-42))	
Outdoor NO_2 at home address at birth ($\mu g/m^3$)	1448 (22.8 (9.2-59.6))	

RESULTS

Study population characteristics are presented in Table 1. Half of the children were boys, 60% had highly educated parents, and 52% had atopic parents. Parents who reported any SHS exposure from pregnancy till age 17 (N=823) were less often atopic and less often highly educated than parents who did not report any exposure (Table E1). Asthma prevalence ranged from 5% at age 17 to 8% at age 4 (Figure E3). Prevalence was 7%, 3%,4% and 1% for early onset, intermediate, persistent, adolescent onset asthma respectively. Baseline characteristics were similar for the study population and the excluded population except for higher prevalence of high parental education and breastfeeding and a lower prevalence of pet ownership in the study population (Table E2).

A decreasing trend in SHS exposure was noted. Of the 1454 children, 11% were exposed prenatally through maternal active smoking. 7% and 4% of the children were always passively exposed during primary school and secondary school time windows, respectively (Figure 1). In the secondary school time window, 13% of the children had taken up smoking themselves. The number of cigarettes smoked per household was small (median number ranged from 5 to 10 cigarettes per day from pregnancy till age 17) and SHS exposure outside

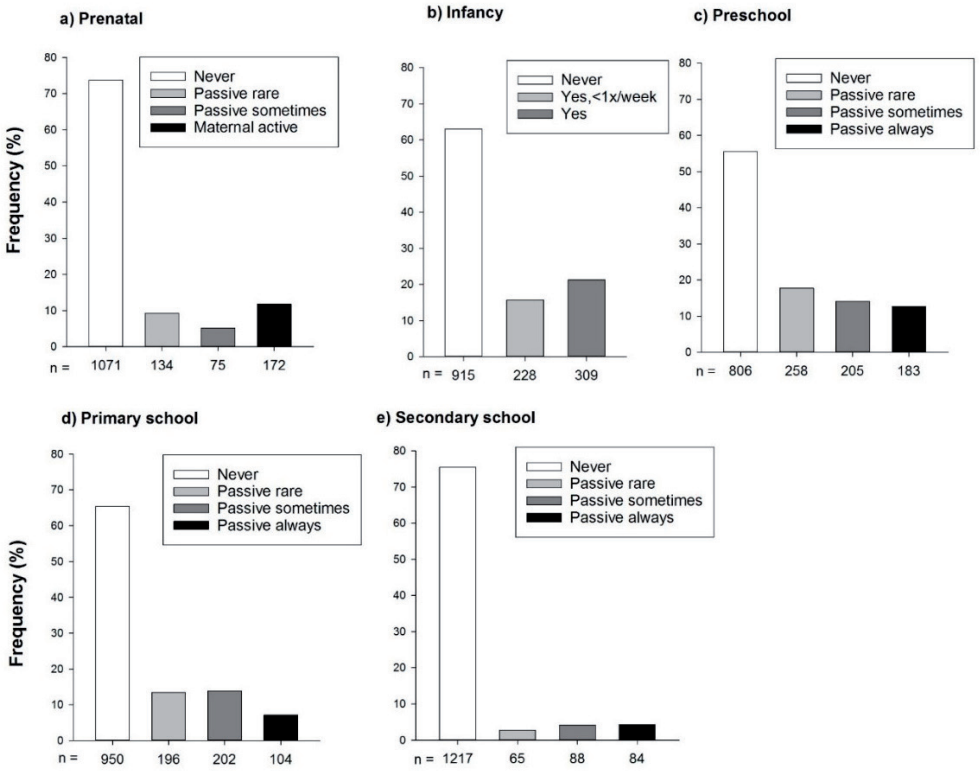


Figure 1. Frequency distribution of time-window SHS exposure.

the child's home was infrequent (6-7% of the participants were sometimes exposed and 2% always exposed outside the home at ages 1 to 4 years) and was therefore not considered in the analysis.

A total of 631 children had a sum score of 0 points, 24 children were assigned the maximum number of 26 points. Four distinct longitudinal patterns of SHS exposure patterns from pregnancy to age 17 were identified: "persistent very low" (67.4%) representing individuals with very low probability of exposure throughout the follow-up; "persistent low " (11.6%) representing children with a low probability of exposure throughout the follow-up; "early high" (8.6%) representing children with a high probability of exposure from birth until around age 8; and "persistent high" (11.5%) representing children with a high probability of exposure during almost the entire follow-up (Figure 2).

The correlation between the different exposure variables was low to moderate ranging from 0.29 for the correlation between the secondary school time window exposure and cumulative scores to 0.73 for the correlation between the preschool time window and the cumulative scores.

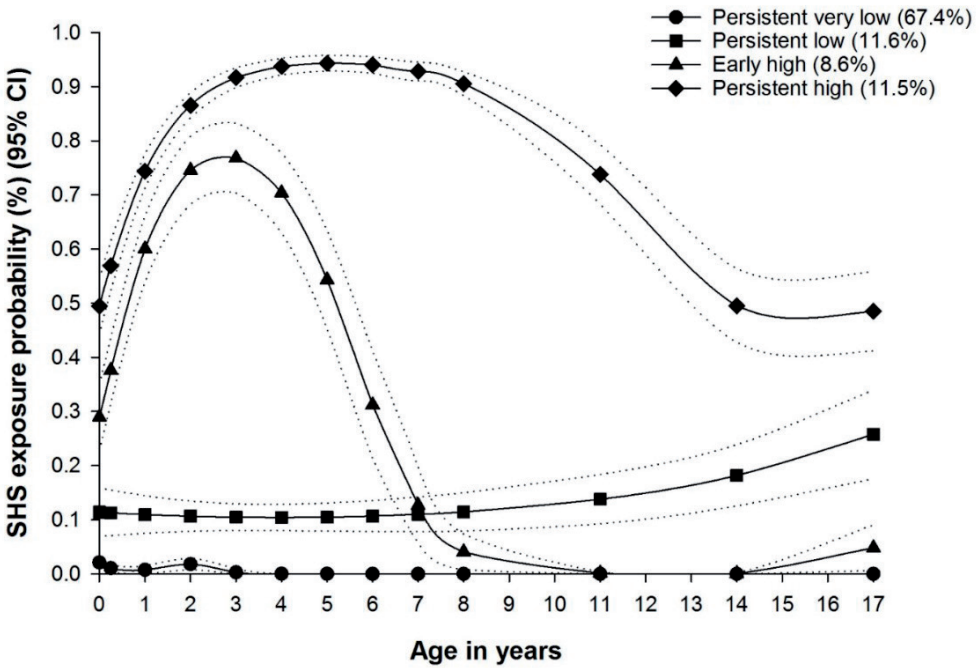
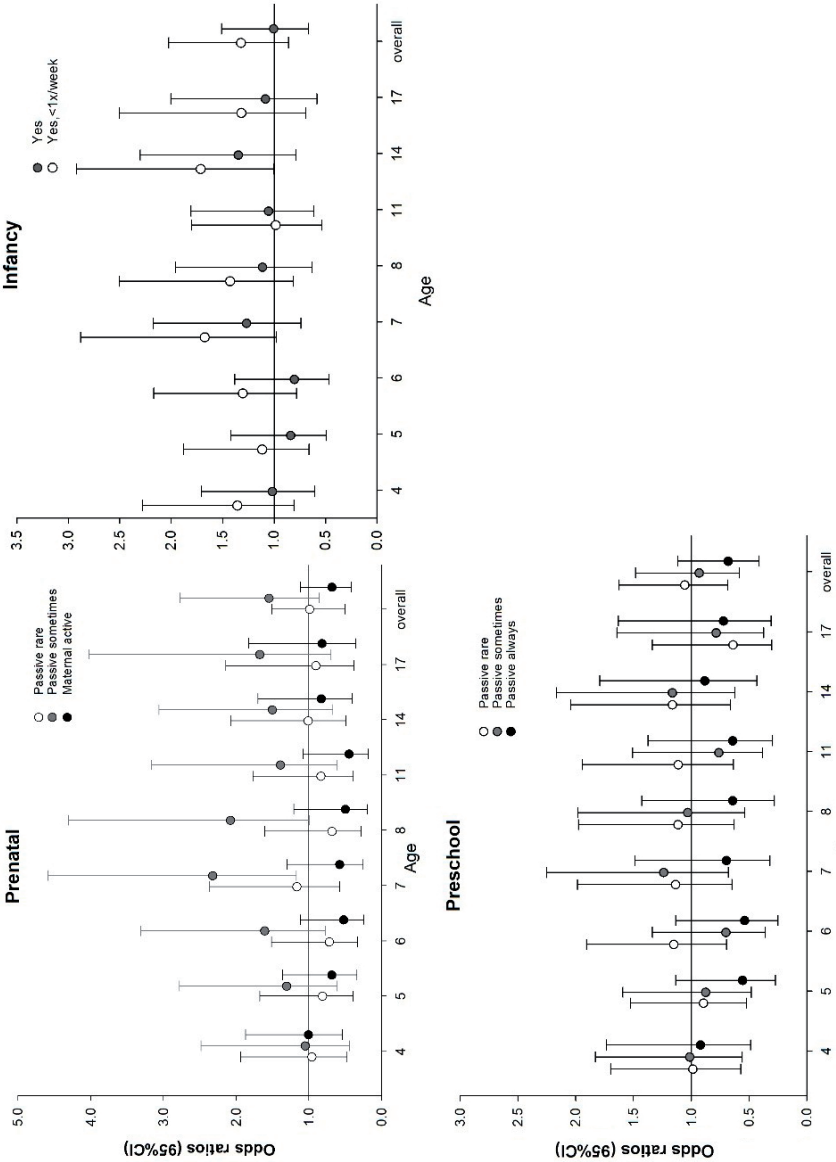


Figure 2. Longitudinal patterns for SHS exposure from birth until age 17.

Adjusted associations between the different methods of SHS exposure assessment and asthma are presented in Figures 3 and 4. Unadjusted odds ratios (Table E3) and adjusted odds ratios for the overall associations were generally similar. There were no statistically significant associations between asthma at ages 4 to 17 years and early-life time windows exposure except for an increased risk of asthma at ages 7 and 8 years in children of mothers who were sometimes exposed to maternal passive smoking during pregnancy. Similarly, we did not see associations at age 17 for the primary and secondary school exposures, cumulative exposures, and longitudinal patterns of exposure. In the same line, we did not observe any significant associations between asthma phenotypes and SHS exposure (Table E4).

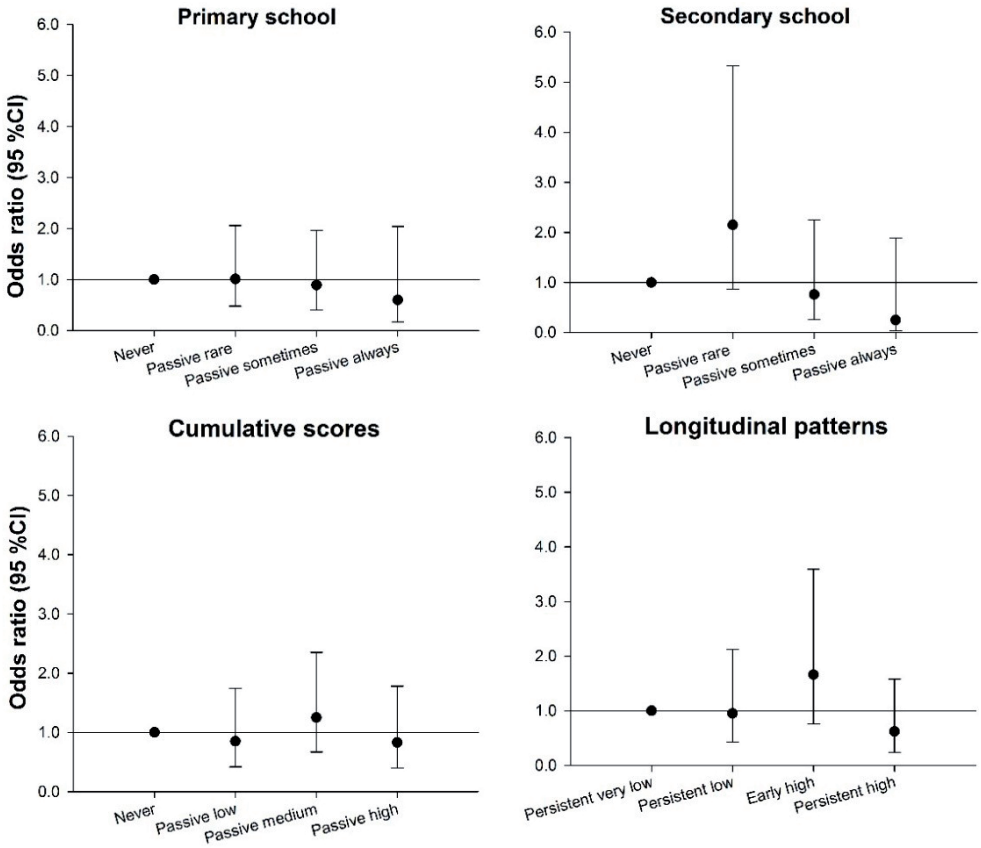
In sensitivity analyses, extending populations did not change results for the time windows as well as the associations between asthma and patterns (Figures E4-E6). Similarly, defining time-varying confounders based on data from the most recent questionnaire or additional adjustment for low birth weight did not influence results (Figure E7 and E8). We also did not see any changes from the main analysis when we excluded active smokers Figure E9)

Stratified analyses by parental atopy, presence of pets, sex, and parental education did not provide any evidence for a modification of the association between SHS and asthma by these factors. Figures E10-E13 show results of these stratified analyses for the preschool time window, results for the prenatal and infancy windows were similar.



* Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months, presence of mould at 1 year, outdoor NO_2 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age

Figure 3. Adjusted * overall and age-specific association of SHS exposure with asthma at ages 4 to 17 for prenatal, infant and preschool time-window-specific exposures.



* Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months, presence of mould at 1 year, outdoor NO2 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age.

Figure 4. Adjusted association of SHS exposure with asthma at age 17 for primary and secondary school time windows and lifetime exposures.

DISCUSSION

We characterized lifetime SHS exposure using three different methods: by time windows, by cumulative scores, and by longitudinal patterns. With none of the methods, we observed an association with asthma in childhood and adolescence in this cohort of children followed since pregnancy.

Many studies have focused on exposure during pregnancy as well as infancy[2,6,11] and attempts have been made to combine time-point specific exposures to characterize cumulative SHS exposure in longitudinal studies.[24] To our knowledge, there is limited literature on characterizing lifetime SHS exposure using data from every follow-up in longitudinal studies with longer follow-up periods. In this study, we used three different

methods using data from all 13 follow-ups. Each of these methods covers a different aspect of lifetime exposure: Time- window-specific analyses were used to determine the role of the timing of exposure; cumulative scores were used to quantify cumulative SHS exposure throughout the follow-up, and longitudinal patterns described lifetime SHS exposure patterns including changes in exposure over time. Four patterns were deduced in our population: persistent very low, persistent low, early high and persistent high exposure.

Few other studies have assessed associations between prenatal and postnatal SHS exposure with asthma during adolescence.[6,10,11] A longitudinal study from Australia[6] found a positive association between maternal active smoking during pregnancy and asthma in 14 year-olds [OR (95% CI) 1.84 (1.16 to 2.92)]. The MUSP study, another Australian cohort[10] observed positive associations of prenatal and postnatal (at 6 months) maternal heavy smoking (≥20 cigarettes/day) and asthma at age 14 in girls [OR (95% CI) 1.98 (1.25 to 3.33)], but not in boys. No associations were found with lower numbers of cigarettes smoked in that study. Stratified analysis by sex in our study did not reveal any differences in the association between SHS exposure and asthma between boys and girls. Thacher *et al*.,[11] explored associations of prenatal (maternal active) smoking and postnatal parental smoking with asthma in the first 16 years of life in the Swedish BAMSE cohort. Associations of prevalent and incident asthma until age 16 with SHS exposure in pregnancy, infancy (2 months after birth) were investigated adjusting for parental smoking throughout childhood. Asthma until age 4, but not at later ages, was associated with maternal smoking during pregnancy. We did not observe such an association between early SHS exposure and asthma at age 4 in the age-specific analyses, nor in the analyses with asthma phenotypes in our study. The lack of association between parental smoking throughout childhood and asthma at age 16 in the Swedish cohort, however, is consistent with our findings. The generally low numbers of cigarettes smoked in the present study as compared with the Australian studies may explain the difference between the present study and the two Australian studies. The two mentioned Australian studies defined maternal smoking as smoking at any stage of pregnancy, which is comparable to our definition of smoking in at least the first 4 weeks of pregnancy and therefore, differences in exposure definitions likely do not explain the lack of association in our study. In contrast to our study, which assessed lifelong SHS exposure from birth until age 17, most of these prospective studies focused on exposure at one or few specific ages. Cross-sectional studies investigating the relationship between SHS exposure and adolescent asthma have also been conducted. Findings of these studies are as conflicting. A cross-sectional study from Hong Kong observed associations between current SHS exposure and asthma symptoms in 15 year-old adolescents [OR (95% CI) 1.45 (1.17 to 1.81)].[25] In contrast, two other cross-sectional studies[26,27] did not find significant associations between current SHS exposure and current asthma in children aged 11-15 years. However, cross-sectional studies have major limitations; recall bias may occur when assessing past exposures and a temporal relationship with asthma cannot be established. Apart from the

Swedish study, none of these studies investigated lifetime SHS exposure and its relationship with asthma in adolescence. Our results are therefore not directly comparable to the results from the above-mentioned studies.

We consider the use of different methods to define lifetime SHS exposure that cover different aspects of exposure i.e. lifetime exposure or the importance of the timing of exposure as the major strength of our study. Longitudinal trajectories are also useful descriptive tools in characterizing the population and identifying detailed patterns. SHS exposure data has not been extensively studied in this way to deduce distinct patterns. Other researchers can use this method with other exposures to detect patterns and explore their relationship with an outcome of interest. The prospective nature of the study also allowed small liability of recall bias from parental reports of SHS exposure.

Our findings should be interpreted considering the following limitations: We acknowledge that the cumulative scores were assigned arbitrarily, therefore, we cannot rule out that the use of the scores may have resulted in exposure misclassification. However, the choice of the method was based on the well-documented dose-response relationship between SHS exposure and health outcomes including asthma and scores were categorized using tertiles as cut-offs. Therefore, we believe that bias is no major concern. The number of cigarettes smoked was small in our population and decreased over time. As such we cannot rule out adverse effects of heavy smoking on asthma up to adolescence. The emphasis by health care providers on the health risks associated with SHS exposure may explain the observed decrease in SHS exposure prevalence over time however this is not a unique phenomenon to our study as prevalence of smoking has generally decreased in the Netherlands. In addition, in our study population, there were more atopic parents in the non-exposed group than in the exposed group. The reason for this may be that atopic parents may already smoke less than non-atopic parents in pregnancy and that children of atopic parents have a genetically increased risk of asthma and that they may be less likely to be exposed as parents of asthmatic children have been found to be more inclined to smoke less because of their child's asthma.[28] We, therefore, investigated the potential modification of the SHS effect by parental atopy but did not find any differences in the association between children born to atopic and non-atopic parents. Absence of exposure due to parental atopy is, therefore, unlikely the explanation of the lack of association between SHS exposure and asthma in our study. Another potential explanation for the lack of association could be the use of parental self-reported data, which could lead to differential exposure misclassification as parents of asthmatic children may tend to underreport their household smoking because of knowledge on the harmful consequences of SHS exposure. A validation study comparing SHS exposure self-reports with measured air nicotine levels in a subset of the PIAMA population, however, suggests self-reported information about SHS exposure generally provides valid estimates of residential exposure. [29] Therefore, the reasons for the lack of association between lifetime SHS exposure and asthma in our study remain unclear.

However, more longitudinal studies could investigate effects of lifetime secondhand smoke exposure on asthma in later life.

CONCLUSION

We investigated associations of the timing of secondhand smoke exposure as well as secondhand smoke exposure patterns from birth till age 17 with asthma till age 17. Asthma was not associated with any of the exposure metrics in this study.

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SUPPLEMENTARY MATERIAL

Table E1. Baseline characteristics comparisons: exposed vs non-exposed participants **(N=1454)***′

	Exposed (N=823)		**Non-Exposed (N=631)**		
Characteristic	**N**	**%**	**N**	**%**	**P-value**
Parental atopy (Yes)	383/823	46.5	366/631	58.0	<0.000
Sex (Boys)	407/823	49.4	314/631	49.7	0.907
Presence of pets at 3 months (Yes)	439/823	53.4	235/631	37.2	<0.000
Presence of mould at 1 year(Yes)	217/823	26.3	169/631	26.7	0.858
Breastfeeding >12 weeks (Yes)	385/819	47.0	387/631	61.4	<0.000
Overweight at 3 years					
Yes	56/823	6.8	39/582	6.1	0.239
No	622/823	75.5	500/582	79.2	
Unknown	145/823	17.6	92/582	14.5	
Gas cooking at 3 months (Yes)	670/821	81.6	534/627	85.1	0.073
Older siblings (Yes)	395/823	48.0	332/631	52.6	0.080
Education	823		630		<0.000
Low	99	12.1	26	4.1	
Intermediate	296	35.9	168	26.6	
High	428	52.0	436	69.2	
Region	823		631		0.08
North	256	31.1	198	31.3	
Middle	342	41.5	291	46.1	
Western	225	27.3	142	22.5	
Ethnicity (Dutch)	745/814	91.5	577/621	92.9	0.332
Active smokers (14/17 years)	144/823	17.5	49/631	7.7	<0.000
	N	**Mean (Range)**	**N**	**Mean (Range)**	
Maternal age (years)	812	31.0 (18-42)	626	31.2 (21-42)	0.320
NO_2 at home address at birth (μg/m^3)	819	22.8 (9.2-59.6)	629	22.7 (9.5-45.1)	0.604

* Groups were compared using Chi-square tests for categorical variables and t-tests for continuous variables. Exposed= any positive response of SHS any exposure during any follow up, Non-exposed= no positive response of exposure on all follow ups.

Table E2. Baseline characteristics comparisons: Baseline population compared with the study population.*

	Baseline population (N=3963)		**Study population (N=1454)**		
Characteristic	**N**	**%**	**N**	**%**	**P-value**
Parental atopy (Yes)	2038/3963	51.4	749/1454	51.5	0.954
Sex (Boys)	2054/3963	51.8	721/1454	49.5	0.143
Presence of pets at 3 months (Yes)	2024/3941	51.3	674/1454	46.3	0.001
Presence of mould at 1 years (Yes)	1047/3963	26.4	386/1454	26.5	0.924
Breastfeeding >12 weeks (Yes)	1703/3963	43.7	772/1449	53.2	<0.000
Overweight at 3 years					<0.000
Yes	206/3963	5.1	95/1454	6.5	
No	2765/3963	69.8	1122/1454	77.1	
Unknown	992/3963	25.1	237/1454	16.3	
Gas cooking (Yes)	3247/3963	82.7	1204/1448	83.1	0.742
Older siblings (Yes)	1986/3963	50.1	727/1454	50.0	0.908
Education					<0.000
Low	484/3812	12.7	125/1453	8.6	
Intermediate	1420/3812	37.2	464/1453	31.9	
High	1908/3812	50.1	864/1453	59.5	
Region					0.015
North	1231/3963	31.1	454/1454	31.2	
Middle	1586/3963	40.0	633/1454	43.5	
Western	1146/3963	28.9	367/1454	25.2	
Ethnicity (Dutch)	3327/3684	90.3	1322/1435	92.1	0.043
Active smokers(14/17 years)	216/1639	13.1	193/1454	13.2	<0.000
	N, Mean (Range)		**N, Mean (Range)**		
Maternal age at birth (years)	3871, 30.3 (17-42)		1438, 31.1 (18-42)		0.03
NO_2 at home address at birth ($\mu g/m^3$)	3937, 23.2 (8.7-59.6)		1448, 22.8 (9.2-59.6)		

* Groups were compared using Chi-square tests for categorical variables and t-tests for continuous variables.

Table E3. Unadjusted (N=1454) odds ratios (OR) with 95% confidence intervals (CI) for the association between tobacco smoke exposure and asthma until age 17.*

Prenatal	**Unadjusted OR (95% CI)**
Never	Ref
Passive rare	0.97 (0.63 to 1.47)
Passive sometimes	0.91 (0.58 to 1.42)
Maternal active	0.73 (0.45 to 1.17)
Infancy	
Never	Ref
Yes, <1x/week	1.26 (0.81 to 1.94)
Yes	1.07 (0.73 to 1.56)
Preschool	
Never	Ref
Passive rare	0.88 (0.51 to 1.52)
Passive sometimes	1.55 (0.83 to 2.85)
Passive always	0.70 (0.43 to 1.15)
Primary school	
Never	Ref
Passive rare	0.94 (0.47 to 1.90)
Passive sometimes	0.72 (0.34 to 1.55)
Passive always	0.52 (0.16 to 1.70)
Secondary school	
Never	Ref
Passive rare	2.12 (0.88 to 5.13)
Passive sometimes	0.99 (0.35 to 2.80)
Passive always	0.22 (0.03 to 1.64)
Cumulative scores	
Never	Ref
Passive low	0.78 (0.40 to 1.57)
Passive medium	1.17 (0.65 to 2.12)
Passive high	0.71 (0.34 to 1.47)
Longitudinal patterns	
Persistent very low	Ref
Persistent low	0.74(0.28 to 1.96)
Early high	1.63 (0.77 to 3.42)
Persistent high	0.60 (0.25 to 1.44)

* Odds ratio estimates reported for prenatal, infancy and preschool times windows are longitudinal overall point estimates. Primary school, secondary school, cumulative scores and longitudinal patterns estimates are for asthma at age 17.

Table E4. Adjusted (N=1454) odds ratios (OR) with 95% confidence intervals (CI) for the association between SHS exposure and asthma phenotypes.[γ]

	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
Prenatal*	**Passive smoking**	**Maternal active**	
Persistent	0.95 (0.45 to 1.99)	0.40 (0.13 to 1.15)	
Adolescent onset	0.63 (0.18 to 2.25)	1.01 (0.33 to 3.13)	
Intermediate	1.39 (0.61 to 3.15)	0.83 (0.28 to 2.44)	
Early transient	1.28 (0.72 to 2.25)	1.75 (0.99 to 3.08)	
Infancy	**Yes, <1x/week**	**Yes**	
Persistent	1.70 (0.88 to 3.27)	1.22 (0.64 to 2.32)	
Adolescent onset	0.61 (0.17 to 2.12)	0.98 (0.38 to 2.47)	
Intermediate	1.35 (0.61 to 2.98)	1.39 (0.64 to 3.03)	
Early transient	0.87 (0.48 to 1.58)	0.98 (0.59 to1.64)	
Preschool	**Passive rare**	**Passive sometimes**	**Passive always**
Persistent	1.02 (0.52 to 1.99)	0.81 (0.36 to 1.82)	0.60 (0.24 to 1.51)
Adolescent onset	0.36 (0.08 to1.61)	1.20 (0.42 to 3.42)	1.27(0.43 to 3.72)
Intermediate	1.93 (0.93 to 4.00)	1.29 (0.50 to 3.30)	2.18 (0.87 to 5.46)
Early transient	0.65 (0.34 to 1.22)	1.29 (0.74 to 2.24)	1.16 (0.63 to 2.13)

γ: Odds ratios are interpreted in reference to the 'Never' exposed group and to 'Never' asthma phenotype.
*: Passive smoking categories combined in the prenatal time window due to low frequency cells. Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months , presence of mould at 1 year, outdoor NO_2 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age.

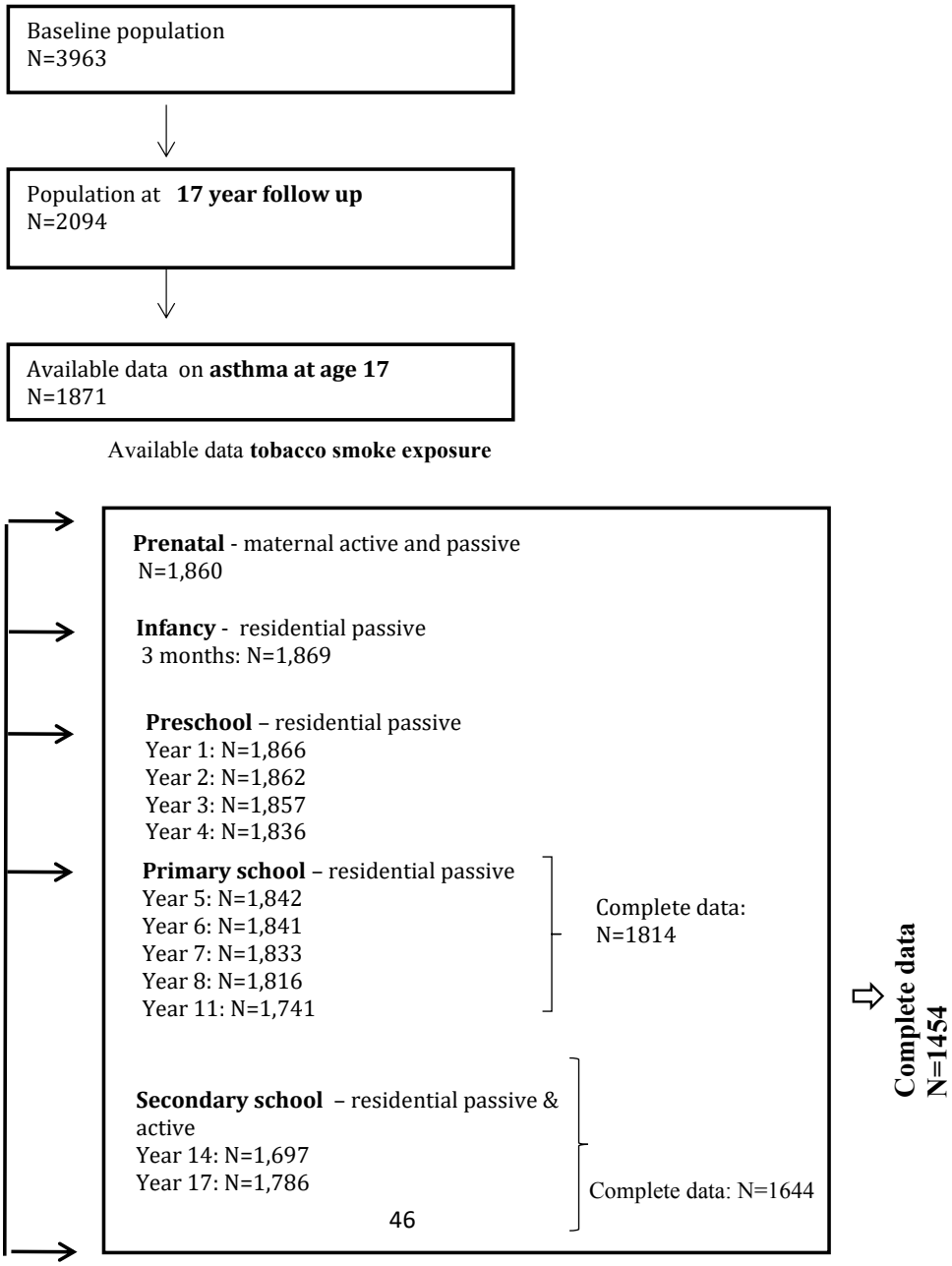


Figure E1. Breakdown of available SHS exposure information until age 17 for children with data on asthma until age 17.

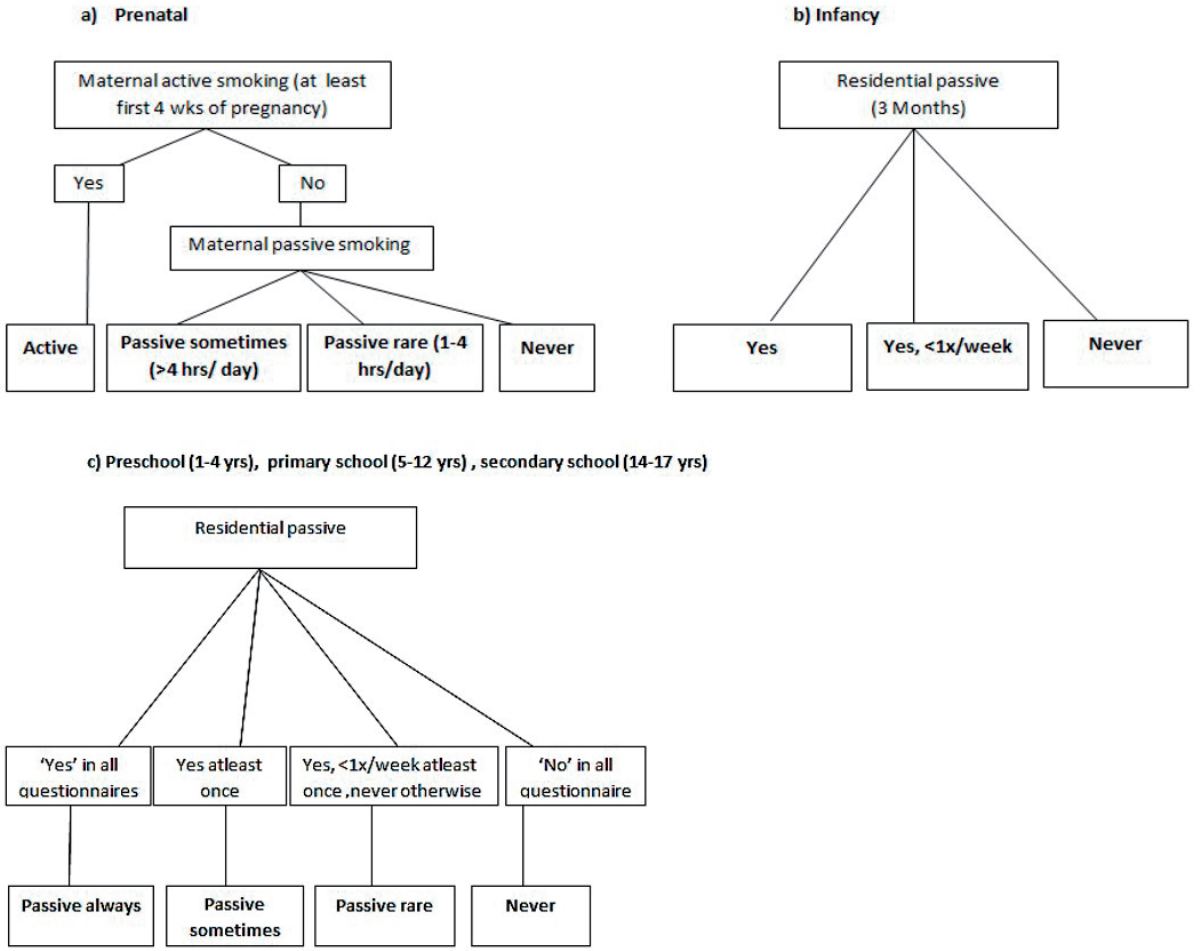


Figure E2. Flowcharts describing the definition of SHS exposure categories for the different time windows.

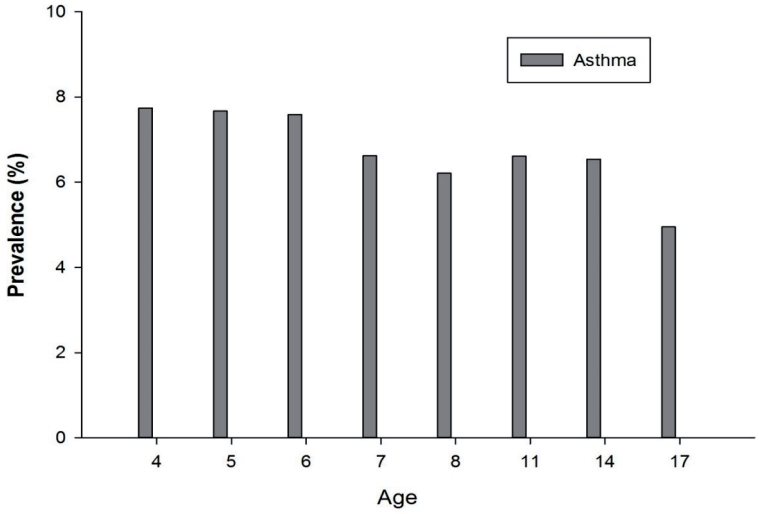
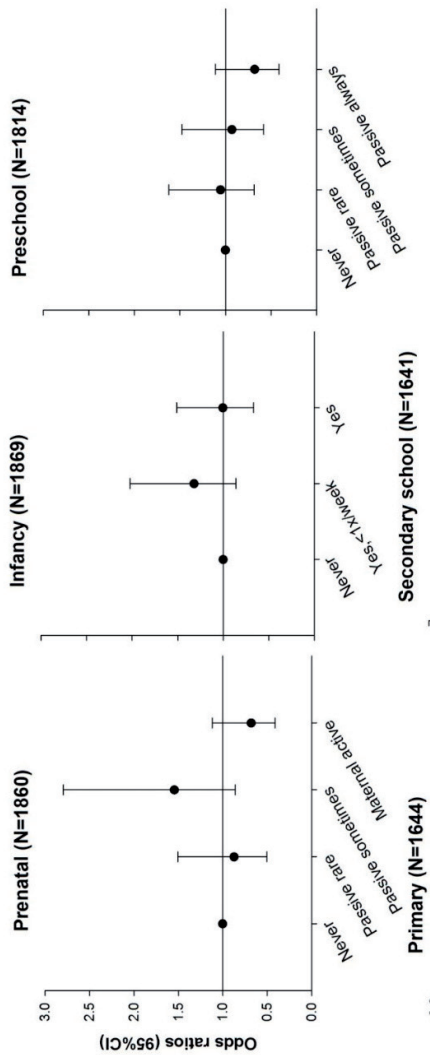


Figure E3. Prevalence of asthma from age 4 to age 17.



¥ Associations are overall associations with asthma at ages 4 to 17 years from GEEs for prenatal, infancy and preschool exposure, associations with asthma at age 17 from cross-sectional analyses for primary and secondary school windows. Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months, presence of mould at 1 year, outdoor N02 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age.

Figure E4. Adjusted association of SHS exposure with asthma until age 17 for extended populations per time window.¥

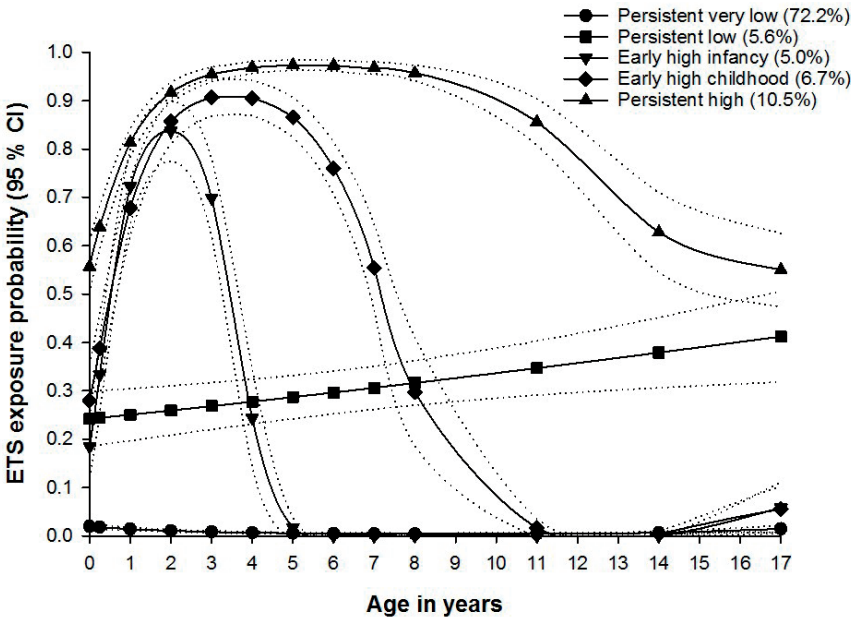
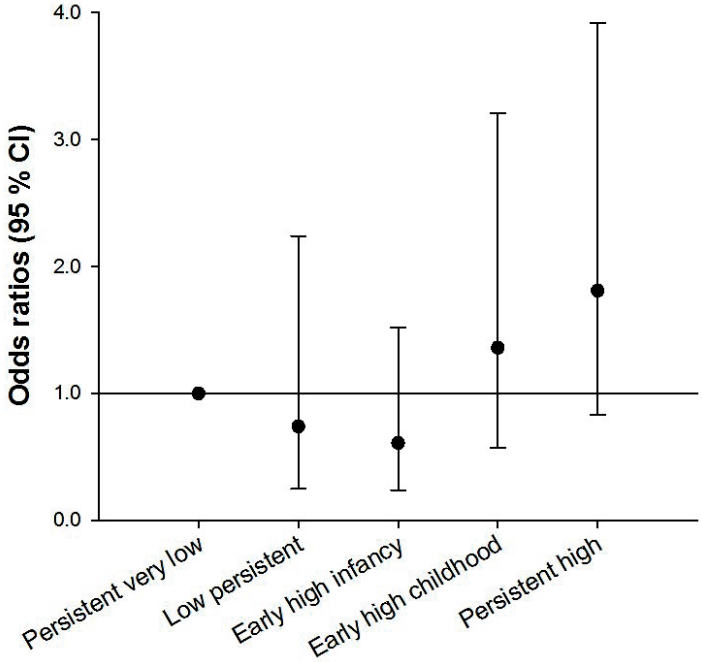
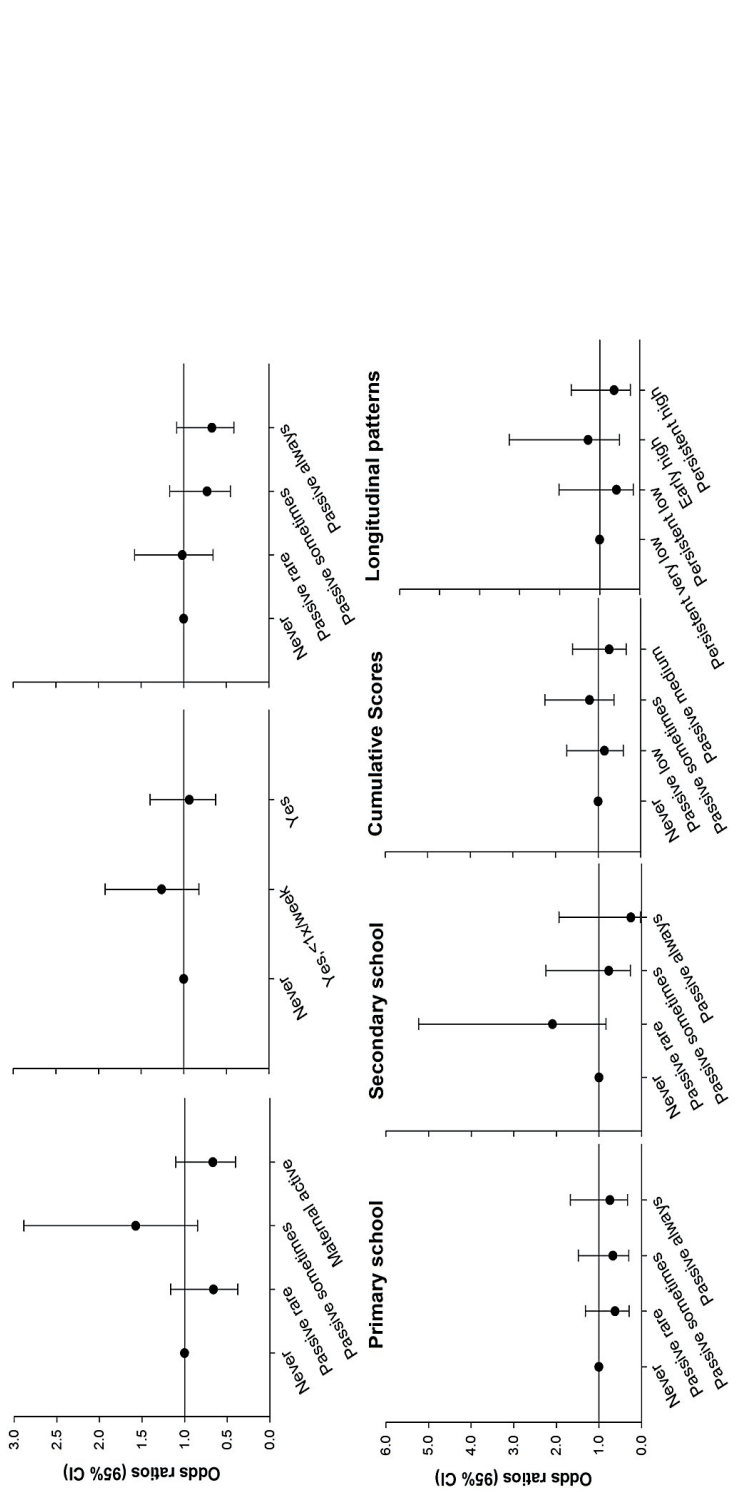


Figure E5. Longitudinal patterns for extended population of children with asthma data but incomplete SHS exposure data (N=1871).



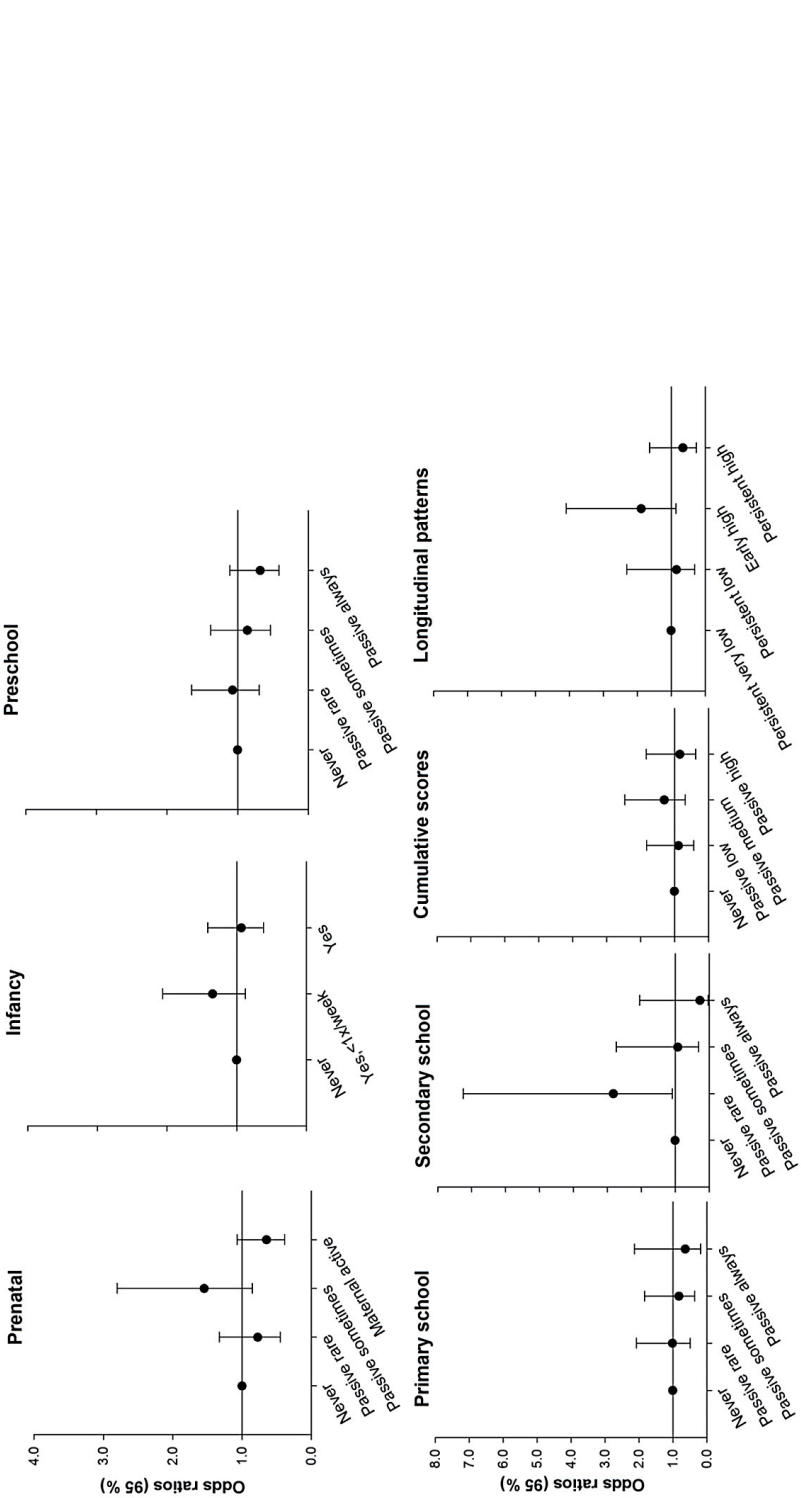
‡ Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months , presence of mould at 1 year , outdoor NO_2 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age.

Figure E6. Adjusted odds rations for association of SHS exposure and with asthma at age 17 with longitudinal patterns for extended population of children with asthma data at age 17 but incomplete SHS exposure data (N=1871). ‡



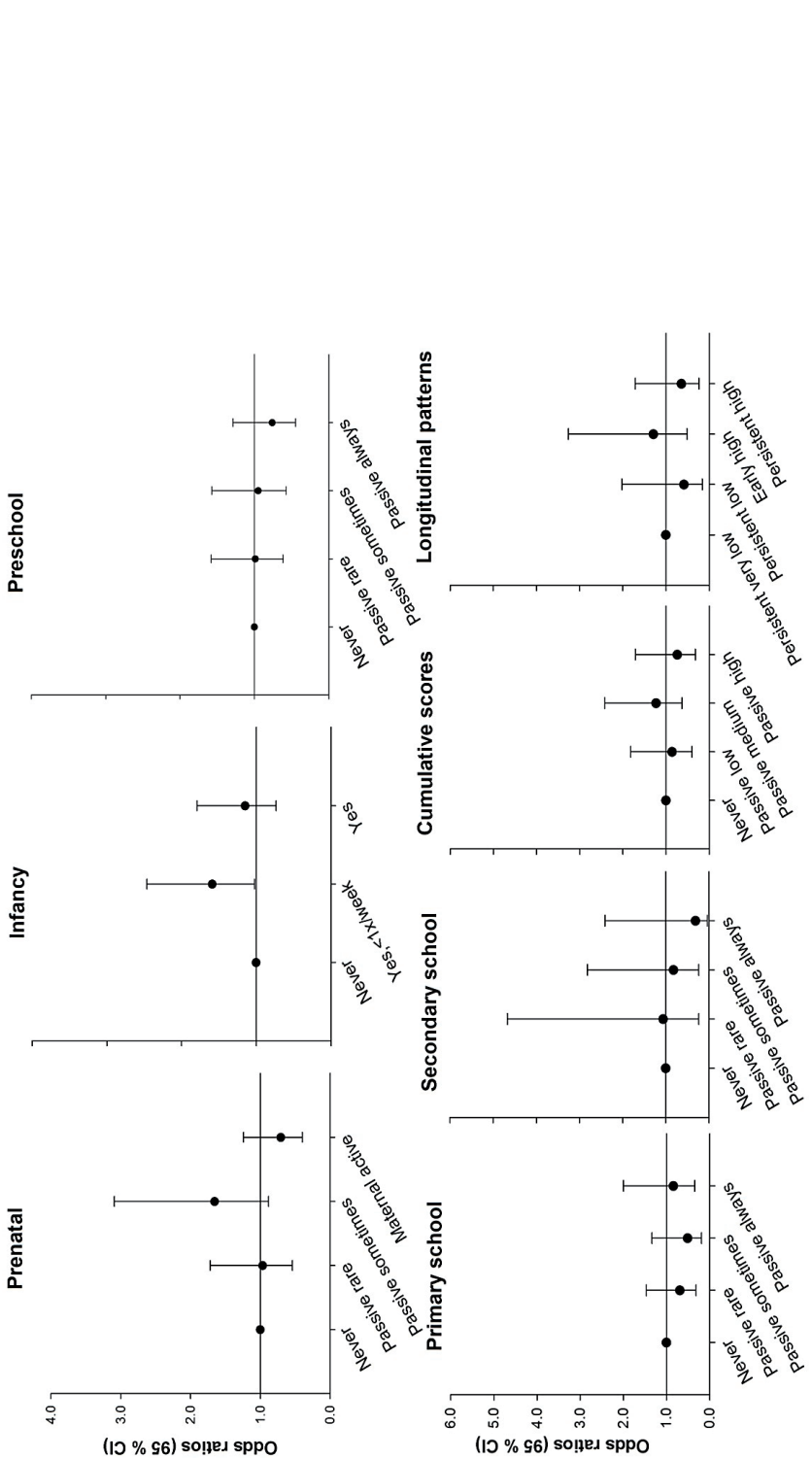
† Associations are overall associations with asthma at ages 4 to 17 years from GEEs for prenatal, infancy and preschool exposure, associations with asthma at age 17 from cross-sectional analyses for primary and secondary school windows. Adjusted for gas cooking, overweight, presence of pets and presence of mould at 17 years, outdoor NO_2 exposure at home address at 14 years, sex, breastfeeding, active smoking, older siblings at birth, parental atopy, parental education, region and maternal age at birth.

Figure E7. Adjusted odds ratios for association of SHS exposure and asthma until 17, adjusted for time varying confounders defined at age 17 years instead of early-life.[†]



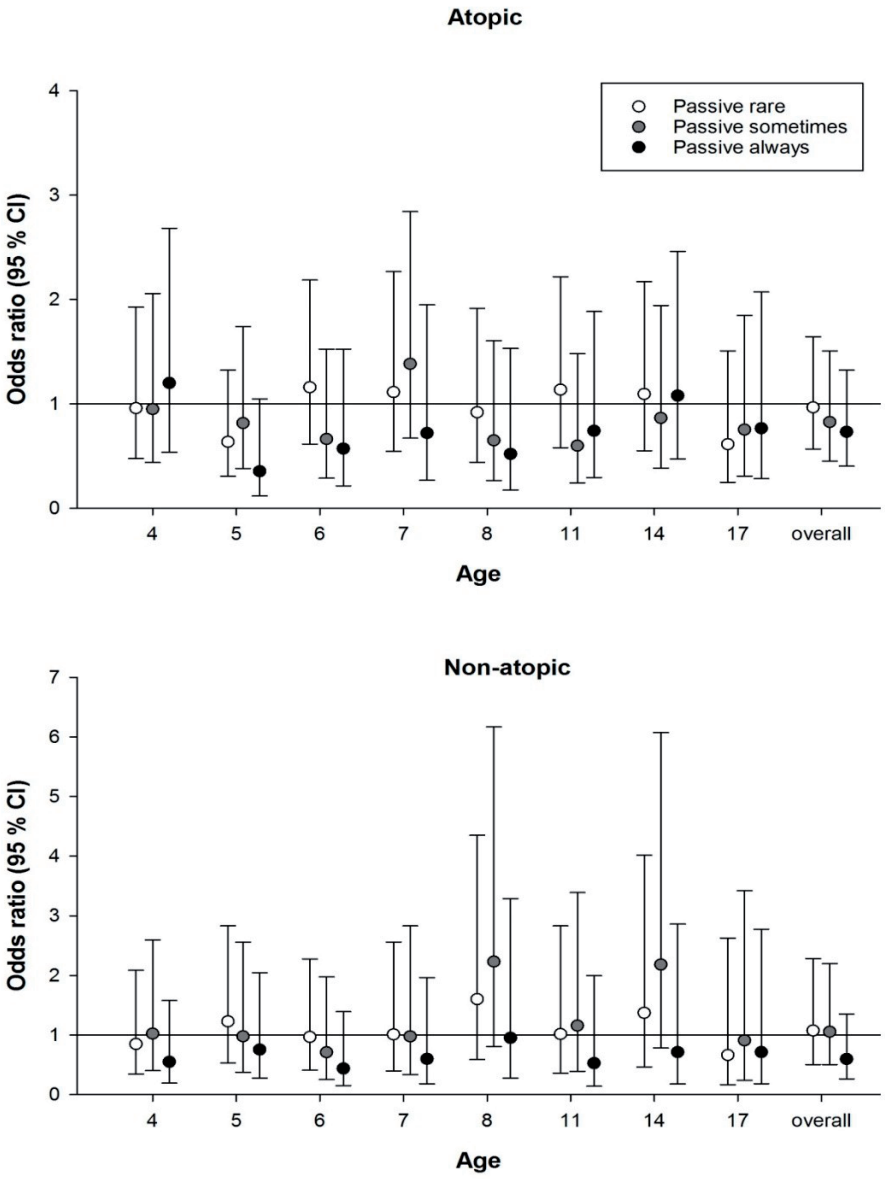
* Associations are overall associations with asthma at ages 4 to 17 years from GEEs for prenatal, infancy and preschool exposure, associations with asthma at age 17 from cross-sectional analyses for primary and secondary school windows. Adjusted for gas cooking, overweight, presence of pets and presence of mould at 17 years , outdoor NO_2 exposure at home address at 14 years, sex, breastfeeding, active smoking, older siblings at birth, low birthweight, parental atopy, parental education, region and maternal age at birth.

Figure E8. Adjusted odds ratios for association of SHS exposure and asthma until 17, for time windows, additionally adjusted for low birth weight.*



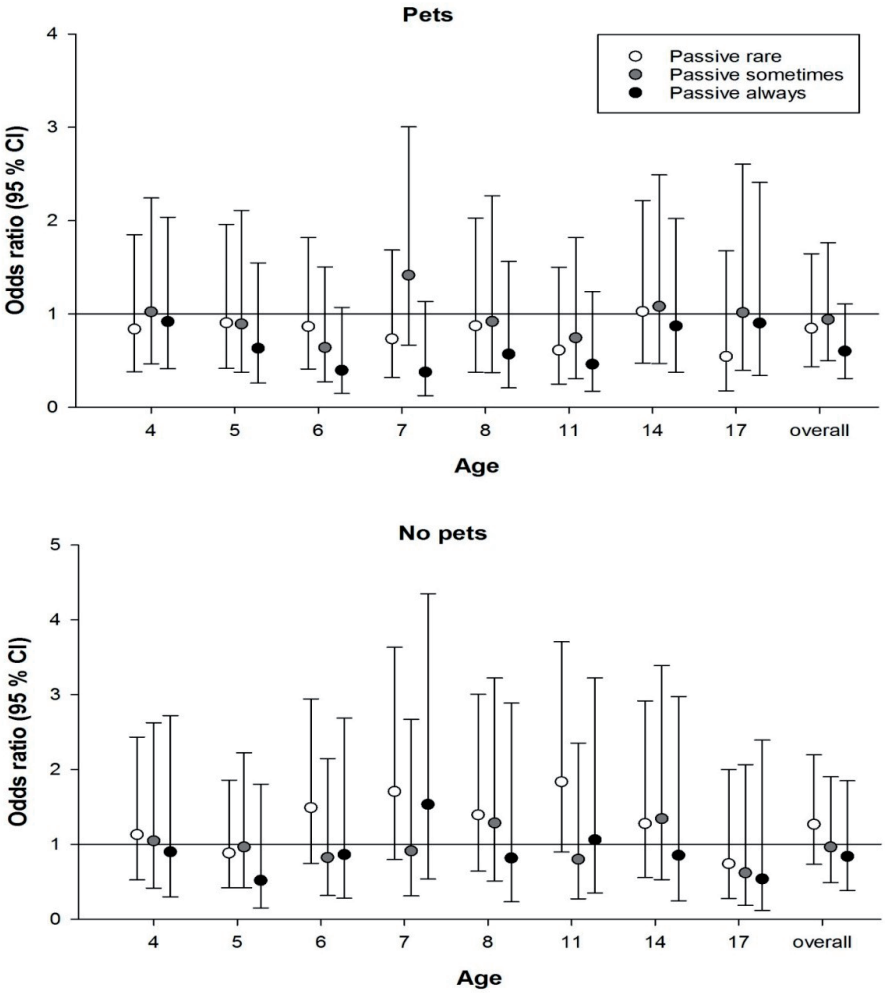
+ Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months , presence of mould at 1 year, outdoor NO_2 exposure at home address at birth, sex, active smoking, breastfeeding, older siblings at birth, parental atopy, parental education, region and maternal age. Overall longitudinal estimates reported for prenatal, infancy and preschool time windows.

Figure E9. Adjusted odds ratios for association of SHS exposure and asthma until age 17 excluding active smokers.[+]



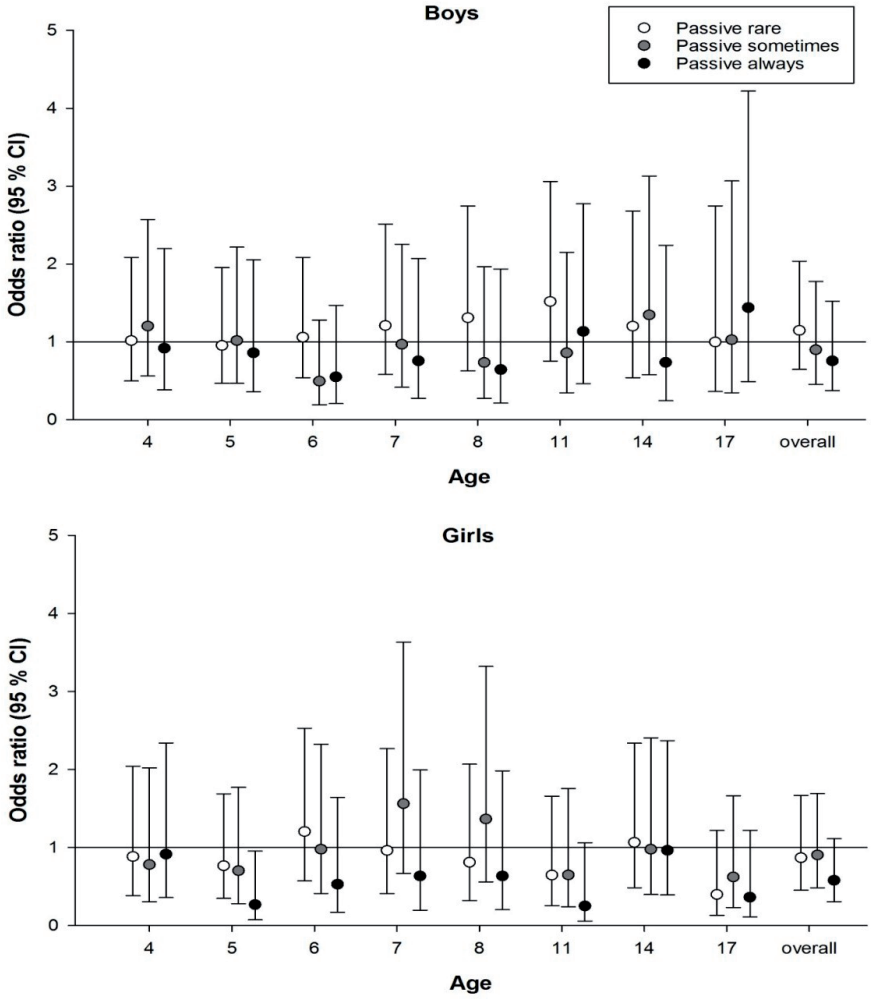
α Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months , presence of mould at 1 year , outdoor NO_2 exposure at home address at birth, sex, breastfeeding, active smoking, older siblings at birth, parental education, region and maternal age. Reference group= Never exposed.

Figure E10. Adjusted odds ratios for association of SHS exposure and asthma until age 17 stratified by atopy preschool time window-specific exposure. [α]



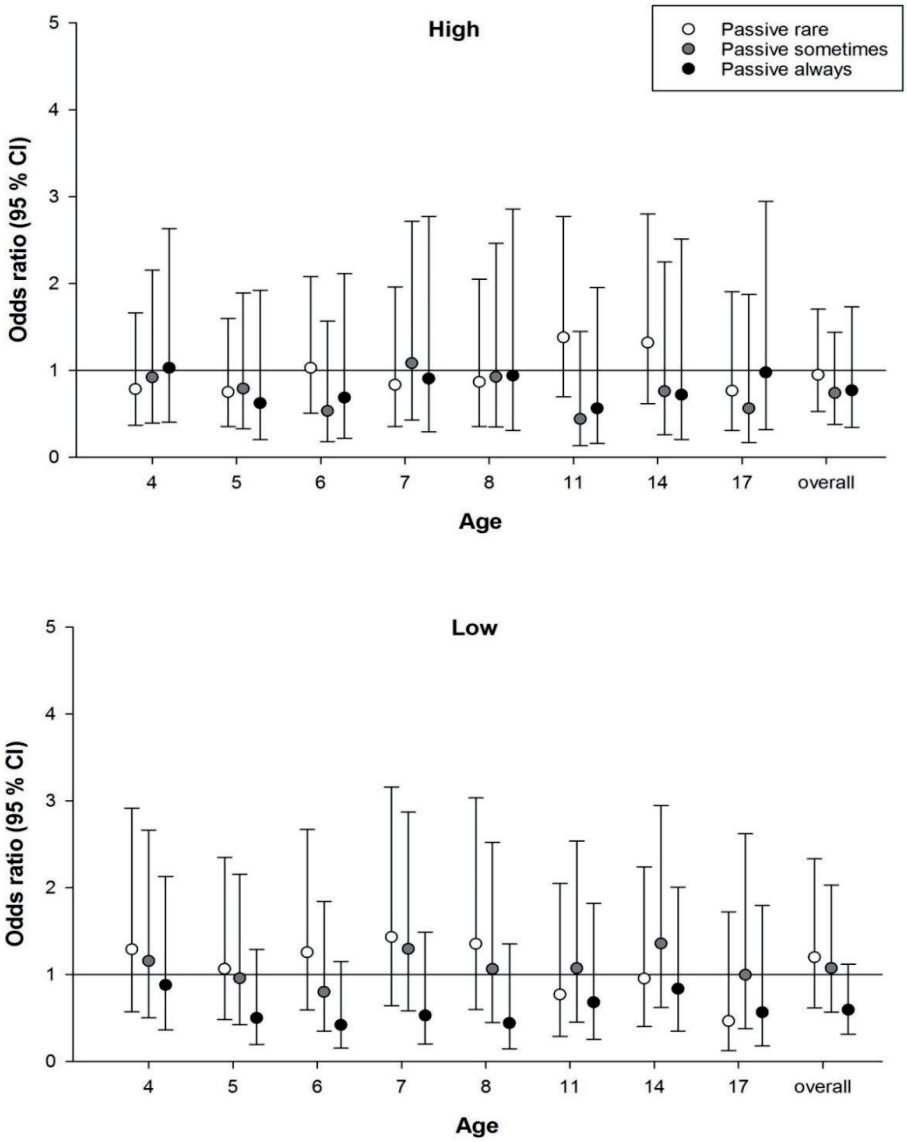
Γ Adjusted for gas cooking at 3 months, overweight at 3 years, presence of mould at 1 year, outdoor NO_2 exposure at home address at birth, sex, breastfeeding, active smoking, older siblings at birth, parental atopy, parental education, region and maternal age. Reference group= Never exposed.

Figure E11. Adjusted odds ratios for association of SHS exposure and asthma until age 17 stratified by presence of pets for preschool time window-specific exposure. Γ



θ Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months, presence of mould at 1 year , outdoor NO_2 exposure at home address at birth, breastfeeding, active smoking, older siblings at birth, parental atopy, region and maternal age. Reference group= Never exposed.

Figure E12. Adjusted odds ratios for association of SHS exposure and asthma until age 17 stratified by sex for preschool time window-specific exposure. [θ]



β Adjusted for gas cooking at 3 months, overweight at 3 years, presence of pets at 3 months, presence of mould at 1 year outdoor NO_2 exposure at home address at birth, sex breastfeeding, active smoking, older siblings at birth, parental atopy, region and maternal age. Reference group= Never exposed.

Figure E13. Adjusted odds ratios for association of SHS exposure and asthma until age 17 stratified by parental education for preschool time window-specific exposure.[β]

CHAPTER 3

3

Role of timing of exposure to pets and dampness or mould on asthma and sensitization in adolescence

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Clinical and Experimental Allergy (2019): Oct; 49(10):1352-1361

ABSTRACT

Background: Pet and dampness or mould exposure are considered risk factors for asthma and sensitization. It is unclear whether timing of exposure to these factors is differentially associated with asthma risk and sensitization in adolescence.

Objective: We investigated the role of timing of pet and dampness or mould exposure in asthma and sensitization in adolescence. Understanding this role is essential to build targeted prevention strategies.

Methods: We used data from 1871 participants of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort. Residential exposure to pets, dampness or mould was assessed by repeated parental questionnaires. We used asthma data from the 17-year questionnaire and sensitization data from the 16-year medical examination. We characterized timing using longitudinal exposure patterns from pregnancy until age 17 using longitudinal latent class growth modelling. We used logistic regression models to analyse associations of exposure patterns with asthma at age 17 and sensitization at age 16.

Results: For none of the time windows, exposure to pets and dampness or mould was associated with asthma at age 17, but a lower sensitization risk at age 16 was suggested, e.g. the odds ratio (95% confidence interval) for sensitization was 0.63 (0.35 to 1.11) and 0.69 (0.44 to 1.08) for early-life and persistently high pet exposure, respectively, compared with very low exposure. An inverse association was also suggested for sensitization and moderate early childhood dampness or mould exposure [0.71 (0.42 to 1.19)].

Conclusion and clinical relevance: Different timing of pet and dampness or mould exposure was not associated with asthma, but lower risk of sensitization in adolescence was suggested, which could be partly attributable to reversed causation. Current findings are not sufficient to recommend pet avoidance to prevent allergic disease. More prospective studies are needed to obtain insights that can be used in clinical practice.

BACKGROUND

The impact of exposure to pets on asthma and sensitization in children has been shown previously.[1,2] Associations of dampness or mould with asthma have been demonstrated[3-5] but not with sensitization. [6] It has been suggested that environmental exposures during important windows of immune development play a role in the risk of subsequent allergic disease development. [7,8]

Systematic reviews of the association between pet exposure and asthma present inconsistent evidence. While some studies suggest that pet exposure is associated with a higher risk of asthma,[2,9-11] others suggest a lower asthma risk in exposed individuals. [7,12,13] A pooled analysis of 11 European cohorts found no association between keeping furry pets early in life and asthma in children aged 6-10 years.[14] Another study investigated associations of pet exposure during different periods of childhood with asthma and also found no association of early, past and current pet exposure with asthma in schoolchildren.[15] Sensitization to inhalant allergens is considered an important risk factor for the development of asthma [16] and exposure to pets in early-life has been consistently associated with lower risk of sensitization during childhood,[1,14,17] but it is unknown whether this inverse relationship persists into adolescence and whether exposure during other periods is relevant.

Reviews of the epidemiological evidence for respiratory and allergic health effects of dampness or mould exposure have consistently suggested higher risks of asthma in exposed children.[3,4,18] However, limited evidence exists on the associations of dampness or mould exposure with sensitization though a higher risk of sensitization has been observed in exposed children.[19] Few studies have assessed associations of dampness or mould exposure with asthma or sensitization beyond childhood into adolescence. A study that addressed this gap [20] reported a higher risk of asthma up to 16 years in relation to exposure to dampness or mould during infancy, but no association with sensitization was observed.

Existing literature on associations of pet or dampness or mould exposure with asthma and sensitization has either focused on exposure in early-life and/or asthma and sensitization in early-life and childhood. Therefore, not much evidence exists on the associations of life course exposure and the relevance of timing of exposure during different periods until adolescence. Investigating the timing of exposure is essential as exposure during different time periods in the life course may differentially affect development of asthma and sensitization and may thus have consequences for the timing and type of preventive measures. [16]

It is possible that the effect of pet or dampness or mould exposure may differ in different phases of the development of the immune system. The perinatal time window is crucial as the infant's immune system is vulnerable, and the development of the immune response is ongoing. [21] And in childhood, there is a shift from Th2 cells dominated immune response to Th1 dominated responses.[22] As such, as age advances, the immune system also undergoes

profound remodelling and decline, which may have impact on life course health outcomes.[23] We hypothesize that exposure to pets and dampness or mould during different stages of childhood would differentially affect asthma and sensitization prevalence in adolescence. We, therefore, used longitudinal patterns of exposure from pregnancy to adolescence, to investigate the relevance of timing of pet and dampness or mould exposure for the prevalence of asthma at age 17 and sensitization at age 16.

METHODS

Study design and population

We used data from the Dutch PIAMA birth cohort that has been described in detail elsewhere.[24] The cohort recruited pregnant women between 1996-97 in the Northern, Central and Western regions of the Netherlands. Information on lifestyle, health, and environmental exposure characteristics were collected using parental questionnaires that were administered during pregnancy, at 3 months, annually until age 8, and then at ages 11, 14, 16 (for the subgroup that participated in the medical examination) and 17. The study population consists of all participants with data on sensitization at age 16 and/or asthma at age 17, and data on exposure to pets or dampness or mould from at least one follow-up (N=1871). The PIAMA study was approved by the institutional review boards of participating institutes and written informed consent was obtained from parents or legal guardians of all participants.

Exposure assessment

Exposure was assessed from pregnancy (pets) and 3 months (dampness or mould) until age 17.

Pet exposure

The question '*Do you keep a dog/cat/rodent indoors?*' (*yes, no)* was used to assess exposure to furry pets. The question was asked separately for each pet.

Dampness or mould exposure

The question *'Have you seen any moisture stains or mould on the ceiling or walls in the last 12 months?'* (*yes, no)* was used to assess dampness or mould exposure. Assessment was restricted to presence of dampness or mould in the living room and the child's bedroom because this is where participants are expected to spend most of their time.

Longitudinal patterns of exposure

We characterized time-varying binary exposures into longitudinal patterns using latent class growth modelling procedure (LCGM, TRAJ in SAS 9.4, Cary, USA) as in previous analyses.[25]

We used this approach unlike using distinct time windows (e.g. prenatal, preschool, primary, secondary school time windows) because it allocates individuals based on probability of exposure rather than a subjective definitive assignment of individuals into classes and it can handle missing data while using all available data.[26,27] In addition, it is a data-driven procedure that displays subpopulations of individuals with different patterns of life course exposure indicating exposure during specific phases of follow-up. All participants with available data on pet or dampness or mould exposure from at least one of the repeated questionnaire surveys were included in the latent class modelling procedure, i.e. all available data were used. Table E1 presents the frequencies of questionnaire surveys with missing values for the different exposures. We used questionnaires from 13 waves of follow-up. Only 6% and 9% of the study population had missing data of more than two waves for pet and dampness or mould exposure respectively. The different patterns obtained in the procedure were used as exposure variables in statistical analyses of exposure-health relationships.

Outcomes

Asthma at age 17 was defined by positive answers to at least two out of the following three questions as described by the MeDALL protocol [28]: doctor-diagnosed asthma ever, wheezing in the past 12 months, and prescription of asthma medication in the past 12 months.

Sensitization at age 16 was assessed in a subgroup of participants that participated in the medical examination (N=682) and defined as a specific IgE level ≥ 0.35 IU/mL for at least one of the following allergens: house dust mite (HDM, *Dermatophagoides pteronyssinus*), cat allergen, birch, and cocksfoot (*Dactylis glomerata)*. Specific IgE levels were measured with a Radioallergosorbent test-like method (Sanquin Laboratories, Amsterdam, The Netherlands). 0.35 IU/mL was chosen as the primary cut-off point because it is commonly used in epidemiological research and clinical practice.

Confounders

The following factors were considered as potential confounders: sex, parental education (maximum of maternal and paternal education, low/medium/high), maternal and paternal allergy (defined as positive if the father and/or mother ever had asthma, were allergic to house dust, house dust mite or pets, or had hay fever), breastfeeding at 12 weeks (yes/no), parental country of birth (Netherlands, yes/no), maternal smoking during pregnancy (yes/no), secondhand smoke (SHS) exposure in the child's home at 1 year (yes/no), active smoking (at 17 years, yes/no), gas cooking at 3 months (yes/no), the presence of older siblings (yes/no), respiratory infections (serious cold or flu, infection of the throat, otitis media, sinusitis, bronchitis or pneumonia) in the first 4 years of life and antibiotic use in the first 4 years of life (never, at least once*)*. In addition, we adjusted for furry pets in the home at 1 year (yes/no) in analyses with dampness or mould exposure and for dampness or mould in the home at 1 year (yes/no) in models with pet exposure.

Statistical analyses

We used logistic regression to assess crude and adjusted associations of different patterns of exposure with asthma at age 17 and sensitization at age 16. All models were adjusted for the previously mentioned potential confounders. Observations were weighted by posterior probabilities produced by the latent class modelling procedure to account for uncertainties in the allocation of longitudinal exposure patterns.[29]

A number of sensitivity analyses were performed. We investigated associations of the exposures of interest with allergic sensitization to specific inhalant allergens i.e. cat, house dust mite, birch and cocksfoot allergens to explore how different timing of exposure may be associated with sensitization to the specific inhalant allergens. We performed stratified analyses by parental allergy as predisposition to asthma and allergy may influence the risk of disease and (avoidance of) exposure to pets.[30] Consequently, to investigate if pet avoidance behaviour distorted associations of pet exposure with asthma and sensitization, we repeated pet exposure analyses after excluding parents who reported getting rid of a pet at any point during follow up due to an allergy of a family member (N=246). We also analysed associations of exposure to different pets (cats, dogs, rodents) with asthma and sensitization separately as different pets have been suggested to have different effects on asthma/sensitization.[21] To assess if weighting observations by posterior probabilities influenced our results, we repeated the main analyses without using weights, i.e. by allocating subjects to the exposure trajectory with the highest posterior probability. We also assessed associations of the exposures of interest with sensitization using a higher IgE cut-off of 0.7 IU/mL to investigate the influence of a different cut off point. Moreover, we investigated associations of pet and dampness or mould exposure with mono- and polysensitization (i.e. sensitization to only one allergen and more than one allergen) versus no sensitization using multinomial logistic regression.

RESULTS

Table 1 shows characteristics of the study population. Twenty-nine percent of the participants had an allergic mother and 31% had an allergic father. Thirteen percent had mothers who smoked during pregnancy and 22% were exposed to SHS at home; 42% were exposed to pets and 8% were exposed to dampness or mould in the first year of life. Five percent of the population was asthmatic at age 17 and 48% was sensitized to at least one of the inhalant allergens tested at age 16. Participants in the study population were more often breastfed for more than 12 weeks, less often exposed to SHS at home at 1 year and to maternal smoking during pregnancy, and more often had highly educated parents than the excluded population (Table E2).

Table 1. Study population characteristics.

	Study population (N=1871)	
Covariates	**N/n**	**(%)**
Parental allergy ¶		
Allergic mother	551/1871	29.4
Allergic father	585/1871	31.2
Boys	926/1871	49.4
Presence of pets at 1 year	792/1862	42.5
Presence of mould at 1 year	152/1827	8.3
Breastfeeding >12 weeks	1044/1861	56.1
Gas cooking at 3 months	1550/1864	83.1
Maternal smoking during pregnancy	245/1857	13.2
SHS exposure in the home at 1 year	411/1866	22.0
Respiratory infection in the first 4 years of life ¥	1469/1843	79.7
Antibiotics use in the first 4 years of life	989/1854	53.3
Parental education		
Low	177/1865	9.4
Medium	600/1865	32.2
High	1088/1865	58.4
Active smokers at 17 years	155/1871	8.3
Older siblings at birth	919/1871	49.1
Parental country of birth (Netherlands)	1763/1845	95.6
Health outcomes		
Asthma at age 17	96/1871	5.1
Allergic sensitization at age 16, IgE ≥ 0.35 IU/L		
Sensitization to at least one allergen	328/682	48.1
Sensitization to cat	97/682	14.2
Sensitization to house dust mite (*D. pteronyssinus*)	260/682	38.1
Sensitization to birch	114/682	16.7
Sensitization cocksfoot (*Dactylis glomerata)*	193/682	28.3
Mono-sensitization ‡	126/682	18.5
Poly-sensitization β	202/682	29.6
Allergic sensitization at age 16, IgE ≥ 0.70 IU/L		
Sensitization to at least one allergen	296/682	43.4
Sensitization to cat	79/682	11.6
Sensitization to house dust mite (*D. pteronyssinus*)	230/682	33.7
Sensitization to birch	96/682	14.1
Sensitization cocksfoot (*Dactylis glomerata*)	175/682	25.6

¶ ever had asthma, allergic to house dust, house dust mite or pets, or had hay fever

¥ - Respiratory and/or throat-, nose,- ear infections, such as cold, infection of the throat, infection of the middle ear, sinusitis, bronchitis or pneumonia

‡ sensitization to only one allergen, specific IgE level ^{3} 0.35 IU/mL

β sensitization to more than one allergen, specific IgE level ^{3} 0.35 IU/mL

Figure 1 shows the longitudinal patterns of pet and dampness or mould exposure from pregnancy (pets) or 3 months (dampness or mould) to 17 years. The mean posterior probabilities per pattern ranged from 0.90 - 0.97 for pet exposure and 0.75 – 0.92 for dampness or mould exposure indicating reliable classification of membership (Table E3).

Five distinct patterns of pet exposure reflecting timing of exposure were identified as follows: very low (28%) indicating very low probability of exposure throughout follow-up, early-life (11.1%) indicating high probability of exposure in early-life, mid-childhood (14%) indicating high probability of exposure in mid-childhood, late childhood (14%) indicating high probability of exposure later in childhood and persistently high exposure (31%) showing high probability of high exposure during the entire follow up. We identified three patterns of dampness or mould exposure: very low (79%) characterized by a very low probability of exposure throughout follow-up, moderate early childhood (11%) with only moderate probability of exposure in early-life and moderate late childhood (9%) with moderate probability of exposure in late childhood. Distributions of study characteristics among patterns of exposure are presented in Tables E4 and E5. The very low pet exposure pattern was characterized by more children with allergic and highly educated parents and fewer participants exposed to maternal smoking during pregnancy and SHS in the home. The persistently high pattern was characterized by fewer participants with allergic parents and less highly educated parents and more participants exposed to maternal smoking during pregnancy and SHS in the home. Study characteristics were evenly distributed between dampness or mould exposure patterns.

Figure 2 shows adjusted associations of longitudinal patterns of exposure with asthma at age 17 and sensitization at age 16. Crude and adjusted odds ratios were generally similar (Table E6). We did not observe consistent associations of any of the pet exposure patterns with the risk of asthma at age 17 compared with very low exposure, but a higher risk of asthma was suggested for early-life pet exposure [OR (95% CI) 1.66 (0.86 to 3.19)]. All patterns of pet exposure, however, tended to be consistently associated with a lower risk of sensitization at age 16 [0.63 (0.35 to 1.11)] for early-life pet exposure and [0.69 (0.44 to 1.08)] for persistently high pet exposure as compared with very low exposure. No significant associations with patterns of dampness or mould exposure were observed for asthma, but a tendency of a lower risk of sensitization was also observed (Table E6).

Sensitivity analyses

When we assessed associations of pet and dampness or mould exposure with allergic sensitization to specific allergens, early-life, late childhood and persistently high pet exposure were associated with lower risk of sensitization to birch, house dust mite and cocksfoot allergens at age 16 (Table E7). Dampness or mould exposure was also significantly associated with a lower risk of cat [0.15 (0.03 to 0.64) for moderate late childhood exposure]

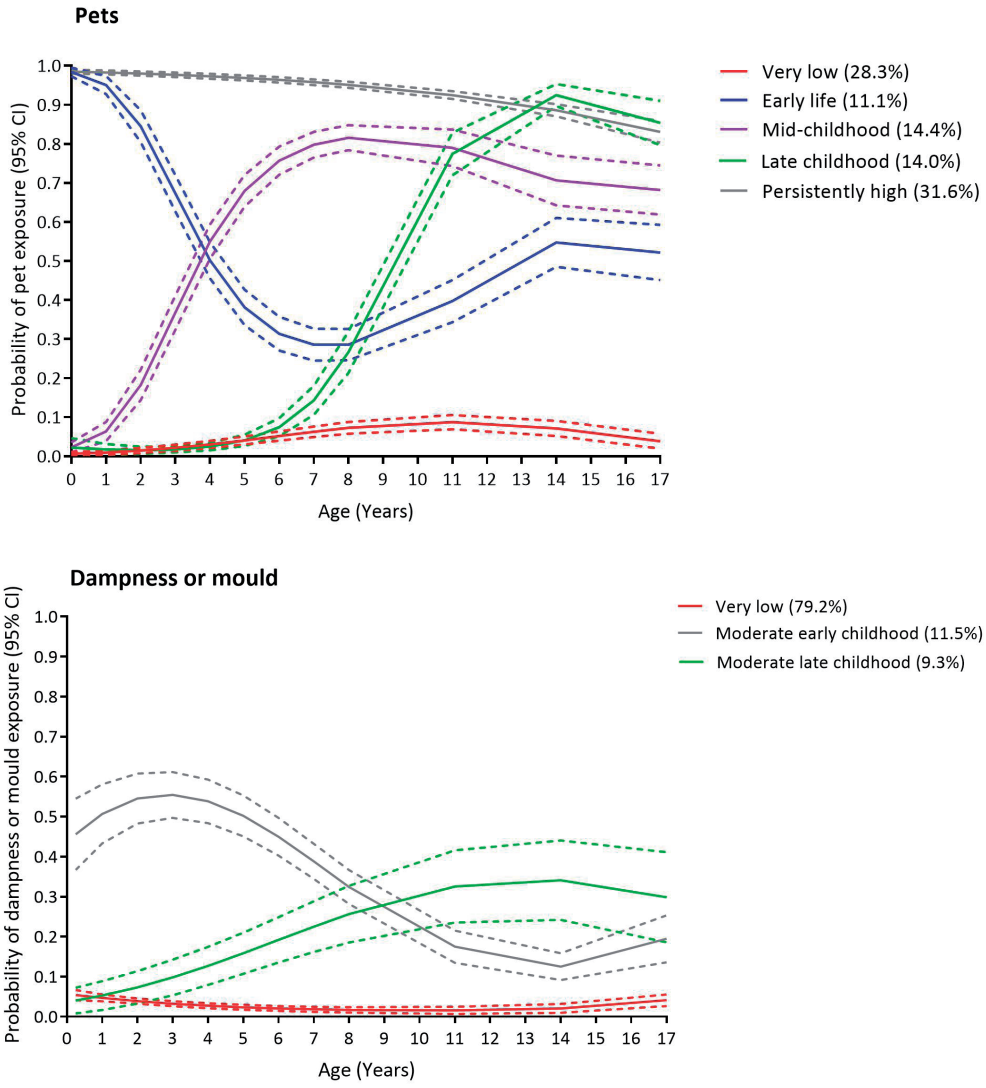


Figure 1. Longitudinal patterns of pet and dampness or mould exposure.

and house dust mite allergen sensitization [0.55 (0.32 to 0.96) for moderate early childhood exposure] (Table E7).

Stratification by parental allergy showed similar associations as in the main analyses and there were no differences in associations between children born to allergic and non-allergic parents (Table E8). Excluding participants whose parents reported getting rid of pets at any point during follow-up due to an allergy of a family member did not change results though a higher risk of asthma was suggested. (Table E9).

Non- significant lower risks of sensitization were consistently observed with cat and dog exposure, but not with exposure to rodents in analyses with patterns of exposure to separate pets. We did not observe any associations with exposure to specific pets for asthma. (Figure E2 and Figure E3). When we repeated the main analyses without weighting by posterior probabilities, the weighted and unweighted analyses produced similar estimates (Table E10).

Likewise, estimates were similar in sensitivity analyses using a higher cut off value (0.7 IU/mL) for sensitization except for a significant inverse association between early-life pet exposure and sensitization to at least one allergen and stronger associations of early-life pet exposure with sensitization to specific allergens (Table E11). Lower risk of both, polysensitization and monosensitization was suggested for all time windows of pet and dampness or mould exposure (Table E12), but few associations were statistically significant as numbers became small.

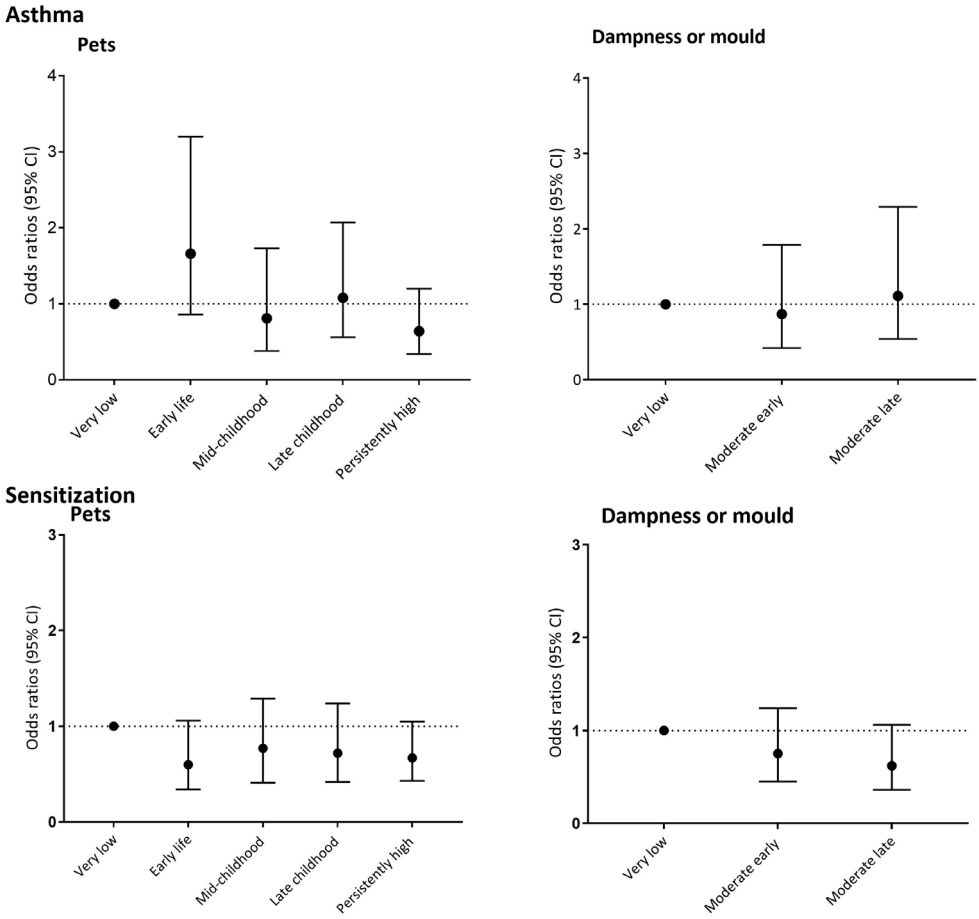
DISCUSSION

In our prospective birth cohort, we did not find associations of different timing of pet and dampness or mould exposure from pregnancy/birth till adolescence, with asthma at age 17 compared with very low exposure, but any pet and dampness or mould exposure during the life course tended to be consistently associated with a lower risk of sensitization at age 16.

Timing of pet exposure

Studies have shown both higher[2,9,10,31,32] and lower risks[1,12,13,17] of asthma and sensitization among those exposed to pets. A pooled analysis of 11 European birth cohorts including our own did not find an association between pet exposure in the first two years and asthma at ages 6-10 but observed a lower risk of sensitization.[14] To our knowledge no other study has investigated the relevance of the timing of pet exposure in associations with asthma and sensitization in adolescence. We did not observe significant associations of any time window of pet exposure with asthma in adolescence but risk of asthma was suggested for early-life pet exposure partly in line with studies that have reported higher risk of asthma in relation to early-life pet exposure. [31] Consistent inverse associations were suggested for sensitization when different timing patterns were compared with low exposure.

Separate analyses of the associations of allergic sensitization to specific allergens with pet exposure suggested that compared with very low exposure, early-life, late childhood and persistently high pet exposure may be associated with lower risks of sensitization to house dust mite, cocksfoot and birch allergen. Results of a previous analysis within our cohort showed inverse associations of pet exposure with sensitization and null associations



Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 week, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 year, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year, and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

Figure 2. Adjusted associations of longitudinal patterns of pet and dampness or mould exposure with asthma at age 17 (N = 1747) and with sensitization at age 16 (N = 637).

with asthma at age 8.[33] Our current findings extend the exposure period until adolescence and taken together, our set of findings suggests that in our cohort, pet exposure from birth until adolescence is not associated with asthma in adolescence and that the inverse associations with sensitization persist into adolescence.

An important issue regarding the current findings concerns (reverse) causality. Children with allergic parents were over-represented among participants with a very low

probability of exposure during the entire follow-up, suggesting that avoidance behaviour may at least partly explain the suggested inverse association. Children born to allergic parents are predisposed to develop asthma or become sensitized, and allergic parents are more likely to avoid keeping pets in the home. Consequently, such avoidance behaviour can be a source of bias in estimating the associations between pet exposure and allergic outcomes. We investigated the impact of avoidance of pets by allergic parents in stratified analyses by parental allergy and by excluding participants whose parents got rid of pets during follow-up because of allergies of a family member. We found similar associations for children of allergic and non-allergic parents. However in analyses where we excluded participants whose parents got rid of pets during follow-up a higher risk of asthma was suggested. Therefore, while our results indicate that it is unlikely that the suggested inverse associations are driven by avoidance of pets by allergic parents, reverse causation cannot be completely ruled out.

The suggested lower risk of sensitization to at least one allergen tested and allergic sensitization to specific inhalant allergens observed in our study is in line with the findings of another study that reported inverse associations of early-life pet exposure with total IgE levels among allergic individuals up to 18 years old,[34] and is in line with the so-called hygiene hypothesis. The hygiene hypothesis links a favourable maturation of the immune system with exposure to microbes in childhood [35,36] and is supported by studies reporting lower risks of sensitization in children growing up on farms with farm animals as compared with children growing up without farm animals.[37,38] The associations of proximity to farm animals are however less consistent with asthma.[39] The mechanisms underlying the inverse association are not clear. For example, it has been suggested that exposure to cat allergens may reduce the risk of asthma and sensitization due to a modified Th2 response characterized by the production of IgG4 antibodies produced in response to cat allergen exposure.[40,41] Alternatively, the presence of endotoxins, which is associated with the presence of pets in the home[42-44] may explain the suggested lower risks of allergic sensitization among those exposed to pets. Endotoxin exposure early in life might promote Th1 cell differentiation, which might reduce the risk of any allergen sensitization.[45,46]

Timing of dampness or mould exposure

Higher risks of asthma and allergic sensitization in relation to dampness or mould exposure have been reported in several studies,[3,4,18,47,48] while null associations have been reported in others.[31,49] A meta-analysis of eight European birth cohorts including our own, reported a positive association of early exposure to visible mould and/or dampness with asthma, but not with sensitization against inhalant allergens at early school age.[48] Few studies have been able to assess associations between dampness and/or mould and asthma or sensitization beyond childhood into adolescence. We found no evidence of an association between different timing of exposure to dampness or mould and asthma in the current study, but a

tendency towards a lower risk of sensitization in adolescence among participants moderately exposed in early-life and late childhood was suggested. A study like ours investigated the association of dampness or mould exposure in early-life with asthma and sensitization in adolescence and reported a higher risk of asthma up to age 16, but no associations with sensitization in contrast with our findings.[20] However, that study only investigated early-life exposure and not different timing of exposure. The lower risk of sensitization in relation to dampness or mould exposure suggested in our study may be explained by the presence of mould derived agents such as β (1,3)-glucans, which may be associated with a lower risk of sensitization to inhalant allergens.[18,50] While we did not observe positive associations with asthma, multiple reviews have suggested that dampness or mould exposure is associated with a higher risk of asthma.[4,47] Biological mechanisms including inflammatory and immunosuppressive responses to exposure to mould spores and components of microbial agents have been suggested[3] though the wide variety of health effects associated with dampness or mould cannot be explained by a single mechanism.[4]

Strengths and limitations

An important strength of our study is the availability of detailed information about exposure from birth till age 17. This allowed us to characterize longitudinal patterns of exposure over time and therefore investigate the timing of exposure in relation to asthma and sensitization in adolescence. Few other studies so far have (included) exposure data beyond childhood. The prospective design of our study implied small liability of recall bias. We were also able to investigate reverse causation due to allergy of family members which is a common problem in studies assessing associations of pet exposure with allergic outcomes.

We acknowledge several limitations of our study. We relied on parental reports as proxies of pet and dampness or mould exposure assessment which can introduce misclassification of exposure as parents may underreport exposure leading to underestimation of exposure estimates. However, we expect that this misclassification is likely non-differential. Collecting dust samples from homes and analysing these samples e.g. for their contents of allergens, endotoxin and other biocontaminants could be a more objective assessment for pet exposure, but it is costly for a large study like ours and reflects exposure at one or more specific points in time rather than life course exposure. Visible mould reports, however, have been reported to be highly correlated with airborne concentrations of fungal spores[51] suggesting self-reports of dampness or mould are a good exposure indicator. We were also unable to include factors which might alter some of our observed associations, e.g. frequency and type of contact between children and pets outside the child's home. We only assessed residential indoor exposure and it may be possible that the indoor environment is less important in the aetiology of asthma in adolescence than it is in childhood [31] with children spending less time in the home as they grow older. However, exposure outside the home was beyond the scope of this study. A potential limitation of the latent trajectory modelling

procedure is that classification of individuals depends on the study population and therefore not exactly the same set of classes may be replicated in a different study population with different exposure patterns. We are not aware of other studies that used this method to classify exposure, but this method has been used for classification of trajectories of atopic dermatitis and wheeze and similar trajectories have been found in different cohorts,[52,53] which suggests that replication may be possible in comparable settings. Another limitation is that asthma status was assessed from questionnaires and not based on lung function tests. However, the questionnaire-based outcome is used in large birth cohort studies[54,55] and it offers data for many subjects, while lung function measurements are more costly and therefore often not feasible for all participants.

There were more highly educated parents, fewer mothers who smoked during pregnancy and fewer participants breastfed and exposed to secondhand smoke in the study population than in the excluded PIAMA population. This may affect generalizability, given that highly educated parents may be less likely to keep pets and less likely to smoke. However, we assume that the associations of potential predictors of pet and dampness or mould exposure with asthma and sensitization, would not be different in the general population with comparable levels of pet ownership. Generalizability may be limited beyond the Dutch population with different levels of pet ownership because varying prevalence of asthma and sensitization, pet ownership rates across countries and varying cultural/lifestyle differences may present different associations.[56] For example, the higher/lower the frequency of pet ownership in a given community the higher/lower the degree of allergen dispersal in pet-free homes.[57]

In conclusion, we found no evidence of a difference in risk of asthma in adolescents with different timing of pet or dampness or mould exposure as compared with those with very low exposure. Lower risk of sensitization was suggested for all time windows of pet and dampness or mould exposure, but may partly be attributable to reversed causation. While this study adds to the evidence that the risk of sensitization in adolescence might be lower among those with exposure to pets, current evidence from the literature is not strong enough to recommend parents of (young) children to acquire pets to reduce risk of developing allergies. On the other hand, there seems to be no evidence for couples to get rid of pets when expecting a child. More prospective studies establishing a temporal link between pet exposure and asthma and sensitization in adolescence are needed get more insights into this relationship that can then be used in clinical practice when advising parents about acquiring pets in the home.

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SUPPLEMENTARY MATERIAL

Table E1. Number of surveys from birth until age 17 with missing data on exposure to pets and dampness or mould.

Number of surveys with missing data	N (%)		N (%)
Pets		**Dampness or mould**	
0	1524 (81.4)	0	1242 (66.4)
1	232 (12.4)	1	442 (23.6)
2	76 (4.1)	2	132 (7.1)
3	21 (1.0)	3	37 (1.9)
4	10 (0.5)	4	11(0.6)
5	5 (0.2)	5	6 (0.3)
6	2 (0.1)	6	1 (<0.1)
7	1 (< 0.1)		

Table E2. Study population and excluded population: comparison of characteristics. †

Characteristics	Study population (N=1871)		Excluded population (N=2092)		P-value
	N	%	N	%	
Parental allergy ¶					
Allergic mother	551/1871	29.4	686/2092	32.8	0.023
Allergic father	585/1871	31.2	632/2086	30.3	0.509
Boys	926/1871	49.4	1128/2092	53.9	0.012
Presence of pets at 1 year	792/1862	42.5	927/1923	48.2	<0.001
Dampness or moulds at 1 year	152/1827	8.3	160/1871	8.5	0.799
Breastfeeding > 12 weeks	1044/1861	56.1	848/2035	41.6	<0.000
Gas cooking at 3 months	1550/1864	83.1	1697/2059	82.4	0.542
Maternal smoking during pregnancy	245/1857	13.2	458/2063	22.2	<0.001
SHS exposure in the home at 1 year	411/1866	22.0	702/2066	33.9	<0.001
Respiratory infection in the first 4 years of life ¥	1469/1843	79.7	1599/1907	83.8	0.104
Antibiotics use in the first 4 years of life	989/1854	53.3	1099/1843	59.6	0.057
Parental education					<0.001
Low	177/1865	9.4	325/1947	16.1	
Intermediate	600/1865	32.2	802/1947	41.1	
High	1088/1865	58.4	820/1947	42.1	
Older siblings at birth	919/1871	49.1	1067/2087	51.1	0.207
Parental country of birth (Netherlands)	1763/1845	95.5	1722/1855	92.1	<0.001

†Groups compared using Chi-square tests for categorical variables

¶ asthma ever, allergy against house dust, house dust mite or pets, or rhinitis or hay fever

¥ Serious cold or flu, infection of the throat, otitis media, sinusitis, bronchitis or pneumonia

Table E3. Distribution of posterior probabilities by pattern of pet and dampness or mould exposure.

	Posterior probabilities	
Pets	**Mean (SD)**	**(Min, Max)**
Very low	0.95 (0.09)	(0.45, 0.99)
Early-life	0.93 (0.11)	(0.52, 0.99)
Mid-childhood	0.91 (0.14)	(0.40, 0.99)
Late childhood	0.90 (0.12)	(0.40, 0.99)
Persistently high	0.97 (0.08)	(0.39, 0.99)
Dampness or mould		
Very low	0.92 (0.10)	(0.40, 0.98)
Moderate early childhood	0.87 (0.16)	(0.34, 0.99)
Moderate late childhood	0.75 (0.16)	(0.41, 0.99)

Table E4. Study characteristics by pet exposure patterns. †

Characteristic	**Very low (%)**	**Early-life (%)**	**Late childhood (%)**	**Mid childhood (%)**	**Persistently high (%)**	**P-Value**
Parental allergy¶						
Allergic mother	37.6	30.6	30.1	27.3	22.3	<0.001
Allergic father	34.5	33.4	34.9	29.8	26.5	<0.001
Sex (Boys)	49.8	49.7	49.1	52.4	47.9	0.824
Maternal smoking during pregnancy	7.3	18.2	10.2	11.9	18.6	<0.001
Presence of moulds at 1 year	5.3	8.2	8.8	11.1	9.5	0.036
Breastfeeding >12 weeks	61.6	54.8	60.7	58.6	48.3	<0.001
SHS exposure in the home at 1 year(Yes)	15.7	26.3	15.6	20.1	30.0	<0.001
Gas cooking at 3 months	81.8	87.5	84.4	81.0	83.2	0.314
Asthma at age 17	6.5	8.1	6.1	3.1	3.0	0.011
Respiratory infections in the first 4 years of life ¥	77.5	81.8	83.1	79.5	79.5	0.404
Antibiotics use in the first 4 years of life	50.7	50.2	53.8	57.6	54.6	0.329
Sensitization at age 16	54.1	42.5	46.8	47.2	45.1	0.308
Parental education						<0.001
Low	6.9	9.5	7.2	7.8	13.6	
Medium	27.6	37.3	26.2	29.0	38.5	
High	65.3	53.2	66.6	63.2	47.9	
Older siblings at birth	47.0	44.0	53.9	66.0	42.9	<0.001
Parental country of birth (Netherlands)	93.7	95.7	93.6	97.7	97.0	0.011

† Groups compared using Chi-square tests

¶ asthma ever, allergy against house dust, house dust mite or pets, or rhinitis or hay fever

¥ Serious cold or flu, infection of the throat, otitis media, sinusitis, bronchitis or pneumonia

Table E5. Study characteristics by dampness or mould exposure patterns. †

Characteristic	Very low (%)	Moderate early childhood (%)	Moderate late childhood (%)	P-value
Parental allergy¶				
Allergic mother	29.5	30.1	27.9	0.890
Allergic father	31.5	25.4	35.4	0.106
Sex (Boys)	48.4	52.3	55.2	0.156
Maternal smoking during pregnancy	12.6	15.8	14.6	0.386
Presence of pets at 1 year	58.5	51.8	55.5	0.135
Breastfeeding >12 weeks	54.4	62.9	61.9	0.019
SHS exposure in the home at 1 year	21.1	26.7	23.9	0.211
Gas cooking at 3 months(Yes)	82.9	86.1	81.8	0.523
Asthma at age 17	5.3	5.2	4.1	0.783
Sensitization at age 16	50.2	41.2	39.4	0.09
Respiratory infection in the first 4 years of life ¥	79.6	79.5	80.2	0.983
Antibiotics use in the first 4 years of life	53.3	49.7	57.9	0.285
Parental education				0.947
Low	9.4	9.7	9.3	
Medium	32.6	30.2	30.9	
High	57.9	60.0	59.6	
Older siblings at birth	52.5	44.4	45.3	0.023
Parental country of birth (Netherlands)	95.5	96.2	95.8	0.834

†Groups compared using Chi-square tests

¶ asthma ever, allergy against house dust, house dust mite or pets, or rhinitis or hay fever

¥ Serious cold or flu, infection of the throat, otitis media, sinusitis, bronchitis or pneumonia

Table E6. Crude and adjusted associations of longitudinal patterns of pets and dampness or mould exposure with asthma at age 17 and sensitization to at least one allergen at age 16. †

	Odds Ratio (95% Confidence Interval)			
	Asthma at age 17		**Sensitization to at least one allergen at age 16**	
Pets	**Crude (N=1871)**	**Adjusted (N=1747)**	**Crude (N=682)**	**Adjusted (N=637)**
Early-life vs Very low	1.27 (0.69 to 2.32)	1.66 (0.86 to 3.19)	0.62 (0.37 to 1.05)	0.63 (0.35 to 1.11)
Mid-childhood vs Very low	0.55 (0.27 to 1.13)	0.80 (0.38 to 1.72)	0.75 (0.47 to 1.20)	0.78 (0.47 to 1.30)
Late childhood vs Very low	0.93 (0.50 to 1.71)	1.08 (0.56 to 2.09)	0.74 (0.45 to 1.21)	0.72 (0.42 to 1.24)
Persistently high vs Very low	0.45 (0.25 to 0.80)	0.64 (0.34 to 1.21)	0.69 (0.46 to 1.03)	0.69 (0.44 to 1.08)
Dampness or mould	**Crude (N=1870)**	**Adjusted (N=1773)**	**Crude (N=682)**	**Adjusted (N=647)**
Moderate early childhood vs Very low	0.78 (0.38 to 1.58)	0.94 (0.45 to 1.94)	0.69 (0.43 to 1.12)	0.71 (0.42 to 1.19)
Moderate late childhood vs Very low	0.99 (0.48 to 2.02)	1.01 (047 to 2.18)	0.64 (0.39 to 1.07)	0.65 (0.37 to 1.12)

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year .

Table E7. Adjusted associations of longitudinal patterns of pets and dampness or mould exposure with sensitization to specific allergens: cat, house dust mite (*Dermatophagoides pteronyssinus*), birch and Cocksfoot (*Dactylis glomerata)* at age 16. †

	Odds Ratio (95% Confidence Interval)			
	Sensitization to cat	**Sensitization to house dust mite**	**Sensitization to cocksfoot**	**Sensitization to birch**
Pets (N=651)				
Early-life vs Very low	1.00 (0.44 to 2.22)	**0.49 (0.27 to 0.90)**	0.61 (0.33 to 1.14)	0.47 (0.22 to 1.01)
Mid-childhood vs Very low	0.96 (0.46 to 2.00)	0.70 (0.42 to 1.19)	0.67 (0.39 to 1.16)	0.52 (0.27 to 1.00)
Late childhood vs Very low	0.89 (0.42 to 1.88)	0.86 (0.50 to 1.47)	**0.54 (0.30 to 0.99)**	**0.39 (0.19 to 0.82)**
Persistently high vs Very low	0.75 (0.40 to 1.42)	0.69 (0.44 to 1.08)	**0.50 (0.31 to 0.82)**	**0.46 (0.26 to 0.82)**
Dampness or mould (N=651)				
Moderate early childhood vs Very low	0.72 (0.34 to 1.56)	**0.55 (0.32 to 0.96)**	0.76 (0.43 to 1.36)	0.68 (0.33 to 1.40)
Moderate late childhood vs Very low	**0.15 (0.03 to 0.64)**	0.76 (0.43 to 1.33)	0.55 (0.28 to 1.07)	0.71 (0.33 to 1.51)

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

Table E8. Adjusted associations of longitudinal patterns of pets and dampness or mould exposure with asthma at age 17 and sensitization to at least one allergen at age 16 stratified by parental allergy. †

	Odds Ratio (95% Confidence Interval)					
	Asthma at age 17			**Sensitization to at least one allergen at age 16**		
Pets	**Allergic parents (N=894)**	**Non-allergic parents (N=853)**	**P-Value***	**Allergic parents (N=358)**	**Non-allergic parents (N=279)**	**P-Value***
Early-life vs Very low	1.60 (0.76 to 3.40)	1.60 (0.43 to 5.97)	0.966	0.52 (0.25 to 1.08)	0.59 (0.23 to 1.48)	0.804
Mid-childhood vs Very low	0.75 (0.30 to 1.82)	0.75 (0.19 to 3.06)	0.979	0.76 (0.38 to 1.53)	0.78 (0.36 to 1.68)	0.890
Late childhood vs Very low	1.03 (0.48 to 2.20)	1.10 (0.30 to 3.96)	0.964	0.95 (0.47 to 1.92)	0.38 (0.14 to 1.00)	0.133
Persistently high vs Very low	0.60 (0.28 to 1.30)	0.55 (0.18 to 1.74)	0.952	0.77 (0.42 to 1.40)	**0.49 (0.25 to 0.97)**	0.353
Dampness or mould						
Moderate early childhood vs Very low	0.80 (0.31 to 2.09)	1.37 (0.44 to 4.27)	0.386	0.50 (0.23 to 1.05)	0.94 (0.45 to 1.95)	0.376
Moderate late childhood vs Very low	1.25 (0.54 to 2.88)	0.43 (0.06 to 3.34)	0.430	0.59 (0.28 to 1.21)	0.89 (0.39 to 2.01)	0.250

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year. *Interaction p-value

Table E9. Adjusted associations of longitudinal patterns of pets and dampness or mould exposure with asthma at age 17 and sensitization to at least one allergen at age 16 excluding participants whose parents got rid of a pet due to allergy of a family member. †

	Odds ratio (95 % Confidence Interval)	
	Asthma at age 17 (N=1518)	**Sensitization to at least one allergen at age 16 (N=553)**
Pets		
Early-life vs Very low	1.92 (0.85 to 4.35)	0.55 (0.29 to 1.05)
Mid-childhood vs Very low	1.28 (0.55 to 3.00)	0.71 (0.41 to 1.22)
Late childhood vs Very low	1.14 (0.51 to 2.55)	0.57 (0.31 to 1.03)
Persistently high vs Very low	0.80 (0.38 to 1.69)	0.70 (0.44 to 1.12)

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

Table E10. Unweighted * adjusted associations of longitudinal patterns of pet and dampness or mould exposure with asthma, sensitization to at least one allergen and sensitization to specific allergens (cat, house dust mite (*Dermatophagoides pteronyssinus*, cocksfoot (*Dactylis glomerata)* and birch*)* at age 16. †

	Odds Ratio (95% Confidence Interval)					
	Asthma at age 17	**Sensitization to to at least one allergen**	**Sensitization to cat**	**Sensitization to house dust mite**	**Sensitization to cocksfoot**	**Sensitization to birch**
Pets	**N=1747**	**N=637**	**N=637**	**N=637**	**N=637**	**N=637**
Early-life vs Very low	1.67 (0.87 to 3.20)	0.63 (0.36 to 1.12)	1.00 (0.45 to 2.23)	**0.49 (0.27 to 0.90)**	0.61 (0.33 to 1.14)	**0.47 (0.22 to 1.01)**
Mid-childhood vs Very low	0.80 (0.38 to 1.72)	0.79 (0.47 to 1.31)	0.96 (0.46 to 1.99)	0.70 (0.42 to 1.19)	0.67 (0.39 to 1.16)	0.52 (0.27 to 1.00)
Late childhood vs Very low	1.08 (0.56 to 2.09)	0.72 (0.42 to 1.24)	0.89 (0.42 to 1.88)	0.86 (0.50 to 1.47)	**0.54 (0.30 to 0.99)**	**0.39 (0.19 to 0.82)**
Persistently high vs Very low	0.64 (0.34 to 1.21)	0.70 (0.45 to 1.09)	0.75 (0.39 to 1.42)	0.69 (0.44 to 1.08)	**0.50 (0.31 to 0.82)**	**0.46 (0.26 to 0.82)**
Dampness or mould	**N=1747**	**N=647**	**N=647**	**N=647**	**N=647**	**N=647**
Moderate early childhood vs Very low	0.94 (0.45 to 1.94)	0.71 (0.43 to 1.19)	0.72 (0.34 to 1.56)	**0.55 (0.32 to 0.96)**	0.76 (0.43 to 1.36)	0.68 (0.33 to 1.40)
Moderate late childhood vs Very low	1.01 (0.47 to 2.18)	0.65 (0.37 to 1.12)	**0.15 (0.03 to 0.64)**	0.76 (0.43 to 1.33)	0.55 (0.28 to 1.07)	0.71(0.33 to 1.35)

*: Assigning subjects to the exposure trajectory with the highest posterior probability instead of weighting observations by posterior probabilities

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

Table E11. Adjusted associations of longitudinal patterns of pet and dampness or mould exposure with sensitization to at least one allergen and sensitization to specific allergens using a cut-off of 0.7 IU/mL to define sensitization. †

	Odds Ratio (95% Confidence Interval)				
	Sensitization to at least one allergen	**Sensitization to cat**	**Sensitization to house dust mite**	**Sensitization to cocksfoot**	**Sensitization to birch**
Pets (N=637)					
Early-life vs Very low	**0.49 (0.27 to 0.89)**	0.92 (0.39 to 2.18)	**0.39 (0.20 to 0.76)**	0.53 (0.28 to 1.01)	**0.42 (0.18 to 0.99)**
Mid-childhood vs Very low	0.84 (0.50 to 1.40)	0.95 (0.44 to 2.06)	0.71 (0.41 to 1.21)	0.66 (0.38 to 1.15)	0.50 (0.24 to 1.02)
Late childhood vs Very low	0.64 (0.37 to 1.11)	0.85 (0.38 to 1.90)	0.81 (0.46 to 1.42)	**0.44 (0.24 to 0.83)**	0.47 (0.22 to 1.00)
Persistently high vs Very low	0.74 (0.47 to 1.16)	0.59 (0.29 to 1.22)	0.65 (0.41 to 1.05)	**0.48 (0.29 to 0.79)**	**0.39 (0.20 to 0.74)**
Dampness or mould (N=647)					
Moderate early childhood vs Very low	**0.51 (0.30 to 0.88)**	0.71 (0.30 to 1.66)	**0.53 (0.29 to 0.95)**	0.62 (0.33 to 1.16)	0.52(0.22 to 1.23)
Moderate late childhood vs Very low	0.62 (0.35 to 1.09)	**0.10 (0.01 to 0.71)**	0.66 (0.37 to 1.22)	**0.50 (0.25 to 1.02)**	0.75(0.33 to 1.68)

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

Table E12. Adjusted associations of longitudinal patterns of pets and dampness or mould exposure with polysensitization, monosensitization vs non-sensitization.

	Mono- sensitization vs non- sensitization	**Poly-sensitization vs non-sensitization**
Pets	**N=637**	**N=637**
Early-life vs Very low	0.79 (0.38 to 1.66)	0.52 (0.26 to 1.03)
Mid-childhood vs Very low	0.90 (0.46 to 1.73)	0.71 (0.39 to 1.29)
Late childhood vs Very low	0.82 (0.41 to 1.64)	0.66 (0.35 to 1.24)
Persistently high vs Very low	0.88 (0.49 to 1.55)	**0.58 (0.34 to 0.98)**
Dampness or mould	**N=647**	**N=647**
Moderate early childhood vs Very low	1.02 (0.55 to 1.89)	**0.50 (0.25 to 0.97)**
Moderate late childhood vs Very low	0.78 (0.39 to 1.57)	0.55 (0.28 to 1.08)

†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings. Analyses with pet exposure were additionally adjusted for dampness or mould exposure in the home at 1 year and analyses of dampness or mould exposure were adjusted for furry pets in the home at 1 year.

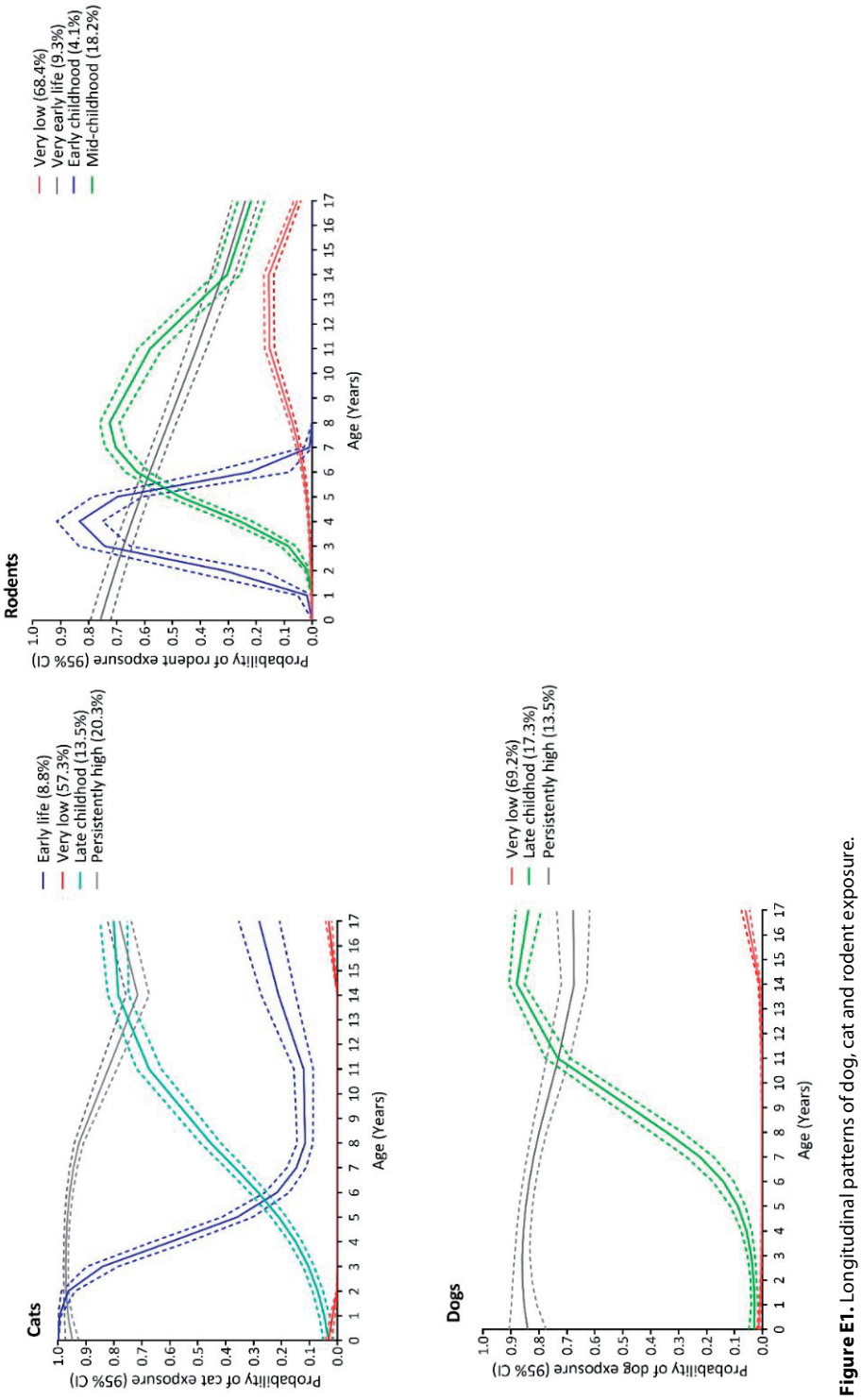
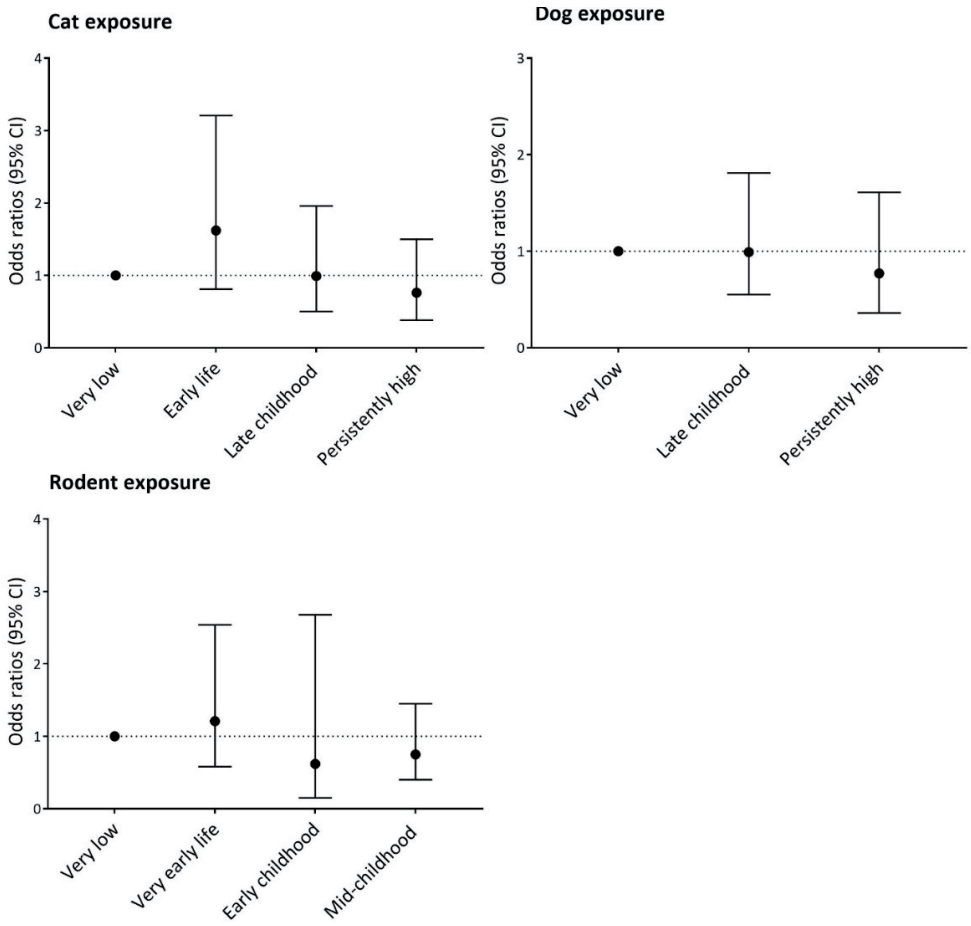
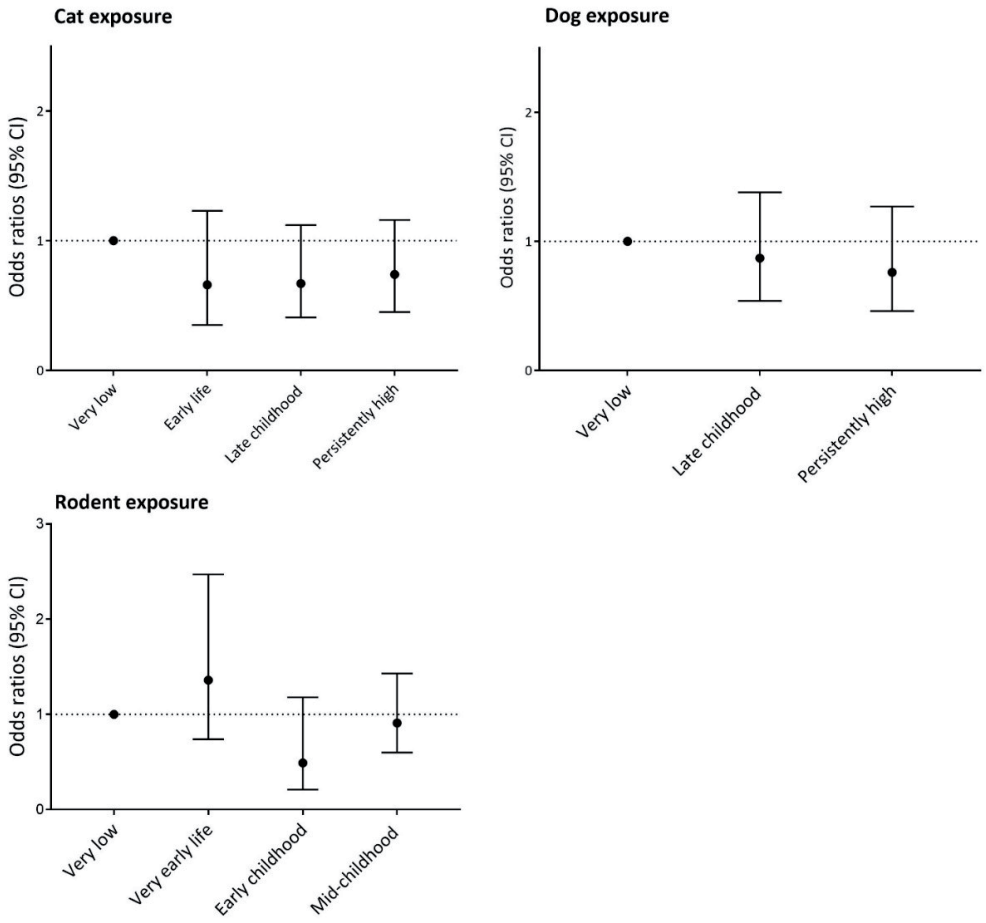


Figure E1. Longitudinal patterns of dog, cat and rodent exposure.



†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings, and dampness or mould exposure in the home at 1 year.

Figure E2. Adjusted associations of longitudinal exposure to cats, dogs and rodents separately with asthma at age 17.†



†: Associations adjusted for: sex, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, parental country of birth, maternal smoking during pregnancy, any smoking in the child's home at 1 year, active smoking at 17 years, gas cooking at 3 months, respiratory infections and antibiotic use in the first 4 years of life, and the presence of older siblings and dampness or mould exposure in the home at 1 year.

Figure E3. Adjusted associations of longitudinal exposure to cats, dogs and rodents separately with sensitization at age 16. †

CHAPTER 4

4

Considerations in the use of different spirometers in epidemiological studies

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Environmental Health (2019) Apr 25;18(1):39.

ABSTRACT

Background: Spirometric lung function measurements have been proven to be excellent objective markers of respiratory morbidity. The use of different types of spirometers in epidemiological and clinical studies may present systematically different results affecting interpretation and implication of results. We aimed to explore considerations in the use of different spirometers in epidemiological studies by comparing forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) measurements between the Masterscreen pneumotachograph and EasyOne spirometers. We also provide a correction equation for correcting systematic differences using regression calibration.

Methods: Forty-nine volunteers had lung function measured on both spirometers in random order with at least three attempts on each spirometer. Data were analysed using correlation plots, Bland and Altman plots and formal paired t-tests. We used regression calibration to provide a correction equation.

Results: The mean (SD) FEV_1 and FVC was 3.78 (0.63) L and 4.78 (0.63) L for the Masterscreen pneumotachograph and 3.54 (0.60) L and 4.41 (0.83) L for the EasyOne spirometer. The mean FEV_1 difference of 0.24 L and mean FVC difference of 0.37 L between the spirometers (corresponding to 6.3% and 8.4% difference, respectively) were statistically significant and consistent between younger (<30 years) and older volunteers (>30 years) and between males and females. Regression calibration indicated that an increase of 1 L in the EasyOne measurements corresponded to an average increase of 1.032 L in FEV_1 and 1.005 L in FVC in the Masterscreen measurements.

Conclusion: Use of different types of spirometers may result in significant systematic differences in lung function values. Epidemiological researchers need to be aware of these potential systematic differences and correct for them in analyses using methods such as regression calibration.

BACKGROUND

Spirometry is a commonly used test of lung function, an important tool in the diagnosis, and monitoring of respiratory diseases and is frequently used in epidemiological and clinical research.[1] Results of spirometry tests depend on several factors including technical factors such as the type of spirometer used, personal factors such as a subject's posture, and the cooperation between the subject and the technician, which need to be considered in clinical and epidemiological studies.

Despite potential differences between spirometers, there may be compelling reasons to use different spirometers in clinical and epidemiological research. In large-scale multicentre studies for example, for efficiency reasons, more than one spirometer of the same type or different spirometers of different types may be used in different centres. In follow-up studies, there may be need to replace older spirometers by newer spirometers.

Comparisons between different types of spirometers as well as similar types of spirometers have been performed in several studies.[2-5] Systematic differences between different types of spirometers have been reported.[2,4] Such differences can bias exposure-health relationships in studies where the use of a specific spirometer is associated with exposure, e.g. in multi-centre studies of effects of ambient air pollution where different spirometers are used in different study regions with different levels of exposure. Adjustment for type of spirometer is one possibility to account for systematic differences between spirometers. However, this may result in over-adjustment if the region is also an important determinant of exposure. Methods such as regression calibration are more suitable in such situations, but require data on the comparability of devices. [6]

In this study, we compared FEV_1 and FVC measurements from two widely used spirometers - the Masterscreen pneumotachograph and the EasyOne spirometer that were simultaneously used in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. We also investigated comparability between two EasyOne spirometers. We used the obtained measurements to provide a correction equation to adjust for differences between the spirometers in an epidemiological study.

METHODS

Comparison study design and study population

Two series of spirometry tests were performed in volunteers by trained research staff between April and May 2017. In the first test series that we consider to be our main comparison performed at the University Medical Centre Groningen, we compared the Masterscreen pneumotachograph with an EasyOne spirometer (referred to here as EasyOne1). Two highly experienced and trained technicians conducted spirometry measurements in the first test

series (one for the Masterscreen pneumotachograph and one for the EasyOne1). We let each technician use a different spirometer by design to reflect a real-life multicentre research setting where different spirometers are used in different centres by different technicians. In the second series, one of the technicians involved in the first test series performed the tests at Utrecht University, and the EasyOne1 from the first series was compared with a second EasyOne spirometer of the same generation, referred to as EasyOne2 (both purchased in 2008). In both series, all volunteers performed tests on both spirometers in random order but in immediate succession to eliminate confounding by individual characteristics. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured in sitting position while wearing a nose clip. Measurements that fulfilled the ATS/ERS criteria[1] were included in the analysis (n=45 for each of the series). In addition, test results were included which did not meet these criteria (difference between the largest and next largest value ≤ 150 mL for FEV_1 and FVC), but which were obtained from otherwise technically acceptable flow-volume curves with the largest and next largest values for FEV_1 and FVC ≤ 200 mL, (n=4 for each of the two series) as in previous analyses.[7] Zero flow was established before each measurement with both devices. For each test series, the final study population consisted of 49 volunteers. Information on ethnicity, self-reported weight, height and age of volunteers was also collected.

The PIAMA cohort

The PIAMA birth cohort is a Dutch population-based study that started in 1996/97 with 3963 new-borns and has been extensively described elsewhere.[8] Follow-up was conducted from pregnancy, 3 months, and yearly until age 8, then at ages 11, 14, 16 and 17. Medical examinations measuring lung function including FEV_1 and FVC and anthropometric characteristics such as weight and height were conducted at ages 8, 12 and 16. At age 16, lung function measurements were obtained in 721 participants. Both the Masterscreen pneumotachograph (CareFusion, Yorba Linda, CA, USA) and Easy One spirometers (NDD Medical Technologies, Inc, Switzerland) were used to measure FEV_1 and FVC at age 16 in two centres, Groningen and Utrecht respectively. We applied the correction equation in the current study to lung function data from the PIAMA cohort measured at age 16.

Ethical approval of the current study was obtained from medical ethical review board from University Medical Center Groningen (ref no. M17.220613) and all volunteers provided consent to participate.

Spirometers

We used two EasyOne spirometers (NDD Medical Technologies, Inc, Switzerland) and the Jaeger Masterscreen pneumotachograph spirometer (CareFusion, Yorba Linda, CA, USA).

Masterscreen pneumotachograph is one of the most widely used pulmonary function systems. It measures lung volumes indirectly with a pneumotachograph using the pressure

difference over a small, fixed-resistance, offered by a fine metal mesh.[9] In brief, it measures the pressure drop when a patient blows into the device. The pressure drop divided by the resistance of the pneumotachograph yields the flow, which can be transformed into a volume by time integration [10]. It is sensitive to temperature, humidity and atmospheric pressure of surrounding air and therefore requires constant calibration.

The EasyOne spirometer is a handheld standalone flow-sensing instrument that requires no calibration though calibration can be checked with a syringe.[11] Unlike the Masterscreen pneumotachograph, the EasyOne spirometer incorporates an ultrasonic flow sensor to measure the flow of air in and out of the patients' lungs. Ultrasonic flow measurements are independent of gas composition, pressure, temperature, and humidity and therefore inaccuracy is reduced due to the mentioned factors.[12]

Statistical analyses

Sample size calculations were performed based on a standard deviation (SD) for FEV_1 of 0.5 L. With a significance level of 0.05, 44 volunteers were required to detect a mean difference of 0.3 L between the spirometers with 80% power.

Correlations and agreement between spirometry measurements performed with the different spirometers were assessed with scatterplots, Pearson correlation coefficients and Bland and Altman plots [13]. Significance of differences between spirometers (within persons) was tested with paired t-tests.

In the absence of a gold standard, we computed the percent predicted FEV_1 and FVC according to sex, age, height, and ethnicity-based on reference regression equations as developed by the Global Lung Function Initiative (GLI)[14] to assess which of the two spirometers most likely gives a better estimate of the lung function.

Moreover, we used the data from the first test series to provide a correction equation by regressing measurements from the Masterscreen pneumotachograph on the measurements obtained by the EasyOne1 spirometer as follows:

$$FEV_{1}\_Masterscreen = \alpha + \beta * FEV_{1}EasyOne1$$
$$FVC\_Masterscreen = \alpha + \beta * FVCEasyOne1$$

The regression coefficients can be used to correct for systematic differences in epidemiological analyses and we showed this by applying the equation to lung function data at age 16 from the PIAMA birth cohort. Data were analysed using SAS version 9.4 (The SAS Institute, Cary, NC, USA).

RESULTS

Table 1 shows characteristics of the volunteers that participated in the two series of spirometer comparisons. On average, the FEV_1 and FVC as measured by the Masterscreen pneumotachograph were significantly higher than the FEV_1 and FVC as measured by the EasyOne1 spirometer (FEV_1: 3.78 L vs 3.54 L, mean difference 0.24 L, p-value < 0.0001; FVC: 4.78 L vs 4.41 L, mean difference 0.37 L, p-value < 0.0001). The 0.24 L and 0.37 L mean differences, correspond to a 6.3% decrease in FEV_1 switching from the Masterscreen pneumotachograph to the EasyOne1 spirometer and 8.4% decrease in FVC switching from the Masterscreen pneumotachograph to the EasyOne1 spirometer respectively. Differences in FEV_1 and FVC between the two EasyOne spirometers were small i.e. FEV_1: 3.50 L vs 3.46 L with a mean difference of 0.03 L, p-value < 0.003 and FVC: 4.31 L vs 4.27 L mean difference, 0.04 L, p-value < 0.003, respectively. The mean differences correspond to a 1.1 % decrease in FEV_1 switching from the EasyOne1 to the EasyOne2 spirometer and 0.9% decrease in FVC switching from the EasyOne1 to the EasyOne2 spirometer (Tables 1 and 2). The observed differences between the spirometers were similar in males and females and in younger and older volunteers (Table 2).

Measurements were highly correlated (r = 0.98 for the first test series and r = 0.99 for the second test series for both FEV_1 and FVC) indicating a strong linear relationship, which deviates from identity (Figure 1) for FEV_1 (but not FVC) in the first test series, but not for the second test series. The Bland and Altman plots show that the mean differences are consistently larger than zero indicating a systematic difference between the two spirometers with the Masterscreen pneumotachograph consistently producing higher values than the EasyOne1. There was no systematic difference between the two EasyOne1 and EasyOne2 measurements (Figure 2).

Using the GLI reference equations, the percent predicted for the Masterscreen pneumotachograph was close to 100% (98.3% for FEV_1 and 103.7% for FVC), but less so for the EasyOne1 (92.3% for FEV_1 and 95.5% for FVC).

Regression of the measurements from the Masterscreen pneumotachograph on the EasyOne1 measurements produced the following regression equations (Figure 1):

$$FEV_1 Masterscreen = 0.114\ (0.05) + 1.032\ (0.01) * FEV_1 EasyOne1$$
$$FVC Masterscreen = 0.357\ (0.05) + 1.005 (0.01) * FVC EasyOne1$$

The above regression equations indicate that an increase of 1 L in the EasyOne1 measurements is associated with an estimated average increase of 1.032 L for the FEV_1 and 1.005 L for the FVC in the Masterscreen pneumotachograph measurements.

Table 1. Study population characteristics.

Masterscreen vs EasyOne1	**Overall (N=49)**	**Males (N=15)**	**Females (N=34)**
Age (years) – mean (SD)	30.2 (10.9)	29.2 (10.8)	30.8 (11.1)
Age ≤ 30 years – N (%)	32 (65)	12 (66)	29 (67)
Ethnicity-N (%)			
Caucasian	49 (100)	15 (100)	34 (100)
Weight (Kg) – mean (SD)	68.9 (11.3)	72.6 (11.9)	66.9 (10.6)
Height (m) – mean (SD)	1.74 (8.32)	1.81 (6.46)	1.70 (6.57)
FEV_1Masterscreen (L) – mean (SD)	3.78 (0.63)	4.38 (0.62)	3.51(0.43)
FEV_1EasyOne 1 – mean (SD)	3.54 (0.60)	4.11 (0.57)	3.29 (0.42)
FVC Masterscreen (L) – mean (SD)	4.78 (0.85)	5.77 (0.76)	4.35 (0.42)
FVCEasyOne1 (L) – mean (SD)	4.41 (0.83)	5.35 (0.74)	4.01 (0.44)
FEV_1Masterscreen mean (SD) percent predicted	98.3 (11.1)	93.6 (10.5)	100.4 (10.8)
FEV_1EasyOne1 mean (SD) percent predicted	92.3 (10.8)	87.9 (9.91)	94.2 (10.7)
FVC Masterscreen mean (SD) percent predicted	103.7 (10.5)	101.2 (11.4)	104 (10.1)
FVC EasyOne1 mean (SD) percent predicted	95.5 (10.5)	93.8 (12.2)	96.2 (9.87)
EasyOne1 vs EasyOne2	**Overall (N=49)**	**Males (N=17)**	**Females (N=32)**
Age (years) – mean (SD)	35.1 (11.4)	32.8 (10.4)	37.4 (12.1)
Age ≤ 30 years – N (%)	23 (46)	11 (47)	12 (52)
Ethnicity- N (%)			
Caucasian	43 (88)	15 (88)	28 (88)
Asian	4 (8)	2 (12)	2 (6)
Other mixed	2 (4)	0 (0)	2 (6)
Weight (Kg) – mean (SD)	68.1 (10.1)	76.1 (9.3)	63.7 (7.7)
Height (m) – mean (SD)	1.71 (0.11)	1.82 (0.72)	1.65 (0.86)
FEV_1EasyOne 1(L) – mean (SD)	3.50 (0.85)	4.33 (0.63)	3.05 (0.58)
FEV_1EasyOne 2 (L) – mean (SD)	3.46 (0.84)	4.27 (0.62)	3.03 (0.58)
FVCEasyOne1(L) – mean (SD)	4.31 (1.05)	5.45 (0.64)	3.71 (0.65)
FVCEasyOne2 (L) – mean (SD)	4.27 (1.04)	5.38 (0.65)	3.68 (0.66)
FEV_1 EasyOne1 mean (SD) percent predicted	95.8 (11.1)	92.8 (12.2)	97.4 (10.2)
FEV_1 EasyOne2 mean (SD) percent predicted	94.8 (11.2)	91.5 (12.1)	96.5 (10.5)
FVCEasyOne1mean (SD) percent predicted	97.4 (9.9)	96.1 (11.1)	98.1 (9.3)
FVCEasyOne2mean (SD) percent predicted	96.5 (10.2)	94.8 (11.1)	97.4 (9.7)

Table 3 shows the mean of FEV_1 and FVC as measured in the PIAMA birth cohort at the age of 16 years, before and after correction for the systematic differences.

The mean difference reduces from 0.37 L to 0.13 L for FEV_1 and 0.44 L to 0.07 L for FVC after correction.

Table 2. Mean differences (with confidence intervals): Masterscreen vs EasyOne1 and EasyOne1 vs EasyOne2, overall and by age and sex.

		FEV1 (L)		FVC (L)	
	N	**Mean diff.**	**95 % CI**	**Mean diff.**	**95% CI**
Masterscreen –EasyOne1					
Overall	49	0.24	(0.19 ;0.26)	0.37	(0.33; 0.41)
≤30 years	32	0.23	(0.18; 0.27)	0.37	(0.31; 0.42)
>30 years	17	0.23	(0.17; 0.29)	0.38	(0.33; 0.44)
Males	15	0.26	(0.18; 0.35)	0.42	(0.31; 0.53)
Females	34	0.21	(0.18; 0.24)	0.35	(0.31; 0.39)
EasyOne1 –EasyOne2					
Overall	49	0.03	(0.01 ;0.06)	0.04	(0.01; 0.06)
≤30 years	23	0.03	(-0.00; 0.08)	0.04	(0.00; 0.08)
>30 years	26	0.03	(0.00; 0.06)	0.03	(-0.00; 0.07)
Males	17	0.06	(0.00; 0.11)	0.06	(0.01; 0.12)
Females	32	0.02	(0.00; 0.05)	0.02	(-0.00;0.05)

Table 3. Means of corrected lung function measurements from PIAMA lung function data.

	Uncorrected Mean (95% CI)	**Corrected Mean (95% CI)**
Overall FEV1 (L)	3.81 (3.75 to 3.86)	3.94 (3.89 to 4.00)
FEV1_EasyOne1 (L)	3.65 (3.58 to 3.72)	3.88 (3.81 to 3.95)
FEV1_Masterscreen (L)	4.03 (3.95 to 4.11)	4.03 (3.95 to 4.11)
Mean difference (L)	**-0.37 (-0.47 to -0.26)**	**-0.13 (-0.24 to -0.03)**
Overall FVC (L)	4.48 (4.42 to 4.55)	4.70 (4.64 to 4.77)
FVC_EasyOne1 (L)	4.30 (4.21 to 4.38)	4.67 (4.59 to 4.76)
FVC_Masterscreen (L)	4.74 (4.64 to 4.84)	4.74 (4.64 to 4.84)
Mean difference (L)	**-0.44 (-0.56 to -0.31)**	**-0.07 (-0.19 to 0.07)**

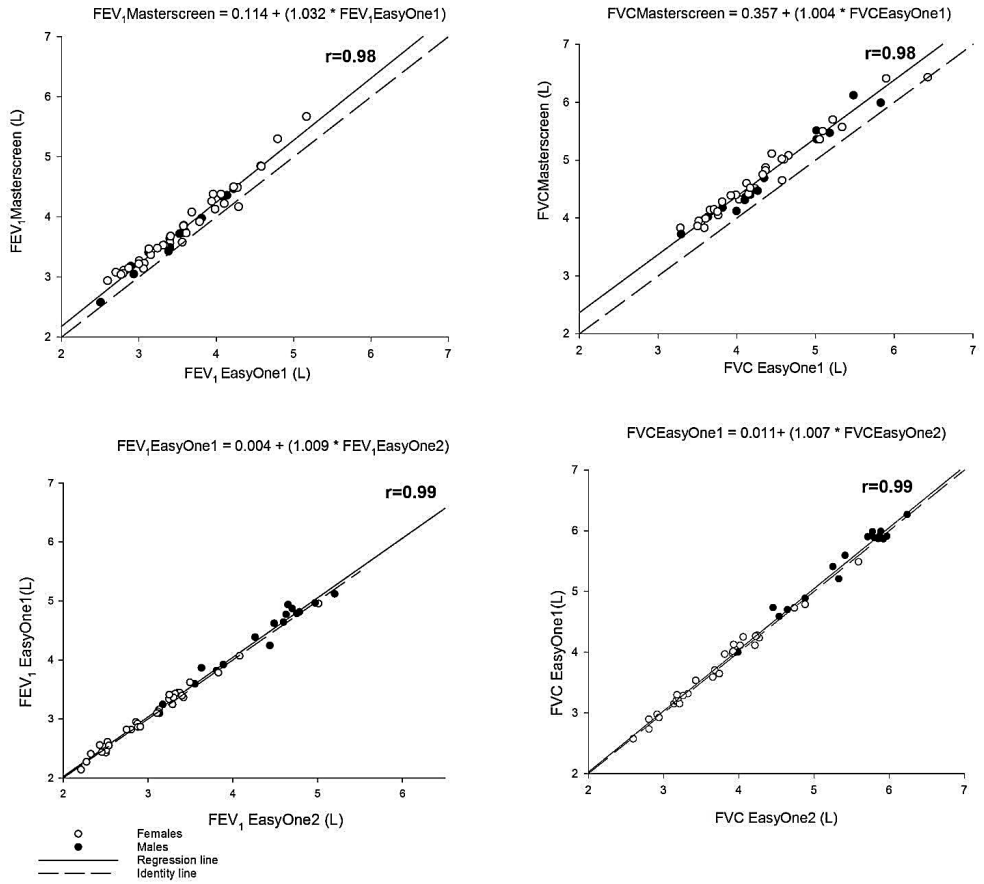

Figure 1. Correlation between measurements from the first comparison series (Masterscreen and EasyOne1 spirometer, upper panels) and the second series (EasyOne1 spirometer from the first series to another EasyOne2 spirometer of the same generation, lower panel).

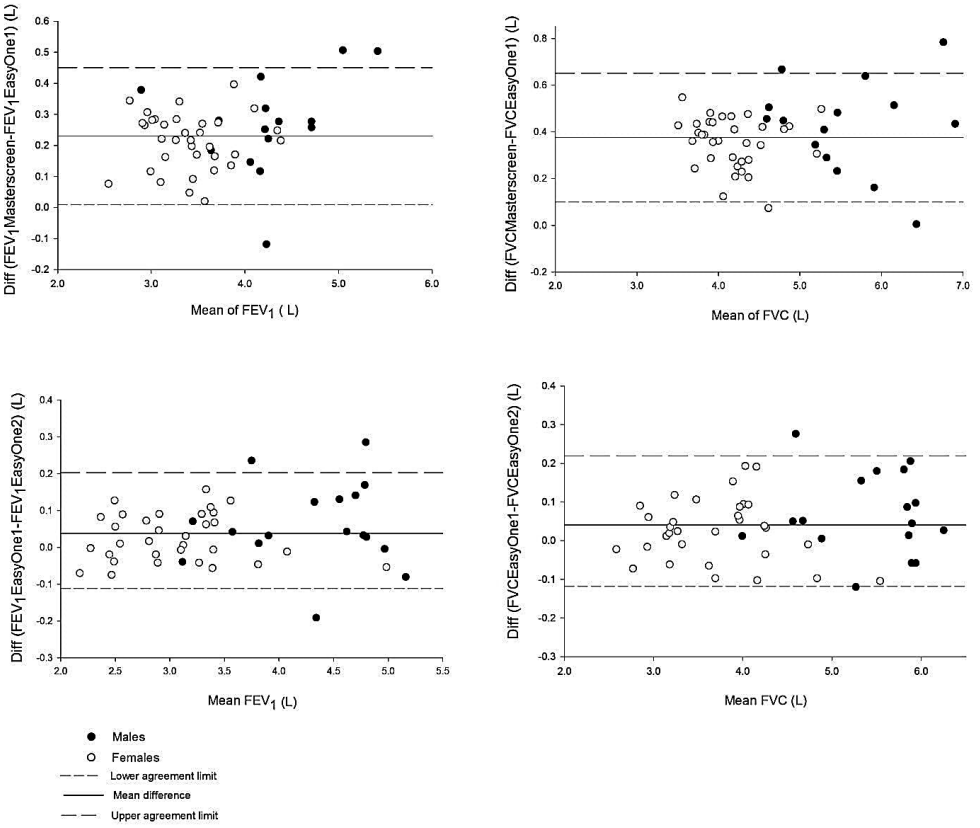

Figure 2. Bland and Altman plots of FEV_1 and FVC measurements from the first test series: Masterscreen and EasyOne1 spirometer (upper panels) and the second test series:EasyOne1 spirometer from the first series to another EasyOne2 spirometer of the same generation (lower panel).

DISCUSSION

We compared FEV_1 and FVC measurements from two different, widely used spirometers, the EasyOne and Masterscreen pneumotachograph and found that the EasyOne spirometer provided on average systematically lower measurements than the Masterscreen pneumotachograph. We also investigated the agreement between two EasyOne spirometers of the same generation and found that measurements were comparable, but with a small significant difference.

In epidemiological studies, lung function measurements can be performed using more than one spirometer of the same type or different types. This study showed a systematic difference between two types of spirometers used in the PIAMA birth cohort study.[15] We conducted this experiment in healthy volunteers for which the mean percent predicted FEV_1 and FVC was expected to be close to 100%. Based on reference equations provided by the GLI,[14] for none of the spirometers the mean percent FEV_1 and FVC was exactly 100%, but percentages were closer to 100% for the Masterscreen pneumotachograph than the EasyOne1 especially for FEV_1. The lower percent predicted lung function for the EasyOne1 suggests that the EasyOne spirometer may be more likely to overestimate the percentage of subjects with a clinically low lung function in a setting where different spirometers are used. This has been previously demonstrated in a comparison involving the EasyOne spirometer and a water-sealed spirometer (Collins, Stead-Wells) where underestimated values of both FEV_1 and FVC from the EasyOne spirometer and consequently higher prevalence rates of airway obstruction were observed.[16] It is important to note that the GLI reference equations are not universally applicable. However, these equations are based on an extensive database and studies in the Netherlands have shown that measurements in the Dutch population generally agree with the GLI references values in adults.[17] We, therefore, believe these equations are most likely suitable for our current study population as the Masterscreen-EasyOne comparison population was 100% Dutch. It is advised that regardless of which reference equations are used, clinical decisions should never be based solely on lung function test results but backed up with complementary laboratory clinical and physical findings.[18]

Several studies have conducted similar experiments comparing different types of spirometers, handheld/office and standard laboratory spirometers both in clinical and research settings, [2-4,19-22] with the comparisons also used as a quality control procedure in international multicentre epidemiological studies.[23,24] High correlations were observed throughout these studies, but significant systematic differences between spirometers in some of the studies [2,19,20] suggest that measurements from different spirometers are not always comparable. Kunzli et al., [4] conducted a study comparing eight flow sensing spirometers of the same type (Sensormedics 2200) and found that the new generation of Sensormedics (V_{max}) gave systematically lower results than the older generation. Based on

this comparison, an informed decision on the choice of spirometers to use for their follow up study was made by excluding the new generation spirometers in the SALPADIA cohort. Similar practical changes were made in another study based on a similar comparison.[23] Small systematically lower FVC and FEV_1 at follow-up, may eventually translate into erroneous deficits of lung function in the studied population, leading to erroneous conclusions about the effect of environmental, biologic or life-style factors on lung function changes.[2] Use of different types of lung function spirometers in the same study can be less detrimental if comparability is established and if necessary any systematic differences corrected.

The source of the observed differences between the Masterscreen pneumotachograph and the EasyOne spirometer is unclear. The Masterscreen pneumotachograph was routinely calibrated for each session as per requirement. The EasyOne spirometers are made to require no calibration but were occasionally checked using a calibration syringe. Both spirometers were therefore thoroughly checked as regards calibration such that chance that the observed differences are due to calibration differences are minimal. However, the following limitations should be considered; two experienced technicians performed the first test series (one for the Masterscreen pneumotachograph and one for the EasyOne) and one of them performed all measurements of the second test series. We designed the comparison of the Masterscreen pneumotachograph and EasyOne spirometers such that different technicians operated the different spirometers to imitate a real multicentre study. While the technicians were highly trained and experienced, due to the study design it was impossible to disentangle differences between spirometers from differences between technicians. Consequently, part of the observed difference between spirometers may be attributable to differences between technicians. The provided correction equation thus simultaneously corrects for the technician and device effect and may not be generalizable to other studies where different technicians are involved. However, it is expected that the calibration method can be applied accordingly. We were not able to assess the external validity of the correction for spirometry measurements outside the PIAMA population, but it has been used before to correct spirometry measurements [6] and the method has been validated in other fields of epidemiology.[25] We used self-reported instead of measured height and weight for the 98 (in total) volunteers that participated in the comparisons of the spirometers. Since spirometers were compared within persons, and consequently height and weight did not differ between the spirometers that were compared within a series, this does not affect the observed differences between spirometers. Self-reported height might be a source of bias in the GLI equations as height values may be over/underreported. Weight is not used in the GLI equations to estimate percent predicted lung function and therefore poses no risk of bias. Studies of the agreement between self-reported and measured weight and height provided inconsistent results, some suggested good agreement,[26,27] while others reported significant discrepancies mainly in overweight/obese individuals.[28,29] It is also not clear to what extent the systematic differences between the two spirometers

can be attributed to hardware as computer software has been identified as another major source of discrepancies between spirometers.[30]

The strength of this study is that the order of the spirometers was randomized to minimize influences of personal characteristics and differences due to study design. We observed high precision of the regression parameter estimates, which highly suggests that the sample size in our experiment is not a concern.

CONCLUSION

We observed systematic differences between lung function measurements from two spirometers of different types. Epidemiological researchers need to be aware of these potential systematic differences and correct for them in the analyses using methods such as regression calibration.

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CHAPTER 5

5

Timing of secondhand smoke, pet, dampness or mould exposure and lung function in adolescence

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Thorax. (2019) Nov 20. thoraxjnl-2019-213149

ABSTRACT

Background: The relevance of timing of exposure in the associations of secondhand tobacco smoke (SHS), pets, and dampness or mould exposure with lung function is unclear. We investigated the relevance of timing of these exposures for lung function in adolescence.

Methods: We used data from participants of the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort with spirometric measurements at ages 12 and 16 (N=552). Data on residential exposure to SHS, pets, and dampness or mould were obtained by repeated parental questionnaires. We characterized timing of exposure through longitudinal patterns using latent class growth modelling and assessed associations of these patterns with forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) at ages 12 and 16 and FEV_1 and FVC growth between ages 12 and 16 using linear regression models.

Results: Childhood SHS exposure was associated with reduced FEV_1 growth/year (95% Confidence Interval) [-0.34 % (-0.64 to -0.04%)]. Late childhood and early-life pet exposure were associated with increased FEV_1 growth [0.41 % (0.14 to 0.67%) and reduced FVC growth [-0.28% (-0.53 to -0.03%)] respectively compared with very low exposure. Early-life dampness or mould exposure was associated with reduced lung function growth. All time windows of SHS exposure tended to be associated with lower level of attained lung function and pet exposure tended to be associated with higher FEV_1.

Conclusion: SHS exposure during childhood could lead to reduced lung function growth and lower level of attained lung function in adolescence. While pet exposure in late childhood may not adversely affect lung function, early childhood pet exposure may slow down FVC growth in adolescence.

BACKGROUND

Household environmental exposures such as secondhand tobacco smoke (SHS), pets, and dampness or mould are modifiable risk factors for adverse respiratory health effects and lung function deficits in children.[1]

Associations of SHS exposure with lung function in children have been reported across cross-sectional and longitudinal studies,[2-8] but evidence is inconsistent. While some studies have reported associations of early-life SHS exposure with lower lung function,[9,10] another study has reported no adverse associations of current SHS exposure between 9-15 years with lung function in adolescents aged 9-15 except in wheezing children.[11] SHS exposure during infancy was associated with reduced growth of pulmonary function in children aged 8-17 years[12] and in adolescent girls only [9] in some studies, but another study has also reported no associations of SHS exposure with lung function growth except in male children with lower lung function at baseline aged 5 -15 years.[13]

Few studies have investigated associations of pet exposure with lung function in childhood and adolescence. The Avon Longitudinal Study of Parents and Children (ALSPAC) study showed no association between pet exposure and forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) at 8 years,[14] but current pet exposure was associated with lower FEV_1 and FVC in 11-year-olds in the Seven Northeastern Cities (SNEC) study.[15] Another study investigated association of pet exposure with lung function in adolescence and found dog and/or cat exposure to be associated with higher lung function in asthmatic girls.[1] None of these studies, however, assessed associations of pet exposure with lung function growth.

There is limited literature on associations of dampness or mould exposure with lung function. Small reductions in lung function have been reported for current dampness or mould exposure in children aged 6-12 years.[16] No study has investigated associations of dampness or mould exposure with lung function growth in adolescence.

The inconsistency observed in the above-mentioned studies could be attributed to different ranges of ages studied, different exposure assessments and different study designs. Currently, focus on associations of longitudinal patterns of SHS, pet, and dampness or mould exposures with lung function and lung function growth is rare. However, it may give insights into relevance of timing of exposure, potential windows of susceptibility and consequently windows of opportunity for prevention of lung function growth deficits, which have long-term health consequences beyond adolescence.[17]

We aimed to investigate associations of timing of SHS, pets, and dampness or mould exposure with lung function growth from ages 12 to 16 and lung function level attained at ages 12 and 16 using longitudinal patterns of exposure from pregnancy till 12 years. Potential modifications of associations by sex were explored as these have been suggested for SHS[9] and pet exposure.[15]

METHODS

Data were obtained from the Dutch population-based Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort that has been described previously in detail.[18] In brief, pregnant women were recruited and baseline study population consisted of 3963 children born between 1996/97. Information on residential exposures, health and lifestyle characteristics was obtained by parental questionnaires completed during pregnancy, 3 months after birth, annually from age 1 to 8, and then at ages 11, 14, 16 (children who participated in the medical examination) and 17. Medical examinations were performed at ages 8, 12 and 16. Current study population consists of children with lung function measurements at both ages 12 and 16, and data on SHS, pet and/or dampness or mould exposure (N=552). Ethical approval was obtained from participating institutes (Ethical approval numbers: Rotterdam, MEC 132.636/1994/39 and 137.326/1994/130; Groningen, MEC 94/08/92; Utrecht, MEC-TNO 95/50) and informed consent was obtained from parents, or legal guardians and participants.

Exposure assessment

Exposure was defined based on questionnaires administered from pregnancy (SHS and pets) or from 3 months (dampness or mould) until age 12.

Secondhand smoke

SHS exposure during pregnancy was defined as maternal smoking during the first 4 weeks of pregnancy. After birth until age 12, SHS exposure was defined as any smoking in the home, assessed by the question '*Does anyone smoke in the house*' (*yes, yes but less than once a week, never)* dichotomized as yes (for all yes responses), and no (never)).

Pet exposure

The question '*Do you keep a dog/cat/rodent indoors?*' (*yes, no)* asked separately for each pet was used to assess exposure to pets.

Dampness or mould

The question *'Have you seen any moisture stains or mould on the ceiling or walls in the last 12 months?*' (*yes, no)* was used to assess dampness or mould exposure. Assessment was restricted to presence of dampness or mould in the living room or child's bedroom because this is where children are expected to spend most of their time.

Longitudinal patterns of exposure

Time-varying responses to questions on SHS, pets, and dampness or mould exposures were characterized into longitudinal exposure patterns from pregnancy until age 12 using

Latent Class Growth Modelling (TRAJ procedure in SAS 9.4, Cary, USA).[19] The procedure allocates individuals into patterns based on posterior probabilities. To establish number of exposure patterns, we first assumed one constant pattern by specifying the intercept and added additional patterns until model performance according to the Bayesian Information Criterion (BIC) was no longer improved. Final choice of number of patterns was based on model with smallest BIC, and practical plausibility, e.g. groups with less than 2% class membership or groups with similar shapes were combined as these did not provide new information regarding exposure patterns. All children with data on exposure for at least one time point (missing data for one or more time points) were included in the latent class modelling procedure.

Outcome

Lung function

Lung function (forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)) measurements were performed during medical examinations at ages 12 and 16. Details of the measurements have been described elsewhere.[20] 1292 and 721 children had successful lung function measurements at ages 12 and 16, respectively, and 552 had measurements at both ages (Figure E1). Percentage of annual lung function growth was calculated by taking log of the difference in lung function between age 12 and 16 and then dividing this difference by time (in years) between the two measurements. We used EasyOne spirometers (NDD Medical Technologies, Inc, Switzerland) at age 12 and both Jaeger Masterscreen pneumotachograph (CareFusion, Yorba Linda, California, USA) and EasyOne spirometers at age 16.[21] All measurements were performed following American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.[22] At least three acceptable manoeuvres were required for each child. We also included measurements, which did not meet these criteria (difference between largest and next largest value ≤ 150 mL for FEV_1 and FVC) but were obtained from technically acceptable trials with differences between largest and next largest values for FEV_1 and FVC ≤ 200 mL (N=125 at age 12 and N=67 at age 16).

Confounders

The following a priori selected variables that were obtained during the medical examination and from parental questionnaires were considered as potential confounders based on evidence from literature on their relationship with lung function and/or the respective exposures: sex, height, weight and age at the time of medical examination were included as predictors of lung function;[22] height, weight and age were log-transformed as described elsewhere [23,24] in view of the strongly non-linear relationships between lung function and these factors. Maternal and paternal allergy (defined as positive if the father and/or mother ever had asthma, were allergic to house dust, house dust mite or pets, or had hay fever) was

adjusted for as it predisposes to asthma and allergic disease and may be associated with (avoidance of) exposure.[25,26] We also adjusted for respiratory infections during the 3 weeks before lung function measurement as this may influence lung function, breastfeeding at 12 weeks (yes/no) because breastfeeding has been shown to enhance lung volume in children,[27] parental country of birth (Netherlands, yes/no) to account for ethnicity differences in lung function,[22] as well as gas cooking at 3 months (yes/no), estimated annual average NO_2 concentrations at the birth address, and birth weight because these are considered/ known risk factors of lower lung function.[21,28-30] Models with patterns of pet exposure were additionally adjusted for maternal smoking during pregnancy, SHS exposure and presence of dampness or mould in the child's home during the first year; models with patterns of SHS exposure were additionally adjusted for pets and dampness or mould in the child's home during the first year, and models with patterns of dampness or mould exposure were additionally adjusted for maternal smoking during pregnancy, SHS and pets in the child's home during the first year. These mutual adjustments of the respective exposures (defined as binary variables, yes/no) were performed to take into account the relationship of an exposure and lung function in the presence of the other two respective exposures.

Statistical analyses

We used linear regression models to assess associations of longitudinal exposure patterns with growth in FEV_1 and FVC between 12 and 16 years and attained levels of FEV_1 and FVC at age 12 and 16. FEV_1 and FVC were log-transformed because of their strong non-linear relationships with age, height and weight as reported in the Harvard Six cities study[23] and used in other studies including our own.[20,24,31] Longitudinal exposure patterns were included as independent variables. Associations with lung function growth are expressed as percent difference in growth per year and associations with attained level of lung function at age 12 and 16 are expressed as percent difference, and both relate to geometric mean lung function variables calculated from estimated regression coefficients b as $(e^b-1) \times 100$. Exposure patterns defined as 'very low' were used as reference categories. To account for uncertainty in allocation of patterns, we created multiple records for each participant (one for each exposure pattern) and weighted records by respective posterior probabilities in all analyses. Crude models assessing lung function growth were adjusted for sex, log transformations of differences in height, weight and age between lung function measurements and crude models of attained level of FEV_1 or FVC at age 12 and 16 were adjusted for sex, log transformations of height, weight and age. All models were further adjusted for all other mentioned confounders in adjusted analyses. We used the STROBE cohort reporting guidelines [32]. Statistical analyses were performed in SAS 9.4 (Cary, USA) at 0.05 level of significance.

Sensitivity analyses

As part of sensitivity analyses, we investigated sex interactions as development and progression of certain common respiratory diseases has been found to differ by sex.[33,34] We also performed stratified analyses by parental allergy status. We excluded children who reported respiratory infection in the previous 3 weeks before lung function measurements. We also repeated analyses after excluding both, childhood asthmatics until age 8 and children whose parents had removed pets because of any family member allergies (N=129) as it has been suggested that childhood asthma may influence pet avoidance and this may distort associations of pet exposure.[35] In addition, we repeated analyses excluding active smokers at either age 14 or 16 (N=44).

RESULTS

Table 1 shows study population characteristics. 12.4% of the children were exposed to maternal smoking during pregnancy; 41.1% owned pets, and 9.1% were exposed to dampness or mould in the first year of life. Mean (SD) FEV_1 was 2733 (434) mL and 3939 (705) mL at ages 12 and 16, respectively. Mean (SD) FVC was 3244 (511) mL and 4710 (847) mL at ages 12 and age 16, respectively (Table 2). Boxplots indicating distribution of lung function values across different patterns of exposures of interest have been presented in Figures E2-E4 of the supplementary file. There were fewer children who owned pets, who were exposed to maternal smoking during pregnancy and more children with highly educated parents in the study population than in the baseline population (Table E1). Compared with the study population, excluded population of children with lung function measurements at age 12, but not at age 16 had more boys, fewer children breastfed for 12 weeks or more, fewer children exposed to gas cooking, fewer children of low educated parents and more children exposed to SHS during the first year (Table E1). Table E2 presents frequencies of surveys with missing exposure data. More than 92% of the children had complete SHS and pet exposure data from pregnancy till age 12; 76% of the children had complete dampness or mould exposure data from 3 months till age 12 and 22% had one missing value for that period. For all three exposures, no more than 1% of the children had 3 or more missing values.

We identified four (SHS and dampness or mould) and five (pets) exposure patterns including for every exposure; a very low probability of exposure pattern throughout childhood, higher probability of exposure in early-life and higher probability of childhood exposure (Figure 1). We also identified a persistently low exposure pattern of SHS exposure. Univariate associations of patterns of exposure with selected participant characteristics are presented in Table E2. Very low exposure patterns were generally characterized with children with higher odds of having allergic and/or highly educated parents while high persistent exposure patterns were characterized by children with low educated and/or less allergic parents.

Table 1. Study population characteristics.

Characteristics	Study population (N=552)	
	(n/N)	(%)
Parental allergy		
Allergic mother	178/552	32.2
Allergic father	187/364	33.9
Boys	251/552	45.4
Presence of pets at 1 year	226/489	41.1
Dampness/mould at 1 year	49/540	9.1
Breastfeeding > 12 weeks	330/552	59.7
Gas cooking at 3 months	471/549	85.8
Maternal smoking during pregnancy	68/548	12.4
Indoor SHS exposure at 1 year	109/551	19.7
Parental education		
Low	38/552	6.8
Intermediate	167/552	30.2
High	347/552	62.8
Parental country of birth (Netherlands)	530/545	97.2
Asthma until 8 years	129/552	23.3
Respiratory infections 3 weeks before lung function measurement		
12 years	182/552	32.9
16 years	233/552	42.2
Active smokers at age 14/16 years	44/552	7.9

Table 2. Age, anthropometric measures and lung function measurements.

Variable	Age 12 (Mean, SD)	Age 16 (Mean, SD)	(Mean Difference, SD)
Age (years)	12.6 (0.3)	16.3 (0.2)	3.7 (0.4)
Weight (kg)	48.2 (8.6)	64.1 (9.9)	15.9 (7.4)
Height (cm)	160.4 (7.5)	175.5 (8.5)	15.1 (7.9)
FEV_1 (mL)	2733 (434)	3939 (705)	328 (163)
FVC (mL)	3244 (511)	4710 (847)	400 (191)
Girls (N=301)			
Age (years)	12.7 (0.4)	16.3 (0.2)	3.6 (0.5)
Weight (kg)	48.7 (8.6)	60.8 (8.7)	12.1(5.9)
Height (cm)	160.8 (7.2)	170.2 (6.1)	9.4 (4.8)
FEV_1 (mL)	2751 (422)	3517 (440)	766 (316)
FVC (mL)	3218 (509)	4170 (517)	952 (370)
Boys (N=251)			
Age (years)	12.6 (0.3)	16.3 (0.2)	3.7 (0.5)
Weight (kg)	47.4 (8.5)	67.9 (9.9)	20.5 (6.4)
Height (cm)	159.9 (7.9)	181.7 (6.5)	21.8 (5.3)
FEV_1 (mL)	2711 (448)	4444 (626)	1733 (435)
FVC (mL)	3274 (513)	5359 (697)	2085 (506)

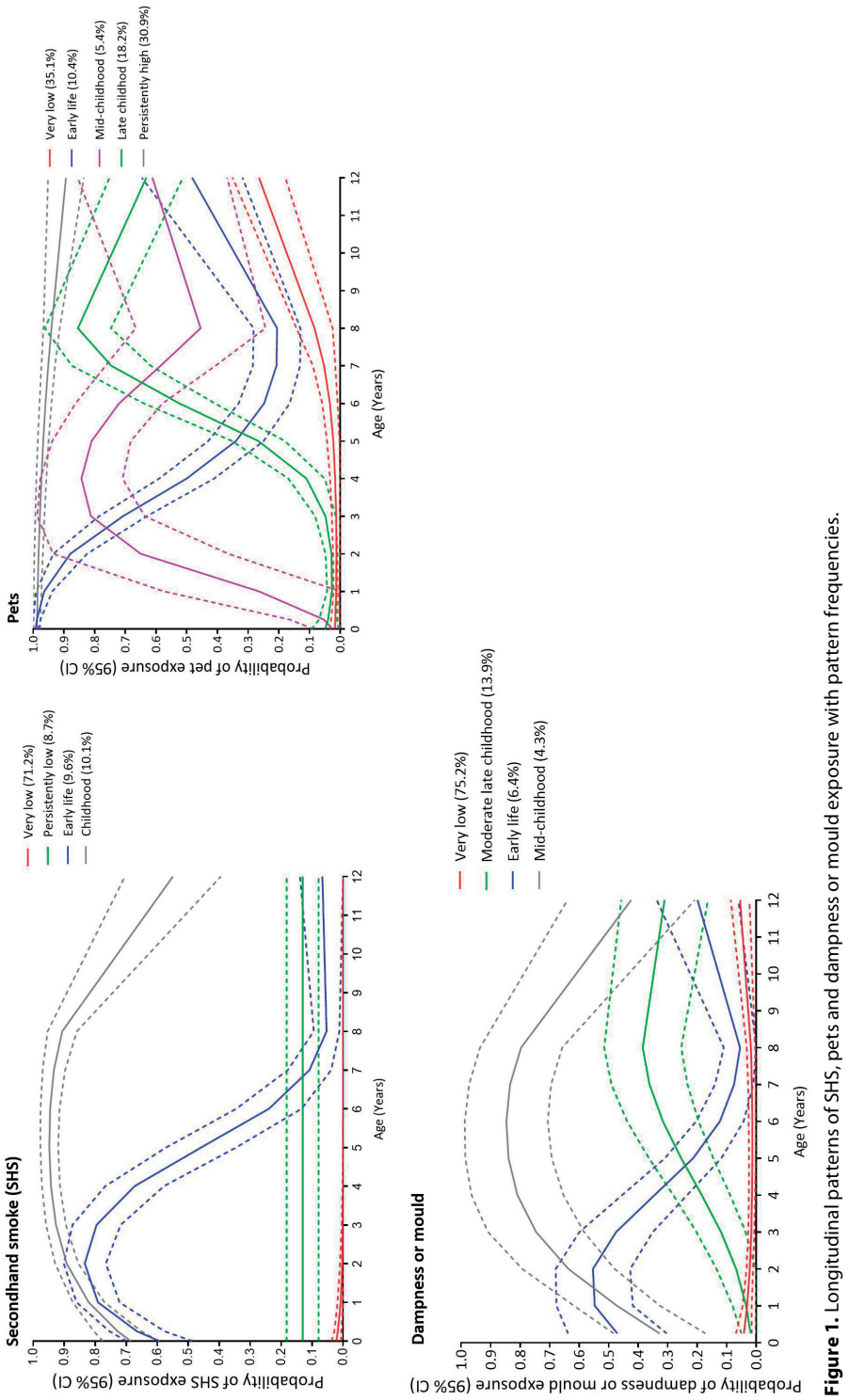


Figure 1. Longitudinal patterns of SHS, pets and dampness or mould exposure with pattern frequencies.

Associations of exposure patterns with lung function growth

Crude associations of exposure patterns with lung function growth and attained level of lung function were generally similar to adjusted associations (Tables 3 and E4).

Higher probability of childhood SHS exposure was associated with reduced FEV_1 growth between ages 12 and 16 [percent difference in growth/year (95% confidence interval) -0.34 % (-0.64 to -0.04%) compared with very low exposure (Table 3). In contrast, higher probability of early-life and persistently low SHS exposure were not negatively associated with lung function growth.

Higher probability of late childhood pet exposure was associated with increased FEV_1 growth [0.41 % (0.14 to 0.67 %) compared with very low exposure while persistently high and early-life pet exposure were associated with reduced FVC growth; [-0.33 % (-0.53 to -0.14 %)] and [-0.28% (-0.53 to -0.03%)] respectively.

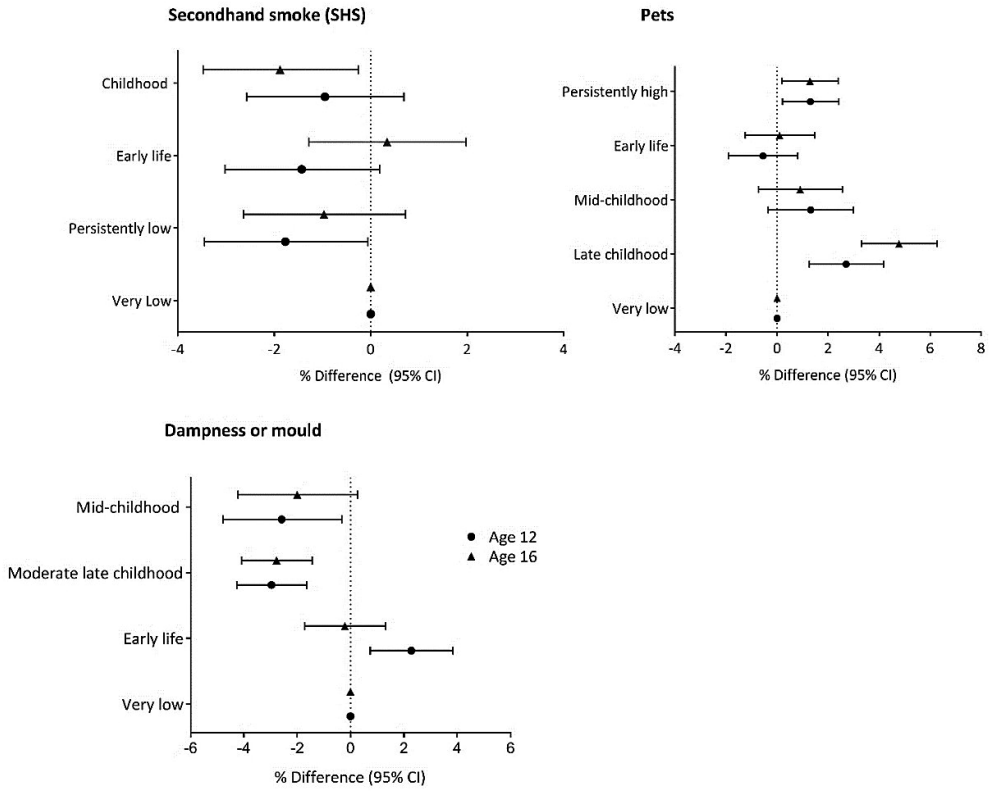
Higher probability of early-life dampness or mould exposure was associated with both reduced FEV_1 and FVC growth (Table 3).

Associations of exposure patterns with lung function level

We observed lower lung function levels in children with childhood SHS exposure e.g. percent difference (95% confidence interval) -1.88% (-3.47 to -0.26 %) for FEV_1 at age 16, as well persistently low exposure -1.77% (-3.45 to -0.06 %) for FEV_1 at age 12. (Figure 2, Table E4). Higher probability of SHS exposure in early-life was associated with lower attained levels of FVC especially at age 12 compared with very low SHS exposure (Figure 3).

Exposure to pets during mid and late childhood was associated with higher attained levels of lung function, e.g. percent difference 4.78% (3.32 to 6.27%) in FEV_1 at age 16 and 2.21 % (0.98 to 3.45%) in FVC at age 16 for late childhood exposure. All other pet exposure patterns (i.e. probability of early-life exposure and persistently high exposure also tended to be associated with higher attained level of FEV_1 and FVC at both 12 and 16 years (Figures 2 and 3, Table E4). Moderate late childhood and mid-childhood dampness or mould exposure were associated with lower FEV_1 and FVC at ages 12 and 16. In contrast, we observed higher FEV_1 and FVC at age 12 with early-life dampness or mould exposure (Figures 2 and 3, Table E4).

Figure 4 shows distinct patterns of exposure to cats, dogs and rodents separately. In general, similar patterns of exposure were observed across different pets. Associations of individual pet exposure patterns with lung function were complex. Higher probability of cat and rodent exposure in early-life was associated with reduced FEV_1 and FVC growth but late childhood exposure to these pets was generally associated with higher level of attained FEV_1 and FVC. Dog exposure was generally associated with lower lung function and reduced lung function growth (Table 4, Figures 5 and 6).



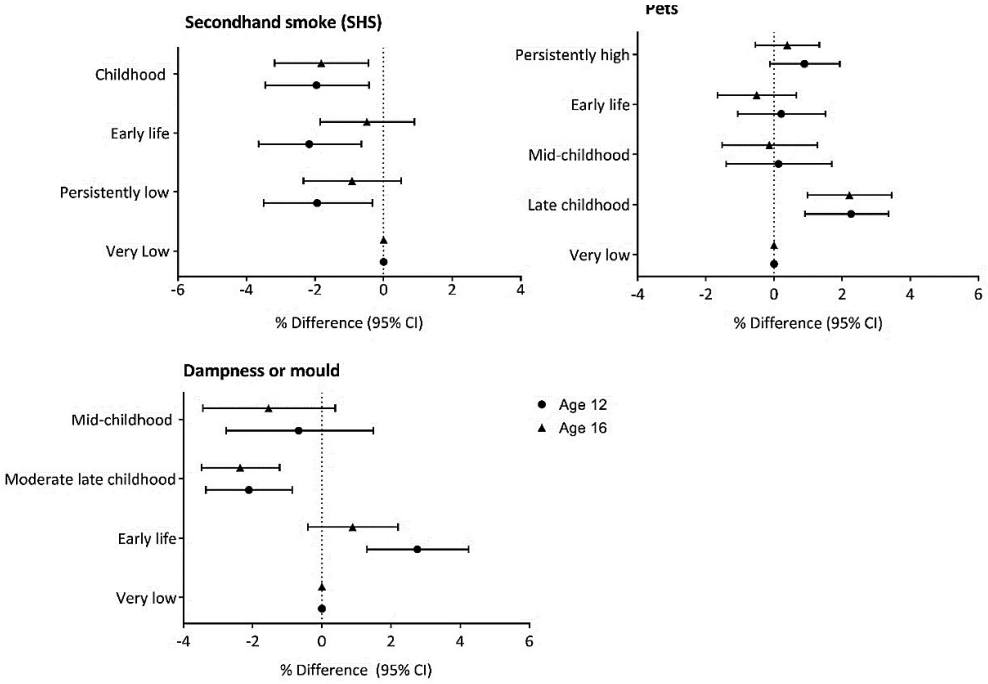
α Adjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Figure 2. Associations of longitudinal patterns of SHS, pets and dampness or mould exposure with FEV_1 level (% difference) at age 12 and 16.[α]

Sensitivity analyses

Associations between all exposures of interest and lung function growth were inconsistent between boys and girls (Table E5). Both boys and girls tended to have lower FEV_1 and FVC at age 12 with early-life exposure to SHS though weaker in girls. Boys tended to have higher FEV_1 at age 16 with late childhood pet exposure (e.g. p-value of interaction <0.001, Table E6).

We did not observe different associations with SHS exposure patterns for children of allergic and non-allergic parents, except for stronger associations of SHS exposure with lower attained level of FVC in children of allergic parents (Tables E7 and E8). All patterns of pet



α Adjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Figure 3. Associations of longitudinal patterns of SHS, pets and dampness or mould exposure with FVC level (% difference) at age 12 and 16.[α]

exposure were consistently associated with a higher attained level of lung function at ages 12 and 16 in children of non-allergic parents. Late childhood pet exposure was associated with increased FEV_1 growth in children of allergic parents, but persistently high exposure was associated with reduced FEV_1 and FVC growth in this group. There were generally no differences in association for dampness or mould exposure (Tables E7 and E8).

Excluding children who had respiratory infections during the 3 weeks before lung function measurements did not change results (Tables E9-E10). Results were similar when we excluded asthmatics and children of parents who reported removal of pets due to family allergies (Table E11), but a stronger reduction in the attained level of FVC for pet exposure was observed when we excluded active smokers (Table E12).

Table 3. Associations of longitudinal patterns of SHS, pets and dampness or mould exposure with percent difference in annual growth of FEV1 and FVC from age 12 to 16.

	% difference in FEV1 growth/year (95% CI)		% difference in FVC growth/year (95% CI)	
	Crude (N=552)$^{\alpha}$	**Adjusted (N=525)$^{\beta}$**	**Crude (N=552)$^{\alpha}$**	**Adjusted (N=525)$^{\beta}$**
SHS				
Persistently low vs Very low	0.16 (-0.14 to 0.47)	0.30 (-0.01 to 0.62)	0.20 (-0.11 to 0.50)	0.31 (0.00 to 0.64)
Early-life vs Very low	0.15 (-0.13 to 0.44)	0.31 (0.01 to 0.61)	0.06 (-0.22 to 0.35)	0.32 (0.03 to 0.62)
Childhood vs Very low	-0.48 (-0.76 to -0.19)	-0.34 (-0.64 to -0.04)	-0.28 (-0.56 to -0.00)	-0.04 (-0.33 to 0.26)
Pets				
Early-life vs Very low	-0.03 (-0.28 to 0.21)	0.02 (-0.24 to 0.27)	-0.34 (-0.58 to -0.10)	-0.28 (-0.53 to -0.03)
Mid-childhood vs Very low	-0.23 (-0.52 to 0.07)	-0.09 (-0.40 to 0.21)	-0.16 (-0.45 to 0.13)	-0.04 (-0.34 to 0.26)
Late childhood vs Very low	0.18 (-0.07 to 0.43)	0.41 (0.14 to 0.67)	-0.46 (-0.70 to -0.21)	-0.14 (-0.39 to 0.12)
Persistently high vs Very low	-0.32 (-0.51 to -0.14)	-0.26 (-0.46 to -0.06)	-0.43 (-0.61 to -0.25)	-0.33 (-0.53 to -0.14)
Dampness or mould				
Early-life vs Very low	-0.74 (-1.01 to -0.46)	-0.77 (-1.05 to -0.49)	-0.53 (-0.81 to -0.26)	-0.56 (-0.83 to -0.28)
Moderate late childhood vs Very low	0.10 (-0.14 to 0.35)	0.05 (-0.19 to 0.30)	0.05 (-0.19 to 0.30)	-0.02 (-0.26 to 0.23)
Mid-childhood vs Very low	0.10 (-0.32 to 0.51)	0.04 (-0.37 to 0.46)	-0.18 (-0.59 to 0.23)	-0.23 (-0.63 to 0.18)

α Adjusted for sex, log transformations of differences in height, weight and age between the 12 and 16-year lung function measurements. **β** Adjusted for sex, log transformations of differences in height, weight and age between the 12 and 16-year lung function measurements, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, annual average NO_2 concentration at the birth address, birth weight, dampness or mould in the child's home at 1 year (except in models with dampness or mould exposure), pets in the home at 1 year (except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Table 4. Adjusted associations of longitudinal patterns of cats, dogs and rodents exposure with annual percent growth of FEV_1 and FVC from age 12 to 16.

n=524	**% difference in FEV_1 growth /year (95 % CI)**	**% difference in FVC growth /year) (95 % CI)**
Cats		
Early-life vs Very low	-0.09 (-0.38 to 0.20)	-0.71 (-0.99 to -0.42)
Late childhood vs Very low	0.41 (0.05 to 0.77)	0.08 (-0.25 to 0.43)
Persistently high vs Very low	-0.46 (-0.71 to -0.22)	-0.33 (-0.55 to -0.08)
Dog		
Late childhood vs Very low	-0.18 (-0.61 to 0.26)	-0.34 (-0.76 to 0.08)
Persistently high vs Very low	-0.26 (-0.56 to 0.05)	-0.11 (-0.40 to 0.19)
Rodent		
Early childhood vs Very low	-0.75 (-1.15 to -0.35)	-0.80 (-1.19 to -0.41
Late childhood vs Very low	0.25 (0.01 to 0.48)	0.35 (0.12 to 0.58)
Mid-childhood vs Very low	0.13 (-0.27 to 0.52)	0.28 (-0.10 to 0.67)

Adjusted for sex, log transformations of differences in height, weight and age between the 12 and 16 year lung function measurements, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year, use of gas for cooking at 3 months, annual average NO_2 concentration at the birth address, birth weight, dampness or mould in the child's home at 1 year, respiratory infections in the past 3 weeks before medical examination.

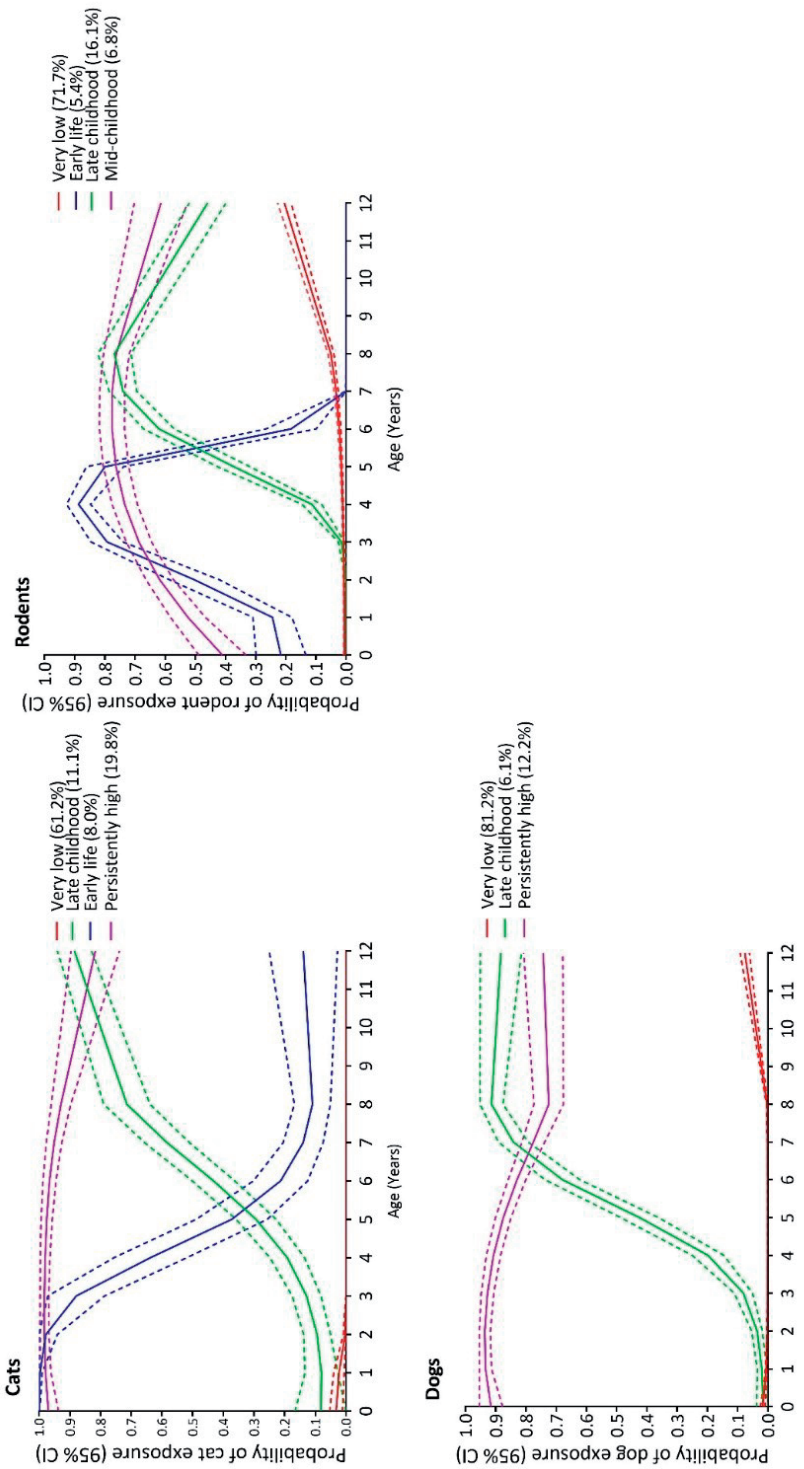
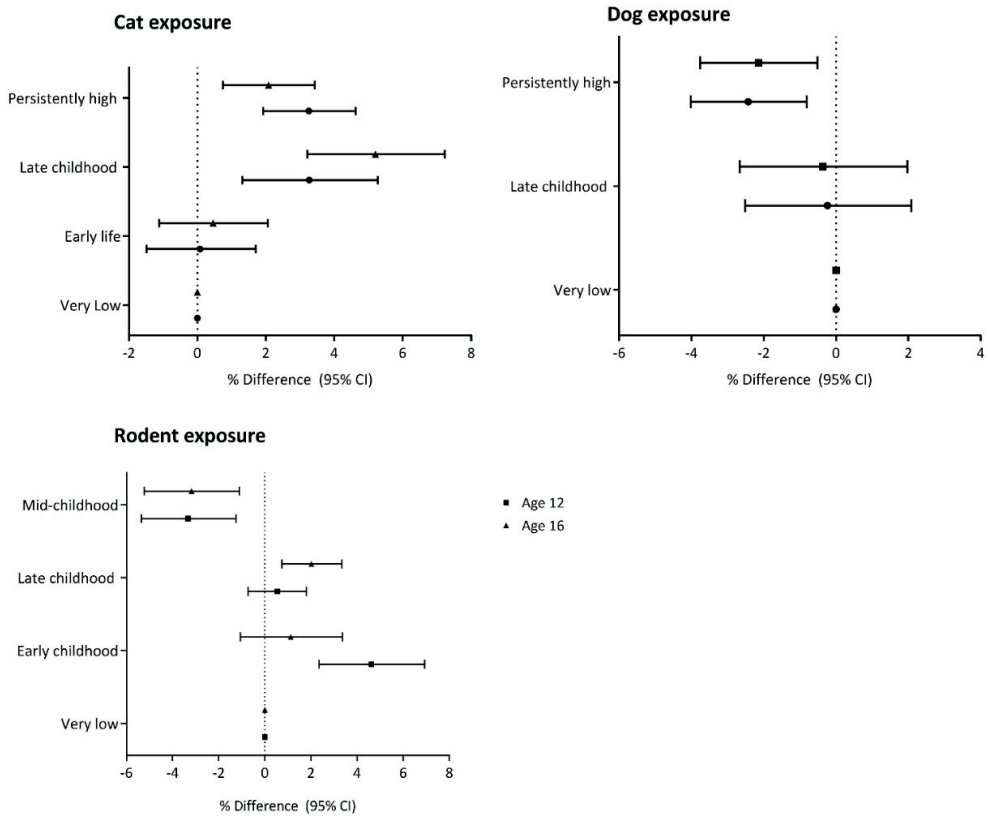
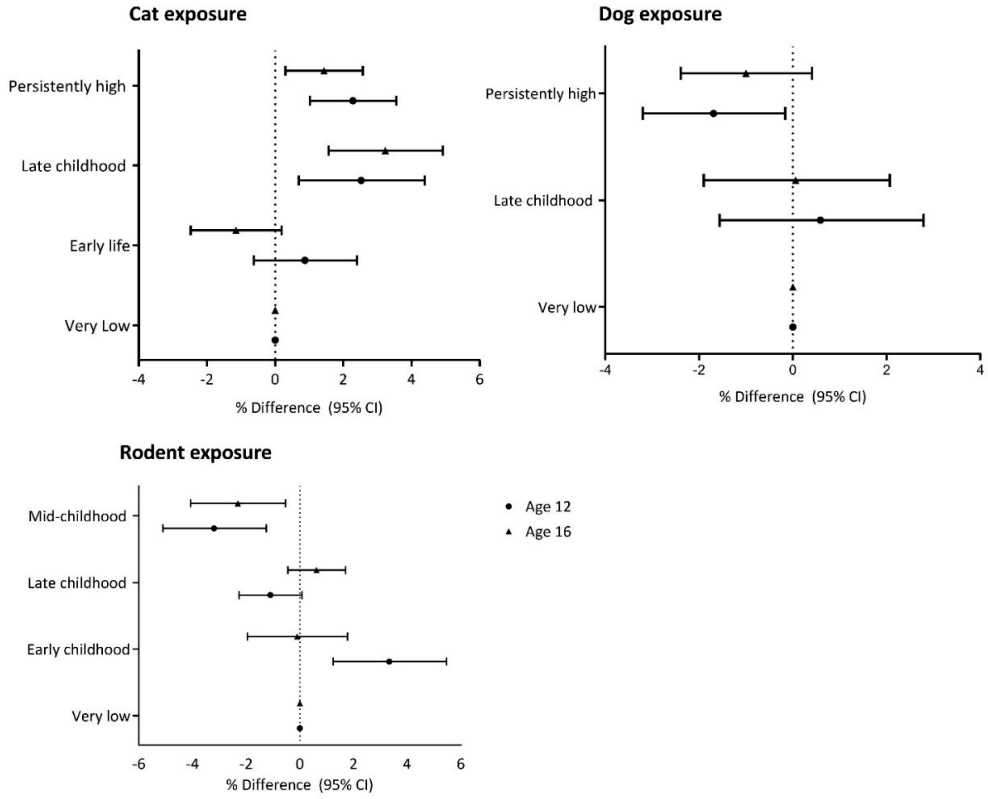


Figure 4. Longitudinal patterns of cats, dogs and rodents exposure with pattern frequencies.



βAdjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year, use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year, respiratory infections in the past 3 weeks before medical examination.

Figure 5. Associations of longitudinal patterns of cats, dogs and rodents exposure with FEV_1 level (percent difference) at age 12 and 16.[β]



βAdjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, indoor SHS at 1 year, use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year, respiratory infections in the past 3 weeks before medical examination.

Figure 6. Associations of longitudinal patterns of cats, dogs and rodents exposure with FVC level (% difference) at age 12 and 16.[β]

DISCUSSION

In our prospective birth cohort, we assessed the role of different timing of exposure in relation to lung function (growth) using longitudinal exposure patterns. Higher childhood SHS exposure was associated with reduced lung function growth and all periods of SHS exposure until age 12 tended to be associated with lower level of lung function attained in adolescence. Late childhood and early-life pet exposure was associated with increased FEV_1 growth and reduced FVC growth respectively, while all pet exposure periods tended to be associated with higher attained level of lung function compared with very low pet exposure. Early-life exposure to dampness or mould was associated with reduced lung function growth and in contrast early-life dampness or mould exposure was associated with higher level of lung function at both ages 12 and 16.

Lung function and secondhand smoke

Our findings suggest that continued exposure to SHS from birth until childhood may lead to reduced FEV_1 growth and lower attained level of lung function in adolescence, indicating possible airway obstruction and reduced lung volume. The associations of continuous SHS childhood exposure with reduced FEV_1 growth are consistent with findings of other longitudinal studies that have studied similar associations.[9,12,13] SHS exposure was associated with lower attained level of FEV_1 at age 16 as reported in multiple studies.[9,34,36] However only one study addressed different timing of SHS exposure and lung function in adolescence [8] and reported, in contrast to our study, no significant associations of SHS exposure at 3 months or at age 16 years with lung function at age 16. Likewise, another study reported no significant associations of current SHS exposure between ages 9-15 with FEV_1 or FVC in children 9-15 years old.[11] We contribute to the increasing body of evidence that suggests that effects of continued SHS exposure during childhood on lung function can persist throughout childhood and into adolescence.[2,6,37] The observed positive associations of early-life SHS exposure with lung function growth in adolescence may point to the possibility that the lungs of children whose parents smoked in early-life but quit in early years of the child may benefit from sustained parental smoking abstinence.

Exact mechanisms by which SHS affects lung function are unclear, but altered organ maturation and immune function have been suggested though mechanisms may vary across phases of lung growth and development, extending from *in utero* to completion of lung growth in late adolescence.[38]

Lung function and pets

Few studies have investigated associations of pet exposure with childhood or adolescent lung function while none have investigated pet exposure and lung function growth. Existing evidence is conflicting as pet exposure has been associated with lower[15] and higher

lung function.[1] Null associations have also been reported.[14] Pet exposure in late childhood was associated with increased FEV_1 growth and higher FEV_1 and FVC in adolescence in our study pointing to either beneficial effects or selection/reverse causation as allergic parents whose children have an increased risk of being allergic may avoid pets (Table E2). Further investigations on pet avoidance due to early childhood respiratory symptoms showed no association between childhood asthma and rhinitis and pattern membership (Table E13) suggesting that asthma and rhinitis in early and mid-childhood were not reasons for parents to avoid pets until late childhood in our cohort. In contrast, early-life and persistently high exposure to pets were associated with reduced FVC growth which may be partly in line with studies that have reported lower lung function in relation to pet exposure. One study reported associations of cat, dog and rodent exposure with higher lung function in adolescents [1] and in children.[14] We observed similar associations with cats and attained level of FEV_1, but early-life cat, rodent and all patterns of dog exposure were associated with reduced and lower FVC (growth). Studies have suggested that IgE-associated inflammation responses could be responsible for allergic lung inflammation due to pet exposure,[39] but this remains controversial as IgE related mechanisms are also attributed to protective effects of asthma and it is unclear what role this could play in improved lung function. The majority of the parents kept one type of pet at a time but some parents kept more than one type of pet (Figure E5). This raises the possibility of pet-pet interactions in relation to lung function, but numbers are too small to explore these interactions in the present study population.

Until now, existing literature focused on pet exposure and lung function in childhood. Our study extends into adolescence and our findings suggest that late childhood pet exposure may be a relevant exposure period in our study but also that high persistent and early-life exposure from birth into adolescence may have adverse effects on FVC growth. Presence of pets in the home which has been linked to higher concentration of endotoxins has also been linked to reduced risks of allergic sensitization and less consistently, asthma.[40-42] However, the relationship with lung function is unclear. A recent review[43] reported weak reductions in FEV_1 and FVC in relation to endotoxin exposure, but the evidence was from occupational studies and in adults. This warrants more studies on pet exposure and lung function (growth) towards adolescence.

Lung function and dampness or mould

Associations with dampness or mould exposure have been reported for respiratory symptoms in children, but rarely with lung function in adolescence. Current dampness exposure was weakly associated with lower FEV_1 in Dutch 8-12 year-olds,[16] but no associations were observed in 6-10-year-old Danish children.[44] Scarcity of evidence for associations between dampness or mould exposure and lung function (growth) in adolescents limits comparisons of our findings though we did not observe consistent associations. Higher lung function at age 12 was observed for early-life exposure but reduced growth between age

12 and 16 was also observed, as well as lower attained level of lung function for moderate and mid-childhood exposure patterns. It has been suggested that complex interactions of factors which are set in motion after inhaling mould fragments, toxins or spores can induce airway inflammation[45] leading to the restricted function of the lungs.

Strengths and limitations

We consider the characterization of exposure from pregnancy/birth until adolescence through longitudinal patterns, investigation of timing of exposure based on repeated exposure assessments and assessment of associations of these longitudinal patterns with lung function growth in adolescence as major strengths and novelty of our study.

Several limitations are considered. Exposure was assessed through self-reports and parents may under- or over-report exposures due to knowledge of negative health effects. However, a multi-cohort validation study (including a subset of our cohort) comparing SHS exposure self-reports and measured air nicotine concentrations showed that self-reported SHS exposure provided valid estimates of reported residential exposure. [46] Visible mould reports have also been shown to be highly correlated with airborne concentrations of fungal spores[47] suggesting self-reported dampness or mould is a good exposure indicator. We performed analyses with raw spirometric data adjusting for age, sex, height and ethnicity. Alternatively, z-scores such as those provided by the Global Lung Initiative, taking into account age, sex, height and ethnicity, might have been used. Z-scores might be better in adjusting for age, sex, height and ethnicity,[48] but their interpretation is less straightforward. We adjusted all models for co-exposures in early-life only and did not take into account co-exposures at different time points as confounders which may result in residual confounding. We used questionnaire responses to assess probability of exposure over time as actual levels of exposure were unknown. It has been shown that parental self-reported exposure is highly correlated with measured nicotine levels,[46], therefore, the effect of lack of levels of exposure data on our findings is likely small.

The 16-year lung function measurements were performed using two different spirometers in two different centres for logistical reasons. We performed a comparison study in healthy volunteers, using the two spirometers to establish a calibration equation which we used to correct for systematic differences.[49] We observed very high correlation between measurements from the two spirometers (0.98-0.99), moreover, we do not expect exposure patterns to be different for measurements performed by either of the spirometers so that effect of using different spirometers is likely very small. There were more children with highly educated parents, fewer children whose parents owned pets, fewer children who were exposed to SHS and more children who were breastfed for more than 12 weeks in the study population than in the baseline population due to loss to follow-up. Highly educated parents may be less likely to keep pets and less likely to smoke affecting generalizability of our findings. However, we do not expect the associations between predictors of the

exposures of interest with lung function to be different from the entire PIAMA cohort. Generalization beyond the Dutch population may, however, be limited in settings with different pet-keeping habits.

In conclusion, our study suggests that for lung function (growth), all time windows of exposure until age 12 may be relevant time windows for SHS exposure and pet exposure. Continued SHS exposure during childhood until age 12 could lead to reduced lung function growth and lower attained level of lung function, but pet exposure in late childhood may not adversely affect lung function. However, early-life pet and dampness or mould exposure could lead to FVC growth deficits in adolescence. While observed effect sizes were small, these may cumulatively add up over time, and translate into important clinical lung function deficits/increments at the population level.

This study advances our understanding of the relevance of the timing of exposure and could provide guidance on the timing and structure of interventions to improve respiratory health.

ACKNOWLEDGEMENTS

The authors would like to thank PIAMA participants who contributed to the study

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Table E1. Characteristics comparisons between baseline population and study population.*

	Baseline (N=3963)		Study population (N=552)		P-value$^{\Omega}$	Lung function data at age 12, but not at age 16 (N=740)$^{\alpha}$		P-value$^{\mu}$
Characteristics	**N**	**%**	**N**	**%**		**N**	**%**	
Parental allergy								
Allergic mother	1237/3963	31.2	178/552	32.2	0.624	252/740	34.0	0.495
Allergic father	1217/3957	30.7	187/364	33.9	0.130	229/740	30.9	0.255
Boys	2054/3963	51.8	251/552	45.4	0.002	383/740	51.1	0.025
Presence of pets at 1 year	1719/3785	45.4	226/489	41.1	0.009	314/650	42.6	0.600
Dampness or mould at 1 year	312/3386	8.4	49/540	9.1	0.243	47/723	6.5	0.087
Breastfeeding > 12 weeks	1892/3896	48.5	330/552	59.7	<0.001	392/737	53.2	0.018
Gas cooking at 3 months	3247/3923	82.7	471/549	85.8	0.076	599/737	81.2	0.032
Maternal smoking during pregnancy	703/3920	17.8	68/548	12.4	0.003	118/736	16.0	0.065
Indoor SHS at 1 year	1059/3963	27.7	109/552	19.7	<0.001	187/739	25.3	0.014
Parental education					<0.001			0.001
Low	502/3812	13.1	38/552	6.8		81/739	10.9	
Intermediate	140 2/3812	36.7	167/552	30.2		263/739	35.5	
High	1908/3812	50.1	347/552	62.8		395/739	53.4	
Parental country of birth (Netherlands)	3485/3700	94.1	530/545	97.2	0.007	699/735	95.1	0.056

Ω: Comparison between baseline population and study population

α: Excluded from population with successful lung function measurements at age 12 (N=1292) because of lack of lung function data at age 16

μ : Comparison between study population and excluded population with lung function data at age 12, but not at age 16

*Characteristics compared using Chi-Square tests

Table E2. Completeness of exposure data from repeated surveys from birth until age 12.

Number surveys with missing exposure data	N (%)
SHS	
0	511 (92.6)
1	33 (6.0)
2	5 (0.9)
3	3 (0.5)
Pets	
0	515 (93.3)
1	25 (4.5)
2	8 (1.4)
3	3 (0.5)
5	1 (0.2)
Dampness or mould	
0	417 (75.5)
1	119 (21.6)
2	10 (1.8)
3	5 (0.9)
4	1 (0.2)

Table E3. Univariate associations of longitudinal exposure patterns with selected study characteristics presented as odds ratios (95% confidence interval).

Characteristics	Pets			SHS	Dampness or mould	
Low education vs High education	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	0.9 (0.3 to 3.1)	Early-life	2.9 (1.1 to 8.1)	Early-life	0.9 (0.3 to 2.9)
	Mid-childhood	0.8 (0.1 to 3.16)	Persistently low	3.1 (1.2 to 8.6)	Moderate late childhood	0.8 (0.3 to 2.1)
	Early-life	0.8 (0.2 to 3.1)	Childhood	5.3 (2.1 to 13.8)	Mid-childhood	0.5 (0.0 to 4.2)
	Persistently high	2.2 (1.0. to 4.8)				
Intermediate education vs high education	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	0.9 (0.5 to 1.8)	Early-life	1.5 (0.8 to 2.9)	Early-life	1.0 (0.5 to 1.9)
	Mid-childhood	1.2 (0.5 to 2.5)	Persistently low	1.2 (0.6 to 2.43)	Moderate late childhood	0.6 (0.3 to 1.1)
	Early-life	1.9 (1.1 to 3.4)	Childhood	2.9 (1.5 to 5.3)	Mid-childhood	0.8 (0.3 to 2.1
	Persistently high	2.1 (1.3 to 3.3)				
Parental allergy (Yes vs No)	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	0.7 (0.4 to 1.2)	Early-life	0.5 (0.3 to 0.9)	Early-life	0.7 (0.4 to 1.3)
	Mid-childhood	0.5 (0.2 to 1.0)	Persistently low	0.9 (0.4 to 1.6)	Moderate late childhood	0.8 (0.5 to 1.4)
	Early-life	0.9 (0.5 to 1.6)	Childhood	0.5 (0.3 to 0.9)	Mid-childhood	1.0 (0.4 to 2.4)
	Persistently high	0.5 (0.3 to 0.8)				
SHS exposure at 1 year (Yes vs No)	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	1.3 (0.6 to 2.7)	Early-life		Early-life	1.2 (0.6 to 2.3)
	Mid-childhood	1.4 (0.5 to 3.3)	Persistently low		Moderate late childhood	1.0 (0.5 to 1.8)
	Early-life	3.0 (1.6 to 5.6)	Childhood		Mid-childhood	0.8 (0.2to 2.4)
	Persistently high	1.8 (1.1 to 3.1)				
Maternal smoking during pregnancy (Yes vs No)	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	0.7 (0.2 to 2.3)	Early-life		Early-life	1.2 (0.5 to 2.6)
	Mid-childhood	1.8 (0.7 to 5.1)	Persistently low		Moderate late childhood	1.1 (0.5 to 2.3)
	Early-life	3.7 (1.7 to 0.7)	Childhood		Mid-childhood	1.5 (0.5 to 4.5)
	Persistently high	2.0 (1.1 to 4.1)				
Gas cooking (Yes vs No)	Very low	1.0	Very low	1.00	Very low	1.00
	Late childhood	0.9 (0.4 to 2.1)	Early-life	2.0 (0.7 to 5.8)	Early-life	1.4 (0.5 to 3.4)
	Mid-childhood	0.8 (0.3 to 2.1)	Persistently low	0.6 (0.3 to 1.3)	Moderate late childhood	0.8 (0.4 to 1.5)
	Early-life	0.9 (0.4 to 2.0)	Childhood	1.0 (0.4 to 2.2)	Mid-childhood	0.8 (0.2 to 2.4)
	Persistently high	1.2 (0.6 to 2.3)				

Table E4. Crude and adjusted associations of longitudinal patterns of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at ages 12 and 16 presented as percent difference.

	AGE 12		AGE 16	
% Difference in FEV_1 (95% CI)	**CRUDE α**	**ADJUSTED β**	**CRUDE α**	**ADJUSTED β**
SHS	N=552	N=526	N=552	N=526
Early-life vs Very low	-1.02 (-2.53 to 0.52)	-1.43 (-3.02 to 0.18)	0.45 (-1.09 to 2.01)	0.34 (-1.28 to 1.98)
Persistently low vs Very low	-1.69 (-3.29 to -0.05)	-1.77 (-3.45 to -0.06)	-1.16 (-2.76 to 0.46)	-0.97 (-2.64 to 0.72)
Childhood vs Very low	-0.92 (-2.43 to 0.61)	-0.95 (-2.57 to 0.69)	-2.32 (-3.80 to -0.82)	-1.88 (-3.47 to -0.26)
Pets	N=552	N=524	N=552	N=524
Early-life vs Very low	-0.85 (-2.14 to 0.46)	-0.55 (-1.91 to 0.82)	-0.49 (-1.78 to 0.83)	0.10 (-1.25 to 1.48)
Mid-childhood vs Very low	0.83 (-0.77 to 2.45)	1.32 (-0.34 to 3.00)	-0.08 (-1.65 to 1.52)	0.91 (-0.73 to 2.57)
Late childhood vs Very low	2.30 (0.94 to 3.68)	2.71 (1.26 to 4.18)	3.66 (2.29 to 5.05)	4.78 (3.32 to 6.27)
Persistently high vs Very low	0.99 (-0.01 to 2.01)	1.31 (0.21 to 2.42)	0.55 (-0.47 to 1.57)	1.29 (0.19 to 2.39)
Dampness or mould	N=552	N=534	N=552	N=534
Early-life vs Very low	2.10 (0.59 to 3.64)	2.28 (0.74 to 3.84)	-0.32 (-1.80 to 1.19)	-0.21 (-1.72 to 1.32)
Moderate late childhood vs Very low	-3.00 (-4.29 to -1.70)	-2.96 (-4.26 to -1.64)	-2.64 (-3.94 to -1.33)	-2.77 (-4.08 to -1.44)
Mid-childhood vs Very low	-2.97 (-5.12 to -0.76)	-2.58 (-4.78 to -0.33)	-2.00 (-4.20 to 0.24)	-2.00 (-4.22 to 0.26)
% Difference in FVC (95% CI)				
SHS	N=552	N=526	N=552	N=526
Early-life vs Very low	-1.67 (-3.08 to -0.24)	-2.17 (-3.65 to -0.67)	-0.69 (-1.98 to 0.62)	-0.49 (-1.85 to 0.89)
Persistently low vs Very low	-2.10 (-3.60 to -0.57)	-1.94 (-3.50 to -0.33)	-1.21 (-2.56 to 0.17)	-0.82 (-2.25 to 0.62)
Childhood vs Very low	-1.54 (-2.95 to -0.11)	-1.96 (-3.45 to -0.43)	-2.33 (-3.59 to -1.06)	-1.82 (-3.18 to -0.44)
Pets	N=552	N=524	N=552	N=524
Early-life vs Very low	0.12 (-1.11 to 1.37)	0.21 (-1.07 to 1.51)	-0.91 (-2.01 to 0.20)	-0.51 (-1.66 to 0.66)
Mid-childhood vs Very low	-0.25 (-1.74 to 1.26)	0.13 (-1.41 to 1.69)	-0.91 (-2.24 to 0.43)	-0.14 (-1.52 to 1.27)
Late childhood vs Very low	2.36 (1.08 to 3.66)	2.26 (0.91 to 3.36)	1.34 (0.20 to 2.50)	2.21 (0.98 to 3.45)
Persistently high vs Very low	0.71 (-0.24 to 1.66)	0.89 (-0.13 to 1.93)	-0.27 (-1.12 to 0.60)	0.39 (-0.54 to 1.33)
Dampness or mould	N=552	N=534	N=552	N=534
Early-life vs Very low	2.44 (1.00 to 3.89)	2.76 (1.31 to 4.24)	0.62 (-0.65 to 1.90)	0.89 (-0.41 to 2.20)
Moderate late childhood vs Very low	-2.10 (-3.32 to -0.86)	-2.11 (-3.35 to -0.86)	-2.15 (-3.26 to -1.03)	-2.36 (-3.48 to -1.22)
Mid-childhood vs Very low	-0.95 (-3.03 to 1.17)	-0.67 (-2.77 to 1.48)	-1.26 (-3.14 to 0.66)	-1.54 (-3.44 to 0.39)

α Adjusted for sex, log transformations of height, weight and age at medical examination, **β** Adjusted for log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year (except in models with dampness or mould exposure), pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Table E5. Adjusted associations of longitudinal patterns of SHS, pet, and dampness or mould exposure with FEV_1 and FVC growth between age 12 and 16 stratified by sex. Associations are presented as annual percent growth of FEV_1 and FVC

	% difference in FEV_1 Growth/year (95% CI)			**% difference in FVC Growth/year (95% CI)**		
	Girls	Boys	P-value*	Girls	Boys	P-value*
SHS	N=283	N=241		N=283	N=241	
Early-life vs Very low	0.08 (-0.28 to 0.43)	0.68 (0.21 to 1.15)	0.198	-0.07 (-0.44 to 0.31)	0.86 (0.42 to 1.30)	0.118
Persistently low vs Very low	-0.08 (-0.45 to 0.29)	0.61 (0.11 to 1.12)	0.084	0.10 (-0.29 to 0.50)	0.59 (0.12 to 1.07)	0.679
Childhood vs Very low	-0.56 (-0.89 to -0.24)	-0.12 (-0.65 to 0.41)	0.104	-0.50 (-0.84 to -0.15)	0.53 (0.03 to 1.04)	0.000
Pets	N=283	N=241		N=283	N=241	
Early-life vs Very low	0.24 (-0.05 to 0.53)	0.08 (-0.33 to 0.49)	0.599	-0.01 (-0.32 to 0.30)	-0.39 (-0.78 to 0.01)	0.213
Mid-childhood vs Very low	-0.28 (-0.63 to 0.08)	0.14 (-0.34 to 0.61)	0.005	0.01 (-0.31 to 0.34)	-0.07 (-0.52 to 0.39)	0.952
Late childhood vs Very low	0.36 (0.05 to 0.66)	0.94 (0.53 to 1.36)	0.004	-0.02 (-0.40 to 0.36)	0.04 (-0.36 to 0.43)	0.818
Persistently high vs Very low	0.02 (-0.21 to 0.24)	-0.21 (-0.54 to 0.13)	0.215	0.01 (-0.23 to 0.25)	-0.33 (-0.65 to -0.01)	0.082
Dampness or mold	N=283	N=241		N=283	N=241	
Early-life vs Very low	-0.31 (-0.63 to 0.00)	-1.02 (-1.47 to -0.57	0.003	-0.21 (-0.54 to 0.12)	-0.76 (-1.19 to -0.34)	0.014
Moderate late childhood vs Very low	-0.22 (-0.53 to 0.09)	0.48 (0.10 to 0.85)	0.015	-0.23 (-0.55 to 0.10)	0.29 (-0.07 to 0.65)	0.004
Mid-childhood vs Very low	-0.25 (-0.78 to 0.28)	0.22 (-0.39 to 0.83)	0.743	0.66 (-1.22 to -0.10	0.06 (-0.52 to 0.64)	0.237

Adjusted for log transformations of differences in height, weight and age between the 12 and 16 year lung function measurements, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination

*P-value of interaction terms

Table E6. Adjusted associations of longitudinal patterns of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at ages 12 and 16 stratified by sex.

	AGE 12		**P-value***	**AGE 16**		P-value*
% Difference in FEV_1 (95% CI)	Girls	Boys		Girls	Boys	
SHS	N=283	N=241		N=283	N=241	
Early-life vs Very low	-0.91 (-2.99 to 1.22)	-2.23 (-4.64 to 0.23)	0.278	0.42 (-1.73 to 2.61)	0.69 (-1.74 to 3.18)	0.735
Persistently low vs Very low	-2.39 (-4.55 to -0.18)	0.15 (-2.49 to 2.87)	0.401	-3.87 (-6.00 to -1.70)	2.83 (0.17 to 5.55)	0.004
Childhood vs Very low	-1.71 (-3.61 to 0.23)	-1.01 (-3.83 to 1.89)	0.904	-3.35 (-5.25 to -1.40)	-0.75 (-3.48 to 2.05)	0.217
Pets	N=283	N=241		N=283	N=241	
Early-life vs Very low	-1.87 (-3.52 to -0.19)	0.31 (-1.88 to 2.54)	0.139	-0.63 (-2.33 to 1.10)	0.89 (-1.24 to 3.07)	0.136
Mid-childhood vs Very low	1.73 (-0.44 to 3.94)	2.60 (0.00 to 5.27)	0.226	0.02 (-2.12 to 2.20)	3.20 (0.71 to 5.76)	0.040
Late childhood vs Very low	1.57 (-0.25 to 3.42)	3.88 (1.59 to 6.23)	0.047	2.56 (0.67 to 4.49)	8.42 (6.14 to 10.74)	<0.001
Persistently high vs Very low	1.82 (0.48 to 3.18)	0.20 (-1.57 to 2.01)	0.322	1.79 (0.41 to 3.18)	0.69 (-1.06 to 2.47)	0.463
Dampness or mould	N=283	N=241		N=283	N=241	
Early-life vs Very low	1.94 (0.04 to 3.88)	2.47 (-0.00 to 5.01)	0.688	0.33 (-1.58 to 2.27)	-0.52 (-2.87 to 1.89)	0.668
Moderate late childhood vs Very low	-2.86 (-4.63 to -1.06)	-3.04 (-4.98 to -1.06)	0.013	-3.95 (-5.72 to -2.15)	-1.89 (-3.84 to 0.11)	0.135
Mid-childhood vs Very low	-4.20 (-7.25 to -1.05)	-1.82 (-5.01 to 1.48)	0.023	-3.98 (-7.13 to -0.72)	-0.20 (-3.41 to 3.11)	0.079
% Difference in FVC (95% CI)						
SHS	(N=253	(N=213)		(N=253	(N=213)	
Early-life vs Very low	0.06 (-1.97 to 2.13)	-5.15 (-7.28 to -2.97)	0.001	0.59 (-1.27 to 2.49)	-1.83 (-3.83 to 0.20)	0.089
Persistently low vs Very low	-2.00 (-4.10 to 0.14)	-0.81 (-3.20 to 1.64)	0.760	-2.20 (-4.08 to -0.28)	1.31 (-0.89 to 3.56)	0.147
Childhood vs Very low	-1.11 (-2.95 to 0.78)	-3.98 (-6.47 to -1.42)	0.007	-2.62 (-4.29 to -0.93)	-1.54 (-3.82 to 0.80)	0.555
Pets	(N=254)	(N=212)		(N=254)	(N=212)	
Early-life vs Very low	-0.60 (-2.23 to 1.05)	1.16 (-0.86 to 3.22)	0.340	-0.31 (-1.79 to 1.20)	-0.29 (-2.09 to 1.54)	0.530
Mid-childhood vs Very low	1.55 (-0.55 to 3.70)	-0.43 (-2.75 to 1.94)	0.490	1.12 (-0.75 to 3.04)	-0.28 (-2.34 to 1.83)	0.305
Late childhood vs Very low	1.99 (0.22 to 3.80)	1.86 (-0.20 to 3.96)	0.767	1.67 (0.04 to 3.32)	3.15 (1.29 to 5.04)	0.198
Persistently high vs Very low	0.81 (-0.48 to 2.12)	0.68 (-0.96 to 2.34)	0.989	0.81 (-0.38 to 2.00)	0.25 (-1.24 to 1.76)	0.780
Dampness or mould	(N=260)	(N=216)		(N=260)	(N=216)	
Early-life vs Very low	2.16 (0.31 to 4.03)	2.95 (0.68 to 5.28)	0.343	1.03 (-0.63 to 2.72)	0.79 (-1.20 to 2.83)	0.872
Moderate late childhood vs Very low	-2.87 (-4.58 to -1.13)	-1.69 (-3.49 to 0.14)	0.396	-3.88 (-5.41 to -2.33)	-1.49 (-3.14 to 0.19)	0.022
Mid-childhood vs Very low	-2.91 (-5.90 to 0.17)	0.41 (-2.57 to 3.49)	0.162	-4.26 (-6.98 to -1.47)	0.55 (-2.16 to 3.34)	0.005

Adjusted for log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination. *P-value of interaction terms

Table E7. Associations of SHS, pet, and dampness or mould exposure with FEV_1 and FVC growth between age 12 and 16 stratified by parental allergy. Association are presented as annual percent growth of FEV_1 and FVC.

	FEV1 growth % / ye_{ar} (95% CI)			**FVC growth % / year (95% CI)**		
	Non- allergic parents	**Allergic parents**	**P-value***	**Non-allergic parents**	**Allergic parents**	P-**value***
SHS	N=235	N=291		N=235	N=291	
Early-life vs Very low	0.23 (-0.18 to 0.65)	0.38 (-0.09 to 0.85)	0.979	0.20 (-0.18 to 0.58)	0.44 (-0.03 to 0.91)	0.241
Persistently low vs Very low	-0.04 (-0.52 to 0.43)	0.48 (0.05 to 0.91)	0.095	0.01 (-0.43 to 0.46)	0.52 (0.08 to 0.95)	0.984
Childhood vs Very low	-0.41 (-0.82 to -0.01)	-0.31 (-0.76 to 0.15)	0.985	0.09 (-0.28 to 0.47)	-0.19 (-0.65 to 0.28)	0.486
Pets	N=234	N=290		N=234	N=290	
Early-life vs Very low	-0.00 (-0.41 to 0.40)	-0.08 (-0.41 to 0.25)	0.385	-0.37 (-0.74 to 0.01)	-0.24 (-0.58 to 0.09)	0.902
Mid-childhood vs Very low	-0.33 (-0.75 to 0.09)	0.22 (-0.22 to 0.66)	0.078	-0.49 (-0.88 to -0.10)	0.47 (0.02 to 0.92)	0.001
Late childhood vs Very low	0.13 (-0.25 to 0.51)	0.74 (0.38 to 1.11)	0.035	-0.25 (-0.60 to 0.10)	-0.01 (-0.38 to 0.36)	0.545
Persistently high vs Very low	0.11 (-0.18 to 0.41)	-0.60 (-0.88 to -0.31)	0.000	-0.22 (-0.49 to 0.05)	-0.38 (-0.66 to -0.09)	0.173
Dampness or mould	N=241	N=293		N=241	N=293	
Early-life vs Very low	-0.73 (-1.12 to -0.34)	-0.79 (-1.19 to -0.39)	0.581	-0.56 (-0.92 to -0.20)	-0.46 (-0.86 to -0.05)	0.710
Moderate late childhood vs Very low	0.19 (-0.18 to 0.55)	-0.05 (-0.40 to 0.30)	0.385	0.10 (-0.23 to 0.44)	-0.17 (-0.52 to 0.19)	0.470
Mid-childhood vs Very low	-0.04 (-0.66 to 0.58)	0.05 (-0.51 to 0.61)	0.862	-0.46 (-1.03 to 0.12)	-0.13 (-0.69 to 0.44)	0.307

Adjusted for log transformations of differences in height, weight and age at medical examination, parental education, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year (except in models with dampness or mould exposure), pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination. *P-value of interaction terms

Table E8a. Adjusted associations of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at age 12 stratified by parental allergy. Associations are presented as percent difference.

	Non-allergic parents	**Allergic parents**	**P-value***
% Difference in FEV_1	**12 years**		
SHS	N=235	N=292	
Early-life vs Very low	-0.25 (-2.33 to 1.87)	-3.26 (-5.72 to -0.75)	0.055
Persistently low vs Very low	-0.58 (-3.01 to 1.91)	-1.92 (-4.24 to 0.46)	0.471
Childhood vs Very low	-1.13 (-3.17 to 0.95)	-0.63 (-3.15 to 1.97)	0.860
% Difference in FVC			
Early-life vs Very low	-0.52 (-2.41 to 1.41)	-4.48 (-6.79 to -2.12)	0.001
Persistently low vs Very low	0.20 (-2.03 to 2.48)	-3.64 (-5.82 to -1.42)	0.006
Childhood vs Very low	-2.40 (-4.23 to -0.54)	-0.96 (-3.36 to 1.50)	0.587
Pets	N=234	N=291	
% Difference in FEV_1			
Early-life vs Very low	2.40 (0.32 to 4.52)	-2.05 (-3.83 to -0.23)	0.001
Mid-childhood vs Very low	4.23 (2.00 to 6.51)	-1.10 (-3.49 to 1.35)	0.000
Late childhood vs Very low	4.05 (2.03 to 6.11)	1.64 (-0.37 to 3.69)	0.253
Persistently high vs Very low	1.64 (0.14 to 3.16)	0.99 (-0.59 to 2.60)	0.372
% Difference in FVC			
Early-life vs Very low	3.92 (2.01 to 5.87)	-1.92 (-3.63 to -0.17)	<0.001
Mid-childhood vs Very low	2.32 (0.34 to 4.34)	-2.17 (-4.44 to 0.15)	0.000
Late childhood vs Very low	3.63 (1.80 to 5.48)	0.91 (-1.00 to 2.86)	0.045
Persistently high vs Very low	1.77 (0.40 to 3.15)	-0.10 (-1.60 to 1.43)	0.037
Dampness or mould.	N=241	N=294	
% Difference in FEV_1			
Early-life vs Very low	0.80 (-1.20 to 2.84)	3.53 (1.24 to 5.86)	0.036
Moderate late childhood vs Very low	-4.37 (-6.17 to -2.54)	-1.34 (-3.22 to 0.57)	0.018
Mid-childhood vs Very low	-0.16 (-3.39 to 3.18)	-4.74 (-7.66 to -1.72)	0.006
% Difference in FVC			
Early-life vs Very low	3.30 (1.45 to 5.18)	2.29 (0.12 to 4.51)	0.494
Moderate late childhood vs Very low	-3.32 (-4.96 to -1.65)	-0.68 (-2.50 to 1.18)	0.017
Mid-childhood vs Very low	2.04 (-0.93 to 5.11)	-2.45 (-5.33 to 0.51)	0.021

Adjusted for log transformations of height, weight and age at medical examination, parental education, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Table E8b. Adjusted associations of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at age 16 stratified by parental allergy. Associations are presented as percent difference.

	Non-allergic parents	**Allergic parents**	**P-value***
% Difference in FEV_1	**16 years**		
SHS	N=235	N=292	
Early-life vs Very low	2.41 (0.10 to 4.76)	-2.16 (-4.52 to 0.26)	0.005
Persistently low vs Very low	-0.15 (-2.77 to 2.54)	-1.27 (-3.45 to 0.96)	0.643
Childhood vs Very low	-2.22 (-4.36 to -0.03)	-0.75 (-3.16 to 1.72)	0.641
% Difference in FVC			
Early-life vs Very low	1.93 (0.00 to 3.89)	-3.00 (-5.00 to -0.96)	0.000
Persistently low vs Very low	0.90 (-1.32 to 3.17)	-2.35 (-4.19 to -0.47)	0.002
Childhood vs Very low	-1.76 (-3.57 to 0.08)	-1.12 (-3.17 to 0.98)	0.871
Pets	N=234	N=291	
% Difference in FEV_1			
Early-life vs Very low	3.09 (0.80 to 5.43)	-1.61 (-3.31 to 0.11)	0.000
Mid-childhood vs Very low	3.85 (1.46 to 6.29)	-1.30 (-3.54 to 1.00)	0.001
Late childhood vs Very low	4.74 (2.56 to 6.96)	5.23 (3.26 to 7.24)	0.664
Persistently high vs Very low	2.77 (1.11 to 4.46)	-0.00 (-1.48 to 1.50)	0.007
% Difference in FVC			
Early-life vs Very low	3.56 (1.63 to 5.52)	-2.25 (-3.71 to -0.78)	<0.001
Mid-childhood vs Very low	1.97 (0.01 to 3.98)	-1.76 (-3.70 to 0.21)	0.009
Late childhood vs Very low	3.58 (1.78 to 5.41)	1.17 (-0.47 to 2.84)	0.096
Persistently high vs Very low	2.15 (0.77 to 3.55)	-0.78 (-2.05 to 0.50)	0.003
Dampness or mould.	N=241	N=294	
% Difference in FEV_1			
Early-life vs Very low	-1.72 (-3.83 to 0.43)	1.37 (-0.78 to 3.56)	0.057
Moderate late childhood vs Very low	-3.38 (-5.33 to -1.38)	-2.38 (-4.19 to -0.54)	0.444
Mid-childhood vs Very low	0.37 (-3.10 to 3.97)	-4.42 (-7.25 to -1.50)	0.018
% Difference in FVC			
Early-life vs Very low	1.31 (-0.50 to 3.16)	0.82 (-1.01 to 2.68)	0.816
Moderate late childhood vs Very low	-2.91 (-4.56 to -1.24)	-1.70 (-3.26 to -0.12)	0.171
Mid-childhood vs Very low	0.04 (-2.86 to 3.02)	-2.85 (-5.32 to -0.31)	0.165

Adjusted for log transformations of height, weight and age at medical examination, parental education, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year except in models with dampness or mould exposure, pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination. *P-value of interaction terms

Table E9. Adjusted associations of SHS, pet, and dampness or mould exposure with FEV_1 and FVC growth between age 12 and 16 excluding participants with respiratory infections during the 3 weeks preceding lung function measurements. Associations are presented as annual percent growth of FEV_1 and FVC.

N=212	**% FEV_1 growth (95% CI)**	**% in FVC growth (95% CI)**
SHS		
Early-life vs Very low	0.15 (-0.33 to 0.64)	0.52 (0.04 to 1.01)
Persistently low vs Very low	0.54 (-0.00 to 1.09)	0.64 (0.10 to 1.19)
Childhood vs Very low	-1.04 (-1.50 to -0.58)	-0.87 (-1.32 to -0.41)
Pets		
Early-life vs Very low	0.73 (0.37 to 1.09)	-0.15 (-0.50 to 0.20)
Mid-childhood vs Very low	-0.11 (-0.58 to 0.36)	-0.46 (-0.93 to 0.01)
Late childhood vs Very low	0.57 (0.18 to 0.96)	-0.12 (-0.50 to 0.27)
Persistently high vs Very low	-0.29 (-0.57 to -0.01)	-0.32 (-0.60 to -0.04)
Dampness or mould N=215		
Early-life vs Very low	-0.71 (-1.13 to -0.29)	-0.04 (-0.46 to 0.38)
Moderate late childhood vs Very low	0.24 (-0.10 to 0.57)	0.06 (-0.26 to 0.40)
Mid-childhood vs Very low	0.29 (-0.44 to 1.02)	-0.07 (-0.78 to 0.65)

Associations adjusted for sex, log transformation of differences in height, weight and age between the 12 and 16 year lung function measurements, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year, use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year, pets in the home at 1 year.

Table E10. Adjusted associations of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at ages 12 and 16 excluding participants with respiratory infections during the 3 weeks preceding lung function measurements.

	% Difference in FEV1 (95% CI)		% Difference in FVC (95% CI)	
	AGE 12	**AGE 16**	**AGE 12**	**AGE 16**
SHS	N=350	N=307	N=350	N=307
Early-life vs Very low	-1.34 (-3.27 to 0.62)	-0.63 (-2.60 to 1.39)	-3.33 (-5.20 to -1.43)	-1.03 (-2.77 to 0.73)
Persistently low vs Very low	-1.80 (-3.87 to 0.31)	-2.43 (-4.50 to -0.32)	-0.71 (-2.78 to 1.41)	-2.13 (-3.96 to -0.27)
Childhood vs Very low	-0.35 (-2.24 to 1.58)	-2.69 (-4.79 to -0.54)	-0.00 (-1.89 to 1.92)	-1.84 (-3.71 to 0.07)
Pets	N=370	N=319	N=370	N=319
Early-life vs Very low	-1.33 (-2.82 to 0.19)	-0.25 (-1.92 to 1.45)	-0.70 (-2.19 to 0.82)	-2.23 (-3.68 to -0.77)
Mid-childhood vs Very low	0.17 (-1.85 to 2.22)	-0.78 (-2.88 to 1.36)	-2.72 (-4.66 to -0.73)	-2.42 (-4.24 to -0.57)
Late childhood vs Very low	3.94 (2.26 to 5.65)	2.23 (0.38 to 4.11)	2.69 (1.04 to 4.37)	-0.40 (-1.99 to 1.22)
Persistently high vs Very low	1.31 (0.04 to 2.59)	1.07 (-0.26 to 2.42)	0.70 (-0.55 to 1.98)	-0.16 (-1.32 to 1.01)
Dampness or mould	N=356	N=311	N=356	N=311
Early-life vs Very low	2.63 (0.86 to 4.43)	-1.44 (-3.42 to 0.59)	3.03 (1.26 to 4.82)	-0.49 (-2.27 to 1.32)
Moderate late childhood vs Very low	-3.46 (-4.86 to -2.04)	-2.29 (-3.83 to -0.72)	-2.74 (-4.14 to -1.32)	-2.95 (-4.31 to -1.57)
Mid-childhood vs Very low	-3.93 (-7.04 to -0.72)	-5.83 (-8.41 to -3.18)	-1.43 (-4.60 to 1.85)	-4.21 (-6.54 to -1.83)

Associations adjusted for sex, difference in height, weight and age between the 12 and 16 year lung function measurements, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year, use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year, pets in the home at 1 year.

Table E11. Adjusted associations of longitudinal patterns of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at ages 12 and 16 and FEV_1 and FVC growth between 12 and 16 years excluding childhood asthmatics (until age 8) and those who avoided pets due to family allergies (N=129).

	% Difference in FEV1 (95% CI)		**% FEV1 growth (95% CI)**
	AGE 12	**AGE 16**	
SHS			
Early-life vs Very low	-1.63 (-3.48 to 0.25)	0.17 (-1.66 to 2.03)	0.42 (0.05 to 0.81)
Persistently low vs Very low	-2.34 (-4.13 to -0.50)	-1.35 (-3.12 to 0.46)	0.29 (-0.08 to 0.66)
Childhood vs Very low	-0.74 (-2.47 to 1.03)	-1.75 (-3.42 to -0.05)	-0.53 (-0.88 to -0.18)
Pets			
Early-life vs Very low	-0.25 (-1.85 to 1.37)	1.05 (-0.51 to 2.64)	0.19 (-0.12 to 0.50)
Mid-childhood vs Very low	1.66 (-0.15 to 3.49)	1.38 (-0.38 to 3.16)	-0.17 (-0.51 to 0.18)
Late childhood vs Very low	2.24 (0.73 to 3.77)	4.03 (2.53 to 5.54)	0.38 (0.09 to 0.67)
Persistently high vs Very low	0.88 (-0.31 to 2.09)	1.13 (-0.04 to 2.31)	-0.14 (-0.37 to 0.09)
Dampness or mould			
Early-life vs Very low	2.64 (0.96 to 4.35)	0.20 (-1.42 to 1.84)	-0.74 (-1.05 to -0.43)
Moderate late childhood vs Very low	-3.11 (-4.52 to -1.68)	-2.29 (-3.69 to -0.88)	0.15 (-0.13 to 0.43)
Mid-childhood vs Very low	-2.01 (-4.35 to 0.39)	-2.00 (-4.28 to 0.35)	-0.28 (-0.73 to 0.18)
	% Difference in FVC (95% CI)		**% FVC growth (95% CI)**
	AGE 12	**AGE 16**	
SHS			
Early-life vs Very low	-3.77 (-5.52 to -1.99)	-1.87 (-3.48 to -0.23)	0.46 (0.09 to 0.82)
Persistently low vs Very low	-1.98 (-3.73 to -0.20)	-1.78 (-3.37 to -0.17)	0.06 (-0.30 to 0.41)
Childhood vs Very low	-1.29 (-2.95 to 0.41)	-1.59 (-3.10 to -0.06)	-0.35 (-0.68 to -0.01)
Pets			
Early-life vs Very low	0.30 (-1.26 to 1.88)	0.14 (-1.27 to 1.56)	-0.16 (-0.47 to 0.14)
Mid-childhood vs Very low	1.11 (-0.64 to 2.87)	0.79 (-0.79 to 2.39)	-0.19 (-0.53 to 0.15)
Late childhood vs Very low	1.08 (-0.37 to 2.54)	1.42 (0.10 to 2.75)	-0.07 (-0.35 to 0.21)
Persistently high vs Very low	0.23(-0.29 to 1.39)	-0.15 (-1.20 to 0.90)	-0.32 (-0.55 to -0.10)
Dampness or mould			
Early-life vs Very low	3.01 (1.38 to 4.68)	1.31 (-0.16 to 2.80)	-0.57 (-0.87 to -0.26)
Moderate late childhood vs Very low	-2.00 (-3.38 to -0.61)	-2.07 (-3.32 to -0.80)	-0.02 (-0.29 to 0.25)
Mid-childhood vs Very low	1.46 (-0.89 to 3.86)	-1.04 (-3.11 to 1.08)	-0.73 (-1.17 to -0.29)

Adjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, , maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year (except in models with dampness or mould exposure), pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Table E12. Adjusted associations of longitudinal patterns of SHS, pet, and dampness or mould exposure with FEV_1 and FVC at ages 12 and 16 and FEV_1 and FVC growth between age 12 and 16 excluding smokers(N=44).

N=463	**% Difference in FEV1 (95% CI)**		**% FEV1 growth (95% CI)**
SHS	**AGE 12**	**AGE 16**	
Early-life vs Very low	-1.20 (-2.85 to 0.48)	0.78 (-0.90 to 2.48)	0.34 (0.02 to 0.66)
Persistently low vs Very low	-2.23 (-4.14 to -0.28)	-1.25 (-3.14 to 0.68)	0.21 (-0.16 to 0.58)
Childhood vs Very low	-0.77 (-2.54 to 1.02)	-1.13 (-2.87 to 0.64)	-0.38 (-0.71 to -0.05)
Pets			
Early-life vs Very low	-0.05 (-1.51 to 1.43)	0.46 (-0.99 to 1.92)	-0.03 (-0.30 to 0.24)
Mid-childhood vs Very low	1.60 (-0.11 to 3.35)	1.05 (-0.63 to 2.76)	-0.10 (-0.41 to 0.21)
Late childhood vs Very low	2.75 (1.23 to 4.29)	4.84 (3.32 to 6.39)	0.38 (0.10 to 0.65)
Persistently high vs Very low	1.42 (0.26 to 2.60)	1.00 (-0.15 to 2.16)	-0.30 (-0.51 to -0.09)
Dampness or mould			
Early-life vs Very low	2.48 (0.84 to 4.14)	0.35 (-1.25 to 1.98)	-0.71 (-1.01 to -0.42)
Moderate late childhood vs Very low	-2.97 (-4.36 to -1.56)	-2.97 (-4.36 to -1.56)	0.01 (-0.25 to 0.28)
Mid-childhood vs Very low	-1.05 (-3.44 to 1.40)	-0.24 (-2.65 to 2.22)	-0.08 (-0.52 to 0.37)
	% Difference in FVC (95% CI)		**% FVC growth (95% CI)**
	AGE 12	**AGE 16**	
SHS			
Early-life vs Very low	-2.37 (-3.91 to -0.80)	-0.86 (-2.27 to 0.57)	0.25 (-0.06 to 0.56)
Persistently low vs Very low	-1.68 (-3.50 to 0.18)	-0.63 (-2.26 to 1.03)	0.17 (-0.20 to 0.53)
Childhood vs Very low	-2.00 (-3.65 to -0.32)	-1.23 (-2.72 to 0.28)	-0.05 (-0.38 to 0.28)
Pets			
Early-life vs Very low	0.70 (-0.69 to 2.11)	-0.10 (-1.34 to 1.14)	-0.29 (-0.56 to -0.03)
Mid-childhood vs Very low	0.30 (-1.31 to 1.93)	0.07 (-1.37 to 1.52)	0.00 (-0.31 to 0.31)
Late childhood vs Very low	2.31 (0.87 to 3.76)	2.19 (0.91 to 3.48)	-0.17 (-0.44 to 0.10)
Persistently high vs Very low	1.08 (-0.02 to 2.19)	0.24 (-0.74 to 1.23)	-0.37 (-0.57 to -0.16)
Dampness or mould			
Early-life vs Very low	3.03 (1.48 to 4.61)	1.09 (-0.28 to 2.48)	-0.60 (-0.89 to -0.31)
Moderate late childhood vs Very low	-2.29 (-3.62 to -0.95)	-3.05 (-4.22 to -1.85)	-0.14 (-0.40 to 0.12)
Mid-childhood vs Very low	0.72 (-1.58 to 3.08)	-0.20 (-2.25 to 1.88)	-0.60 (-0.89 to -0.31)

Adjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, , maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year (except in models with SHS exposure), use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year (except in models with dampness or mould exposure), pets in the home at 1 year(except in models with pet exposure), respiratory infections in the past 3 weeks before medical examination.

Table E13. Association of childhood asthma and rhinitis during the first 8 years of life with exposure pattern membership vs late childhood membership, for pet exposure and separately for cats and rodent exposure. Associations are presented as odds ratios.

	Odds ratios (95% CI)	
	Any asthma	**Any rhinitis**
Pets	(N=525)	(N=256)
Very low vs Late childhood	2.10 (0.70 to 6.36)	1.50 (0.51 to 4.37)
Early-life vs Late childhood	2.79 (0.82 to 9.49)	2.60 (0.71 to 9.45)
Mid-childhood vs Late childhood	1.09 (0.23 to 5.24)	0.57 (0.11 to 2.91)
Persistently high vs Late childhood	3.17 (1.03 to 9.75)	0.51 (0.15 to 1.74)
Cats	(N=525)	(N=256)
Very low vs Late childhood	5.61 (0.74 to 42.37)	4.07 (0.47 to 34.78)
Early-life vs Late childhood	3.81 (0.42 to 33.29)	4.59 (0.40 to 51.06)
Persistently high vs Late childhood	5.94 (0.73 to 48.08)	1.75 (0.17 17.2)
Rodents	(N=525)	(N=256)
Very low vs Late childhood	1.10 (0.54 to 2.24)	2.39 (1.00 to 5.70)
Early-life vs Late childhood	1.23 (0.34 to 4.40)	1.06(0.16 to 6.82)
Mid-childhood vs Late childhood	0.99 (0.25 to 3.96)	2.93 (0.56 to 15.34)

Adjusted for sex, log transformations of height, weight and age at medical examination, parental education, maternal and paternal allergy, breastfeeding at 12 weeks, maternal smoking during pregnancy, average annual NO_2 exposure at birth address, birthweight, indoor SHS at 1 year, use of gas for cooking at 3 months, dampness or mould in the child's home at 1 year, respiratory infections in the past 3 weeks before medical examination.

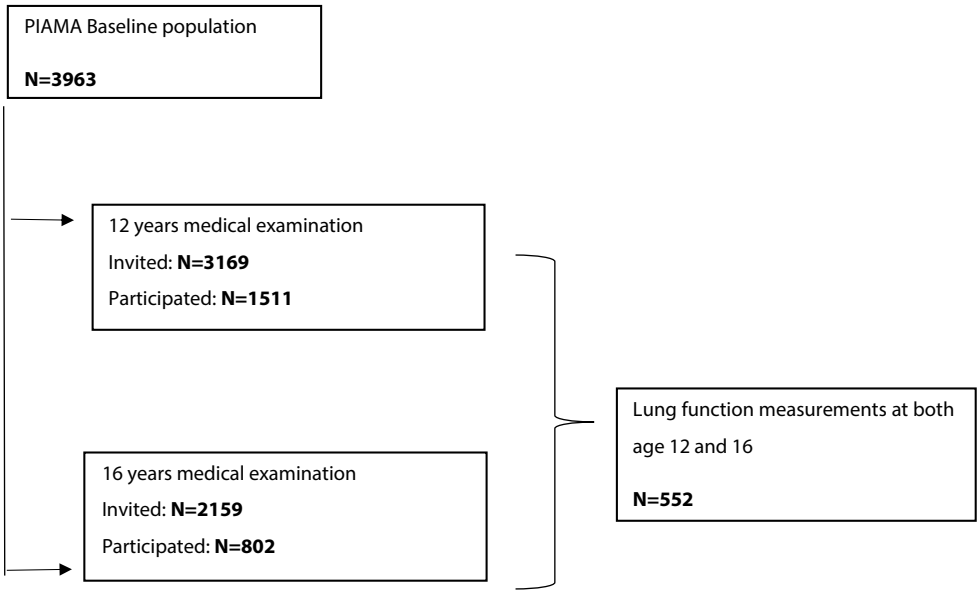
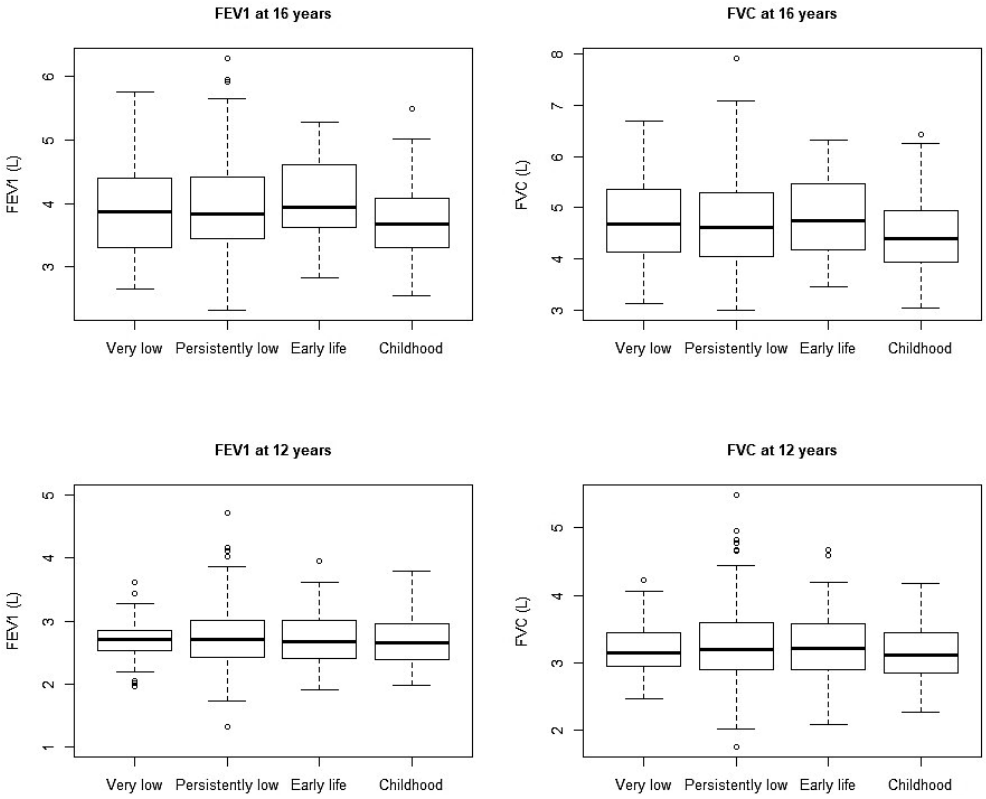
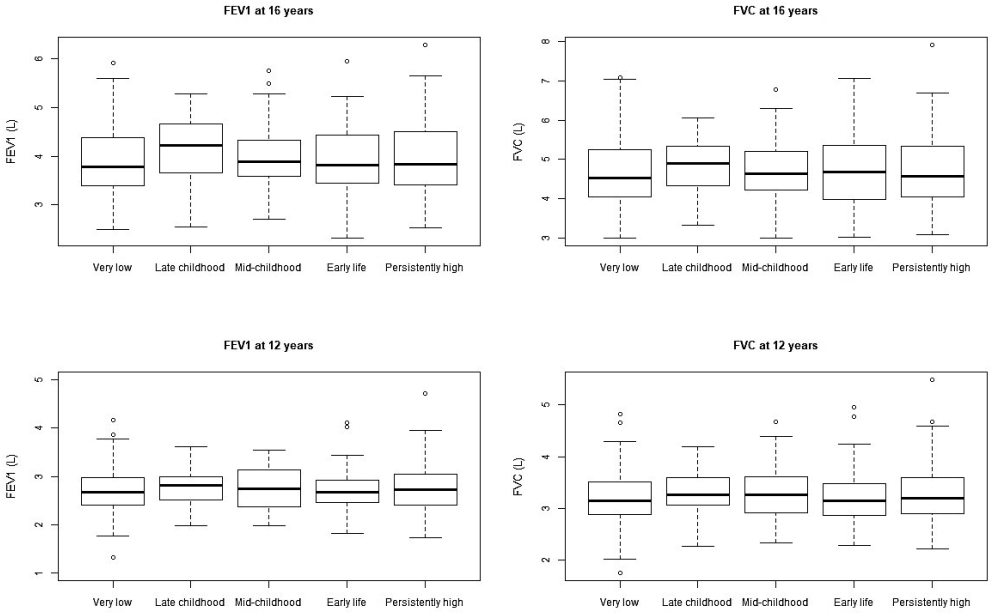


Figure E1. Flow diagram of study population from the baseline population



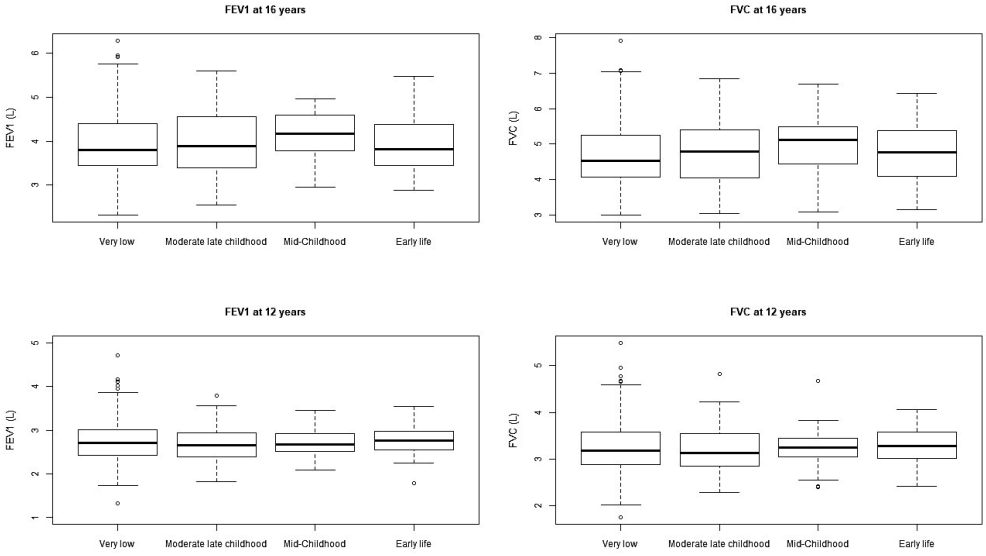
Allocation to exposure pattern based on the largest posteriori probability

Figure E2. Boxplots of FEV_1 and FVC values vs patterns of secondhand smoke (SHS) exposure.



Allocation to exposure pattern based on the largest posteriori probability

Figure E3. Boxplots of FEV_1 and FVC values vs pattern of for pet exposure.



Allocation to exposure pattern based on the largest posterior probability

Figure E4. Boxplots of FEV1 and FVC values vs pattern of dampness or mould exposure.

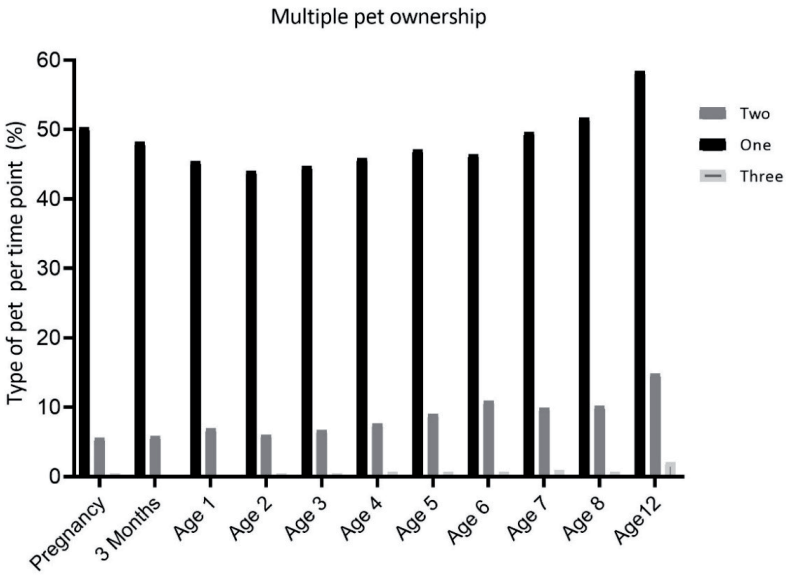


Figure E5. Number of different pets (cat, dog, rodent) owned per time point.

CHAPTER 6

6

Air pollution and lung function until age 16: The PIAMA birth cohort study

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European Respiratory Journal (2018) Sep 27;52(3)

ABSTRACT

Background: Evidence for effects of air pollution exposure on lung function growth into adolescence is scarce. We investigated associations of air pollution exposure with lung function and lung function growth until age 16.

Methods: We conducted both longitudinal (N=915) and cross-sectional (N=721) analyses of associations of air pollution exposure with forced expiratory volume in 1s (FEV_1) and forced vital capacity (FVC) growth from ages eight to 16 and FEV_1 and FVC at age 16. We estimated residential concentrations of nitrogen dioxide (NO_2), "soot", and particulate matter with diameters < 2.5 ($PM_{2.5}$), < 10 (PM_{10}), and 2.5–10 µm (PM_{coarse}) during the preschool, primary school and secondary school time windows by land use regression models. Associations with (growth in) FEV_1 and FVC by were analysed linear (mixed effects) regression.

Results: Higher air pollution exposure was associated with reduced FEV_1 growth, e.g. adjusted difference (95% confidence interval) -0.26% (-0.49 to -0.03%) per interquartile range increase in secondary school $PM_{2.5}$ and lower FEV_1, adjusted difference -2.36% (95% CI -3.76 to -0.94%), but was not adversely associated with FVC. Associations with FEV_1 were stronger in boys than girls and were not modified by asthma status.

Conclusions: Higher air pollution exposure may lead to increased airway obstruction, but not reduced lung volume in adolescence.

BACKGROUND

Air pollution exposure has been shown to adversely affect the respiratory health of children.[1] The role of air pollution exposure in lung function growth has also been determined.[2-5] However, effects of air pollution exposure over the whole lifetime have rarely been investigated. Understanding the effects of lifetime air pollution exposure on health can provide essential insights into the relevance of exposure during different time windows and provide guidance on the timing and structure of interventions to successfully improve respiratory health.[6]

Until now, mainly air pollution exposure during distinct age ranges has been investigated. Several studies in children and adolescents aged 8-16 years[7-10] have reported adverse associations of exposure with lung function within first three years of life, while a recent multi-cohort study reported associations with current exposures in the range 6-8 years. [11]

There are currently few longitudinal studies of the association between air pollution exposure and lung function in adolescence. [2,10,12] The Children's Health Study (CHS) [2,5] in Southern California has presented strong evidence for an association between air pollution exposure and lung function growth from 10–18 years. Higher exposure from study entry (~10 years) was associated with reduced growth in FEV_1 by age 18.[2] Similarly, the Swedish Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE) study,[10] reported an association between early-life air pollution exposure and lower lung function at age 16.

Lung function reaches its maximum in early adulthood. Reduced growth resulting in low maximum attained level of lung function in early adulthood, may be associated with an increased risk of developing chronic obstructive pulmonary disease (COPD) later in life.[13,14] This makes research on persistence of air pollution effects since birth an essential health interest. Therefore, we investigated associations of air pollution exposure from birth with lung function growth from ages 8 to 16 and lung function at age 16. Since differences in associations of air pollution with lung function have been suggested between boys and girls and between asthmatics and non-asthmatics [2,7,12] we also explored possible effect modification by sex and asthma status. Currently, evidence for potential interactions with sex and asthma is mixed and therefore there is no expected direction for these interactions.

METHODS

Study design and study population

This study was performed within the Dutch population-based Prevention and Incidence of Asthma and Mite Allergy birth cohort study (PIAMA).[15] Briefly, pregnant women were recruited in 1996/1997 from northern, western and central regions of the Netherlands. The

cohort started with 3963 new-borns. Data on lifestyle, household and health characteristics were collected through questionnaires completed by parents during pregnancy, at 3 months, annually till age 8, and at ages 11, 14, and 16. At ages 8, 12 and 16, lung function was measured as part of medical examinations. The current study populations consist of participants with air pollution exposure data and 1) at least two lung function measurements throughout follow-up for longitudinal analyses (N=915) and 2) lung function measurements at age 16 for cross-sectional analyses (N=721, Figure E1). Ethical approval was obtained from ethical review boards of participating institutes and written informed consent was obtained from participants as well as their parents/legal guardians.

Lung function measurements

Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured by spirometry at ages 8, 12 and 16. At ages 8 and 12, 1058 and 1292 participants from all three regions of the country had successful lung function measurements, respectively. At age 16, participants from the northern and central regions of the Netherlands (N=2159) were invited for medical examination. Successful lung function measurements were obtained in 721 participants. We used Jaeger pneumotachograph (Viasys Healthcare, USA) at age 8 and Easy One spirometers (NDD Medical Technologies, Inc, Switzerland) at age 12. Both Jaeger Masterscreen pneumotachograph (CareFusion, Yorba Linda, CA, USA) and the EasyOne spirometer were used at age 16. All measurements were performed following recommendations of the American Thoracic Society (ATS)/European Respiratory Society (ERS).[16] For each participant, at least three acceptable manoeuvres were required. In addition, test results were included, which did not meet these criteria (difference between the largest and next largest value ≤ 150 mL for FEV_1 and FVC), but which were obtained from otherwise technically acceptable flow- volume curves with differences between the largest and next largest values for FEV_1 and FVC ≤ 200 mL, (N=190 at age 12 and N=76 at age 16), as in previous analyses.[17] Since different spirometers were used at age 16, we compared the spirometers in 49 volunteers in a separate experiment and calibrated measurements performed with the EasyOne spirometer using regression equations obtained from the comparison, as delineated in equations 1 and 2 below (values in parentheses (0.05/0.01) are the standard errors of the regression coefficients preceding them).

$$FEV_1Masterscreen = 0.114\ (0.05) + 1.032\ (0.01) * FEV_1EasyOne \quad (1)$$

$$FVCMasterscreen = 0.357\ (0.05) + 1.005(0.01) * FVCEasyOne \quad (2)$$

Air pollution exposure assessment

We used land use regression (LUR) models to estimate annual average concentrations of nitrogen dioxide (NO_2), particulate matter with aerodynamic diameters of < 2.5 μm ($PM_{2.5}$), < 10 μm (PM_{10}), and 2.5-10 μm (PM_{coarse}), and $PM_{2.5}$ absorbance ("soot", defined as reflectance

of $PM_{2.5}$ filters) at the participants' home addresses throughout follow-up as described elsewhere.[18,19] Concisely, three two-week measurements of nitrogen dioxide (NO_2) were performed at 80 sites in the study area between October 2008 and February 2010. Simultaneous measurements of $PM_{2.5}$, PM_{10}, $PM_{coarse,}$ and $PM_{2.5}$ absorbance were performed at 40 of these sites. The three measurements were averaged to obtain the annual average concentration for each site [20,21]. Variables such as nearby traffic, household density, and land use derived from Geographic Information Systems (GIS) were used as predictor variables in LUR model development.[18,19] Substantial variability in annual average concentrations was explained for NO_2, $PM_{2.5}$, $PM_{10,}$ and $PM_{2.5}$ absorbance (R^2_{LOOCV}=0.61–0.89), but not for PM_{coarse} (R^2_{LOOCV} =0.38). [18,19]

We used complete residential histories from birth until the 16-year lung function measurements to estimate average air pollution exposures during different time windows as follows: preschool (birth–4 years), primary school (5–12 years), and secondary school (13–16 years). Participant's occupancy at an address was used as weight in calculation of time window-specific average concentrations.

Confounders

Information on potential confounders was obtained from parental-completed questionnaires. The following set of *a priori* selected potential confounders was considered: age, sex, weight, height, parental education (maximum of either maternal or paternal education), maternal and paternal atopy, breastfeeding, respiratory infections in the last 3 weeks before lung function measurement, Dutch nationality (both parents born in the Netherlands), maternal smoking during pregnancy; indoor tobacco smoke exposure in the home, furry pets in the home, mould in the home, gas cooking during the first year of life; and average air pollution concentrations during the 7 days preceding lung function measurements retrieved from the Dutch National Air Quality Monitoring Network.

Statistical analyses

Lung function was log-transformed in all analyses because of the strongly non-linear relationships between lung function, age, height and weight.[22,23] Associations with different pollutants were assessed in separate models with concentrations as continuous variables assuming a linear dose-response relationship without threshold. Both longitudinal and cross-sectional analyses were initially adjusted for sex, age, log-transformed height and weight at time of lung function measurements; and then additionally adjusted for all other potential confounders. Associations are presented as percent differences in lung function and 1-year lung function growth for an interquartile range (IQR) increase in exposure to facilitate comparison of estimates between pollutants. The same IQRs were used in all analyses. Percent differences were calculated from estimated regression coefficients b as $(e^{b \times IQR} - 1) \times 100$. We performed complete case analyses and excluded participants with

missing data for one or more potential confounders from additionally adjusted longitudinal (n=44) and cross-sectional analyses (n=50). Results of crude analyses did not differ between all participants and the subset with complete information of all potential confounders (data not shown).

Longitudinal analyses of lung function growth from age 8 to 16

We used linear mixed effects models with random subject intercepts and exposure-age interaction terms to assess associations of air pollution exposure with lung function growth from age 8 to 16. The interaction terms can be directly interpreted as the association of air pollution exposure with the annual rate of change in lung function. Only the preschool time window was used in longitudinal analyses to ensure that exposure precedes outcome.

Cross-sectional analyses of lung function at age 16

We used linear regression to assess associations of air pollution exposure with lung function at age 16. Associations with exposure during different window were assessed in separate models.

Sensitivity analyses

We performed stratified analyses by sex and asthma status. We also explored the independence of associations of exposure during different time windows in movers with lung function at age 16 using multi-time window models. We only included preschool and secondary school time window exposures in one model because models with other combinations of time windows led to multicollinearity problems (Variance Inflation Factors >3). All analyses were performed using SAS V9.4 (Cary, USA) with significance levels of 0.05.

RESULTS

Population characteristics

Study population characteristics for longitudinal and cross-sectional analyses and distributions of lung function variables are presented in Table 1. More than 60% of the participants had at least one highly educated parent, more than 30% had an atopic father and more than 30% had an atopic mother. Of the participants, 20% were exposed to indoor tobacco smoke exposure in early-life. At age 16, the mean (SD) was 3.9 (0.7) L for FEV_1 and 4.7 (0.8) L for FVC (Table 2). Characteristics of study populations and the PIAMA baseline cohort were generally similar, except for a higher percentage of participants with an atopic mother in the longitudinal analysis population and higher percentage of highly educated parents in both study populations (Table E1). Of the participants, 62% changed addresses (movers)

at any time between preschool time window and the 16-year lung function measurements. Population characteristics were not different between movers and non-movers (Table E2).

Air pollution exposure

The distributions of estimated average air pollution levels for longitudinal and cross-sectional analyses populations were similar (Tables 3 and E3). Air pollution levels were consistent between time windows with means slightly decreasing over time for NO_2 and $PM_{2.5}$ absorbance. Consequently, exposure during secondary school time window was slightly lower than exposure during the preschool time window in movers (Table E4). Variation in exposure levels was larger for NO_2 and $PM_{2.5}$ absorbance than for PM mass. NO_2, $PM_{2.5}$ absorbance, $PM_{2.5}$ and PM_{10} concentrations were moderately to highly correlated within time windows (r=0.53–0.96, Tables E5 and E6). Moderate to high correlations were also observed for concentrations of the same pollutant between time windows (r=0.65–0.96) and these correlations were similar for movers (r=0.48–0.95, Table E7).

Longitudinal analyses of lung function growth from age 8 to 16

Exposure during preschool time window was associated with reduced growth in FEV_1 for all pollutants, e.g. difference in 1-year growth in FEV_1 (95 % confidence interval) was -0.31% (-0.47 to -0.14%) per 7.8 μg/m^3 increase in NO_2 and -0.26% (-0.49 to -0.03%) per 1.2 μg/m^3 increase in $PM_{2.5}$. Growth in FVC was not associated with air pollution exposure except for a positive association with $PM_{2.5}$ [0.24% (0.03 to 0.45%) per 1.2 μg/m^3] (Table 4).

Cross-sectional analyses of lung function at age 16

Associations of FEV_1 and FVC at age 16 with air pollution exposure are shown in Figure 1 and Table E8. We observed lower FEV_1 at age 16, with higher air pollution exposure e.g. percent difference (95% CI) was -2.14% (-3.53 to -0.73%) per 1.2 μg/m^3 increase in preschool $PM_{2.5}$ and -1.29% (-2.31 to -0.26%) per 0.9 μg/m^3 increase in secondary school PM_{10}. Association estimates were consistently negative for FVC at age 16, but none of the associations was statistically significant, e.g. -0.63% (-1.68 to 0.44%) per 0.3 10^{-5}/m increase in primary school $PM_{2.5}$ absorbance and -0.64% (-1.54 to 0.26%) per 0.5μg/m^3 increase in secondary school PM_{coarse}.

Sensitivity analyses

Negative associations of air pollution exposure with FEV_1 growth and FEV_1 at age 16 were stronger in boys than in girls, whereas associations with FVC were negative in boys and mostly positive in girls (Figure 2, Tables E9 and E10). Associations of air pollution with FEV_1 and FVC (growth) were not significantly different between asthmatics and non-asthmatics but positive estimates for FEV_1 in asthmatics at age 16 were observed (Figure 2, Tables E9 and E11).

Table 1. Population characteristics for the two study populations.[α]

	Cross-sectional analyses population			Longitudinal analyses population		
Characteristic	**Total (N=721)**	**Boys (N=338)**	**Girls N=(383)**	**Total (N=915)**	**Boys (N=434)**	**Girls N=(481)**
Parental atopy						
Atopic mother, n(%)	232 (32.1)	111 (32.8)	121 (31.5)	434 (47.4)	211 (48.6)	223 (46.3)
Atopic father, n(%)	242 (33.6)	114 (33.7)	128 (33.5)	306 (33.5)	144 (33.1)	162 (33.7)
Boys, n(%)	338 (46.8)			434 (47.4)		
Presence of pets at 3 months, n(%)	302 (41.8)	134 (39.4)	168 (43.8)	409 (44.9)	182 (41.9)	212 (44.0)
Presence of mould at 3 months, n(%)	193 (27.2)	112 (36.6)	144 (41.5)	243 (27.1)	143 (36.4)	169 (38.9)
Breastfeeding > 12 weeks, n(%)	434 (60.2)	191 (56.5)	243 (63.4)	534 (58.4)	246 (56.6)	288 (59.8)
Gas cooking at 3 months, n(%)	613 (85.3)	281 (83.1)	332 (87.3)	771 (84.7)	355 (82.2)	416 (87.0)
Maternal smoking during pregnancy, n(%)	92 (12.8)	38 (11.3)	54 (14.1)	124 (13.6)	54 (12.5)	70 (14.6)
Indoor tobacco smoke exposure at 3 months, n(%)	146 (20.2)	69 (20.4)	77 (20.1)	192 (20.9)	89 (20.5)	103 (21.4)
Parental education						
Low, n(%)	53 (7.3)	27 (7.9)	26 (6.7)	75 (8.2)	40 (9.2)	35 (7.3)
Intermediate, n(%)	215 (29.8)	97 (28.7)	118 (30.8)	282 (30.8)	127 (29.2)	155 (32.3)
High, n(%)	453 (62.8)	214 (63.3)	239 (62.4)	557 (60.9)	267(61.5)	290 (60.4)
Dutch nationality	686 (97.1)	323 (97.3)	363 (96.8)	872 (96.5)	413 (96.0)	459 (96.9)
Asthma at age 16, n(%)	59 (8.5)	32 (9.8)	27 (7.3)	55 (9.1)	29 (10.2)	26 (8.0)
Respiratory infections 3 weeks before the lung function measurement	303 (42.1)	128 (37.8)	175 (45.7)	266 (42.1)	111 (38.1)	155 (45.3)
Age 16, n(%)				298 (33.7)	121 (28.8)	177 (38.1)
Age 12, n(%)				102	65 (21.5)	95 (28.8)
Age 8, n(%)				(5.3)		

α: N smaller than indicated in some variables due to missing values

Table 2. Distribution of age, height, weight, body mass index, FEV_1 and FVC for the two study populations.[α]

	Cross-sectional analyses population			Longitudinal analyses population		
	Total (N=721)	**Boys (N=338)**	**Girls N=(383)**	**Total (N=915)**	**Boys (N=434)**	**Girls (N=481)**
16 years		**(mean (SD)**		**(mean (SD)**		
Age (years)	16.3 (0.2)	16.3 (0.2)	16.3 (0.2)	16.3 (0.2)	16.3 (0.2)	16.3 (0.2)
Weight (kg)	64.2 (10.1)	68.1 (10.1)	60.7 (8.8)	64.1 (10.0)	67.8 (9.9)	60.9 (8.8)
Height (cm)	175.5 (8.6)	181.7 (6.7)	169.9 (6.0)	175.3 (8.5)	181.5 (6.4)	170.0 (6.0)
BMI (kg/m^2)	20.8 (2.7)	20.6 (2.7)	20.9 (2.6)	20.8 (2.6)	20.5 (2.6)	21.0 (2.6)
FEV_1 (L)	3.9 (0.7)	4.4 (0.6)	3.4 (0.4)	3.9 (0.7)	4.4 (0.6)	3.5 (0.4)
FVC (L	4.7 (0.8)	5.3 (0.7)	4.1 (0.5)	4.7 (0.8)	5.3 (0.6)	4.1 (0.5)
12 years						
Age (years)				12.6 (0.3)	12.6 (0.3)	12.6 (0.3)
Weight (kg)				40.6 (7.3)	47.6 (9.2)	48.8 (8.9)
Height (cm)				152.1 (6.9)	159.5 (7.8)	160.5 (7.2)
BMI (kg/m^2)				18.7 (2.6)	18.6 (2.6)	18.8 (2.6)
FEV_1 (L)				2.7 (0.4)	2.7 (0.4)	2.7 (0.4)
FVC (L)				3.2 (0.5)	3.2 (0.5)	3.2 (0.5)
8 years						
Age (years)				8.1 (0.3)	8.0 (0.3)	8.1 (0.3)
Weight (kg)				28.5 (4.3)	29.0 (4.4)	29.0 (4.9)
Height (cm)				133.4 (5.2)	133.4 (5.4)	132.9 (5.7)
BMI (kg/m^2)				16.3 (1.8)	16.2 (1.7)	16.3 (1.9)
FEV_1 (L)				1.7 (0.2)	1.8 (0.2)	1.7 (0.2)
FVC (L)				2.0 (0.3)	2.0 (0.2)	1.9 (0.2)

α: N smaller than indicated in some variables due to missing values

Table 3. Distribution of annual average exposure concentrations for time windows of exposure and short-term exposures (cross-sectional analyses population, N=721).

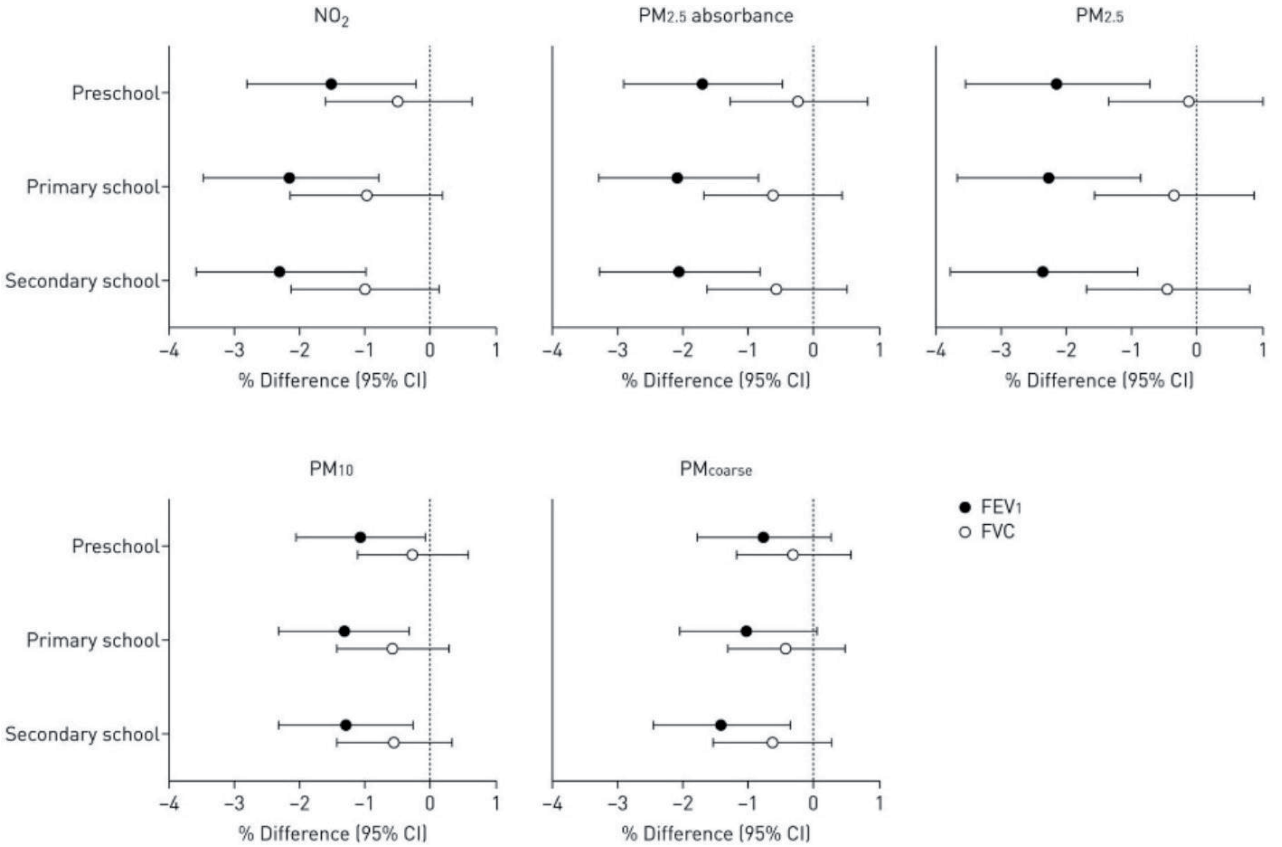
	Min	Median	Mean (SD)	IQR	75th Percentile	Max
Preschool						
NO_2 (μg/m³)	10.3	21.5	21.3 (5.5)	7.4	24.8	44.4
$PM_{2.5}$ absorbance (10^{-5}/m)	0.8	1.2	1.2 (0.2)	0.3	1.2	2.1
$PM_{2.5}$ (μg/m³)	14.9	16.4	16.2 (0.7)	1.2	16.6	19.4
PM_{10} (μg/m³)	23.7	24.4	24.6 (0.7)	0.9	24.8	28.6
PM_{coarse} (μg/m³)	7.5	8.1	8.2 (0.5)	0.5	8.3	11.1
Primary school						
NO_2 (μg/m³)	10.3	20.9	20.7 (5.2)	7.6	24.3	44.4
$PM_{2.5}$ absorbance (10^{-5}/m)	0.8	1.1	1.1 (0.2)	0.3	1.2	1.9
$PM_{2.5}$ (μg/m³)	14.9	16.4	16.2 (0.7)	1.2	16.6	19.4
PM_{10} (μg/m³)	23.7	24.3	24.5 (0.7)	0.8	24.8	28.5
PM_{coarse} (μg/m³)	7.5	7.9	8.1 (0.4)	0.5	8.3	10.7
Secondary school						
NO_2 (μg/m³)	10.3	20.9	20.6 (5.3)	7.9	24.3	44.4
$PM_{2.5}$ absorbance(10^{-5}/m)	0.8	1.1	1.1 (0.2)	0.3	1.2	1.8
$PM_{2.5}$ (μg/m³)	14.8	16.4	16.2 (0.6)	1.2	16.6	18.7
PM_{10} (μg/m³)	23.7	24.3	24.5 (0.7)	0.8	24.7	27.7
PM_{coarse} (μg/m³)	7.5	7.9	8.1 (0.4)	0.5	8.3	10.7
Short term exposures[α]						
NO_2	24.1	14.7	15.9	9.6	20.1	42.7
PM_{10}	8.3	15.8	17.8	6.8	20.3	46.5

αAverage concentrations for the 7 days preceding the 16-year lung function measurement.
IQR = interquartile range

Table 4. Additionally adjusted associations of preschool time window average air pollution exposure with lung function growth from age 8 to 16 (N=871).[α]

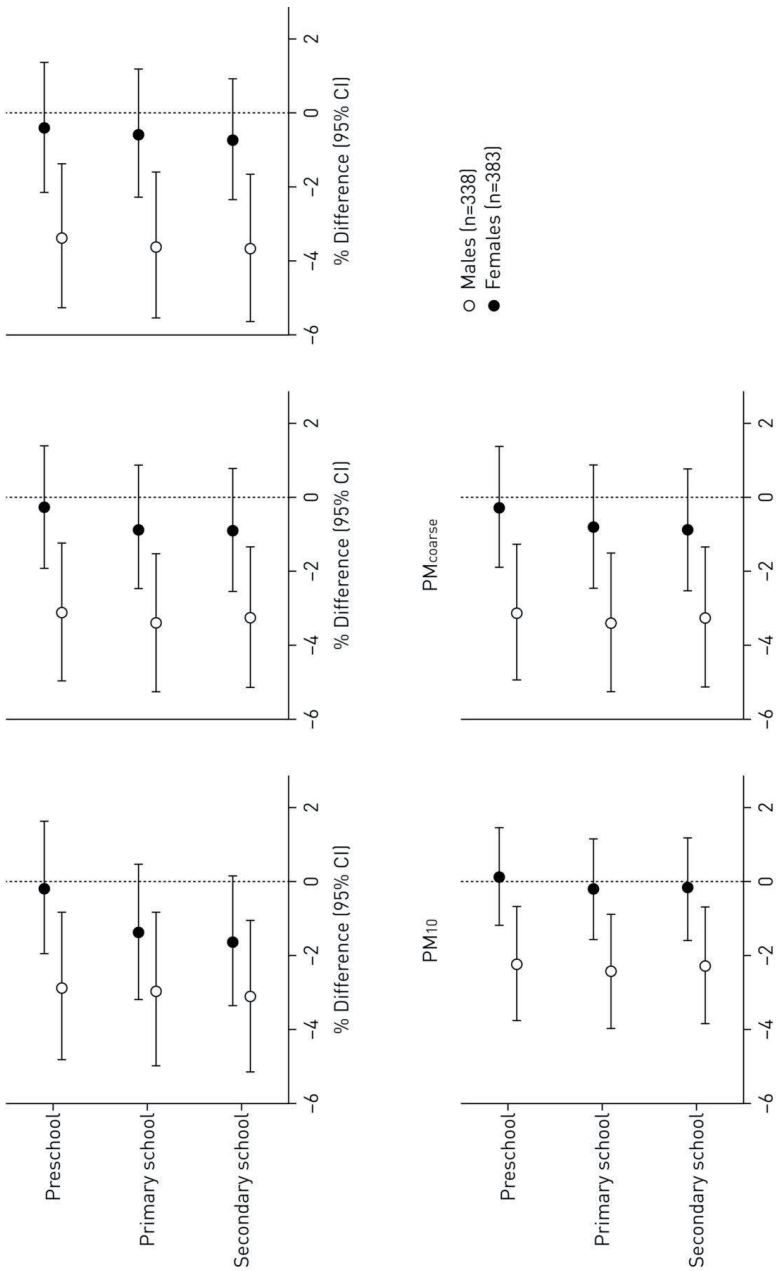
	Increment	Difference in FEV_1 % (95% CI)	Difference in FVC % (95% CI)
NO_2	7.8 μg/m³	-0.31 (-0.47 to -0.14)	0.01 (-0.14 to 0.16)
$PM_{2.5}$ absorbance	0.3 10^{-5}/m	-0.33 (-0.51 to -0.16)	0.05 (-0.11 to 0.22)
$PM_{2.5}$	1.2 μg/m³	-0.26 (-0.49 to -0.03)	0.24 (0.03 to 0.45)
PM_{10}	0.9 μg/m³	-0.20 (-0.33 to -0.08)	-0.02 (-0.13 to 0.09)
PM_{coarse}	0.5 μg/m³	-0.17 (-0.28 to -0.06)	-0.01 (-0.11 to 0.09)

α: Estimates interpreted as the percent difference in 1-year growth (95% confidence intervals) in FEV_1 (FVC) for an interquartile range (IQR) increase in exposure. Adjusted for sex, age, and log-transformations of weight, and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks (prior to the medical examination), Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets in the home at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. N smaller than indicated due to missing data.



Adjusted for sex, age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement.

Figure 1. Additionally adjusted associations of time window average air pollution exposure with lung function at age 16 years (N=674).



Adjusted for age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement.

Figure 2. Adjusted associations of time window average air pollution exposure with forced expiratory volume in 1s at age 16, stratified by sex.

Analyses with mutual adjustment of time windows showed lower FEV_1, with higher air pollution exposure mainly with secondary school time window exposure but estimates were attenuated for the preschool time window (Table E12).

DISCUSSION

In the PIAMA birth cohort, we found that higher air pollution exposure from birth until age 16 was associated with reduced FEV_1 growth from age 8 to 16 and lower FEV_1 at age 16, but not with reduced FVC and FVC growth.

Our findings concur with longitudinal studies that have reported associations of air pollution exposure with reduced lung function growth. The longitudinal CHS from the USA has demonstrated reduced lung function growth with increasing air pollution exposure in adolescents.[2,5] Over an 8-year period, exposure to NO_2 and $PM_{2.5}$ was associated with reduced growth in FEV_1 and FVC.[2] However, the CHS assessed exposure from study entry (~10 years) and not exposure from birth. The Swedish BAMSE cohort study [10] addressed this gap and found that exposure to nitrogen oxides (NO_x) and PM_{10} at the participants' home addresses during the first year of life was not associated with reduced lung function growth in contrast to our findings.

This study and the BAMSE study are, to our knowledge, the only two studies that have studied the association of air pollution exposure from birth to adolescence with lung function growth into adolescence. More longitudinal studies are warranted to confirm and add to these findings. Better understanding of the effects of air pollution exposure on subsequent lung function growth extending into adolescence and adulthood is imperative for preventive and management strategies in reducing the burden of respiratory diseases.[2]

Associations of air pollution exposure with lung function have been reported in several studies in pre- and primary school children aged 5-11years followed from birth, [7-9,11,25] but analyses linking air pollution exposure to lung function until adolescence are scarce. A recent BAMSE study investigated the association of air pollution exposure from birth into adolescence and lung function in adolescents and reported associations of traffic-related air pollution exposure during the first year of life with lower FEV_1 at age 16, but not during later time periods.[10] The German GINI/LISA study[12] performed a similar analysis and reported no association of air pollution exposure at birth, 10 and 15-year addresses with lung function at 15 years, in contrast to our study. We previously reported that higher exposure to air pollution at the current address was associated with lower FEV_1 at ages 8[11] and 12, [17] which suggests the importance of mid-childhood exposures in our cohort. Opportunities to compare air pollution concentrations between studies are limited as different methods have been used in different studies. However, comparisons can be made for the Swedish BAMSE and

German GINI/LISA cohorts, because exposures have been estimated using a standardized methodology recently.[11] Compared with the Netherlands, air pollution levels in Sweden, are considerably lower but comparable to Germany (Table E13). Comparison of pollutant concentrations with other studies such as CHS is not straightforward because different exposure assessment methods were used. The present findings extend into adolescence and add that air pollution exposure has an impact on lung function until adolescence. Together with findings of the BAMSE study, our results strengthen the conclusions of a recent review that air pollution exposure in early-life, later life and exposure over the entire age range could be relevant. [26]

We found higher air pollution exposure to be associated with lower FEV_1 and reduced growth in FEV_1, but no indication for an adverse effect on FVC. This has also been observed in other studies. [7,27,28] Our results suggest that air pollution exposure affects the diameter of the airways and hence increases airway obstruction, but does not affect lung volume. However, current evidence is not sufficient to conclude that the effects of air pollution are limited to specific spirometric measures.[29]

We observed associations of air pollution exposure with both reduced FEV_1 growth and lower FEV_1 in boys, but not in girls, consistent with CHS and BAMSE findings.[2,10] Literature suggests that boys already present with a higher total number of alveoli and alveolar surface area at birth than girls and that boys may have a pulmonary phenotype more susceptible to adverse effects of air pollution exposure in early childhood since they have narrower airways between infancy and adolescence.[30] However, the current literature is inconsistent with regard to a possible air pollution-sex interaction as a number of studies in children have shown stronger associations of air pollution and lung function in girls[7,31] and other studies have found no differences.[12,25] The results of these studies are summarized in Table E13. We observed reduced FEV_1 growth in asthmatics and non-asthmatics with increasing air pollution exposure and lower FEV_1 in non-asthmatics but not in asthmatics; however, the differences were not statistically significant. Associations with air pollution tended to be negative for FVC and FVC growth in both asthmatics and non-asthmatics. Although stronger negative associations with FEV_1 have been reported in non-asthmatics than in asthmatics in the CHS and BAMSE cohorts [5,10] we need to acknowledge that our study had few asthmatics and therefore was possibly underpowered to identify significant differences by asthma status. Earlier reports of interactions of air pollution with asthma are as inconsistent (Table E13). Most studies that investigate effect modification by asthma are likely underpowered to detect a difference and literature seldom reports consistent stronger associations in asthmatics or weaker effects in asthmatics.[26]

In mutually adjusted time window analyses, associations with exposure during the secondary school time window were more pronounced than associations with preschool exposure, suggesting that later life exposure could play a key role in the level of lung function of adolescents in our study.

An important strength of our study is the availability of repeated objective measurements of lung function and detailed individual exposure data from birth, allowing us to investigate effects of lifetime exposure. We also consider the analysis of lung function growth into adolescence with exposure from birth as a major strength as to the extent of our knowledge, only one other study [10] has done this.

This study also has several important limitations. Two different spirometers were used to measure lung function during the 16-year medical examination. We acknowledge that systematic differences between measurements obtained by the two spirometers may affect the estimated air pollution exposure-lung function relationships. We, therefore, conducted a separate experiment in healthy volunteers and derived calibration equations to correct for systematic differences. The correlation between the readings from the two instruments was very high (0.98-0.99) and the calibration factor was estimated with great precision so that after calibration, the impact of the use of different instruments on our findings is likely small.

We used spatial exposure models based on an air pollution measurement campaign performed in 2008–2010 to assess air pollution exposure from 1996/1997 (when children were born) until 2013/2014 when 16-year lung function measurements were conducted assuming constant spatial contrasts in air pollution levels since birth. Several studies have demonstrated the validity of LUR models over several years [32-34] supporting our assumption of constant spatial contrasts. In addition, measurement data from the Dutch National Air Quality Monitoring Network also show that annual average concentrations of NO_2 and PM_{10} have not changed substantially between 2000 and 2007.[35] We did not account for long-term temporal trends in air pollution levels since the beginning of the cohort. Therefore, the stronger associations with more recent exposure than with early-life exposure could be partly attributed to measurement error which may be larger for early-life exposures because of larger time difference with the LUR models measurement campaign.

Another limitation is that we used estimated residential exposure disregarding other sources exposures e.g. school address exposures. However, the correlation between home and school address exposures was moderate to high for NO_2, $PM_{2.5}$, $PM_{2.5}$ absorbance and PM_{10} in our cohort (r=0.68–0.88 for primary school exposure and r=0.36–0.73 for secondary school exposure, Table E14). Therefore, measurement error resulting from reliance on residential exposure is likely small.

We acknowledge the difficulty to disentangle effects in the different time windows because pollution levels during the different time windows were correlated. Therefore, effects of exposure in later life may reflect effects of earlier life exposures. We, however, attempted to disentangle preschool from secondary school time window exposures in movers and results suggested greater importance of later life exposures for lung function in adolescence.

The prevalence of maternal atopy was higher in the longitudinal study population than in the source population due to the overrepresentation of children of atopic mothers

invited for lung function measurements at age 8. There were also more children from highly educated parents in the study population compared with the baseline PIAMA population, which may limit generalizability of our findings to the full PIAMA cohort and to the general population.

CONCLUSION

In conclusion, higher air pollution exposure may lead to increased airway obstruction, but not to reduced lung size in adolescence. We contribute to limited knowledge on the potential impact of air pollution exposure on lung function development throughout childhood into adolescence.

ACKNOWLEDGEMENTS

The authors thank all the children and parents for their participation, all the field workers and laboratory personnel involved for their efforts and Marjan Tewis (Institute for Risk Assessment Sciences (IRAS), Utrecht University) for data management.

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SUPPLEMENTARY MATERIAL

Table E1. Comparison of characteristics between baseline population and study populations. [α]

	Baseline population		Cross-sectional analyses population			Longitudinal analyses population		
	(N=3963)		(N=721)			(N=915)		
Characteristic	**n/N**	**%**	**n/N**	**%**	**P-Value[β]**	**n/N**	**%**	**P-value[γ]**
Parental atopy								
Atopic mother	1237/3963	31.2	232/720	32.1	0.607	434/915	47.4	0.000
Atopic father	1217/3957	30.7	242/720	33.6	0.128	306/914	33.5	0.041
Boys	2054/3963	51.8	338/721	46.8	0.014	434/915	47.4	0.002
Presence of pets at 3 months	1923/3947	48.7	312/718	43.4	0.009	409/911	44.9	0.008
Presence of mould at 1 year	1047/3702	28.3	193/708	27.2	0.579	243/900	27.1	0.326
Breastfeeding > 12 weeks	1892/3896	48.5	434/721	60.2	<0.000	534/915	58.4	<0.000
Gas cooking at 3 months	3247/3923	82.7	613/718	85.3	0.085	771/910	84.7	0.074
Maternal smoking during pregnancy	700/3926	17.8	92/716	12.8	0.001	72/863	13.6	0.000
Indoor tobacco smoke exposure at 3 months	1129/3963	28.4	146/721	20.2	<0.001	124/910	20.9	<0.001
Parental education					<0.001			<0.001
Low	502/3812	13.1	53/721	7.3		7 5/914	8.2	
Intermediate	1402/3812	36.7	215/721	29.8		282/914	30.8	
High	1908/3812	50.1	453/721	62.8		557/914	60.9	
Dutch nationality	3485/3700	94.1	686/707	97.1	0.002	872/904	96.5	0.000
Asthma at age 16	66/767	8.6	59/691	8.5	0.649	55/607	9.1	0.085

α Continuous characteristics compared by t-tests and categorical characteristics compared using Chi-Square tests

β: Comparison of baseline population and the cross-sectional analyses data

γ: Comparison of baseline population and the longitudinal analyses data.

Table E2. Comparison of characteristics between non- movers and movers in cross-sectional analyses study population (N=721).[α]

	Non-Movers		**Movers**		
	(N=268)		**(N=453)**		
Characteristic	**n/N**	**(%)**	**n/N**	**(%)**	**P-Value**
Parental atopy					
Atopic mother	83/268	30.9	149/453	32.8	0.593
Atopic father	91/268	33.9	151/452	33.5	0.880
Boys	139/268	51.8	199/453	43.9	0.039
Presence of pets at 3 months	118/268	44.1	94/450	43.1	0.810
Presence of mould at 1 year	72/258	27.3	121/445	27.2	0.957
Breastfeeding > 12 weeks	159/268	59.3	275/453	60.7	0.714
Gas cooking at 3 months	221/265	16.6	392//453	86.5	0.250
Maternal smoking during pregnancy	32/266	12.1	60/450	13.3	0.614
Indoor tobacco smoke exposure at 3 months	54/268	20.1	92/453	20.3	0.958
Parental education					0.000
Low	26/268	9.7	27/453	5.9	
Intermediate	99/268	36.9	116/453	25.7	
High	143/268	53.3	310/453	68.4	
Dutch nationality	254/261	97.3	432/446	96.7	0.729
Respiratory infections in the 3 weeks before the lung function measurement	109/268	40.6	194/453	42.8	0.571
N, Mean (SD)					
Age (years) β	268, 16.3 (0.2)		453, 16.3 (0.2)		0.643
Weight (kg)β	268, 64.9 (10.3)		453, 63.8 (10.1)		0.160
Height (cm)β	268, 175.9 (8.2)		453, 175.7 (8.9)		0.246
FEV1 (L)β	268, 4.1 (0.7)		453, 3.9 (0.7)		0.037
FVC (L)β	268, 4.7 (0.8)		453, 4.6 (0.8)		0.111

α: Continuous characteristics compared by t-tests and categorical characteristics compared using Chi-Square tests.
β: at the time of the 16-year lung function measurements

Table E3. Distribution of annual average concentrations of air pollution for different time windows of exposure, and short-term exposures for longitudinal analyses population, N=915.

	Min	Median	Mean (SD)	IQR	75th Percentile	Max
Preschool						
NO2 (µg/m3)	**10.3**	**22.6**	**22.4 (6.2)**	**8.2**	**26.[3]**	**52.4**
PM2.5 absorbance (10-5/m)	0.8	1.2	1.2 (0.2)	0.3	1.3	2.6
PM2.5 (µg/m3)	15.2	16.4	16.3 (0.6)	1.5	16.7	19.4
PM10 (µg/m3)	23.7	24.5	24.7 (0.9)	0.9	25.0	29.8
PMcoarse (µg/m3)	7.6	8.1	8.3 (0.6)	0.7	8.5	12.3
Primary school						
NO2 (µg/m3)	10.3	22.0	21.8 (5.8)	8.4	25.8	47.7
PM2.5 absorbance (10-5/m)	0.8	1.2	1.1 (0.2)	0.3	1.3	2.0
PM2.5 (µg/m3)	14.9	16.4	16.2 (0.6)	1.1	16.7	19.4
PM10 (µg/m3)	23.7	24.4	24.6 (0.8)	0.9	24.9	29.8
PMcoarse (µg/m3)	7.6	8.0	8.2 (0.6)	0.6	8.43	11.4
Secondary school						
NO2 (µg/m3)	10.0	21.8	21.5 (5.8)	8.6	25.56	47.7
PM2.5 absorbance (10-5/m)	0.8	1.1	1.1 (0.2)	0.3	1.30	2.0
PM2.5 (µg/m3)	14.9	16.4	16.2 (0.6)	1.1	16.6	18.6
PM10 (µg/m3)	23.7	24.4	24.5 (0.8)	0.8	24.8	29.8
PMcoarse (µg/m3)	7.60	8.0	8.2 (0.6)	0.6	8.3	11.4
Short term exposure[α]						
NO2 (µg/m3)						
8 year med. exam	2.7	19.2	21.4 (10.3)	14.6	28.7	55.7
12 year med. exam	4.0	15.6	20.1 (9.9)	11.1	22.8	62.1
16 year med. exam	4.1	14.7	15.9 (6.9)	9.7	20.2	42.8
PM10 (µg/m3)						
8 year med. exam	12.5	25.5	28.0 (9.5)	11.1	32.3	74.7
12 year med. exam	8.8	20.8	23.1 (9.6)	11.1	27.7	70.5
16 year med. exam	8.3	16.1	18.1 (6.8)	7.2	20.7	46.5

α: average concentrations of 7 days preceding the respective lung function measurements.

Table E4. Mean difference between preschool and secondary school exposure for movers (N=453).

	Mean (SD)	(Min, Max)
NO_2 (µg/m^3)	-1.65 (5.1)	-19.2, 17.5
$PM_{2.5}$ absorbance (10^{-5}/m)	-0.05 (0.1)	-1.2,0.6
$PM_{2.5}$ (µg/m^3)	-0.08 (0.6)	-3.6 ,2.7
PM_{10} (µg/m^3)	-0.26 (0.9)	-5.1, 3.3
PM_{coarse} (µg/m^3)	-0.14 (0.6)	-2.3, 1.8

Table E5. Correlation matrix of time window average concentrations of air pollution for the cross-sectional analyses population (N=721).[α]

	NO_2			$PM_{2.5}$ absorbance			$PM_{2.5}$			PM_{10}			PM_{coarse}		
	Birth–4	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**
NO_2															
Birth–4	1.00	**0.89**	**0.80**	0.70	0.58	0.52	0.73	0.63	0.56	**0.87**	0.78	0.71	0.71	0.65	0.61
5-12		1.00	**0.94**	0.60	0.67	0.63	0.63	0.72	0.69	0.79	**0.86**	**0.82**	0.67	0.71	0.69
13–16			1.00	0.51	0.62	0.67	0.54	0.67	0.72	0.71	**0.80**	**0.85**	0.61	0.67	0.69
$PM_{2.5}$ abs															
Birth–4				**0.86**	0.70	0.61	0.72	0.64	0.55	1.00	**0.89**	0.80	**0.91**	**0.82**	0.75
5-12				0.72	**0.85**	0.78	0.62	0.72	0.66		1.00	**0.93**	0.83	**0.91**	**0.86**
13–16				0.61	0.77	**0.84**	0.54	0.67	0.70			1.00	0.74	**0.86**	**0.89**
$PM_{2.5}$															
Birth–4				0.67	0.56	0.46	0.56	0.52	0.44				1.00	**0.90**	**0.82**
5-12				0.57	0.65	0.61	0.49	0.57	0.51					1.00	**0.96**
13–16				0.48	0.59	0.64	0.43	0.53	0.53						1.00
PM_{10}															
Birth–4				1.00	0.78	0.65	0.77	0.63	0.52						
5-12					1.00	**0.91**	0.61	0.75	0.67						
13–16						1.00	0.52	0.68	0.72						
PM_{coarse}															
Birth–4							1.00	**0.82**	0.66						
5-12								1.00	**0.90**						
13–16									1.00						

α: Birth–4=**Preschool**, 5-12=**Primary school**, 13–16=**secondary school**. High correlations are presented in bold font

Table E6. Correlation matrix of time window average concentrations of air pollution for the longitudinal analyses population (N=915).[α]

	NO_2			$PM_{2.5}$ absorbance			$PM_{2.5}$			PM_{10}			PM_{coarse}		
	Birth–4	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**
NO_2															
Birth–4	1.00	**0.92**	**0.84**	0.74	0.67	0.62	0.76	0.67	0.57	**0.90**	**0.83**	0.76	0.75	0.67	0.59
5-12		1.00	**0.95**	0.70	0.72	0.69	0.68	0.73	0.67	**0.83**	**0.89**	**0.85**	0.68	0.74	0.69
13–16			1.00	0.64	0.69	0.70	0.60	0.68	0.70	0.76	**0.85**	**0.88**	0.62	0.71	0.73
$PM_{2.5}$ abs															
Birth–4				1.00	**0.90**	**0.81**	0.65	0.56	0.46	**0.89**	**0.82**	0.73	0.58	0.50	0.42
5-12					1.00	**0.95**	0.56	0.62	0.57	0.79	**0.88**	**0.84**	0.49	0.54	0.49
13–16						1.00	0.48	0.57	0.61	0.71	**0.83**	**0.87**	0.44	0.51	0.51
$PM_{2.5}$															
Birth–4							1.00	**0.86**	0.70	**0.87**	0.76	0.66	**0.80**	0.68	0.57
5-12								1.00	**0.91**	0.75	0.85	**0.80**	0.69	0.76	0.70
13–16									1.00	0.63	0.78	**0.85**	0.58	0.70	0.74
PM_{10}															
Birth–4										1.00	**0.91**	**0.81**	0.75	0.65	0.56
5-12											1.00	**0.95**	0.66	0.72	0.66
13–16												1.00	0.60	0.69	0.70
PM_{coarse}															
Birth–4													1.00	**0.85**	0.72
5-12														1.00	**0.93**
13–16															1.00

α: Birth–4=**Preschool**, 5-12=**Primary school**, 13–16=**secondary school.** High correlations are presented in bold

Table E7. Correlation matrix of time window average concentrations of air pollution for participants who changed address since birth (N=453).[α]

	NO_2			$PM_{2.5}$ absorbance			$PM_{2.5}$			PM_{10}			PM_{coarse}		
	Birth–4	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**	**Birth–4**	**5-12**	**13–16**
NO_2															
Birth–4	1.00	**0.83**	0.67	**0.88**	0.67	0.62	0.70	0.59	0.57	0.67	0.56	0.41	0.69	0.62	0.47
5-12		1.1.00	**0.87**	0.76	**0.83**	0.78	0.67	0.69	0.68	0.59	0.69	0.59	0.63	0.74	0.66
13–16			1.00	0.62	**0.80**	**0.85**	0.56	0.67	0.69	0.43	0.56	0.68	0.47	0.62	0.75
$PM_{2.5}$abs															
Birth–4				1.00	0.74	0.67	**0.88**	0.69	0.65	**0.81**	0.66	0.44	0.69	0.61	0.45
5-12					1.00	**0.91**	0.72	**0.90**	**0.84**	0.57	0.78	0.75	0.52	0.67	0.66
13–16						1.00	0.66	**0.84**	**0.88**	0.49	0.67	**0.84**	0.47	0.61	0.69
$PM_{2.5}$															
Birth–4							1.00	0.79	0.74	0.67	0.56	0.36	0.56	0.52	0.39
5-12								1.00	**0.95**	0.45	0.60	0.58	0.42	0.54	0.51
13–16									1.00	0.39	0.52	0.61	0.38	0.50	0.53
PM_{10}															
Birth–4										1.00	0.77	0.48	0.76	0.61	0.39
5-12											1.00	0.77	0.60	0.74	0.57
13–16												1.00	0.39	0.57	0.71
PM_{coarse}															
Birth–4													1.00	**0.83**	0.51
5-12														1.00	0.76
13–16															1.00

α: Birth–4=**Preschool**, 5-12=**Primary school**, 13–16=**secondary school**. ≥0.80=high correlation

Table E8. Associations of time window average air pollution exposure with FEV_1 and FVC at age 16.

	Difference in FEV_1: % (95% CI)		Difference in FVC: % (95% CI)	
Exposure	**Minimally adjusted (N=719)[α]**	**Additionally adjusted (N=671)[β]**	**Minimally adjusted (N=719)[α]**	**Additionally adjusted (N=671)[β]**
Preschool				
NO_2	-0.92 (-2.04 to 0.20)	-1.52 (-2.81 to -0.22)	0.02 (-0.94 to 1.00)	-0.50 (-1.61 to 0.63)
$PM_{2.5}$ absorbance	-1.37 (-2.51 to -0.21)	-1.71 (-2.91 to -0.49)	-0.00 (-1.00 to 1.00)	-0.23 (-1.28 to 0.83)
$PM_{2.5}$	-1.79 (-3.11 to -0.44)	-2.14 (-3.53 to -0.73)	0.07 (-1.10 to 1.25)	-0.15 (-1.37 to 1.08)
PM_{10}	-0.88 (-1.81 to 0.06)	-1.07 (-2.05 to -0.08)	-0.12 (-0.92 to 0.70)	-0.27 (-1.12 to 0.58)
PM_{coarse}	-0.56 (-1.49 to 0.37)	-0.76 (-1.77 to 0.27)	-0.16 (-0.96 to 0.64)	-0.32 (-1.19 to 0.56)
Primary school				
NO_2	-1.34 (-2.50 to -0.16)	-2.15 (-3.48 to -0.80)	-0.35 (-1.36 to 0.67)	-0.99 (-2.14 to 0.18)
$PM_{2.5}$ absorbance	-1.74 (-2.90 to -0.57)	-2.08 (-3.29 to -0.86)	-0.43 (-1.45 to 0.59)	-0.63 (-1.68 to 0.44)
$PM_{2.5}$	-1.94 (-3.27 to -0.60)	-2.27 (-3.66 to -0.87)	-0.20 (-1.36 to 0.98)	-0.36 (-1.57 to 0.87)
PM_{10}	-1.12 (-2.08 to -0.16)	-1.32 (-2.31 to -0.32)	-0.46 (-1.29 to 0.37)	-0.58 (-1.43 to 0.29)
PM_{coarse}	-0.82 (-1.81 to 0.17)	-1.02 (-2.05 to 0.03)	-0.25 (-1.10 to 0.60)	-0.43 (-1.32 to 0.47)
Secondary school				
NO_2	-1.53 (-2.66 to -0.38)	-2.30 (-3.59 to -0.99)	-0.42 (-1.41 to 0.58)	-1.01 (-2.14 to 0.13)
$PM_{2.5}$ absorbance	-1.75 (-2.92 to -0.57)	-2.07 (-3.29 to -0.83)	-0.38 (-1.40 to 0.66)	-0.56 (-1.63 to 0.52)
$PM_{2.5}$	-2.08 (-3.41 to -0.72)	-2.36 (-3.76 to -0.94)	-0.30 (-1.48 to 0.89)	-0.46 (-1.69 to 0.78)
PM_{10}	-1.10 (-2.09 to -0.11)	-1.29 (-2.31 to -0.26)	-0.41 (-1.26 to 0.44)	-0.55 (-1.43 to 0.34)
PM_{coarse}	-1.27 (-2.27 to -0.27)	-1.41 (-2.45 to -0.36)	-0.42 (-1.29 to 0.45)	-0.64 (-1.54 to 0.26)

α:Adjusted for sex, age, log-transformations of weight and height **β**: Adjusted for sex, age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. Exposure increments: 7.8 $\mu g/m^3$ for NO_2, 0.3 $10^{-5}/m$ for $PM_{2.5}$ absorbance, 1.2 $\mu g/m^3$ for $PM_{2.5}$, 0.9 $\mu g/m^3$ for PM_{10} and 0.5 $\mu g/m^3$ for PM_{coarse}).

Table E9. Association of preschool time window average air pollution exposure with FEV_1 and FVC growth stratified by asthma status and sex.

Exposure	Non-asthmatic (N=607)	Asthmatic(N=55)	P-Value*	Boys (N=333)	Girls (N=383)	P-Value*
Difference in FEV_1: % (95% CI)						
NO_2	-0.22 (-0.43 to 0.00)	-0.33 (-1.04 to 0.39)	0.56	-0.52 (-0.77 to -0.27)	-0.07 (-0.26 to 0.11)	0.05
$PM_{2.5}$ absorbance	-0.20 (-0.42 to 0.03)	-0.40 (-1.15 to 0.36)	0.44	-0.60 (-0.88 to -0.32)	-0.06 (-0.25 to 0.14)	0.07
$PM_{2.5}$	-0.13 (-0.40 to 0.14)	-0.44 (-1.37 to 0.49)	0.31	-0.53 (-0.90 to -0.16)	-0.03 (-0.27 to 0.22)	0.15
PM_{10}	-0.18 (-0.37 to 0.01)	-0.26 (-0.89 to 0.37)	0.85	-0.42 (-0.61 to -0.22)	0.01 (-0.12 to 0.14)	0.07
PM_{coarse}	-0.14 (-0.30 to 0.02)	-0.13 (-0.66 to 0.40)	0.34	-0.37 (-0.54 to -0.20)	0.05 (-0.06 to 0.16)	0.70
Difference in FVC: % (95% CI)						
NO_2	0.02 (-0.18 to 0.22)	0.07 (-0.64 to 0.78)	0.69	-0.14 (-0.36 to 0.08)	0.18 (-0.01 to 0.37)	0.43
$PM_{2.5}$ absorbance	0.11 (-0.09 to 0.32)	0.04 (-0.70 to 0.79)	0.54	-0.17 (-0.41 to 0.07)	0.30 (0.10 to 0.50)	0.42
$PM_{2.5}$	0.26 (0.02 to 0.51)	0.18 (-0.73 to 1.09)	0.73	-0.03 (-0.43 to 0.36)	0.49 (0.24 to 0.74)	0.38
PM_{10}	-0.02 (-0.19 to 0.15)	-0.13 (-0.74 to 0.49)	0.26	-0.17 (-0.34 to -0.00)	0.15 (0.01 to 0.28)	0.51
PM_{coarse}	-0.01 (-0.15 to 0.13)	-0.06 (-0.57 to 0.46)	0.83	-0.14 (-0.29 to 0.01)	0.15 (0.03 to 0.26)	0.71

Adjusted for sex (except for sex stratified analyses), age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. Exposure increments: 7.8 $\mu g/m^3$ for NO_2, 0.3 $10^{-5}/m$ for $PM_{2.5}$ absorbance, 1.2 $\mu g/m^3$ for $PM_{2.5}$, 0.9 $\mu g/m^3$ for PM_{10} and 0.5 $\mu g/m^3$ for PM_{coarse}).
*P-value for interaction

Table E10. Association of time window average air pollution exposure with FEV_1 and FVC at age 16 stratified by sex.

	Difference in FEV_1: % (95% CI)			Difference in FVC: % (95% CI)		
Exposure	**Boys (N=338)**	**Girls (N=383)**	**P-Value***	**Boys (N=338)**	**Girls (N=383)**	**P-Value***
Preschool						
NO_2	-2.85 (-4.81 to -0.85)	-0.18 (-1.94 to 1.61)	0.03	-1.52 (-3.20 to 0.19)	0.59 (-0.93 to 2.13)	0.01
$PM_{2.5}$ absorbance	-3.13 (-4.96 to -1.26)	-0.27 (-1.90 to 1.39)	0.02	-1.83 (-3.41 to -0.22)	1.17 (-0.27 to 2.62)	0.00
$PM_{2.5}$	-3.66 (-5.77 to -1.50)	-0.45 (-2.35 to 1.49)	0.02	-2.05 (-3.88 to -0.18)	1.65 (-0.02 to 3.36)	0.00
PM_{10}	-2.23 (-3.74 to -0.69)	0.12 (-1.19 to 1.44)	0.03	-1.32 (-2.61 to -0.00)	0.65 (-0.49 to 1.80)	0.03
PM_{coarse}	-1.74 (-3.32 to -0.12)	0.18 (-1.16 to 1.54)	0.19	-1.50 (-2.84 to -0.14)	0.79 (-0.38 to 1.97)	0.02
Primary school						
NO_2	-2.95 (-5.00 to -0.85)	-1.36 (-3.17 to 0.47)	0.17	-1.71 (-3.46 to 0.08)	-0.36 (-1.93 to 1.23)	0.06
$PM_{2.5}$ absorbance	-3.40 (-5.26 to -1.50)	-0.83 (-2.46 to 0.84)	0.03	-2.24 (-3.83 to -0.61)	0.74 (-0.70 to 2.20)	0.00
$PM_{2.5}$	-3.92 (-6.05 to -1.74)	-0.61 (-2.47 to 1.29)	0.01	-2.55 (-4.39 to -0.68)	1.59 (-0.05 to 3.26)	0.00
PM_{10}	-2.44 (-3.97 to -0.89)	-0.24 (-1.58 to 1.12)	0.03	-1.64 (-2.95 to -0.31)	0.40 (-0.77 to 1.58)	0.02
PM_{coarse}	-2.04 (-3.72 to -0.33)	-0.17 (-1.53 to 1.20)	0.13	-1.72 (-3.14 to -0.28)	0.63 (-0.55 to 1.83)	0.02
Secondary school						
NO_2	-3.12 (-5.13 to -1.06)	-1.62 (-3.35 to 0.15)	0.17	-1.67 (-3.40 to 0.09)	-0.43 (-1.94 to 1.10)	0.05
$PM_{2.5}$ absorbance	-3.27 (-5.15 to -1.36)	-0.90 (-2.54 to 0.77)	0.04	-2.15 (-3.76 to -0.51)	0.80 (-0.65 to 2.27)	0.00
$PM_{2.5}$	-4.00 (-6.15 to -1.81)	-0.63 (-2.52 to 1.30)	0.01	-2.81 (-4.66 to -0.93)	1.70 (0.03 to 3.40)	0.00
PM_{10}	-2.28 (-3.83 to -0.71)	-0.22 (-1.59 to 1.17)	0.03	-1.72 (-3.04 to -0.39)	0.54 (-0.66 to 1.75)	0.01
PM_{coarse}	-2.38 (-4.06 to -0.67)	-0.48 (-1.82 to 0.88)	0.08	-2.12 (-3.54 to -0.67)	0.49 (-0.68 to 1.68)	0.00

Adjusted for age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. Exposure increments: 7.8 $\mu g/m^3$ for NO_2, 0.3 $10^{-5}/m$ for $PM_{2.5}$ absorbance, 1.2 $\mu g/m^3$ for $PM_{2.5}$, 0.9 $\mu g/m^3$ for PM_{10} and 0.5 $\mu g/m^3$ for PM_{coarse}. *P-value for interaction

Table E11. Association of time window average air pollution exposure with FEV1 and FVC at age 16 stratified by asthma status.

	Difference in FEV_1: % (95% CI)			Difference in FVC: % (95% CI)		
Exposure	**Non-asthmatic (N=632)**	**Asthmatic (N=55)**	**P-Value***	**Non-asthmatic (N=632)**	**Asthmatic (N=55)**	**P-Value***
Preschool						
NO_2	-1.80 (-3.16 to -0.42)	2.96 (-3.18 to 9.49)	0.36	3.46 (-0.58 to 7.66)	-0.51 (-1.73 to 0.73)	0.93
$PM_{2.5}$ absorbance	-1.95 (-3.24 to -0.64)	2.24 (-3.98 to 8.87)	0.48	-1.03 (-4.65 to 2.72)	-0.03 (-1.20 to 1.16)	0.53
$PM_{2.5}$	-2.39 (-3.86 to -0.90)	2.30 (-4.10 to 9.12)	0.49	-1.48 (-5.57 to 2.78)	-0.06 (-1.40 to 1.30)	0.45
PM_{10}	-1.14 (-2.24 to -0.04)	1.49 (-5.77 to 9.30)	0.87	-1.07 (-3.79 to 1.73)	0.06 (-0.92 to 1.05)	0.37
PM_{coarse}	-0.87 (-1.99 to 0.27)	1.70 (-5.25 to 9.16)	0.58	0.38 (-2.88 to 3.76)	-0.06 (-1.07 to 0.95)	0.70
Primary school						
NO_2	-2.53 (-3.94 to -1.10)	1.76 (-5.03 to 9.03)	0.57	1.70 (-2.34 to 5.91)	-1.20 (-2.47 to 0.08)	0.97
$PM_{2.5}$ absorbance	-2.49 (-3.77 to -1.19)	1.32 (-5.38 to 8.50)	0.34	-1.67 (-5.31 to 2.12)	-0.56 (-1.72 to 0.62)	0.67
$PM_{2.5}$	-2.69 (-4.13 to -1.23)	0.33 (-3.70 to 4.53)	0.24	-1.36 (-5.39 to 2.85)	-0.41 (-1.72 to 0.93)	0.82
PM_{10}	-1.59 (-2.68 to -0.50)	1.33 (-3.20 to 7.08)	0.61	-1.88 (-4.64 to 0.97)	-0.40 (-1.38 to 0.59)	0.48
PM_{coarse}	-1.23 (-2.38 to -0.07)	0.69 (-4.00 to 5.61)	0.45	-0.80 (-4.28 to 2.81)	-0.27 (-1.30 to 0.77)	0.75
Secondary school						
NO_2	-2.83 (-4.19 to -1.45)	0.72 (-4.03 to 5.70)	0.27	2.63 (-1.18 to 7.59)	-1.22 (-2.45 to 0.03)	0.77
$PM_{2.5}$ absorbance	-2.53 (-3.81 to -1.23)	1.82 (-3.02 to 7.91)	0.27	-0.97 (-4.78 to 2.99)	-0.49 (-1.66 to 0.69)	0.77
$PM_{2.5}$	-2.80 (-4.26 to -1.33)	4.12 (-1.19 to 9.71)	0.20	-0.57 (-4.61 to 3.65)	-0.54 (-1.87 to 0.81)	0.48
PM_{10}	-1.61 (-2.72 to -0.48)	4.01 (-1.68 to 10.03)	0.47	-1.11 (-3.98 to 1.84)	-0.37 (-1.37 to 0.65)	0.62
PM_{coarse}	-1.67 (-2.82 to -0.50)	2.30 (-2.68 to 7.53)	0.43	-0.13 (-3.18 to 3.03)	-0.57 (-1.60 to 0.48)	0.95

Adjusted for sex, age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. Exposure increments: 7.8 $\mu g/m^3$ for NO_2, 0.3 $10^{-5}/m$ for $PM_{2.5}$ absorbance, 1.2 $\mu g/m^3$ for $PM_{2.5}$, 0.9 $\mu g/m^3$ for PM_{10} and 0.5 $\mu g/m^3$ for PM_{coarse}.*P-value for interaction

Table E12. Associations of time window average air pollution exposure during preschool and the secondary school time window with FEV_1 and FVC at age 16 in movers with and without mutual adjustment.

	Difference in FEV_1: % (95% CI)		Difference in FVC: % (95% CI)	
Exposure	**Single time-window in model**	**Preschool and secondary school time-windows in model**	**Single time-window in model**	**Preschool and secondary school time-windows in model**
Preschool				
NO_2	-0.85 (-2.43 to 0.76)	0.92 (-1.17 to 3.05)	0.07 (-1.35 to 1.51)	0.41 (-1.17 to 2.02)
$PM_{2.5}$ absorbance	-0.89 (-2.35 to 0.60)	0.41 (-1.59 to 2.46)	0.65 (-0.68 to 2.00)	0.15 (-1.31 to 1.64)
$PM_{2.5}$	-1.18 (-2.87 to 0.54)	0.04 (-2.34 to 2.47)	0.91 (-0.64 to 2.48)	0.48 (-1.23 to 2.22)
PM_{10}	-0.39 (-1.54 to 0.78)	0.67 (-13.0 to 16.49)	0.40 (-0.64 to 1.45)	-0.21 (-1.19 to 0.78)
PM_{coarse}	-0.37 (-1.58 to 0.85)	0.52 (-0.88 to 1.93)	0.07 (-1.01 to 1.17)	-0.29 (-1.42 to 0.86)
Secondary school				
NO_2	-1.96 (-3.55 to -0.34)	-2.60 (-4.66 to -0.50)	-0.67 (-2.12 to 0.79)	-0.93 (-2.62 to 0.78)
$PM_{2.5}$ absorbance	-1.43 (-2.93 to 0.09)	-1.75 (-3.76 to 0.30)	0.18 (-1.19 to 1.57)	0.05 (-1.59 to 1.71)
$PM_{2.5}$	-1.49 (-3.20 to 0.26)	-1.55 (-3.90 to 0.87)	0.44 (-1.13 to 2.04)	0.10 (-1.81 to 2.05)
PM_{10}	-0.62 (-1.85 to 0.62)	-0.65 (-2.02 to 0.73)	0.08 (-1.02 to 1.20)	0.16 (-1.02 to 1.34)
PM_{coarse}	-1.39 (-2.63 to -0.13)	-1.57 (-2.99 t -0.13)	-0.41 (-1.54 to 0.73)	-0.33 (-1.51 to 0.88)

Adjusted for sex, age, log-transformations of weight and height, parental education, maternal atopy, paternal atopy, breastfeeding, respiratory infections in the last 3 weeks, Dutch nationality, indoor tobacco smoke exposure in the home at 3 months, maternal smoking in pregnancy, furry pets at 3 months, mould in the home at 1 year, gas cooking at 3 months, and average air pollution concentrations for the 7 days preceding the lung function measurement. Exposure increments: 7.8 $\mu g/m^3$ for NO_2, 0.3 $10^{-5}/m$ for $PM_{2.5}$ absorbance, 1.2 $\mu g/m^3$ for $PM_{2.5}$, 0.9 $\mu g/m^3$ for PM_{10} and 0.5 $\mu g/m^3$ for PM_{coarse}).

Table E13. Summary of relevant studies assessing associations between air pollution exposure and lung function (growth)

Study	Age group	Exposure assessed	Pollutant levels (Range)	Results	Effect modification by sex	Effect modification by Asthma
Lung function growth in adolescence						
Gauderman et al 2000, CHS (USA) [1]	9-10	Annual average PM_{10}, $PM_{2.5}$, PM_{10}–$PM_{2.5}$, NO2,O_3, inorganic acid vapor assessed at study entry	PM_{10}: 18-70 µg/m^3 $PM_{2.5:}$ 7- 36 µg/m^3 NO_2: 5- 43 ppb	PM_{10}, $PM_{2.5}$, PM_{10}–$PM_{2.5}$, NO_2, and inorganic acid vapor associated with reduced FEV_1 and FVC growth,	No effect modification	No effect modification
Gauderman et al 2002, CHS (USA)[2]	9-10	Annual average NO_2,PM_{10}, $PM_{2.5}$, O_3, acid vapor, elemental carbon assessed at study entry	PM_{10}: 10-73 µg/m^3 $PM_{2.5:}$ 7-30 µg/m^3 NO_2: 3-40 ppb	Acid vapor associated with reduced FEV_1 but not FVC growth	No effect modification	No effect modification
Gauderman et al 2004, CHS (USA)[3]	10-18	Annual average NO_2,PM_{10}, $PM_{2.5}$, O_3, acid vapor, elemental carbon assessed at study entry	PM_{10}: 10-63 µg/m^3 $PM_{2.5:}$ 7- 28 µg/m^3 NO_2: 3- 40 ppb	NO_2,$PM_{2.5}$, O_3, acid vapor, elemental carbon associated with reduced FEV_1 growth	Stronger associations in boys, but no statistically significant differences with girls	Significant associations in subgroups of children with no history of asthma
Gauderman et al 2007, CHS (USA)[4]	10-18	Traffic proximity at study entry	-	Associated with FEV_1 but not FVC growth	Stronger associations in boys than in girls, non-significant differences	Significant effects in subgroups of children who never had asthma
Schultz et al 2016, BAMSE (Sweden)* [5]	16	Time weighted annual averages of NO_X and PM_{10} since birth	$PM_{10:}$ 0.2-22 µg /m3 Median: 4.5 µg/m^3	Exposure to NO_X and PM_{10} not associated with FEV_1 growth from 8-16 years	Significant associations in boys	Associations in non-asthmatics
Lung function level						
Rice et al, 2016, (USA) [6]	7	Lifetime exposure to $PM_{2.5}$ and black carbon since birth	$PM_{2.5:}$ 18-28 µg/m^3 Median: 10.7 µg/m^3	$PM_{2.5}$ and black carbon associated with FVC but not FEV_1 and	No effect modification	No effect modification
Schultz et al 2012, BAMSE (Sweden)* [7α]	8	PM_{10}, NOx assessed from birth	PM_{10}: 6-30.9 µg/m^3 Mean: 15.7 µg/m^3	First year of life PM_{10} exposure associated with lower FEV_1	Stronger associations in boys	

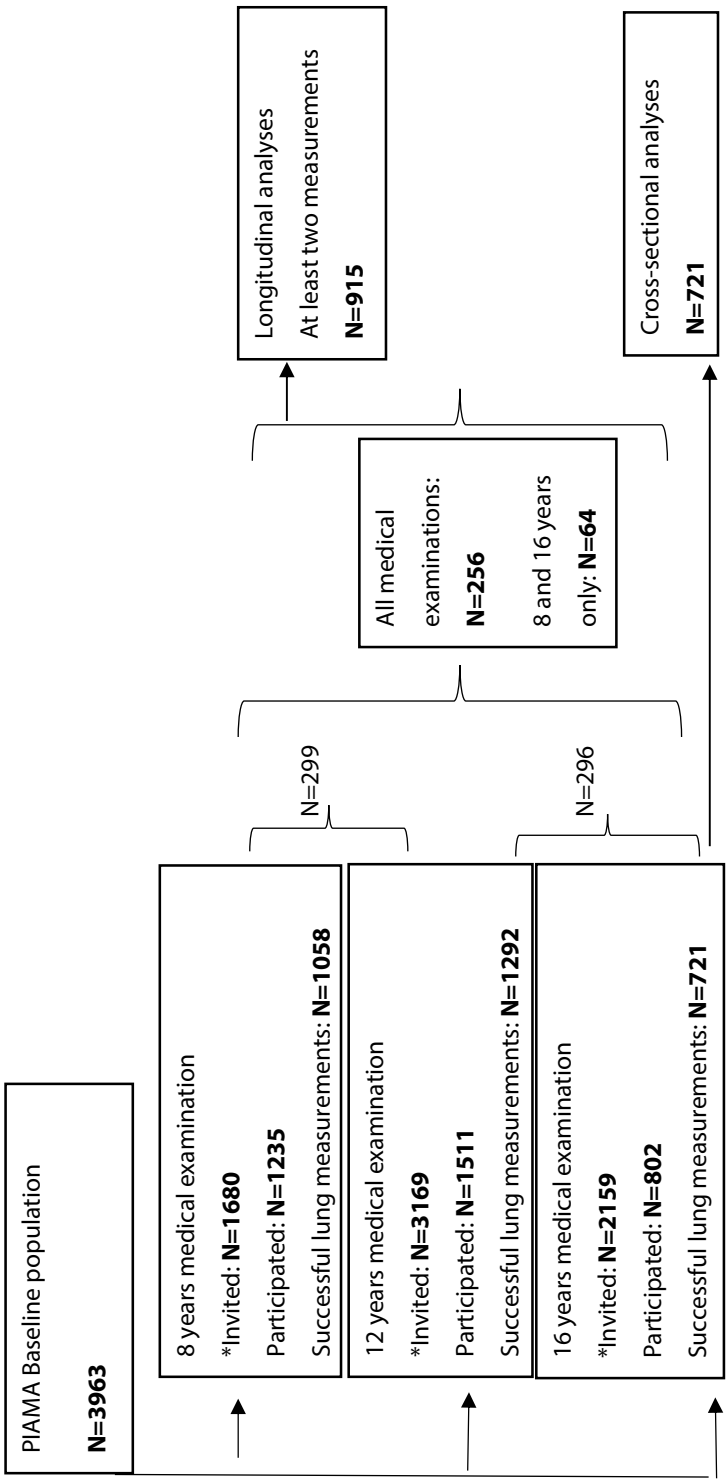
Gehring et al 2013, PIAMA (Netherlands) [8]*	8	Annual average NO_2, NOx,$PM_{2.5}$, PM_{10}, and PMcoarse, $PM_{2.5}$ absorbance	PM_{10}: 23.7-29.8 µg/m^3 Mean: 24.8 µg/m^3 $PM_{2.5}$:14.9- 19.3 µg/m^3 Mean: 16.3 µg/m^3 NO_2: 9.4–52.1 µg/m^3 Mean: 22.2 µg/m^3 $PM_{2.5}$ abs: 0.8- 2.1 10^{-5}/m Mean: 1.2 µg/m^3 PM_{coarse}: 7.6-11.2 µg/m^3 Mean: 8.3 µg/m^3	NO_2, NOx, $PM_{2.5}$ absorbance, and $PM_{2.5}$ at current address associated with FEV_1	No effect modification
Oftedal et al,2008,Oslo (Norway)[9]	9-10	Lifetime exposures to NO_2,$PM_{2.5}$, PM_{10}	PM_{10}: 10-17 µg/m^3 Mean: 14.5 µg/m^3 $PM_{2.5}$: 5- 19 µg/m^3 Mean: 12.3 µg/m^3 NO_2: 3- 65 µg/m^3 Mean: 29.0 µg/m^3	Early-life and lifetime exposure to NO_2,$PM_{2.5}$, PM_{10} associated with FVC, PEF,FEF	Stronger associations in girls
Peters et al 1999,CHS (USA) [10]	9-16	24 h/-week averages of PM_{10}, $PM_{2.5}$,O_3 and NO_2 , acid vapor assessed at study entry	PM_{10}: 13-70.7 µg/m^3 Mean: 34.9 µg/m^3 $PM_{2.5}$: 6.7- 31.5 µg/m^3 Mean: 15.1 µg/m^3 NO_2: 2.7- 42.6 ppb Mean: 21.5 ppb	PM_{10}, $PM_{2.5}$, and NO_2 significantly associated with lower FVC, FEV_1	Stronger associations in girls
Fuertes et al (2015) GINI/LISA (Germany)*[11]	15	Annual averages of NO_2, PM_{10},$PM_{2.5}$ and $PM_{2.5}$absorbance	NO_2: 11.4-59.7 µg/m^3 Median: 21.9 µg/m^3 PM_{10}: 14.8 -32.7 g/m^3 Median: 22.3 5µg/m^3 $PM_{2.5}$: 10.6-21.3 µg/m^3 Median: 14.2 µg/m^3 $PM_{2.5}$abs: 0.9-3.1 10^{-5}/m Median: 1.4 10^{-5}/m^{-1}	No associations observed for either FEV_1 or FVC for all pollutants	No consistent trends in the associations between sexes in stratified analyses
Schultz et al 2016, BAMSE (Sweden)*[5]	16	Time weighted annual averages of NO_x and PM_{10} since birth	PM_{10}: 0.2-22 µg /m3 Median: 4.5 µg/m^3 [α] NO_x: 0.5-50 µg/m^3 Median: 6 µg/m^3	Early-life exposure to NO_x but not PM_{10} associated with FEV_1 at age 16 but not FVC	Significant associations in boys

α BAMSE exposures assessed using dispersion models. Only traffic pollution considered as source of PM_{10}

Table E14. Correlation between exposure at school and home addresses for primary and secondary school time windows air pollution exposure (N=627).

	Home address									
	NO_2		**$PM_{2.5}$ absorbance**		**$PM_{2.5}$**		**PM_{10}**		**PM_{coarse}**	
School address	**primary**	**secondary**	**primary**	**secondary**	**primary**	**secondary**	**primary**	**secondary**	**primary**	**secondary**
NO_2										
Primary	**0.88**									
Secondary		0.69								
$PM_{2.5}$ abs										
Primary			**0.82**							
Secondary				0.62						
$PM_{2.5}$										
Primary					**0.85**					
Secondary						0.73				
PM_{10}										
Primary							0.68			
Secondary								0.35		
PM_{coarse}										
Primary									0.76	
Secondary										0.36

≥0.80=high correlation



*Participants invited for the extensive medical follow-up including lung function measurements

Figure E1. Flow diagram of study population from the baseline population

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CHAPTER 7

7

Detrimental effects of air pollution on adult lung function

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European Respiratory Journal.(2019) Jul 25;54(1) (Invited editorial)

Air pollution, including ambient and household air pollution, is considered one of the world's largest environmental health threats and was estimated to account for 4.9 million deaths and 147 million years of healthy life lost around the world in 2017.[1] Compelling evidence for adverse effects of air pollution on a vast array of health outcomes, in particular, cardiovascular and respiratory health, has been established. [2,3]

Ambient air pollution has been shown to adversely affect lung function through the course of life. Many cross-sectional studies and several longitudinal studies have reported lower lung function and slower lung function growth as a result of exposure to air pollution in children[4-6] and adolescents.[7-9] Fewer studies have assessed associations of air pollution with lung function in adults, but increasing evidence from individual cohort studies from Europe,[10-12] the US[13] and Asia[14] as well as a recent multi-cohort study of five European cohorts within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project,[15] suggest adverse effects on adult lung function too. Studies of effects of air pollution on lung function decline in adulthood are scarce and findings are mixed. An accelerated age-related decline in lung function among participants with higher exposure to air pollution was reported by a large study in Asia,[14] but not in an ESCAPE multi-cohort study.[14] Additional evidence comes from cohort studies that reported slower declines in lung function in relation to reductions in air pollution exposure.[13,16]

Although our understanding of the long-term effects of air pollution on lung function has improved in recent years, the role of air pollution in chronic obstructive pulmonary disease (COPD) development is still unclear. COPD is an inflammatory airway disease that affects the small airways[17] and is characterized by progressive and not fully reversible airflow limitation.[18] It is the fourth leading cause of death worldwide and has been predicted to become the third leading cause by 2030[19] making it a major public health concern. Since COPD risk is jointly determined by lung growth during childhood and adolescence leading to the maximally attained level of lung function in early adulthood as well as the timing and rate of decline of lung function later in life,[20] and these have been shown to be associated with air pollution, a link between air pollution exposure and COPD development seems plausible, but is not well investigated.[21] Evidence from the few studies that investigated this link directly across Europe as well as other geographical settings [14,21-23] is suggestive, but not conclusive.

In this month's issue of *the European Respiratory Journal*, Doiron *et al.*[24] explored associations of long-term air pollution exposure with lung function and COPD in adults aged 40-69 years. The study is unique with a sample size of 303,887 participants, largely from urban areas of England, Wales and Scotland, within the UK Biobank National Cohort Study. In this study, higher levels of Land Use Regression (LUR)-based estimates of air pollutants [nitrogen dioxide (NO_2), particulate matter with aerodynamic diameters <10 μm (PM_{10}) and <2.5 μm ($PM_{2.5}$), and between 2.5 μm and 10 μm (PM_{coarse})] at participants' baseline (2006-2010) home address, were associated with lower pre-bronchodilation spirometry metrics

of forced expiratory volume in 1s (FEV_1) and forced vital capacity (FVC), a lower FEV_1/FVC ratio and a higher risk of COPD (defined as FEV_1/FVC < lower limit of normal). Findings of this study corroborate the findings of previous research suggesting that adults exposed to higher concentrations of air pollution experience significant reductions in lung function and have a higher risk of COPD.

Placing the findings by Doiron *et al.*[24] into perspective by comparing absolute changes in lung function for standardized exposure increments of the ESCAPE study, the observed association estimates are larger than those reported from studies that used similar exposure assessment methods, i.e. LUR models from the ESCAPE project. For example, for a 10µg/m^3 increase in NO_2, Adam *et al.*[15] reported a 13.9mL (95% Confidence Interval: -25.8 to -2.1mL) reduction in FEV_1, Jong *et al.*[11] reported a 18mL (-30 to -7 mL) reduction in FEV_1, whereas Doiron *et al.*[24] reported a 33 mL (-36.3 to -31.3 mL) reduction in FEV_1 although air pollution levels were generally lower than those in the two aforementioned studies. The authors attribute the stronger estimates to the large sample size, harmonized spirometer use and protocols and reduced air pollution exposure misclassification.[24] Estimates for NO_2 reported by Doiron *et al.*[24], however, are comparable to those reported by Forbes *et al.* [-32 mL (-39 to -24 mL) per 10µg/m^3 increase in NO_2][12] from another British study that did not use ESCAPE LUR models, but air dispersion models for air pollution exposure assessment.

Determining susceptible subgroups within the population is important for developing targeted interventions to reduce the respiratory disease burden. Therefore, the assessment of potential subgroups with increased susceptibility to the adverse effects of air pollution on lung function and COPD by Doiron *et al.* presents an interesting perspective. The extraordinary large sample size, ensuring sufficient statistical power to conduct subgroups analyses, is a major strength of this study over previous studies. The stronger associations of air pollution exposure with lower lung function in subjects with a lower income and obese individuals were in line with findings of previous studies.[15,25] Associations between air pollution and lung function were found to be stronger in males, while associations between air pollution and COPD were stronger in females.[24] Current evidence on sex as a modifier of the association between air pollution and lung function is mixed. A review of the role of sex in air pollution epidemiology concluded that in later childhood, adolescence and adulthood, effects tended to be stronger for females than for males, while effects tended to be stronger for boys in early-life.[26] However, a more recent review reported uncertainty around evidence for sex effect modification of air pollution effects on lung function in childhood. [4]

Of particular interest are also the subgroup analyses by occupation presented by Doiron *et al.*,[24] as not many studies have the statistical power to reliably assess this effect modification. Significant associations with lung function, but not with risk of COPD were found for participants with current employment in one out of 14 jobs with an increased risk of COPD, compared with participants with other occupations. The authors argue that the lack of effect modification by occupation for COPD might be attributable to a healthy

worker effect, i.e. participants with COPD being less likely to work in high-risk occupations. The lack of data on occupational history hinders further considerations regarding duration of occupation and past occupations. However, supportive evidence comes from another study that has investigated a similar effect modification in relation to mortality and has reported a higher relative risk of death in relation to air pollution exposure among participants exposed to dust or fumes in the workplace.[27] More studies with adequate data are required to explore and contribute to these findings.

Despite the impressive sample size, objective spirometry measurements, and standardized exposure assessment, there are some limitations to the work by Doiron *et al*.[24] Firstly, the cross-sectional nature of the study limits the ability to establish temporal causal links of air pollution exposure with lung function and COPD in this study. Also, it is uncertain whether the lower adult lung function and higher risk of COPD are the result of changes in lung development during childhood that resulted in lower maximum achieved lung function in early adulthood and persisted into adulthood[28] or the result of an accelerated age-related decline in lung function during adulthood as suggested by some studies.[13,14,29] Continued follow-up of spirometry measurements from the UK Biobank cohort could provide important contributions to the knowledge gap regarding age-related decline.

The assessment of the consequences of air pollution effects on children's lungs for later COPD development remains challenging. Not many birth and children's cohorts currently have sufficiently long follow-ups to assess life course associations of air pollution with lung function and COPD from childhood through adolescence into adulthood. However, several cohort studies with detailed lung function data and exposure histories from childhood are well on their way but will need few more decades to assess the impact of air pollution on lung function and COPD through the course of life. Another potential limitation of the study by Doiron *et al*.[24] concerns air pollution exposure assessment. LUR models developed for the Thames Valley region were transferred to the UK Biobank study areas to predict air pollution concentrations. Transferability of the LUR models may be a source of measurement error as it has been shown that models tend to perform less well outside the Thames Valley region and prior to 2007[29] and therefore bias cannot be ruled out. Furthermore, Doiron *et al*.[24] did not account for spatial autocorrelation of observations, which may have introduced additional bias.

The Air Quality Pollutant Inventories report for England, Scotland, Wales, and Northern Ireland recently reported that as of 2017, UK air quality was much better than at any time since the industrial revolution.[30] However, this study by Doiron *et al*.,[24] one of the largest to date to examine associations of air pollution exposure and lung function and COPD in adults, still demonstrates adverse effects even at declining air pollution levels. This presents important evidence that can inform policymaking, highlighting the necessity for more comprehensive efforts in reducing air pollution and the need to mitigate the burden of air pollution on respiratory health.

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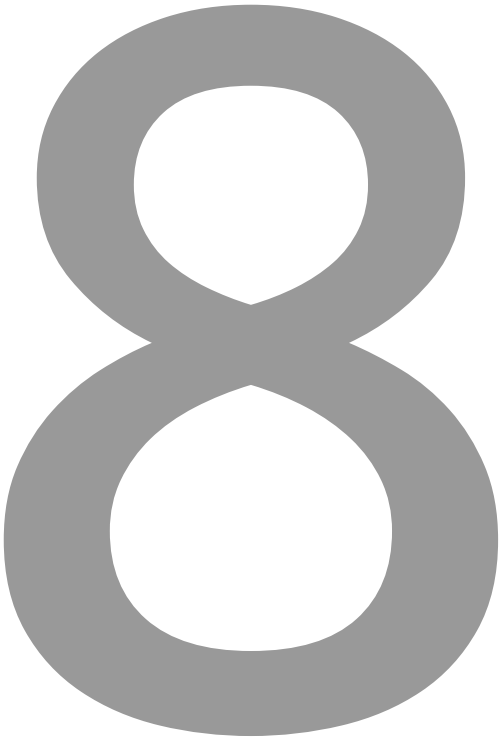
CHAPTER 8



General Discussion

In this thesis, it was hypothesized that different timing of secondhand smoke, air pollution, pet, and dampness or mould exposure can differentially affect asthma and lung function in adolescence. Specifically, associations of these exposures during different time periods with asthma at 17 years and lung function and lung function growth up to 16 years were investigated using data from the Dutch prospective PIAMA birth cohort study. Several approaches were used to characterize the timing of exposure over the life course including specific time windows (**chapters 2 and 6**), cumulative exposure scores **(chapter 2),** and longitudinal exposure patterns **(chapters 2, 3 and 5)**. In **chapter 3**, associations with sensitization at 16 years were also investigated. This chapter summarizes and discusses the overall findings, their interpretations, contributions to the literature and implications for future research.

MAIN FINDINGS

In **chapter 2**, associations of the timing of SHS exposure with asthma in childhood and adolescence are described. Three approaches were used to characterize exposure: time windows, cumulative exposure scores, and longitudinal latent exposure patterns using Latent Class Growth Models (LCGM). Different timing of SHS exposure was not associated with an increased risk of asthma in childhood and adolescence in this study. This was consistent across all methods.

In exploring associations of pet and dampness or mould exposure with asthma and sensitization, in **chapter 3**, longitudinal patterns of exposure were used to characterize time-varying exposures. Different timing of pet and dampness or mould exposure as reflected by the patterns was not associated with asthma at age 17, but a lower risk of sensitization at age 16 for pet and dampness exposure was suggested.

For the 16-year medical examinations, due to operational constraints, two different types of spirometers, the Jaeger Masterscreen pneumotachograph and EasyOne spirometers were used to measure lung function. A short experiment was performed to explore potential systematic differences in lung function measurements between different spirometers. It was observed that the use of different types of spirometers may result in significant systematic differences in lung function values (**chapter 4**). The calibration equations obtained from this study were used to correct for systematic differences between the spirometers used in the 16-year medical examinations of the PIAMA population, and the corrected FEV_1 and FVC measurements were used in chapters 5 and 6 where the role of timing of the different exposures on lung function in adolescence was investigated.

A study investigating the importance of the timing of exposure to SHS, pets and dampness or mould for lung function in adolescence is described in **chapter 5**. Childhood SHS exposure was associated with reduced FEV_1 growth between ages 12 and 16 and lower

attained level of lung function at these ages. Late childhood pet exposure was consistently associated with faster FEV_1 growth and higher attained level of lung function at 12 and 16 years, but early-life and persistently high pet exposure were associated with reduced FVC growth. Early-life exposure to dampness or mould was associated with reduced lung function growth and moderate late and mid-childhood exposures were associated with lower attained lung function.

In **chapter 6**, exposure to air pollution during preschool, primary school, and secondary school time windows was distinguished to determine the relevance of timing of air pollution exposure for lung function growth from age 8 to 16 and attained lung function levels at age 16. Higher preschool air pollution exposure was associated with reduced FEV_1 growth. Similarly, higher air pollution exposure during preschool, primary and secondary school time windows was associated with lower lung function levels at age 16.

Mutual adjustment of preschool and secondary school time window exposure resulted in stronger associations with lower attained level of lung function for the secondary school time window than for the preschool time window. This indicated potentially a stronger relevance of secondary school time window (13-16 years) or later life exposures over early-life exposure.

Chapter 7 is an invited editorial on a study that investigated associations of air pollution exposure with lung function and COPD in adults from the UK Biobank cohort and it is further discussed in line with results presented in chapter 6.

CONTRIBUTION TO SCIENTIFIC LITERATURE

Characterization of time-varying exposure

An important methodological contribution of this research is the life course approach to characterize exposure over long periods of follow-up. Most prospective studies with exposure data from repeated follow-up use single exposure measurements at a single time point in relation to a later life outcome. This cross-sectional approach may not fully utilize the power and information that the data can provide. In this thesis, in order to identify critical time windows and to investigate the relevance of the timing of exposure to environmental exposures, three approaches were explored to utilize data from all follow-ups effectively:

1. Time windows

Distinct time windows were defined to mark different phases of childhood, i.e. preschool, primary school, and secondary school. This method of characterizing the timing of exposure assumes that there are one or more critical windows of exposure, over the life course during which exposure causes deficits in health later in life. In this case, the research question can be stated as follows: "Is the exposure associated with the health outcome if it occurs in time window 1, 2, …, or *n*? or several of these time windows?".[1] Exposure during each of the time windows is separately characterized by a summary measure (e.g. time-weighted average) as in chapter 6 or time window specific combined questionnaire responses, as in chapter 2. The summary measure is included in the statistical model as the measure of exposure. The advantage of this method is that it is intuitive. It allows for the possibility to attribute observed associations to particular windows of exposure that match settings already established as standard prevention settings e.g. prenatal health clinics, daycare, and youth care in primary and secondary schools. Alternatively, time windows can be divided into either equal periods or periods where prior/biological evidence suggests their importance. The main disadvantage of this approach is that it does not directly address the possibility that the effects of exposure during a particular time window may be influenced by effects of exposure from the preceding time window(s) e.g. effects of air pollution seen in later life may be influenced by air pollution exposure at birth. Another disadvantage is that exposures during different windows may be highly correlated. Multiple time windows can be mutually adjusted in one regression model but correlation can be too high to allow for mutual adjustment.

2. Cumulative exposure scores

To quantify cumulative exposure over time, scores were calculated taking into account questionnaire responses on exposure throughout follow-up, where positive responses to exposure were assigned higher scores and negative responses received lower scores. This approach allowed us to investigate a possible dose-response relationship between

cumulative exposure and the outcomes of interest. However, the study-specific distribution of the scores may limit its use, as comparability of results with other studies may be difficult.

3. Longitudinal patterns of exposure

Longitudinal patterns of exposure determined using group-based trajectory models[2] known as latent class growth models (LCGM) were used to describe different patterns reflecting the timing of exposure. These models identify underlying unobserved subgroups within the population based on the probability of exposure during follow up.[3] Unlike time windows and cumulative scores, the LCGM provide an objective metric, the posterior probabilities, to evaluate the precision of group membership thereby minimizing the subjectivity of allocation of individuals to groups. Therefore, apart from reflecting the timing of exposure over time, this data-driven procedure also serves as an important explorative tool for time-varying factors and health outcomes measured at multiple time points. In the group-based LCGM individuals within the same class are assumed to have the same pattern of exposure i.e. within-group variation is constrained to zero, therefore the latent classes are viewed as a possible explanatory variable for the observed correlation between time points.[4]

One important consideration in the use of LCGM is the incorporation of uncertainty in the allocation of individuals to groups as assuming that group membership is exact rather than approximate will result in biased parameter estimates and standard errors potentially compromising inference.[5] There are several methods for accounting for uncertainty in class membership e.g. weighing observations in any subsequent analysis using posterior probabilities produced by the procedure, e.g. regression analysis, weighted by posterior probabilities of class membership as performed in this thesis. Alternatively, covariates can be incorporated in the latent class modelling procedure to reduce classification error but this has been demonstrated to lead to loss of efficiency.[5] An important limitation of LCGM models is related to model selection. While there are indices used for model selection such as the Bayesian Information Criteria (BIC) and Akaike Information Criteria (AIC), the final model selection needs to be guided by both statistical and biological plausibility as well as complexity and interpretability of the classes[6] e.g. the final selected model can have multiple similar patterns with few cases in each, which may hinder further statistical analyses and may also not necessarily provide new information regarding the underlying patterns within the population.

It should be mentioned that formulating the scientific research question in terms of discrete time windows is advantageous from a clinical and practical perspective but considering the timing of exposure in a continuous manner may be more advantageous from an analytical standpoint.[7]

While group-based latent class modelling has become popular in health research, different fields employ a variety of statistical methods to identify distinctive growth trajectories or critical phases during the life course.

Multilevel/random effects mixed models (and variations thereof) remain one of the most common statistical methods to analyse longitudinal trends. For each individual, repeated measures of time-varying exposure are first modelled as a function of time (e.g. using random slopes and intercepts) and subsequently estimates of these random coefficients are used as predictors in standard regression models.[1] This method is extensively implemented across different disciplines in health research, e.g. in anthropometric growth studies of body weight and BMI.[8-11] Life course path analysis, a method that can jointly estimate associations between outcomes at different time points and health outcomes throughout life by decomposing the time-varying variables into indirect and direct effects is another approach used.[12] It has been used to determine life course trajectories of childhood growth and cardiometabolic markers as demonstrated in multiple studies.[13-15] Other alternatives for summarizing time-varying variables to relate to later outcomes include distributed lag models[16] and structural equations modelling.[17] The decision on which methods to use relies upon, among other things, the relevant research question and the available statistical technical expertise.

IS THE TIMING OF EXPOSURE IMPORTANT?

SHS exposure

Asthma

The findings presented in **chapter 2** do not point to the relevance of a particular time window of SHS exposure in relation to asthma. The effect of SHS exposure on asthma in children and to some extent, adolescents, is well recognized.[18-22] Maternal smoking in pregnancy and early-life has been extensively studied[23,24](Table E8.1), and with the accumulation of evidence, these have been established as critical time windows for the presence of asthma. As such, evidence on the importance of exposure during other periods of the life course is currently limited.

A potential explanation for the lack of association presented in chapter 2 is exposure avoidance. Exposed children in this study were significantly less likely to have allergic parents compared to non-exposed children (p-value <0.001). Similarly, a study in the PIAMA cohort reported that a considerable proportion of allergic parents (53%) reported that their allergy was taken into consideration when they furnished homes and they smoked significantly less often in their homes, [OR (95% CI) 0.46 (0.27 to 0.75)].[25] Therefore, it is plausible to state that in this study, children of allergic parents -who are predisposed to develop allergic symptoms themselves -were less exposed to SHS than their non-predisposed peers.

There is evidence that prevalence of smoking in the Netherlands has declined considerably, e.g. the prevalence of smoking at home in the presence of children was 48% in 1996 vs 10% in 2009[26] and this has been attributed to the reinforcement of smoking

bans and strict tobacco policies.[27] In our study, the prevalence of smoking in the home ranged from 5% (secondary school time window) to 20% (infancy), which is similar to the prevalence reported in infancy in the Swedish BAMSE cohort (21%)[28], a study similar to the study presented in chapter 2. In general, it is unclear whether the declining prevalence of SHS exposure has resulted in a decline in asthma cases in the Netherlands though such a decline has been demonstrated in other countries[29] and for other health outcomes.[30] It is also possible that the lack of association between SHS exposure and asthma is attributable to the low prevalence of SHS exposure and asthma in the studied cohort and the consequently limited statistical power, which limits the certainty around the observed associations.

One of the important questions relevant to public health and the scientific community is whether a multi-faceted disease such as asthma can be prevented by reducing SHS exposure levels in children at different stages of childhood. Studies on effects of interventions involving parental smoking cessation on asthma in children report mixed findings[31,32], and while smoke cessation is highly recommended, based on published literature, it is still unclear to what extent timing of smoking cessation in the home can effectively contribute to the reduction of asthma cases in childhood and into adolescence.

Lung function

Longitudinal cohort studies have shown that lung function development can be disrupted by environmental exposures.[33,34] Findings presented in **chapter 5** of this thesis indicate that a persistent high probability of exposure to SHS from birth throughout childhood is associated with a lower attained level of lung function at ages 12 and 16 and slows down lung function growth between these ages, pointing to the importance of exposure in all phases of childhood up to 12 years of age. Currently, evidence from published studies is considered sufficient to infer a causal relationship between SHS exposure in early-life including maternal smoking during pregnancy and lower lung function during childhood (between >1 -12 years),[18] but due to lack of studies and inconsistent findings, the causal relationship is unclear for lung function (growth), when the children attain adolescence. In one of the pioneer studies on the associations between SHS exposure and lung function growth, Tager *et al*.,[35] projected reductions in lung function growth in children aged 5-9 years followed for 7 years into adolescence. In this study, it was estimated that between two children with the same initial FEV_1, age, height, and personal cigarette-smoking history, of which one has been exposed to maternal smoking throughout his/her life, the difference in FEV_1 between the exposed child, as compared to the unexposed child, would approximately be 28, 51, and 101 ml after one, two, and five years, representing a reduction of 10.7, 9.5, and 7.0% in the expected FEV_1 increase, respectively.[35] Other studies that have studied lung growth in childhood have attributed slow lung growth to a lasting consequence of *in utero* exposure from maternal smoking than postnatal exposure.[36-39] Children with a high probability of early-life exposure in our study were also likely to be exposed to maternal

smoking during pregnancy, but no adverse effects of SHS exposure on lung function growth in this group were observed. This is in contrast with previous studies (Table E8.3) that reported, for example, a 154ml (95% CI 4 to 304 ml) reduction in FEV_1 growth in 18-year-old girls in an Australian cohort in relation to maternal smoking in the first 26 days of life[40] and a difference of −0.9% (95% CI −1.3 to −0.5%) in FEV_1 in relation to maternal smoking during pregnancy reported in a pooled analysis.[41]

Considering that lung development is completed at the end of adolescence,[34] a slowing down of lung function growth from birth to adolescence will likely lead to lower maximum attained lung function levels in young adulthood that predispose to respiratory morbidities such as COPD.[42,43] While it has been shown in adult active smokers that smoking cessation can attenuate the rate of decline in lung function comparable to that of never smokers,[44] it is unclear if children who are no longer exposed to SHS experience catch up growth in lung function towards adolescence to attain the maximum level of lung function comparable to those that were never exposed.

Pet exposure

Asthma and sensitization

Timing of pet exposure in **chapter 3** was not associated with risk of asthma in adolescence, but a lower risk of sensitization was suggested for all time windows of exposure as compared to the very low exposed group. This suggests that pet exposure at any time between birth and age 17 may not pose an increased risk of asthma, nor protect against asthma in adolescence and that children may have a lower risk of sensitization in adolescence regardless of the time window during which they were exposed to pets. Existing literature on these associations focuses on pet exposure as well as asthma and sensitization in childhood (Table E8.2). The work presented in chapter 3 extends this into adolescence, providing an opportunity to examine how different timing of exposure to pets over the life course, contributes to asthma and sensitization in adolescence. Reviews have reported that exposure to pets is associated with a higher risk of asthma and allergy, [45,46] yet others have concluded that early childhood exposure to cat or dog does not have an impact on the development of asthma up to school age.[47] The null associations observed for asthma are consistent with earlier findings from the PIAMA cohort up to 8 years[48] e.g. [OR (95% CI) 1.08 (0.82 to 1.41) and 0.75 (0.52 to 1.07)] for dog and cat ownership, respectively]. Similarly, a lower risk of sensitization at age 8 was observed in the PIAMA cohort, [OR (95%CI) 0.78 (0.58 to 1.04) and 0.62 (0.42 to 0.91)] for cat and dog ownership, respectively].[48] The inverse associations of pet exposure with sensitization especially with sensitization to inhalant allergens are consistent with previous studies that have observed lower risks of sensitization with pet exposure in children aged 4-13 years, mostly reported for early-life pet exposure.[49-53] However, one other study has reported a higher risk of sensitization in 8-15-year-olds with pet exposure[54] and null associations have also been reported.[55] Overall, the inconsistent conclusions in

literature that pet exposure may be beneficial or detrimental for asthma, sensitization and other allergic outcomes make the question of effects of pet exposure for allergic disease development a long-standing debate as any view on these associations can be supported by substantial evidence from literature.[45,47,56] Therefore the role of pet exposure in asthma and sensitization remains unclear.[57] The interesting, but contrasting reports undoubtedly pose confusion among families that seek advice on acquiring pets in their homes to prevent allergies in their children. Although no adverse associations between pet exposure and asthma were observed and inverse associations with sensitization were observed, the current evidence from all studies so far are not enough to advocate for pet ownership as a preventive measure for allergic diseases.

Lung function

There was an indication of higher levels of lung function at ages 12 and 16 and faster lung function growth in relation to pet exposure in **chapter 5.** This is in contrast to the findings of another study that reported lung function deficits in children up to 16 years in relation to pet exposure[58] (Table E8.3) but in line with a study that reported higher percent predicted FEV_1 and FVC among 6-7-year-old boys exposed to a cat or dog in the first years of life[49] and higher FEV_1 in 8-16-year-old asthmatic girls exposed to a cat or dog at time of study.[59] To my knowledge, this is the first study that has assessed different timing of pet exposure in relation to lung function growth in adolescence. Results from the ALSPAC cohort on the role of different timing of pet exposure during childhood (at any time up to 7 years) and the level of attained lung function at 8 years do not support clear associations of cat and dog ownership with lung function. However, beneficial associations of rodent exposure at age 3 or later, but not before that age with FEV_1 were observed [mean difference in FEV_1 z-score 0.078 (0.0 to 0.14), Table E8.3][60] which the authors, attributed to chance. Considering the consistency of the significant positive associations between late childhood pet exposure and lung function (growth) in adolescence in chapter 5, it is unlikely that this is a chance finding but pet exposure avoidance, nevertheless, may play a role, as discussed in the paragraph below. In separate analyses with exposure to specific pets, i.e. cats, dogs, and rodents, early-life exposures to cats and rodents showed adverse effects on lung function growth, but mid and late-childhood exposures to these pets consistently showed positive associations with lung function (growth) especially for cats and rodents. This suggests that early-life pet exposure to any pet may pose adverse effects on lung function and therefore it might be reasonable to avoid pet ownership during that period, however, pet ownership later in childhood may not be a concern.

Avoidance effect

An important discussion with regard to the associations between pet exposure and health outcomes concerns the factors that influence families to keep pets or not. For example,

allergic families or parents whose children have experienced respiratory symptoms and have allergies may be more likely to avoid keeping pets than their non-allergic peers. Consequently, it may appear that keeping pets is associated with a lower risk of asthma or sensitization or a better lung function, as it is the individuals that are at a lower risk of developing adverse effects that are more likely to keep and therefore, to be exposed to pets, a phenomenon that is called reverse causation and results in reversed association estimates.[61] Many epidemiological studies fail to account for reasons for (avoidance of) pet ownership. In the PIAMA study, parents were asked if they got rid of a pet because of an allergy of a family member. Taking this into account and performing stratified analyses by parental allergy in chapter 3 and 5 did not show evidence that the beneficial associations observed for sensitization and lung function were a consequence of avoidance. However, a possible role of avoidance cannot be completely dismissed.

Dampness or mould exposure

Asthma and sensitization

Findings from **chapter 3** do not point to a critical time window of dampness or mould exposure that may increase the risk of asthma in adolescence. This is in contrast to a similar study which reported that exposure to dampness or mould during infancy (defined as the first 2 months) increased the risk of asthma up to 16 years of age[62] and also in contrast to systematic reviews and metanalyses that have demonstrated higher risk of allergic outcomes [63-66] especially in children up to school age (Table E8.2). One recent systematic review on the impact of residential dampness and mould exposure on the risk of developing asthma concluded that dampness and mould in the home increases the risk of developing asthma but when stratified by age there was barely any association between dampness or mould exposure and the risk of asthma in children up to 16 years [OR (95% CI) 1.03 (0.68 to 1.55)].[67] Partly in line with findings reported in chapter 3, several studies in children have also reported no association between dampness or mould and asthma.[68-70] There was generally low prevalence of asthma and dampness or mould exposure in the PIAMA cohort; 79% of participants had very low probability of being exposed to either dampness or mould throughout the study period which might explain the null associations for any of the exposure time windows reported in chapter 3.

Limited literature exists on associations between dampness or mould exposure and the risk of sensitization in adolescents and none on the relevance of the timing of exposure. Null associations were observed for any sensitization but exposure both in early and late childhood was associated with a lower risk of sensitization to specific inhalant allergens i.e. cat and HDM in chapter 3. Findings of a meta-analysis of cohorts including PIAMA reported no associations between early exposure to visible mould and/or dampness and sensitization against inhalant allergens in children aged 6-8 years [OR 1.05 (95% CI, 0.89 to 1.24)].[64] Likewise, a study similar to chapter 3, from the BAMSE cohort reported no associations between

dampness or any mould exposure in the first 2 months with sensitization to any inhalant or food allergens in 16-year-olds.[62] A multicentre cross-sectional study in 6-7 year-olds across 20 countries, however, found a positive association between dampness or mould exposure and a higher risk of HDM sensitization [OR (95% CI) 1.16 (1.03 -1.32)].[71] The differences in reports may be attributed to different age ranges studied, design of the studies and the exposure periods considered (Table E8.2). However, earlier studies within the PIAMA cohort have also shown null associations of dampness or mould exposure in early-life with asthma at 8 years of age, and a lower risk of sensitization at the same age was suggested,[72] consistent with findings reported in chapter 3 suggesting that the null associations and lower risk of sensitization persist into adolescence in this cohort.

Lung function

Associations between dampness or mould exposure and respiratory symptoms such as cough and wheeze have been assessed but studies assessing associations with lung function are scarce. The few that have assessed the impact of dampness or mould exposure in the home on lung function in childhood do not report clear associations[70,73] and there is no study so far on associations with lung function growth and in adolescence. **In chapter 5,** early-life dampness or mould exposure was associated with reduced lung function growth and late and mid-childhood exposure was associated with lower attained levels of lung function at ages 12 and 16 pointing to the relevance of these time windows. A cross-sectional study in Dutch 6-12-year-olds reported no associations between reported presence of damp stains and mould in the home in childhood and FEV_1 [percent difference (95% CI) 1.0% (-1.1 to 3.2 %) and -0.8% (-3.6 to 1.9%) for damp stains and mould respectively]. A similar study also reported no associations with damp stains and mould [percent difference (95% CI) in FEV_1 -1.6% (-4.5 to 1.3 %) and -1.0 % (-4.2 to 2.1%) for damp stains and mould respectively].[74] A more recent study also reported no associations between home dampness and lung function.[75] These studies are, however, few and evidence from more studies is lacking. Apart from these few studies in children, there are studies that have assessed associations of dampness or mould with lung function in adults. A longitudinal study from the European Community Respiratory Health Survey across 23 countries reported and stronger lung function decline in adults aged 20-44 exposed to dampness or mould in the home as compared to those unexposed.[76] It is indisputable that there is insufficient evidence on the role of dampness or mould exposure in relation to lung function, especially in children and adolescents. Therefore, there is still much to understand about whether dampness and mould exposure is associated with adverse lung function outcomes. Investigations from other birth cohorts will aid in bridging the gap in the knowledge of the importance of dampness or mould exposure in relation to lung function beyond childhood into adolescence.

Air pollution exposure and lung function

Findings presented in **chapter 6** demonstrate the relevance of early-life air pollution exposure (from birth to 4 years) for lung function growth between 8-16 years and the relevance of exposure during each time window until age 16 for levels of FEV_1 and FVC at age 16. Taking into account the whole lifespan from birth allowed us to identify time windows contributing to lung function and lung function growth in adolescence. Besides the study presented in chapter 6, there were only two other published studies [77,78] which investigated lung function in adolescence in relation to air pollution exposure since birth (Table E8.4). Only one of them investigated lung function growth in adolescence[78] and found no adverse associations with lung function growth between 8 and 16 years and no clear associations with attained level of lung function at age 16, e.g. mean change in FEV_1 (95% CI) per 0.9 µg/m^3 increase in PM_{10} was -34.3ml (-86.6 to 17.9 ml) for exposure at 0-1 years, -27.2 (-75.1 to 20.7 ml) for exposure between 1-8 years and -13.5ml (- 52.4 to 25.4 ml) for exposure between 8-16 years. The other study by Fuertes *et al*[77] consistently found no significant associations of air pollution exposure at the birth address, at the 10-year address and at the 15-year address with level of lung function in 15-year-olds (Table E8.4).

While findings in chapter 6 provide supportive evidence for adverse effects of air pollution exposure on lung function in adolescents, there is also growing evidence supporting associations of air pollution with lower lung function in adults as highlighted in the editorial in chapter 7. Considering that adverse associations of air pollution with lung function have been demonstrated for different phases of the life course and into adulthood,[79-82] it is plausible to suggest that air pollution exposure during an individual's whole lifespan, from birth, childhood, adolescence and up to adulthood, has impact on lung function growth and lung function level up to adulthood. It is, however, uncertain if there are levels of air pollution exposure which can be considered safe, and whether such effect thresholds would depend on the timing of air pollution exposure.

An unfolding discussion regarding air pollution exposure and lung function is the extent to which our lungs benefit from reduced air pollution exposure. The Children's Health Study from the USA is one of the major studies assessing the impact of air pollution on children's lungs that has also demonstrated effects of air pollution exposure on lung function growth in adolescents.[83] Additionally, this study has also demonstrated that reductions in air pollution exposure during adolescence may have beneficial effects on lung function level[84,85] e.g. the proportions of children with clinically low FEV_1 (defined as <80% of the predicted value) at 15 years of age declined significantly from 7.9% to 6.3% to 3.6% across three periods of exposure (1994–1997, 1997–2000 and 2007–2010) as the air quality improved.[85] Lung function improvements in relation to decreases in air pollution exposure have also been reported in adults.[86] Small changes in exposure due to changes in residential addresses were observed in chapter 6 but it was not possible to investigate lung function improvements in relation to reduced air pollution exposure over time. This was mainly due to the limitation in the use

of purely spatial LUR models for air pollution exposure assessment in chapter 6 that do not account for differences in temporal trends of air pollution levels.[87] A further limitation of the use of these models concerns exposure contrasts that may be underestimated for earlier years of follow-up and overestimated for more recent years as NO_2 and PM_{10} concentrations have decreased in the Netherlands over the last decades, [88,89] which may cause some bias in the observed exposure-response relationships.

Mechanisms of action of environmental exposures in asthma and lung function

The mechanisms through which environmental exposures operate to affect asthma and lung function are not entirely clear, but likely complex and may depend on the timing of exposure.[90,91]

Both SHS and air pollution exposure are known to cause oxidative stress, inflammatory responses, and tissue remodelling.[92,93] Inflammation plays a central role in asthma and it is also a hallmark of lung function decline and eventually COPD in adults.[94] Inflammation of cells leads to narrowing of the airways through deposition of connective tissue in the airway walls resulting in obstruction, contraction of the smooth airway muscles and vasodilation of airway vessels leading to asthma symptoms, respiratory distress as well as reductions in lung function parameters such as FEV_1 and FVC.[95-97]

Potential mechanisms explaining inverse associations of exposure to pet and dampness or mould-related agents with allergic outcomes have been proposed in the framework of the so-called hygiene hypothesis.[98] The hygiene hypothesis, in short, suggests that exposure to microbial environment, which includes endotoxin, and glucans early in life has a protective effect on the development of allergic disease.[99,100] Therefore, it has been hypothesized that pet exposure, which is associated with higher levels of endotoxin,[101] early in life could induce a modified Th2 response that is non-allergic in nature.[102] Protective effects of environmental microbial exposures emanating from dampness or mould exposure have been suggested to be mediated through the production of protective metabolites such as short-chain fatty acids or polysaccharides. Additionally, pathogen-associated molecular patterns from microbes might stimulate innate immune responses and thereby protect against allergies.[103]

The mechanisms by which pet exposure would benefit lung function are unknown. It is possible that mechanisms similar to those that have been suggested to protect from allergic disease also operate in relationships of pet exposure with lung function. Since this is one of the novel findings of this research, more studies are required to contribute to this evidence and to elucidate possible mechanisms and biological pathways.

The interplay between environmental factors and genes has been acknowledged to play a role in the development of asthma and lung function through epigenetics specifically through DNA methylation.[104,105] Epigenetics is simply defined as the alteration in the gene expression profile of a cell that is not caused by changes in the DNA sequence.[106] DNA methylation is the most studied phenomenon of epigenetics and it is a dynamic process

seen as a linkage of the genome to the environment with respect to health and disease.[107] Studies which PIAMA was part of [108,109] have provided novel findings concerning DNA methylation in relation to asthma in childhood suggesting the presence of CPG sites as potential markers for the risk of developing asthma. In the PIAMA study, pet exposure in the secondary school time window was demonstrated to affect DNA methylation in the nasal epithelium, which may have protective effects on the risk of asthma and rhinitis in adolescents.[110] Moreover, earlier findings from the PIAMA study have suggested that an interaction between *in utero* cigarette smoke exposure and the asthma gene ADAM33 results in reduced lung function and bronchial hyperresponsiveness.[111] This builds up from evidence suggesting that environmentally-induced disruptions to the normal epigenetic characteristics of DNA within distinct cells during lung development can lead to alterations in gene expression and thus, changes in lung development.[112] However, whether the mechanisms involve genetic changes first and subsequent environmental exposure leads to increased susceptibility or whether exposure to environmental exposures causes genetic changes that result in increased susceptibility to lower (reduced) lung function (growth) or asthma is still unclear.

Recommendations for future research

Findings presented in this thesis lead to the following recommendations for future research:

- At the heart of environmental epidemiologic analyses are methodological considerations that have not been fully explored in the context of repeated exposure data. The methods exemplified in this thesis may be of great use in future environmental epidemiologic studies determined to; a) elucidate the complex relationships between life course environmental exposures and later life health exposures; and b) assess underlying distinct unobserved patterns of time-varying exposure.
- The LCGMs used in this thesis are specifically recommended for analysis of time-varying exposures as they have flexible statistical properties such as the ability to summarize each individual's entire exposure trajectory by a single variable, easy interpretation, and the ability to accommodate the time-varying nature of the exposure. These features make it easy for application in epidemiological analyses.
- The results on life course pet exposure and asthma, sensitization and lung function reported in this thesis are not sufficient to provide recommendations to clinical practice on whether families should acquire or get rid of pets in the home to prevent allergic disease despite that keeping pets may not adversely affect lung function in later childhood. Life course SHS and air pollution were associated with lower and reduced lung function growth and efforts to reduce exposure to these factors should be intensified.

- Collaborative efforts of multidisciplinary research teams are required to understand the different contributions of environmental exposures and how they affect respiratory outcomes beyond childhood, the underlying mechanisms and how targeted preventive strategies can be integrated within clinical practice. This also includes understanding the interplay between genetics and timing of environmental exposures.

CONCLUSION

While much has been learned about associations of environmental exposures with asthma and lung function, much remains unclear especially throughout the life course as the preceding chapters have demonstrated. This thesis has demonstrated that the timing of exposure to secondhand smoke, pets, dampness or mould, and air pollution, plays a role in lung function, and lung function growth. There was no indication for specific relevant windows of exposure for asthma. It is evident that answering the question of the importance of timing of exposure requires long follow-ups, detailed exposure assessment, and efficient exposure characterization. Epidemiological longitudinal studies with continued follow-ups from birth into adulthood are needed to investigate life course effects of environmental exposures on asthma and lung function and to further develop the evidence presented in this thesis. The studies presented in this thesis address a vital public health problem and together with other studies that have investigated effects of environmental exposures on asthma and lung function, they contribute to the evidence that later exposures as well as continued exposure through the life course, in addition to early-life exposure, may play a role in respiratory health. This knowledge can be used to inform effective respiratory disease burden management throughout the life course.

STUDIES AND SYSTEMATIC REVIEWS COMPARABLE TO STUDIES PRESENTED IN THIS THESIS

Table E8.1 Secondhand smoke and asthma

Author/Study	Study type	Exposure type and age range	Outcome type and age range	Results (Regression estimates /Odds ratios)		Conclusion
Thacher *et al.* (2014)[28]	Birth cohort	Passive smoking in pregnancy, infancy, until age 16,	Asthma development up to 16 years	Pregnancy 1.45 (1.12 to 1.87) Up to age 16 1.07 (0.89 to 1.28)	Infancy 1.26 (0.99 to 1.61)	Early SHS exposure, *in utero* associated with development of asthma in adolescence
Hollams *et al.* (2014)[23]	Birth cohort	Maternal smoking in pregnancy	Current asthma at age 14	1.84 (1.16 to 2.92)		Exposure to maternal smoking during pregnancy associated with asthma in adolescence
Alati *et al.* (2006)[24]	Birth cohort	Maternal smoking in pregnancy, 6 months 5 years,14 years	Asthma at age 14 Boys Girls	Pregnancy 1.06 (0.67 to 1.66) 5 years 0.96 (0.70 to 1.32) Pregnancy 1.76 (1.16 to 2.66) 5 years 1.24 (0.89 to 1.73)	6 months 1.10 (0.81 to 1.50) 14 years 0.92 (0.68 to 1.27) 6 months 1.53 (1.10 to 2.13) 14 years 1.14 (0.81 to 1.59)	Exposure to maternal smoking during pregnancy and early-life associated with asthma in adolescence in girls
Silvestri *et al.*(2015)[22]	Systematic review and metanalysis	Prenatal maternal/ postnatal smoking exposure	Current asthma in 0-6 years and 7-18-year-olds	Prenatal 0-6 years Not reported 7-18 years 1.22 (1.03 to 1.44)	Postnatal 0-6 years 1.30 (1.13 to 1.51) 7-18 years Not estimated	Prenatal and postnatal SHS exposure associated with asthma

Table E8.2 Pets, dampness or mould and asthma and sensitization

Pets and asthma						
Author/Study	**Study type**	**Exposure type and age range**	**Outcome type and age range**	**Results (Odds ratios)**		**Conclusion**
Al-Tamprouri *et al.* (2019)[113]	Birth cohort	Cat/dog first year and after first year of life	Asthma (13 years)	Cat (first year) 1.03(0.58 to 1.82) Cat (after first year) 0.68 (0.37 to 1.26)	Dog (first year) 0.29 (0.04 to 3.34) Dog (after first year) 0.89 (0.50 to 1.61)	Cat/dog exposure not associated asthma in adolescence
Lødrup *et al.* (2012)[114]	Pooled birth cohorts	Exposure to cat/ dog in first 2 years of life	Current asthma in 6-10 year olds	Cat 1.00 (0.78 to 1.28)	Dog 0.77 (0.58 to 1.03)	Cat and dog exposure not associated asthma
Ayuk *et al.* (2018) [115]	Cross-sectional	Cat/dog exposure (13-14 years)	Asthma (13-14 years)	Cat 1.13 (1.03 to 1.23)	Dog 1.06 (0.97 to 1.16)	Cat but not dog exposure associated asthma
Kerkhof *et al.* (2009)[48]	Birth cohort	Cat/dog exposure at 3 months	Asthma at age 8	Cat 1.08 (0.82 to 1.41)	Dog 0.75 (0.52 to 1.07)	Exposure to cat or dog not associated asthma
Collin *et al.* (2015)[116]	Birth cohort	Any pet owned 0-7 years <3 years >3 years <3> years	Doctor-diagnosed/ current asthma at 7 years	Doctor diagnosed asthma 1.54 (1.00 to 2.36) 0.85 (0.64 to 1.13) 0.94 (0.74 to 1.18)	Current asthma 1.07 (0.63 to 1.81) 0.67 (0.47 to 0.94) 0.72 (0.55 to 0.95)	Pet ownership not associated with current/doctor diagnosed asthma
Apelberg *et al.* (2001)[45]	Systematic review	Early-life pet exposure	current asthma	Pooled estimate 1.19 (1.02 to 1.56)		Early-life pet exposure associated asthma
McConnell *et al.* (2002)[117]	Follow-up	Any pet exposure	Asthma in 12 to 21 year-olds	1.1 (0.6 to 2.0)		Pet exposure not associated with asthma

Pets and sensitization

Author/Study	Study type	Exposure type and age range	Outcome type and age range	Results (Odds ratios)		Conclusion
Al-Tamprouri et al. (2019)[113]	Birth cohort	Cat/dog first year and after first year of life	Sensitization at 13 years	Cat (first year) 0.58(0.35 to 0.94) Cat (after first year) 0.54(0.33to 0.87)	Dog (first year) 0.58(0.37-0.92) Dog (after first year) 0.69 (0.43 to 1.11)	Dog/cat ownership in the first year of life/childhood associated with lower risk of sensitization
Ownby et al (2002)[49]	Birth cohort	Cat/dog first year of life	Sensitization (6-7 years)	1 dog/cat 1.01 (0.61 to 1.67)	≥2dogs/cats 0.31 (0.14 to 0.72)	Exposure to ≥2 dogs/cats associated with lower risk of sensitization
Henriksen et al (2001)[52]	Cross-sectional	Cat ownership ever	Current sensitization (13-19 years)	Cat 0.5 (0.3 to 0.9)		Early-life pet exposure not significantly associated with suggested lower risk of sensitization
Wegienka et al (2010)[53]	Birth cohort	Cat/dog (first year of life	Sensitization at age 18	1 pet 1.04 (0.88 to 1.22)	≥2 pets 0.83 (0.65 to 1.06)	Early-life pet exposure not significantly associated with suggested lower risk of sensitization
Lodge et al (2012)[55]	Birth cohort	Cat/dog exposure at birth	Cat dander and house dust mite sensitization from 2 to 12 years	Cat dander 1.27 (0.74 to 2.16)	House dust mite 0.83 (0.56 to 1.23)	Pet exposure not associated with sensitization from 2 to 12 years
Al-Mousawi et al (2004)[54]	Case-control	Cat exposure in 8-15 year olds	Sensitization in 8-15 year olds	3.32 (1.19 to 9.25)		Cat exposure associated with cat sensitization
Kerkhof et al (2009)[48]	Birth cohort	Cat/dog exposure at 3 months	Sensitization at age 8	Cat 0.78 (0.58 to 1.04)	Dog 0.62 (0.42 to 0.91)	Exposure to dog associated with lower risk of sensitization

Dampness and sensitization					
Author/Study	**Study type**	**Exposure type and age range**	**Outcome type and age range**	**Results (Odds ratios)**	**Conclusion**
Thacher *et al.* (2017)[62]	Birth cohort	Dampness or mould at 2 months	Sensitization at 16 years	0.93 (0.77 to 1.15)	No associations were observed between any mould or dampness indicator and IgE sensitization to inhalant allergens
Weinmayr *et al.* (2013)[71]	Cross-sectional	Current dampness/ mould	Sensitization at 6-7 years	1.16 (1.03 to 1.32)	Dampness or mould exposure associated with sensitization
Tischer *et al.* (2011)[64]	Metana-lysis	Dampness or mould in the first 2 years	Sensitization to inhalant allergens at 6-8 years	1.05 (0.89 to 1.24)	Dampness or mould not associated with sensitization

Table E8.3 SHS, pet, dampness or mould and lung function

Secondhand smoke and lung function (Regression coefficients, percent difference reported unless specified otherwise)						
Author/ Study	**Study type**	**Exposure type and age range**	**Outcome type and age range**	**Results (Regression estimates /Odds ratios)**		**Conclusion**
Dai *et al*. (2017)[40]	Birth cohort	Parental smoking in early-life (26 days after birth)	FEV_1, FVC at 18 years, FEV_1 FVC growth 12-18 years	Boys	Girls	Early smoke exposure associated with reduced FEV_1 growth in adolescent girls
			FEV_1	26ml (-202 to 255ml) -70ml(-336 to 195ml)	-272ml(-438 to -107ml) -162ml (-344 to 22ml)	
			FVC	145ml (-58 to 349ml) 68ml (-174 to 311ml)	-154ml (-304 to -4ml) -42ml (-235 to 151ml)	
			FEV_1 growth			
			FVC growth			
Hu *et al*. (2017) [118]	Cross-sectional	Maternal active smoking in pregnancy, SHS in first 2 years, current SHS	FEV_1 at ~11.6 years	Boys *OR (95% CI)* Maternal 4.12 (1.52 to 11.17) SHS first 2 yrs 1.16 (0.87 to 1.55) Current SHS 1.78 (1.39 to 2.27) Maternal 6.46(2.58 to 16.17) SHS first 2 yrs	Girls *OR (95% CI)* Maternal 1.28 (0.38 to 4.27) SHS first 2 yrs 1.24 (0.93 to 1.66) Current SHS 1.36 (1.05 to 1.75) Maternal 2.16 (0.96 to 4.88) SHSfirst2yrs	SHS exposure associated with lung function deficits
			FVC at ~11.6 years	1.04 (0.79 to 1.37) Current SHS 1.60 (1.27 to 2.01)	1.46 (1.15 to 1.85) Current SHS 1.53 (1.23 to 1.89)	
Cook *et al*. (1998)[36]	Systematic review and metanalysis	SHS exposure in school-aged children	FEV_1 and FVC in school-aged children	FEV1 (% diff) −1.4% (−1.9 to −1.0%)	FVC (% diff) −0.4% (−0.8 to 0.0%)	SHS exposure associated with lower FEV_1

Thacher *et al.* (2018)[119]	Birth cohort	Maternal smoking during pregnancy SHS exposure during infancy SHS exposure at 16 years	FEV_1 and FVC at 16 years	FEV1 -32.7ml (-91.9 to 26.4ml) -2.4ml (-49.6 to 44.8ml) 35.1ml (-22.7 to 92.8ml)	FVC 16.1ml (-54.0 to 86.2ml) 20.2ml (-35.0 to 75.4ml) 65.0ml (-3.9 to 133.9ml)	No association between early-life/ adolescent passive smoke exposure with lung function in adolescence
Pets and lung function						
Collin *et al.* (2015)[60]	Birth cohort	Cat/dog/rodent / bird(0-7 years) <3 years >3 years <3> years	FEV_1 (FVC) (8 years)	FEV1 *Mean z-scores* -0.06(-0.24 to 0.11) 0.0 (-0.0 to 0.11) 0.01 (-0.07 to 0.1)	FVC *Mean z-scores* -0.04(-0.21 to 0.12) -0.01(-0.12 to 0.08) 0.00 (-0.07 to 0.09)	No association with lower lung function
Hu *et al.* (2017)[58]	Cross-sectional	Any pet Pregnancy First 2 years Current	FEV_1(FVC)<85 % predicted(6-16 years)	FEV1 Pregnancy 0.9 (0.61 to 1.33) First 2 years 1.15(0.87 to 1.51) Current 1.57(1.29 to 1.93)	FVC Pregnancy 1.13 (0.82 to 1.56) First 2 years 1.28 (1.01 to 1.63) Current 1.35 (1.12 to 1.62)	Postnatal pet exposure associated with lung function deficits
Dampness and lung function						
Brunekreef (1992)[70]	Cross-sectional	Dampness or mould in the last 2 years in 6-12-year-olds	FEV_1 and FVC 6-12-year-olds	FEV1 Damp stains 1.0%(-1.1 to 3.2%) Mould -0.8% (-3.6 to 1.9%	FVC Damp stains 1.4%(-0.6 to 3.4%) Mould 0.5%(-2.0 to 3.1%)	No associations with lung function

Cuijpers *et al.* (1995)[74]	Cross-sectional	Damp stains and mould growth 6-12-year-olds	6-12 years olds FEV_1	Boys Damp stains -1.6%(-4.5 to 1.3%) Mould growth -1.0%(-4.2 to 2.1%)	Girls Damp stains 0.9%(-2.3 to 4.0%) Mould growth -0.9%(-3.6 to 1.8%)	No associations between dampness or mould and lung function
			FVC	Damp stains -1.0%(-3.6 to1.6%) Mould growth 0.2%(-2.6 to 3.0%)	Damp stains 0.4%(-2.8 to 3.6%) Mould growth 0.2%(-2.6 to 3.0%)	
Holst *et al.* (2016)[75]	Cross-sectional	Bedroom dampness	FEV_1 and FVC in 6–10-year-olds	FEV1 0.06 (-0.33 to 0.46)	FVC 0.07 (-0.33 to 0.47)	Bedroom dampness not associated with lung function

Table E8.4 Air pollution and lung function

Author /Study	Study type	Exposure type and age range	Outcome type and age range	Results (Regression estimates /Odds ratios)			Conclusion
Schultz *et al.* (2016)[78]	Birth cohort	PM_{10} exposure years 1-8 years 8- 16 years	FEV_1 at 16 years FVC at 16 years FEV_1 growth (8-16 years)	years -34.3 (-86.6 to 17.9ml) -7.9ml (-66.9 to 51.2ml) 1.4ml (-29.0 to 31.9ml)	1-8 years -27.2 (-75.1 to 20.7ml) -9.3ml (-63.7 to 45.0ml) 19.1ml (-20.7 to 59.0 ml)	8-16 years -13.5ml (-52.4 to 25.4ml) -17.9ml (-61.9 to 26.0ml) 23.4 ml (-14.7 to 61.6ml)	No significant associations of PM_{10} exposure with FEV_1 and FEV_1 growth
Fuertes *et al.* (2015)[77]	Birth cohort	NO_2,PM_{10},$PM_{2.5}$, PM absorbance at birth,10 year and 15-year addresses	FEV_1 at 15 years FVC at 15 years	Birth **NO_2** 3.3ml (-18.2 to 24.9 ml) **PM_{10}** -4.8ml (-51.9 to 42.1 ml) **$PM_{2.5}$** 56.0ml (-29.7 to 141.7ml) **$PM_{2.5}$ abs** 20.3ml (-24.9 to 65.5ml) **NO2** 12.8ml(-10.8 to 36.5ml) **PM_{10}** 21.7ml (29.7 to 73.35ml) **$PM_{2.5}$** 70.0ml (23.9 to 164.0ml) **$PM_{2.5}$ abs** 17.8ml (31.7 to 67.4ml)	10 years **NO_2** -6.5ml (-32.7 to 19.6 ml) **PM_{10}** -8.3ml (-56.9 to 40.2 ml) **$PM_{2.5}$** 37.4ml (-57.3 to 132.2ml) **$PM_{2.5}$ abs** 14.4ml (-31.4 to 60.3ml) **NO2** 3.1ml (-25.5 to 31.8ml) **PM_{10}** -6.8ml (-60.0 to 46.3ml) **$PM_{2.5}$** 44.6ml (59.2 to 148.4ml) **$PM_{2.5}$ abs** -1.1ml (-51.4 to 49.1ml)	15 years **NO_2** –3.1 ml (–29.2 to 22.9ml) **PM_{10}** 4.6ml (–42.6 to 52.0 ml) **$PM_{2.5}$** 40.0ml (–54.4 to 134.5ml) **$PM_{2.5}$ abs** 31.1ml [–13.7 to 75.9ml) **NO_2** 11.5ml (–17.0 to 40.0ml) **PM_{10}** 15.3ml (–36.4 to 67.2ml) **$PM_{2.5}$** 63.0ml (–40.3 to 166.5ml) **$PM_{2.5}$ abs** 33.8ml (–15.1 to 82.9ml)	No associations observed between exposure and lung function in adolescence

Author /Study	Study type	Exposure type and age range	Outcome type and age range	Results (Regression estimates /Odds ratios)			Conclusion
Gauderman *et al.* (2004)[83]	Cohort	At study entry ~9-10 years NO_2 PM_{10} $PM_{2.5}$	FEV_1 growth FVC growth	NO2 -101.4ml (-164.5 to -38.4ml) -95.0ml (-189.4 to -0.6ml)	PM10 -82.1ml (-176.9 to 12.8ml) -60.2ml (-190.6 to 70.3ml)	PM2.5 -79.7ml (-153.0 to -6.4ml) -60.1ml (-166.1 to 45.9ml)	NO_2 and $PM_{2.5}$ exposure associated with reduced lung function growth

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APPENDICES

A

Appendices

Summary

BACKGROUND

Exposure to modifiable environmental exposures such as secondhand smoke (SHS), air pollution, pets, and dampness or mould has been linked to adverse respiratory health effects, such as asthma and lung function deficits. Asthma is one of the most prevalent chronic respiratory diseases in children worldwide and a low level of lung function in childhood could be a precursor for chronic lung diseases such as chronic obstructive pulmonary disease in adulthood. While evidence for a role of these environmental exposures in asthma and lung function development in children exists, evidence for such a relationship in adolescents is limited and the importance of the timing to these exposures over the life course is unclear. Uncovering critical time windows of increased vulnerability to environmental exposures is a complex question that requires sophisticated statistical data analysis methods that take into account the time-varying nature of the exposure. The aims of this thesis were to explore, methods that can be used to characterize longitudinal time-varying environmental exposures and to determine the relevance of the timing of exposure to secondhand smoke, pet, dampness or mould, and air pollution in the associations with asthma and lung function in adolescence. The relationships of pet and dampness or mould exposure with sensitization were also explored.

METHODS

The data analysed in this thesis were obtained from the Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort, a birth cohort established in 1996/97 that enrolled pregnant women and recruited 3963 new-borns. Participants were followed from birth and parents completed questionnaires at 3 months, annually from 1 year until age 8, and then at ages 11, 14,16 and 17. At ages, 8, 12 and 16 medical examinations were performed where measurements such as anthropometric measures, lung function measures, and IgE measurements were taken. Data on lifestyle, health, environmental exposures, and household characteristics was collected. Questions on environmental exposures were used to assess exposure to secondhand smoke, pets, and dampness or mould exposure. Air pollution exposure was determined using Land Use Regression models.

The main outcomes assessed in this thesis were asthma in adolescence, lung function growth in childhood, adolescence, and level of attained lung function in adolescence

In order to investigate the relevance of the timing of exposure, the following methods

were used: distinct time windows, where exposure occurring during specific time windows was assumed to be more associated with a health outcome in later life than exposure during other time windows. This method was used to assess the role of SHS exposure on asthma at age 17 and air pollution exposure on lung function up to age 16. Time windows of pregnancy (for SHS), preschool, primary school, and secondary school were distinguished in line with established prevention settings in health care. Cumulative scores were created by assigning scores to responses about residential SHS exposure. Specifically, these were used to determine the magnitude of cumulative exposure over the life course and explore a dose-response relationship with asthma at age 17. Longitudinal patterns of exposure were characterized using latent class growth models (LCGM). These models distinguish underlying patterns of exposure within a study population using the probability of exposure over time. These were used to assess the timing of SHS, pet dampness or mould exposure in relation to asthma, lung function, and sensitization.

MAIN RESULTS AND DISCUSSION

Three approaches in characterizing time-varying exposures were explored. These methods were aimed at leveraging the power that longitudinal data from prospective studies provide by utilizing repeated data from all follow-ups. The LCGM models characterizing longitudinal patterns of exposure were used for the first time to assess patterns of environmental exposures in this thesis. With advantages such as intuitive interpretation, explorative tools and ease of application in epidemiological analyses, these LCGM models can be also be applied by other researchers in environmental epidemiology.

None of the approaches used to characterize life course SHS exposure and to assess its associations with asthma up to 17 years support the relevance of a specific period for SHS exposure in **chapter 2**. The lack of association could be attributed to exposure avoidance, as exposed children in this study were significantly less likely to have allergic parents, who might be more likely to avoid SHS exposure because of their allergies. In **chapter 3**, longitudinal patterns of pet exposure were not associated with asthma at age 17, but a lower risk of sensitization was suggested for all patterns indicating that pet exposure at any time over the life course may not increase risk of asthma, but may possibly lower the risk of sensitization in adolescence. Exposure to dampness or mould at any time was not associated with asthma, but lower risk of sensitization to specific inhalant allergens was suggested.

Results in **chapter 4** showed systematic differences between two spirometers used to measure lung function during the 16-year medical examination. The regression equations obtained were used to correct for these systematic differences in lung function measurements at age 16 in the PIAMA study. The results also point to the need for epidemiological researchers to be aware of potential systematic differences between instruments and to correct for them accordingly.

In **chapter 5**, SHS exposure throughout childhood was associated with reduced lung function growth between ages 12 and 16 and lower levels of lung function at these ages. These findings strengthen the evidence that associations of continued SHS exposure from birth throughout childhood with lung function can persist into adolescence. While early-life pet exposure was associated with reduced lung function growth, late childhood pet exposure was associated with higher attained level of lung function at ages 12, 16 and a faster lung growth between 12 and 16 years. This could possibly point to effects of pet avoidance behaviour but sensitivity analyses did not support this. Childhood exposure to dampness or mould was generally associated with lower levels of lung function at 12 and 16 years and early-life exposure to dampness was associated with reduced lung function growth between ages 12 and 16.

Higher air pollution exposure during the preschool time window was associated with reduced lung function growth between ages 8 to 16 in **chapter 6** and higher exposure in preschool, primary school and secondary school time windows was associated with lower attained level of lung function at age 16. These findings contribute to the evidence that air pollution exposure in early-life, later life and exposure over the entire age range could be relevant for lung function.

CONCLUSION

Establishing critical windows of vulnerability to environmental exposures is essential in children's health research. The studies presented in this thesis and together with existing literature on effects of environmental exposures on asthma and lung function, contribute to the evidence that later exposures as well as continued exposure throughout the life course, in addition to early-life exposure, may be relevant for asthma and lung function. The lack of clarity in the literature regarding the relationships of environmental exposures with asthma and lung function reflects the need for more extensive studies with advanced statistical tools to enhance understanding of the associations, effects and possible mechanisms throughout the life course.

Nederlands samenvatting

ACHTERGROND

Blootstelling aan omgevingsfactoren zoals passief roken, luchtverontreiniging, huisdieren en vocht of schimmel wordt in verband gebracht met astma en longfunctiestoornissen. Astma is wereldwijd één van de meest voorkomende chronische aandoeningen bij kinderen. Een verminderde longfunctie op kinderleeftijd vergroot de kans op chronische longaandoeningen, zoals chronische obstructieve longziekte (COPD), op latere leeftijd. Hoewel een effect van deze omgevingsfactoren op de ontwikkeling van astma en longfunctie bij kinderen is aangetoond, is er amper bewijs voor een dergelijke relatie bij adolescenten. Ook is het belang van het moment van blootstellen gedurende de levensloop nog onduidelijk. Het aantonen van kritieke periodes met een verhoogde gevoeligheid voor de effecten van milieublootstellingen is een complexe vraag waarvoor geavanceerde statistische methoden vereist zijn die rekening houden met de verandering van de blootstelling in de tijd. De doelstellingen van dit proefschrift zijn het verkennen van methoden die kunnen worden gebruikt om in de tijd varierende omgevingsblootstellingen te karakteriseren, en het bepalen van de relevantie van het *moment* van blootstelling aan passief roken, huisdieren, vocht of schimmel en luchtverontreiniging voor het ontstaan van astma, allergische sensitisatie en longfunctiestoornissen bij adolescenten.

METHODEN

De in dit proefschrift geanalyseerde gegevens zijn afkomstig van het Nederlandse PIAMA (Preventie en Incidentie van Astma en Mijt Allergie) cohort, een geboortecohort dat in 1996/97 werd gestart met 3963 pasgeborenen. De deelnemers werden vanaf hun geboorte gevolgd door hun ouders vragenlijsten in te laten vullen toen de deelnemers 3 maanden en 1 jaar oud waren, daarna jaarlijks tot en met de leeftijd van 8 jaar en vervolgens op de leeftijden van 11, 14, 16 en 17 jaar. Op de leeftijden van 8, 12 en 16 jaar werden medische onderzoeken uitgevoerd waarbij onder meer antropometrische gegevens werden verzameld, longfunctiemetingen werden gedaan en bloedmonsters werden verzameld. In het bloed zijn later IgE-antilichamen tegen voedsel- en inhalatieallergenen gemeten. Met behulp van de vragenlijsten werden gegevens over leefstijl, gezondheid, blootstelling aan omgevingsfactoren en kenmerken van het huishouden werden verzameld. In dit proefschrift werden de vragen over omgevingsfactoren gebruikt om de blootstelling aan tabaksrook, huisdieren, en vochtigheid of schimmel te beoordelen. Blootstelling aan

luchtverontreiniging werd bepaald met behulp van zogenaamde 'Land Use Regression' modellen.
De belangrijkste uitkomstmaten in dit proefschrift zijn astma in bij adolescenten, groei van de longfunctie in de kindertijd en pubertijd en het niveau van longfunctie in pubertijd.
Om het belang van het tijdstip van de blootstelling te onderzoeken, werden de volgende methoden gebruikt: blootstelling gedurende verschillende tijdvensters, waarbij wordt aangenomen dat blootstelling gedurende één of meerdere specifieke tijdvensters sterker geassocieerd is met een gezondheidsuitkomst later in het leven dan blootstelling tijdens andere tijdvensters. Deze methode werd gebruikt om de rol van passief roken bij astma op 17-jarige leeftijd en de rol van blootstelling aan luchtverontreiniging op de longfunctie tot 16 jaar te onderzoeken. De volgende tijdvensters werden onderscheiden om aan te sluiten bij de preventieve gezondheidszorg in Nederland gedurend verschillende levensfasen zoals verloskundigen, consultatiebureau's en schoolartsen: zwangerschap (passief roken) kleuterschool, basisschool en middelbare school. Cumulatieve blootstellingsscores werden gecreëerd door scores toe te kennen aan antwoorden over blootstelling aan tabaksrook in de woonomgeving. Met behulp van die cumulatieve scores kan de omvang van de totale blootstelling gedurende de levensloop bepaald worden alsmede een mogelijke dosis-responsrelaties met astma op 17-jarige leeftijd. Longitudinale blootstellingspatronen werden beschreven met behulp van latente klasse groeimodellen (LCGM). Deze modellen onderscheiden onderliggende blootstellingspatronen binnen een onderzoekspopulatie met behulp van de (verandering van de) waarschijnlijkheid van blootstelling in de loop van de tijd. In dit proefschrift werden deze modellen gebruikt om blootstellingspatronen te karakteriseren voor passief roken, huisdieren en vochtigheid of schimmel en deze vervolgens aan astma, longfunctie en allergische sensitisatie te relateren.

DE BELANGRIJKSTE BEVINDINGEN EN DISCUSSIE

Drie methoden voor het karakteriseren van blootstellingen variërend in de tijd werden onderzocht. Deze methoden zijn gericht op het optimale gebruik van de longitudinale gegevens uit prospectieve studies door herhaalde gegevens uit alle follow-ups te gebruiken. De LCGM-modellen voor het beschrijven van longitudinale blootstellingspatronen werden in dit proefschrift voor het eerst toegepast op milieublootstellingen. LCGM-modellen zijn geschikt om blootstelingspatronen te verkennen, ze zijn intuïtief en eenvoudig in het gebruik en ze kunnen daarom ook worden toegepast door andere onderzoekers in de milieu-epidemiologie.

In **hoofdstuk 2** worden benaderingen beschreven om de blootstelling aan passief roken tijdens de zwangerschap en na de geboorte tot en met de adolescentie te karakteriseren. Geen van deze wijst een specifieke periode aan waarin blootstelling aan tabaksrook is

geassocieerd met astma tot en met 17 jaar. Het vermijden van blootstelling aan passief roken bij kinderen met een verhoogd risico op astma zou een mogelijke verklaring kunnen zijn voor het ontbreken van een verband. Blootgestelde kinderen in deze studie hebben namelijk minder vaak allergische ouders, die waarschijnlijk eerder blootstelling vermijden vanwege hun eigen allergieën. In **hoofdstuk 3** werden geen associaties gevonden tussen longitudinale patronen van blootstelling aan huisdieren en astma op 17-jarige leeftijd. Wel wordt er een lager risico op allergische sensitisatie in adolescentie gesuggereerd voor alle patronen waarbij sprake is van blootstelling aan huisdieren op enig moment gedurende het leven. Ook blootstelling aan vocht of schimmel werd op geen enkel moment in verband gebracht met astma. Maar ook voor blootstelling aan vocht of schimmel werd een lager risico op sensitisatie tegen inhalatieallergenen gesuggereerd.

De resultaten in **hoofdstuk 4** tonen systematische verschillen aan tussen twee spirometers die werden gebruikt om de longfunctie te meten tijdens het medische onderzoek op 16-jarige leeftijd. De zo verkregen regressievergelijkingen werden gebruikt om voor de resulterende systematische verschillen in longfunctiemetingen op 16-jarige leeftijd in het PIAMA-onderzoek te corrigeren. De resultaten benadrukken dat epidemiologische onderzoekers zich bewust moeten zijn van mogelijke systematische verschillen tussen instrumenten. Resultaten van onderzoek moeten hiervoor worden gecorrigeerd.

In **hoofdstuk 5** is blootstelling aan passief roken gedurende de kindertijd geassocieerd met een verminderde toename in longfunctie tussen de leeftijd van 12 en 16 jaar en lagere niveaus van longfunctie op deze leeftijden. Dit bevestigt dat associaties van voortdurende blootstelling aan tabaksrook vanaf de geboorte gedurende de kindertijd met longfunctie kunnen aanhouden tot in de pubertijd. Hoewel blootstelling aan huisdieren gedurende de eerste levensjaren was geassocieerd met een verminderde toename van de longfunctie, werd de blootstelling aan huisdieren in de late kindertijd geassocieerd met een betere longfunctie op de leeftijden van 12 en 16 jaar en een snellere longgroei tussen deze leeftijden. Dit kan wijzen op vermijding van huisdieren door ouders van kinderen met gevoelige longen, maar nadere analyses ondersteunen dit niet. Blootstelling van kinderen aan vocht of schimmel was over het algemeen geassocieerd met lagere longfunctie op 12 en 16 jaar en vroege blootstelling aan vocht of schimmels was geassocieerd met een verminderde toenamen van de longfunctie tussen 12 en 16 jaar.

In **hoofdstuk 6** wordt beschreven dat een hogere blootstelling aan luchtverontreiniging tijdens het voorschoolse tijdvenster is geassocieerd met verminderde toename van de longfunctie tussen 8 en 16 jaar. Daarnaast was een hogere blootstelling aan luchtverontreiniging in de voorschoolse,basisschool-en middelbare school-tijdvensters geassocieerd met een lagere longfunctie op 16-jarige leeftijd. Deze bevindingen dragen bij aan het bewijs dat blootstelling aan luchtverontreiniging gedurende het hele leven negatieve effecten kan hebben op de longfunctie.

CONCLUSIE

Het vaststellen van periodes gedurende de levensloop waarin een verhoogde gevoeligheid bestaat voor de effecten van milieublootstellingen is essentieel in het gezondheidsonderzoek van kinderen. De in dit proefschrift gepresenteerde onderzoeken, samen met bestaande literatuur over de effecten van milieublootstellingen op astma en longfunctie, laten zien dat latere blootstellingen evenals voortdurende blootstelling gedurende de levensloop, naast vroeg blootstelling tijdens de eerste jaren, relevant kunnen zijn voor het ontstaan van astma en de ontwikkeling van de longfunctie. Nieuwe studies met geavanceerde statistische hulpmiddelen kunnen ons inzicht in het verband tussen milieu blootstellingen en de ontwikkeling van astma en longfunctie bij kinderen verder verbeteren.

ABBREVIATIONS USED IN THESIS

ALSPAC	Avon Longitudinal Study of Parents and Children
BAMSE:	Children, Allergy, Milieu, Stockholm, Epidemiological Survey
CHS:	Children's Health Study
CI:	Confidence interval
$FEV_{1:}$	Forced expiratory volume in 1 second
FVC :	Forced vital capacity
GINI/LISA	German Infant study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development/Influence of Life style factors on the development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics.
HDM:	House dust mite
LCGM:	Latent class growth models
LUR:	Land-use regression
NO_2:	Nitrogen dioxide
NO_x:	Nitrogen oxides
OR:	Odds ratio
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy
PM_{coarse}:	Particulate matter (aerodynamic diameter between 2·5-10 µm)
PM_{10}:	Particulate matter (aerodynamic diameter <10µm)
$PM_{2·5}$:	Particulate matter (aerodynamic diameter <2·5µm)

$PM_{2·5}$ absorbance: Reflectance of $PM_{2·5}$ filters

SHS:	Secondhand Smoke

Acknowledgements

If it were not for God I would not have been here today. This is all for His glory. Likewise, if it were not for so many people this would have been all but a dream.

Dr Ulrike Gehring, the guidance, mentorship and supervision you gave me for the entire 4.5 years I worked with you is immeasurable and no words can express how I am grateful to you. You left your door open for me all the time regardless of your busy schedule. You made it a point to attend all the presentations I ever did and you guided my steps not by always giving out instructions on what to do but also pose questions to me to think through what to do and why I would do it. You gave me time to work through analyses even when it seemed trivial and I did not know what and how to do it, you still trusted me to do it eventually. Your faith in me -from the Skype interviews, to giving me a PhD position and getting the work done - has propelled me into the academic that I am today. Time and again you also set time aside to help me debug SAS codes just to realize it was a colon missing from the code. I have grown to realise that as a statistician I will run into this so many times and it happens to the rest of us but thanks to you I know a few tricks. Besides my academic needs you were also always there to offer moral support and to encourage me mentally, allowing me to take time off work when I needed to and constantly checking how my parents and siblings were doing. Thank you for very much for everything. You will always remain a significant person in my entire academic and professional career and I know your door will always be open for me whenever I come running back.

Prof. Bert Brunekreef, from day one I arrived in Utrecht, I have known you as a man of "few but mighty words". Your overarching supervision for my PhD and also staying in the lead to make crucial and concrete decisions allowed smooth progression of my PhD. Your boundless experience in the field and the amount that you poured into my PhD is immense. I know I will always have a great mentor in you and I am very grateful for the support and kindness you showed me the entire time I worked with you and in general throughout my stay in Utrecht. And may I also take this opportunity to wish you a happy retirement.

Prof. Smit (Jet), apart from your important contributions during the monthly PIAMA meetings, the PIAMA project meetings in Zwolle and throughout my project in general, you also made it a point to constantly check on me and showed me so much kindness throughout my stay, I will never forget. The PIAMA midnight summer BBQs were my favourite. I hope that both you and Bert will go into retirement knowing you have transformed lives of so many people, mine inclusive.

To the rest of the PIAMA PIs, Gerrard, Alet and Judith, thank you for your guidance and contributions during my PhD. I would not have been able to do it without you.

To my fellow PIAMA PhDs, Joseph, Lizan, Linda, Annemarijn and Tom. You made this

journey bearable and you all in one way or another contributed to me being able to navigate through my PhD in ways you may or may not know. Thank you for the friendship and know that I never took your presence and assistance for granted. Marjan and Marieke, thank you both for your support.

To room 3.19b Calvin, Marije, Juls, Daniel and Ilse thank you for everything- game nights, BBQs, trips to the keukoenhof and all the efforts to make sure I loosened up a little and realize there is life outside the PhD.

To Ingrid Dahmen. Thank you for being the focal point person and also just being able to coordinate through my needs and logistics during my PhD. Your selflessness is greatly appreciated

To the IRAS department for providing an environment that enabled me to effectively carry out my duties and indeed excel in doing so. Thank you all for your lovely companionship during my stay in Utrecht.

A number of people made Utrecht home away from home for me. My family from the English Mass Community in Utrecht, Julie, George, Irene, Sunny, Farida, Ancille, Jean, Ruth and John. You were all a significant part of my life in Utrecht. Thank you for being my family and I wish you all God's blessings.

Ndifanji, true definition of partner in crime. This is yours as it is mine.

To my dad, and my siblings (aka G8). For some reason I have a feeling you all always knew I would be here even before I did myself. Your unwavering support and faith in me are undoubtedly the fuel that keeps me going. I love you and I would not trade you all for anything. Elasma, I will forever be grateful to you. I wouldn't be here if it wasn't for you.
And lastly, to mum, who sadly is not here to witness this today, but will forever remain my biggest cheerleader. This is for you and I know you are proud.

List of publications

PUBLICATIONS IN THIS THESIS

Milanzi EB, Brunekreef B, Koppelman GH, Wijga AH, van Rossem L, Vonk JM Smit. H.A. U.Gehring. Lifetime secondhand smoke exposure and childhood and adolescent asthma: findings from the PIAMA cohort. *Environmental Health*, 2017; 16: 14.

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Curriculum Vitae

Edith Milanzi was born on 11th July 1990 in Lilongwe, Malawi. She studied Statistics and Biology at Chancellor College, University of Malawi. After her undergraduate degree she briefly worked as a research field supervisor at Malaria Alert Centre under the College of Medicine in Malawi after which she proceeded to study for a Masters degree in Biostatistics at Hasselt University in Belgium. As soon as she completed her masters, she joined IRAS in Utrecht taking a PhD position focusing on environmental exposures and respiratory health and statistical methods of summarizing longitudinal exposure data. Currently she works as a medical statistician at MRC Clinical Trials Unit at University College London (UCL) in London.