Air Pollution Exposure and Pregnancy Complications

The Generation R Study

Edith van den Hooven

ACKNOWLEDGEMENTS

The general design of the Generation R Study was made possible by financial support from the Erasmus Medical Center Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMW), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport, and the Ministry of Youth and Families.

The printing of this thesis has been financially supported by the Generation R Study Group, the Department of Obstetrics and Gynaecology, and the Department of Epidemiology of the Erasmus Medical Center Rotterdam; TNO Urban Environment and Safety; and the Erasmus University Rotterdam.

Further financial support was kindly provided by the J.E. Jurriaanse Stichting, Nederlandse Vereniging voor Obstetrie en Gynaecologie (NVOG), Stichting Astma Bestrijding, Chiesi Pharmaceuticals B.V., BMA B.V. (Mosos), and GE Healthcare.

Cover design: Elvira 't Hart, elvirathart.nl
Lay-out: Legatron Electronic Publishing, Rotterdam
Printing: Ipskamp Drukkers, Enschede

ISBN: 978-94-6191-230-5

Air Pollution Exposure and Pregnancy Complications

The Generation R Study

Blootstelling aan luchtverontreiniging en zwangerschapscomplicaties

Het Generation R Onderzoek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
donderdag 19 april 2012 om 13.30 uur

door

Edith Henriëtte van den Hooven

geboren te Zwolle

ERASMUS UNIVERSITEIT ROTTERDAM

PROMOTIECOMMISSIE

Promotoren	Prof.dr. A. Hofman
	Prof.dr. E.A.P. Steegers
Overige leden	Prof.dr.ir. A. Burdorf
	Prof.dr. I. Reiss
	Prof.dr. J. Sunyer
Copromotoren	Dr. V.W.V. Jaddoe
	Dr. F.H. Pierik

Paranimfen	MSc L.L. de Jonge
	MSc J.C. Kiefte-De Jong

CONTENTS

Manuscripts that form the basis of this thesis

Chapter 2

van den Hooven EH, Pierik FH, van Ratingen SW, Zandveld PYJ, Meijer EW, Hofman A, Miedema HME, Jaddoe VWV, de Kluizenaar Y. Air pollution exposure estimation using dispersion modelling and continuous monitoring data in the Generation R Study. *Environ Health 2012; 11:9*

Chapter 3.1

van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, Lindemans J, Russcher H, Steegers EAP, Miedema HME, Jaddoe VWV. Air pollution exposure during pregnancy and maternal and fetal C-reactive protein levels. The Generation R Study. *Environ Health Perspect 2012; doi: 10.1289/ehp.1104345*

Chapter 3.2

van den Hooven EH, Pierik FH, de Kluizenaar Y, Hofman A, van Ratingen SW, Zandveld PYJ, Russcher H, Lindemans J, Miedema HME, Steegers EAP, Jaddoe VWV. Air pollution exposure and markers of placental growth and function. The Generation R Study. *Submitted for publication*

Chapter 3.3

van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, Mackenbach JP, Steegers EAP, Miedema HME, Jaddoe VWV. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy. The Generation R Study. *Hypertension 2011; 57:406-412*

Chapter 3.4

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, Zandveld PYJ, Mackenbach JP, Steegers EAP, Miedema HME, Jaddoe VWV. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environ Health Perspect 2012; 120:150-156*

Chapter 3.5

van den Hooven EH, Jaddoe VWV, de Kluizenaar Y, Hofman A, Mackenbach JP, Steegers EAP, Miedema HME, Pierik FH. Residential traffic exposure and pregnancy-related outcomes in mother and child: A prospective birth cohort study. *Environ Health 2009; 8:59*

List of abbreviations

AC	Abdominal circumference
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CO	Carbon monoxide
CRP	C-reactive protein
DBP	Diastolic blood pressure
DWTD	Distance-weighted traffic density
EFW	Estimated fetal weight
EU	European Union
FL	Femur length
GA	Gestational age
GIS	Geographic Information System
HC	Head circumference
hs-CRP	High-sensitive C-reactive protein
LMP	Last menstrual period
LUR	Land-use regression model
NO_2	Nitrogen dioxide
O_3	Ozone
OR	Odds ratio
PI	Pulsatility index
PlGF	Placental growth factor
$PM_{2.5}$	Particulate matter with an aerodynamic diameter <2.5 µm
PM_{10}	Particulate matter with an aerodynamic diameter <10 µm
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Standard deviation score
SES	Socioeconomic status
sFlt-1	Soluble fms-like tyrosine kinase-1
SGA	Small size for gestational age at birth
SO_2	Sulfur dioxide

Chapter 1

Introduction

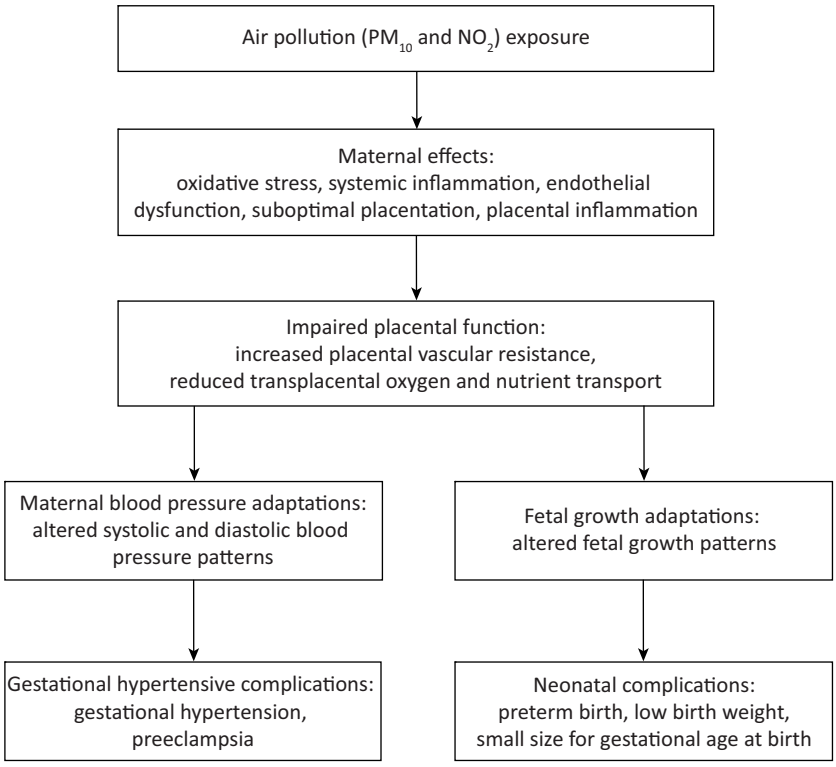
BACKGROUND

In the winter of 1952, London experienced an episode of dense smog. The heavy increase in air pollution during these days resulted in thousands of excess deaths [1-2]. This event has illustrated the potential impact of air pollution on human health, and has initiated research in the field of air pollution epidemiology. Since then, a wide range of epidemiological, clinical, and toxicological studies have provided evidence that air pollution exposure is associated with cardiovascular and respiratory morbidity and mortality [3-9]. In response, authorities such as the World Health Organization and the European Union have defined guidelines for air quality in order to protect human health [10]. These guidelines include limit values for the most important air pollutants. Particulate matter (PM_{10}) and nitrogen dioxide (NO_2) are two pollutants that have been associated with several adverse health effects, and often exceed the limit values at locations near heavy traffic. Governmental and municipal initiatives to improve air quality in the Netherlands have led to a gradual decrease in PM_{10} and NO_2 levels in the last decade. However, at several locations near air pollution sources, concentrations still exceed the limit values [11]. Furthermore, mounting evidence indicates that air pollution adversely affects health at concentrations even below these limit values [9].

Certain subgroups of the populations might be more susceptible to the adverse effects of air pollution. It has been suggested that pregnant women and their unborn children may constitute a more vulnerable subgroup [12-13]. The last two decades have seen a growing number of studies linking air pollution exposure during pregnancy with neonatal complications, such as preterm birth, intrauterine growth restriction, and low birth weight. These outcomes are strongly associated with neonatal morbidity and mortality [14-15] and also seem to be associated with problems in later life, such as increased risks of cardiovascular disease and diabetes [16-18]. Recent studies indicated that air pollution may also contribute to the development of gestational hypertensive complications, including gestational hypertension and preeclampsia. These complications are not only associated with adverse maternal and neonatal outcomes, but also with an increased risk of future cardiovascular disease [19].

Many studies have been conducted on the impact of air pollution on neonatal complications, but results are inconsistent [20-21]. Furthermore, not much is known about the underlying mechanisms through which air pollution affects the course of pregnancy. Air pollution is hypothesized to induce oxidative stress and systemic inflammation [3], possibly resulting in suboptimal placentation or impaired placental function [22-23], thereby reducing the nutrient and oxygen transport to the fetus. This may eventually result in the development of maternal and neonatal complications (Figure 1). Thus far, only few studies have addressed specific outcomes such as maternal blood pressure, placental function, inflammation markers, and fetal growth, which may be implicated in the pathways through which air pollution contributes to maternal and neonatal complications.

Figure 1. Proposed pathway leading from air pollution exposure during pregnancy to maternal and neonatal complications.



OBJECTIVES

This thesis aims to reveal pathways underlying the associations of air pollution exposure during pregnancy with maternal and neonatal complications.

The main objectives were:

1. To examine the associations of air pollution exposure during pregnancy with the risks of gestational hypertensive complications and neonatal complications.
2. To examine the mechanisms that underlie the associations of air pollution exposure during pregnancy with maternal and neonatal complications. Mechanisms of interest are placental function, inflammatory responses, maternal blood pressure, and fetal growth.

SETTING

All studies described in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from pregnancy onward in Rotterdam, the Netherlands. The Generation R Study was designed to identify early environmental and genetic determinants of growth, development, and health during fetal life and childhood [24]. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in the study. The aim was to enrol participants during pregnancy, but enrolment was allowed until the birth of the child. In total, 9778 women were included, of whom 8880 were enrolled in the prenatal part of the study. The largest ethnic groups were the Dutch, Surinamese, Turkish, and Moroccan. Data on pregnancy were collected on the basis of questionnaires, physical examinations, fetal ultrasound examinations, biological samples, and medical records completed by midwives and obstetricians. Physical assessments were performed in early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18-25 weeks), and late pregnancy (gestational age ≥25 weeks), but the individual time schemes depended on the specific gestational age at enrolment [24]. Postnatal data was obtained by community health centers and questionnaires. From the age of 5 years onward, regular detailed hands on assessments are performed in all children and their parents in a research center [24]. The Generation R study has been approved by the Medical Ethical Committee of the Erasmus Medical Center Rotterdam. All participants provided written informed consent.

OUTLINE OF THESIS

In **Chapter 2**, we describe the methodology of the air pollution exposure assessment for participants of the Generation R Study. These individual air pollution exposure estimates have been applied in a number of studies described in the next chapter.

Chapter 3 presents different studies that examine the associations of air pollution with maternal and neonatal outcomes. In **Chapter 3.1**, we studied whether maternal air pollution exposure was associated with maternal and fetal inflammatory responses. **Chapter 3.2** addresses the associations of maternal air pollution exposure with markers of placental growth and function. In **Chapter 3.3**, we studied whether maternal air pollution exposure was associated with blood pressure patterns during pregnancy and the risks of gestational hypertensive complications. **Chapter 3.4** presents the associations of maternal air pollution exposure with fetal growth patterns and the risks of neonatal complications. In **Chapter 3.5**, we assessed whether residential proximity to traffic, as an indicator of air pollution exposure, was associated with the risks of maternal and neonatal complications.

Finally, **Chapter 4** provides a general discussion of our and previous studies performed on maternal air pollution exposure and pregnancy complications, and describes the implications, methodological considerations, and suggestions for future research.

REFERENCES

1. Bell ML, Davis DL. Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. *Environ Health Perspect.* 2001; 109 Suppl 3:389-94.
2. Logan WP. Mortality in the London fog incident, 1952. *Lancet.* 1953; 1(6755):336-8.
3. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
4. Kunzli N, Kaiser R, Medina S, Studnicka M, Chanel O, Filliger P, et al. Public-health impact of outdoor and traffic-related air pollution: a European assessment. *Lancet.* 2000; 356(9232):795-801.
5. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, et al. Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med.* 2007; 356(5):447-58.
6. Nawrot TS, Perez L, Kunzli N, Munters E, Nemery B. Public health importance of triggers of myocardial infarction: a comparative risk assessment. *Lancet.* 2011; 377(9767):732-40.
7. Pope CA, 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc.* 2006; 56(6):709-42.
8. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation.* 2010; 121(25):2755-65.
9. World Health Organization: Health aspects of air pollution. Results from the WHO project "Systematic review of health aspects of air pollution in Europe". 2004. Available: http://www.euro.who.int/_data/assets/pdf_file/0003/74730/E83080.pdf
10. European Commission: Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. 2008. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:152:0001:0044:EN:PDF
11. National Institute for Public Health and the Environment [RIVM]: Progress of the National Air Quality Cooperation Programme (NSL) [Monitoringsrapportage NSL. Stand van zaken 2010 Nationaal Samenwerkingsprogramma Luchtkwaliteit]. 2010. RIVM report 68712002/2010. Available: http://www.rivm.nl/bibliotheek/rapporten/680712002.html
12. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
13. Wang L, Pinkerton KE. Air pollutant effects on fetal and early postnatal development. *Birth Defects Res C Embryo Today.* 2007; 81(3):144-54.
14. Wilcox AJ, Skjaerven R. Birth weight and perinatal mortality: the effect of gestational age. *Am J Public Health.* 1992; 82(3):378-82.
15. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med.* 1999; 340(16):1234-8.
16. Barker DJ. Fetal origins of coronary heart disease. *BMJ.* 1995; 311(6998):171-4.
17. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation.* 1996; 94(12):3246-50.
18. Leon DA, Lithell HO, Vagero D, Koupilova I, Mohsen R, Berglund L, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *BMJ.* 1998; 317(7153):241-5.
19. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstet Gynecol.* 2009; 114(5):961-70.
20. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
21. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
22. Dejmek J, Selevan SG, Benes I, Solansky I, Sram RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ Health Perspect.* 1999; 107(6):475-480.

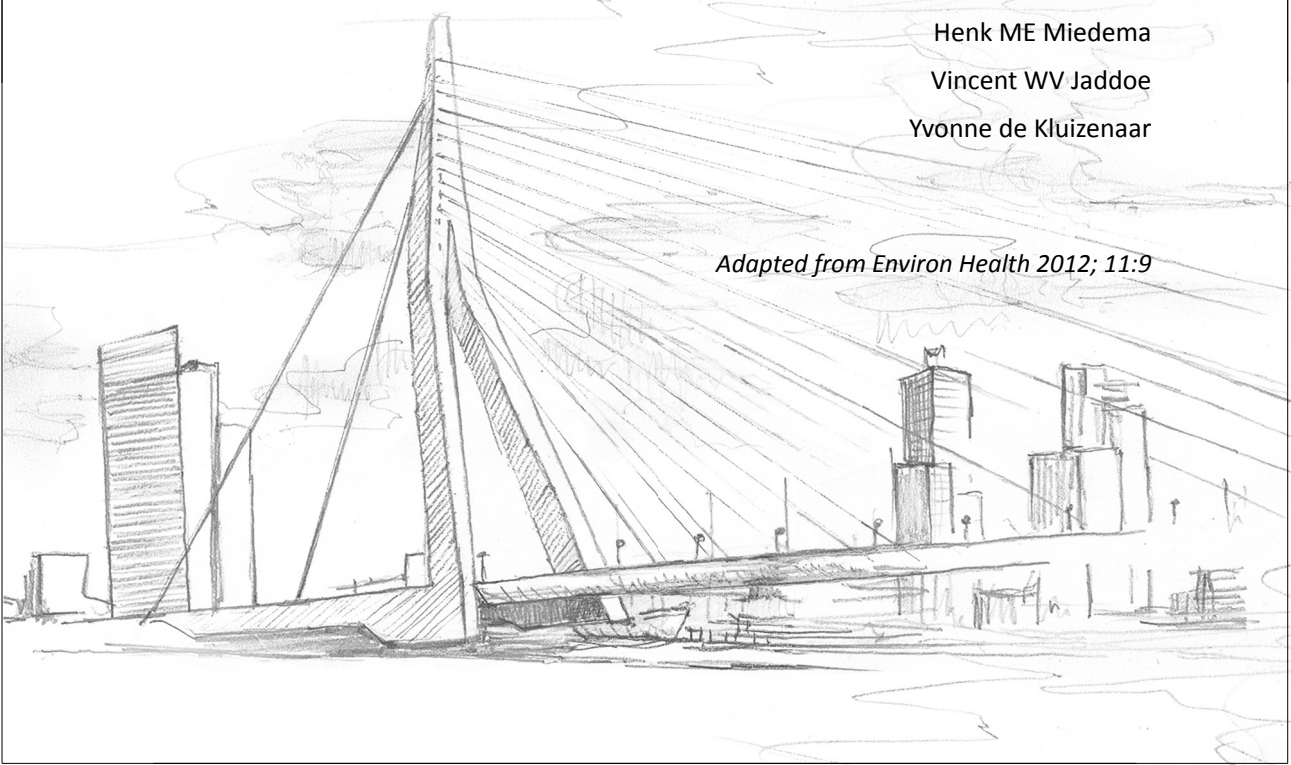
23. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
24. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.

Chapter 2

Methodology of the air pollution exposure assessment

Edith H van den Hooven
Frank H Pierik
Sjoerd W van Ratingen
Peter YJ Zandveld
Ernst W Meijer
Albert Hofman
Henk ME Miedema
Vincent WV Jaddoe
Yvonne de Kluizenaar

Adapted from Environ Health 2012; 11:9



ABSTRACT

Background: Previous studies suggest that pregnant women and children are particularly vulnerable to the adverse effects of air pollution. A prospective cohort study in pregnant women and their children enables identification of the specific effects and critical periods. This paper describes the design of air pollution exposure assessment for participants of the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in 9778 women in the Netherlands.

Methods and Results: Individual exposures to PM_{10} and NO_2 levels at the home address were estimated for mothers and children, using a combination of advanced dispersion modelling and continuous monitoring data, taking into account the spatial and temporal variation in air pollution concentrations. Full residential history was considered. We observed substantial spatial and temporal variation in air pollution exposure levels.

Conclusions: The Generation R Study provides unique possibilities to examine effects of short- and long-term air pollution exposure on various maternal and childhood outcomes and to identify potential critical windows of exposure.

INTRODUCTION

Air pollution exposure has been associated with several adverse health effects, such as cardiovascular disease, respiratory disease, and total mortality [1-4]. Certain subgroups of the population, including pregnant women and their unborn children, have been suggested to be more susceptible to the adverse effects of air pollution [5, 6]. Literature on the specific effects of air pollution exposure in pregnant women on outcomes such as inflammation markers, placental function, and blood pressure, is scarce. In contrast, research on the impact of air pollution exposure on birth outcomes has increased in the last decade, which has led to a number of reviews summarizing the available evidence [7, 8]. Most routinely measured air pollutants (e.g., PM_{10}, NO_2, CO, O_3, SO_2) have been linked to increased risks of adverse birth out-comes [6]. However, results are not consistent between studies, with respect to the specific air pollutants, relevant exposure periods, and specific birth outcomes [7, 8]. Recommendations for future research are to improve exposure assessment by incorporating detailed information on spatial and temporal patterns in air pollution concentrations and to consider a greater variety of reproductive outcomes [9]. Furthermore, it is of interest to include noise exposure data in studies on traffic-related air pollution exposure and health, since traffic is a major shared source for both air pollution and noise [10-13].

Dispersion models are applied to estimate air pollution concentrations in a study area, using data on emissions, meteorological conditions, and topography [14]. Despite the relatively costly data input, dispersion modelling is a promising method to obtain air pollution estimates for epidemiological studies, as it allows consideration of both spatial and temporal variation without the need for extensive air pollution monitoring. Dispersion models are increasingly used in combination with geographic information system (GIS) based methods. This introduces the possibility for spatial linkage of geographically referenced data, such as residential addresses, road networks, pollution sources, and street characteristics, which further enhances the quality of the modelling approach [14, 15].

In this paper we describe the design of studies focused on the effects of air pollution exposure on various health outcomes in mothers and children in the Generation R Study. We describe the assessment of individual exposures to particulate matter with an aerodynamic diameter <10 μm (PM_{10}) and nitrogen dioxide (NO_2) at the home address, using a combination of continuous monitoring data and GIS based dispersion modelling techniques, taking into account both the spatial and temporal variation in air pollution. In addition, we present the distribution of exposure levels for various relevant exposure periods in the prenatal and postnatal phase, and we present exposure levels according to maternal and infant characteristics.

METHODS

Study design

The Generation R Study is a population-based prospective cohort study from pregnancy onwards, which was designed to identify early environmental and genetic causes of normal and abnormal growth, development, and health during fetal life, childhood and adulthood. It has been described previously in detail [16, 17]. In brief, the cohort includes mothers and children of different ethnicities living in the city of Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age <18 weeks), but was allowed until the birth of the child. Out of the total number of eligible children in the study area, 61 percent participated in the study at birth. In total, 9778 mothers with a delivery date between April 2002 and January 2006 were enrolled in the study. Extensive assessments have been carried out in mothers and fathers and are currently performed in their children, who form a prenatally recruited birth cohort that will be followed until young adulthood. Data collection included questionnaires, detailed physical and ultrasound examinations, behavioural observations, and biological samples. Assessments in pregnancy were performed in each trimester. Assessments in the children in the preschool period (birth to age of 4 years) included a home-visit, questionnaires, and visits to the routine child health centres. From the age of 5 years onward, regular detailed hands on assessments are performed in all children and their parents in a research center. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Air pollution exposure assessment

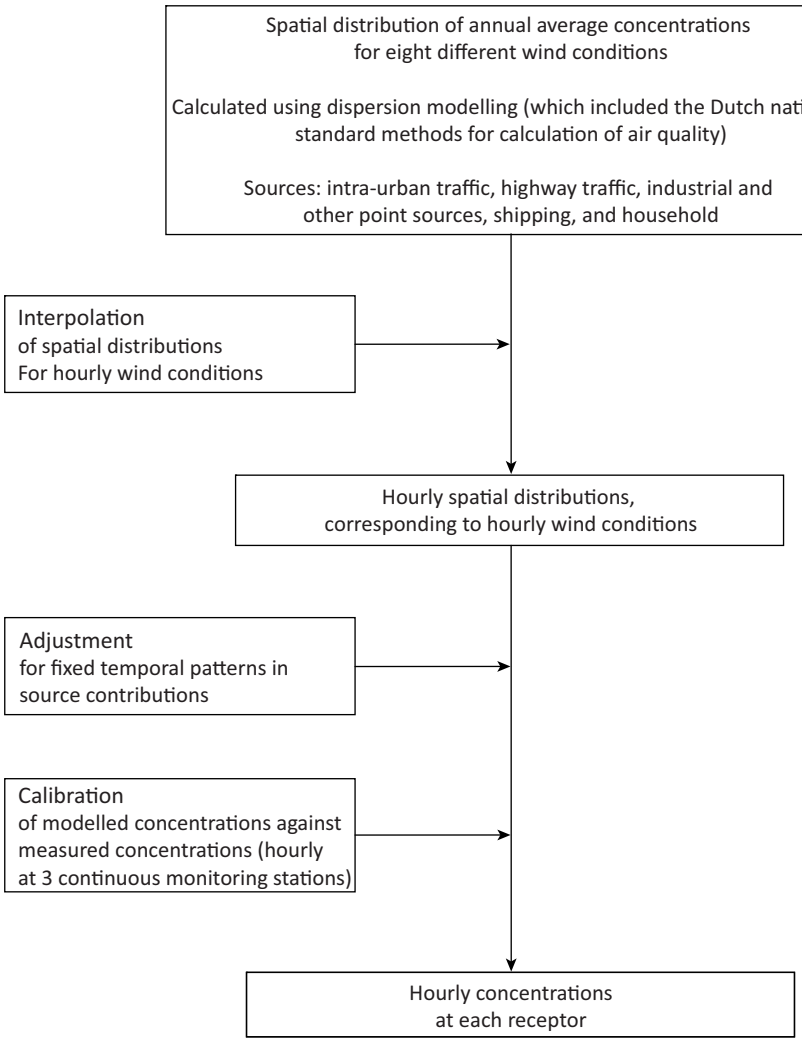
Hourly concentrations of PM_{10} and NO_2 at all addresses in the study area (northern part of Rotterdam) were assessed for the years 2001-2008, using advanced spatiotemporal dispersion modelling techniques in combination with hourly air pollution measurements at three continuous monitoring sites. A flow chart of the exposure assessment approach is presented in Figure 1. We took the following steps to assign exposure estimates to the addresses in this area.

Spatial pattern

First, approximately 800,000 digital calculation points, further referred to as 'receptors', were assigned to the façades of all dwellings in the study area. For all receptors, annual average concentrations of PM_{10} and NO_2 for the years 2001-2008 were calculated using GIS and the three Dutch national standard methods for the calculation of air quality. These standard methods have been established by the Dutch government, and are designated to calculate the contribution of intra-urban road traffic, traffic on highways, and industrial and other point sources (standard calculation method 1, 2 and 3, respectively) [18]. Subsequently, in order to obtain spatiotemporal patterns, spatially resolved annual concentrations were calculated for eight different wind conditions (wind direction: north/

east/south/west; wind velocity: light/strong), resulting in an averaged spatially resolved concentration pattern for each wind class.

Figure 1. Flow chart of the air pollution exposure assessment.



Input data for the calculations were traffic characteristics (including annual traffic intensities, traffic composition, and traffic speed), road characteristics, buildings and ground characteristics, and annual emissions from traffic, shipping, industry, and households. Detailed digital maps with information on geographic locations and traffic characteristics for roads in the study area were obtained from the local authorities of Rotterdam. Traffic intensity data was supplied by the DCMR Environmental Protection

Agency Rijnmond (DCMR). Emission sources and emission data were obtained from the National Institute for Public Health and the Environment (RIVM) and the DCMR. Hourly meteorological data was obtained from observations at Rotterdam The Hague Airport, performed by the Royal Netherlands Meteorological Institute (KNMI).

Temporal pattern

To account for temporal variation due to different wind conditions, for each hour we derived the corresponding spatial distribution for the prevailing wind direction and wind speed at that specific hour, by means of interpolation between the eight characteristic spatial distributions. Subsequently, the spatial distributions that corresponded to the hourly wind conditions were adjusted for fixed temporal patterns of source activities. In this way, we accounted for temporal fluctuations in the contribution of air pollution sources during the month, week (e.g., working days and weekend days), and day (e.g., morning and evening rush hour). The adjustment for temporal patterns was performed for traffic and household emissions. Traffic is the source with the strongest fluctuations in emissions within 24 hours. This 24h-pattern is fairly stable for working days and weekend days. Hence, the contribution of traffic was scaled using an average hourly traffic intensity pattern (based on traffic counts), thereby deriving hourly intensities. We also considered the time dependence of household emissions, by applying a 24h-pattern, and we applied a function for outdoor temperature dependence to account for seasonal fluctuations. These functions were derived from energy use statistics. In this way, hourly household emissions were estimated from annual household emissions. Emissions from industrial sources do not contribute significantly to small-scale variations in air pollution concentrations. Emissions from shipping are quite stable over time and also display relatively small temporal fluctuations. Therefore, these emissions were not adjusted for fixed temporal patterns. Nevertheless, even if some small-scale variations had occurred as a result of these emissions, the difference would have been corrected for in the next step (adjustment for hourly background concentrations).

Adjustment for background concentrations

The modelled hourly concentrations were adjusted for background concentrations, in order to take into account the temporal fluctuations in background concentrations. This was done using continuous hourly monitoring data from three monitoring stations in the study area. The measured air pollution concentrations at these stations are considered as the sum of the background concentration and the contribution from local emission sources. We modelled the contribution of local emission sources to the PM_{10} and NO_2 concentrations at the three monitoring stations. Subsequently, we subtracted the hourly modelled contributions from the hourly measured concentrations at the stations, thereby deriving an hourly estimate for the background concentrations. The hourly estimates for the background concentrations at the three stations were averaged, which yielded an average hourly background concentration for the study area. In the adjustment

procedure, this average hourly background concentration was added to the modelled hourly contributions at the home addresses, in order to take into account the background concentration.

Continuous air pollution monitoring data was provided by DCMR. Missing values for PM_{10} concentrations at the three monitoring stations were imputed with hourly concentrations derived from the large-scale concentration database for air pollution in the Netherlands (generally referred to as 'GCN map') published by the Netherlands Environmental Assessment Agency (PBL), the national institute for policy analysis in the field of environment, nature and spatial planning. The hourly concentrations in the GCN database are estimated on the basis of hourly measurements from the National Air Quality Monitoring Network (LML), emission data, and modelling. The developed nationwide concentration maps are updated annually and provide a best currently available estimate of large-scale air quality [19].

Modelling performance

As described above, the first step in our modelling procedure involved the assessment of annual average PM_{10} and NO_2 concentrations, using a combination of the three Dutch standard methods. The performance of this modelling procedure based on (a combination of) the three standard methods has been evaluated by two previous studies in the same study area. These studies reported a good agreement between predicted annual average PM_{10} and NO_2 concentrations and concentrations measured at monitoring stations [20, 21]. More specifically, Beelen et al., who performed the most extensive validation study, found a correlation of 0.77 between modelled and measured NO_2 concentrations [20]. Our dispersion modelling approach, resulting in hourly average concentrations, is a refinement of this former modelling procedure. An additional validation study of this refined modelling procedure was not feasible within the scope of this project.

Exposure assignment

For each dwelling, the receptor at the most exposed façade was selected, and the corresponding air pollution values were assigned to the address. We obtained full residential history of the participants by combining the address data collected by questionnaires with data from the local authorities of Rotterdam. It was ascertained that the residential history covered the period from pregnancy onward. We calculated exposure estimates for the participants using the following approach. Derived from the hourly concentrations of PM_{10} and NO_2, we constructed a database containing daily averages (24h) for every address, for the years 2001-2008. Allowing for residential mobility, air pollution exposure estimates were linked to the different home addresses of the participants throughout the study period. This yielded a database with individual exposures, which can be used to derive average exposure estimates for any period between 2001 and 2008, depending on the specific research question. For the present paper, we describe air pollution exposure estimates for a number of pregnancy and childhood periods, to illustrate the distribution

of exposure levels in participants in these potential sensitive periods. More specifically, we derived exposures for the following periods: first trimester, second trimester, third trimester, total pregnancy, birth until 6 months postnatally, and 7 until 12 months postnatally. Exposures were only calculated for periods with less than 20-25% of the daily averages missing. For the other periods, air pollution exposures were set to missing.

Statistical analyses

Descriptive analyses were performed for all air pollution exposure averages, including the evaluation of the Pearson correlation coefficients between the different exposure averages. In addition, we examined mean maternal PM_{10} and NO_2 exposure levels during total pregnancy according to maternal characteristics and infant characteristics. Information on these characteristics was obtained from questionnaires in pregnancy and from medical records, as described in the following Chapters of this thesis. Road traffic noise exposure was assessed at the home address at delivery in accordance with requirements of the European Union (EU) Environmental Noise Directive [22]. Supplementary File S1 and Supplementary Table S1 provide more detailed information on the rationale to assess noise exposure levels, the assessment procedure, and the distribution of noise exposure levels in our study cohort. Information on average neighbourhood income was obtained from Statistics Netherlands as neighbourhoods' average disposable income per income receiver in the year 2004, and classified into: low (<1400 euro/month), moderate (1400-2200 euro/month), and high (>2200 euro/month). Season of conception and season of birth were categorized as winter (December to February), spring (March to May), summer (June to August), and fall (September to November). For all maternal and infant characteristics, we performed a one-way ANOVA followed by Bonferroni's post hoc comparison tests to examine the differences in mean air pollution exposure levels compared with the reference group. All statistical analyses were performed using PASW version 17.0 for Windows (PASW Inc., Chicago, IL, USA).

RESULTS

Air pollution exposure in the study cohort

Of the 9778 women, exposure estimates could not be calculated for 149 mothers because they had an abortion (n=29) or intrauterine death (n=75), or were lost-to-follow up (n=45), and consequently no information was available on the date of conception and delivery. For the remaining 9629 women (and their 9748 children), 12188 addresses were available for the time period presented here (conception until the first year postnatally). Of all women, 74% did not move in this period, 25% changed residence once, and less than 1% moved two or three times. Of the 12188 addresses, 10518 (86%) could be linked to the air pollution exposure database, and 1938 addresses could not be linked. This was either due to missing address information, incompatible street number suffices, or to addresses

situated outside of the study area of the Generation R Study [16]. As a result, air pollution exposure estimates for the present paper were available for 8810 mothers and 8921 children.

Table 1 presents the distribution of maternal PM_{10} and NO_2 levels for a number of illustrative prenatal and postnatal periods. The number of participants with available exposure data varied for the specific periods. On average, PM_{10} and NO_2 exposure levels during first trimester were higher than during second and third trimester, and postnatal exposure levels were lower than prenatal exposure levels. This can be explained by the decreasing trend in air pollution levels throughout the study period. Mean air pollution exposure levels during pregnancy were 30.2 $\mu g/m^3$ (range 23.1 to 39.9) for PM_{10} and 39.7 $\mu g/m^3$ (range 25.3 to 56.9) for NO_2 (Table 1). On average, these levels are below the European Union annual limit values (40 $\mu g/m^3$ for both PM_{10} and NO_2) that are defined for protection of human health [23], but a substantial proportion of the women was exposed to levels higher than these limit values. Moreover, it has been acknowledged that significant health effects may occur even below the current limit values [24].

Table 1. Distribution of maternal PM_{10} and NO_2 exposure levels for different prenatal and postnatal periods.

	N	Minimum	25th percentile	Mean	Median	75th percentile	Maximum
PM_{10} exposure ($\mu g/m^3$)							
Prenatal							
First trimester	7894	22.0	27.7	30.6	30.5	33.4	43.1
Second trimester	8311	21.3	26.2	30.1	29.5	33.3	45.6
Third trimester	8438	22.0	26.6	29.8	29.8	32.0	43.5
Total pregnancy	7877	23.1	27.7	30.2	29.9	32.8	39.9
Postnatal							
Month 0-6	8381	22.7	27.3	29.5	29.3	31.4	39.9
Month 7-12	8082	22.8	27.0	28.8	28.7	30.5	39.3
NO_2 exposure ($\mu g/m^3$)							
Prenatal							
First trimester	7893	21.4	36.9	40.2	40.6	43.5	58.5
Second trimester	8310	20.2	35.2	39.6	40.5	43.9	59.7
Third trimester	8434	21.3	35.4	39.3	39.9	43.2	58.8
Total pregnancy	7889	25.3	37.0	39.7	39.5	42.2	56.9
Postnatal							
Month 0-6	8389	24.2	36.3	39.4	39.5	42.5	59.3
Month 7-12	8082	24.1	35.5	38.6	38.6	41.6	58.0

Air pollution exposure was estimated for different prenatal and postnatal periods: first trimester (0-18 weeks), second trimester (18-25 weeks), third trimester (25 weeks-delivery), total pregnancy, month 0-6 postnatally, and month 7-12 postnatally.

Epidemiological studies often evaluate associations for air pollution exposure levels in different periods, in order to examine the relevant exposure periods, which is informative only if the correlations among these exposure levels are not too high. Table 2 shows that Pearson correlation coefficients between the different air pollution exposure averages for the present paper varied between 0.02 and 0.83. Correlations among exposure averages for the first, second, and third trimester were moderate (PM_{10}: r=0.31 to 0.48, NO_2: r=0.17 to 0.48). Correlations between exposure averages for the separate trimesters with exposure averages for the total pregnancy period were higher (PM_{10}: r=0.73 to 0.83, NO_2: r=0.43 to 0.51). Correlations between prenatal and postnatal exposure averages were low for PM_{10} (r=0.13 to 0.29), and somewhat higher for NO_2 (r=0.22 to 0.78). PM_{10} and NO_2 exposures averages for the same period were moderately correlated (r=0.58 to 0.66).

There was substantial spatial and temporal variation in air pollution exposure levels (Figures 2 and 3). Figure 2 presents maps of the spatial distribution of PM_{10} and NO_2 concentrations in the study area, demonstrating differences in annual average concentrations up to 4-8 $\mu g/m^3$ between urban and suburban areas. Figure 3 presents the temporal variation in PM_{10} and NO_2 exposure levels estimated at two different locations in the study area (one situated in the city center and one situated in a suburb of Rotterdam). Especially for NO_2, substantial differences were observed between the two locations.

For illustrative purposes, we present mean maternal air pollution exposure during total pregnancy according to maternal characteristics (Table 3) and infant characteristics (Table 4). Table 3 shows that PM_{10} and NO_2 exposure levels were higher for mothers who were younger than 25 years, of non-Dutch ethnicity, nulliparous, were exposed to higher noise levels, lived in a low neighbourhood income area, and whose conception occurred in summer or fall. In addition, NO_2 exposure was slightly higher in women who continued smoking, and PM_{10} exposure was higher in women who continued to consume alcohol during pregnancy. There was a clear decrease in air pollution exposure over time: women whose conception fell between 2001 and 2003 were exposed to higher PM_{10} and NO_2 levels during pregnancy than women with a conception date in 2004 or 2005. Table 4 shows that mothers were exposed to higher PM_{10} and NO_2 levels when they gave birth in spring or summer, compared with winter or fall. Mean exposure levels according to the year of birth also showed a decreasing trend in air pollution concentrations between 2002 and 2006.

Table 2. Correlation coefficients between period-specific PM_{10} and NO_2 exposure averages.

	PM_{10}						NO_2					
	First trimester	**Second trimester**	**Third trimester**	**Total pregnancy**	**Month 0-6 postnatally**	**Month 7-12 postnatally**	**First trimester**	**Second trimester**	**Third trimester**	**Total pregnancy**	**Month 0-6 postnatally**	**Month 7-12 postnatally**
PM_{10}												
First trimester	1											
Second trimester	0.48	1										
Third trimester	0.31	0.46	1									
Total pregnancy	0.83	0.74	0.73	1								
Month 0-6 postnatally	0.19	0.13	0.34	0.29	1							
Month 7-12 postnatally	0.11	0.02	0.01	0.06	0.21	1						
NO_2												
First trimester	0.59	0.36	0.19	0.51	0.28	0.01	1					
Second trimester	0.26	0.58	0.41	0.48	0.15	0.24	0.45	1				
Third trimester	0.17	0.24	0.63	0.43	0.25	0.36	0.17	0.48	1			
Total pregnancy	0.49	0.47	0.53	0.64	0.32	0.26	0.77	0.76	0.73	1		
Month 0-6 postnatally	0.48	0.21	0.22	0.42	0.66	0.27	0.66	0.22	0.30	0.57	1	
Month 7-12 postnatally	0.17	0.29	0.44	0.37	0.26	0.63	0.34	0.68	0.78	0.77	0.39	1

Values reflect Pearson correlation coefficients between air pollution exposure estimates for different prenatal and postnatal periods.

Figure 2. Maps illustrating the spatial distribution of PM_{10} and NO_2 concentrations in the study area.

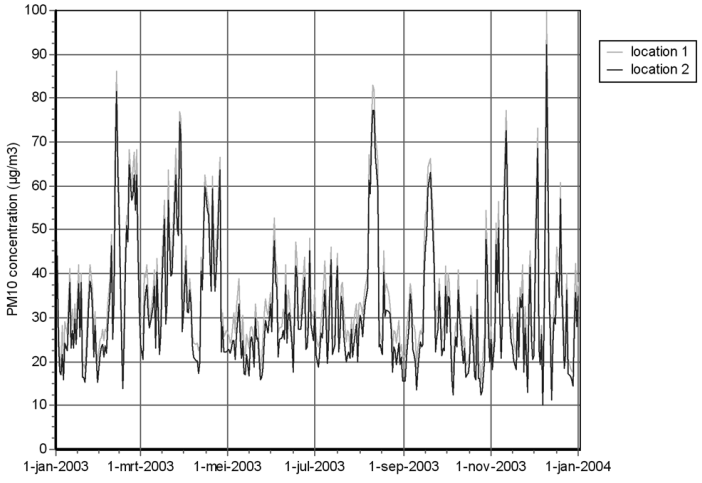
a. PM_{10} concentration

b. NO_2 concentration

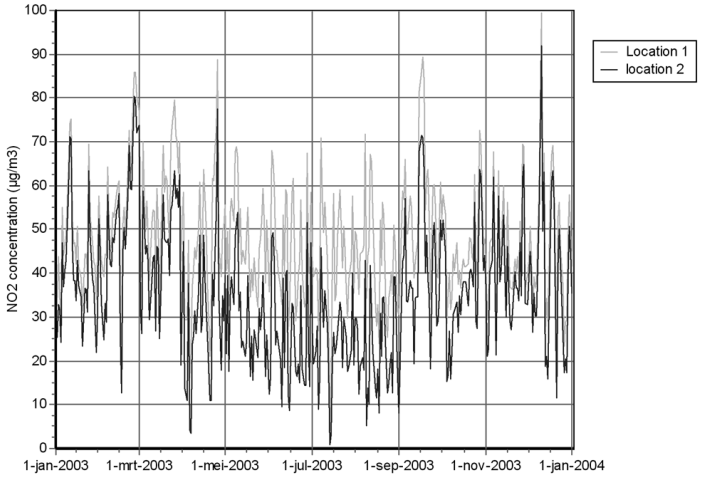
The maps show the surface water (in blue) and the spatial distribution of PM_{10} and NO_2 concentrations (see legends) in the study area. Areas without addresses remain white on the maps. The presented PM_{10} and NO_2 concentrations are annual average concentrations in 2003 for the addresses in the study area. These figures are only presented as an illustration, since daily average concentrations in 2001-2008 were used to calculate individual exposures.

Figure 3. Illustration of the temporal variation in of PM_{10} and NO_2 exposure levels in the study area.

a. PM_{10} concentration



b. NO_2 concentration



Estimated PM_{10} and NO_2 concentrations in 2003 at two different locations in the study area. Location 1 is located in the city center, whereas location 2 is situated in a suburb of Rotterdam.

Table 3. Maternal air pollution exposure during pregnancy according to maternal characteristics.

Maternal characteristics	**N**	**PM_{10} exposure** ($\mu g/m^3$) Mean (SD)	**NO_2 exposure** ($\mu g/m^3$) Mean (SD)
Age			
<25 years	1446	30.5 (3.2) *	40.4 (3.8) *
25-30 years (Reference)	2051	30.2 (3.1)	39.8 (4.2)
30-35 years	2998	30.1 (3.2)	39.5 (4.4) *
>35 years	1395	30.0 (3.2)	39.5 (4.3)
Body mass index			
<20 kg/m^2	627	30.5 (3.2)	40.3 (4.2)
20-25 kg/m^2 (Reference)	3714	30.3 (3.2)	39.8 (4.2)
25-30 kg/m^2	1843	30.3 (3.1)	39.8 (4.1)
>30 kg/m^2	972	30.0 (3.2)	39.6 (4.0)
Missing	734	29.1 (3.1) **	38.6 (4.7) **
Ethnicity			
Dutch/Caucasian (Reference)	4268	30.1 (3.2)	39.4 (4.5)
Turkish	622	30.1 (3.0)	40.2 (3.5) **
Moroccan	489	30.2 (3.0)	40.1 (3.5) *
Surinamese	619	30.6 (3.2) *	40.2 (4.0) **
Other	1151	30.4 (3.3) *	40.3 (4.1) **
Missing	741	29.8 (3.0)	40.1 (4.0) **
Educational level			
No education/primary	757	30.3 (3.1)	40.0 (3.6)
Secondary	3102	30.3 (3.2)	39.7 (4.3)
Higher (Reference)	3132	30.1 (3.2)	39.6 (4.4)
Missing	899	29.8 (3.0)	40.1 (4.0) *
Parity			
Nulliparous (Reference)	4129	30.3 (3.2)	40.0 (4.3)
Multiparous	3528	30.1 (3.1) *	39.5 (4.1) **
Missing	233	29.4 (3.1) **	38.8 (4.5) **
Smoking in pregnancy			
No (Reference)	4616	30.2 (3.2)	39.7 (4.2)
First trimester only	527	30.5 (3.3)	40.1 (4.6)
Continued	1059	30.5 (3.2)	40.2 (4.2) *
Missing	1688	29.6 (2.9) **	39.5 (4.2)
Alcohol use in pregnancy			
No (Reference)	3022	30.2 (3.2)	39.8 (4.1)
First trimester only	820	30.2 (3.2)	39.6 (4.4)
Continued	2415	30.4 (3.2) *	39.9 (4.3)
Missing	1633	29.7 (2.9) **	39.5 (4.2)

Table 3. *Continued*

Maternal characteristics	**N**	**PM_{10} exposure** ($\mu g/m^3$) Mean (SD)	**NO_2 exposure** ($\mu g/m^3$) Mean (SD)
Noise exposure			
<50 dB(A)	2985	29.6 (3.0) **	37.9 (3.3) **
50-65 dB(A) (Reference)	4016	30.2 (3.1)	39.8 (3.6)
>65 dB(A)	791	32.2 (3.5) **	46.0 (4.3) **
Missing	91	29.8 (3.1)	40.0 (4.0)
Neighbourhood income			
Low	1141	30.9 (2.9) **	41.0 (3.2) **
Moderate (Reference)	4678	30.0 (3.1)	39.6 (4.2)
High	1945	30.2 (3.2)	39.6 (4.5)
Missing	126	28.4 (3.2) **	35.2 (5.5) **
Season of conception			
Winter (Reference)	2184	29.9 (3.8)	38.8 (4.5)
Spring	1850	39.7 (2.6)	38.9 (4.1)
Summer	1810	30.5 (2.4) **	41.1 (3.8) **
Fall	2046	30.5 (3.4) **	40.3 (3.9) **
Year of conception			
2001 (Reference)	345	34.6 (1.3)	39.6 (3.4)
2002	2161	33.1 (1.6) **	41.8 (3.8)
2003	2468	29.5 (3.0) **	39.9 (4.2) **
2004	2460	28.0 (2.0) **	38.2 (3.9) **
2005	456	28.4 (1.2) **	37.4 (4.1) **

** P< 0.01; * P<0.05

Values are mean PM_{10} and NO_2 exposure levels for the total pregnancy period. P-values are based on One-way ANOVA followed by Bonferroni's post hoc comparison tests to examine the differences in means compared with the Reference group.

Table 4. Maternal air pollution exposure during total pregnancy according to infant characteristics.

Child characteristics	**N**	**PM_{10} exposure** ($\mu g/m^3$) Mean (SD)	**NO_2 exposure** ($\mu g/m^3$) Mean (SD)
Gestational age at birth			
<37 weeks	463	30.4 (3.3)	40.0 (4.5)
37-42 weeks (Reference)	6871	30.2 (3.1)	39.7 (4.2)
≥42 weeks	556	30.1 (3.3)	39.7 (4.1)
Birth weight			
<2500 grams	359	30.4 (3.1)	40.0 (4.4)
2500-4500 grams (Reference)	7194	30.2 (3.2)	39.7 (4.2)
>4500 grams	337	30.0 (3.2)	39.6 (4.3)
Season of birth			
Winter (Reference)	1856	29.7 (2.7)	38.9 (4.1)
Spring	1781	30.4 (2.3) **	41.0 (3.8) **
Summer	2098	30.5 (3.4) **	40.4 (4.0) **
Fall	2155	30.0 (3.8)	38.7 (4.5)
Year of birth			
2002 (Reference)	696	33.6 (1.7)	39.6 (3.5)
2003	2406	33.2 (1.6) **	41.9 (3.9) **
2004	2548	27.6 (2.4) **	39.0 (4.2) *
2005	2214	28.8 (1.5) **	38.3 (3.9) **
2006	26	27.8 (1.3) **	36.8 (4.1) *

** P< 0.01; * P<0.05
Values are mean PM_{10} and NO_2 exposure levels for the total pregnancy period. P-values are based on One-way ANOVA followed by Bonferroni's post hoc comparison tests to examine the differences in means compared with the Reference group.

DISCUSSION

For the participants of this large population-based cohort study, we assessed individual air pollution exposure at the home address using advanced state-of-the-art methods. By using a combination of GIS based dispersion modelling and continuous monitoring data, we were able to take into account the spatial and temporal variation in air pollution concentrations. The individual exposure estimates can be used in further epidemiological studies that examine air pollution effects in this population of mothers and children.

Air pollution exposure

In our air pollution exposure assessment procedure, we were able to consider fine spatial and temporal contrasts in exposure by using a combination of dispersion modelling and continuous monitoring. The high temporal resolution enables investigation of

relatively short exposure windows (e.g., total pregnancy, trimesters, or months) that are particularly of interest in pregnant women and children. It also facilitates identification of critical windows of exposure. These short-term exposure windows cannot be examined in studies with only annual average concentrations. In examination of the different exposure windows, the (possibly) moderate to high correlations among some of the exposure averages need to be considered when interpreting the results. Next to a high temporal resolution, detailed information on spatial contrasts in air pollution exposure is required, since ambient air pollutants display significant small-scale spatial variation. This intra-urban spatial variation has been documented especially for traffic-related pollutants such as NO_2, black smoke, elemental carbon, ultrafine particles, and to a lesser extent for PM_{10} and $PM_{2.5}$ [25, 26]. Our exposure estimates have been used in the studies presented in this thesis, which suggest that exposure to air pollution during pregnancy may affect maternal and fetal health (see Chapters 3.1 to 3.4).

We explored whether air pollution exposure levels were differentially distributed according to maternal and infant characteristics. Associations between air pollution exposure and health may be subject to confounding, if sociodemographic and lifestyle-related factors are associated both with air pollution exposure and with health. Our illustrative findings suggest that in our cohort, air pollution exposure may be differentially distributed according to age, ethnicity, parity, neighbourhood income area, smoking, and alcohol consumption. This stresses the importance to account for these factors when analyzing the associations between air pollution exposure and health.

Rotterdam is the second largest city in the Netherlands with a high population density and the largest port of Europe. It is characterized by high emissions from road traffic, shipping, households, and industry. A few recent European studies assessed air pollution exposure in pregnant women using land-use regression modelling approaches that also considered spatiotemporal variation in exposure [27-30]. In these studies, mean NO_2 exposure levels estimated for the entire pregnancy were slightly lower than those obtained in our cohort (i.e., around 36-37 $\mu g/m^3$ compared with 40 $\mu g/m^3$ in our cohort). None of the studies assessed PM_{10} exposure. The differences in exposure levels can be explained by various factors, including the geographic location and urbanization degree of the study area, study period (season and year), modelling approach, input data, climate, meteorological conditions, and pollution sources.

Traffic-related air pollution is a complex mixture of several pollutants. We assessed exposure to PM_{10} and NO_2 in our cohort, because these pollutants have been routinely measured in the National Air Quality Monitoring Network during the study period, and they often exceed the air quality standards at locations near heavy traffic. Furthermore, PM_{10} and NO_2 can be regarded as markers for the traffic-related air pollution mixture and have been associated with several adverse health effects [1, 2, 9, 31-33]. Other components that may be relevant for health ($PM_{2.5}$, black smoke) have not been monitored during the study period and could therefore not be assessed. Up to now, we have assessed air pollution exposure until the year 2008, and we are planning to update this data for future

years when the relevant monitoring data will be available (for PM_{10}, NO_2, and specific components). In addition, exposure to other 'criteria' air pollutants such as SO_2 and CO could be estimated in the future using the same modelling procedure.

Assigning exposures based on the home address at time of delivery may introduce exposure misclassification as a number of women change their address during pregnancy [34], and are thus exposed to different levels of air pollution. We obtained full residential history of the participants, which showed that 26% of the women moved at least once in the period between conception and the first year postnatally. Air pollution exposure estimates were assessed for the different prenatal and postnatal addresses. There can still be misclassification of air pollution exposure, since exposure levels were estimated at the home address, and people do not spend all of their time at home. Indoor, occupational, or commuting sources of air pollution have not been captured in our modelling procedures. The extent of the possible misclassification may be minor in this specific population, as pregnant women are likely to spend more time at home than non-pregnant individuals, especially in the last stage of pregnancy [35].

There is increasing awareness of the importance to incorporate information on noise exposure in studies on traffic-related air pollution exposure and health [10-13]. Thus far, few studies have included both air pollution and noise when investigating health outcomes [10, 36-38]. In our studies on air pollution and pregnancy outcomes, we included information on noise exposure, in order to adjust for its potential confounding effect (see Chapters 3.1 to 3.4).

Conclusion

Detailed air pollution exposure levels are available for mothers, fathers, and children in the Generation R Study and efforts are ongoing to update these exposures. The individual exposure estimates can be used in further epidemiological studies focused on the effects of prenatal and postnatal air pollution exposure on various health outcomes in mothers and children, including reproductive outcomes, growth and development, cognitive function, respiratory function, and cardiovascular outcomes. The combination with other detailed data (noise levels, biomarkers, and genetics) enables in-depth investigations and identification of critical windows of exposure.

REFERENCES

1. Pope CA, 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc.* 2006; 56(6):709-42.
2. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
3. Kampa M, Castanas E. Human health effects of air pollution. *Environ Pollut.* 2008; 151(2):362-7.
4. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation.* 2010; 121(25):2755-65.
5. Wang L, Pinkerton KE. Air pollutant effects on fetal and early postnatal development. *Birth Defects Res C Embryo Today.* 2007; 81(3):144-54.
6. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
7. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
8. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
9. Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. Meeting report: atmospheric pollution and human reproduction. *Environ Health Perspect.* 2008; 116(6):791-8.
10. de Kluizenaar Y, Gansevoort RT, Miedema HM, de Jong PE. Hypertension and road traffic noise exposure. *J Occup Environ Med.* 2007; 49(5):484-92.
11. Allen RW, Davies H, Cohen MA, Mallach G, Kaufman JD, Adar SD. The spatial relationship between traffic-generated air pollution and noise in 2 US cities. *Environ Res.* 2009; 109(3):334-42.
12. Davies HW, Vlaanderen JJ, Henderson SB, Brauer M. Correlation between co-exposures to noise and air pollution from traffic sources. *Occup Environ Med.* 2009; 66(5):347-50.
13. Foraster M, Deltell A, Basagana X, Medina-Ramon M, Aguilera I, Bouso L, et al. Local determinants of road traffic noise levels versus determinants of air pollution levels in a Mediterranean city. *Environ Res.* 2011; 111(1):177-83.
14. Jerrett M, Arain A, Kanaroglou P, Beckerman B, Potoglou D, Sahsuvaroglu T, et al. A review and evaluation of intraurban air pollution exposure models. *J Expo Anal Environ Epidemiol.* 2005; 15(2):185-204.
15. Bellander T, Berglind N, Gustavsson P, Jonson T, Nyberg F, Pershagen G, et al. Using geographic information systems to assess individual historical exposure to air pollution from traffic and house heating in Stockholm. *Environ Health Perspect.* 2001; 109(6):633-9.
16. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
17. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007; 22(12):917-23.
18. Netherlands Ministry of Infrastructure and the Environment: Air Quality Decree 2007 (Regeling beoordeling Luchtkwaliteit 2007). 2007. Available: http://wetten.overheid.nl/BWBR0022817
19. Velders G, Aben J, Diederen H, Drissen E, Geilenkirchen G, Jimmink B, et al.: Large-scale Air Quality Concentration Maps in the Netherlands. Report 2010 [Concentratiekaarten voor grootschalige luchtverontreiniging in Nederland. Rapportage 2010]. Netherlands Environmental Assessment Agency (PBL), Report 500088006. 2010. Available: http://www.rivm.nl/bibliotheek/rapporten/500088006.pdf
20. Beelen R, Voogt M, Duyzer J, Zandveld P, Hoek G. Comparison of the performances of land use regression modelling and dispersion modelling in estimating small-scale variations in long-term air pollution concentrations in a Dutch urban area. *Atmos Environ.* 2010; 44(36):4614-4621.
21. Keuken M, Zandveld P, van den Elshout S, Janssen NAH, Hoek G. Air quality and health impact of PM10 and EC in the city of Rotterdam, the Netherlands in 1985-2008. *Atmos Environ.* 2011; 45(30):5294-5301.
22. European Commission: Directive 2002/49/EC, relating to the assessment and management of environmental noise. 2002. Available: http://ec.europa.eu/environment/noise/directive.htm

23. European Commission: Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. 2008. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:152:0001:0044:EN:PDF
24. World Health Organization: Health aspects of air pollution. Results from the WHO project "Systematic review of health aspects of air pollution in Europe". 2004. Available: http://www.euro.who.int/_data/assets/pdf_file/0003/74730/E83080.pdf
25. Fischer PH, Hoek G, van Reeuwijk H, Briggs DJ, Lebret E, van Wijnen JH, et al. Traffic-related differences in outdoor and indoor concentrations of particles and volatile organic compounds in Amsterdam. *Atmos Environ.* 2000; 34(22):3713-3722.
26. Lewne M, Cyrys J, Meliefste K, Hoek G, Brauer M, Fischer P, et al. Spatial variation in nitrogen dioxide in three European areas. *Sci Total Environ.* 2004; 332(1-3):217-30.
27. Aguilera I, Sunyer J, Fernandez-Patier R, Hoek G, Aguirre-Alfaro A, Meliefste K, et al. Estimation of outdoor NO(x), NO(2), and BTEX exposure in a cohort of pregnant women using land use regression modeling. *Environ Sci Technol.* 2008; 42(3):815-21.
28. Slama R, Morgenstern V, Cyrys J, Zutavern A, Herbarth O, Wichmann HE, et al. Traffic-related atmospheric pollutants levels during pregnancy and offspring's term birth weight: a study relying on a land-use regression exposure model. *Environ Health Perspect.* 2007; 115(9):1283-1292.
29. Iniguez C, Ballester F, Estarlich M, Llop S, Fernandez-Patier R, Aguirre-Alfaro A, et al. Estimation of personal NO2 exposure in a cohort of pregnant women. *Sci Total Environ.* 2009; 407(23):6093-9.
30. Gehring U, Wijga AH, Fischer P, de Jongste JC, Kerkhof M, Koppelman GH, et al. Traffic-related air pollution, preterm birth and term birth weight in the PIAMA birth cohort study. *Environ Res.* 2011; 111(1):125-35.
31. Peters A. Particulate matter and heart disease: evidence from epidemiological studies. *Toxicol Appl Pharmacol.* 2005; 207(2 Suppl):477-82.
32. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
33. World Health Organization: Air quality guidelines. Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulphur dioxide. 2006. Available: http://whqlibdoc.who.int/hq/2006/WHO_SDE_PHE_OEH_06.02_eng.pdf
34. Fell DB, Dodds L, King WD. Residential mobility during pregnancy. *Paediatr Perinat Epidemiol.* 2004; 18(6):408-14.
35. Nethery E, Brauer M, Janssen P. Time-activity patterns of pregnant women and changes during the course of pregnancy. *J Expo Sci Environ Epidemiol.* 2009; 19(3):317-24.
36. Tobias A, Diaz J, Saez M, Alberdi JC. Use of poisson regression and box-jenkins models to evaluate the short-term effects of environmental noise levels on daily emergency admissions in Madrid, Spain. *Eur J Epidemiol.* 2001; 17(8):765-71.
37. Selander J, Nilsson ME, Bluhm G, Rosenlund M, Lindqvist M, Nise G, et al. Long-term exposure to road traffic noise and myocardial infarction. *Epidemiology.* 2009; 20(2):272-9.
38. Beelen R, Hoek G, Houthuijs D, van den Brandt PA, Goldbohm RA, Fischer P, et al. The joint association of air pollution and noise from road traffic with cardiovascular mortality in a cohort study. *Occup Environ Med.* 2009; 66(4):243-50.

SUPPLEMENTARY MATERIAL

Supplementary File S1. Road traffic noise exposure assessment

There is increasing awareness of the importance to incorporate information on noise exposure in studies on traffic-related air pollution exposure and health [1-4]. Thus far, few studies have included both air pollution and noise when investigating health outcomes [4-7]. The exact mechanisms through which air pollution and noise contribute to adverse health effects may differ, however some pathways may be related. Noise is hypothesized to induce stress responses, which may result in altered function of the sympathetic autonomic nervous system, endocrine systems, and immune system [8, 9], leading to alterations in cardiovascular, haematological, and immunological parameters [8]. Substantial evidence indicates an effect of traffic-related noise exposure on cardiovascular endpoints such as hypertension and ischemic heart disease [4, 10, 11].

We assessed road traffic noise exposure at all addresses in the study area (northern part of Rotterdam). The method has been described previously in more detail [4, 12]. Briefly, noise exposure was assessed using Standard Noise Mapping Method 2 (SKM2) [13], which is the sophisticated version of the Dutch standard method for noise modelling and producing noise maps, in accordance with requirements of the EU Environmental Noise Directive [14]. Noise exposure levels were expressed in the standard noise metric L_{den} (day, evening, night), a measure of annual average sound levels. Input data for the calculations were detailed digital maps with information on the geographic location of buildings and ground characteristics, and a digital map describing the geographic location of roads and the traffic characteristics for each road segment. The latter was provided by the local authorities of Rotterdam for the current situation at time of the study (base year 2004). This data can be reasonably applied to adjacent years, as the road network is assumed to be relatively constant, with only small (if any) but equal changes in noise exposure across the population. We assessed the road traffic noise level at the most exposed façade of the dwelling of every address in the study area. Very low levels of noise exposure (<45 dB(A)) were recoded as 45 dB(A) because this is considered a lower limit of ambient noise in an urban surrounding. To each participant, we assigned the noise exposure level calculated at the home address at time of the specific measurement (during pregnancy or at delivery).

In Supplementary Table S1, we present the distribution of the noise exposure levels based on the home address at time of conception and at time of delivery. Both at time of conception and delivery, approximately 10% of the women was exposed to noise values above 65 dB(A), which is considered as unacceptable by the European Commission [15]. Noise exposure levels at time of conception and at time of delivery were highly correlated (Pearson correlation coefficient r=0.90). This was expected, as the estimated noise levels reflect long-term exposures. Any observed differences in the distributions of the two variables are merely the result of residential changes during pregnancy, and hence a different number of participants with available address information. Correlations between

noise exposure levels and air pollution exposure averages were low to moderate (PM_{10}: r=0.15 to 0.28, NO_2: r=0.37 to 0.56, results not shown).

Noise exposure was calculated with a detailed model that takes into account the small-scale intra-urban contrasts in the study area. This approach reduces the misclassification of noise exposure that may occur in studies where exposure is based for example on calculations for a coarse grid or on subjective information such as questionnaire data. Furthermore, both air pollution and noise exposure were assessed using the same spatial input data, and for the same locations (the façades of participants' home addresses). This minimizes any potential bias that could arise from differences in spatial resolution. Nevertheless, we cannot exclude the possibility that the correlations between air pollution and noise exposure estimates were underestimated, since both exposures were derived from modelling procedures [3].

Supplementary Table S1. Distribution of maternal noise exposure levels (L_{den}) in the study cohort.

Noise exposure (dB(A))	**N**	**Minimum**	**25th percentile**	**Mean**	**Median**	**75th percentile**	**Maximum**
Based on home address at conception	7724	45.0	47.6	54.3	52.7	60.8	76.5
Based on home address at delivery	8608	45.0	47.5	54.2	52.6	60.6	76.5

REFERENCES (Supplementary Material)

1. Allen RW, Davies H, Cohen MA, Mallach G, Kaufman JD, Adar SD. The spatial relationship between traffic-generated air pollution and noise in 2 US cities. *Environ Res.* 2009; 109(3):334-42.
2. Davies HW, Vlaanderen JJ, Henderson SB, Brauer M. Correlation between co-exposures to noise and air pollution from traffic sources. *Occup Environ Med.* 2009; 66(5):347-50.
3. Foraster M, Deltell A, Basagana X, Medina-Ramon M, Aguilera I, Bouso L, et al. Local determinants of road traffic noise levels versus determinants of air pollution levels in a Mediterranean city. *Environ Res.* 2011; 111(1):177-83.
4. de Kluizenaar Y, Gansevoort RT, Miedema HM, de Jong PE. Hypertension and road traffic noise exposure. *J Occup Environ Med.* 2007; 49(5):484-92.
5. Tobias A, Diaz J, Saez M, Alberdi JC. Use of poisson regression and box-jenkins models to evaluate the short-term effects of environmental noise levels on daily emergency admissions in Madrid, Spain. *Eur J Epidemiol.* 2001; 17(8):765-71.
6. Selander J, Nilsson ME, Bluhm G, Rosenlund M, Lindqvist M, Nise G, et al. Long-term exposure to road traffic noise and myocardial infarction. *Epidemiology.* 2009; 20(2):272-9.
7. Beelen R, Hoek G, Houthuijs D, van den Brandt PA, Goldbohm RA, Fischer P, et al. The joint association of air pollution and noise from road traffic with cardiovascular mortality in a cohort study. *Occup Environ Med.* 2009; 66(4):243-50.
8. Babisch W. Traffic Noise and Cardiovascular Disease: Epidemiological Review and Synthesis. *Noise Health.* 2000; 2(8):9-32.
9. Passchier-Vermeer W, Passchier WF. Noise exposure and public health. *Environ Health Perspect.* 2000; 108 Suppl 1:123-31.
10. van Kempen EE, Kruize H, Boshuizen HC, Ameling CB, Staatsen BA, de Hollander AE. The association between noise exposure and blood pressure and ischemic heart disease: a meta-analysis. *Environ Health Perspect.* 2002; 110(3):307-17.
11. Babisch W, Kamp I. Exposure-response relationship of the association between aircraft noise and the risk of hypertension. *Noise Health.* 2009; 11(44):161-8.
12. de Kluizenaar Y, Salomons EM, Janssen SA, van Lenthe FJ, Vos H, Zhou H, et al. Urban road traffic noise and annoyance: the effect of a quiet facade. *J Acoust Soc Am.* 2011; 130(4):1936-42.
13. Netherlands Ministry of Infrastructure and the Environment: Prescribed measurement and calculation methods for noise annoyance 2006 (Meet- en rekenvoorschrift geluidshinder 2006). 2006. Available: http://www.infomil.nl/onderwerpen/hinder-gezondheid/geluid/wet-geluidhinder/rekenen-meten
14. European Commission: Directive 2002/49/EC, relating to the assessment and management of environmental noise. 2002. Available: http://ec.europa.eu/environment/noise/directive.htm
15. European Commission: The Green Paper on Future Noise Policy (COM(96) 540). 1996. Available: http://ec.europa.eu/environment/noise/pdf/com_96_540.pdf

Chapter 3

Air pollution exposure and pregnancy complications

Chapter 3.1

Air pollution and maternal and fetal C-reactive protein levels

Edith H van den Hooven
Yvonne de Kluizenaar
Frank H Pierik
Albert Hofman
Sjoerd W van Ratingen
Peter YJ Zandveld
Jan Lindemans
Henk Russcher
Eric AP Steegers
Henk ME Miedema
Vincent WV Jaddoe

Adapted from Environ Health Perspect 2012; doi: 10.1289/ehp.1104345

ABSTRACT

Background: Exposure to air pollution has been associated with higher C-reactive protein (CRP) levels, suggesting an inflammatory response. Not much is known about this association in pregnancy. We investigated the associations of air pollution exposure during pregnancy with maternal and fetal CRP levels in a population-based cohort study in the Netherlands.

Methods: PM_{10} and NO_2 levels were estimated at the home address using dispersion modelling for different averaging periods preceding the blood sampling (1 week, 2 weeks, 4 weeks, and total pregnancy). High-sensitivity CRP levels were measured in maternal blood samples in early pregnancy (n=5067) and in fetal cord blood samples at birth (n=4450).

Results: As compared to the lowest quartile, higher PM_{10} exposure levels for the prior one and two weeks were associated with elevated maternal CRP levels (>8 mg/L) in the first trimester (odds ratio (OR) 1.32, 95% confidence interval (CI) 1.08 to 1.61 for the fourth PM_{10} quartile for the prior week, and OR 1.28, 95% CI 1.06 to 1.56 for the third PM_{10} quartile for the prior two weeks), however, no clear dose-response relationships were observed. PM_{10} and NO_2 exposure levels for one, two, and four weeks preceding delivery were not consistently associated with fetal CRP levels at delivery. Higher long-term PM_{10} and NO_2 exposure levels (total pregnancy) were associated with elevated fetal CRP levels (>1 mg/L) at delivery (OR 2.18, 95% CI 1.08 to 4.38 and OR 3.42, 95% CI 1.36 to 8.58 for the fourth quartiles of PM_{10} and NO_2, respectively; P-values for trend <0.05).

Conclusions: Our results suggest that exposure to air pollution during pregnancy may lead to maternal and fetal inflammatory responses.

INTRODUCTION

C-reactive protein (CRP) is an acute phase reactant and a frequently used marker of low grade systemic inflammation, which levels increase in response to both infectious and non-infectious stimuli [1]. CRP levels have been suggested to increase during pregnancy, due to the maternal inflammatory response to the pregnancy [2, 3]. Among pregnant women, elevated CRP levels have been associated with adverse outcomes such as preterm delivery, preeclampsia, and fetal growth restriction [4-8]. Additionally, elevated CRP levels in umbilical cord blood have been reported in infants being born small for gestational age [9, 10].

CRP levels might increase in response to air pollution exposure. Previous studies have linked air pollution exposure to increased CRP levels in various populations, including healthy adults, diseased subjects, and elderly subjects, but results have been inconsistent [11-21]. Only one study investigated the associations of air pollution exposure with CRP levels in pregnant women [22]. Associations of maternal air pollution exposure with fetal CRP levels have not yet been examined. This is of interest, since induction of systemic inflammation has been proposed as one potential biological mechanism through which air pollution could result in adverse pregnancy outcomes [23, 24].

Therefore, we investigated the associations of maternal exposure to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) during pregnancy with maternal and fetal CRP levels in a population-based cohort study among 6508 mother-child pairs living in an urban area in the Netherlands.

METHODS

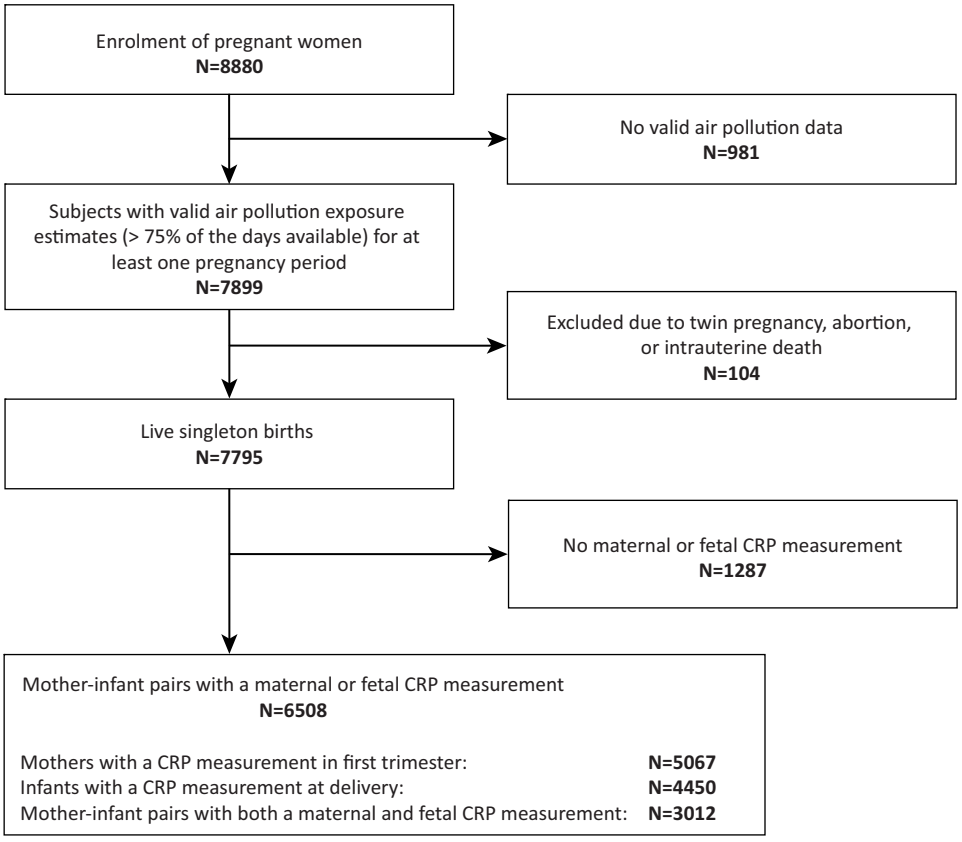
Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in the city of Rotterdam, the Netherlands, which has been described previously in detail [25]. Mothers enrolled between 2001 and 2005. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all mothers.

Of the 8880 prenatally enrolled women, air pollution exposure estimates were available for 7899 mothers (89%). For 981 mothers, air pollution exposure data could not be assessed due to incomplete address history, or because they had moved outside the study area before delivery [25]. Mothers with a twin pregnancy (n=85), abortion (n=7), or intrauterine death (n=12) were excluded. Of the mothers with live singleton births and their infants, a CRP measurement in maternal blood and/or cord blood was available for 6508 mother-infant pairs. Median gestational age at enrollment was 13.1 weeks (range 5.1 to 38.4). We excluded mothers and infants with extremely high CRP values (>100 mg/L, n=4 and >20mg/L, n=8, respectively), as these concentrations are likely to reflect acute

inflammatory processes due to specific infectious causes. Associations between air pollution exposure and CRP levels were analyzed in 5067 mothers with a maternal CRP measurement in the first trimester and in 4450 infants with a fetal CRP measurement at delivery (see Figure 1 for a flow chart).

Figure 1. Population for analysis.



Air pollution exposure

Individual exposures to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) during pregnancy were assessed at the home address, using a combination of dispersion modelling techniques and continuous monitoring data, taking into account both the spatial and temporal variation in air pollution. A detailed description and a flow chart of the exposure assessment are presented in Chapter 2. In brief, annual average concentrations of PM_{10} and NO_2 for the years 2001-2006 were assessed for all addresses in the study area, using the three Dutch national standard methods for air quality modelling [26]. Hourly concentrations of PM_{10} and NO_2 were derived, taking into account hourly wind conditions and fixed temporal patterns in the contribution of air pollution sources. Subsequently,

the hourly concentrations were adjusted for background concentrations, using hourly air pollution measurements from three continuous monitoring stations. We obtained full residential history of the participants, which showed that approximately 13% of the women moved at least once during pregnancy. Based on participants' home addresses, we derived average exposure estimates for different periods preceding the day of blood sampling (in first trimester or at delivery): one week (day 1-7), two weeks (day 1-14), and four weeks (day 1-28). The consideration of different averaging periods was decided a priori and based on the previous study on air pollution and CRP levels in pregnant women [22]. Additionally, we estimated average exposure for the total pregnancy period (conception until delivery).

High-sensitivity C-reactive protein levels

Maternal venous blood samples were collected in early pregnancy (median 13.2 weeks of gestation, range 4.5 to 17.9). Sampling of venous umbilical cord blood was carried out by midwives and obstetricians immediately after delivery (median 40.1 weeks of gestation, range 27.6 to 43.6). Blood samples were transported to the regional laboratory for processing and storage at -80^{o}C [27]. High-sensitivity CRP (hs-CRP) concentrations were measured in EDTA plasma samples at the Department of Clinical Chemistry of the Erasmus Medical Center in 2009. We measured high-sensitivity CRP since traditional clinically used CRP methods lack the sensitivity in low ranges needed for predicting future risk of events in apparently healthy individuals [28]. Hs-CRP levels were analyzed using an immunoturbidimetric assay on the Architect System (Abbot Diagnostics B.V., Hoofddorp, the Netherlands). The total precision (inter-assay variation) for hs-CRP was 0.9% at 12.9 mg/L and 1.3% at 39.9 mg/L. The lowest level of detection was 0.2 mg/L. Elevated maternal CRP concentrations were defined as >8 mg/L (~83th percentile), a cut-off point that has been associated with adverse pregnancy outcomes in previous studies [6, 29]. Elevated fetal CRP levels were defined as >1 mg/L (~97th percentile), a threshold that has been associated with neonatal infection [30].

Covariates

Medical records were used to obtain information on date of birth, gestational age at birth, fetal sex, and birth weight. Information on maternal age, educational level, ethnicity, parity, and first trimester infectious or inflammatory disease (doctor-consulted) was obtained by a questionnaire at enrolment. As there were no differences in observed results when ethnicity was categorized into five groups instead of two groups, we reclassified ethnicity as: European, or non-European. Maternal anthropometrics were assessed at time of enrolment. Maternal smoking and alcohol consumption before and during pregnancy were assessed by questionnaires in each trimester, and were categorized as: no, until pregnancy was known, or continued during pregnancy. Month of conception and month of birth were categorized into seasons: winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Road traffic noise exposure

was assessed at the home address (in first trimester and at delivery) in accordance with requirements of the EU Environmental Noise Directive, as described in the Supplementary Material of Chapter 2. To each participant, we assigned the noise exposure level calculated at the home address at time of the blood sampling (first trimester or delivery).

Statistical analysis

Air pollution exposures in each period were categorized into quartiles. The lowest quartile of PM_{10} and NO_2 exposure was used as the reference group. First, unadjusted and adjusted linear regression models were run to analyze the associations for an interquartile range increase in air pollution exposure in different periods preceding the first trimester measurement with maternal CRP levels. Maternal CRP concentrations were log-transformed (using the natural log) to obtain a normally distributed outcome variable. We present coefficients from the linear regression analyses for the log-transformed CRP concentrations, multiplied by 100, which can be interpreted in units of percentage differences [31]. Second, the associations of air pollution exposure quartiles for different periods preceding the first trimester measurement with elevated maternal CRP levels (>8 mg/L) were estimated using unadjusted and adjusted logistic regression models. Third, unadjusted and adjusted logistic regression models were run to estimate the associations of air pollution exposure quartiles for different periods preceding delivery with elevated fetal CRP levels (>1 mg/L). Logistic regression models in which air pollution exposure was included as a continuous variable (per 10 $\mu g/m^3$ increase) were considered as test for trend. All models were adjusted for known determinants of CRP levels (maternal age, body mass index, ethnicity, education, parity, smoking, alcohol consumption, and gestational age at measurement) and for road traffic noise exposure (based on home address in first trimester for models on maternal CRP levels or on home address at delivery for models on fetal CRP levels). Models with maternal CRP levels were additionally adjusted for season of conception, and models with fetal CRP levels were additionally adjusted for season of birth. The percentages of missing values within the population for analysis were lower than 1% for continuous data and lower than 15% for categorical data. We applied multiple imputation for missing data in covariates. All measures of association are presented with their 95% confidence intervals. All statistical analyses were performed using PASW version 17.0 for Windows (PASW Inc., Chicago, IL, USA).

RESULTS

Subject and exposure characteristics

The median age of the participants was 30.4 years (Table 1). The majority of the women was nulliparous, and 41.2% had completed high education. Median maternal CRP concentration was 4.4 (range 0.2 to 93.8) mg/L, and 1309 women had an elevated CRP concentration (>8 mg/L). Of the neonates, 3485 (53.5%) had a CRP concentration below the detection limit of 0.2 mg/L. 72 neonates had an elevated CRP concentration (>1 mg/L).

Mean maternal exposure levels for the prior week were 30.6 μg/m^3 for PM_{10} and 40.3 μg/m^3 for NO_2 in early pregnancy, and 29.6 μg/m^3 for PM_{10} and 39.5 μg/m^3 for NO_2 at delivery (Supplementary Table S1). Mean air pollution exposure levels for the total pregnancy period were 30.3 μg/m^3 (range 23.2 to 40.9) for PM_{10} and 39.9 μg/m^3 (range 26.5 to 56.9) for NO_2. On average, these levels are below the European Union annual limit values (40 μg/m^3 for both PM_{10} and NO_2) that are defined for protection of human health [32], but a substantial proportion (46%) of the women was exposed to NO_2 levels higher than this limit value. Correlations among exposure averages for the prior one, two, and four weeks were moderate to strong (PM_{10}: Pearson correlation coefficient r=0.58 to 0.83, NO_2: r=0.74 to 0.89). Correlations between exposure averages for the prior one, two, and four weeks with exposure averages for the total pregnancy period were lower (PM_{10}: r=0.27 to 0.48, NO_2: r=0.36 to 0.51). PM_{10} and NO_2 levels averaged for the same period were moderately correlated (r=0.35 to 0.54).

Table 1. Subject characteristics (N=6508).

	Mean ± SD, median (95% range), or number (percentage)
Maternal characteristics	
Age at enrolment (yr)	30.4 (15.4, 46.3)
Gestational age at enrolment (wks)	13.1 (5.1, 38.4)
Height (cm)	167.4 ± 7.5
Weight at enrolment (kg)	67.0 (37.0, 142.0)
Body mass index at enrolment (kg/m^2)	23.7 (15.2, 51.2)
Parity – n (%)	
Nulliparous	3592 (55.2)
Multiparous	2854 (43.9)
Missing	62 (1.0)
Ethnic background – n (%)	
European	3624 (55.7)
Non-European	2483 (38.2)
Missing	401 (6.2)
Highest completed educational level – n (%)	
No education/primary	626 (9.6)
Secondary	2701 (41.5)
Higher	2680 (41.2)
Missing	501 (7.7)
Smoking in pregnancy – n (%)	
No	4192 (64.4)
First trimester only	492 (7.6)
Continued	1002 (15.4)
Missing	822 (12.6)

Table 1. *Continued*

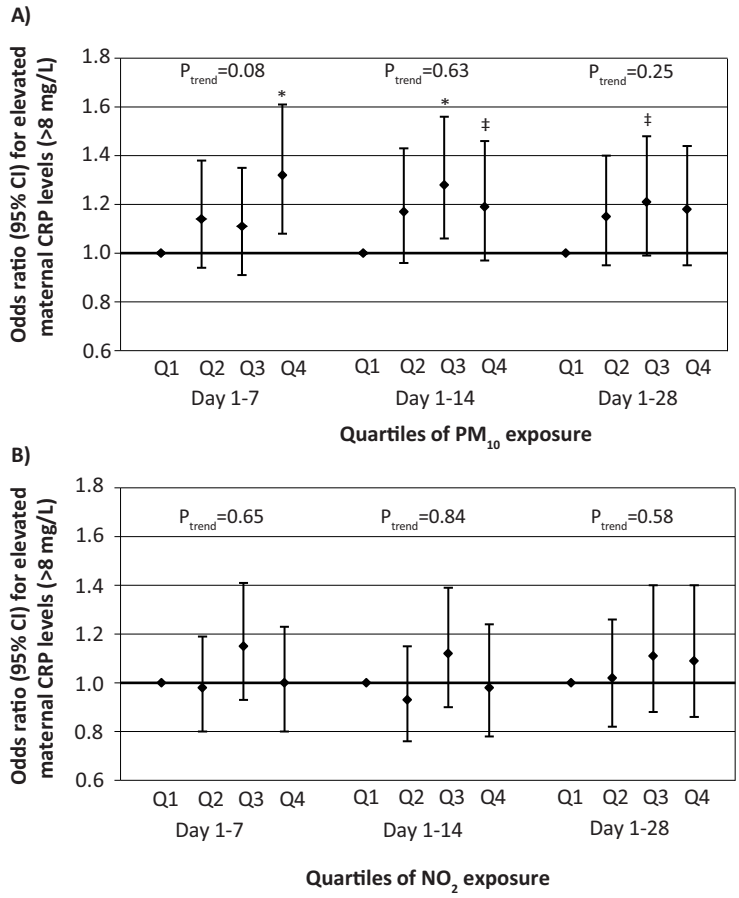
	Mean ± SD, median (95% range), or number (percentage)
Alcohol consumption in pregnancy – n (%)	
No	2695 (41.4)
First trimester only	779 (12.0)
Continued	2259 (34.7)
Missing	775 (11.9)
Season of conception – n (%)	
Winter	1781 (27.4)
Spring	1516 (23.3)
Summer	1521 (23.4)
Fall	1690 (26.0)
Noise exposure based on home address in first trimester (dB(A))	53.1 (45.0, 76.0)
Noise exposure based on home address at delivery (dB(A))	52.7 (45.0, 76.0)
Gestational age at blood sampling (wks)	13.2 (4.5, 17.9)
C-reactive protein concentration (mg/L)	4.4 (0.2, 93.8)
C-reactive protein concentration > 8.0 mg/L – n (%)	1309 (24.8)
Child characteristics	
Gestational age at birth (wks)	40.1 (27.6, 43.6)
Birth weight (g)	3460.7 ± 502.5
C-reactive protein concentration > 1.0 mg/L – n (%)	69 (1.5)

Values are means ± SD, or medians (range) for variables with a skewed distribution, and number of subjects (%) in case of categorical variables.

Air pollution and maternal CRP levels

We observed non-significant, negative percentage changes in maternal CRP levels per interquartile range increase in air pollution exposure preceding the first trimester measurement in the unadjusted models. Adjustment for covariates attenuated the effect estimates towards the null (Supplementary Table S2). Compared to the lowest quartile, the highest quartile of PM_{10} exposure for the prior week was associated with elevated maternal CRP levels (>8 mg/L) (odds ratio (OR) 1.32, 95% confidence interval (CI) 1.08 to 1.61) (Figure 2A). The third and fourth quartiles of PM_{10} exposure for the prior two weeks were also associated with elevated CRP (OR 1.28, 95% CI 1.06 to 1.56 and OR 1.19, 95% CI 0.97 to 1.46, respectively). However, ORs were comparable for all quartiles, and tests for trend were not significant. Associations of PM_{10} exposure levels for the prior four weeks with maternal CRP levels in early pregnancy did not reach statistical significance (Figure 2A). NO_2 exposure levels for the prior one, two, and four weeks were not associated with maternal CRP levels in early pregnancy (Figure 2B). When we performed analyses

Figure 2. Associations of maternal air pollution exposure with the risks of elevated maternal C-reactive protein levels in early pregnancy (N=5067).



* p<0.05; ‡ p<0.10

Values are odds ratios (95% CI) and reflect the risk for elevated maternal CRP levels (>8 mg/L) for each quartile of **A) PM_{10} exposure** and **B) NO_2 exposure** in different periods preceding the first trimester measurement compared with the reference group (lowest quartile). Cut-off values for categorization of PM_{10} exposure were <24.6, 24.6-28.8, 28.8-33.9, >33.9 µg/m^3 for the prior week, <25.4, 25.4-28.8, 28.8-33.7, >33.7 µg/m^3 for the prior two weeks, and <26.3, 26.3-29.4, 29.4-33.8, >33.8 µg/m^3 for the prior four weeks. Cut-off values for NO_2 exposure were <33.9, 33.9-39.9, 39.9-46.0, >46.0 µg/m^3 for the prior week, <35.2, 35.2-40.5, 40.5-45.3, >45.3 µg/m^3 for the prior two weeks, and <35.8, 35.8-40.8, 40.8-44.5, >44.5 µg/m^3 for the prior four weeks. Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous term (per 10 µg/m^3 increase) in the model. Number of subjects classified as having elevated CRP levels are indicated in Supplementary Table S3. Models are adjusted for gestational age at measurement, maternal age, body mass index, parity, ethnicity, education, smoking, alcohol consumption, noise exposure, and season of conception.

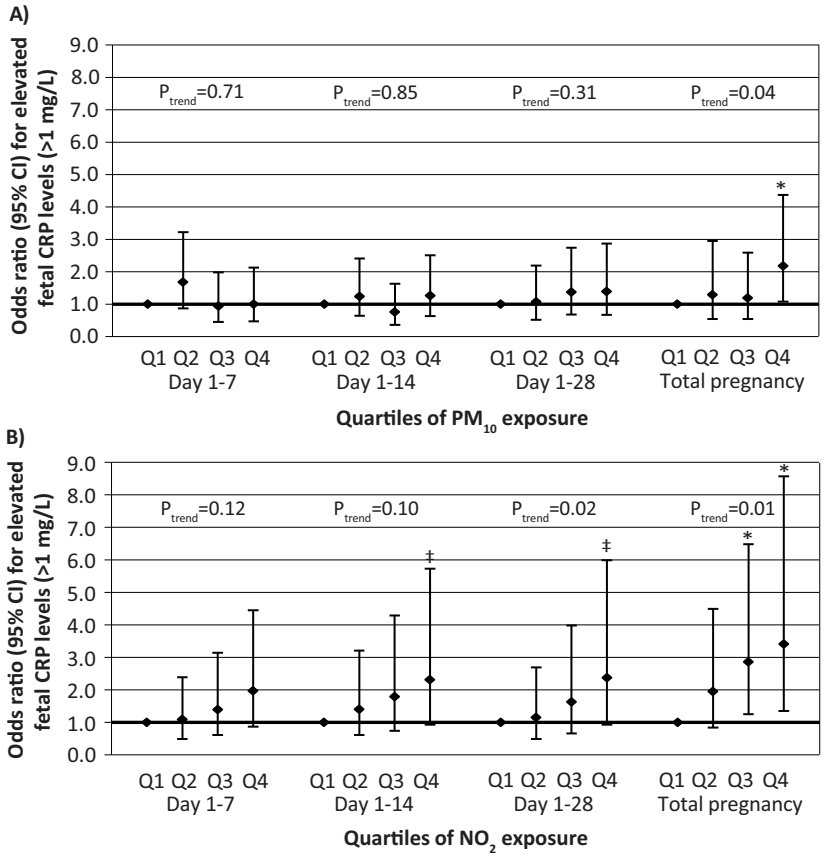
with different cut-off points for CRP (>10 and >5 mg/L, n=915 and n=2316 classified as elevated, respectively), results were comparable (i.e., the same patterns of associations

were observed) (results not shown). When we restricted the analyses to 2403 women with an early CRP measurement (before gestational week 13), we observed similar patterns of associations, although p-values were larger for the associations of PM_{10} exposure for the prior two and four weeks with CRP levels. When we excluded women with pre-existing conditions (diagnosed hypertension, diabetes, high cholesterol, chronic heart disorders, and systemic lupus erythematosus; N=179), the results did not change. Results from the sensitivity analyses in non-smoking women (N=4192) or in women without illnesses in the first trimester that could indicate a possible infection or inflammation (N=5792) were similar. Additional adjustment for maternal passive smoking or meteorological conditions on the day of the measurement (24h-average temperature, maximum temperature, relative humidity, and barometric pressure) did not influence the results either. Associations were comparable when the analyses were restricted to women with a normal body mass index (<25 kg/m^2; N=4103) (results not shown). The unadjusted models showed smaller effect estimates with larger p-values (Supplementary Table S3).

Air pollution and fetal CRP levels

No consistent associations with fetal CRP levels were observed for maternal PM_{10} exposure for one, two, and four weeks preceding delivery in the adjusted models (Figure 3A). Compared to the lowest quartile, the fourth quartile of PM_{10} exposure during total pregnancy was associated with elevated fetal CRP levels (>1 mg/L) at delivery (OR 2.18, 95% CI 1.08 to 4.38), and a positive trend (*P*=0.04) was observed as well. Positive, but non-significant associations were observed for NO_2 exposure for the prior one and two weeks with fetal CRP levels at delivery (Figure 3B), with a monotonic increase in ORs. A positive trend was observed for NO_2 exposure for the prior four weeks and elevated fetal CRP levels (*P*=0.02). Elevated fetal CRP levels were associated with the third and fourth quartiles of NO_2 exposure during total pregnancy (OR 2.85, 95% CI 1.25 to 6.47, and OR 3.42, 95% CI 1.36 to 8.58, respectively), with a monotonic increase in ORs (*P*=0.01). When we performed analyses with different cut-off points for fetal CRP (>0.8 and >0.4 mg/L, n=85 and n=127 classified as elevated, respectively), results were comparable (results not shown). The same patterns of associations were observed in the sensitivity analyses in non-smoking women and in mothers without illnesses in first trimester. Additional adjustment for mode of delivery, maternal passive smoking, or meteorological conditions on the day of the measurement did not change the results. When we restricted the analyses to women with a normal body mass index, we observed slightly larger effect estimates for the associations between air pollution and fetal CRP levels (e.g., OR 3.46, 95% CI 1.18 to 10.10 and OR 3.69, 95% CI 1.04 to 12.98 for the highest quartiles of total pregnancy PM_{10} and NO_2 exposure, respectively). Unadjusted associations for air pollution exposure with elevated fetal CRP were largely similar, although smaller effect estimates with larger p-values were observed for total pregnancy exposure (Supplementary Table S4). We did not observe consistent associations between the maternal and fetal CRP response to air pollution (results not shown).

Figure 3. Associations of maternal air pollution exposure with the risks of elevated fetal C-reactive protein levels at delivery (N=4450).



* $p < 0.05$; ‡ $p < 0.10$

Values are odds ratios (95% CI) and reflect the risk for elevated fetal CRP levels (>1 mg/L) for each quartile of **A) PM_{10} exposure** and **B) NO_2 exposure** in different periods preceding delivery compared with the reference group (lowest quartile). Cut-off values for categorization of PM_{10} exposure were <23.9, 23.9-27.7, 27.7-32.8, >32.8 μg/m³ for the prior week, <24.7, 24.7-28.0, 28.0-32.1, >32.1 μg/m³ for the prior two weeks, <25.6, 25.6-28.5, 28.5-32.8, >32.8 μg/m³ for the prior four weeks, and <27.8, 27,8-30.0, 30.0-32.9, >32.9 μg/m³ for total pregnancy. Cut-off values for NO_2 exposure were <33.2, 33.2-39.3, 39.3-45.6, >45.6 μg/m³ for the prior week, <34.1, 34.1-39.8, 39.8-44.7, >44.7 μg/m³ for the prior two weeks, <34.7, 34.7-40.2, 40.2-44.1, >44.1 μg/m³ for the prior four weeks, and <37.2, 37.2-39.6, 39.6-42.3, >42.3 μg/m³ for total pregnancy. Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous term (per 10 μg/m³ increase) in the model. Number of subjects classified as having elevated CRP levels are indicated in Supplementary Table S4. Models are adjusted for gestational age at birth, season of birth, maternal age, body mass index, parity, ethnicity, education, smoking, alcohol consumption, and noise exposure.

DISCUSSION

In this large population-based prospective cohort study from early pregnancy onwards, we observed weak associations for short-term average PM_{10} exposure levels (prior one and two weeks) with elevated maternal CRP levels in first trimester of pregnancy. Higher long-term average PM_{10} and NO_2 exposure levels (total pregnancy) were associated with elevated fetal CRP levels at delivery. This study extends previous epidemiological research on air pollution and markers of systemic inflammation in various populations, and suggests that maternal air pollution exposure may promote inflammatory processes in the mother and fetus.

Air pollution and C-reactive protein levels during pregnancy

In normal pregnancy, maternal CRP levels slightly increase as a result of the inflammatory response to the pregnancy [2, 3]. This systemic inflammatory response, which is part of the innate immune system, generally peaks during the third trimester [33]. Among pregnant women, a further elevation of CRP levels has been reported in pregnancies complicated by fetal growth restriction, preeclampsia, and preterm delivery [4, 6-8]. In addition, increased CRP levels in cord blood have been observed in neonates that were born preterm, small for gestational age, or with a low birth weight [9, 10]. Two recent studies in our population showed that elevated maternal CRP levels in first trimester were associated with reductions in third trimester estimated fetal weight and birth weight, and with small size for gestational age at birth [5]. In addition, maternal CRP levels were positively associated with diastolic blood pressure, and elevated CRP levels were associated with gestational hypertension and preeclampsia, but these associations attenuated towards the null after adjustment for maternal body mass index [34]. These findings indicate a possible link between an enhanced systemic inflammatory response and adverse pregnancy outcomes.

Potential biological pathways through which air pollution, especially particulate matter, may influence pregnancy are induction of oxidative stress and translocation of particles directly in the blood, both resulting in systemic inflammation [35]. It has been hypothesized that an enhanced systemic inflammatory response may lead to placental inflammation and alterations in maternal immunity [23], or suboptimal placentation [36], which could predispose to the development of adverse pregnancy outcomes. A number of routinely measured air pollutants (e.g., PM_{10}, $PM_{2.5}$, NO_2, CO, O_3, SO_2) have been linked to adverse pregnancy outcomes such as preterm birth, low birth weight, and intrauterine growth restriction [37-39], although results differ among studies. In our previous work in the same population, we have shown that maternal exposure to PM_{10} and NO_2 during pregnancy was associated with measures of fetal growth retardation and a reduced birth weight [40]. Also, elevated PM_{10} exposure levels were associated with increased risks of preterm birth, small size for gestational age at birth [40] and gestational hypertension [41].

In the present study, no statistically significant percentage changes in maternal CRP levels in early pregnancy were observed for an interquartile range increase in PM_{10} or

NO_2 exposure. In contrast, weak associations were observed for short-term average PM_{10} exposure with elevated maternal CRP levels (>8 mg/L). NO_2 exposure was not associated with elevated maternal CRP levels. Possibly, air pollution-induced increases in maternal CRP levels, if any, might be difficult to detect, since CRP levels already increase in response to the pregnancy.

Thus far, only one previous study has examined associations of maternal air pollution exposure with CRP levels during pregnancy. This study was conducted in 1696 women in the United States, and observed a tendency towards higher risks for elevated CRP levels (>8 mg/L) for an interquartile range increase in PM_{10} and $PM_{2.5}$ exposure for the prior 22- and 29-days (odds ratios ranging from 1.18 to 1.32) [22]. Effect estimates were generally larger in non-smokers only. Positive, but non-significant associations were observed for exposure to ozone (O_3), whereas no associations were observed for exposure to NO_2, carbon monoxide (CO), and sulfur dioxide (SO_2). However, the consideration of the spatial variability of air pollutants was limited in this study, since exposure estimates were based on monitoring stations only. Several other studies estimated the impact of air pollution exposure on CRP levels in non-pregnant adults. Positive associations with CRP levels were observed for exposure to PM_{10} [12, 18], NO_2 [42], or markers of primary combustion including $PM_{2.5}$ [16, 42], but other studies reported only weak associations [14, 19] or were not able to detect associations with particles or NO_2 [11, 15, 17, 20, 21].

Considering fetal CRP levels, in the present study elevated fetal CRP levels at delivery were observed in association with higher exposure to PM_{10} and NO_2 during total pregnancy. No consistent associations were observed for air pollution exposure in shorter exposure periods (one week, two weeks, or four weeks), although odds ratios increased monotonically with higher NO_2 levels. To our knowledge, this study is the first to examine the associations of maternal air pollution exposure with fetal CRP levels. Since CRP does not cross the placenta [43], elevated CRP levels are considered to reflect hepatic synthesis by the fetus [44]. The underlying mechanism through which maternal air pollution exposure may lead to an enhanced inflammatory response in the fetus is unclear. It is possible that it might involve systemic and placental inflammation at the maternal side. Alternatively, air pollution might provoke an inflammatory response directly in the fetus, due to either short- or long-term exposure. We did not observe consistent associations between the maternal and fetal CRP response to air pollution (i.e., whether the air pollution effect in the mother was related to the air pollution effect in the fetus). This may be related to the different timing of the measurements (early pregnancy versus delivery). We could not examine the possibility that acute maternal infections contributed to elevated fetal CRP levels, as information on third trimester maternal infections was not available. Future studies are needed to confirm our findings and to explore the underlying mechanisms.

CRP increases rapidly following an inflammatory trigger. Most previous studies on air pollution and CRP levels estimated associations with relatively acute exposures (same day or multiday averages). Information on the impact of longer averages of air pollution is limited. Possibly, exposure to high air pollution concentrations during a few weeks or

months may cause chronically elevated CRP levels in mother and fetus. Effect estimates for the associations between air pollution and elevated fetal CRP levels were slightly stronger in the subgroup of women with a normal BMI. It is known that body mass index is an important determinant of CRP levels in pregnant women, and previous studies have reported increased levels of inflammatory markers (including CRP) in overweight and obese women [45, 46]. The increased inflammatory response in overweight and obese women possibly masks the effects of air pollution on maternal and fetal CRP levels.

This study was performed in an urban area that is characterized by high emissions from road traffic, shipping, households, and industry. No information was available on pollutants other than PM_{10} and $NO_{2.}$ Mean exposure levels in previous studies that examined the associations between air pollution and CRP levels varied substantially. Compared to our study, reported PM_{10} levels were lower in studies in the United Kingdom and the United States [15, 18, 22], similar in another study in Rotterdam, the Netherlands [17], and higher in studies in Taiwan and Israel [12, 20]. Reported NO_2 exposure levels were (slightly) lower in previous studies in Taiwan, Israel, the United Kingdom, and the United States [12, 14, 15, 20, 22], similar in the other Dutch study [17], and higher in a study in Los Angeles, United States [42]. However, these comparisons should be considered with caution because of the different averaging periods. Furthermore, adverse health effects associated with PM_{10} and NO_2 exposure are not necessarily caused by these pollutants, but may be caused by other compounds present in the complex air pollution mixture, which may differ between geographic locations.

Methodological considerations

An important strength of this study is the population-based cohort, which included a large number of participants studied from early pregnancy onwards. Furthermore, we collected detailed information on many potential confounding factors, such as maternal educational level, ethnicity, body mass index, smoking, alcohol consumption, and noise exposure. However, residual confounding due to unmeasured variables might still be an issue.

Many previous studies have not addressed both intra-urban and temporal contrasts in air pollutants. A few earlier studies on CRP levels in non-pregnant adults did consider spatiotemporal variation, either by controlling exposure in an exposure chamber or by measuring personal, indoor-home, or outdoor-home concentrations [11, 13, 14, 16, 18, 21]. However, these studies were based on relatively small sample sizes ($n<150$), and were often conducted in elderly or diseased subjects [13, 14, 18]. In our study, we were able to consider detailed spatial and temporal variation in exposure by using a combination of dispersion modelling and continuous monitoring. Moreover, we were able to account for residential mobility of the women during pregnancy.

We should still acknowledge the possibility of misclassification of air pollution exposure, as exposures were only estimated at the home address, and study participants did not spend all of their time at home. No information was available on other locations

or other types of exposure (e.g., occupational, commuting, or indoor sources). This limitation should be taken into account when interpreting the results. Ideally, information on time-activity patterns should be considered when examining the associations for air pollution with health outcomes [38, 47], but unfortunately this information was not available. Whether and in which direction this possible misclassification has affected our effect estimates is not clear. Nevertheless, since pregnant women are likely to spend more time at home than non-pregnant individuals, especially in the last stage of pregnancy [47], the extent of the possible misclassification may be less than in non-pregnant adults.

The present study was based on single blood measurements, whereas CRP levels are known to have substantial within-individual variability. Future studies that repeatedly assess CRP levels during pregnancy in relation to air pollution exposure are recommended.

Conclusion

In a population-based prospective cohort study in the Netherlands, we showed that short-term maternal PM_{10} exposure was modestly associated with elevated maternal CRP levels in early pregnancy, and that long-term maternal PM_{10} and NO_2 exposure during pregnancy was associated with elevated fetal CRP levels at delivery. Our results suggest that air pollution exposure may lead to maternal and fetal inflammatory responses. More research is needed to confirm these findings, to examine the underlying mechanisms, and to explore the consequences.

REFERENCES

1. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* 1999; 340(6):448-54.
2. Thornton CA. Immunology of pregnancy. *Proc Nutr Soc.* 2010; 69(3):357-65.
3. von Versen-Hoeynck FM, Hubel CA, Gallaher MJ, Gammill HS, Powers RW. Plasma levels of inflammatory markers neopterin, sialic acid, and C-reactive protein in pregnancy and preeclampsia. *Am J Hypertens.* 2009; 22(6):687-92.
4. Tjoa ML, van Vugt JM, Go AT, Blankenstein MA, Oudejans CB, van Wijk IJ. Elevated C-reactive protein levels during first trimester of pregnancy are indicative of preeclampsia and intrauterine growth restriction. *J Reprod Immunol.* 2003; 59(1):29-37.
5. Ernst GD, de Jonge LL, Hofman A, Lindemans J, Russcher H, Steegers EA, et al. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. *Am J Obstet Gynecol.* 2011; 205:132.e1-8.
6. Pitiphat W, Gillman MW, Joshipura KJ, Williams PL, Douglass CW, Rich-Edwards JW. Plasma C-reactive protein in early pregnancy and preterm delivery. *Am J Epidemiol.* 2005; 162(11):1108-13.
7. Guven MA, Coskun A, Ertas IE, Aral M, Zencirci B, Oksuz H. Association of maternal serum CRP, IL-6, TNF-alpha, homocysteine, folic acid and vitamin B12 levels with the severity of preeclampsia and fetal birth weight. *Hypertens Pregnancy.* 2009; 28(2):190-200.
8. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum C-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. *Clin Biochem.* 2007; 40(5-6):330-5.
9. Amarilyo G, Oren A, Mimouni FB, Ochshorn Y, Deutsch V, Mandel D. Increased cord serum inflammatory markers in small-for-gestational-age neonates. *J Perinatol.* 2011; 31(1):30-2.
10. Trevisanuto D, Doglioni N, Altinier S, Zaninotto M, Plebani M, Zanardo V. High-sensitivity C-reactive protein in umbilical cord of small-for-gestational-age neonates. *Neonatology.* 2007; 91(3):186-9.
11. Brauner EV, Moller P, Barregard L, Dragsted LO, Glasius M, Wahlin P, et al. Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. *Part Fibre Toxicol.* 2008; 5:13.
12. Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *Am J Respir Crit Care Med.* 2007; 176(4):370-6.
13. Delfino RJ, Staimer N, Tjoa T, Polidori A, Arhami M, Gillen DL, et al. Circulating biomarkers of inflammation, antioxidant activity, and platelet activation are associated with primary combustion aerosols in subjects with coronary artery disease. *Environ Health Perspect.* 2008; 116(7):898-906.
14. Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. *Environ Health Perspect.* 2006; 114(7):992-8.
15. Forbes LJ, Patel MD, Rudnicka AR, Cook DG, Bush T, Stedman JR, et al. Chronic exposure to outdoor air pollution and markers of systemic inflammation. *Epidemiology.* 2009; 20(2):245-53.
16. Riediker M, Cascio WE, Griggs TR, Herbst MC, Bromberg PA, Neas L, et al. Particulate matter exposure in cars is associated with cardiovascular effects in healthy young men. *Am J Respir Crit Care Med.* 2004; 169(8):934-40.
17. Rudez G, Janssen NA, Kilinc E, Leebeek FW, Gerlofs-Nijland ME, Spronk HM, et al. Effects of ambient air pollution on hemostasis and inflammation. *Environ Health Perspect.* 2009; 117(6):995-1001.
18. Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, Cherrie J, et al. Particulate air pollution and the blood. *Thorax.* 1999; 54(11):1027-32.
19. Diez Roux AV, Auchincloss AH, Astor B, Barr RG, Cushman M, Dvonch T, et al. Recent exposure to particulate matter and C-reactive protein concentration in the multi-ethnic study of atherosclerosis. *Am J Epidemiol.* 2006; 164(5):437-48.
20. Steinvil A, Kordova-Biezuner L, Shapira I, Berliner S, Rogowski O. Short-term exposure to air pollution and inflammation-sensitive biomarkers. *Environ Res.* 2008; 106(1):51-61.

21. Zuurbier M, Hoek G, Oldenwening M, Meliefste K, Krop E, van den Hazel P, et al. In-Traffic Air Pollution Exposure and CC16, Blood Coagulation, and Inflammation Markers in Healthy Adults. *Environ Health Perspect.* 2011; 119(10):1384-9.
22. Lee PC, Talbott EO, Roberts JM, Catov JM, Sharma RK, Ritz B. Particulate air pollution exposure and C-reactive protein during early pregnancy. *Epidemiology.* 2011; 22(4):524-31.
23. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
24. Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. Meeting report: atmospheric pollution and human reproduction. *Environ Health Perspect.* 2008; 116(6):791-8.
25. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
26. Netherlands Ministry of Infrastructure and the Environment: Air Quality Decree 2007 (Regeling beoordeling Luchtkwaliteit 2007). 2007. Available: http://wetten.overheid.nl/BWBR0022817
27. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007; 22(12):917-23.
28. Roberts WL, Moulton L, Law TC, Farrow G, Cooper-Anderson M, Savory J, et al. Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Part 2. *Clin Chem.* 2001; 47(3):418-25.
29. Catov JM, Bodnar LM, Ness RB, Barron SJ, Roberts JM. Inflammation and dyslipidemia related to risk of spontaneous preterm birth. *Am J Epidemiol.* 2007; 166(11):1312-9.
30. Kordek A, Halasa M, Podraza W. Early detection of an early onset infection in the neonate based on measurements of procalcitonin and C-reactive protein concentrations in cord blood. *Clin Chem Lab Med.* 2008; 46(8):1143-8.
31. Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. *Stat Med.* 2000; 19(22):3109-25.
32. World Health Organization: Air quality guidelines. Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulphur dioxide. 2006. Available: http://whqlibdoc.who.int/hq/2006/WHO_SDE_PHE_OEH_06.02_eng.pdf
33. Redman CW, Sargent IL. Preeclampsia and the systemic inflammatory response. *Semin Nephrol.* 2004; 24(6):565-70.
34. de Jonge LL, Steegers EA, Ernst GD, Lindemans J, Russcher H, Hofman A, et al. C-reactive protein levels, blood pressure and the risks of gestational hypertensive complications: The Generation R Study. *J Hypertens.* 2011; 29(12):2413-21.
35. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
36. Dejmek J, Selevan SG, Benes I, Solansky I, Sram RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ Health Perspect.* 1999; 107(6):475-480.
37. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
38. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
39. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
40. van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environ Health Perspect.* 2012; 120(1):150-156.
41. van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PY, et al. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy: the Generation R Study. *Hypertension.* 2011; 57(3):406-12.

42. Delfino RJ, Staimer N, Tjoa T, Gillen DL, Polidori A, Arhami M, et al. Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. *Environ Health Perspect.* 2009; 117(8):1232-8.
43. Jaye DL, Waites KB. Clinical applications of C-reactive protein in pediatrics. *Pediatr Infect Dis J.* 1997; 16(8):735-46; quiz 746-7.
44. Raio L, Ghezzi F, Mueller MD, McDougall J, Malek A. Evidence of fetal C-reactive protein urinary excretion in early gestation. *Obstet Gynecol.* 2003; 101(5 Pt 2):1062-3.
45. Madan JC, Davis JM, Craig WY, Collins M, Allan W, Quinn R, et al. Maternal obesity and markers of inflammation in pregnancy. *Cytokine.* 2009; 47(1):61-4.
46. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA.* 1999; 282(22):2131-5.
47. Nethery E, Brauer M, Janssen P. Time-activity patterns of pregnant women and changes during the course of pregnancy. *J Expo Sci Environ Epidemiol.* 2009; 19(3):317-24.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Distribution of PM_{10} and NO_2 exposure levels for different periods.

	Minimum	25th percentile	Mean	Median	75th percentile	Maximum
Early pregnancy						
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-7	16.3	24.6	30.6	28.8	33.9	66.2
Prior day 1-14	18.8	25.4	30.6	28.8	33.7	58.0
Prior day 1-28	20.2	26.3	30.6	29.4	33.8	49.5
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-7	16.6	33.9	40.3	39.9	46.0	73.5
Prior day 1-14	16.9	35.2	40.4	40.5	45.3	67.4
Prior day 1-28	19.8	35.8	40.4	40.8	44.5	65.5
Delivery						
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-7	15.2	23.9	29.6	27.7	32.8	62.5
Prior day 1-14	16.9	24.7	29.5	28.0	32.1	53.6
Prior day 1-28	20.1	25.6	29.6	28.5	32.8	44.5
Total pregnancy	23.2	27.8	30.3	30.0	32.9	40.9
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-7	13.0	33.2	39.5	39.3	45.6	69.1
Prior day 1-14	15.3	34.1	39.5	39.8	44.7	67.2
Prior day 1-28	17.6	34.7	39.5	40.2	44.1	62.8
Total pregnancy	26.5	37.2	39.9	39.6	42.3	56.9

Air pollution exposure was estimated for different periods preceding blood sampling: one week (day 1-7), two weeks (day 1-14), and four weeks (day 1-28). Additionally, exposure was estimated for the total pregnancy period (conception until delivery).

Supplementary Table S2. Unadjusted and adjusted percentage changes in maternal C-reactive protein levels in early pregnancy for an interquartile range increase in maternal air pollution exposure (N=5067).

	IQR ($\mu g/m^3$)	**N** [a]	**Maternal CRP levels** Unadjusted percentage change [b] (95% range)	**Maternal CRP levels** Adjusted percentage change [c] (95% range)
PM_{10}				
Day 1-7	9.23	5057	0.0 (-2.7, 2.6)	0.9 (-1.6, 3.4)
Day 1-14	8.32	5057	-2.1 (-5.0, 0.6)	-1.1 (-3.8, 1.6)
Day 1-28	7.45	5037	-1.8 (-5.1, 1.5)	0.3 (-2.9, 3.6)
NO_2				
Day 1-7	12.11	5065	-0.9 (-4.4, 2.5)	0.4 (-3.1, 3.9)
Day 1-14	10.07	5057	-1.3 (-4.6, 2.1)	-0,4 (-4.1, 3.3)
Day 1-28	8.69	5047	-1.2 (-4.5, 2.2)	-0.4 (-4.4, 3.6)

Values are log-transformed regression coefficients and reflect the percent change (95% range) in maternal CRP levels in early pregnancy per interquartile range increase in air pollution exposure in different periods preceding the first trimester measurement.

[a] Differences in the number of subjects are due to missing air pollution data for the specific periods.

[b] Models are adjusted for gestational age at measurement.

[c] Models are adjusted for gestational age at measurement, maternal age, body mass index, parity, ethnicity, education, smoking, alcohol consumption, noise exposure, and season of conception.

Supplementary Table S3. Unadjusted associations of maternal air pollution exposure with the risk of elevated maternal C-reactive protein levels in early pregnancy (N=5067).

	Risk of elevated maternal CRP levels (>8 mg/L) per PM_{10} quartile Odds ratio (95% CI) *(n of cases)*	**Risk of elevated maternal CRP levels (>8 mg/L) per NO_2 quartile** Odds ratio (95% CI) *(n of cases)*
Day 1-7	N=5057	N=5065
1st quartile	*Reference (n=290)*	*Reference (n=323)*
2nd quartile	1.14 (0.95, 1.37) *(n=320)*	0.92 (0.77, 1.11) *(n=303)*
3rd quartile	1.06 (0.88, 1.28) *(n=304)*	1.01 (0.84, 1.21) *(n=324)*
4th quartile	1.25 (1.04, 1.50) * *(n=344)*	0.94 (0.78, 1.13) *(n=307)*
Trend test [a]	*1.04 (0.97, 1.12)*	*0.98 (0.91, 1.05)*
P for trend	*0.23*	*0.54*
Day 1-14	N=5057	N=5057
1st quartile	*Reference (n=294)*	*Reference (n=323)*
2nd quartile	1.12 (0.93, 1.34) *(n=318)*	0.89 (0.74, 1.07) *(n=296)*
3rd quartile	1.18 (0.98, 1.41) ‡ *(n=331)*	1.04 (0.87, 1.25) *(n=334)*
4th quartile	1.08 (0.90, 1.30) *(n=311)*	0.91 (0.76, 1.09) *(n=299)*
Trend test [a]	*1.10 (1.07, 1.14)*	*0.96 (0.89, 1.05)*
P for trend	*0.87*	*0.39*
Day 1-28	N=5037	N = 5047
1st quartile	*Reference (n=295)*	*Reference (n=315)*
2nd quartile	1.11 (0.93, 1.34) *(n=321)*	0.96 (0.80, 1.15) *(n=306)*
3rd quartile	1.12 (0.94, 1.35) *(n=321)*	(0.84, 1.21) *(n=319)*
4th quartile	1.05 (0.88, 1.27) *(n=309)*	0.97 (0.81, 1.17) *(n=308)*
Trend test [a]	*1.01 (0.90, 1.12)*	*0.97 (0.88, 1.07)*
P for trend	*0.93*	*0.54*

* p<0.05; ‡ p<0.10

Values are odds ratios (95% CI) and reflect the risk for elevated maternal C-reactive protein levels (>8 mg/L) for each quartile of air pollution exposure in different periods preceding the first trimester measurement compared with the reference group (lowest quartile). Cut-off values for categorization of PM_{10} exposure were <24.6, 24.6-28.8, 28.8-33.9, >33.9 μg/m^3 for the prior week, <25.4, 25.4-28.8, 28.8-33.7, >33.7 μg/m^3 for the prior two weeks, and <26.3, 26.3-29.4, 29.4-33.8, >33.8 μg/m^3 for the prior four weeks. Cut-off values for NO_2 exposure were <33.9, 33.9-39.9, 39.9-46.0, >46.0 μg/m^3 for the prior week, <35.2, 35.2-40.5, 40.5-45.3, >45.3 μg/m^3 for the prior two weeks, and <35.8, 35.8-40.8, 40.8-44.5, >44.5 μg/m^3 for the prior four weeks. Differences in the number of subjects are due to missing air pollution data for the specific periods. Models are adjusted for gestational age at measurement. [a] Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous term (per 10 μg/m^3 increase) in the model.

Supplementary Table S4. Unadjusted associations of maternal air pollution exposure with the risk of elevated fetal C-reactive protein levels at delivery (N=4450).

	Risk of elevated fetal CRP levels (>1 mg/L) per PM_{10} quartile Odds ratio (95% CI) *(n of cases)*	**Risk of elevated fetal CRP levels (>1 mg/L) per NO_2 quartile** Odds ratio (95% CI) *(n of cases)*
Day 1-7	N=4422	N=4420
1st quartile	*Reference* *(n=15)*	*Reference* *(n =13)*
2nd quartile	1.62 (0.85, 3.10) *(n=25)*	1.20 (0.57, 2.54) *(n=15)*
3rd quartile	0.90 (0.43, 1.87) *(n=14)*	1.45 (0.70, 3.01) *(n=17)*
4th quartile	0.96 (0.46, 2.00) *(n=14)*	1.87 (0.94, 3.72) ǂ *(n=23)*
Trend test [a]	*0.92 (0.68, 1.24)*	*1.24 (0.95, 1.62)*
P for trend	*0.58*	*0.11*
Day 1-14	N=4410	N=4421
1st quartile	*Reference* *(n=17)*	*Reference* *(n=12)*
2nd quartile	1.19 (0.62, 2.29) *(n=20)*	1.41 (0.66, 2.99) *(n=16)*
3rd quartile	0.73 (0.35, 1.54) *(n=12)*	1.71 (0.82, 3.53) *(n=19)*
4th quartile	1.14 (0.59, 2.20) *(n=19)*	1.88 (0.92, 3.84) ǂ *(n=21)*
Trend test [a]	*0.98 (0.70, 1.39)*	*1.26 (0.93, 1.70)*
P for trend	*0.91*	*0.13*
Day 1-28	N=4398	N=4413
1st quartile	*Reference* *(n=15)*	*Reference* *(n =14)*
2nd quartile	1.06 (0.52, 2.16) *(n=16)*	1.04 (0.50, 2.20) *(n=14)*
3rd quartile	1.30 (0.66, 2.58) *(n=19)*	1.38 (0.68, 2.78) *(n=18)*
4th quartile	1.19 (0.60, 2.37) *(n=18)*	1.66 (0.85, 3.27) *(n=22)*
Trend test [a]	*1.09 (0.71, 1.69)*	*1.32 (0.94, 1.87)*
P for trend	*0.69*	*0.11*

Supplementary Table S4. *Continued*

	Risk of elevated fetal CRP levels (>1 mg/L) per PM_{10} quartile Odds ratio (95% CI) *(n of cases)*	**Risk of elevated fetal CRP levels (>1 mg/L) per NO_2 quartile** Odds ratio (95% CI) *(n of cases)*
Total pregnancy	N=4123	N=4121
1st quartile	*Reference (n=13)*	*Reference (n=9)*
2nd quartile	0.90 (0.40, 2.01) *(n=11)*	1.70 (0.75, 3.87) *(n=16)*
3rd quartile	1.03 (0.48, 2.20) *(n=14)*	2.10 (0.95, 4.63) ‡ *(n=20)*
4th quartile	1.85 (0.94, 3.63) ‡ *(n=25)*	1.85 (0.83, 4.13) *(n=18)*
Trend test [a]	*2.07 (0.95, 4.55)*	*1.42 (0.79, 2.54)*
P for trend	*0.07*	*0.25*

* p<0.05; ‡ p<0.10

Values are odds ratios (95% CI) and reflect the risk for elevated fetal C-reactive protein levels (>1 mg/L) for each quartile of air pollution exposure in different periods preceding delivery compared with the reference group (lowest quartile). Cut-off values for categorization of PM_{10} exposure were <23.9, 23.9-27.7, 27.7-32.8, >32.8 μg/m³ for the prior week, <24.7, 24.7-28.0, 28.0-32.1, >32.1 μg/m³ for the prior two weeks, <25.6, 25.6-28.5, 28.5-32.8, >32.8 μg/m³ for the prior four weeks, and <27.8, 27,8-30.0, 30.0-32.9, >32.9 μg/m³ for total pregnancy. Cut-off values for NO_2 exposure were <33.2, 33.2-39.3, 39.3-45.6, >45.6 μg/m³ for the prior week, <34.1, 34.1-39.8, 39.8-44.7, >44.7 μg/m³ for the prior two weeks, <34.7, 34.7-40.2, 40.2-44.1, >44.1 μg/m³ for the prior four weeks, and <37.2, 37.2-39.6, 39.6-42.3, >42.3 μg/m³ for total pregnancy. Differences in the number of subjects are due to missing air pollution data for the specific periods. Models are adjusted for gestational age at birth.

[a] Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous term (per 10 μg/m³ increase) in the model.

Chapter 3.2

Air pollution and markers of placental growth and function

Edith H van den Hooven
Frank H Pierik
Yvonne de Kluizenaar
Albert Hofman
Sjoerd W van Ratingen
Peter YJ Zandveld
Henk Russcher
Jan Lindemans
Henk ME Miedema
Eric AP Steegers
Vincent WV Jaddoe

Submitted for Publication

ABSTRACT

Background: Air pollution exposure during pregnancy might affect placental growth and function, which may lead to maternal and fetal complications. We prospectively evaluated the associations of maternal air pollution exposure with markers of placental growth and function among 7801 pregnant women in the Netherlands.

Methods: PM_{10} and NO_2 levels were estimated at the home address for different periods using dispersion modelling techniques. Pro- and anti-angiogenic factors (PlGF and sFlt-1) were measured in first and second trimester maternal blood samples and in fetal cord blood samples at delivery. Pulsatility index of the uterine and umbilical arteries was measured by Doppler ultrasound in second and third trimester. The presence of notching was assessed in third trimester. Information on placental and birth weight was obtained from medical records.

Results PM_{10} and NO_2 exposure in different periods were associated with lower second trimester maternal sFlt-1 and PlGF levels. In addition, higher PM_{10} and NO_2 exposure during total pregnancy were associated with higher sFlt-1 levels and lower PlGF levels in fetal cord blood, reflecting an anti-angiogenic state. We did not observe consistent associations of PM_{10} and NO_2 exposure with placental resistance indices in second or third trimester. Higher NO_2 exposure tended to be associated with increased risks of third trimester notching (odds ratio 1.33, 95% CI 0.99 to 1.78 per 10 $\mu g/m^3$ increase in the prior two months). Higher PM_{10} and NO_2 exposure were associated with lower placenta weight (differences -11.8g, 95% CI -20.9 to -2.7 and -10.7, 95% CI -19.0 to -2.4, respectively, per 10 $\mu g/m^3$ increase in the prior two months), but not with placental to birth weight ratio.

Conclusions Our results suggest that maternal air pollution exposure may influence markers of placental growth and function. Future studies are needed to confirm these findings and to explore the maternal and fetal consequences.

INTRODUCTION

Air pollution exposure during pregnancy has been linked to fetal growth restriction, low birth weight, and preterm birth [1-3]. Previous studies have also reported associations of maternal air pollution exposure with increased risks of developing preeclampsia and gestational hypertension [4, 5]. These fetal and maternal complications have to a large extent their origin in abnormal early placentation, which is characterised by impaired trophoblast invasion and lack of modification of the spiral arteries. As a result, the arteries maintain a higher vascular resistance, which could eventually lead to impaired uteroplacental perfusion and development of maternal and fetal complications [6-9].

It is unknown whether suboptimal placental growth and function underlies the previously shown associations of air pollution exposure with intrauterine growth restriction and the risks of pregnancy complications. Air pollution is suggested to induce systemic and placental inflammation [10, 11]. Not much is known about the effect of air pollution on placental growth and function. Placental growth factor (PlGF) is a pro-angiogenic protein that stimulates trophoblast invasion and vascular remodelling, and soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic protein that inhibits the activity of PlGF. Other measures including placental vascular resistance and placenta weight reflect (ab) normal placentation, hemodynamic placental function, and placental growth [8, 12]. If suboptimal placental function underlies the associations of air pollution with pregnancy complications, then air pollution may result in altered angiogenesis, increased placental resistance, and decreased placenta weight.

Therefore, we investigated the associations of maternal exposure to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) during pregnancy with maternal and fetal angiogenic factors, placental vascular resistance indices, and placenta weight in a population-based cohort study among 7801 subjects living in an urban area in the Netherlands.

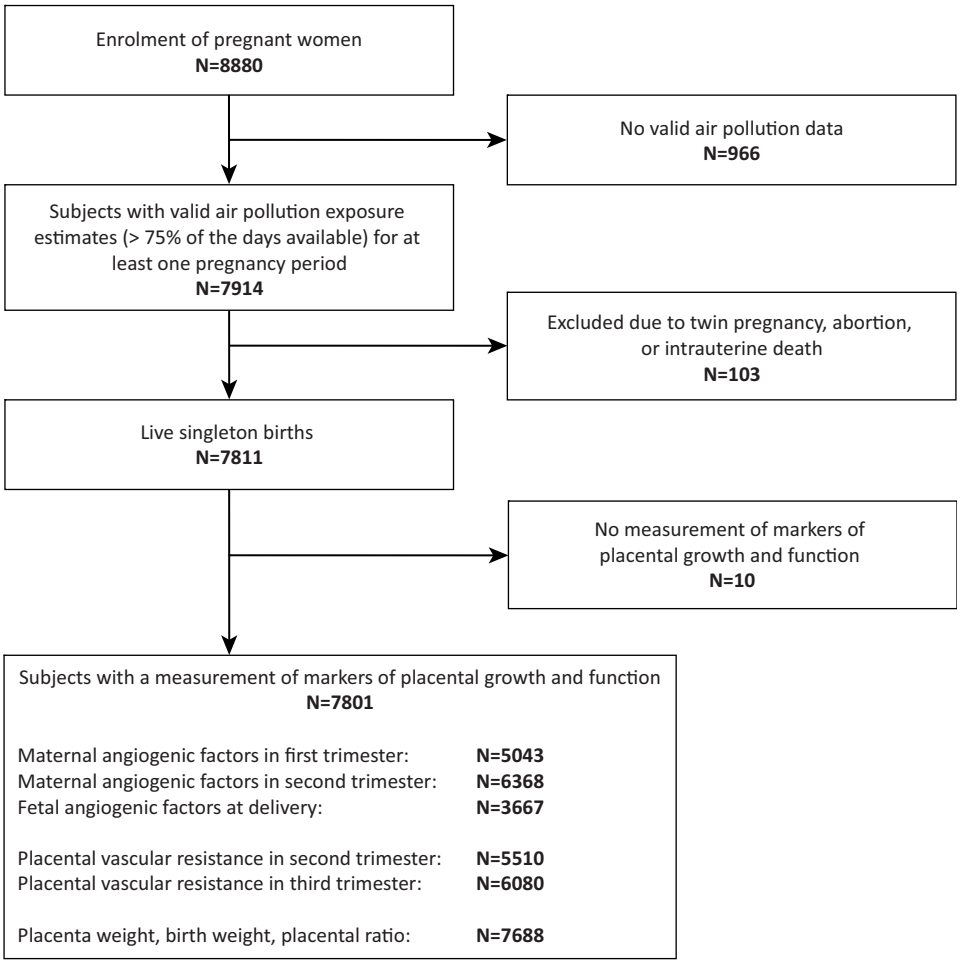
METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in the city of Rotterdam, the Netherlands [13]. Mothers were enrolled between 2001 and 2005. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all mothers. Of the 8880 prenatally enrolled women, air pollution exposure estimates were available for 7914 mothers (89%). For 966 mothers, air pollution exposure data could not be assessed due to incomplete address history, or because they had moved outside the study area before delivery [13]. Mothers with a twin pregnancy (n=84), abortion (n=7), or intrauterine death (n=12) were excluded. Of the mothers with singleton live births, no measurement of angiogenic factors, placental

vascular resistance, or placenta weight was available for 10 subjects. The associations of air pollution exposure with markers of placental growth and function were analyzed in the remaining 7801 subjects (see Figure 1 for a flow chart). The timing of the different measurements is illustrated in Supplementary Figure S1.

Figure 1. Population for analysis.



Air pollution exposure

Individual exposures to PM_{10} and NO_2 during pregnancy were assessed at the home address, using a combination of dispersion modelling techniques and continuous monitoring data, taking into account both the spatial and temporal variation in air pollution. A detailed description and a flow chart of the exposure assessment are presented in Chapter 2. In brief, annual average concentrations of PM_{10} and NO_2 for the years 2001-2006 were assessed

for all addresses in the study area, using the three Dutch national standard methods for air quality modelling [14]. Hourly concentrations of PM_{10} and NO_2 were derived, taking into account hourly wind conditions and fixed temporal patterns in the contribution of air pollution sources. Subsequently, the hourly concentrations were adjusted for background concentrations, using hourly measurements from three continuous monitoring stations. Based on participants' home addresses, we derived individual exposure estimates for different periods preceding the measurements, in order to examine the effects of both short- and longer-term exposures: two weeks (day 1-14), two months (day 1-60), and the specific pregnancy period (conception until measurement).

Angiogenic factors

Maternal non-fasting venous blood samples were collected in first trimester (median 13.2 weeks, 95% range 9.5-17.5) and second trimester (median 20.4 weeks, 95% range 18.5-23.5). Sampling of umbilical cord blood was carried out by midwives and obstetricians immediately after delivery (median 40.1 weeks, 95% range 35.4 to 42.3). All blood samples were transported to the regional laboratory for processing and storage at -80°C [15]. Concentrations of sFlt-1 and PlGF were measured in EDTA plasma samples at the Department of Clinical Chemistry of the Erasmus Medical Center between 2008 and 2010, using a two-step chemiluminescent microparticle immunoassay (CMIA) technology on the Architect System (Abbot Diagnostics B.V., Hoofddorp, the Netherlands). The between-run coefficients of variation for plasma sFlt-1 were 2.8 % at 5.5 ng/ml and 2.3% at 34.0 ng/ml, and the coefficients for plasma PlGF were 4.7% at 24 pg/ml, and 3.8% at 113 pg/ml. The highest level of detection was 150 ng/ml for sFlt-1 and 1500 pg/ml for PlGF.

Placental vascular resistance

Placental vascular resistance was evaluated with flow velocity waveforms from the uterine and umbilical arteries in second trimester (median 20.5 weeks, 95% 18.7-23.3) and third trimester (median 30.3 weeks, 95% range 28.4 to 32.9). Raised uterine and umbilical artery pulsatility indices indicate increased uteroplacental and fetoplacental resistances, respectively [16]. Uterine artery pulsatility index was measured in the right and left uterine artery near the crossover with the external iliac artery, and the mean value was calculated. The presence or absence of third trimester notching (unilateral or bilateral) was assessed in the uterine arteries and reflects an abnormal waveform resulting from increased blood flow resistance. Umbilical artery pulsatility index was measured in a free-floating loop of the umbilical cord. For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. The mean of three measurements was used for further analysis.

Placenta weight and placental ratio

Medical records completed by midwives and obstetricians were used to obtain information on placenta weight and birth weight. The placenta was weighed fresh, with membrane

and umbilical cord attached, within one hour after delivery. Placental ratio was calculated as (placenta weight/birth weight)*100%.

Covariates

Information on date of delivery, gestational age at delivery, and infant sex was obtained from medical records. Information on maternal age, parity, educational level, ethnicity, and folic acid supplementation use was obtained by a questionnaire at enrolment. Because there were no differences in observed results when ethnicity was categorized into five groups instead of two groups, we classified ethnicity as European or non-European. Maternal anthropometrics were assessed at time of enrolment. Maternal smoking and alcohol consumption before and during pregnancy were assessed by questionnaires in each trimester, and were categorized into: no, until pregnancy was known, or continued during pregnancy. Month of conception and month of birth were categorized into seasons: winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Road traffic noise exposure was assessed at the home address in accordance with requirements of the EU Environmental Noise Directive, as described in the Supplementary Material of Chapter 2.

Statistical analysis

First, maternal (first and second trimester) and fetal sFlt-1 and PlGF levels were log-transformed (using the natural log) to obtain normally distributed outcome variables. To prevent the introduction of missing values in transformed variables, concentrations of 0 ng/ml for fetal sFlt-1 (N=29; 0.8%) and 0 pg/ml for fetal PlGF (N=154; 0.1%) were imputed by random draws from the left tail of a normal distribution [17] (corresponding to values <0.024 ng/ml and <3.5 pg/ml, respectively). Imputing these values only marginally influenced the patterns of associations of air pollution with fetal sFlt-1 and PlGF levels, which was probably due to larger numbers. Next, we used linear regression models to analyze the associations of air pollution exposure with maternal and fetal sFlt-1 and PlGF levels. We present coefficients for the log-transformed concentrations, multiplied by 100, which can be interpreted as percentage changes [18]. Furthermore, we used linear regression models to assess the associations of air pollution exposure with placental vascular resistance indices (in SD values). Logistic regression models were performed to examine the associations of air pollution exposure with the risk of third trimester notching. Finally, we performed linear regression models to examine the associations of air pollution exposure with placenta weight, birth weight, and placental ratio. All models were adjusted for potential confounding factors that were chosen a priori and based on previous literature. This included gestational age at measurement, maternal age, body mass index, parity, ethnicity, education, smoking, alcohol consumption, folic acid supplementation use, and infant sex. Models on birth weight were additionally adjusted for maternal height. In addition, we evaluated the potential confounding effect of noise exposure and season of conception/delivery. Based on a ≥10% change in effect estimates,

we included noise exposure and season of conception in the models on placenta weight, birth weight, and placental ratio. In additional sensitivity analyses, for all models we restricted the analyses to non-smoking women, European women, non-obese women, and women without pregnancy complications. The percentages of missing values within the population for analysis were lower than 1% for continuous data and lower than 15% for categorical data, except for folic acid supplementation use (26%). We applied multiple imputation for missing data in covariates [19]. All statistical analyses were performed using PASW version 17.0 for Windows (PASW Inc., Chicago, IL, USA).

RESULTS

Subject, exposure and outcome characteristics

Table 1 presents the maternal and birth characteristics. Descriptives on air pollution exposure levels in different periods are presented in Supplementary Table S1. Mean exposure levels during pregnancy were 30.3 μg/m^3 for PM_{10} and 39.9 μg/m^3 for NO_2 (Table S1). Pearson correlation coefficients between the different exposure averages varied between 0.25 and 0.93. Correlations among exposure averages for the prior two weeks and two months were moderate to strong (PM_{10}: r=0.58 to 0.61, NO_2: r=0.78 to 0.80).

Maternal sFlt-1 levels in first and second trimester were strongly correlated (r=0.77), and PlGF levels in first and second trimester were moderately correlated (r=0.46). No correlations were observed for maternal sFlt-1 and PlGF levels in first and second trimester with fetal sFlt-1 and PlGF levels at delivery (r=-0.01 to 0.04). sFlt-1 and PlGF levels measured at the same time (first trimester, second trimester, or at delivery) were weakly correlated (r=0.13 to 0.24). Second and third trimester pulsatility indices were moderately correlated for both the uterine artery (r=0.31) and umbilical artery (r=0.56). Lower correlations were observed among pulsatility indices of the uterine and umbilical artery assessed at the same time (r=0.07 to 0.10). Placenta weight was strongly correlated with birth weight (r=0.63).

Table 1. Subject characteristics (N=7801).

	Mean ± SD, Median (95% range), or Number (percentage)
Maternal characteristics	
Age at enrolment (yr)	30.4 (19.2-39.3)
Gestational age at enrolment (weeks)	14.4 (10.2-29.5)
Height (cm)	167.1 ± 7.5
Weight (kg)	67.0 (50.0-103.0)
Body mass index (kg/m^2)	23.8 (18.7-36.3)

Table 1. *Continued*

	Mean ± SD, Median (95% range), or Number (percentage)
Parity – n (%)	
Nulliparous	4290 (55.0)
Multiparous	3420 (43.8)
Missing	91 (1.2)
Ethnic background – n (%)	
European	4140 (53.1)
Non-European	3088 (39.6)
Missing	573 (7.3)
Highest completed educational level – n (%)	
No education/primary	814 (10.4)
Secondary	3219 (41.3)
Higher	3071 (39.4)
Missing	697 (8.9)
Smoking in pregnancy – n (%)	
No	4987 (63.9)
First trimester only	574 (7.4)
Continued	1174 (15.0)
Missing	1066 (13.7)
Alcohol consumption in pregnancy – n (%)	
No	3295 (42.2)
First trimester only	905 (11.6)
Continued	2592 (33.2)
Missing	1009 (12.9)
Folic acid supplementation use – n (%)	
Preconceptional	2340 (29.5)
First ten weeks of pregnancy	1793 (23.0)
None	1679 (21.5)
Missing	2025 (26.0)
Season of conception – n (%)	
Winter	2198 (28.2)
Spring	1783 (23.0)
Summer	1771 (22.7)
Fall	2036 (26.1)
Noise exposure based on home address at delivery (dB(A))	52.7 (45.0-68.2)

Table 1. *Continued*

	Mean ± SD, Median (95% range), or Number (percentage)
Angiogenic factors	
First trimester, gestational age at visit (weeks) (n=5043)	13.2 (9.6-17.5)
Maternal sFlt-1 (ng/ml)	5.1 (1.9-14.3)
Maternal PlGF (pg/ml)	42.2 (14.6-188.4)
Second trimester, gestational age at visit (weeks) (n=6368)	20.6 (18.5-23.5)
Maternal sFlt-1 (ng/ml)	5.0 (1.5-17.4)
Maternal PlGF (pg/ml)	201.2 (73.8-623.7)
Delivery, gestational age (weeks) (n=3667)	40.1 (36.6-42.3)
Fetal sFlt-1 (ng/ml)	0.5 (0.1-5.9)
Fetal PlGF (pg/ml)	8.6 (0.0-21.9)
Placental vascular resistance	
Second trimester, gestational age at visit (weeks) (n=5510)	20.5 (18.7-23.3)
Uterine artery pulsatility index	0.90 ± 0.27
Umbilical artery pulsatility index	1.20 ± 0.19
Third trimester, gestational age at visit (weeks) (n=6080)	30.3 (28.4-32.9)
Uterine artery pulsatility index	0.74 ± 0.19
Umbilical artery pulsatility index	0.98 ± 0.17
Presence of unilateral uterine artery notching – n (%)	303 (6.8)
Presence of bilateral uterine artery notching – n (%)	141 (3.2)
Birth characteristics	
Gestational age at birth (weeks) (n=7688)	40.1 (35.7-42.4)
Placenta weight (g)	635 ± 146
Birth weight (g)	3414 ± 559
Placental ratio (%)	18.7 ± 3.5

Values are means ± SDs, or medians (95% range) for variables with a skewed distribution, and number of subjects (%) for categorical variables.

Air pollution and angiogenic factors

Table 2 shows that PM_{10} and NO_2 exposure in different periods were not associated with first trimester maternal sFlt-1 levels, but with lower second trimester sFlt-1 levels (differences -4.3%, 95% confidence interval (CI) -7.4 to -1.1 and -2.7%, 95% CI -5.1 to -0.2% per 10 μg/m^3 increase in PM_{10} and NO_2 in the prior two months, respectively). Higher PM_{10} and NO_2 exposure during total pregnancy were associated with higher fetal sFlt-1 levels at delivery (differences 35.8%, 95% confidence interval (CI) 25.6 to 45.9 and 8.9%, 95% CI 0.6 to 17.3 per 10 μg/m^3 increase in PM_{10} and NO_2, respectively). PM_{10} exposure for the prior two months and for the specific pregnancy period was associated with higher

maternal PlGF levels in first trimester. NO_2 exposure for the prior two months and the specific pregnancy period was associated with reduced PlGF levels in second trimester. Inverse associations were observed for PM_{10} and NO_2 exposure for the prior two months and during total pregnancy with fetal PlGF levels at delivery (differences -16.3%, 95% CI -21.9 to -10.7 and -14.6%, 95% CI -19.3 to -10.0 for PM_{10} and NO_2 during total pregnancy, respectively). The unadjusted associations were consistent with the adjusted associations, although weaker effect estimates were observed for NO_2 with second trimester sFlt-1 levels (Supplementary Table S2).

Table 2. Associations of maternal air pollution exposure with percent changes in angiogenic factors in first and second trimester and at delivery.

	Maternal sFlt-1 Percent change (95% CI)		**Fetal sFlt-1** Percent change (95% CI)
	First trimester N=4993	**Second trimester** N=6365	**Delivery** N=3629
PM_{10} (per 10 µg/m^3)			
Prior two weeks	0.1 (-1.7, 2.0)	-2.7 (-4.7, -0.7) *	-0.6 (-5.2, 3.9)
Prior two months	-0.2 (-3.1, 2.7)	-4.3 (-7.4, -1.1) *	-2.8 (-10.1, 4.4)
Total pregnancy period [a]	1.1 (-2.3, 4.6)	-4.5 (-8.4, -0.5) *	35.8 (25.6, 45.9) **
NO_2 (per 10 µg/m^3)			
Prior two weeks	1.3 (-0.5, 3.1)	-1.8 (-3.7, 0.1) ‡	5.0 (0.6, 9.5) *
Prior two months	0.7 (-1.7, 3.0)	-2.7 (-5.1, -0.2) *	3.4 (-2.0, 8.9)
Total pregnancy period [a]	0.7 (-2.0, 3.4)	-2.1 (-5.1, 1.0)	8.9 (0.6, 17.3) *
	Maternal PlGF Percent change (95% CI)		**Fetal PlGF** Percent change (95% CI)
	First trimester N=5024	**Second trimester** N=6365	**Delivery** N=3224
PM_{10} (per 10 µg/m^3)			
Prior two weeks	0.0 (-1.6, 1.7)	-1.5 (-3.1, 0.2) ‡	-0.5 (-3.1, 2.1)
Prior two months	3.0 (0.4, 5.6) *	-1.9 (-4.5, 0.7)	-14.4 (-18.3, -10.5) **
Total pregnancy period [a]	3.6 (0.5, 6.7) *	-1.1 (-4.3, 2.1)	-16.3 (-21.9, -10.7) **
NO_2 (per 10 µg/m^3)			
Prior two weeks	0.5 (-1.1, 2.1)	-1.0 (-2.6, 0.5)	-4.1 (-6.5, -1.8) *
Prior two months	0.4 (-1.7, 2.5)	-2.7 (-4.7, -0.7) *	-10.4 (-13.3, -7.5) **
Total pregnancy period [a]	0.2 (-2.2, 2.6)	-2.8 (-5.3, -0.3) *	-14.6 (-19.3, -10.0) **

** p<0.001; * p<0.05 ; ‡ p<0.10

Values are regression coefficients and reflect the percent change (95% range) in log-transformed sFlt-1 and PlGF levels per 10 µg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement, fetal sex, maternal age, body mass index, parity, ethnicity, education, smoking, alcohol consumption, and folic acid supplementation use.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until first trimester measurement, from conception until second trimester measurement, or from conception until delivery.

Air pollution and placental vascular resistance

We did not observe consistent associations of air pollution exposure with uterine and umbilical artery pulsatility indices in second and third trimester (Table 3). We only observed that higher PM_{10} exposure for the prior two months and the specific pregnancy period was associated with a reduced umbilical artery pulsatility index in second trimester (differences -0.07 SD, 95% CI -0.13 to -0.02, and -0.10 SD, 95% CI -0.17 to -0.03 per 10 μg/m^3 increase, respectively). Relative to the adjusted models, the unadjusted models showed similar effect estimates (Supplementary Table S3). Table 4 shows that PM_{10} exposure was not associated with notching, but higher NO_2 exposure for the prior two weeks and prior two months tended to be associated with increased risks of bilateral notching (odds ratio (OR) 1.22, 95% CI 0.97 to 1.53 and 1.33, 95% CI 0.99 to 1.78, respectively). The unadjusted models showed stronger associations for NO_2 exposure with the risks of bilateral notching (Supplementary Table S4); other effect estimates were similar to the adjusted models.

Air pollution and placenta weight

Table 5 shows that PM_{10} and NO_2 exposure in the two months preceding delivery were associated with a lower placenta weight (differences -11.8g, 95% CI -20.9 to -2.7, and -10.7, 95% CI -19.0 to -2.4 per 10 μg/m^3 increase, respectively), but no associations were observed for other periods. PM_{10} and NO_2 exposure in different periods were associated with reductions in birth weight (differences -34.6, 95% CI -66.3 to -2.9, and -39.3, 95% CI -69.1 to -9.6 per 10 μg/m^3 increase in PM_{10} and NO_2 during total pregnancy, respectively). PM_{10} exposure for the prior two months was associated with a reduced placental ratio, but no associations were observed for other periods or for NO_2 exposure. Relative to the adjusted models, the unadjusted models showed similar results, although weaker associations were observed for PM_{10} and NO_2 exposure in the two months preceding delivery with placenta weight (Supplementary Table S5).

Table 3. Associations of maternal air pollution exposure with uteroplacental and fetoplacental vascular resistance in second and third trimester.

	Uterine artery Pulsatility Index (SD) Difference (95% CI)	
	Second trimester N=3432	**Third trimester** N=3511
PM_{10} (per 10 μg/m^3)		
Prior two weeks	0.00 (-0.05, 0.05)	-0.03 (-0.08, 0.02)
Prior two months	0.03 (-0.05, 0.11)	-0.01 (-0.08, 0.07)
Total pregnancy period [a]	0.02 (-0.08, 0.11)	-0.06 (-0.17, 0.04)
NO_2 (per 10 μg/m^3)		
Prior two weeks	0.01 (-0.03, 0.06)	-0.03 (-0.07, 0.02)
Prior two months	0.02 (-0.04, 0.08)	-0.02 (-0.07, 0.03)
Total pregnancy period [a]	0.02 (-0.05, 0.09)	-0.04 (-0.11, 0.04)
	Umbilical artery Pulsatility Index (SD) Difference (95% CI)	
	Second trimester N=5443	**Third trimester** N=6026
PM_{10} (per 10 μg/m^3)		
Prior two weeks	-0.02 (-0.06, 0.01)	0.01 (-0.03, 0.04)
Prior two months	-0.07 (-0.13, -0.02) *	0.04 (-0.02, 0.10)
Total pregnancy period [a]	-0.10 (-0.17, -0.03) *	-0.01 (-0.09, 0.06)
NO_2 (per 10 μg/m^3)		
Prior two weeks	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)
Prior two months	-0.02 (-0.06, 0.02)	0.03 (-0.01, 0.07)
Total pregnancy period [a]	-0.04 (-0.09, 0.01)	0.03 (-0.03, 0.08)

* $p<0.05$

Values are regression coefficients and reflect the difference in SD score of uterine and umbilical artery pulsatility index per 10 μg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement, fetal sex, maternal age, body mass index, parity, ethnicity, educational level, smoking, alcohol consumption, and folic acid supplementation use.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until second trimester measurement or from conception until third trimester measurement.

Table 4. Associations of maternal air pollution exposure with the risks of uterine artery notching in third trimester.

	Unilateral notching Odds ratio (95% CI) N=4244	**Bilateral notching** Odds ratio (95% CI) N=4091
PM_{10} (per 10 μg/m^3)		
Prior two weeks	0.94 (0.77, 1.12)	1.18 (0.92, 1.51)
Prior two months	0.93 (0.70, 1.23)	1.31 (0.88, 1.94)
Total pregnancy period	0.96 (0.66, 1.38)	1.14 (0.67, 1.93)
NO_2 (per 10 μg/m^3)		
Prior two weeks	0.99 (0.85, 1.16)	1.22 (0.97, 1.53) ‡
Prior two months	0.96 (0.79, 1.16)	1.33 (0.99, 1.78) ‡
Total pregnancy period	1.12 (0.85, 1.48)	1.18 (0.79, 1.76)

‡ p<0.10

Values are odds ratios and reflect the risks for unilateral and bilateral uterine artery notching in third trimester per 10 μg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement, fetal sex, maternal age, body mass index, parity, ethnicity, educational level, smoking, alcohol consumption, and folic acid supplementation use.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until third trimester measurement.

Table 5. Associations of maternal air pollution exposure with placenta weight, birth weight, and placental ratio.

	Placenta weight (g) Difference (95% CI) N=5605	**Birth weight** (g) Difference (95% CI) N=7688	**Placental ratio** (%) Difference (95% CI) N=5599
PM_{10} (per 10 μg/m^3)			
Prior two weeks	-1.7 (-7.0, 3.6)	-16.0 (-29.5, -2.4) *	0.0 (-0.1, 0.2)
Prior two months	-11.8 (-20.9, -2.7) *	-37.9 (-61.4, -14.4) *	-0.2 (-0.5, 0.0) *
Total pregnancy	-6.0 (-18.5, 6.4)	-34.6 (-66.3, -2.9) *	-0.1 (-0.4, 0.2)
NO_2 (per 10 μg/m^3)			
Prior two weeks	-2.8 (-8.8, 3.2)	-17.1 (-32.4, -1.8) *	0.1 (-0.1, 0.2)
Prior two months	-10.7 (-19.0, -2.4) *	-24.3 (-45.6, -3.1) *	-0.2 (-0.4, 0.0)
Total pregnancy	-9.3 (-20.9, 2.3)	-39.3 (-69.1, -9.6) *	-0.1 (-0.4, 0.1)

* p<0.05

Values are regression coefficients and reflect the difference in placenta weight, birth weight, and placental ratio ((placenta weight/birth weight)*100%) per 10 μg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at delivery, infant sex, maternal age, body mass index, parity, ethnicity, educational level, smoking, alcohol consumption, folic acid supplementation use, noise exposure, and season of conception. Models on birth weight are additionally adjusted for maternal height.

Sensitivity analyses

Results in non-smoking women were comparable. Associations were slightly stronger when the analyses were restricted to European women or women with a normal body mass index, but the patterns of associations were the same. Restriction to women without pregnancy complications (gestational hypertension, preeclampsia, or gestational diabetes) produced similar results, although we observed smaller effect estimates that did not reach significance for the associations of NO_2 exposure with the risks of notching in third trimester.

DISCUSSION

Results from this large prospective cohort study suggest that maternal air pollution exposure may affect placental growth and function. We observed associations of PM_{10} and NO_2 exposure with changes in fetal sFlt-1 and PlGF levels at delivery. Also, higher PM_{10} and NO_2 exposure were associated with lower placenta weight. However, air pollution exposure was not consistently associated with other markers of placental growth and function, indicating that future research is needed to confirm these findings and to examine the underlying mechanisms.

Air pollution and markers of placental function and growth

Adequate placentation and placental functioning are critical for normal pregnancy. Impairment of these processes, reflected by alterations in markers of placental growth and function, has been linked to the development of maternal and fetal complications [7, 8, 12, 20]. Maternal air pollution exposure may affect pregnancy by inducing oxidative stress and systemic inflammation [10], which could result in suboptimal placentation or placental inflammation [11, 21]. A few animal studies showed adverse effects of air pollution exposure on placenta weight and function [22, 23]. However, not much is known about the associations of air pollution exposure with markers of placental growth and function in humans.

Over the past decades there has been increased interest in the role of angiogenic growth factors in the development of maternal and fetal complications. The growth factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are important for placental development and angiogenesis, whereas soluble fms-like tyrosine kinase 1 (sFlt-1) binds to these proteins and thereby inhibits their activity. Previous studies have demonstrated elevated sFlt-1 levels or reduced PlGF levels in women whose pregnancies were complicated by intrauterine growth restriction, preeclampsia, and gestational hypertension [20, 24-26], and in infants of mothers with preeclampsia [27, 28]. However, other studies, including two recent reviews, reported different results for either sFlt-1 or PlGF [24-26, 29, 30]. To our knowledge, no previous studies have examined the associations of air pollution with angiogenic factors during pregnancy. We observed that

PM_{10} and NO_2 exposure were associated with reduced maternal second trimester sFlt-1 levels, and weakly associated with elevated first trimester PlGF levels and reduced second trimester PlGF levels. At delivery, air pollution exposure was associated with higher fetal sFlt-1 levels and lower PlGF levels. These cord blood levels might reflect placental rather than fetal production [8, 28]. Thus, air pollution contributed to an anti-angiogenic profile in fetal cord blood, but not in maternal first and second trimester blood. In this light, it is interesting that maternal smoking, which shares similarities in biological effects with air pollution, was associated with lower sFlt-1 levels and higher PlGF levels in maternal first and second trimester blood. Previous studies on maternal smoking during pregnancy have also reported reduced sFlt-1 levels, indicating that smoking could promote an angiogenic balance [31, 32]. Our associations of air pollution with angiogenic factors differed according to the stage of pregnancy, which may be related to (trimester-specific) alterations in sFlt-1 and PlGF levels in response to the pregnancy [33]. Also, it has been proposed that elevated sFlt-1 levels could be due to increased trophoblastic placental tissue [34], which may indicate that sFlt-1 and PlGF could merely reflect trophoblast volume rather than pathological processes.

Indices of placental vascular resistance and the presence of uterine artery notching have been used to identify complicated pregnancies [8, 35]. In normal pregnancy, the pulsatility index of the uterine arteries decreases with advancing gestational age, as a result of the trophoblast invasion during the first half of pregnancy. Impaired remodelling of the arteries leads to maintenance of high arterial resistance, with subsequent inadequate uteroplacental blood flow [6]. We observed no consistent associations of air pollution exposure with uterine and umbilical artery pulsatility indices in second and third trimester. However, PM_{10} exposure for the prior two months and the specific pregnancy period was associated with a reduced umbilical artery pulsatility index in second trimester. As we hypothesized that air pollution adversely affects placental function, this latter observation is not in line with our expectations. Furthermore, the results are also in disagreement with previous studies on maternal smoking, of which one was conducted in our population, that reported increased uteroplacental and fetoplacental resistance [36, 37]. Nevertheless, in our study NO_2 exposure in the period preceding the measurement tended to be associated with increased risks of bilateral uterine artery notching, indicating increased arterial resistance. The clinical consequences of these time- and pollutant-specific effects of air pollution on placental vascular resistance need to be further studied.

The placental ratio (placenta weight relative to birth weight) can be considered as a marker of placental function, i.e., the capacity to transport oxygen and nutrients. A high placental ratio is suggested to reflect a less efficient placental function and reduced nutrient supply to the fetus [12, 38]. A low placenta weight and a high placental ratio have been linked to adverse pregnancy outcomes in various studies [39, 40]. Previous experimental studies in mice have reported associations of air pollution exposure during pregnancy with lower placenta weight [22] and with morphological changes of the placenta that suggested impaired placental function [23]. Two previous studies examined

the associations of (indicators of) air pollution exposure with placenta weight or ratio in women. The first study was conducted in Italy and reported a lower placenta weight following exposure to PM_{10} and NO_2 in the two months before delivery (differences -3 and -7g per 10 µg/m^3 increase in PM_{10} and NO_2, respectively) [41]. In the second study, conducted in Japan, living within 200m from a major road (as an indicator of air pollution exposure) was associated with a 13g decrease in placenta weight and a 0.5% increase in placental ratio, as compared to living farther away [42]. In the present study, we observed that PM_{10} and NO_2 exposure in the two months preceding delivery were associated with reductions in placenta weight of 12 and 11g per 10 µg/m^3 increase, respectively, which is in line with the Italian study. In the present paper, birth weight was included as an outcome in order to calculate the placental ratio. PM_{10} and NO_2 exposure for different periods were strongly associated with reductions in birth weight, which we have described previously [3]. We did not observe consistent associations of air pollution exposure with placental ratio, which may indicate that placenta weight and birth weight were affected to a similar degree by air pollution.

Methodological considerations

A main strength of our study is the availability of different markers of placental growth and function, enabling investigation of this possible pathway for air pollution effects on pregnancy outcomes. A wide range of potential confounders was available. Nevertheless, residual confounding due to unmeasured variables (e.g., quality of housing) might still be an issue.

Air pollution is a complex mixture of several pollutants. PM_{10} and NO_2 can be regarded as indicators of this mixture rather than the definite causative factors of adverse health effects. Air pollution exposure was estimated using a combination of dispersion modelling and continuous monitoring, which enabled consideration of detailed spatial and temporal variation in exposure. Still, the variation in exposure levels might be relatively small in our study population, which may have limited the ability to detect associations of air pollution with markers of placental growth and function. We were able to allow for residential mobility of the women during pregnancy. There can still be misclassification of air pollution exposure, since exposure levels were estimated at the home address. No information was available on indoor, occupational, or commuting sources of air pollution, or on time-activity patterns of the women. However, the magnitude of the possible misclassification is probably less than in non-pregnant individuals, as pregnant women generally spend more time at home, especially in the last stage of pregnancy [43].

Conclusion

In this prospective population-based cohort study, maternal PM_{10} and NO_2 exposure were associated with changes in fetal sFlt-1 and PlGF levels at delivery, resulting in an anti-angiogenic state, and with lower placenta weight. Our results suggest that air pollution exposure may influence placental growth and function. Future studies are needed to confirm these findings, to examine the underlying mechanisms, and to explore the maternal and fetal consequences.

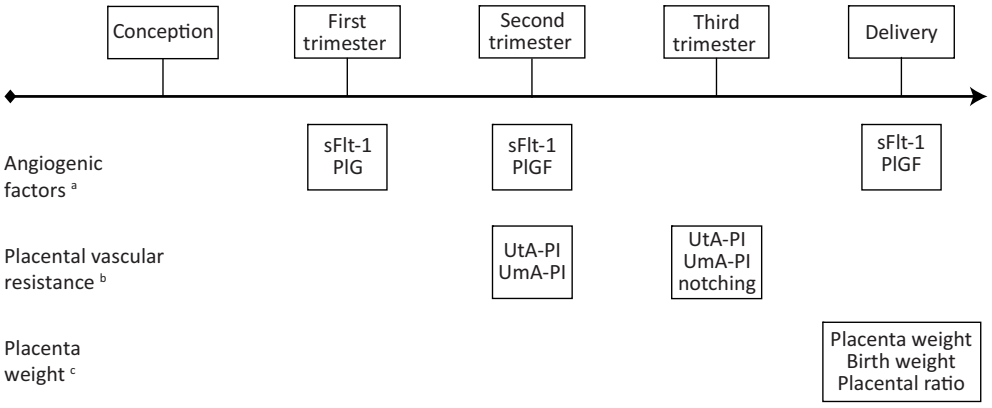
REFERENCES

1. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
2. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
3. van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, et al. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environ Health Perspect.* 2012; 120(1):150-156.
4. van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, et al. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy: The Generation R Study. *Hypertension.* 2011; 57(3):406-12.
5. Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B. Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. *Environ Health Perspect.* 2009; 117(11):1773-9.
6. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod.* 2003; 69(1):1-7.
7. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstet Gynecol.* 2006; 195(1):40-9.
8. Schlembach D, Wallner W, Sengenberger R, Stiegler E, Mortl M, Beckmann MW, et al. Angiogenic growth factor levels in maternal and fetal blood: correlation with Doppler ultrasound parameters in pregnancies complicated by pre-eclampsia and intrauterine growth restriction. *Ultrasound Obstet Gynecol.* 2007; 29(4):407-13.
9. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet.* 2010; 376(9741):631-44.
10. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
11. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
12. Salafia CM, Charles AK, Maas EM. Placenta and fetal growth restriction. *Clin Obstet Gynecol.* 2006; 49(2):236-56.
13. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
14. Netherlands Ministry of Infrastructure and the Environment: Air Quality Decree 2007 (Regeling beoordeling Luchtkwaliteit 2007). 2007. Available: http://wetten.overheid.nl/BWBR0022817
15. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007; 22(12):917-23.
16. Miller J, Turan S, Baschat AA. Fetal growth restriction. *Semin Perinatol.* 2008; 32(4):274-80.
17. Helsel DR. *Nondetects and Data Analysis; Statistics for censored environmental data*. 2005. Hoboken, New Jersey: John Wiley & Sons Inc.
18. Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. *Stat Med.* 2000; 19(22):3109-25.
19. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res.* 2007; 16(3):219-42.
20. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med.* 2004; 350(7):672-83.
21. Dejmek J, Selevan SG, Benes I, Solansky I, Sram RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ Health Perspect.* 1999; 107(6):475-480.
22. Rocha e Silva IR, Lichtenfels AJ, Amador Pereira LA, Saldiva PH. Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. *Fertil Steril.* 2008; 90(5):1921-4.

23. Veras MM, Damaceno-Rodrigues NR, Caldini EG, Maciel Ribeiro AA, Mayhew TM, Saldiva PH, et al. Particulate urban air pollution affects the functional morphology of mouse placenta. *Biol Reprod.* 2008; 79(3):578-84.
24. Asvold BO, Vatten LJ, Romundstad PR, Jenum PA, Karumanchi SA, Eskild A. Angiogenic factors in maternal circulation and the risk of severe fetal growth restriction. *Am J Epidemiol.* 2011; 173(6):630-9.
25. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab.* 2004; 89(2):770-5.
26. Smith GC, Crossley JA, Aitken DA, Jenkins N, Lyall F, Cameron AD, et al. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. *Obstet Gynecol.* 2007; 109(6):1316-24.
27. Catarino C, Rebelo I, Belo L, Rocha S, Castro EB, Patricio B, et al. Fetal and maternal angiogenic/anti-angiogenic factors in normal and preeclamptic pregnancy. *Growth Factors.* 2009; 27(6):345-51.
28. Staff AC, Braekke K, Harsem NK, Lyberg T, Holthe MR. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol.* 2005; 122(1):33-9.
29. Jacobs M, Nassar N, Roberts CL, Hadfield R, Morris JM, Ashton AW. Levels of soluble fms-like tyrosine kinase one in first trimester and outcomes of pregnancy: a systematic review. *Reprod Biol Endocrinol.* 2011; 9:77.
30. Widmer M, Villar J, Benigni A, Conde-Agudelo A, Karumanchi SA, Lindheimer M. Mapping the theories of preeclampsia and the role of angiogenic factors: a systematic review. *Obstet Gynecol.* 2007; 109(1):168-80.
31. Jeyabalan A, Powers RW, Durica AR, Harger GF, Roberts JM, Ness RB. Cigarette smoke exposure and angiogenic factors in pregnancy and preeclampsia. *Am J Hypertens.* 2008; 21(8):943-7.
32. Mehendale R, Hibbard J, Fazleabas A, Leach R. Placental angiogenesis markers sFlt-1 and PlGF: response to cigarette smoke. *Am J Obstet Gynecol.* 2007; 197(4):363 e1-5.
33. Hirashima C, Ohkuchi A, Arai F, Takahashi K, Suzuki H, Watanabe T, et al. Establishing reference values for both total soluble Fms-like tyrosine kinase 1 and free placental growth factor in pregnant women. *Hypertens Res.* 2005; 28(9):727-32.
34. Bdolah Y, Lam C, Rajakumar A, Shivalingappa V, Mutter W, Sachs BP, et al. Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia? *Am J Obstet Gynecol.* 2008; 198(4):428 e1-6.
35. Cnossen JS, Morris RK, ter Riet G, Mol BW, van der Post JA, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *CMAJ.* 2008; 178(6):701-11.
36. Geelhoed JJ, El Marroun H, Verburg BO, van Osch-Gevers L, Hofman A, Huizink AC, et al. Maternal smoking during pregnancy, fetal arterial resistance adaptations and cardiovascular function in childhood. *BJOG.* 2011; 118(6):755-62.
37. Machado Jde B, Plinio Filho VM, Petersen GO, Chatkin JM. Quantitative effects of tobacco smoking exposure on the maternal-fetal circulation. *BMC Pregnancy Childbirth.* 2011; 11:24.
38. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993; 341(8850):938-41.
39. Lao TT, Wong WM. Placental ratio and intrauterine growth retardation. *Br J Obstet Gynaecol.* 1996; 103(9):924-6.
40. Mayhew TM, Ohadike C, Baker PN, Crocker IP, Mitchell C, Ong SS. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta.* 2003; 24(2-3):219-26.
41. Pesatori AC, Bonzini M, Carugno M, Giovannini N, Signorelli V, Baccarelli A, et al. Ambient air pollution affects birth and placental weight. A study from Lombardy (Italy) region. *Epidemiology.* 2008; 19(6):S178-S179.
42. Yorifuji T, Naruse H, Kashima S, Murakoshi T, Tsuda T, Doi H, et al. Residential proximity to major roads and placenta/birth weight ratio. *Sci Total Environ.* 2012; 414:98-102.
43. Nethery E, Brauer M, Janssen P. Time-activity patterns of pregnant women and changes during the course of pregnancy. *J Expo Sci Environ Epidemiol.* 2009; 19(3):317-24.

SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Timing of measurements of angiogenic factors, placental vascular resistance, and placenta weight.



[a] sFlt-1 and PlGF were measured in maternal blood in first trimester (median 13.2 weeks of gestation, 95% range 9.6 to 17.5) and second trimester (median 20.6 weeks of gestation, 95% range 18.5 to 23.5), and in fetal cord blood at delivery (median 40.1 weeks of gestation, 95% range 36.6 to 42.3).
[b] Uterine and umbilical artery pulsatility index were measured in second trimester (median 20.5 weeks of gestation, 95% range 18.7 to 23.3) and third trimester (median 30.3 weeks of gestation, 95% range 28.4 to 32.9), and unilateral or bilateral uterine artery notching was assessed in third trimester.
[c] Placenta weight and birth weight were measured at delivery (median 40.1 weeks of gestation, 95% range 35.7 to 42.4), and placental ratio was calculated.

Supplementary Table S1. Distribution of PM_{10} and NO_2 exposure levels for different periods.

	Minimum	25th percentile	Mean	Median	75th percentile	Maximum
			First trimester [a]			
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-14	18.8	25.4	30.6	28.8	33.7	57.4
Prior day 1-60	21.4	26.9	30.7	30.5	33.6	46.2
Total pregnancy period	22.0	27.7	30.9	30.8	33.7	44.0
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-14	16.9	35.2	40.4	40.5	45.2	70.5
Prior day 1-60	20.2	36.7	40.4	41.0	44.1	59.3
Total pregnancy period	21.0	37.2	40.5	41.0	44.0	59.8
			Second trimester [b]			
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-14	17.4	25.0	30.3	28.7	33.3	56.4
Prior day 1-60	21.5	26.5	30.5	30.3	33.3	48.1
Total pregnancy period	22.6	27.9	30.6	30.5	33.4	43.2
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-14	15.1	34.6	40.2	40.2	45.0	66.9
Prior day 1-60	20.3	36.0	40.0	40.8	44.0	59.6
Total pregnancy period	22.7	37.0	40.2	40.4	43.5	59.3
			Third trimester [c]			
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-14	18.8	25.4	30.6	28.8	33.7	58.0
Prior day 1-60	20.2	26.3	30.6	29.4	33.8	49.5
Total pregnancy period	22.7	27.5	30.0	30.0	32.4	41.5
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-14	16.9	35.2	40.4	40.5	45.3	67.4
Prior day 1-60	19.8	35.8	40.4	40.8	44.5	65.5
Total pregnancy period	25.6	36.8	39.8	39.6	42.5	58.2
			Delivery			
PM_{10} exposure ($\mu g/m^3$)						
Prior day 1-14	16.8	24.7	29.6	28.1	32.2	58.0
Prior day 1-60	21.4	26.1	29.7	29.5	32.4	45.8
Total pregnancy period	23.2	27.8	30.3	30.0	32.9	40.9
NO_2 exposure ($\mu g/m^3$)						
Prior day 1-14	15.3	34.1	39.5	39.9	44.7	67.2
Prior day 1-60	18.5	35.1	39.4	40.3	43.6	65.3
Total pregnancy period	26.5	37.2	39.9	39.6	42.2	59.3

Air pollution exposure was estimated for two weeks (day 1-14) and two months (day 1-60) preceding the different measurements. Additionally, air pollution exposure was estimated for specific pregnancy periods (conception until measurement).

[a] Air pollution exposure averages were calculated prior to the day of blood sampling (for measurement of angiogenic factors) in first trimester.

[b] Air pollution exposure averages were calculated prior to the day of blood sampling (for measurement of angiogenic factors) in second trimester. The ultrasound visit in second trimester was planned on the same day in the majority of the women. The corresponding exposure averages prior to this visit are not shown in the table.

[c] Air pollution exposure averages were calculated prior to the day of the ultrasound visit in third trimester.

Supplementary Table S2. Unadjusted associations of maternal air pollution exposure with percent changes in angiogenic factors in first and second trimester and at delivery.

	Maternal sFlt-1 Percent change (95% CI)		**Fetal sFlt-1** Percent change (95% CI)
	First trimester N=4993	**Second trimester** N=6365	**Delivery** N=3629
PM_{10} (per 10 μg/m^3)			
Prior two weeks	0.2 (-1.7, 2.1)	-2.2 (-4.3, -0.2) *	-0.6 (-5.1, 4.0)
Prior two months	-0.3 (-3.3, 2.7)	-3.7 (-6.9, -0.4) *	-2.4 (-9.7, 4.8)
Total pregnancy period [a]	1.4 (-2.1, 4.9)	-3.5 (-7.6, 0.5) ‡	35.9 (25.8, 46.0) **
NO_2 (per 10 μg/m^3)			
Prior two weeks	1.9 (0.0, 3.7) *	-0.7 (-2.7, 1.3)	5.4 (1.0, 9.8) *
Prior two months	1.6 (-0.8, 4.0)	-0.8 (-3.4, 1.7)	3.9 (-1.6, 9.3)
Total pregnancy period [a]	2.2 (-0.5, 4.9)	0.7 (-2.4, 3.8)	9.8 (1.6, 18.1) *
	Maternal PlGF Percent change (95% CI)		**Fetal PlGF** Percent change (95% CI)
	First trimester N=5024	**Second trimester** N=6365	**Delivery** N=3224
PM_{10} (per 10 μg/m^3)			
Prior two weeks	0.2 (-1.5, 1.9)	-1.1 (-2.8, 0.6)	-0.5 (-3.1, 2.1)
Prior two months	3.7 (1.0, 6.4) *	-0.8 (-3.4, 1.9)	-14.6 (-18.5, -10.7) **
Total pregnancy period [a]	4.4 (1.2, 7.6) *	0.1 (-3.3, 3.5)	-16.6 (-22.2, -10.9) **
NO_2 (per 10 μg/m^3)			
Prior two weeks	1.0 (-0.7, 2.6)	-0.1 (-1.7, 1.5)	-4.3 (-6.7, -2.0) **
Prior two months	1.2 (-1.0, 3.4)	-0.9 (-0.3, 1.2)	-10.7 (-13.6, -7.8) **
Total pregnancy period [a]	1.2 (-1.3, 3.6)	-0.5 (-3.0, 2.1)	-15.5 (-20.2, -10.9) **

** p<0.001; * p<0.05; ‡ p<0.10

Values are regression coefficients and reflect the percent change (95% range) in log-transformed sFlt-1 and PlGF levels per 10 μg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until first trimester measurement, from conception until second trimester measurement, or from conception until delivery.

Supplementary Table S3. Unadjusted associations of maternal air pollution exposure with uteroplacental and fetoplacental vascular resistance in second and third trimester.

	Uterine artery Pulsatility Index (SD) Difference (95% CI)	
	Second trimester N=3432	**Third trimester** N=3511
PM_{10} (per 10 µg/m^3)		
Prior two weeks	0.00 (-0.05, 0.06)	-0.04 (-0.09, -0.02)
Prior two months	0.03 (-0.05, 0.11)	-0.01 (-0.09, 0.07)
Total pregnancy period [a]	0.02 (-0.08, 0.11)	-0.08 (-0.18, 0.03)
NO_2 (per 10 µg/m^3)		
Prior two weeks	0.02 (-0.03, 0.06)	-0.03 (-0.07, 0.01)
Prior two months	0.03 (-0.03, 0.09)	-0.03 (-0.08, 0.03)
Total pregnancy period [a]	0.03 (-0.04, 0.10)	-0.05 (-0.12, 0.03)
	Umbilical artery Pulsatility Index (SD) Difference (95% CI)	
	Second trimester N=5443	**Third trimester** N=6026
PM_{10} (per 10 µg/m^3)		
Prior two weeks	-0.02 (-0.06, 0.02)	0.01 (-0.03, 0.04)
Prior two months	-0.06 (-0.12, -0.01) *	0.04 (-0.02, 0.10)
Total pregnancy period [a]	-0.08 (-0.15, -0.01) *	-0.01 (-0.09, 0.07)
NO_2 (per 10 µg/m^3)		
Prior two weeks	-0.01 (-0.04, 0.03)	-0.01 (-0.02, 0.01)
Prior two months	-0.01 (-0.05, 0.03)	0.03 (-0.01, 0.07)
Total pregnancy period [a]	-0.02 (-0.07, 0.03)	0.03 (-0.02, 0.09)

* $p<0.05$

Values are regression coefficients and reflect the difference in SD score of uterine and umbilical artery pulsatility index per 10 µg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until second trimester measurement or from conception until third trimester measurement.

Supplementary Table S4. Unadjusted associations of maternal air pollution exposure with the risks of uterine artery notching in third trimester.

	Unilateral notching Odds ratio (95% CI) N=4244	**Bilateral notching** Odds ratio (95% CI) N=4091
PM_{10} (per 10 µg/m^3)		
Prior two weeks	0.93 (0.77, 1.12)	1.17 (0.92, 1.49)
Prior two months	0.94 (0.71, 1.25)	1.32 (0.89, 1.94)
Total pregnancy period	0.96 (0.67, 1.38)	1.14 (0.68, 1.92)
NO_2 (per 10 µg/m^3)		
Prior two weeks	1.02 (0.87, 1.19)	1.27 (1.02, 1.58) *
Prior two months	1.00 (0.82, 1.21)	1.39 (1.04, 1.84) *
Total pregnancy period	0.98 (0.87, 1.10)	1.34 (0.92, 1.95)

* p<0.05

Values are odds ratios and reflect the risks for unilateral and bilateral uterine artery notching in third trimester per 10 µg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at measurement.

[a] Air pollution exposure for the total pregnancy period was estimated as average exposure for the period from conception until third trimester measurement.

Supplementary Table S5. Unadjusted associations of maternal air pollution exposure with placenta weight, birth weight, and placental ratio.

	Placenta weight (g) Difference (95% CI) N=5605	**Birth weight** (g) Difference (95% CI) N=7688	**Placental ratio** (%) Difference (95% CI) N=5599
PM_{10} (per 10 µg/m^3)			
Prior two weeks	-0.6 (-5.8, 4.6)	-13.8 (-28.2, 0.5) ‡	0.1 (-0.1, 0.2)
Prior two months	-8.3 (-16.8, 0.2) ‡	-36.9 (-60.3, -13.4) *	-0.2 (-0.4, 0.0)
Total pregnancy	-7.4 (-19.5, 4.7)	-54.0 (-87.0, -20.9) *	0.0 (-0.3, 0.3)
NO_2 (per 10 µg/m^3)			
Prior two weeks	-1.0 (-5.9, 3.8)	-14.2 (-27.2, -1.2) *	0.0 (-0.1, 0.2)
Prior two months	-2.6 (-8.5, 3.3)	-16.4 (-32.4, -0.5) *	0.0 (-0.2, 0.1)
Total pregnancy	-5.6 (-14.8, 3.5)	-55.4 (-80.5, -30.3) **	0.1 (-0.1, 0.3)

* p<0.05; ‡ p<0.10

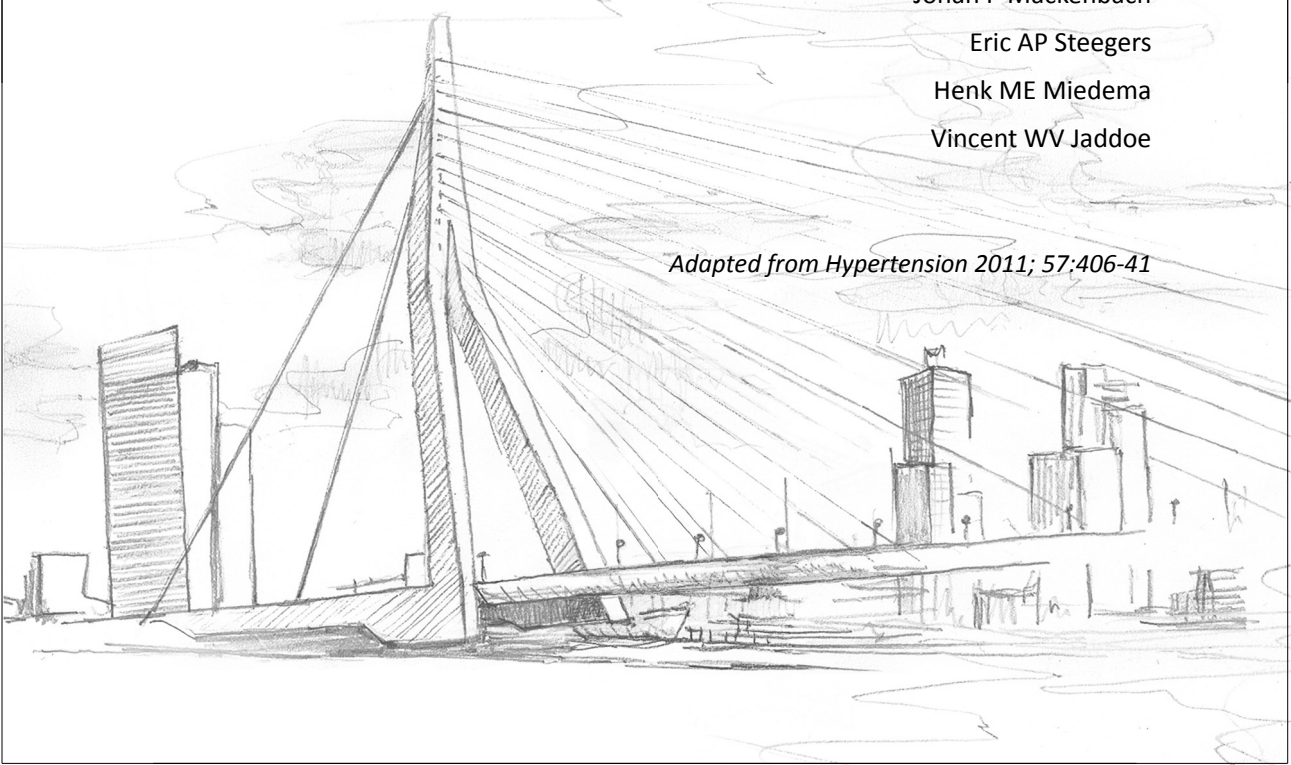
Values are regression coefficients and reflect the difference in placenta weight, birth weight, and placental ratio ((placenta weight/birth weight)*100%) per 10 µg/m^3 increase in air pollution exposure. Models are adjusted for gestational age at delivery.

Chapter 3.3

Air pollution, maternal blood pressure and gestational hypertensive complications

Edith H van den Hooven
Yvonne de Kluizenaar
Frank H Pierik
Albert Hofman
Sjoerd W van Ratingen
Peter YJ Zandveld
Johan P Mackenbach
Eric AP Steegers
Henk ME Miedema
Vincent WV Jaddoe

Adapted from Hypertension 2011; 57:406-41



ABSTRACT

Background: Exposure to air pollution is associated with elevated blood pressure and cardiovascular disease. We assessed the associations of exposure to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) levels with blood pressure measured in each trimester of pregnancy and the risks of gestational hypertension and preeclampsia in 7006 women participating in a prospective cohort study in the Netherlands.

Methods: PM_{10} and NO_2 levels were estimated at the home address for different periods using dispersion modelling techniques. Systolic and diastolic blood pressure were assessed in each trimester of pregnancy. Information on gestational hypertensive disorders was obtained from medical records.

Results: PM_{10} exposure was not associated with first trimester systolic and diastolic blood pressure, but a 10 $\mu g/m^3$ increase in PM_{10} levels was associated with a 1.11 mm Hg (95% CI 0.43 to 1.79) and 2.11 mm Hg (95% CI 1.34 to 2.89) increase in systolic blood pressure in second and third trimester, respectively. Longitudinal analyses showed that elevated PM_{10} exposure levels were associated with a steeper increase in systolic blood pressure throughout pregnancy ($P<0.01$), but not with diastolic blood pressure patterns. Elevated NO_2 exposure was associated with higher systolic blood pressure levels in first, second, and third trimester ($P<0.05$), and with a more gradual increase when analyzed longitudinally ($P<0.01$). PM_{10} exposure, but not NO_2 exposure, was associated with an increased risk of gestational hypertension (odds ratio 1.72, 95% CI 1.12 to 2.63 per 10 $\mu g/m^3$ increase).

Conclusions: Our results suggest that air pollution may affect maternal cardiovascular health during pregnancy. The effects might be small, but relevant on a population level.

INTRODUCTION

Air pollution exposure has been associated with cardiovascular morbidity and mortality [1-3]. Several potential mechanisms for this association have been proposed, including alterations in the autonomic nervous system, induction of pulmonary and systemic inflammation and oxidative stress, endothelial dysfunction, and increased blood coagulability [1, 2, 4, 5]. Elevated blood pressure is a known risk factor for cardiovascular disease, and may be implicated in the association between air pollution and cardiovascular morbidity and mortality. Although results differ among studies [6-18], there is increasing evidence for a relationship between air pollution exposure and elevated blood pressure levels [19]. Pregnant women are a susceptible group for hypertensive disorders, since changes in pregnancy lead to increased stress on the cardiovascular system [20]. A few previous studies observed higher risks of preeclampsia following exposure to air pollution [21-23]. However, the associations of air pollution exposure with blood pressure patterns during pregnancy and gestational hypertension have not yet been examined.

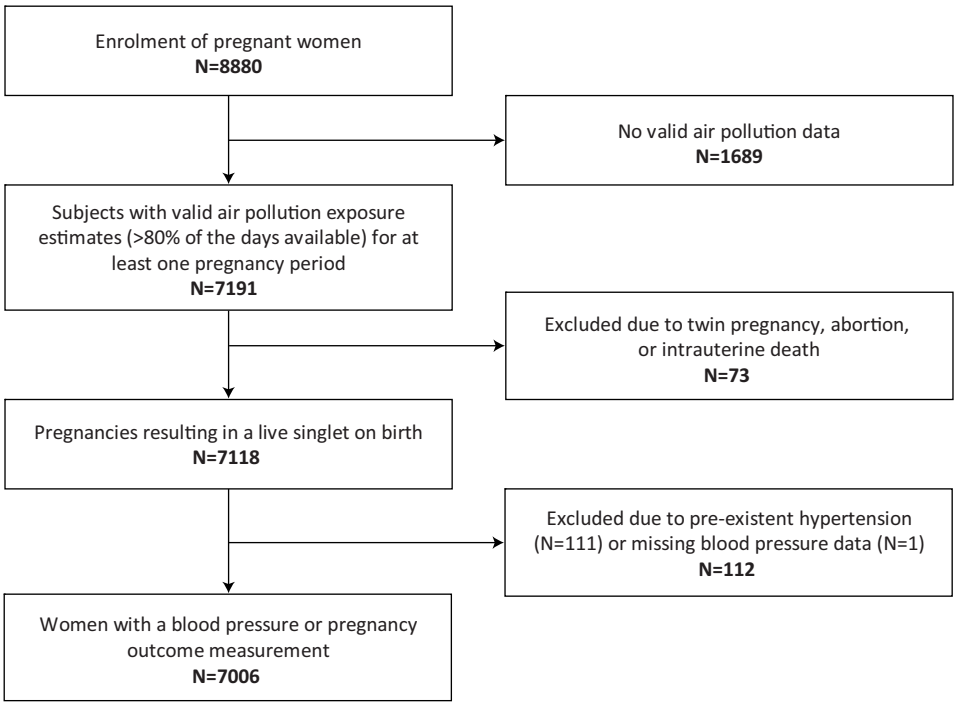
We investigated the associations of particulate matter (PM_{10}) and nitrogen dioxide (NO_2) exposure levels during pregnancy with repeatedly measured blood pressure and the risks of gestational hypertension and preeclampsia in a population-based cohort study among 7006 pregnant women in the Netherlands.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from pregnancy onwards in the city of Rotterdam, the Netherlands, which has been described previously in detail [24]. Mother enrolled between 2001 and 2005. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written consent was obtained from all participants. Of the 8880 prenatally enrolled women, air pollution exposure estimates were available for 7191 mothers (81%). For 1689 mothers, air pollution exposure data could not be assessed due to incomplete address history, or because they had moved outside the study area before delivery [24]. Women with a twin pregnancy (n=73), diagnosed pre-existent hypertension (n=111), or no data on blood pressure during pregnancy (n=1) were excluded. The associations of air pollution exposure with blood pressure levels and gestational hypertensive disorders were analyzed in the remaining 7006 women (see Figure 1 for a flow chart).

Figure 1. Population for analysis.



Air pollution exposure

Individual exposures to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) during pregnancy were assessed at the home address, using a combination of continuous monitoring data and dispersion modelling techniques, taking into account both the spatial and temporal variation in air pollution. A detailed description and a flow chart of the exposure assessment are presented in Chapter 2. In brief, annual average concentrations of PM_{10} and NO_2 for the years 2001-2006 were assessed for all addresses in the study area, using the three Dutch national standard methods for air quality modelling [25]. Hourly concentrations of PM_{10} and NO_2 were derived, taking into account hourly wind conditions and fixed temporal patterns in the contribution of air pollution sources. Subsequently, the hourly concentrations were adjusted for background concentrations, using hourly measurements from three continuous monitoring stations. Based on participants' home addresses, we derived individual exposure estimates for different periods in pregnancy: conception until first blood pressure measurement (median 13.2 weeks of gestation, 95% range 9.6 to 17.5); conception until second blood pressure measurement (median 20.4 weeks of gestation, 95% range 18.5 to 23.6); conception until third blood pressure measurement (median 30.2 weeks of gestation, 95% range 28.4 to 32.9); and conception until delivery (median 40.1 weeks of gestation, 95% range 35.7 to 42.4).

Blood pressure in different trimesters of pregnancy

Blood pressure was measured with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands). All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm, a larger cuff (32~42 cm) was used. The mean value of two blood pressure readings over a 60-second interval was documented for each participant.

Gestational hypertension and preeclampsia

Information on gestational hypertension and preeclampsia was derived from hospital registries. Women suspected of pregnancy complications based on these registries were crosschecked with the original medical records [26]. The diagnosis of gestational hypertension and preeclampsia was based on the following criteria: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24h urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia. Women with hemolysis elevated liver enzymes and low platelets (HELLP) syndrome were included in the preeclampsia group. Women with gestational hypertension were coded as missing for preeclampsia, and women with preeclampsia were coded as missing for gestational hypertension.

Covariates

Medical records completed by midwives and obstetricians were used to obtain information about date and gestational age at delivery. Information on maternal age, educational level, ethnicity, parity, and folic acid supplementation use was obtained by a questionnaire at enrolment. As there were no differences in observed results when ethnicity was categorized into five groups instead of two groups, we reclassified ethnicity into European and non-European. Height and weight were assessed at enrolment. Weight was repeatedly measured at subsequent visits. Maternal smoking and alcohol consumption before and during pregnancy were assessed by questionnaires in each trimester. Road traffic noise exposure was assessed at the home address in accordance with requirements of the EU Environmental Noise Directive, as described in the Supplementary Material of Chapter 2.

Statistical analysis

First, the associations of air pollution exposure with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models. These models take into account the correlation between repeated measurements of the same subject, and allow for incomplete outcome data [27]. The

best fitting models were constructed using fractional polynomials of gestational age [28]. Air pollution exposure averaged over total pregnancy (PM_{10} or NO_2, in quartiles) was included in these models as intercept and as interaction term with gestational age. Second, with multivariate linear regression models, we estimated the associations of PM_{10} and NO_2 levels in the relevant time periods with systolic and diastolic blood pressure in first, second, and early third trimester. Third, the associations of air pollution exposure during pregnancy with gestational hypertension and preeclampsia were assessed using multivariate logistic regression models. In the linear and logistic regression models, air pollution was included as a 10 μg/m^3 increase in exposure. We also conducted analyses with air pollution exposure categorized in quartiles. Additional tests for trend were performed by including the standard deviation value of PM_{10} and NO_2 exposure as a continuous term in the model. All models were adjusted for known determinants of blood pressure patterns during pregnancy (maternal age, height, weight, ethnicity, education, parity, folic acid supplementation use, smoking, and alcohol consumption) and for road traffic noise exposure (as a continuous term). Models with blood pressure patterns were adjusted for weight at measurement and were additionally adjusted for gestational age at measurement. Models with hypertensive complications were adjusted for weight at enrolment. The percentages of missing values within the population for analysis were lower than 1% for continuous data and lower than 15% for categorical data, except for folic acid supplementation use (25%). We applied multiple imputation for missing data in covariates [29]. The repeated measurement analyses were performed using the Statistical Analysis System version 8.2 (SAS, Institute Inc. Gary NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject and exposure characteristics

Table 1 presents the subject characteristics. Blood pressure was measured in 4853 women in first trimester, 6361 women in second trimester, and 6488 women in early third trimester. In total, 17702 blood pressure measurements were available for analyses. Of all women, 3.6% was diagnosed with gestational hypertension, and 2.0% developed preeclampsia or HELLP. The women were evenly distributed by conception season. Supplementary Figure S1 presents mean period-specific PM_{10} and NO_2 exposure levels in the study population, showing that mean exposure levels during pregnancy were 30.3 (SD 3.2) μg/m^3 for PM_{10} and 39.8 (SD 4.2) μg/m^3 for NO_2. Correlations between exposure averages in different pregnancy periods were moderate to strong (PM_{10}: Pearson correlation coefficient r=0.76 to 0.96, NO_2: r=0.68 to 0.94). PM_{10} and NO_2 levels averaged over the same pregnancy period were moderately correlated (r=0.57 to 0.63) (Supplementary Table S1).

Table 1. Subject characteristics (N=7006).

	Mean ± SD, median (95% range), or number (percentage)
Maternal characteristics	
Age at enrolment (yr)	30.5 (19.3-39.4)
Gestational age at enrolment (wks)	14.4 (10.2-28.5)
Height (cm)	167.2 ± 7.4
Weight at enrolment (kg)	67.0 (50.0-102.0)
Body mass index at enrolment (kg/m^2)	23.8 (18.7-36.0)
Parity – n (%)	
Nulliparous	3770 (53.8)
Multiparous	3158 (45.1)
Missing	78 (1.1)
Ethnic background – n (%)	
European	3790 (54.1)
Non-European	2734 (39.0)
Missing	482 (6.9)
Highest completed educational level – n (%)	
No education/primary	720 (10.3)
Secondary	2870 (41.0)
Higher	2825 (40.3)
Missing	591 (8.4)
Smoking in pregnancy – n (%)	
No	5070 (72.4)
Yes	1103 (15.7)
Missing	833 (11.9)
Alcohol consumption in pregnancy – n (%)	
No	3747 (53.5)
Yes	2540 (36.3)
Missing	719 (10.3)
Folic acid supplementation use – n (%)	
Preconceptional	2146 (30.6)
First ten weeks of pregnancy	1617 (23.1)
None	1466 (20.9)
Missing	1777 (25.4)
Noise exposure at delivery (dB(A))	52.5 (45.0-68.2)
Blood pressure measurements	
First trimester (n=4853)	
Gestational age at visit (wks)	13.2 (6.9-17.9)
Weight at visit (kg)	66.0 (50.0-100.9)

Table 1. *Continued*

	Mean ± SD, median (95% range), or number (percentage)
Systolic blood pressure (mm Hg)	115.2 ± 12.0
Diastolic blood pressure (mm Hg)	67.9 ± 9.3
Second trimester (n=6361)	
Gestational age at visit (wks)	20.4 (18.6-23.5)
Weight at visit (kg)	70.0 (52.5-103.0)
Systolic blood pressure (mm Hg)	116.5 ± 11.7
Diastolic blood pressure (mm Hg)	67.0 ± 9.2
Third trimester (n=6488)	
Gestational age at visit (wks)	30.2 (28.5-32.8)
Weight at visit (kg)	75.0 (56.9-108.0)
Systolic blood pressure (mm Hg)	117.8 ± 11.8
Diastolic blood pressure (mm Hg)	68.8 ± 9.2
Gestational hypertensive complications	
Gestational hypertension – n (%)	250 (3.6)
of which nulliparous – n (%)	183 (2.6)
Preeclampsia/HELLP – n (%)	141 (2.0)
of which nulliparous – n (%)	109 (1.6)

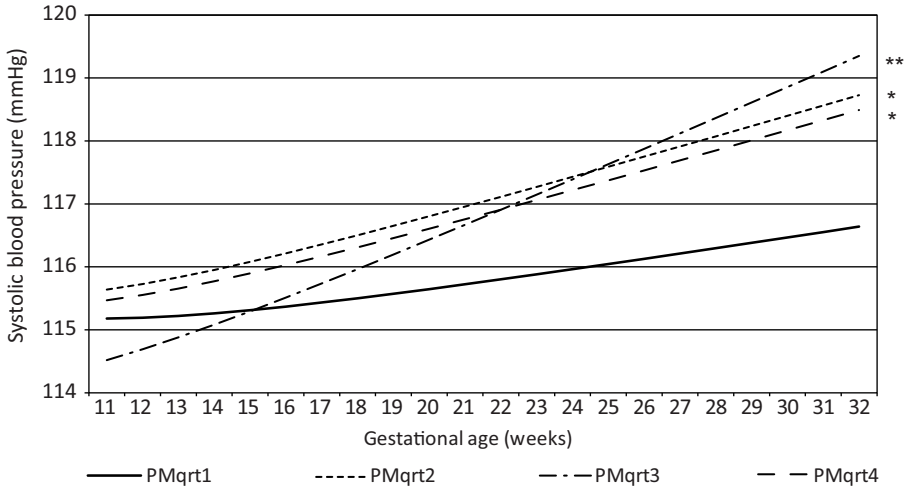
Values are means ± SD, or medians (95% range) for variables with a skewed distribution, and number of subjects (%) in case of categorical variables.

Air pollution and longitudinally measured blood pressure patterns during pregnancy

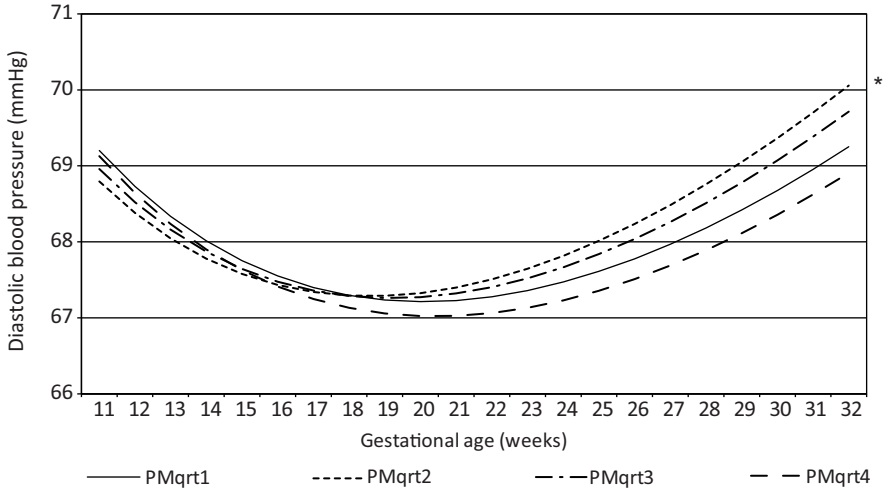
Systolic blood pressure increased throughout pregnancy (Figures 2A and 2C). Since there were no major differences in results before and after adjustment for covariates, we present results for the unadjusted models. Compared to the lowest quartile of PM_{10} exposure, the three higher quartiles showed a steeper increase for systolic blood pressure throughout pregnancy. Compared to the lowest quartile of NO_2 exposure, the three higher quartiles showed a higher systolic blood pressure in early pregnancy, and more gradual increases thereafter. In all air pollution exposure groups, diastolic blood pressure showed a mid-pregnancy dip, with an increase thereafter (Figures 2B and 2D). Compared to the reference group, the second quartile of PM_{10} exposure showed a steeper increase for diastolic blood pressure throughout pregnancy. The diastolic blood pressure patterns for the third and fourth quartiles of PM_{10} exposure were not significantly different from the reference group. Compared to the lowest quartile of NO_2 exposure, the three highest quartiles showed higher mid-pregnancy diastolic blood pressure levels, and more gradual increases thereafter. Effect estimates from these repeated measurement regression analyses are presented in Supplementary Table S2.

Figure 2. Systolic and diastolic blood pressure patterns in different air pollution exposure categories.

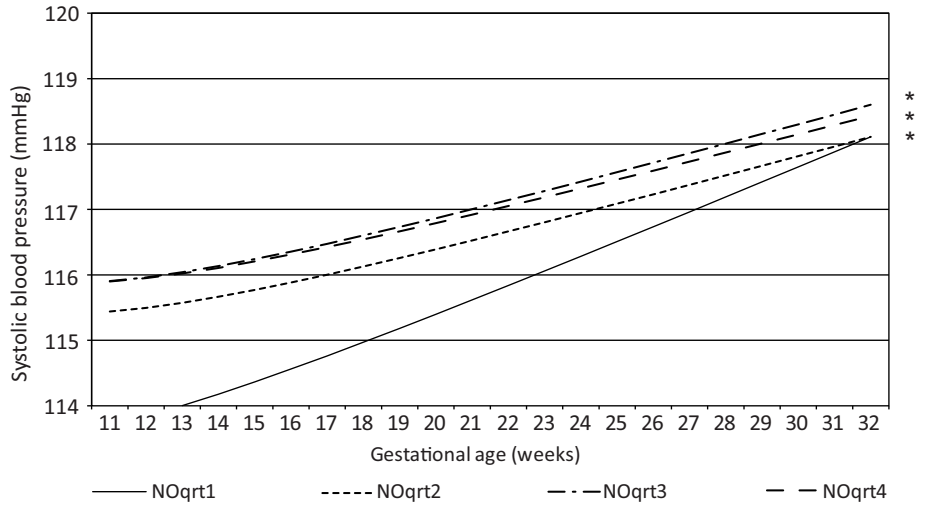
a. Systolic blood pressure in PM_{10} exposure quartiles



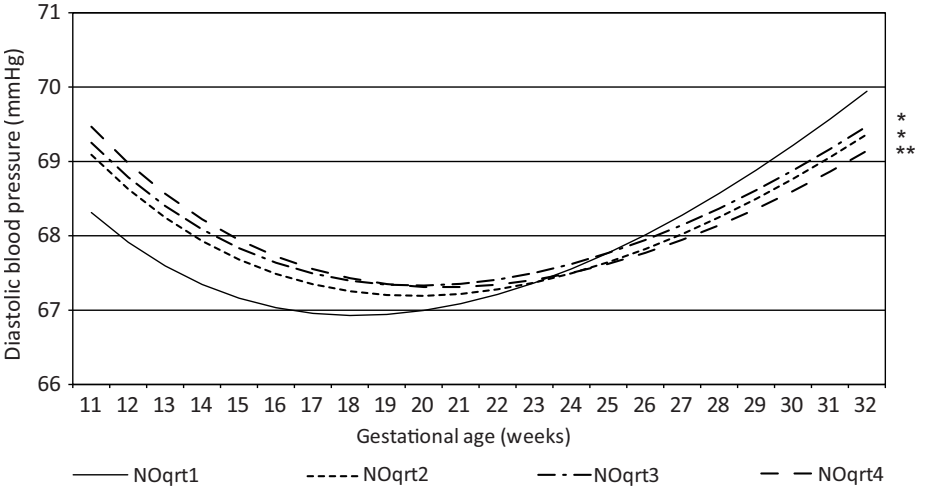
b. Diastolic blood pressure in PM_{10} exposure quartiles



c. Systolic blood pressure in NO_2 exposure quartiles



d. Diastolic blood pressure in NO_2 exposure quartiles



** p<0.001; * p<0.05

Curves represent systolic and diastolic blood pressure patterns for women exposed to different air pollution exposure levels during pregnancy (categorized into quartiles), based on repeated measurements analysis. The lowest quartiles of PM_{10} and NO_2 exposure were used as the reference group. The models are further explained in Supplementary Table S2. P-values reflect the significance level of β_4, which reflects the difference in change in blood pressure per week for systolic and diastolic blood pressure between air pollution exposure quartiles.

Air pollution and blood pressure in different trimesters of pregnancy

Table 2 presents the multivariate cross-sectional associations of PM_{10} and NO_2 levels with systolic and diastolic blood pressure in first, second, and third trimester. In first trimester, no association was observed for PM_{10} exposure with systolic blood pressure. In second and third trimester, PM_{10} exposure was associated with higher systolic blood pressure (difference in systolic blood pressure: 1.11 mm Hg (95% CI 0.43 to 1.79) and 2.11 mm Hg (95% CI 1.34 to 2.89) per 10 μg/m^3 increase in PM_{10}, respectively). PM_{10} exposure was not associated with diastolic blood pressure in first, second, and third trimester. NO_2 exposure was associated with an increased systolic blood pressure in first, second, and third trimester (P<0.001, P<0.001, and P<0.05, respectively). NO_2 exposure was not associated with diastolic blood pressure in first, second, and third trimester. Associations for quartiles of PM_{10} and NO_2 exposure are shown in Supplementary Table S3. When both PM_{10} and NO_2 exposure were included in the same model, no major differences in effect estimates were observed (results not shown). Moreover, when we additionally adjusted for meteorological conditions on the day of the measurement (24h-average temperature, maximum temperature, relative humidity, or barometric pressure), results were comparable (see Supplementary Table S4).

Table 2. Associations of air pollution exposure with systolic and diastolic blood pressure in different trimesters of pregnancy.

Air pollution exposure	First trimester		Second trimester		Third trimester	
	Systolic blood pressure β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)
PM_{10} (per 10 μg/m^3)	-0.01 (-0.76, 0.75)	0.10 (-0.50, 0.70)	1.11 (0.43, 1.79) *	-0.09 (-0.65, 0.46)	2.11 (1.34, 2.89) **	0.25 (-0.37, 0.86)
NO_2 (per 10 μg/m^3)	1.19 (0.54, 1.83) **	0.35 (-0.16, 0.86)	1.35 (0.76, 1.95) **	0.37 (-0.11, 0.85)	0.73 (0.04, 1.41) *	-0.05 (-0.59, 0.50)

** p<0.001; * p<0.05

Values are regression coefficients (95% CI) and reflect the difference in systolic and diastolic blood pressure in mm Hg per 10 μg/m^3 increase in air pollution exposure (averaged from conception until measurement). Models are adjusted for maternal weight and gestational age at measurement, maternal age, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, and noise exposure.

Air pollution and the risks of gestational hypertension and preeclampsia

PM_{10} exposure was associated with an increased risk of developing gestational hypertension (odds ratio (OR) 1.72 (95% CI 1.12 to 2.63) per 10 μg/m^3 increase in PM_{10}) (Table 3). No associations were observed for PM_{10} exposure and the risk of preeclampsia. The associations for PM_{10} exposure became stronger when including NO_2 exposure in the model (results not shown). No associations were observed for NO_2 exposure and the risks of gestational hypertension or preeclampsia. Associations for quartiles of PM_{10} and NO_2 exposure are shown in Supplementary Table S5.

Table 3. Associations of air pollution exposure with gestational hypertensive disorders.

Air pollution exposure	**Gestational hypertension** Odds ratio (95% CI) N=6626	**Preeclampsia** Odds ratio (95% CI) N=6518
PM_{10} (per 10 μg/m^3)	1.72 (1.12, 2.63) *	1.34 (0.78, 2.31)
NO_2 (per 10 μg/m^3)	1.21 (0.83, 1.77)	1.23 (0.75, 2.02)

* p<0.05

Values are odds ratios (95% CI) and reflect the risk for gestational hypertensive disorders per 10 μg/m^3 increase in air pollution exposure during pregnancy. Models are adjusted for maternal age, height, weight at enrolment, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, and noise exposure.

DISCUSSION

This large population-based prospective cohort study from early pregnancy onwards suggests that higher PM_{10} exposure levels are associated with a steeper rise in systolic blood pressure throughout pregnancy and an increased risk of gestational hypertension. This study extends previous epidemiological research on air pollution and cardiovascular endpoints in various populations, and suggests that air pollution exposure may affect cardiovascular health in pregnant women.

Air pollution, blood pressure and the risk of hypertensive complications during pregnancy

In normal pregnancy, blood pressure starts to fall during the first trimester, reaching its lowest point in mid-pregnancy, and then gradually returns to prepregnancy levels by term. This pattern is stronger for diastolic than for systolic blood pressure [30, 31]. Previous research indicates that a different pattern is present in women who develop gestational hypertensive disorders [31]. Their blood pressure is stable during the first half of pregnancy and then continuously increases until delivery [31]. Potential pathways for a prohypertensive effect of air pollution have been suggested to include an increase in sympathetic tone and/or modulation of basal systemic vascular tone as a result of increased

endothelin-1 concentrations [8, 16], and impairment of nitric oxide induced vasodilation [4, 6, 8]. Furthermore, exposure to air pollution might result in placental inflammation [32], which could predispose to the development of gestational hypertensive disorders [20, 33]. The development of these disorders are not only associated with adverse maternal and perinatal outcomes [33], but also with an increased risk of future cardiovascular disease [34].

To our knowledge, this study is the first that examined the associations of air pollution exposure with blood pressure levels in pregnant women. We observed associations for PM_{10} exposure levels with elevated systolic blood pressure levels in second and third trimester, and with a steeper rise in systolic blood pressure throughout pregnancy. No consistent effects of PM_{10} exposure on diastolic blood pressure patterns were observed. Higher NO_2 exposure levels were associated with elevated systolic blood pressure levels in first, second and third trimester. Also, higher NO_2 exposure levels were associated with higher mid-pregnancy diastolic blood pressure levels and a more gradual increase thereafter. Although the differences in blood pressure levels are small and within physiologic ranges, they appear to have the same order of magnitude as effects of maternal smoking during pregnancy [35]. The differences are not clinically relevant on an individual level, but might be relevant on a population level.

There is increasing evidence for a relationship between particulate matter exposure and elevated blood pressure levels [19]. Indeed, a number of previous studies reported increases in systolic blood pressure in non-pregnant adults following exposure to PM_{10} [7, 10], particulate matter from different size ranges [6, 12, 16, 17], or specific components of particulate matter such as black smoke and organic carbon [11, 12]. However, other studies were not able to demonstrate positive associations [8, 9, 13-15, 18]. Results on the associations for particulate matter with diastolic blood pressure are heterogeneous [6-13, 15-17]. Only a few studies evaluated the impact of NO_2 exposure on blood pressure levels and showed inconsistent results [7, 10, 13, 17]. We used pregnancy-specific exposure averages to air pollution. Most previous studies investigated the impact of relatively acute exposures. One study, conducted among 5112 adults, used longer-term (30- and 60-day) exposures, and reported stronger associations for $PM_{2.5}$ exposure with systolic blood pressure when using longer-term averages compared to shorter (1-7 days) averages. The authors suggested that cumulative exposure may have a greater impact on health than acute exposure [6]. This explanation is supported by findings from other studies that observed stronger associations for multiday exposure averages compared to more acute exposures [11, 17].

We observed associations for PM_{10} exposure with an increased risk of gestational hypertension. Associations for PM_{10} exposure with the risk of preeclampsia and for NO_2 exposure with the risks of gestational hypertension and preeclampsia were not significant. Only four studies, all based in the United States, evaluated the associations for air pollution exposure and preeclampsia. The first study reported increased risks for preeclampsia in women exposed to elevated levels of PM_{10} and $PM_{2.5}$ [21], whereas the

second study observed an increased risk in relation to elevated exposure levels of carbon monoxide (CO) and sulfur dioxide, but not for PM_{10}, $PM_{2.5}$, and NO_2 [22]. A third study observed no association with the risk of preeclampsia for $PM_{2.5}$ exposure, and suggestive evidence for CO exposure [36]. The fourth study, and the only one described in a full-text paper, reported increased risks of preeclampsia in women exposed to higher levels of $PM_{2.5}$ and nitrogen oxides (NO_x) [23]. Our study provides further evidence that air pollution exposure is related to gestational hypertensive disorders. More research is needed to confirm these findings and to examine the underlying mechanisms.

Air pollution, especially the traffic-related part, is a complex mixture of several pollutants. PM_{10} and NO_2 might act as surrogates for this mixture, and are therefore not necessarily the causative agents in the relation between air pollution and cardiovascular outcomes. The biological plausibility of health effects induced by particulate matter has been well described [1, 2, 5]. In contrast, it has been proposed that health risks associated with NO_2 may result from traffic-related emissions correlating with $NO_{2,}$ chemical reaction products of NO_2, or NO_2 itself [37]. We observed different results regarding PM_{10} and NO_2 exposure. When including both PM_{10} and NO_2 in the models, results for blood pressure did not change. However, the risks for gestational hypertensive disorders increased for PM_{10} exposure when adjusting for NO_2 exposure, suggesting that PM_{10} may act as better surrogate for the toxic components of air pollution. Nevertheless, although some tests for trends were significant, we observed no clear dose-response relationship for any of the outcomes when examining quartiles of exposure. Therefore, the presence of a causal association between the studied pollutants and blood pressure cannot directly be assumed.

Methodological considerations

A main strength of our study is the use of repeated blood pressure measurements during pregnancy, with 17702 blood pressure measurements available for analysis. Since the last blood pressure measurement was scheduled at a gestational age of 30 weeks, our findings in late third trimester should be interpreted with caution.

Previously demonstrated estimates for the incidence of preeclampsia range from 2 to 8% [20, 33, 38] and from 6 to 9% for gestational hypertension [20, 39]. This study was performed in a population-based cohort with a selection towards a relatively highly educated and more healthy study population [24]. The selective participation might have resulted in an underrepresentation of pregnant women with increased risks of pregnancy hypertensive disorders. We used medical chart review for definition of gestational hypertension. In the Netherlands, community midwives often remain responsible for the care of women with a diastolic blood pressure between 90 and 100 mmHg. As a result, our study may have missed some mild cases of gestational hypertension [39]. The low incidences may have resulted in a loss of power to detect a relationship between air pollution and hypertensive complications during pregnancy.

Many previous studies were limited in their ability to consider both the intra-urban gradients and temporal variation in air pollutants. A few earlier studies on blood pressure incorporated this information, either by monitoring personal, indoor-home, or outdoor-home concentrations [11, 12, 14], or by controlling exposure in an exposure chamber [8, 9, 15]. However, these studies were based on relatively small sample sizes (n<100), and were often conducted in elderly subjects or subjects with pre-existing diseases [11, 12, 14]. In our study, we were able to consider detailed spatial and temporal contrasts in exposure. The quality of the assigned exposure estimates was further enhanced by allowing for residential mobility of the women during pregnancy. There might still be misclassification of air pollution exposure. Exposure levels were estimated at the home address, whereas pregnant women do not spend all of their time at home. Other types of exposure (e.g. indoor, occupational, or commuting) were not taken into account. If anything, misclassification in the air pollution exposure assessment is expected to be random, and might have resulted in underestimation of the effects.

Furthermore, we were able to control for many potential confounders not always available in other studies, such as maternal smoking, alcohol consumption, educational level, weight, height, and noise exposure. However, residual confounding due to unmeasured variables might still be an issue.

Conclusion

We showed in a population-based prospective cohort study in the Netherlands that exposure to higher PM_{10} levels was associated with a steeper rise in systolic blood pressure throughout pregnancy and an increased risk for developing gestational hypertension. Our results suggest that air pollution may affect maternal cardiovascular health during pregnancy. The effects might be small, but relevant on a population level. Future studies are needed to replicate the findings and explore the underlying mechanisms.

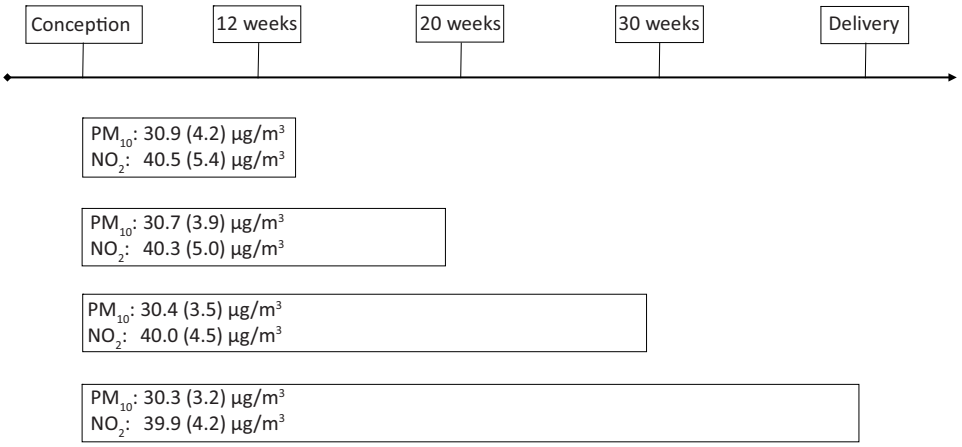
REFERENCES

1. Brook RD. Cardiovascular effects of air pollution. *Clinical Science.* 2008; 115(6):175-87.
2. Pope CA, 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc.* 2006; 56(6):709-42.
3. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation.* 2010; 121(25):2755-65.
4. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
5. Peters A. Particulate matter and heart disease: evidence from epidemiological studies. *Toxicol Appl Pharmacol.* 2005; 207(2 Suppl):477-82.
6. Auchincloss AH, Diez Roux AV, Dvonch JT, Brown PL, Barr RG, Daviglus ML, et al. Associations between recent exposure to ambient fine particulate matter and blood pressure in the Multi-ethnic Study of Atherosclerosis (MESA). *Environ Health Perspect.* 2008; 116(4):486-91.
7. Chuang KJ, Yan YH, Cheng TJ. Effect of air pollution on blood pressure, blood lipids, and blood sugar: a population-based approach. *J Occup Environ Med.* 2010; 52(3):258-62.
8. Urch B, Silverman F, Corey P, Brook JR, Lukic KZ, Rajagopalan S, et al. Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ Health Perspect.* 2005; 113(8):1052-5.
9. Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, et al. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. *Hypertension.* 2009; 54(3):659-67.
10. Choi JH, Xu QS, Park SY, Kim JH, Hwang SS, Lee KH, et al. Seasonal variation of effect of air pollution on blood pressure. *J Epidemiol Community Health.* 2007; 61(4):314-8.
11. Delfino RJ, Tjoa T, Gillen DL, Staimer N, Polidori A, Arhami M, et al. Traffic-related air pollution and blood pressure in elderly subjects with coronary artery disease. *Epidemiology.* 2010; 21(3):396-404.
12. Liu L, Ruddy T, Dalipaj M, Poon R, Szyszkowicz M, You H, et al. Effects of indoor, outdoor, and personal exposure to particulate air pollution on cardiovascular physiology and systemic mediators in seniors. *J Occup Environ Med.* 2009; 51(9):1088-98.
13. Madsen C, Nafstad P. Associations between environmental exposure and blood pressure among participants in the Oslo Health Study (HUBRO). *Eur J Epidemiol.* 2006; 21(7):485-91.
14. Jansen KL, Larson TV, Koenig JQ, Mar TF, Fields C, Stewart J, et al. Associations between health effects and particulate matter and black carbon in subjects with respiratory disease. *Environ Health Perspect.* 2005; 113(12):1741-6.
15. Gong H, Jr., Linn WS, Sioutas C, Terrell SL, Clark KW, Anderson KR, et al. Controlled exposures of healthy and asthmatic volunteers to concentrated ambient fine particles in Los Angeles. *Inhal Toxicol.* 2003; 15(4):305-25.
16. Ibald-Mulli A, Stieber J, Wichmann HE, Koenig W, Peters A. Effects of air pollution on blood pressure: a population-based approach. *Am J Public Health.* 2001; 91(4):571-7.
17. Zanobetti A, Canner MJ, Stone PH, Schwartz J, Sher D, Eagan-Bengston E, et al. Ambient pollution and blood pressure in cardiac rehabilitation patients. *Circulation.* 2004; 110(15):2184-9.
18. de Kluizenaar Y, Gansevoort RT, Miedema HM, de Jong PE. Hypertension and road traffic noise exposure. *J Occup Environ Med.* 2007; 49(5):484-92.
19. Brook RD, Rajagopalan S. Particulate matter, air pollution, and blood pressure. *J Am Soc Hypertens.* 2009; 3(5):332-50.
20. Yoder SR, Thornburg LL, Bisognano JD. Hypertension in pregnancy and women of childbearing age. *Am J Med.* 2009; 122(10):890-5.
21. Mendola P, Tandon R, Parker J, Kravets N, MacKay A. Delivery Hospitalization Complicated by Preeclampsia in Relation to Ambient Particulate Matter Exposure Prior to Admission in the United States, 1999-2005. *Epidemiology.* 2009; 20(6):S62.
22. Woodruff TJ, Morello-Frosch R, Jesdale B. Air Pollution and Preeclampsia Among Pregnant Women in California, 1996-2004. *Epidemiology.* 2008; 19(6):S310.

23. Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B. Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. *Environ Health Perspect.* 2009; 117(11):1773-9.
24. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
25. Netherlands Ministry of Infrastructure and the Environment: Air Quality Decree 2007 (Regeling beoordeling Luchtkwaliteit 2007). 2007. Available: http://wetten.overheid.nl/BWBR0022817
26. Coolman M, de Groot CJ, Jaddoe VW, Hofman A, Raat H, Steegers EA. Medical record validation of maternally reported history of preeclampsia. *J Clin Epidemiol.* 2010; 63(8):932-7.
27. Goldstein H, *Multilevel statistical models*. 2nd ed. 1995, London: Edward Arnold.
28. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol.* 1999; 28(5):964-74.
29. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res.* 2007; 16(3):219-42.
30. Hermida RC, Ayala DE, Mojon A, Fernandez JR, Alonso I, Aguilar MF, et al. Differences in circadian blood pressure variability during gestation between healthy and complicated pregnancies. *Am J Hypertens.* 2003; 16(3):200-8.
31. Hermida RC, Ayala DE, Iglesias M. Predictable blood pressure variability in healthy and complicated pregnancies. *Hypertension.* 2001; 38(3 Pt 2):736-41.
32. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
33. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet.* 2005; 365(9461):785-99.
34. Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstet Gynecol.* 2009; 114(5):961-70.
35. Bakker R, Steegers EA, Mackenbach JP, Hofman A, Jaddoe VW. Maternal smoking and blood pressure in different trimesters of pregnancy: the Generation R study. *J Hypertens.* 2010; 28(11):2210-8.
36. Rudra C, Williams M. A Prospective Study of Periconceptional Ambient Air Pollutant Exposures and Preeclampsia Risk. *Epidemiology.* 2006; 17(6):S104-S105.
37. World Health Organization: Air quality guidelines. Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulphur dioxide. 2006. Available: http://whqlibdoc.who.int/hq/2006/WHO_SDE_PHE_OEH_06.02_eng.pdf
38. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet.* 2010; 376(9741):631-44.
39. Silva L, Coolman M, Steegers E, Jaddoe V, Moll H, Hofman A, et al. Maternal educational level and risk of gestational hypertension: the Generation R Study. *J Hum Hypertens.* 2008; 22(7):483-92.

SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Period-specific PM_{10} and NO_2 exposure levels in the study population.



Values reflect average exposure levels (mean and SD) for different periods in pregnancy: 1) conception until first blood pressure measurement (median 13.2 weeks of gestation, 95% range 9.6 to 17.5); 2) conception until second blood pressure measurement (median 20.4 weeks of gestation, 95% range 18.5 to 23.6); 3) conception until third blood pressure measurement (median 30.2 weeks of gestation, 95% range 28.4 to 32.9); and 4) conception until delivery (median 40.1 weeks of gestation, 95% range 35.7 to 42.4).

Supplementary Table S1. Correlation coefficients between period-specific PM_{10} and NO_2 exposure averages.

	PM_{10}				NO_2			
	Conception – 1st trimester	Conception – 2nd trimester	Conception – 3rd trimester	Total pregnancy	Conception – 1st trimester	Conception – 2nd trimester	Conception – 3rd trimester	Total pregnancy
PM_{10}								
Conception – 1st trimester	1							
Conception – 2nd trimester	0.92	1						
Conception – 3rd trimester	0.84	0.93	1					
Total pregnancy	0.76	0.87	0.96	1				
NO_2								
Conception – 1st trimester	0.57	0.57	0.52	0.47	1			
Conception – 2nd trimester	0.52	0.59	0.58	0.54	0.92	1		
Conception – 3rd trimester	0.43	0.54	0.61	0.60	0.77	0.92	1	
Total pregnancy	0.42	0.50	0.59	0.63	0.68	0.81	0.94	1

Values reflect Pearson correlation coefficients. All p-values testing equality to 0 are <0.001.

Supplementary Table S2. Associations of air pollution exposure and longitudinally measured systolic and diastolic blood pressure.

	Difference in intercept (95% CI)	**p-value**	**Difference in blood pressure increase per week** (95% CI)	**p-value**
PM_{10}	**Systolic blood pressure**		**Systolic blood pressure**	
1st quartile	*Reference*		*Reference*	
2nd quartile	-0.3219 (-1.6763, 1.0325)	0.64	0.0755 (0.0186, 0.1324)	<0.01
3rd quartile	-2.5172 (-3.8533, -1.1811)	<0.001	0.1652 (0.1102, 0.2202)	<0.001
4th quartile	-0.5521 (-1.9011, 0.7968)	0.42	0.0776 (0.0232, 0.1320)	<0.01
PM_{10}	**Diastolic blood pressure**		**Diastolic blood pressure**	
1st quartile	*Reference*		*Reference*	
2nd quartile	-1.0182 (-2.0766, 0.0403)	0.06	0.0582 (0.0137, 0.1026)	<0.05
3rd quartile	-0.6793 (-1.7234, 0.3648)	0.20	0.0377 (-0.0053, 0.0807)	0.09
4th quartile	0.0461 (-1.0493, 1.0585)	0.99	-0.0130 (-0.0511, 0.0340)	0.69
NO_2	**Systolic blood pressure**		**Systolic blood pressure**	
1st quartile	*Reference*		*Reference*	
2nd quartile	2.6624 (1.2866, 4.0383)	<0.001	-0.0818 (-0.1400, -0.0235)	<0.01
3rd quartile	3.3050 (1.9523, 4.6577)	<0.001	-0.0835 (-0.1413, -0.0294)	<0.01
4th quartile	3.3211 (1.9771, 4.6652)	<0.001	-0.0909 (-0.1455, -0.0363)	<0.01
NO_2	**Diastolic blood pressure**		**Diastolic blood pressure**	
1st quartile	*Reference*		*Reference*	
2nd quartile	1.4089 (0.3337, 2.4841)	<0.05	-0.0613 (-0.1068, -0.0158)	<0.01
3rd quartile	1.7041 (0.6471, 2.7611)	<0.01	-0.0653 (-0.1090, -0.0215)	<0.01
4th quartile	2.1231 (1.0731, 3.1732)	<0.001	-0.0890 (-0.1317, -0.0463)	<0.001

Values are based on repeated linear regression models and reflect the difference in systolic blood pressure and diastolic blood pressure patterns in mm Hg (95% CI) for each quartile of air pollution exposure during pregnancy compared to the reference group (lowest quartile).
Models are based on 17702 measurements, and can be written as follows:

- Systolic blood pressure = $\beta_0 + \beta_1$*air pollution + β_2*gestational age + β_3*gestational age^{-2} + β_4*air pollution*gestational age
- Diastolic blood pressure = $\beta_0 + \beta_1$*air pollution + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*air pollution*gestational age

In these models, '$\beta_0 + \beta_1$*air pollution' reflects the intercept, 'β_2*gestational age + β_3*gestational age^{-2}' reflects the slope of change in blood pressure per week for systolic blood pressure, and 'β_2*gestational age + β_3*gestational age$^{0.5}$' reflects the slope of change in blood pressure per week for diastolic blood pressure.
P-values reflect the significance levels of β_1 and β_4, which reflect the difference in intercept, and the difference in change in blood pressure per week for systolic and diastolic blood pressure between different air pollution exposure quartiles, respectively.

Supplementary Table S3. Associations of air pollution exposure with systolic and diastolic blood pressure in different trimesters of pregnancy.

	First trimester		Second trimester		Third trimester	
	Systolic blood pressure β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)
PM_{10}						
1st quartile	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
2nd quartile	1.21 (0.34, 2.09) *	0.41 (-0.28, 1.10)	1.87 (1.13, 2.60) **	0.75 (0.15, 1.34) *	1.98 (1.24, 2.73) **	0.99 (0.40, 1.59) *
3rd quartile	-0.47 (-1.35, 0.41)	0.31 (-0.39, 1.00)	1.09 (0.36, 1.83) *	0.00 (-0.60, 0.60)	2.46 (1.71, 3.20) **	0.77 (0.18, 1.37) *
4th quartile	0.42 (-0.47, 1.31)	0.24 (-0.47, 0.95)	1.35 (0.61, 2.09) **	-0.05 (-0.65, 0.56)	1.92 (1.17, 2.68) **	0.27 (-0.33, 0.87)
Per 1 SD increase	-0.01 (-0.33, 0.31)	0.03 (-0.22, 0.29)	0.43 (0.17, 0.70) *	-0.04 (-0.25, 0.18)	0.73 (0.46, 1.01) **	0.08 (-0.14, 0.30)
P for trend	0.94	0.79	<0.01	0.73	<0.01	0.46
NO_2						
1st quartile	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
2nd quartile	1.27 (0.39, 2.16) *	0.65 (-0.05, 1.34) ‡	1.29 (0.55, 2.03) *	0.30 (-0.30, 0.90)	0.76 (0.00, 1.51) *	0.19 (-0.41, 0.79)
3rd quartile	2.00 (1.11, 2.89) **	0.76 (0.06, 1.47) *	1.50 (0.75, 2.24) **	0.48 (-0.13, 1.09)	0.74 (-0.03, 1.51) ‡	-0.06 (-0.67, 0.56)
4th quartile	1.78 (0.82, 2.74) **	0.62 (-0.14, 1.38)	1.74 (0.92, 2.56) **	0.21 (-0.46, 0.88)	0.85 (0.00, 1.70) ‡	-0.13 (-0.81, 0.55)
Per 1 SD increase	0.63 (0.28, 0.98) **	0.18 (-0.10, 0.45)	0.68 (0.39, 0.98) **	0.18 (-0.06, 0.42)	0.33 (0.02, 0.64) *	-0.03 (-0.28, 0.22)
P for trend	<0.01	0.21	<0.01	0.14	0.04	0.83

** p<0.001; * p<0.05; ‡ p<0.10

Values are regression coefficients (95% CI) and reflect the difference in systolic and diastolic blood pressure in mm Hg for each quartile of air pollution exposure (averaged from conception until measurement) compared with the reference group (lowest quartile). Models are adjusted for maternal weight and gestational age at measurement, maternal age, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, and noise exposure. Tests for trend were performed by including standard deviation values for the exposure variables (observed value/standard deviation value in the study population) as a continuous term in the regression model.

Supplementary Table S4. Associations of air pollution exposure with systolic and diastolic blood pressure in different trimesters of pregnancy, additionally adjusted for meteorological variables.

	First trimester		Second trimester		Third trimester	
	Systolic blood pressure β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)	**Systolic blood pressure** β (95% CI)	**Diastolic blood pressure** β (95% CI)
PM_{10} [a]	-0.01 (-0.76, 0.75)	0.10 (-0.50, 0.70)	1.11 (0.43, 1.79) *	-0.09 (-0.65, 0.46)	2.11 (1.34, 2.89) **	0.25 (-0.37, 0.86)
PM_{10} [b]	-0.26 (-1.02, 0.50)	-0.02 (-0.62, 0.58)	1.15 (0.47, 1.83) *	-0.07 (-0.62, 0.48)	2.54 (1.77, 3.32) **	0.47 (-0.16, 1.09)
PM_{10} [c]	-0.16 (-0.91, 0.60)	0.02 (-0.58, 0.62)	1.27 (0.59, 1.95) **	0.00 (-0.55, 0.55)	2.68 (1.90, 3.46) **	0.55 (-0.08, 1.17) *
PM_{10} [d]	0.05 (-0.72, 0.81)	0.24 (-0.37, 0.85)	1.27 (0.58, 1.97) **	-0.01 (-0.57, 0.56)	2.37 (1.58, 3.16)	0.35 (-0.28, 0.98)
PM_{10} [e]	-0.03 (-0.79, 0.73)	0.12 (-0.48, 0.72)	1.09 (0.40, 1.77) *	-0.09 (-0.65, 0.46)	2.09 (1.32, 2.87) **	0.23 (-0.39, 0.85)
NO_2 [a]	1.19 (0.54, 1.83) **	0.35 (-0.16, 0.86)	1.35 (0.76, 1.94) **	0.37 (-0.11, 0.85)	0.73 (0.04, 1.41) *	-0.05 (-0.59, 0.50)
NO_2 [b]	0.03 (-0.68, 0.74)	-0.27 (-0.83, 0.30)	0.68 (0.08, 1.29) *	-0.02 (-0.52, 0.48)	0.76 (0.08, 1.45) *	-0.02 (-0.57, 0.52)
NO_2 [c]	0.18 (-0.52, 0.88)	-0.22 (-0.78, 0.33)	0.85 (0.25, 1.45) *	0.05 (-0.44, 0.54)	0.90 (0.22, 1.58) *	0.05 (-0.49, 0.60)
NO_2 [d]	1.19 (0.54, 1,83) **	0.35 (-0.16, 0.86)	1.45 (0.86, 2.05) **	0.43 (-0.05, 0.92) *	0.97 (0.26, 1.67) *	0.06 (-0.50, 0.62)
NO_2 [e]	1.16 (0.51, 1.80)	0.37 (-0.14, 0.88)	1.32 (0.73, 1.92) **	0.38 (-0.11, 0.86)	0.70 (0.01, 1.39) *	-0.06 (-0.61, 0.49)

** p<0.001; * p<0.05

Values are regression coefficients (95% CI) and reflect the difference in systolic and diastolic blood pressure in mm Hg per 10 μg/m^3 increase in air pollution exposure (averaged from conception until measurement).

[a] Models are adjusted for maternal weight and gestational age at measurement, maternal age, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, and noise exposure.

[b] Models are additionally adjusted for 24h-average temperature on the day of the measurement.

[c] Models are additionally adjusted for maximum temperature on the day of the measurement.

[d] Models are additionally adjusted for relative humidity on the day of the measurement.

[e] Models are additionally adjusted for barometric pressure on the day of the measurement.

Supplementary Table S5. Associations of air pollution exposure with the risks of gestational hypertensive disorders.

	Gestational hypertension Odds ratio (95% CI) N=6626	**Preeclampsia** Odds ratio (95% CI) N=6518
PM_{10}		
1st quartile	*Reference* n=59	*Reference* n=31
2nd quartile	0.84 (0.56, 1.27) n=44	0.87 (0.51, 1.46) n=26
3rd quartile	1.46 (1.02, 2.10) * n=77	1.31 (0.80, 2.12) n=38
4th quartile	1.37 (0.94, 2.00) ‡ n=67	1.43 (0.88, 2.30) n=44
P for trend	0.01	0.29
NO_2		
1st quartile	*Reference* n=74	*Reference* n=28
2nd quartile	0.77 (0.52, 1.12) n=51	0.97 (0.57, 1.65) n=29
3rd quartile	0.91 (0.63, 1.32) n=60	1.38 (0.83, 2.27) n=42
4th quartile	1.02 (0.67, 1.55) n=62	1.35 (0.77, 2.38) n=40
P for trend	0.32	0.41

* $p<0.05$; ‡ $p<0.10$

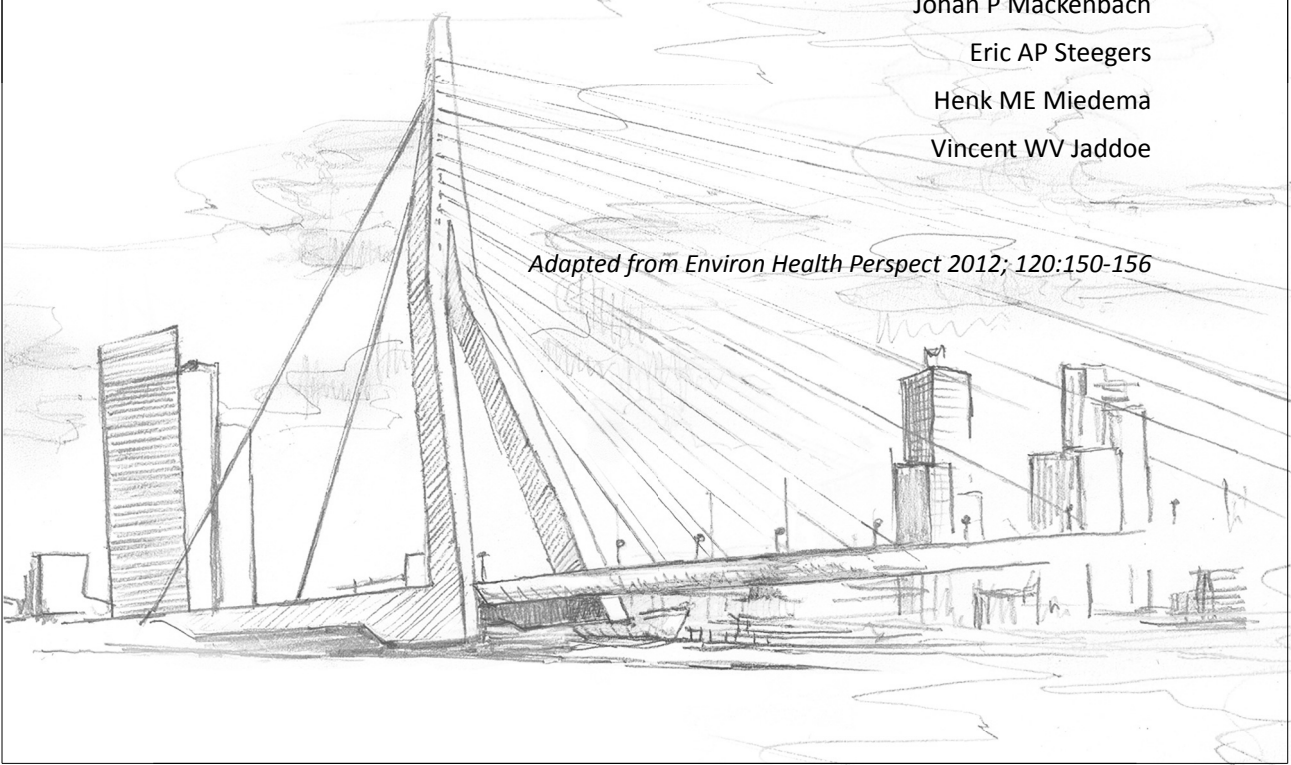
Values are odds ratios (95% CI) and reflect the risk for gestational hypertensive disorders for each quartile of air pollution exposure during pregnancy compared with the reference group (lowest quartile). Tests for trend were performed by including standard deviation values for the exposure variables (observed value/standard deviation value in the study population) as a continuous term in the regression model. Models are adjusted for maternal age, height, weight at enrolment, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, and noise exposure. Women with gestational hypertension were coded as missing for preeclampsia, and women with preeclampsia were coded as missing for gestational hypertension.

Chapter 3.4

Air pollution, fetal growth and neonatal complications

Edith H van den Hooven
Frank H Pierik
Yvonne de Kluizenaar
Sten P Willemsen
Albert Hofman
Sjoerd W van Ratingen
Peter YJ Zandveld
Johan P Mackenbach
Eric AP Steegers
Henk ME Miedema
Vincent WV Jaddoe

Adapted from Environ Health Perspect 2012; 120:150-156



ABSTRACT

Background: Air pollution exposure during pregnancy might have trimester-specific effects on fetal growth. We prospectively evaluated the associations of maternal air pollution exposure with fetal growth characteristics and adverse birth outcomes in 7772 subjects in the Netherlands.

Methods: PM_{10} and NO_2 levels were estimated using dispersion modelling at the home address. Fetal head circumference, length and weight were estimated in each trimester by ultrasound. Information on birth outcomes was obtained from medical records.

Results: In cross-sectional analyses, NO_2 levels were inversely associated with fetal femur length in second and third trimester, and PM_{10} and NO_2 levels both were associated with smaller fetal head circumference in third trimester (difference -0.18 mm, 95% CI -0.24 to -0.12 per 1 $\mu g/m^3$ increase in PM_{10} and -0.12 mm, 95% CI -0.17 to -0.06 per 1 $\mu g/m^3$ increase in NO_2). Average PM_{10} and NO_2 exposure levels during pregnancy were not associated with head circumference and length at birth or neonatally, but were inversely associated with birth weight (difference -3.6g, 95% CI -6.7 to -0.4 per 1 $\mu g/m^3$ increase in PM_{10} and -3.4g, 95% CI -6.2 to -0.6 per 1 $\mu g/m^3$ increase in NO_2). Longitudinal analyses showed similar patterns for head circumference and weight, but for length no associations were observed. The third and fourth quartiles of PM_{10} exposure were positively associated with preterm birth (odds ratio (OR) 1.40, 95% CI 1.03 to 1.89, and OR 1.32, 95% CI 0.96 to 1.79, respectively), relative to the first quartile. The third quartile of PM_{10} exposure, but not the fourth quartile, was associated with an increased risk of small size for gestational age at birth (OR 1.38, 95% CI 1.00 to 1.90). No consistent associations were observed for NO_2 levels and adverse birth outcomes.

Conclusions: Results suggest that maternal air pollution exposure is inversely associated with fetal growth during the second and third trimester and with weight at birth. Elevated PM_{10} exposure was positively associated with preterm birth and small size for gestational age at birth.

INTRODUCTION

Maternal exposure to air pollution during pregnancy has been suggested to be associated with increased risks of adverse birth outcomes such as low birth weight, intrauterine growth restriction, and preterm birth [1]. Thus far, results are not consistent: reported associations (or absence thereof) for specific air pollutants, exposure periods, and birth outcomes have differed between studies [2, 3]. Most previous studies defined fetal growth using measures at birth, such as weight, length, and head circumference [4-7]. However, since impaired growth during early pregnancy may be compensated for in the remaining intrauterine life, the eventual measures at birth can represent both normal and abnormal fetal growth and development. To provide insight into the specific effects of maternal air pollution exposure, and to identify critical windows of exposure, it is of interest to assess fetal growth in different periods of pregnancy rather than only at birth. A small number of studies have examined the impact of air pollution exposure on fetal growth using ultrasound measurements during pregnancy as direct estimates of growth [8-10]. These studies were based on small numbers, did not report measurements in each trimester of pregnancy, or were not able to consider the spatiotemporal variation in air pollution exposure.

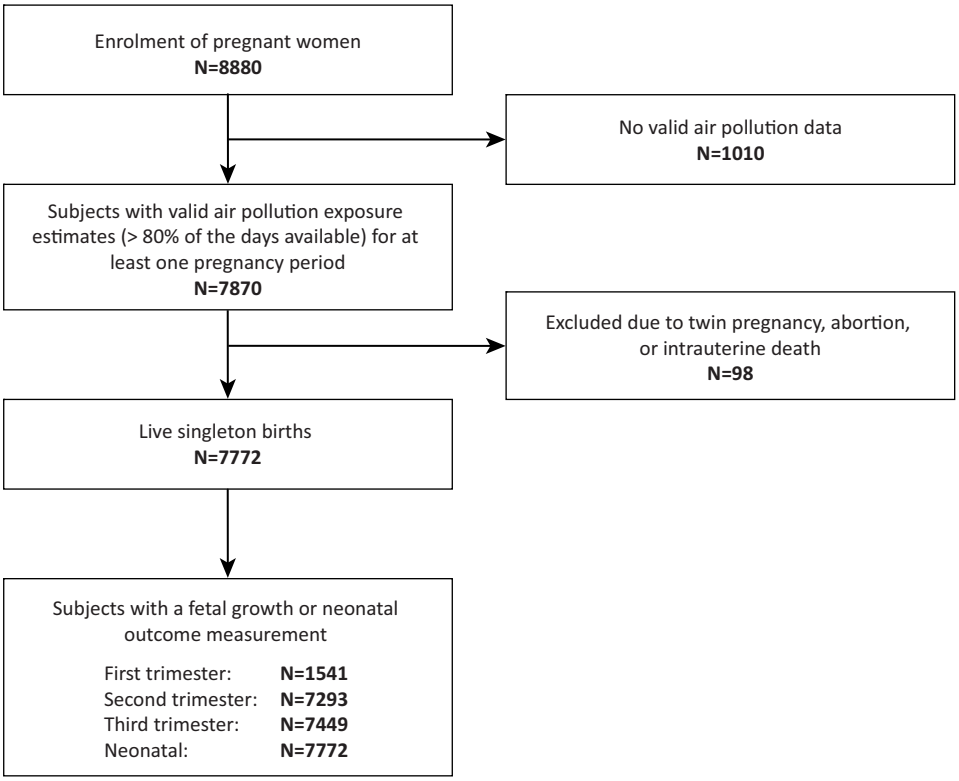
We investigated associations of particulate matter (PM_{10}) and nitrogen dioxide (NO_2) exposure levels during pregnancy with fetal growth characteristics assessed by ultrasound in each trimester of pregnancy and adverse birth outcomes in a population-based cohort study among 7772 pregnant women in the Netherlands.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from pregnancy onwards in Rotterdam, the Netherlands [11]. Mothers enrolled between 2001 and 2005. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written consent was obtained from all participants. Of the 8880 prenatally enrolled women, air pollution exposure estimates were available for 7870 mothers (89%). For 1010 mothers, air pollution concentrations could not be estimated because of incomplete address history, or because they had moved outside the study area before delivery. Women with a twin pregnancy (n=79), abortion (n=7), or intrauterine death (n=12) were excluded. The cohort for analysis consisted of 7772 mothers and singleton live births (see Figure 1).

Figure 1. Population for analysis.



Air pollution exposure

Individual exposures to PM_{10} and NO_2 during pregnancy were assessed at the home address, using a combination of continuous monitoring data and dispersion modelling techniques, taking into account both the spatial and temporal variation in air pollution. A detailed description and a flow chart of the exposure assessment are presented in Chapter 2. In brief, annual average concentrations of PM_{10} and NO_2 for the years 2001-2006 were assessed for all addresses in the study area, using the three Dutch national standard methods for air quality modelling [12]. Hourly concentrations of PM_{10} and NO_2 were derived, taking into account hourly wind conditions and fixed temporal patterns in the contribution of air pollution sources. Subsequently, the hourly concentrations were adjusted for background concentrations, using hourly measurements from three continuous monitoring stations. Based on participants' home addresses, we derived individual exposure estimates for different periods in pregnancy: conception until first trimester ultrasound, conception until second trimester ultrasound, conception until early third trimester ultrasound, and conception until delivery.

Fetal growth characteristics

Fetal ultrasound examinations were performed in each trimester of pregnancy using standardized ultrasound procedures [13, 14]. In the first trimester, we used fetal crown-rump length to assess fetal growth only in mothers with a known date of last menstrual period (LMP) and a regular menstrual cycle of 28 (range 24 to 32) days [15]. Date of LMP was obtained from the community midwife or hospital, and was confirmed orally with the subjects at the ultrasound visit. For growth measurements in the second and third trimester we used gestational age based on ultrasound examination [14], since using LMP has several limitations [16]. Fetal growth measurements used in the present study included head circumference and femur length. Femur length was used as a proxy for fetal body length. The intra- and interobserver reproducibility of fetal biometry measurements was good [17]. Estimated fetal weight was calculated using femur length and head and abdominal circumference using the Hadlock formula [18]. Longitudinal growth curves and gestational age-adjusted standard deviation (SD) scores were constructed for all fetal growth measurements based on reference growth curves of our own study population [14].

Birth outcomes

Medical records and hospital registries were used to obtain information about date of birth, gestational age at birth, fetal sex, birth weight, and birth length. We completed missing data on birth length (16%) with measurements of length from the first visit at the routine child health center within the first two months after birth, which had negligible influence on the results. Head circumference was not routinely measured at birth. Therefore, we used head circumference from the first child health center visit. The regression models with neonatal length or head circumference as the outcome were adjusted for postconceptional age (gestational age for measurements at birth or gestational age + postnatal age for measurements at the child health center). Models with neonatal length were also adjusted for the method of measurement (birth or child health center). Gestational age and sex-adjusted SD scores for birth weight and birth length were constructed based on reference charts from a North-European birth cohort [19]. Postnatal age and sex-adjusted SD scores for neonatal head circumference and length were constructed using reference charts from a nationwide study in the Netherlands [20]. The regression models with SD scores for neonatal head circumference or length were adjusted for gestational age at birth, and models with SD scores for neonatal length were also adjusted for the method of measurement. Adverse birth outcomes were defined as preterm birth (gestational age <37 weeks), low birth weight (birth weight <2500 grams), and small size for gestational age at birth (SGA) (gestational age and sex-adjusted birth weight <5th percentile).

Covariates

Information on maternal age, educational level (no education/primary; secondary; higher), parity (nulliparous; multiparous), folic acid supplementation use (preconceptional; first ten weeks of pregnancy; none) [21], and ethnicity was obtained by a questionnaire at enrolment. Because there were no differences in observed results when ethnicity was categorized into five groups instead of two groups, we classified ethnicity into European and non-European. Maternal smoking and alcohol consumption before and during pregnancy (no; yes) were assessed by questionnaires in each trimester. Maternal and paternal anthropometrics were assessed at enrolment. Road traffic noise exposure was assessed at the home address in accordance with requirements of the EU Environmental Noise Directive, as described in the Supplementary Material of Chapter 2.

Statistical analysis

We used the lowest quartiles of PM_{10} and NO_2 exposure as the reference exposure groups. First, with multivariate linear regression models, we assessed associations between air pollution exposure in quartiles in the relevant time periods (i.e., from conception until measurement) with absolute measures of fetal growth and neonatal parameters. Second, to assess potential non-linear longitudinal effects, we used mixed-effects models with unstructured residual covariance to longitudinally model fetal growth SD scores from 18 weeks of pregnancy until birth by natural cubic splines [22]. We present these results as change in SD score to enable comparison of effect estimates throughout pregnancy. We positioned interior knots of the spline based on moments of data collection (18, 23, 30, 37, and 43.4 weeks for head circumference and weight, and 10.5, 15, 25, 37 and 43.4 weeks for length). The models include a separate spline model for each quartile of air pollution exposure during pregnancy. We performed a multivariate F-test to test for a difference between the splines of each quartile of air pollution exposure compared with the reference group. Third, we assessed the associations between air pollution exposure during pregnancy and adverse birth outcomes using multivariate logistic regression analyses. Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous variable in the linear or logistic regression models. All models were adjusted for known determinants of fetal growth (maternal age, body mass index, height, ethnicity, education, parity, folic acid supplementation use, smoking, alcohol consumption, paternal height), and for road traffic noise exposure. Models of absolute measures of fetal growth, fetal growth SD scores, preterm birth, and low birth weight were additionally adjusted for fetal sex. Models of absolute measures of fetal growth were additionally adjusted for gestational age at measurement. Models of low birth weight were additionally adjusted for gestational age at birth. The percentages of missing values within the population for analysis were lower than 1% for continuous data and lower than 15% for categorical data, except for folic acid supplementation use (26%). We used multiple imputation to impute missing values for covariates [23]. All measures of association are presented with their 95% confidence intervals. Spline regression analyses were performed using SAS version

9.2 (SAS Institute Inc., Cary, NC, USA), and other analyses were performed using PASW version 17.0 for Windows (PASW Inc., Chicago, IL, USA).

RESULTS

Subject and exposure characteristics

Table 1 presents the maternal, paternal, and fetal characteristics. Distributions of PM_{10} and NO_2 exposure levels in our study population are presented in Supplementary Table S1. Mean total exposure levels during pregnancy were 30.3 µg/m^3 for PM_{10} and 39.8 µg/m^3 for NO_2. Correlations among exposure averages in different pregnancy periods were moderate to strong (PM_{10}: Pearson correlation coefficient r=0.76 to 0.96, NO_2: r=0.68 to 0.94). PM_{10} and NO_2 exposure averages for corresponding periods were moderately correlated (r=0.57 to 0.63).

Table 1. Subject characteristics (N=7772).

	Mean ± SD, median (95% range), or number (percentage)
Maternal characteristics	
Age at enrolment (yr)	30.4 (19.2-39.3)
Gestational age at enrolment (wks)	14.4 (10.2-29.5)
Height (cm)	167.1 ± 7.5
Body mass index at enrolment (kg/m^2)	23.8 (18.7-36.3)
Parity – n (%)	
Nulliparous	4267 (54.9)
Multiparous	3414 (43.9)
Missing	91 (1.2)
Ethnic background – n (%)	
European	4132 (53.2)
Non-European	3065 (39.4)
Missing	575 (7.4)
Highest completed educational level – n (%)	
No education/primary	806 (10.4)
Secondary	3207 (41.3)
Higher	3062 (39.4)
Missing	697 (9.0)
Smoking in pregnancy – n (%)	
No	5592 (72.0)
Yes	1236 (15.9)
Missing	944 (12.1)

Table 1. *Continued*

	Mean ± SD, median (95% range), or number (percentage)
No	4175 (53.7)
Yes	2771 (35.7)
Missing	826 (10.6)
Folic acid supplementation use – n (%)	
Preconceptional	2300 (29.6)
First ten weeks of pregnancy	1793 (23.1)
None	1661 (21.4)
Missing	2018 (26.0)
Noise exposure at the home address at delivery (dB(A))	52.7 (45.0-68.2)
Paternal characteristics	
Paternal height (cm)	181.7 ± 7.9
Fetal characteristics	
First trimester (n=1541)	
Gestational age at visit (wks)	13.2 (10.5-17.5)
Crown-rump length (mm)	62.5 ± 12.7
Second trimester (n=7293)	
Gestational age at visit (wks)	20.5 (18.6-23.4)
Head circumference (mm)	179.3 ± 14.6
Femur length (mm)	33.5 ± 3.6
Estimated fetal weight (g)	381 ± 95
Third trimester (n=7449)	
Gestational age at visit (wks)	30.3 (28.3-33.0)
Head circumference (mm)	285.0 ± 12.7
Femur length (mm)	57.4 ± 3.1
Estimated fetal weight (g)	1618 ± 265
Birth outcomes (n=7772)	
Gestational age (wks)	40.1 (35.5-42.4)
Head circumference (cm)	37.6 ± 1.4
Length (cm)	51.0 ± 2.9
Birth weight (g)	3413 ± 560
Preterm birth (<37 wk) – n (%)	412 (5.3)
Low birth weight (<2500 g) – n (%)	371 (4.8)
Small size for gestational age at birth (<5%) – n (%)	385 (5.0)

Values are means ± SD, or medians (95% range) for variables with a skewed distribution, and number of subjects (%) for categorical variables.

Air pollution and fetal growth characteristics

Tables 2 and 3 present the cross-sectional associations for air pollution exposure with fetal growth characteristics. PM_{10} and NO_2 levels were not consistently associated with second trimester or neonatal head circumference, but higher levels were associated with smaller fetal head circumference in third trimester (difference -0.18 mm, 95% confidence interval (CI) -0.24 to -0.12 per 1 $\mu g/m^3$ increase in PM_{10} and -0.12 mm, 95% CI -0.17 to -0.06 per 1 $\mu g/m^3$ increase in NO_2, P-values<0.01). PM_{10} levels were not associated with fetal or neonatal length, but NO_2 levels were inversely associated with fetal femur length in second and third trimester (*P*<0.01). Exposure to PM_{10} was associated with increased estimated fetal weight in second trimester (P<0.05), but PM_{10} and NO_2 levels were associated with a lower birth weight (difference -3.6g, 95% CI -6.7 to -0.4 per 1 $\mu g/m^3$ increase in PM_{10} and -3.4g, 95% CI -6.2 to -0.6 per 1 $\mu g/m^3$ increase in NO_2, P-values<0.05).

When comparing the individual associations of maternal air pollution exposure and smoking during pregnancy with weight by trimester, we observed that inverse associations for air pollution exposure were smaller in magnitude than associations for maternal smoking, but still considerable: smoking compared with no smoking was associated with decreases of 39g and 146g in third trimester weight and birth weight, respectively, whereas elevated PM_{10} and NO_2 exposure levels (highest vs. lowest quartile) were associated with reductions of 13g and 46g in third trimester weight and birth weight, respectively, for PM_{10} and reductions of 20g and 61g in third trimester weight and birth weight, respectively, for NO_2 (results not shown). When including both PM_{10} and NO_2 in the models, the inverse association for PM_{10} exposure with first trimester crown-rump length reached statistical significance, and the associations for PM_{10} exposure with third trimester head circumference and for NO_2 exposure with femur length in second and third trimester persisted (results not shown). The unadjusted associations were consistent with the adjusted associations, although somewhat stronger inverse associations and smaller p-values were observed for PM_{10} exposure with weight in third trimester and at birth, and for NO_2 exposure with head circumference in second and third trimester, length neonatally, and weight in third trimester and at birth (results not shown).

Figure 2 (A-F) presents the associations of PM_{10} and NO_2 exposure with longitudinally measured fetal growth. Compared with the first quartile, the third and fourth quartiles of PM_{10} and NO_2 exposure showed a significant overall difference in head circumference growth during pregnancy (P-values<0.01) (Figures 2A and 2D). No significant associations were observed for air pollution exposure with longitudinally measured fetal length (Figures 2B and 2E). Figures 2C and 2F show significant overall differences in weight growth during pregnancy for the highest quartiles of PM_{10} and NO_2 exposure compared with the first quartile (*P*<0.01 and *P*<0.001 for third and fourth quartiles, respectively).

Table 2. Trimester-specific associations of PM_{10} exposure with measures of fetal growth.

Fetal growth parameter	N	**PM_{10} 2nd quartile** Difference (95% CI) [a]	**PM_{10} 3rd quartile** Difference (95% CI) [a]	**PM_{10} 4th quartile** Difference (95% CI) [a]	*Trend test (per 1µg/m³ increase)*	*P-value for trend*
Head circumference						
Second trimester (mm)	6625	0.65 (0.24, 1.05) *	0.57 (0.16, 0.98) *	0.16 (-0.26, 0.57)	*0.01 (-0.03, 0.05)*	*0.51*
Third trimester (mm)	6723	0.35 (-0.25, 0.94)	-0.43 (-1.02, 0.16)	-1.74 (-2.34, -1.13) **	*-0.18 (-0.24, -0.12)*	*<0.01*
Birth (cm)	4448	-0.01 (-0.09, 0.07)	-0.05 (-0.14, 0.04)	-0.03 (-0.13, 0.06)	*0.00 (-0.02, 0.01)*	*0.39*
Length						
First trimester (mm)	1541	0.42 (-0.58, 1.41)	-0.35 (-1.34, 0.65)	-0.77 (-1.82, 0.28)	*-0.08 (-0.17, 0.00)*	*0.06*
Second trimester (mm)	6646	0.14 (0.02, 0.27) *	-0.04 (-0.16, 0.08)	0.00 (-0.12, 0.12)	*-0.01 (-0.02, 0.00)*	*0.15*
Third trimester (mm)	6778	0.11 (-0.04, 0.26)	0.00 (-0.15, 0.15)	-0.15 (-0.30, 0.00) *	*-0.01 (-0.03, 0.00)*	*0.11*
Birth (cm)	5606	-0.07 (-0.21, 0.07)	-0.12 (-0.27, 0.02)	0.15 (-0.01, 0.30) ‡	*0.02 (0.00, 0.03)*	*0.09*
Weight						
Second trimester (g)	6612	5.9 (2.9, 8.8) *	5.0 (2.1, 8.0) *	3.8 (0.8, 6.8) *	*0.3 (0.0, 0.6)*	*0.05*
Third trimester (g)	6751	11.0 (-1.0, 22.9) ‡	7.4 (-4.7, 19.4)	-11.0 (-23.2, 1.2) ‡	*-0.7 (-1.9, 0.6)*	*0.29*
Birth (g)	7003	-18.1 (-45.2, 9.1)	-25.5 (-52.8, 1.8) ‡	-34.3 (-62.1, -6.4) *	*-3.6 (-6.7, -0.4)*	*0.03*

** p<0.001; * p<0.05; ‡ p<0.10

[a] Values are regression coefficients and reflect the difference in fetal growth parameters for each quartile of PM_{10} exposure (averaged from conception until measurement) compared with the reference group (lowest quartile). Fetal length was measured by ultrasound as crown-rump length in 1st trimester and femur length in 2nd and 3rd trimester, and as body length neonatally. Weight was estimated by ultrasound in 2nd and 3rd trimester of pregnancy, and measured at birth. Cut-off values for categorization of PM_{10} exposure are <27.4, 27.4-30.8, 30.8-33.6, >33.6 µg/m³ for first trimester, <28.0, 28.0-30.6, 30.6-33.6, >33.6 µg/m³ for second trimester, <27.8, 27.8-30.5, 30.5-33.2, >33.2 µg/m³ for third trimester, and <27.8, 27.8-30.0, 30.0-32.9, >32.9 µg/m³ for total pregnancy. Tests for trend were performed by including PM_{10} exposure as a continuous variable (per 1µg/m³ increase) in the model. Models are adjusted for gestational age and noise exposure at measurement, maternal age, body mass index, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, paternal height, and fetal sex. Models with neonatal head circumference or length are additionally adjusted for postconceptional age (gestational age for measurements at birth or gestational age + postnatal age for measurements at the child health center), and models with neonatal length are additionally adjusted for method of measurement.

Table 3. Trimester-specific associations of NO_2 exposure with measures of fetal growth.

Fetal growth parameter	N	NO_2 2nd quartile Difference (95% CI) [a]	NO_2 3rd quartile Difference (95% CI) [a]	NO_2 4th quartile Difference (95% CI) [a]	*Trend test (per 1µg/m³ increase)*	*P-value for trend*
Head circumference						
Second trimester (mm)	6625	0.16 (-0.25, 0.57)	-0.24 (-0.66, 0.17)	-0.23 (-0.69, 0.22)	*-0.02 (-0.05, 0.02)*	*0.36*
Third trimester (mm)	6723	-0.40 (-1.00, 0.20)	-0.81 (-1.42, -0.20) *	-1.28 (-1.96, -0.61) **	*-0.12 (-0.17, -0.06)*	*<0.01*
Birth (cm)	4448	0.04 (-0.05, 0.13)	0.02 (-0.07, 0.12)	0.00 (-0.10, 0.11)	*0.00 (-0.01, 0.01)*	*0.85*
Length						
First trimester (mm)	1541	-0.10 (-1.11, 0.91)	0.54 (-0.50, 1.57)	0.06 (-1.08, 1.20)	0.01 (-0.07, 0.08)	0.87
Second trimester (mm)	6646	-0.08 (-0.20, 0.05)	-0.18 (-0.30, -0.05) *	-0.19 (-0.33, -0.06) *	*-0.02 (-0.03, -0.01)*	*<0.01*
Third trimester (mm)	6778	-0.02 (-0.17, 0.13)	-0.09 (-0.24, 0.06)	-0.33 (-0.50, -0.16) **	*-0.02 (-0.04, -0.01)*	*<0.01*
Birth (cm)	5606	-0.10 (-0.25, 0.05)	-0.01 (-0.17, 0.14)	-0.09 (-0.26, 0.09)	*-0.01 (-0.02, 0.01)*	*0.49*
Weight						
Second trimester (g)	6612	-0.3 (-3.3, 2.6)	-1.4 (-4.4, 1.6)	0.8 (-2.5, 4.1)	*0.1 (-0.2, 0.3)*	*0.67*
Third trimester (g)	6751	-3.7 (-15.8, 8.5)	-7.2 (-19.6, 5.1)	-14.2 (-28.0, -0.5) *	*-0.7 (-1.8, 0.5)*	*0.25*
Birth (g)	7003	2.6 (-25.0, 30.2)	-18.6 (-46.7, 9.6)	-37.6 (-69.7, -5.6) *	*-3.4 (-6.2, -0.6)*	*0.02*

** p<0.001; * p<0.05

[a] Values are regression coefficients and reflect the difference in fetal growth parameters for each quartile of NO_2 exposure (averaged from conception until measurement) compared with the reference group (lowest quartile). Fetal length was measured by ultrasound as crown-rump length in 1st trimester and femur length in 2nd and 3rd trimester, and as body length neonatally. Weight was estimated by ultrasound in 2nd and 3rd trimester of pregnancy, and measured at birth. Cut-off values for categorization of NO_2 exposure are <37.0, 37.0-40.9, 40.9-43.9, >43.9 µg/m³ for first trimester, <37.0, 37.0-40.5, 40.5-43.4, >43.4 µg/m³ for second trimester, <37.0, 37.0-39.8, 39.8-42.8, >42.8 µg/m³ for third trimester, and <37.2, 37.2-39.6, 39.6-42.2, >42.2 µg/m³ for total pregnancy. Tests for trend were performed by including NO_2 exposure as a continuous variable (per 1µg/m³ increase) in the model. Models are adjusted for gestational age and noise exposure at measurement, maternal age, body mass index, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, paternal height, and fetal sex. Models with neonatal head circumference or length are additionally adjusted for postconceptional age (gestational age for measurements at birth or gestational age + postnatal age for measurements at the child health center), and models with neonatal length are additionally adjusted for method of measurement.

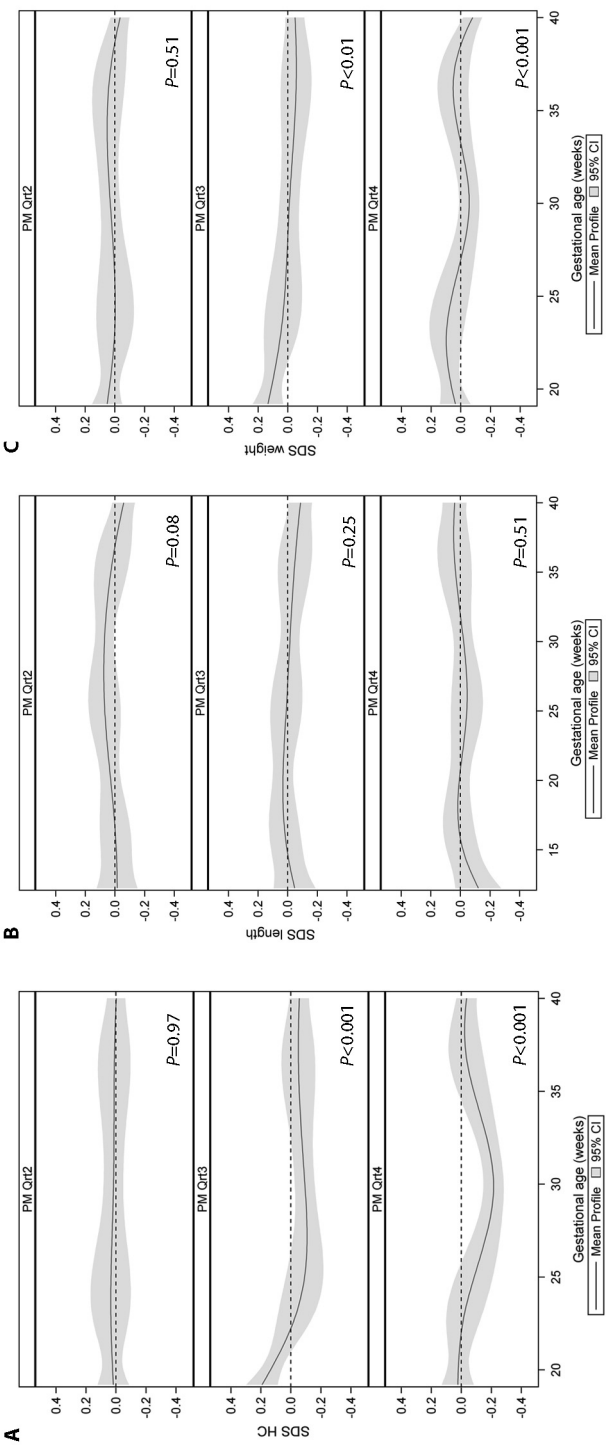
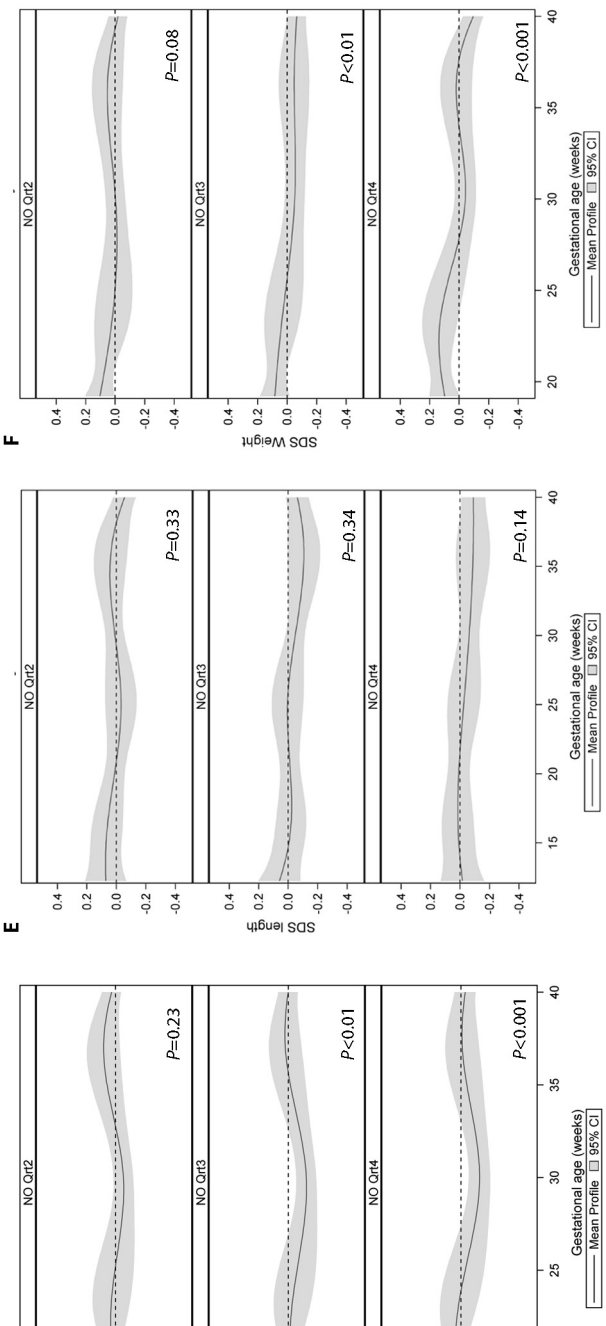


Figure 2. Associations of PM_{10} and NO_2 exposure with longitudinally measured fetal growth characteristics.

Figure 2. *Continued*



Figures are based on spline regression models of longitudinally measured **A+D) head circumference (HC) growth** (17726 observations), **B+E) length growth** (N=20305 observations), and **C+F) weight growth** (N=20395 observations), all expressed in SD scores (SDS), for each quartile (Q) of air pollution exposure during pregnancy (from conception until delivery) compared with the reference group (lowest quartile). Figures A-C present associations for PM_{10} exposure, and figures D-F present associations for NO_2 exposure. Head circumference was estimated by ultrasound in 2nd and 3rd trimester of pregnancy, and measured neonatally. Fetal length was measured by ultrasound as crown-rump length in 1st trimester and femur length in 2nd and 3rd trimester, and as body length neonatally. Weight was estimated by ultrasound in 2nd and 3rd trimester of pregnancy, and measured at birth. P-values are based on multivariate F-tests and reflect the difference between the spline of each air pollution exposure category compared with the reference category. Models are adjusted for maternal age, body mass index, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, noise exposure, paternal height, and fetal sex.

Air pollution and risks of adverse birth outcomes

Compared with mothers in the lowest quartile of PM_{10} exposure levels, exposures in the third and fourth quartiles were positively associated with preterm birth (odds ratio (OR) 1.40, 95% CI 1.03 to 1.89, and OR 1.32, 95% CI 0.96 to 1.79, respectively) (Table 4). The third quartile of PM_{10} exposure was associated with SGA (OR 1.38, 95% CI 1.00 to 1.90), but no significant association was observed for the fourth quartile (OR 1.23, 95% CI 0.89 to 1.70). No consistent associations were observed for NO_2 exposure with adverse birth outcomes. When including both PM_{10} and NO_2 in the models, associations for PM_{10} exposure with preterm birth became larger in magnitude, and larger effect estimates with smaller p-values were observed for associations between PM_{10} exposure and low birth weight (results not shown). Relative to the adjusted models, the unadjusted models showed smaller p-values for the positive associations for PM_{10} exposure with preterm birth and SGA (*P*-values for trend=0.03) and larger effect estimates with smaller p-values for the associations for NO_2 exposure with preterm birth, low birth weight, and SGA (e.g., ORs for SGA: 1.47, 95% CI 1.08 to 2.01 and OR 1.49, 95% CI 1.09 to 2.04 for the third and fourth quartiles of NO_2 exposure, respectively) (results not shown).

Table 4. Associations of PM_{10} and NO_2 exposure with the risks of adverse birth outcomes.

Air pollution exposure	**Preterm birth** (<37 weeks) Odds ratio (95% CI) N=7045	**Low birth weight** (<2500 g) Odds ratio (95% CI) N=7003	**SGA at birth** (<5%) Odds ratio (95% CI) N=6997
PM_{10}			
1st quartile	*Reference* N=78	*Reference* N=74	*Reference* N=73
2nd quartile	0.96 (0.70, 1.33) N=75	0.76 (0.49, 1.20) N=66	1.05 (0.75, 1.47) N=78
3rd quartile	1.40 (1.03, 1.89) * N=106	0.89 (0.58, 1.34) N=93	1.38 (1.00, 1.90) * N=98
4th quartile	1.32 (0.96, 1.79) ‡ N=105	0.91 (0.60, 1.40) N=90	1.23 (0.89, 1.70) N=97
Trend test (per 1μg/m³ increase)	1.03 (1.00, 1.07)	1.00 (0.95, 1.05)	1.03 (0.99, 1.07)
P-value for trend	0.07	0.93	0.13

Table 4. *Continued*

Air pollution exposure	Preterm birth (<37 weeks) Odds ratio (95% CI) N=7045	Low birth weight (<2500 g) Odds ratio (95% CI) N=7003	SGA at birth (<5%) Odds ratio (95% CI) N=6997
NO_2			
1st quartile	*Reference* N=79	*Reference* N=75	*Reference* N=70
2nd quartile	1.10 (0.81, 1.51) N=92	0.84 (0.54, 1.31) N=71	0.93 (0.66, 1.31) N=73
3rd quartile	1.09 (0.79, 1.49) N=95	0.86 (0.55, 1.33) N=88	1.25 (0.90, 1.73) N=101
4th quartile	1.10 (0.77, 1.57) N=99	0.95 (0.58, 1.55) N=89	1.35 (0.94, 1.94) N=102
Trend test (per 1µg/m^3 increase)	1.01 (0.98, 1.04)	1.00 (0.95, 1.04)	1.03 (0.99, 1.06)
P-value for trend	0.43	0.87	0.11

* p<0.05; ǂ p<0.10

Values are odds ratios (95% confidence interval) and reflect the risk for adverse birth outcomes for each quartile of air pollution exposure during pregnancy (from conception until delivery) compared with the reference group (lowest quartile). Cut-off values for categorization are <27.8, 27.8-30.0, 30.0-32.9, >32.9 µg/m^3 for PM_{10} exposure, and <37.2, 37.2-39.6, 39.6-42.2, >42.2 µg/m^3 for NO_2 exposure. Tests for trend were performed by including PM_{10} and NO_2 exposure as a continuous variable (per 1µg/m^3 increase) in the model. Models are adjusted for maternal age, body mass index, height, parity, ethnicity, education, folic acid supplementation use, smoking, alcohol consumption, noise exposure, and paternal height. Models with preterm birth and low birth weight are additionally adjusted for fetal sex, and models with low birth weight are additionally adjusted for gestational age at birth.

DISCUSSION

Results from this large population-based prospective cohort study from early pregnancy onwards suggest that maternal exposure to PM_{10} and NO_2 is inversely associated with fetal growth in second and third trimester and with weight at birth. Elevated PM_{10} exposure levels were also positively associated with preterm birth and SGA at birth.

Air pollution, fetal growth and birth outcomes

A few animal experiments suggested effects of maternal exposure to air pollution on placental function and fetal growth [24, 25]. Several potential biological mechanisms have been described through which air pollution could influence pregnancy, such as induction of systemic inflammation and oxidative stress [26], eventually resulting in suboptimal placentation [27] and increased maternal susceptibility to infections [28]. These alterations

could impair fetal growth. In addition, maternal infections and elevated levels of inflammatory mediators might trigger preterm birth [26, 28, 29].

Thus far, only three studies have examined the associations of maternal air pollution exposure with fetal growth measured by ultrasound during pregnancy. The first study was conducted in Australia among 14734 women and examined 15623 mid-pregnancy ultrasound scans. The researchers observed inverse associations of maternal exposure to PM_{10}, ozone (O_3), and sulfur dioxide (SO_2) during different periods with fetal growth parameters. No significant associations were observed for NO_2 exposure. The authors reported that the observed associations were heterogeneous regarding the specific exposure periods and outcome measures examined [9]. The second study was conducted in France and was based on three ultrasound scans in 271 women. Associations were observed between maternal personal exposure to airborne benzene and smaller fetal biparietal diameter in mid- and late pregnancy, and with head circumference in mid- and late pregnancy and at birth [10]. The third study was conducted in Spain among 562 pregnant women with 1692 scans, and observed no associations for NO_2 exposure with fetal growth parameters in different periods. When the analysis was restricted to women who spent less than 2 hours/day in non-residential outdoor environments, significant associations were observed between exposure to a mixture of aromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylene; BTEX), and biparietal diameter growth during the second trimester, and between NO_2 exposure and SD scores for both size and growth of second and third trimester head circumference, abdominal circumference, biparietal diameter, and estimated fetal weight [8]. Several studies have estimated the impact of air pollution on anthropometric parameters at birth such as head circumference, length, and weight. Inverse associations of maternal exposure to NO_2 [30], polycyclic aromatic hydrocarbons (PAHs) [6], and particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$) [4] with head circumference and length at birth have been reported. A reduction in birth weight has also been linked to air pollutants, including $PM_{2.5}$ [4], PAHs [6], and carbon monoxide (CO) and O_3 [5]. Another study has not detected associations between exposure to NO_2, PM_{10}, O_3, or visibility-reducing particles with head circumference and weight at birth [7]. The present study was based on a larger number of fetal ultrasound measurements than previous studies. We observed an inconsistent pattern of associations for air pollution across gestation, which was reported earlier as well [9]. The clinical relevance of a relative decrease in head and length growth during pregnancy when sizes at birth are within the normal range needs to be further studied, as well as the consequences of a relative increase in weight during pregnancy followed by a relative decrease in weight at birth. However, results from the analyses at different time points should be interpreted carefully, because the number of subjects with available outcome data and hence the statistical power of the analyses varied between measurements in our study. Also, differences in methods and accuracy between fetal and neonatal measurements could explain the heterogeneous results. We estimated small differences in fetal growth parameters. For example, in the third trimester, the highest

PM_{10} and NO_2 exposure quartiles were associated with a reduction in femur length of 0.2 and 0.3 mm and a reduction in head circumference of 1.7 and 1.3 mm, respectively. These differences may not be clinically relevant on an individual level, but could be relevant on a population level. Moreover, although we have previously shown good intra- and interobserver reproducibility of fetal biometry measurements [17], the associations might be underestimated because of random measurement error. Although the overall strength of evidence is still limited, the results of previous studies and our study suggest that air pollution exposure influences fetal growth from the second trimester onwards. We observed associations for PM_{10} exposure, but not for NO_2 exposure, with preterm birth and SGA. The literature on birth outcomes has increased in the last decade, which has led to a number of reviews summarizing the available evidence [2, 3, 31-34]. Most routinely measured air pollutants (e.g., PM_{10}, $PM_{2.5}$, NO_2, CO, O_3, SO_2) have been linked to outcomes such as preterm birth, low birth weight, and intrauterine growth restriction [1], but results differ among studies. In our previous work, residential proximity to traffic – a proxy for traffic-related air pollution – was not consistently associated with birth weight, nor with preterm birth and SGA [35]. In this study, we were able to estimate individual exposure levels that better capture the spatial and temporal variation in air pollution concentrations.

Air pollution, especially the traffic-related part, is a complex mixture of several pollutants. PM_{10} and NO_2 might act as surrogates for this mixture, and are therefore not necessarily the causative agents in the relation between air pollution and adverse fetal growth and birth outcomes. The biological plausibility of health effects induced by particulate matter has been well described [26, 28]. In contrast, it has been proposed that health risks associated with NO_2 may result from traffic-related emissions correlating with NO_2, chemical reaction products of NO_2, or NO_2 itself [36]. When including both PM_{10} and NO_2 in the models, the results did not highlight clearly stronger associations for one pollutant or the other. We acknowledge that the variation in exposure levels is relatively small in our study population. In populations with a larger exposure variability, stronger associations for air pollution exposure with fetal growth parameters and adverse birth outcomes might be detected.

Methodological considerations

Many previous studies assessed exposure to air pollution using only monitoring stations. That approach does not consider intra-urban gradients in pollutants. More recent approaches applying spatial modelling address the spatial variation, but not the temporal variation. Together with a number of recent studies that used temporally adjusted land-use regression models or dispersion models to assess exposure [8, 10, 30, 37], we were able to consider finer spatial and temporal contrasts in exposure by using a combination of dispersion modelling and continuous monitoring. The quality of the assigned exposure estimates was further enhanced by allowing for residential mobility of the women during pregnancy, which overcomes the potential misclassification that could arise when

exposure is based solely on the home address at time of delivery [38]. There might still be non-differential misclassification of air pollution exposure. Exposure levels were estimated at the home address, however, pregnant women do not spend all of their time at home. Other types of exposure (e.g., occupational or commuting) were not taken into account.

Although fetal ultrasound examination is a more reliable basis than the last menstrual period for establishing gestational age [16], this method has the disadvantage that the growth variation of the fetal characteristics used for pregnancy dating is assumed to be zero [14]. Because the early pregnancy characteristics are correlated throughout pregnancy with head circumference, abdominal circumference and femur length, our study may have underestimated the variation in the latter three growth characteristics, resulting in an underestimation of our effect estimates. In addition, the assessment of gestational age could be biased if air pollution exposure shows an early effect of fetal growth [28]. We observed a non-significant inverse association between PM_{10} exposure and crown-rump length. When restricting the analyses to the subgroup of women with a known LMP, adjustment for the LMP-based gestational age rather than the ultrasound-based gestational age resulted in somewhat stronger negative effects of air pollution on fetal growth from the third trimester onward. This suggests that effects of air pollution on fetal growth might be underestimated when gestational age is defined using ultrasounds [39]. However, the observations in this subgroup should be considered with caution because of the relatively small size.

Conclusion

This prospective population-based cohort study in the Netherlands suggests that maternal PM_{10} and NO_2 exposure is inversely associated with fetal growth during the second and third trimester and with weight at birth. Elevated PM_{10} exposure was also associated with preterm birth and SGA at birth. This study further supports previous epidemiological research, and suggests that the associations between maternal exposure to air pollution and fetal growth are trimester- and growth characteristic-specific. Future studies are needed to explore the underlying mechanisms and postnatal consequences of these findings.

REFERENCES

1. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
2. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
3. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
4. Jedrychowski W, Bendkowska I, Flak E, Penar A, Jacek R, Kaim I, et al. Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland. *Environ Health Perspect.* 2004; 112(14):1398-1402.
5. Salam MT, Millstein J, Li YF, Lurmann FW, Margolis HG, Gilliland FD. Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. *Environ Health Perspect.* 2005; 113(11):1638-1644.
6. Choi H, Jedrychowski W, Spengler J, Camann DE, Whyatt RM, Rauh V, et al. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. *Environ Health Perspect.* 2006; 114(11):1744-1750.
7. Hansen C, Neller A, Williams G, Simpson R. Low levels of ambient air pollution during pregnancy and fetal growth among term neonates in Brisbane, Australia. *Environ Res.* 2007; 103(3):383-389.
8. Aguilera I, Garcia-Esteban R, Iniguez C, Nieuwenhuijsen MJ, Rodriguez A, Paez M, et al. Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. *Environ Health Perspect.* 2010; 118(5):705-11.
9. Hansen CA, Barnett AG, Pritchard G. The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. *Environ Health Perspect.* 2008; 116(3):362-9.
10. Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A, et al. Maternal personal exposure to airborne benzene and intrauterine growth. *Environ Health Perspect.* 2009; 117(8):1313-21.
11. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
12. Netherlands Ministry of Infrastructure and the Environment: Air Quality Decree 2007 (Regeling beoordeling Luchtkwaliteit 2007). 2007. Available: http://wetten.overheid.nl/BWBR0022817
13. Royal College of Obstetricians and Gynaecologists. *Routine ultrasound screening in pregnancy: protocol*. 2000. London: RCOG Press.
14. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet Gynecol.* 2008; 31(4):388-96.
15. Mook-Kanamori DO, Steegers EA, Eilers PH, Raat H, Hofman A, Jaddoe VW. Risk factors and outcomes associated with first-trimester fetal growth restriction. *JAMA.* 2010; 303(6):527-34.
16. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. *Ultrasound Obstet Gynecol.* 1996; 8(3):178-85.
17. Verburg BO, Mulder PG, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. Intra- and interobserver reproducibility study of early fetal growth parameters. *Prenat Diagn.* 2008; 28(4):323-31.
18. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements - a prospective study. *Am J Obstet Gynecol.* 1985; 151(3):333-7.
19. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand.* 1991; 80(8-9):756-62.
20. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000; 47(3):316-23.
21. Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr.* 2009; 102(5):777-85.

22. Devlin TF, Weeks BJ. Spline functions for logistic regression modeling. *Proceedings of the 11th Annual SAS Users Group International Conference.* 1986; 11:646-651.
23. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res.* 2007; 16(3):219-42.
24. Rocha e Silva IR, Lichtenfels AJ, Amador Pereira LA, Saldiva PH. Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. *Fertil Steril.* 2008; 90(5):1921-4.
25. Tsukue N, Tsubone H, Suzuki AK. Diesel exhaust affects the abnormal delivery in pregnant mice and the growth of their young. *Inhal Toxicol.* 2002; 14(6):635-51.
26. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
27. Dejmek J, Selevan SG, Benes I, Solansky I, Sram RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ Health Perspect.* 1999; 107(6):475-480.
28. Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. Meeting report: atmospheric pollution and human reproduction. *Environ Health Perspect.* 2008; 116(6):791-8.
29. Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition--a review. *Placenta.* 2003; 24 Suppl A:S33-46.
30. Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A, et al. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. *Environ Health.* 2010; 9:6.
31. Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. *Epidemiology.* 2004; 15(1):36-45.
32. Lacasana M, Esplugues A, Ballester F. Exposure to ambient air pollution and prenatal and early childhood health effects. *Eur J Epidemiol.* 2005; 20(2):183-199.
33. Maisonet M, Correa A, Misra D, Jaakkola JJ. A review of the literature on the effects of ambient air pollution on fetal growth. *Environ Res.* 2004; 95(1):106-115.
34. Sram RJ, Binkova B, Dejmek J, Bobak M. Ambient air pollution and pregnancy outcomes: a review of the literature. *Environ Health Perspect.* 2005; 113(4):375-382.
35. van den Hooven EH, Jaddoe VW, de Kluizenaar Y, Hofman A, Mackenbach JP, Steegers EA, et al. Residential traffic exposure and pregnancy-related outcomes: a prospective birth cohort study. *Environ Health.* 2009; 8:59.
36. World Health Organization: Air quality guidelines. Global update 2005. Particulate matter, ozone, nitrogen dioxide and sulphur dioxide. 2006. Available: http://whqlibdoc.who.int/hq/2006/WHO_SDE_PHE_OEH_06.02_eng.pdf
37. Gehring U, Wijga AH, Fischer P, de Jongste JC, Kerkhof M, Koppelman GH, et al. Traffic-related air pollution, preterm birth and term birth weight in the PIAMA birth cohort study. *Environ Res.* 2011; 111(1):125-35.
38. Fell DB, Dodds L, King WD. Residential mobility during pregnancy. *Paediatr Perinat Epidemiol.* 2004; 18(6):408-14.
39. Slama R, Khoshnood B, Kaminski M. How to control for gestational age in studies involving environmental effects on fetal growth [Letter]. *Environ Health Perspect.* 2008; 116(7):A284-285.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Distribution of PM_{10} and NO_2 exposure levels for different pregnancy periods.

Air pollution levels	Minimum	25th percentile	Mean	Median	75th percentile	Maximum
PM_{10} exposure ($\mu g/m^3$)						
until 1st trimester	21.7	27.4	30.8	30.8	33.6	44.0
until 2nd trimester	22.6	28.0	30.7	30.6	33.6	43.2
until 3rd trimester	22.7	27.8	30.4	30.5	33.2	41.5
total pregnancy	23.2	27.8	30.3	30.0	32.9	40.9
NO_2 exposure ($\mu g/m^3$)						
until 1st trimester	21.0	37.0	40.4	40.9	43.9	59.7
until 2nd trimester	22.7	37.0	40.2	40.5	43.4	58.4
until 3rd trimester	25.6	37.0	40.0	39.8	42.8	58.2
total pregnancy	26.5	37.2	39.8	39.6	42.2	56.9

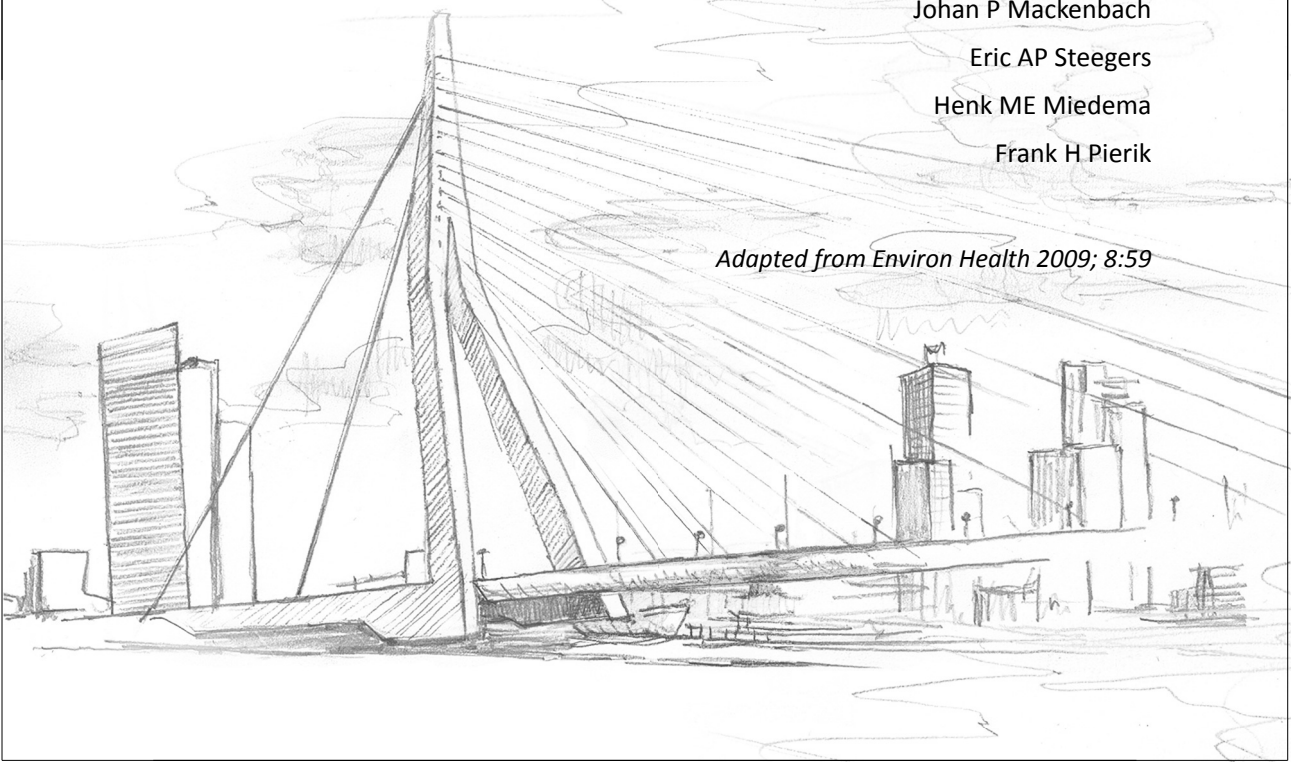
Air pollution exposure was estimated for different periods in pregnancy: conception until first, second, and early third trimester ultrasound, and conception until delivery

Chapter 3.5

Residential proximity to traffic and pregnancy complications

Edith H van den Hooven
Vincent WV Jaddoe
Yvonne de Kluizenaar
Albert Hofman
Johan P Mackenbach
Eric AP Steegers
Henk ME Miedema
Frank H Pierik

Adapted from Environ Health 2009; 8:59



ABSTRACT

Background: The effects of ambient air pollution on pregnancy outcomes are under debate. Previous studies have used different air pollution exposure assessment methods. The considerable traffic-related intra-urban spatial variation needs to be considered in exposure assessment. Residential proximity to traffic is a proxy for traffic-related exposures that takes into account within-city contrasts.

Methods: We investigated the association between residential proximity to traffic and various birth and pregnancy outcomes in 7339 pregnant women and their children participating in a population-based cohort study. Residential proximity to traffic was defined as 1) distance-weighted traffic density in a 150 meter radius, and 2) proximity to a major road. We estimated associations of these exposures with birth weight, and with the risks of preterm birth and small size for gestational age at birth. Additionally, we examined associations with gestational hypertension, (pre)eclampsia, and gestational diabetes.

Results: There was considerable variation in distance-weighted traffic density. Almost fifteen percent of the participants lived within 50m of a major road. Residential proximity to traffic was not associated with birth and pregnancy outcomes in the main analysis and in various sensitivity analyses.

Conclusions: Mothers exposed to residential traffic had no higher risk of adverse birth outcomes or pregnancy complications in this study. Future studies may be refined by taking both temporal and spatial variation in air pollution exposure into account.

INTRODUCTION

Exposure to air pollution has been suggested to adversely affect various birth outcomes. As reported in a number of reviews, outcomes such as low birth weight, intrauterine growth restriction, and preterm birth have been associated with ambient air pollution levels, although effects were not always consistent between studies [1-4]. In large studies, assessing individual exposure to air pollution is often rather demanding for participants and requires extensive resources. Therefore, other approaches have been used to estimate exposure of individuals. Most studies have assessed exposure to air pollution using (an often limited number of) outdoor monitoring stations, either by using the station closest to the mother's home address at time of delivery [5, 6], or by taking averaged concentrations measured at one or multiple monitor sites in a district [7, 8]. Although concentrations of pollutants measured by ambient monitors may correspond to air pollution exposure at regional levels, this may not represent individual exposure [8], particularly for primary pollutants which display higher spatial heterogeneity. This spatial variation in air pollutant concentrations in urban areas, which can be largely attributed to traffic emissions, has been documented for several pollutants, such as nitrogen dioxide, black smoke, elemental carbon, ultrafine particles, and particulate matter ($PM_{2.5}$ and PM_{10}) [9, 10]. Levels of these pollutants are elevated near roads [9, 11, 12], and are correlated with the traffic intensity on these roads [11, 13]. Therefore, intra-urban gradients need to be taken into account.

Indicators of residential proximity to traffic, such as distance to a major road and traffic intensity around a location, are increasingly being used as proxies for long-term exposure to traffic pollutants. Epidemiological studies have linked these indicators to various health outcomes, such as respiratory symptoms [14, 15], cardiovascular diseases [16], mortality rates [17] and childhood cancer [18]. In addition, few studies explored the effects of these indicators on birth and pregnancy outcomes. Associations of proximity to traffic with birth weight [19] and with the risks of preterm birth [20-23], small size for gestational age at birth [19, 20, 24], and low birth weight [20, 21, 24] have been suggested. Previous studies generally obtained information on birth outcomes from birth certificates. This may have reduced their ability to adjust for confounding, as birth records usually include limited information on potential confounding factors [8, 25]. A prospective pregnancy cohort study with detailed exposure and covariate information can overcome this limitation [26, 27].

Several potential biological mechanisms have been described through which air pollution could influence pregnancy outcomes, such as the induction of inflammation (placental, pulmonary, or systemic) and oxidative stress [28], eventually resulting in suboptimal placentation [7] and increased maternal susceptibility to infections [27]. These alterations could lead to adverse birth outcomes and maternal pregnancy complications such as gestational hypertension and preeclampsia.

The aim of the present study was to investigate whether residential proximity to traffic is associated with various birth and pregnancy outcomes in a large population-based cohort study.

METHODS

Design

The present study was embedded in the Generation R Study, a population-based prospective cohort study from pregnancy onwards. The Generation R study is designed to identify early environmental and genetic determinants of growth, development and health and has been described previously in detail [29, 30]. In brief, the cohort includes mothers and children of different ethnicities living in Rotterdam, the Netherlands. Ideally, enrolment in the study took place in early pregnancy (gestational age <18 weeks), but was possible until the birth of the child. Out of the total number of eligible children in the study area, 61 percent participated in the study at birth. In total, 8880 pregnant women with a delivery date between April 2002 and January 2006 entered the prenatal part of the study. The majority of these mothers (75%) was enrolled in early pregnancy (gestational age <18 weeks); 22 percent enrolled in mid-pregnancy (gestational age 18-24 weeks), and 3 percent enrolled in late pregnancy (gestational age >25 weeks) [30]. Data on pregnancy were collected on the basis of physical examinations, fetal ultrasounds, hospital registrations and questionnaires. Assessments were planned for early pregnancy, mid-pregnancy, and late pregnancy, but the individual time schemes depended on the specific gestational age at enrolment [29]. The study protocol was approved by the Medical Ethical Committee of Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Traffic exposure measures

Individual traffic exposure estimates at each participant's home address were assessed using Geographical Information Systems (GIS). The following traffic variables were used: 1) distance-weighted traffic density (DWTD) within a 150 meter radius around the home, and 2) proximity to a major road (with >10,000 vehicles/24h). Input for the traffic exposure calculations was obtained from the local authorities of Rotterdam and included detailed digital maps with information on geographic locations and traffic characteristics for roads in the study area. The digital road maps include highways, arterial roads, main streets, and principal residential streets; the smallest local roads are not included. However, the traffic on these streets contributes only minorly to the total traffic flow in the area, and is therefore believed not to impact our traffic exposure measures. Annual average daily traffic intensities for the year 2004 were attached as attributes to the road segments for a dense network of roads. This data was used to estimate exposure for all pregnancies between 2002 and 2006. Based on index numbers for traffic intensity in the years 2002-2006 [31], it was concluded that the 2004 data could reasonably be applied to adjacent years. We geocoded the mothers' home addresses at time of delivery using ArcGIS (v9, ESRI). All matches were made at the address level. We constructed a 150m radius buffer around the home. Distance-weighted traffic density was calculated using MapInfo Professional (v9.0, Pitney Bowes). To estimate the dispersion of motor vehicle exhaust, we

employed a model that was based on a Gaussian distribution that assumes that 96% of the emitted pollutants disperse up to 150m from the road:

$$Y_i = \frac{1}{0.4\sqrt{2\pi}} \exp\left[\frac{-\frac{1}{2}\left(\frac{D_i}{150}\right)^2}{(0.4)^2} \right]$$

where D_i is the distance from the road segment *i*. This curve was used to weigh the products of the length (in m) and the traffic intensities (in vehicles/24h) of all road segments within the buffer. The weighted values were summed for each subject to obtain the distance-weighted traffic density. As vehicles may use multiple segments in the buffer, the DWTD values can be relatively high (up to millions of vehicles/24h*m). Various definitions of DWTD are being used in the literature. We remark that our method to define DWTD is derived from the method used by Wilhelm & Ritz [21], with the difference that we take into account the length of the roads within the buffer. In addition to DWTD, we identified the nearest major road (with >10,000 vehicles/24h) and calculated the distance to this road, up to a distance of 500m.

Birth and pregnancy outcomes

In mothers who were enrolled in early or mid-pregnancy, gestational age was established on the basis of fetal ultrasound examination during the first ultrasound visit, as the use of the last menstrual period (LMP) has several limitations [32]. In mothers who were enrolled in late pregnancy, the LMP was used for pregnancy dating. Medical records completed by midwives and obstetricians were used to obtain information about date of birth, birth weight, fetal sex, and occurrence of pregnancy complications. Main birth outcomes were birth weight (grams), small size for gestational age (SGA) at birth (<-2.0 SDS birth weight) and preterm birth (gestational age <37 weeks). Gestational age-adjusted standard deviation birth weight scores were based on published reference charts from a North European birth cohort [33], which are based on a large population and include the extremes of the birth weight distribution. Information about maternal pregnancy complications was available, including gestational diabetes mellitus, gestational hypertension, and (pre)eclampsia. This last group consisted of women with eclampsia, preeclampsia or hemolysis elevated liver enzymes and low platelets (HELLP) syndrome. Gestational diabetes was diagnosed according to Dutch midwifery and obstetric guidelines using the following criteria: random glucose level >11.0 mmol/L, fasting glucose >7.0 mmol/l or a fasting glucose between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test, in women with no pre-existing diabetes. Gestational hypertension was defined according to criteria described by the International Society for the Study of Hypertension in Pregnancy (ISSHP): development of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg without proteinuria after 20 weeks of gestation in previously normotensive women [34]. Preeclampsia was defined as development of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in a previously

normotensive woman and proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24h urine collection containing at least 300 mg of protein) [34].

Covariates

Based on previous studies, the following variables were considered as potential confounders: maternal age at enrolment, educational level, ethnicity, body mass index, parity, smoking, alcohol consumption, and fetal sex. Information on maternal age, educational level, ethnicity, and parity was obtained in the first questionnaire at enrolment in the study. The highest educational level achieved by mother was used as an indicator of maternal socioeconomic status (SES) and was reclassified into three categories: no education or primary school, secondary school, and higher education. Parity was classified into two categories: nulliparous and multiparous. Ethnic background of the woman was assessed on the basis of country of birth of her and her parents [30] and reclassified into five categories: Dutch and Caucasian, Turkish, Moroccan, Surinamese, and other. Maternal anthropometrics were assessed at time of enrolment and at subsequent visits. Since the correlation of prepregnancy weight obtained by questionnaire and weight measured at enrolment was high (0.97, $p<0.001$) [35], body mass index was calculated on the basis of maternal weight and height at enrolment. Maternal smoking and alcohol consumption habits were assessed on the basis of three questionnaires (in early, mid-, and late pregnancy) by asking women whether they smoked/used alcohol before or during pregnancy (no/until pregnancy was known/yes). Mothers who reported in the first questionnaire that they did not smoke at all or had smoked until pregnancy was known, but reported smoking in the second or third questionnaire, were reclassified into the continued smoking category [36]. The same approach was followed for maternal alcohol consumption habits.

Population for analysis

For the present analyses, data on all prenatally enrolled women were available (n=8880). We decided to restrict to participants living in the northern part of Rotterdam at time of delivery, since participants living in the southern part of Rotterdam did not participate in the postnatal follow-up study [30]. This yielded 7506 women. We included all live singleton births (n=7431); women who gave birth to twins (n=75) were excluded. We were able to calculate traffic exposure for 7339 of these 7431 women (99%) due to incomplete address data in 92 subjects. The associations between traffic indicators and pregnancy-related outcomes in mother and child were analyzed in the 7339 remaining mothers.

Statistical analysis

Main analyses

Based on a population for analysis of 7000 subjects and a proportion exposed of 10%, we were able to detect a difference of 0.11 SD (type I error of 5%, type II error of 20% (power

80%)) for a continuous normally distributed outcome. Previous studies on air pollution and birth weight showed reductions in birth weight ranging to 140 grams, which is equal to 0.3 SD.

For the statistical analyses, distance-weighted traffic density was divided into quartiles. The distance to a major road was categorized as <50, 50-100, 100-150, 150-200, and >200m. The associations of proximity to traffic with continuously measured birth weight were assessed using multivariate linear regression analyses. The associations between proximity to traffic and dichotomous birth and pregnancy outcomes were assessed using multivariate logistic regression analysis. Models were adjusted for known determinants of birth and pregnancy outcomes (maternal age, maternal ethnicity, maternal education, maternal BMI, parity, maternal smoking, and maternal alcohol consumption). Maternal age and BMI were included in the models as continuous variables. Models with birth weight as outcome were additionally adjusted for gestational age (with a linear term) and fetal sex. Models with SGA at birth and preterm birth were additionally adjusted for fetal sex. Additionally, we included indicator variables for month and year of birth in the models to control for season and long-term trends. Missing data on categorical factors were included in the analyses as a separate category.

Sensitivity analyses

We performed various sensitivity analyses to assess the robustness of our results.

First, to determine whether our findings were sensitive to the categorization of the traffic measures, we examined associations when using different cut-offs (e.g., the 80th, 90th and 95th percentiles of the distributions). Second, analyses were repeated when DWTD was calculated for different buffer radii. Next, to evaluate whether our results would change when we would introduce more contrast in our exposure levels, we calculated the distance to the nearest highway (with >25,000 vehicles/24h) and examined associations with the main outcomes. Furthermore, to evaluate whether the results were sensitive to the method of determining gestational age (ultrasound versus LMP), we repeated the analyses after excluding women who were enrolled in late pregnancy, since only mothers who were enrolled in mid- and late pregnancy were dated on ultrasound. In addition, we repeated analyses in a subsample of women with data available on body mass index before pregnancy, and adjusted these analyses for BMI before pregnancy rather than BMI at enrolment. Furthermore, to evaluate whether our findings were sensitive to the definition of SGA at birth (which was based on reference charts for the North-European population), we repeated the analysis in a subcohort of Dutch participants only. Also, we investigated whether the associations between traffic exposure and pregnancy-related outcomes differed per educational level by performing stratified analyses. Finally, we conducted stratified analyses for residential mobility. We had information available on change of residence (yes/no/missing) in the period between seven months before conception and five months of pregnancy, and repeated the analyses for the different strata. All measures

of association are presented with their 95% confidence intervals. Statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Table 1 shows baseline characteristics of the study population. The median age of the women was 30.5 years. The largest ethnic group was the Dutch and Caucasian (54.1%); other major ethnic groups were the Moroccan, Surinamese, and Turkish women. Of all women, 40.9% had completed high education. A total of 15.5% of the mothers smoked during pregnancy, and 36.5% continued using alcohol. Median gestational age at delivery was 40.1 weeks (90% range 20.5 to 38.0); mean birth weight of the newborns was 3418 grams (SD 561). Of all children, 5.5% were born preterm and 3.5% were born small for gestational age. Among the pregnant women, 3.4% were diagnosed with gestational hypertension, 2.0% developed (pre)eclampsia or HELLP, and 0.7% had gestational diabetes.

Table 1. Baseline characteristics (N=7339).

	Mean ± SD, median (95% range), or number (percentage)
Maternal characteristics	
Age at enrolment (yr)	30.5 (20.5-38.0)
Weight at enrolment (kg)	67.0 (52.0-94.0)
Height (cm)	167.2 ± 7.4
Body mass index at enrolment (kg/m^2)	23.8 (19.3-33.5)
Ethnicity – n (%)	
Dutch – Caucasian	54.1
Turkish	8.3
Moroccan	6.4
Surinamese	8.2
Other	15.6
Missing	7.4
Educational level – n (%)	
No education/primary	10.2
Secondary	39.9
Higher	40.9
Missing	9.0
Parity – n (%)	
Nulliparous	55.0
Multiparous	43.8
Missing	1.2

Table 1. *Continued*

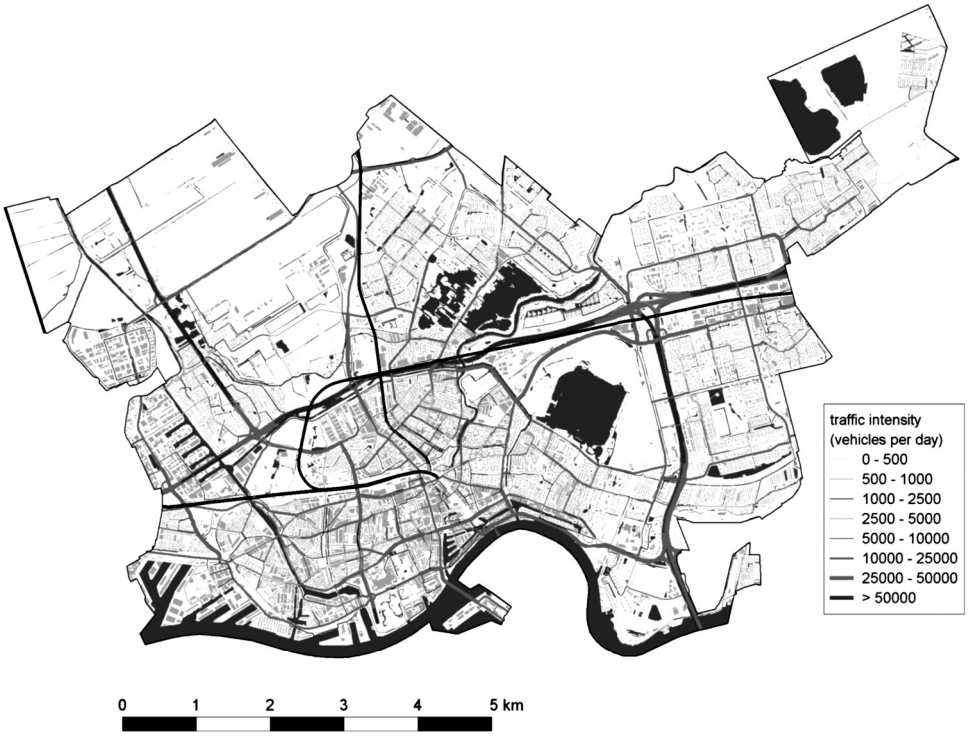
	Mean ± SD, median (95% range), or number (percentage)
Smoking in pregnancy – n (%)	
No	72.1
Yes	15.5
Missing	12.4
Alcohol consumption in pregnancy – n (%)	
No	52.7
Yes	36.5
Missing	10.8
Birth and pregnancy outcomes	
Gestational age at birth (wks)	40.1 (36.9-42.1)
Birth weight (g)	3417.6 ± 561.0
SDS birth weight	-0.10 ± 1.03
Male (%)	50.3
Small size for gestational age at birth (<-2.0 SDS) (%)	3.5
Preterm birth (<37 wk) (%)	5.5
Gestational hypertension (%)	3.4
(Pre)eclampsia or HELLP (%)	2.0
Gestational diabetes (%)	0.7

Values are means ± SD, or medians (90% range) for variables with a skewed distribution, and number of subjects (%) in case of categorical variables
Of the total group, data were missing on maternal weight at enrolment (n=31), maternal height (n=26), body mass index at enrolment (n=56), gestational age at birth (n=2), fetal sex (n=29), birth weight (n=51), SDS birth weight (n=61), SGA at birth (n=61), preterm birth (n=2), gestational hypertension (n=231), (pre)eclampsia/ HELLP (n=231), and gestational diabetes (n=271).

Traffic variables

A map of the study area showing the road network, traffic intensities, and residences is shown in Figure 1. Characteristics of the distributions of distance-weighted traffic density and distance to a major road are shown in Supplementary Table S1. The distribution of DWTD was highly skewed, with a maximum of 18,500,000 vehicles/24h*m. In total, 14.5% of the participants lived within 50m of a major road and 36.0% lived more than 200m from a major road. The correlation between DWTD and distance to a major road was moderate (Spearman rho=0.70). Distance to a highway was only weakly correlated to the other traffic variables (rho=-0.28 and 0.32). In analyses with maternal sociodemographic variables and proximity to traffic, we observed that low body mass index, high educational level, and nulliparity were positively associated with residential traffic exposure, whereas Moroccan women had lower exposure to residential traffic (results not shown).

Figure 1. Map of the study area (Rotterdam North) showing the road network and traffic intensities (see legend), rail network (black lines), residences (in grey), and surface water (in blue).



Proximity to traffic and birth and pregnancy outcomes

There were no substantial differences in traffic exposure between cases and non-cases of adverse birth outcomes or maternal pregnancy complications (results not shown). Crude associations between proximity to traffic and birth outcomes and maternal pregnancy complications are presented in Supplementary Tables S2 and S3. We observed a few significant associations between distance to a major road and birth weight, and between DWTD and preterm birth. No associations with pregnancy complications were detected. Tables 2 and 3 show the results of the linear and logistic regression analyses for associations between proximity to traffic and pregnancy-related outcomes in mother and child, adjusted for covariates. We observed significant associations between the second DWTD quartile and the risk for preterm birth, and between living 100 to 150m from a major road and birth weight. Although not significant, there was some evidence of an exposure-response pattern for SGA at birth across the quartiles of DWTD and categories of distance to a major road. This pattern was less clear for birth weight and preterm birth. No consistent associations were observed between traffic exposure and pregnancy

complications, although we did see a tendency towards higher odds ratios in the highest exposure categories.

Table 2. Covariate-adjusted associations between residential traffic exposure and birth outcomes.

	Birth weight (g) [b]	**Small for gestational age** [c] *(n of cases)*	**Preterm birth** [c] *(n of cases)*
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference (n=61)*	*Reference (n=84)*
158,503 – 546,770	-20 (-47, 8)	0.94 (0.65, 1.36) *(n=60)*	1.37 (1.02, 1.84) * *n=112)*
546,770 – 1,235,384	-9 (-37, 18)	0.99 (0.69, 1.43) *(n=62)*	1.33 (0.98, 1.79) ‡ *(n=110)*
> 1,235,384	6 (-21, 34)	1.12 (0.78, 1.59) *(n=74)*	1.18 (0.87, 1.59) *(n=100)*
Distance to major road (m)			
> 200 *(n=2646)*	*Reference*	*Reference (n=82)*	*Reference (n=134)*
150-200 *(n=1066)*	-21 (-52, 9)	1.00 (0.67, 1.49) *(n=38)*	1.09 (0.79, 1.50) *(n=59)*
100-150 *(n=1258)*	-41 (-69, -12) *	1.01 (0.69, 1.48*)* *(n=44)*	1.13 (0.84, 1.52) *(n=75)*
50-100 *(n=1302)*	8 (-20, 37)	1.12 (0.78, 1.62) *(n=51)*	1.08 (0.80, 1.45) *(n=74)*
0-50 *(n=1067)*	-6 (-36, 24)	1.14 (0.77, 1.68) *(n=42)*	1.15 (0.84, 1.58) *(n=64)*

* p<0.05; ‡ p<0.10

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are regression coefficients (95% confidence interval) and reflect the difference in birth weight for change in traffic parameters. Analyses are based on 7288 subjects. Models are adjusted for maternal age, education, ethnicity, body mass index, parity, smoking, alcohol consumption, gestational age, fetal sex, month of birth, and year of birth.

[c] Values are odds ratios (95% confidence interval) and reflect the risk for adverse birth outcomes for change in traffic parameters. Analyses are based on 7278 subjects for small for gestational age at birth and 7337 subjects for preterm birth. Models are adjusted for maternal age, education, ethnicity, body mass index, parity, smoking, alcohol consumption, fetal sex, month of birth, and year of birth.

Table 3. Covariate-adjusted associations between residential traffic exposure and pregnancy complications.

	Gestational hypertension [b] *(n of cases)*	**(Pre)eclampsia or HELLP** [b] *(n of cases)*	**Gestational diabetes** [b] *(n of cases)*
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference (n=64)*	*Reference (n=34)*	*Reference (n=15)*
158,503 – 546,770	1.00 (0.69, 1.45) *(n=59)*	0.94 (0.57, 1.55) *(n=31)*	0.69 (0.30, 1.57) *(n=10)*
546,770 – 1,235,384	0.90 (0.62, 1.30) *n=59)*	1.12 (0.70, 1.79) *(n=39)*	1.07 (0.51, 2.23) *(n=15)*
> 1,235,384	1.07 (0.75, 1.53) *(n=68)*	1.14 (0.71, 1.82) *(n=40)*	0.79 (0.35, 1.81) *(n=10)*
Distance to major road (m)			
> 200 *(n=2646)*	*Reference (n=93)*	*Reference (n=54)*	*Reference (n=19)*
150-200 *(n=1066)*	0.88 (0.57, 1.36) *(n=29)*	0.74 (0.42, 1.29) *(n=17)*	1.07 (0.47, 2.44) *(n=9)*
100-150 *(n=1258)*	0.94 (0.64, 1.39) *(n=39)*	0.96 (0.59, 1.56) *(n=25)*	0.77 (0.32, 1.88) *(n=7)*
50-100 *(n=1302)*	1.07 (0.75, 1.54) *(n=49)*	0.85 (0.52, 1.38) *(n=24)*	1.13 (0.51, 2.50) *(n=10)*
0-50 *(n=1067)*	1.08 (0.74, 1.60) *(n=40)*	1.03 (0.63, 1.69) *(n=24)*	0.68 (0.25, 1.86) *(n=5)*

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.
[b] Values are odds ratios (95% confidence interval) and reflect the risk for pregnancy complications for change in traffic parameters. Analyses are based on 7108 subjects for gestational hypertension and for (pre)eclampsia or HELLP, and on 7068 subjects for gestational diabetes. Models are adjusted for maternal age, education, ethnicity, body mass index, parity, smoking, alcohol consumption, month of birth, and year of birth.

Sensitivity analyses

Although we observed a few significant associations between proximity to traffic and birth outcomes (Table 2), these could not be reproduced in sensitivity analyses when different categorizations and buffer radii for the traffic measures were chosen. In line with the results from the main analyses, no associations with pregnancy outcomes were observed in the sensitivity analyses. Similarly, when distance to a highway was used as an exposure metric, no significant associations with birth and pregnancy outcomes were observed. Furthermore, results of the analyses did not change after excluding women who were enrolled in late pregnancy and for whom gestational age was determined based on LMP. Results were also comparable when analyses were adjusted for maternal BMI before pregnancy rather than maternal BMI at enrolment. In addition, logistic regression analysis with SGA at birth in the subgroup of Dutch participants yielded similar results (see

Supplementary Table S4). Stratified analyses by educational level did not show different results (see Supplementary Tables S5, S6 and S7 for associations between proximity to traffic and selected outcomes). However, it must be noted that the statistical power for some of these analyses was limited due to small numbers of participants in the subgroups, especially in the lowest educational group. Finally, stratified analyses by residential mobility showed that results were not different across strata (see Supplementary Tables S8 and S9).

DISCUSSION

To our knowledge, this is the first report on residential proximity to traffic and pregnancy-related outcomes that was based on a prospective cohort study. A significant number of examinations were performed in mothers and children, providing information on the relevant potentially confounding variables. We observed no associations between residential proximity to traffic and birth and pregnancy outcomes, also after controlling for potential confounders. Proximity to traffic was defined by means of two variables: distance-weighted traffic density (DWTD) and distance to a major road. These traffic measures are used to capture the spatial variation within a city, and have been applied previously in a small number of register-based studies on birth outcomes [19-24]. These studies did not show conclusive evidence. Two studies, both conducted in California, used distance-weighted traffic density as an exposure metric. The first study observed an association between increased DWTD and the risk of preterm birth, with stronger effects in women living in lower SES areas [21]. The second study reported that DWTD was positively associated with the risk of preterm birth in mothers living in low SES neighbourhoods whose third trimester fell during winter, and in mothers living in moderate SES neighbourhoods [23]. Another study, conducted in Massachusetts, used cumulative traffic density as an exposure metric, which is a more rough exposure metric than DWTD as the products of the traffic intensities and the lengths of the road segments are not weighted. The study observed an association for cumulative traffic density with the risk for SGA at birth, but not with birth weight and preterm birth. Moreover, the researchers also reported evidence for effect modification by socioeconomic status, with stronger effects of proximity to traffic in low educated women and in women living in lower SES areas [19]. Distance to a major road or highway was examined previously in relation to birth outcomes as well. The Massachusetts study observed associations between distance to a primary highway and birth weight, but not with the risks of preterm birth and SGA at birth [19]. In Taiwan, an increased risk of preterm delivery was reported in mothers living within 500m of one particular freeway compared to mothers living between 500-1500m from this freeway [22]. Two recent studies in British Columbia produced different findings. Brauer et al. (2008) observed an increased risk of SGA at birth in mothers living within 50m from an expressway or highway (with a mean of >21,000 vehicles/day), but no

association was found with the risk of preterm birth. Also, no significant associations were detected for those living within 50 or 150m from a road with a mean of 15,000-18,000 vehicles/day [24]. The second study reported that proximity to a highway (with a minimum speed of 70 km/hr) was associated with preterm birth, but not with SGA at birth [20]. Moreover, a higher susceptibility among advantaged mothers was described, in contrast to the American studies. In our study, stratified analyses on SES showed no differences in susceptibility for traffic exposure between the SES groups.

To our knowledge, no previous studies have been conducted on residential proximity to traffic, or on air pollution exposure in the broader sense, and pregnancy complications. Recently, it has been suggested that studying these outcomes may provide insights into the underlying mechanisms [37]. In the present study, no crude or adjusted associations between residential proximity to traffic and pregnancy complications were observed, although we did observe tendencies towards elevated odds ratios in the highest exposure groups.

There are several differences between earlier studies and the present study that may explain the dissimilar findings. First of all, previous studies primarily relied upon birth records, which may have resulted in less complete information on important confounders. In our study, maternal education, ethnicity, body mass index, parity, and smoking were the main predictors in most of the models with birth outcomes, and in some of the models with pregnancy complications. Earlier studies did not have information on all of these covariates [20-24]. As a result, they may have been more susceptible to residual confounding, which could have affected some of the observed associations.

Secondly, the exposure metrics used in the different studies are based on different input data. Also, the classification of roads, calculation methods (e.g. buffer size), and the accuracy and completeness of traffic and road data may vary between studies.

Third, the observed differences between previous studies and the present study may be related to the geographic location of the studies. Rotterdam is the second largest city in the Netherlands and has a high population density. It is characterized by high emissions from road traffic, shipping, households, and industry. In the year 2004, average air pollutant levels derived from ambient monitoring stations in the Rijnmond region (the larger Rotterdam area) were 30.7 $\mu g/m^3$ for PM_{10}, 43.8 $\mu g/m^3$ for nitrogen dioxide (NO_2), and 13.8 $\mu g/m^3$ for sulfur dioxide (SO_2) [38]. These average concentrations are based on both regional background stations and traffic stations, consequently, pollutant levels in the specific (urban) area under study may be even higher. Previous studies on proximity to traffic and pregnancy outcomes have mainly been performed in the United States, Canada and Taiwan. No previous studies on these specific exposure measures and outcomes have been conducted in a European area, where air pollution may differ in terms of composition and concentrations.

This study has some potential limitations. First, there is the potential for misclassification of exposure. Exposure levels were estimated at the home address, whereas pregnant women do not spend all of their time at home. No detailed information

was available about time-activity patterns of the women. However, it has been suggested that outdoor levels of traffic components are well correlated with indoor levels [39, 40] and are good predictors of personal exposure [39, 41]. Furthermore, exposure misclassification may arise from a change in address during pregnancy. As residential mobility during pregnancy has previously been shown to be differential by sociodemographic factors (e.g., maternal age, household income, parity, and ethnicity) [42], it could influence the results of our study. In stratified analyses, we observed that results were not different across those who did/did not change residence in the period between seven months before conception and five months of pregnancy. This indicates that residential mobility did not have a large effect on our effect estimates.

Second, the sample size of our study was smaller than that of previous studies, which were based on birth certificate data and had sample sizes of 37,000-99,000 subjects. Our study had 7339 participants. We were able to detect a difference of 0.11 SD in birth weight, which is smaller than effect sizes observed in previous studies. However, the power to detect a relationship between air pollution and some of the dichotomous outcome measures was lower compared to previous studies, especially for the analyses with pregnancy complications.

Furthermore, gestational age could not be determined based on ultrasound examinations in 3% of the participants, because they were enrolled in late pregnancy. Nevertheless, results were comparable when these women were included or excluded.

Another limitation is related to the traffic density measures. These are derived from digital maps that cover the most important residential roads, but do not include the smallest local roads. As a result, traffic on these streets is not counted in the distance-weighted traffic density.

Finally, traffic measures may be viewed as crude estimates of air pollution. They do not take into account influencing factors such as type of traffic, emission factors, meteorology, and land cover data. Furthermore, they are based on annual averages and do not reflect seasonal, monthly or daily differences in air pollution levels. Ideally, these temporal variations would be taken into account in the exposure assessment, next to the spatial variability. This has been done by a few earlier pregnancy studies, some of them conducted in Europe, in which air pollution concentrations were modelled and subsequently adjusted for temporal variation [24, 25, 43, 44]. Unfortunately, we were not able to take into account temporal variations in our air pollution exposure assessment, but we are planning to do this for future analyses. Despite these limitations, a recent study that assessed the validity of traffic variables showed that measures of (weighted) traffic density can well be used as predictors of measured NO_2, and are therefore good proxies for exposure to road traffic [45]. So, when direct measurements or modelled levels of traffic-related air pollutants are not available, traffic measures are a good alternative, as they are relatively simple measures that are easy to apply and to interpret [24].

Conclusion

The present study is based on a prospective population-based cohort with a large number of subjects studied from early pregnancy onwards. Exposures were estimated at the individual level, and detailed individual information on relevant confounders was available. In the city of Rotterdam, residential proximity to traffic was not associated with birth and pregnancy outcomes, contrary to previous studies. Future studies are needed to further investigate this relationship, preferably with more detailed data on temporal and spatial variation in exposure.

REFERENCES

1. Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. *Epidemiology.* 2004; 15(1):36-45.
2. Lacasana M, Esplugues A, Ballester F. Exposure to ambient air pollution and prenatal and early childhood health effects. *Eur J Epidemiol.* 2005; 20(2):183-199.
3. Maisonet M, Correa A, Misra D, Jaakkola JJ. A review of the literature on the effects of ambient air pollution on fetal growth. *Environ Res.* 2004; 95(1):106-115.
4. Sram RJ, Binkova B, Dejmek J, Bobak M. Ambient air pollution and pregnancy outcomes: a review of the literature. *Environ Health Perspect.* 2005; 113(4):375-382.
5. Lin CM, Li CY, Yang GY, Mao IF. Association between maternal exposure to elevated ambient sulfur dioxide during pregnancy and term low birth weight. *Environ Res.* 2004; 96(1):41-50.
6. Ritz B, Yu F, Chapa G, Fruin S. Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993. *Epidemiology.* 2000; 11(5):502-11.
7. Dejmek J, Selevan SG, Benes I, Solansky I, Sram RJ. Fetal growth and maternal exposure to particulate matter during pregnancy. *Environ Health Perspect.* 1999; 107(6):475-480.
8. Sagiv SK, Mendola P, Loomis D, Herring AH, Neas LM, Savitz DA, et al. A time-series analysis of air pollution and preterm birth in Pennsylvania, 1997-2001. *Environ Health Perspect.* 2005; 113(5):602-606.
9. Fischer PH, Hoek G, van Reeuwijk H, Briggs DJ, Lebret E, van Wijnen JH, et al. Traffic-related differences in outdoor and indoor concentrations of particles and volatile organic compounds in Amsterdam. *Atmos Environ.* 2000; 34(22):3713-3722.
10. Lewne M, Cyrys J, Meliefste K, Hoek G, Brauer M, Fischer P, et al. Spatial variation in nitrogen dioxide in three European areas. *Sci Total Environ.* 2004; 332(1-3):217-30.
11. Janssen NAH, van Vliet PHN, Aarts F, Harssema H, Brunekreef B. Assessment of exposure to traffic related air pollution of children attending schools near motorways. *Atmos Environ.* 2001; 35(22):3875-3884.
12. Zhu Y, Hinds WC, Kim S, Sioutas C. Concentration and size distribution of ultrafine particles near a major highway. *J Air Waste Manag Assoc.* 2002; 52(9):1032-42.
13. Lena TS, Ochieng V, Carter M, Holguin-Veras J, Kinney PL. Elemental carbon and PM(2.5)levels in an urban community heavily impacted by truck traffic. *Environ Health Perspect.* 2002; 110(10):1009-15.
14. Cesaroni G, Badaloni C, Porta D, Forastiere F, Perucci CA. Comparison between various indices of exposure to traffic-related air pollution and their impact on respiratory health in adults. *Occup Environ Med.* 2008; 65(10):683-90.
15. English P, Neutra R, Scalf R, Sullivan M, Waller L, Zhu L. Examining associations between childhood asthma and traffic flow using a geographic information system. *Environ Health Perspect.* 1999; 107(9):761-7.
16. Tonne C, Melly S, Mittleman M, Coull B, Goldberg R, Schwartz J. A case-control analysis of exposure to traffic and acute myocardial infarction. *Environ Health Perspect.* 2007; 115(1):53-7.
17. Beelen R, Hoek G, van den Brandt PA, Goldbohm RA, Fischer P, Schouten LJ, et al. Long-term effects of traffic-related air pollution on mortality in a Dutch cohort (NLCS-AIR study). *Environ Health Perspect.* 2008; 116(2):196-202.
18. Pearson RL, Wachtel H, Ebi KL. Distance-weighted traffic density in proximity to a home is a risk factor for leukemia and other childhood cancers. *J Air Waste Manag Assoc.* 2000; 50(2):175-80.
19. Zeka A, Melly SJ, Schwartz J. The effects of socioeconomic status and indices of physical environment on reduced birth weight and preterm births in Eastern Massachusetts. *Environ Health.* 2008; 7(60):1-13.
20. Genereux M, Auger N, Goneau M, Daniel M. Neighbourhood socioeconomic status, maternal education and adverse birth outcomes among mothers living near highways. *J Epidemiol Community Health.* 2008; 62(8):695-700.
21. Wilhelm M, Ritz B. Residential proximity to traffic and adverse birth outcomes in Los Angeles county, California, 1994-1996. *Environ Health Perspect.* 2003; 111(2):207-216.
22. Yang CY, Chang CC, Chuang HY, Ho CK, Wu TN, Tsai SS. Evidence for increased risks of preterm delivery in a population residing near a freeway in Taiwan. *Arch Environ Health.* 2003; 58(10):649-54.
23. Ponce NA, Hoggatt KJ, Wilhelm M, Ritz B. Preterm birth: the interaction of traffic-related air pollution with economic hardship in Los Angeles neighborhoods. *Am J Epidemiol.* 2005; 162(2):140-8.

24. Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C. A cohort study of traffic-related air pollution impacts on birth outcomes. *Environ Health Perspect.* 2008; 116(5):680-6.
25. Slama R, Morgenstern V, Cyrys J, Zutavern A, Herbarth O, Wichmann HE, et al. Traffic-related atmospheric pollutants levels during pregnancy and offspring's term birth weight: a study relying on a land-use regression exposure model. *Environ Health Perspect.* 2007; 115(9):1283-1292.
26. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
27. Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. Meeting report: atmospheric pollution and human reproduction. *Environ Health Perspect.* 2008; 116(6):791-8.
28. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
29. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007; 22(12):917-23.
30. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. *Eur J Epidemiol.* 2008; 23(12):801-11.
31. Statistics Netherlands: CBS Statline, Index numbers for traffic intensity, year 2002-2005 [in Dutch]. Available: http://statline.cbs.nl/StatWeb
32. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. *Ultrasound Obstet Gynecol.* 1996; 8(3):178-85.
33. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand.* 1991; 80(8-9):756-62.
34. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy.* 2001; 20(1):IX-XIV.
35. Ay L, Kruithof CJ, Bakker R, Steegers EA, Witteman JC, Moll HA, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. *BJOG.* 2009; 116(7):953-63.
36. Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, et al. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. *Paediatr Perinat Epidemiol.* 2008; 22(2):162-71.
37. Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. Methodological issues in studies of air pollution and reproductive health. *Environ Res.* 2009; 109(3):311-20.
38. DCMR Environmental Protection Agency: Air in Numbers 2004. Air quality in the Rijnmond area. [Lucht in cijfers 2004. Luchtkwaliteit in het Rijnmondgebied]. 2004. Available: http://www.dcmr.nl/binaries/publicatie/2005/LUC/luchtincijfers2004.pdf
39. Janssen NA, Lanki T, Hoek G, Vallius M, de Hartog JJ, Van Grieken R, et al. Associations between ambient, personal, and indoor exposure to fine particulate matter constituents in Dutch and Finnish panels of cardiovascular patients. *Occup Environ Med.* 2005; 62(12):868-77.
40. Gotschi T, Oglesby L, Mathys P, Monn C, Manalis N, Koistinen K, et al. Comparison of black smoke and PM2.5 levels in indoor and outdoor environments of four European cities. *Environ Sci Technol.* 2002; 36(6):1191-7.
41. Janssen NA, Hoek G, Brunekreef B, Harssema H, Mensink I, Zuidhof A. Personal sampling of particles in adults: relation among personal, indoor, and outdoor air concentrations. *Am J Epidemiol.* 1998; 147(6):537-47.
42. Canfield MA, Ramadhani TA, Langlois PH, Waller DK. Residential mobility patterns and exposure misclassification in epidemiologic studies of birth defects. *J Expo Sci Environ Epidemiol.* 2006; 16(6):538-43.
43. Aguilera I, Guxens M, Garcia-Esteban R, Corbella T, Nieuwenhuijsen MJ, Foradada CM, et al. Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort. *Environ Health Perspect.* 2009; 117(8):1322-7.

44. Fanshawe TR, Diggle PJ, Rushton S, Sanderson R, Lurz PWW, Glinianaia SV, et al. Modelling spatio-temporal variation in exposure to particulate matter: a two-stage approach. *Environmetrics.* 2008; 19(6):549-566.
45. Rose N, Cowie C, Gillett R, Marks GB. Weighted road density: A simple way of assigning traffic-related air pollution exposure. *Atmos Environ.* 2009; 43(32):5009-5014.

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Distribution of traffic indicators.

	Minimum	25th percentile	Median	75th percentile	Maximum
Distance-weighted traffic density (vehicles/24h*m)	0	$1.6 * 10^5$	$5.5 * 10^5$	$1.2 * 10^6$	$1.9 * 10^7$
Distance to a major road (m)	7	74	143	225	498

Supplementary Table S2. Crude associations between residential traffic exposure and birth outcomes.

	Birth weight (g) [b]	**Small for gestational age** [c] *(n of cases)*	**Preterm birth** [c] *(n of cases)*
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference (n=61)*	*Reference (n=84)*
158,503 – 546,770	-29 (-58, 0) ‡	0.99 (0.69, 1.42) *(n=60)*	1.37 (1.02, 1.84) * *(n=112)*
546,770 – 1,235,384	-19 (-48, 10)	1.02 (0.71, 1.47) *(n=62)*	1.36 (1.01, 1.83) * *(n=110)*
> 1,235,384	-14 (-43, 15)	1.22 (0.87, 1.73) *(n=74)*	1.25 (0.92, 1.69) *(n=100)*
Distance to major road (m)			
> 200 *(n=2646)*	*Reference*	*Reference (n=82)*	*Reference (n=134)*
150-200 *(n=1066)*	-48 (-79, -16) *	1.15 (0.78, 1.70) *(n=38)*	1.13 (0.82, 1.55) *(n=59)*
100-150 *(n=1258)*	-65 (-95, -35) **	1.13 (0.78, 1.65) *(n=44)*	1.20 (0.89, 1.61) *(n=75)*
50-100 *(n=1302)*	-18 (-48, 12)	1.27 (0.89, 1.81) *(n=51)*	1.16 (0.86, 1.56) *(n=74)*
0-50 *(n=1067)*	-28 (-59, 4) ‡	1.28 (0.88, 1.87) *(n=42)*	1.23 (0.90, 1.68) *(n=64)*

** p<0.001; * p<0.05; ‡ p<0.10

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are regression coefficients (95% confidence interval) and reflect the difference in birth weight for change in traffic parameters. Analyses are based on 7288 subjects. Models are adjusted for gestational age and fetal sex.

[c] Values are odds ratios (95% confidence interval) and reflect the risk for adverse birth outcomes for change in traffic parameters. Analyses are based on 7278 subjects for small for gestational age at birth and 7337 subjects for preterm birth. Models are adjusted for fetal sex.

Supplementary Table S3. Unadjusted associations between residential traffic exposure and pregnancy complications.

	Gestational hypertension [b] *(n of cases)*	**(Pre)eclampsia or HELLP** [b] *(n of cases)*	**Gestational diabetes** [b] *(n of cases)*
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference (n=64)*	*Reference (n=34)*	*Reference (n=15)*
158,503 – 546,770	0.92 (0.64, 1.32) *(n=59)*	0.91 (0.56, 1.50) *(n=31)*	0.66 (0.30, 1.48) *(n=10)*
546,770 – 1,235,384	0.92 (0.64, 1.32) *(n=59)*	1.15 (0.73, 1.84) *(n=39)*	1.00 (0.49, 2.05) *(n=15)*
> 1,235,384	1.06 (0.75, 1.50) *(n=68)*	1.18 (0.74, 1.87) *(n=40)*	0.67 (0.30, 1.49) *(n=10)*
Distance to major road (m)			
> 200 (n=2646)	*Reference (n=93)*	*Reference (n=54)*	*Reference (n=19)*
150-200 (n=1066)	0.77 (0.50, 1.17) *(n=29)*	0.78 (0.45, 1.34) *(n=17)*	1.17 (0.53, 2.60) *(n=9)*
100-150 (n=1258)	0.87 (0.60, 1.28) *(n=39)*	0.97 (0.60, 1.56) *(n=25)*	0.76 (0.32, 1.82) *(n=7)*
50-100 (n=1302)	1.07 (0.75, 1.52) *(n=49)*	0.89 (0.55, 1.45) *(n=24)*	1.07 (0.50, 2.31) *(n=10)*
0-50 (n=1067)	1.07 (0.73, 1.55) *(n=40)*	1.10 (0.68, 1.79) *(n=24)*	0.65 (0.24, 1.76) *(n=5)*

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.
[b] Values are odds ratios (95% confidence interval) and reflect the risk for pregnancy complications for change in traffic parameters. Analyses are based on 7108 subjects for gestational hypertension and for (pre) eclampsia or HELLP, and on 7068 subjects for gestational diabetes.

Supplementary Table S4. Adjusted associations between residential traffic exposure and SGA at birth in Dutch children (N=3414).

	Small for gestational age [b] *(n of cases)*
Distance-weighted traffic density (veh/24h*m) [a]	
< 158,503	*Reference (n=27)*
158,503 – 546,770	0.82 (0.46, 1.46) *(n=22)*
546,770 – 1,235,384	1.08 (0.62, 1.87) *(n=28)*
> 1,235,384	0.74 (0.41, 1.34) *(n=21)*
Distance to major road (m)	
> 200	*Reference (n=42)*
150-200	0.52 (0.25, 1.08) ‡ *(n=14)*
100-150	1.11 (0.64, 1.93) (*n=13)*
50-100	0.74 (0.39, 1.41) *(n=20)*
0-50	1.09 (0.58, 2.05) *(n=9)*

‡ $p<0.10$

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are odds ratios (95% confidence interval) and reflect the risk for small for gestational age at birth for change in traffic parameters. Models are adjusted for maternal age, education, body mass index, parity, smoking, alcohol consumption, fetal sex, month of birth, and year of birth.

Supplementary Table S5. Adjusted associations between residential traffic exposure and birth weight, stratified for maternal education.

	Birth weight (g) [b]		
	None/ primary education	**Secondary education**	**Higher education**
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference*	*Reference*
158,503 – 546,770	-26 (-115, 64)	-17 (-60, 26)	-20 (-63, 23)
546,770 – 1,235,384	-56 (-145, 32)	-20 (-63, 22)	10 (-34, 55)
> 1,235,384	43 (-49, 134)	0 (-44, 43)	5 (-38, 47)
Distance to major road (m)			
> 200	*Reference*	*Reference*	*Reference*
150-200	7 (-85, 100)	-32 (-80, 17)	-20 (-68, 28)
100-150	-2 (-94, 90)	-50 (-94, -6) *	-45 (-91, 2) ‡
50-100	32 (-57, 121)	-7 (-52, 37)	16 (-29, 61)
0-50	98 (-5, 201) ‡	-33 (-83, 16)	5 (-41, 50)

* p<0.05; ‡ p<0.10

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are regression coefficients (95% confidence interval) and reflect the difference in birth weight for change in traffic parameters. Models are adjusted for maternal age, ethnicity, body mass index, parity, smoking, alcohol consumption, gestational age, fetal sex, month of birth, and year of birth.

Supplementary Table S6. Adjusted associations between residential traffic exposure and SGA at birth, stratified for maternal education.

	Small for gestational age [b]		
	None/ primary education	**Secondary education**	**Higher education**
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference*	*Reference*
158,503 – 546,770	1.11 (0.38, 3.27)	0.64 (0.36, 1.16)	1.20 (0.64, 2.26)
546,770 – 1,235,384	1.03 (0.35, 3.03)	0.95 (0.56, 1.62)	1.05 (0.53, 2.07)
> 1,235,384	0.54 (0.17, 1.72)	1.18 (0.71, 1.97)	1.05 (0.54, 2.01)
Distance to major road (m)			
> 200	*Reference*	*Reference*	*Reference*
150-200	1.46 (0.49, 4.38)	1.50 (0.84, 2.69)	0.54 (0.24, 1.20)
100-150	1.08 (0.33, 3.58)	0.95 (0.52, 1.76)	0.80 (0.42, 1.56)
50-100	0.94 (0.31, 2.89)	1.55 (0.90, 2.67)	0.95 (0.50, 1.79)
0-50	0.67 (0.18, 2.53)	1.44 (0.79, 2.63)	0.54 (0.25, 1.14)

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are odds ratios (95% confidence interval) and reflect the risk for small size for gestational age at birth for change in traffic parameters. Models are adjusted for maternal age, ethnicity, body mass index, parity, smoking, alcohol consumption, fetal sex, month of birth, and year of birth.

Supplementary Table S7. Adjusted associations between residential traffic exposure and gestational hypertension, stratified for maternal education.

	Gestational hypertension [b]		
	None/ primary education	**Secondary education**	**Higher education**
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference*	*Reference*
158,503 – 546,770	0.44 (0.03, 6.15)	1.38 (0.79, 2.40)	0.86 (0.50, 1.48)
546,770 – 1,235,384	6.00 (0.92, 39.21)	1.06 (0.60, 1.84)	0.62 (0.34, 1.13)
> 1,235,384	3.64 (0.60, 22.07)	1.32 (0.77, 2.29)	0.82 (0.48, 1.40)
Distance to major road (m)			
> 200	*Reference*	*Reference*	*Reference*
150-200	0.54 (0.05, 5.74)	1.25 (0.70, 2.26)	1.07 (0.59, 1.97)
100-150	2.56 (0.44, 15.06)	1.10 (0.64, 1.89)	0.66 (0.34, 1.29)
50-100	4.12 (0.92, 18.49) ‡	1.29 (0.75, 2.23)	0.82 (0.45, 1.50)
0-50	1.06 (0.17, 6.68)	0.66 (0.32, 1.35)	0.94 (0.53, 1.68)

‡ $p<0.10$

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are odds ratios (95% confidence interval) and reflect the risk for gestational hypertension for change in traffic parameters. Models are adjusted for maternal age, ethnicity, body mass index, parity, smoking, alcohol consumption, month of birth, and year of birth.

Supplementary Table S8. Adjusted associations between residential traffic exposure and birth outcomes in non-movers (N=1118).

	Birth weight (g) [b]	**Small for gestational age** [c]	**Preterm birth** [c]
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference*	*Reference*
158,503 – 546,770	-1 (-73, 71)	0.84 (0.35, 2.05)	1.24 (0.58, 2.64)
546,770 – 1,235,384	36 (-37, 110)	0.92 (0.38, 2.22)	1.28 (0.59, 2.77)
> 1,235,384	40 (-33, 113)	0.57 (0.22, 1.51)	1.30 (0.61, 2.80)
Distance to major road (m)			
> 200	*Reference*	*Reference*	*Reference*
150-200	-51 (-132, 31)	1.59 (0.62, 4.05)	1.29 (0.59, 2.81)
100-150	-78 (-154, -3) *	1.03 (0.40, 2.62)	1.11 (0.53, 2.33)
50-100	6 (-70, 80)	1.11 (0.43, 2.85)	0.84 (0.36, 1.93)
0-50	-35 (-115, 45)	0.82 (0.28, 2.43)	1.30 (0.61, 2.79)

* $p<0.05$

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.

[b] Values are regression coefficients (95% confidence interval) and reflect the difference in birth weight for change in traffic parameters. The model is adjusted for maternal age, maternal education, ethnicity, body mass index, parity, smoking, alcohol consumption, gestational age, fetal sex, month of birth, and year of birth.

[c] Values are odds ratios (95% confidence interval) and reflect the risk for adverse birth outcomes for change in traffic parameters. Models are adjusted for maternal age, education, ethnicity, body mass index, parity, smoking, alcohol consumption, fetal sex, month of birth, and year of birth.

Supplementary Table S9. Adjusted associations between residential traffic exposure and pregnancy complications in non-movers (N=1118).

	Gestational hypertension [b]	(Pre)eclampsia or HELLP [b]	Gestational diabetes [b]
Distance-weighted traffic density (veh/24h*m) [a]			
< 158,503	*Reference*	*Reference*	*Reference*
158,503 – 546,770	1.08 (0.39, 3.04)	0.75 (0.15, 3.69)	n/a [c]
546,770 – 1,235,384	0.79 (0.25, 2.46)	1.95 (0.51, 7.41)	n/a [c]
> 1,235,384	1.42 (0.51, 3.90)	1.95 (0.52, 7.35)	n/a [c]
Distance to major road (m)			
> 200	*Reference*	*Reference*	*Reference*
150-200	0.24 (0.03, 1.92)	0.73 (0.14, 3.86)	n/a [c]
100-150	1.11 (0.38, 3.18)	1.10 (0.28, 4.26)	n/a [c]
50-100	1.03 (0.36, 2.92)	0.58 (0.11, 3.14)	n/a [c]
0-50	1.60 (0.59, 4.36)	2.18 (0.64, 7.36)	n/a [c]

[a] Values listed are the <25th, 25-50th, 50-75th and >75th percentiles of the DWTD values.
[b] Values are odds ratios (95% confidence interval) and reflect the risk for pregnancy complications for change in traffic parameters. Models are adjusted for maternal age, ethnicity, body mass index, parity, smoking, alcohol consumption, month of birth, and year of birth.
[c] Not applicable; odds ratios could not be computed because of zero cell counts.

Chapter 4

General discussion

INTRODUCTION

In the last fifteen to twenty years, the number of studies examining the possible impact of air pollution exposure on pregnancy outcomes has substantially increased. Most routinely measured air pollutants (e.g., PM_{10}, $PM_{2.5}$, NO_2, CO, O_3, SO_2) have been associated with neonatal complications such as intrauterine growth restriction, preterm birth, and (low) birth weight [1-3]. Nevertheless, recent reviews concluded that the results are inconsistent, with regard to the examined air pollutants, exposure periods, and neonatal outcomes, and regarding the reported associations [1, 3-5]. The inconsistencies may partly be related to differences in study design and methodological limitations [1, 3]. Most studies were based on birth certificates rather than prospectively collected data, and had limited information on potential confounding variables. Furthermore, many studies assessed exposure to air pollution using (a small number of) outdoor monitoring stations, which restricted their ability to take into account the intra-urban variation in air pollutants. More recent approaches based on spatial modelling considered the spatial, but often not the temporal variation. A number of recommendations for further research have been put forward, including better consideration of spatiotemporal contrasts in exposure and obtaining information on residential mobility during pregnancy [6, 7]. In addition, it has been advocated to examine specific outcomes that may provide insight into the underlying mechanisms, such as fetal growth, inflammatory responses, maternal blood pressure, and gestational hypertensive disorders [6, 7]. Only a few recent studies have examined the associations of maternal air pollution exposure with impaired fetal growth as measured by ultrasound examinations during pregnancy [8-10] or with the development of gestational hypertensive disorders [11-13]. Also, not much is known about the associations of air pollution with blood pressure, inflammatory responses, and placental function.

The studies described in this thesis were initiated to examine the associations of air pollution exposure during pregnancy with maternal and fetal health, and to gain more knowledge about the underlying pathways. All studies were embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, the Netherlands [14, 15]. In this part of the thesis, we describe the main findings of our studies and their interpretation. A schematic overview of the findings is also presented in Figure 1. Furthermore, we discuss some methodological considerations and we give suggestions for future research.

Figure 1. Schematic overview of the statistically significant findings in the studies described in this thesis.

Exposure	Maternal and fetal inflammation	Placental function	Maternal blood pressure	Maternal gestational hypertensive complications	Fetal growth	Neonatal complications
Traffic proximity	n/a	n/a	n/a		n/a	
PM_{10}	↑ mat CRP (1^{st})* ↑ fetal CRP (D)*	↓ mat sFlt-1 (2^{nd}) ↑ fetal sFlt-1 (D)* ↓ fetal PlGF (D)* ↓ PW*	↑ SBP (2^{nd}, 3^{rd})	↑ PIH	↓ HC (3^{rd}) ↑ EFW (2^{nd}) ↓ BW	↑ PTB ↑ SGA
NO_2	↑ fetal CRP (D)	↑ mat PLGF (1^{st})* ↓ mat sFlt-1 (2^{nd})* ↓ mat PLGF (2^{nd})* ↑ fetal sFlt-1 (D)* ↓ fetal PlGF (D) ↓ PW*	↑ SBP (1^{st}, 2^{nd}, 3^{rd})		↓ HC (3^{rd}) ↓ FL (2^{nd}, 3^{rd}) ↓ BW	

n/a (not applicable) indicates that the association was not examined; empty cells indicate that no statistically significant associations were observed;
* indicates that associations were not observed for all examined exposure periods (if different periods were evaluated).
1^{st}: first trimester; 2^{nd}: second trimester; 3^{rd}: third trimester; D: delivery; BW: birth weight; CRP: C-reactive protein; EFW: estimated fetal weight; FL: femur length; HC: head circumference; mat: maternal; PIH: gestational hypertension; PlGF: placental growth factor; PTB: preterm birth; PW: placenta weight; SBP: systolic blood pressure; sFlt-1: soluble fms-like tyrosine kinase-1; SGA: small size for gestational age at birth.

MAIN FINDINGS

Air pollution exposure estimation

Air pollution levels within an urban area display considerable spatial and temporal variation. Many previous studies on air pollution and pregnancy complications were not able to consider both the spatial and temporal variation in their exposure estimation approach. For the participants of the Generation R Study, we assessed individual exposures to PM_{10} and NO_2 at the home address, using a combination of GIS based dispersion modelling techniques and continuous monitoring data, taking into account the spatial and temporal variation in air pollutants. Air pollution exposure averages were derived for different prenatal periods, in order to relate these exposure averages to various outcomes assessed during pregnancy. We observed that on average, participants' exposure levels decreased throughout the study period (2001 to 2006). Illustrative analyses indicated that PM_{10} and NO_2 exposure levels during pregnancy were differentially distributed according to maternal age, ethnicity, parity, neighbourhood income area, smoking, and alcohol consumption. The individual exposure estimates have been applied in the studies presented in this thesis and can be used in future studies focused on the effects of air pollution exposure on health outcomes in mothers and children **(Chapter 2)**.

Maternal and fetal inflammatory response

Previous studies observed associations of air pollution exposure with higher levels of the inflammatory marker C-reactive protein (CRP). Not much is known about this association in pregnant women. We evaluated whether air pollution exposure was related to maternal CRP levels in first trimester of pregnancy and fetal CRP levels at delivery. Short-term average PM_{10} exposure levels were weakly associated with elevated maternal CRP levels in first trimester, which could indicate that short-term peaks in exposure may induce inflammatory processes in the mother. Higher long-term average PM_{10} and NO_2 exposure levels were associated with elevated fetal CRP levels at delivery. Since fetal CRP levels are believed to be produced by the fetus itself [16, 17], this may suggest that cumulative (long-term) exposures are more important in affecting fetal inflammatory processes than short-term exposures. Our findings suggest that air pollution exposure may promote the inflammatory processes in mother and fetus. More research is needed to confirm these findings and to examine the underlying mechanisms (**Chapter 3.1**).

Placental growth and function

One of the hypothesized pathways through which air pollution may lead to maternal and fetal complications is by affecting the placenta [6, 18]. Few studies have investigated the associations of air pollution exposure with markers of placental growth and function. We observed that higher PM_{10} and NO_2 exposure levels were associated with changes in the levels of angiogenic growth factors (sFlt-1 and PlGF) in fetal cord blood, reflecting an anti-angiogenic state. This pattern was not observed with maternal sFlt-1 and PlGF levels in first

and second trimester. Air pollution exposure was not associated with placental vascular resistance indices. Furthermore, we observed associations of PM_{10} and NO_2 exposure with a decreased placental weight, but these associations were not consistent for the different exposure periods. Our results suggest that maternal air pollution exposure may influence placental growth and function. Nevertheless, the evidence is insufficient to infer that this pathway plays a major role in the associations of air pollution with pregnancy complications (**Chapter 3.2**).

Maternal blood pressure and gestational hypertensive disorders

Another potential pathway through which air pollution may affect pregnancy is by acting on the cardiovascular system. Air pollution exposure has been shown to be associated with alterations in blood pressure levels in non-pregnant individuals [19-22]. We examined the associations of air pollution exposure with repeatedly measured blood pressure during pregnancy and the risks of gestational hypertension and preeclampsia. Higher PM_{10} and NO_2 exposure levels were associated with increased systolic blood pressure levels in different trimesters of pregnancy. No consistent associations were observed with diastolic blood pressure levels. Also, higher PM_{10} exposure was associated with an increased risk of gestational hypertension, but not with the risk of preeclampsia. Although the effects on blood pressure were within physiological ranges, the findings suggest that air pollution may affect cardiovascular health in pregnant women (**Chapter 3.3**).

Fetal growth and neonatal complications

Most previous studies linking air pollution to intrauterine growth restriction are based on measures at birth. These measures are informative, but do not provide insight into the effects on fetal growth patterns during pregnancy. We examined whether air pollution exposure was associated with fetal growth characteristics assessed by repeated ultrasound examinations during pregnancy and the risks of neonatal complications. We observed that exposure to higher PM_{10} and NO_2 levels was inversely associated with third trimester fetal head circumference and birth weight, and that higher NO_2 levels were inversely associated with second and third trimester fetal femur length. Air pollution exposure was not consistently associated with neonatal head circumference or length. This inconsistent pattern of associations throughout pregnancy may be related to specific stages in fetal development during pregnancy, and needs further investigation. Furthermore, we observed that higher PM_{10} exposure was associated with increased risks of preterm birth and small size for gestational age at birth. This adds to the epidemiological evidence relating air pollution exposure to the risks of neonatal complications **(Chapter 3.4)**.

Residential proximity to traffic and pregnancy outcomes

When detailed information on individual exposure to air pollution levels is not available, epidemiological studies have used measures of residential proximity to traffic as indicators of long-term exposure to traffic pollutants. This approach considers the intra-urban

variation in air pollution, which is mainly due to traffic emissions. Within the Generation R Study, we evaluated the associations of residential proximity to traffic, defined as distance to a major road and distance-weighted traffic density in a 150 meter radius, with the risks of neonatal complications and gestational hypertensive complications. We observed no consistent associations of residential proximity to traffic with birth weight and the risks of preterm birth, small size for gestational age at birth, gestational hypertension, preeclampsia, and gestational diabetes (**Chapter 3.5**). These findings are not entirely in line with our other study, in which higher PM_{10} levels were associated with increased risks of preterm birth and small size for gestational age at birth (Chapter 3.4). Possible explanations for the discrepancy in findings are discussed below.

INTERPRETATION OF THE FINDINGS

Air pollution

Air pollution is a heterogeneous mixture of different particulates and gases. The composition of this mixture depends on the emission source, location, season, and meteorological conditions. Because the mixture varies between locations, and as many compounds in the mixture are correlated, it is difficult to identify the pollutants that are responsible for the adverse health effects [20, 23, 24]. Studies have linked various pollutants to adverse health outcomes [24, 25].

One of the most studied pollutants, with the largest body of evidence regarding biological plausibility, is particulate matter. Particulate matter consists of solid and liquid particles suspended in air, including elemental and organic carbon, metals, nitrates, and sulfates. The exact composition depends on the source, which can be natural (e.g., mineral dust, sea salt, volcano eruptions) or anthropogenic (fossil fuel combustion), and is also influenced by season and meteorological conditions. Primary particles are directly emitted in the atmosphere, whereas secondary particles are formed in the atmosphere through reactive processes. Particulate matter is classified according to the size of the particles. Particles with a median aerodynamic diameter smaller than 10 µm (PM_{10}) are considered capable of entering the lungs. A further distinction is made between ultrafine particles (diameter <0.1 µm), fine particles (diameter <2.5 µm) and coarse particles (diameter 2.5 to 10 µm). The smaller the particles, the deeper they can penetrate into the lungs. Many studies have focused on the adverse effects of PM_{10}. Increasingly, fine and ultrafine particles are believed to be the most harmful for human health, as they can penetrate into the deeper parts of the respiratory system and possibly other tissues, and because they are often composed of more toxic compounds (depending on the source) [24, 26]. However, although fine particles are proposed to be more harmful, an effect of coarse particles ($PM_{2.5-10}$) should not be dismissed [27, 28].

Nitrogen dioxide is another well-studied pollutant that mainly originates from fossil fuel combustion in motor vehicles and industrial processes. It is a reactive substance,

which is involved in various processes in the atmosphere, including the formation (and conversion) of ozone and the formation of smog. NO_2 is generally regarded as an indicator of the traffic-related air pollution mixture. The impact of NO_2 itself on human health is uncertain. In relatively high concentrations, NO_2 has been shown to affect respiratory function and evoke pulmonary inflammation (reviewed by [29, 30]). Nevertheless, experimental studies observed that at concentrations present in the ambient air, direct effects of NO_2 on the lungs are probably small [23]. Many epidemiological studies have reported associations of ambient NO_2 levels with adverse health effects. However, it is unclear whether the health risks result from traffic-related emissions correlating with NO_2, chemical reaction products of NO_2, or NO_2 itself [23, 30].

Rotterdam is the second largest city in the Netherlands with a high population density and the largest port of Europe. It is characterized by high emissions from road traffic, shipping, households, and industry. This results in relatively high air pollution concentrations. In 2004, annual average air pollution levels derived from ambient monitoring stations in the larger Rotterdam area were 30.7 $\mu g/m^3$ for PM_{10}, 43.8 $\mu g/m^3$ for NO_2, and 13.8 $\mu g/m^3$ for SO_2 [31]. In the study area, PM_{10} and NO_2 concentrations are mainly influenced by background concentrations (originating from various sources, including industry, power generators, refineries, agriculture, and distant traffic) and local contributions from road traffic (i.e., traffic emissions and resuspension of road dust). NO_2 concentrations more strongly reflect local traffic sources than PM_{10} concentrations, which may explain the moderate correlations between the PM_{10} and NO_2 exposure averages in our study population (r=0.58 to 0.66). The chemical composition and source apportionment of particulate matter was evaluated in more detail at different locations in the Netherlands, including two locations in the Rotterdam region (i.e., a traffic location and an urban background location) [32]. At these two locations, the contribution of the coarse fraction mass ($PM_{10-2.5}$) to the total PM_{10} mass was around 60-62%, and the contribution of the fine fraction ($PM_{2.5}$) to the total PM_{10} mass was 38-40%. Furthermore, the observations showed that in the Rotterdam region, PM_{10} was mainly composed of secondary inorganic aerosol (i.e., sulphate, nitrate and ammonium) (35-37%) originating from combustion emissions and agriculture, followed by total carbonaceous matter (26-32%) from biogenic and combustion emissions; sea salt (11-17%); mineral dust (7%) and metals (7-9%) from wear processes, resuspension of road dust and wind-blown soil; and an unexplained part (6-7%) [32]. Compared to other locations in the Netherlands (three rural background locations and one traffic location in the city of Breda), the contributions of nitrate, sulphate, and metals to particulate matter were relatively higher in the Rotterdam region, which probably resulted from industrial emissions and resuspension of road dust. When the urban background location and the traffic location in the region were compared, the PM_{10} concentration was on average 3.5 $\mu g/m^3$ higher at the traffic location. This difference could be attributed to carbonaceous material (mainly elemental carbon), mineral dust and metals from traffic emissions, wear processes, and resuspension of road dust [32].

Although background concentrations are an important determinant of long-term average PM_{10} and NO_2 concentrations, the spatial differences in long-term PM_{10} and NO_2 exposures within our urban study area were largely influenced by local contributions from road traffic. In addition, differences in exposures have arisen as a result of temporal contrasts in pollutants (depending on meteorological factors), because we evaluated pregnancy-specific exposures. As a result, in our population of pregnant women, depending on the length of the specific averaging period we already observed substantial spatial and temporal variation in exposure levels.

Biological mechanisms

The exact mechanisms through which air pollution adversely affects human health remain to be clarified. Based on results from epidemiological, toxicological, and animal studies, several potential pathways have been proposed, mainly for particulate matter exposure. These pathways have been summarized in a number of recent reviews [20, 26, 33-37]. In general, air pollution may provoke short-term (acute) effects that occur within a few hours following exposure and longer-term (less acute/chronic) effects that result from longer exposure. Most evidence for the short-term effects has been derived from animal studies, controlled exposure chamber studies, and time-series studies. The longer-term effects of air pollution have been investigated in epidemiological studies. Nevertheless, it is difficult to make a clear distinction between short- and longer-term effects, as there is substantial overlap among the different pathways and timing of effects [20]. Furthermore, some effects may occur predominantly in susceptible individuals, such as those with genetic predispositions, with pre-existent diseases (cardiovascular conditions, respiratory conditions, or diabetes), elderly persons, or children.

One of the proposed effects of air pollution is the induction of systemic inflammation. Studies have reported increased levels of proinflammatory cytokines, including TNFα and interleukins, following exposure to air pollution. Also alterations in the concentrations of acute-phase proteins, such as CRP, have been linked to air pollution (reviewed by [20, 26, 37]). The systemic inflammation may be either caused by pulmonary inflammation, through 'spill-over' of inflammatory mediators into the circulation, or may be induced directly by (translocation of) inhaled particles or microbial components adsorbed to particles [38] (reviewed by [20, 33]). The subsequent low-grade inflammation may promote atherosclerosis and other cardiovascular risk factors. Second, air pollution is suggested to induce oxidative stress, a condition that is characterized by an imbalance between the production and the detoxification of reactive oxygen species. The resulting excess of reactive oxygen species can damage cell components, as demonstrated by a few studies that reported associations of air pollution exposure with increases in markers of lipid, protein, or DNA oxidation (reviewed by [20, 26, 34, 35, 37]). The pathways of systemic inflammation and oxidative stress are probably closely interconnected and involved in some of the other pathways as well [20, 26]. Third, air pollution has been linked to alterations in blood coagulability and thrombosis. This has been reflected by elevated

levels of fibrinogen, a protein that is involved in blood clotting, and markers of platelet activation (reviewed by [20, 26, 36]). Fourth, inhaled particles may affect the autonomic nervous system, resulting in increased heart rate, increased arrhythmias, and decreased heart rate variability (reviewed by [20, 26, 36, 37]). Finally, there is evidence that elevated air pollution levels affect vascular function. A number of studies have related air pollution to impaired vascular function and diminished endothelium-dependent vasodilation, which may contribute to atherosclerosis. In addition, altered vascular function could result in vasoconstriction and subsequent development of increased blood pressure. Evidence for this pathway comes from several studies that reported associations of both short- and longer-term air pollution exposure with alterations in systolic and diastolic blood pressure (reviewed by [20, 26, 36, 37]).

The impact of air pollution might be different in pregnant women. Pregnancy is characterized by various physiological changes, including adaptations in hemodynamic function, cardiac function, immune system, and metabolism [39-41]. This makes pregnant women a potentially more vulnerable group for the effects of air pollution. Fetuses are also considered more susceptible to air pollution, because their organs, detoxification mechanisms, and immune system are not fully developed yet [5, 42, 43]. It has been hypothesized that air pollution exposure could influence pregnancy via various pathways. First, air pollution could elicit systemic inflammation and oxidative stress, thereby increasing maternal susceptibility to infections. Maternal infections and elevated levels of inflammatory mediators might consequently trigger preterm birth and intrauterine growth retardation [6, 18, 44], and also increased levels of lipid peroxidation in maternal and cord blood may increase the risk of adverse neonatal outcomes such as preterm birth [45]. Second, air pollution could induce placental inflammation, which may impair placental perfusion and nutrient and oxygen transport to the fetus, leading to an increased risk of intrauterine growth retardation [18]. Next, alterations in blood viscosity, fibrinogen levels, and vascular function following air pollution exposure could influence placental-fetal transport (reviewed by [6, 18]). These alterations may also lead to blood pressure elevations in pregnant women, which could precede the development of gestational hypertensive complications [18]. Fifth, air pollution may generate placental DNA damage [18]. This may be caused by air pollution-induced oxidative stress, or by microparticles adsorbed to particulate matter that cross the placenta, such as polycyclic aromatic hydrocarbons (PAHs). Studies have reported increased levels of DNA adducts in maternal blood and the placenta in areas with higher air pollution concentrations. These DNA adducts may impair placental-fetal exchange, and have been linked to intrauterine growth restriction [46-48]. In addition, PAHs have been suggested to directly affect trophoblast invasion, resulting in suboptimal placentation and impaired placental perfusion [46]. The hypothesized impact of air pollution on placental exchange and intrauterine growth is supported by a few experimental studies in mice, which showed adverse effects of exposure to ambient air pollution levels during pregnancy on placental weight, placental function, and fetal growth [49, 50]. Finally, air pollution exposure may evoke epigenetic changes, which are heritable

modifications to the genome that do not change the DNA sequence itself [51-54]. The main mechanisms of epigenetic regulation are DNA methylation (i.e., the addition or removal of methyl groups to the DNA), histone modification, and RNA-associated pathways [54]. Air pollution-induced systemic inflammation and oxidative stress could trigger epigenetic changes, leading to adaptive responses and effects on maternal and fetal health [51].

Implications of the findings

Our results suggest that air pollution exposure during pregnancy may affect maternal and fetal health. We observed consistent associations of higher PM_{10} and NO_2 exposure levels with elevated CRP levels in fetal cord blood, altered levels of angiogenic factors in fetal cord blood, higher maternal blood pressure, gestational hypertension, lower birth weight, and preterm birth and small size for gestational age at birth. We also observed associations, but less consistent, with elevated maternal CRP levels in first trimester, altered levels of maternal angiogenic factors in first and second trimester, lower placenta weight, and reduced fetal growth parameters (Figure 1). Adverse prenatal exposures are suggested to have a differential effect depending on the exposure period, or the 'critical window of exposure'. This is related to the timing of development of the different organ systems of the infant [43]. As a result, often relatively short time windows are considered in studies on reproductive or developmental outcomes [55]. Thus far, the literature is inconclusive regarding the rationale to investigate a specific exposure period, such as trimester or monthly exposure averages. Some previous studies indicated that exposures in first trimester may affect implantation of the fetus and formation of the placenta, whereas exposures in third trimester could affect fetal growth, as this is the period with the largest fetal weight gain [3, 7, 56]. However, it could also be argued that cumulative or long-term exposures during pregnancy are of importance [56, 57]. Depending on the timing of our outcome measures and the hypothesized mechanism (short- or long-term effect of air pollution) for each outcome, we considered different exposure windows. For example, in the studies on fetal growth and maternal blood pressure we examined exposure averages for specific pregnancy periods (i.e., from conception until first, second, or third trimester measurement and until delivery), because we were interested in air pollution effects over the course of pregnancy. Regarding CRP levels, most previous studies on air pollution have reported associations with relatively acute exposures (same day or multiday averages), whereas a few studies observed associations with longer-term averages (varying from 7 to 60 days). For this reason, we evaluated different exposure windows: one week, two weeks, four weeks, and total pregnancy. Furthermore, previous studies on air pollution and markers of placental function and growth were limited. Therefore, we considered a number of exposure windows to cover short- and longer-term exposures: two weeks, two months, and the specific pregnancy periods. We had initially examined other exposure windows (e.g., one week and one month) as well, but we did not present these results because the patterns of associations were similar to the presented findings.

The differences regarding the timing of outcome measurements and exposure windows make it difficult to infer which outcomes are most affected by air pollution. The evidence thus far does not point towards one specific pathway as being responsible for the association of air pollution with pregnancy complications. Our findings indicate a possible role of the systemic inflammatory pathway, reflected by elevated CRP levels as a marker of low-grade inflammation, in the associations of air pollution with pregnancy complications. Also, our observations on maternal blood pressure levels and the risk of gestational hypertension support the hypothesis that air pollution may adversely affect pregnancy by acting on the vascular function. Other outcomes that could be implicated in the underlying pathways, such as blood viscosity and placental DNA damage, have not been examined in our studies. More research is needed to confirm our findings, and to examine a wider range of outcomes potentially involved in the underlying mechanisms. Some studies in this thesis, including the studies focused on CRP levels and on markers of placental function and growth, may be considered as hypothesis-generating, and may stimulate further research on the impact of air pollution on pregnancy outcomes.

We observed rather small differences in fetal growth parameters and maternal blood pressure levels. The highest quartiles of PM_{10} and NO_2 exposure were associated with reductions in third trimester fetal femur length of 0.2 to 0.3 millimeter and reductions in third trimester fetal head circumference of 1.3 to 1.7 millimeter, respectively. In addition, an increase in PM_{10} and NO_2 exposure levels with 10 $\mu m/m^3$ was associated with an increase in maternal systolic blood pressure of 1 to 2 millimeters of mercury. These physiological changes may not be clinically relevant on an individual level, but they provide insight into the underlying pathways. Also, the changes may not immediately result in an adverse outcome, but they could increase the risk of adverse outcomes [20]. Furthermore, the changes may be relevant on a population level. Air pollution is an exposure that affects many inhabitants in a city or neighbourhood. If air pollution concentrations experienced at current ambient levels adversely affect the health of many pregnant women and infants, this could have a substantial impact from the public health perspective. Moreover, we also observed associations of air pollution exposure with clinical outcome measures such as preterm birth and gestational hypertension. In the Netherlands, approximately 7% of all infants have a low birth weight and approximately 8% are born preterm [58, 59]. These outcomes are associated with neonatal morbidity and mortality and with future health effects [60-63]. Gestational hypertensive complications affect about 6-8% of all pregnancies and are major causes of maternal and perinatal morbidity and mortality [64, 65]. Thus, even a small effect of air pollution on the risk of pregnancy complications could have important consequences for neonatal and maternal (future) health.

The results of previous studies and our study suggest that air pollution may affect the health of pregnant women and their unborn children, even at concentrations below the current limit values. This should be taken into consideration when setting air quality standards. More efforts are needed to reduce air pollution concentrations. This will not only benefit pregnant women and their children, but also the general population. However,

even when the limit values will be lowered, people will continue to be exposed to air pollution. If further research indicates that pregnant women are indeed more susceptible to air pollution, it will be important to increase awareness of the harmful effects of air pollution among the general population, and particularly among pregnant women and healthcare givers. Possibly, air pollution exposure needs to be considered along with other risk factors (e.g., nulliparity, smoking, or inadequate nutrition) in identifying women at risk for pregnancy complications.

We only had information on exposure to PM_{10} and NO_2 levels. The observed health risks associated with PM_{10} and NO_2 exposure may result from PM_{10} and NO_2 itself, from pollutants correlating with them, or from a combination of different pollutants. The differences in findings between our study on air pollution exposure and neonatal complications (Chapter 3.4) compared to the study on residential proximity to traffic and neonatal complications (Chapter 3.5) suggest that traffic proximity measures are not able to capture the spatial and temporal variation in exposure as well as our estimates based on dispersion modelling. This could have resulted in less precise effect estimates (i.e., wider confidence intervals) for the associations of traffic proximity measures with neonatal complications. Traffic proximity measures are crude estimates of exposure to traffic-related air pollution. They do not take into account the type of traffic, emission factors, meteorology (i.e., wind direction, wind speed, and temperature), dispersion processes, chemical reactions in the atmosphere, and they do not consider measured air pollution concentrations. Furthermore, because they are based on annual averages, they do not consider temporal variations in air pollution levels. This is an important limitation, as pollutants display substantial temporal heterogeneity. In other words, pregnant women in our studies who lived at the same location would have a similar estimated proximity to traffic, regardless of the period of their pregnancy. In contrast, their estimated exposure to air pollution levels may be very different depending on the period of pregnancy as a result of meteorological, seasonal and source-dependent factors. These temporal contrasts in pollutants have been taken into account in the pregnancy-specific air pollution exposure averages. To evaluate the relation between traffic proximity indicators and PM_{10} and NO_2 exposure averages in our study population, we assessed the correlations between the different exposure measures. The correlation between distance to a major road (with 10,000 vehicles/day) and distance-weighted traffic density (in a 150m buffer) was -0.71 (p <0.01). Distance to a major road and distance-weighted traffic density were weakly correlated with average PM_{10} exposure during total pregnancy (r=-0.17 and r=0.21, respectively, p-values <0.01). Correlations for distance to a major road and distance-weighted traffic density with average NO_2 exposure during total pregnancy were moderate (r=-0.38 and r=0.48, respectively, p-values <0.01). Thus, traffic proximity indicators showed higher correlations with NO_2 than with PM_{10} exposure averages. Correlations for traffic proximity measures with PM_{10} and NO_2 exposure averages were smaller when shorter averaging periods for air pollution exposure were considered. This reflects the fact that traffic proximity measures are annual averages, whereas air pollution exposures have been estimated for shorter (pregnancy-specific) periods.

METHODOLOGICAL CONSIDERATIONS

The previous chapters of the thesis have addressed the strengths and limitations of the specific studies. The next section presents a discussion of some general methodological issues related to study design, selection bias, information bias, confounding, and external validity. These issues should be considered when interpreting the results of our observational studies.

Study design

The Generation R Study is a prospective closed cohort study, which means that a group of participants is followed over time and information on exposures and health outcomes is collected. This design allows estimation of the effect of an exposure on the probability to develop a specific outcome. It also enables examination of the temporality of the relationship between the exposure and the outcome, in other words, whether the exposure precedes the outcome [66, 67]. As information is collected on various exposures and outcomes, usually multiple hypotheses are evaluated. An important limitation of a prospective cohort study is that it is not possible to directly infer causality. If an exposure is demonstrated to precede a disease, the exposure can be said to be *associated* with the disease, but it does not necessarily *cause* the disease. In observational studies, the evidence for causality can be evaluated using the Bradford-Hill criteria, which encompass several domains: strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, and analogy [68, 69]. These criteria were not designed to proof causality, but can be helpful in attempting to distinguish causal from non-causal associations.

Another disadvantage is that it is inefficient when rare outcomes are studied [55, 66, 67]. In all types of observational studies, including cohort studies, both internal and external validity are important aspects. Internal validity refers to the strength of the inferences drawn from the study, i.e., whether the observed effect can be attributed to the exposure and is not caused by other causes or systematic error [55, 70]. Most violations to internal validity are the result of selection bias, information bias, or confounding. External validity refers to the ability to generalize the observed associations to other populations [55, 70]. These different issues will be discussed below.

Selection bias

Selection bias could arise if the association between the exposure and the outcome is different between participants and the source population [69]. This may occur if the decision to participate is associated with social, educational, or health conditions, and these conditions are related to certain risk factors [71]. The Generation R Study is a population-based study with a selection towards a relatively highly educated and more healthy study population [15]. For example, household income and educational level of the participants are somewhat higher than that of the source population. Also, the

percentages of infants born preterm or with a low birth weight are smaller than expected on the basis of national and regional statistics. In contrast, the ethnic distribution differed only slightly from that of the source population [15]. The selection towards somewhat healthier women may have resulted in lower incidences of pregnancy complications, and consequently lower statistical power to detect associations of air pollution exposure with these complications. In addition, although previous cohort studies have demonstrated that selective participation at baseline had no large effect on a number of illustrative effect estimates [71], selection bias could lead to different effect estimates among participants than in the source population. For example, it is possible that non-participants of the Generation R Study were exposed to higher levels of air pollution, and that the associations for air pollution with pregnancy-related outcomes were stronger in this subgroup. In that case, the associations in our studies would be underestimated. This possibility should be kept in mind in the interpretation of our findings.

Also, selection bias might occur in our studies from the differential availability of blood samples. Although the majority of the participating mothers enrolled in early pregnancy (69%), a part of the women enrolled in mid-pregnancy (19%), late pregnancy (3%) or at birth of the infant (9%) [15]. Maternal blood was collected in early, mid-, and late pregnancy, and fetal cord blood was obtained at delivery. As a result, fewer blood samples could be obtained in mothers who were enrolled late in pregnancy or at birth. Although the timing of enrolment is probably not related to the outcome, this can not be excluded. Furthermore, cord blood samples were more often missing in neonates with complications including low birth weight and preterm birth [14]. This may have affected our estimates for the associations of air pollution exposure with cord blood levels of CRP and angiogenic factors.

Information bias

Information bias or misclassification is any bias arising from errors in the classification of the exposure or disease status of the study participants [69]. In air pollution research, an important concern is correct assessment of the exposure [55]. As air pollution concentrations vary in time and in space, both the temporal and spatial variation should be taken into account in the exposure estimation. Ideally, exposure to air pollution would be assessed using personal monitors, as this provides the most accurate estimate of the true exposure [72]. However, in large epidemiological studies, (long-term) personal monitoring would require extensive resources. Therefore, other approaches have been used to estimate exposure of individuals. These approaches are usually based on participants' home addresses, thereby assuming that ambient levels at the home address reflect personal exposure levels. Different methods have been applied to estimate exposure at the home address. Most commonly, studies derived exposure estimates from outdoor monitoring stations, by using the station closest to the participant's home address or by averaging the concentrations measured at multiple stations in the study area. Although the air pollution concentrations measured by ambient monitors may

correspond to exposure at regional levels, this may not represent individual exposure [57, 73], as pollutants may display substantial spatial variation in urban areas. This spatial variation has been documented particularly for traffic-related (primary) pollutants such as NO_2, black smoke, elemental carbon, $PM_{0.1}$, and to a smaller extent for PM_{10} and $PM_{2.5}$ [74, 75]. Recent epidemiological studies have improved the exposure assessment by assigning individual exposure estimates rather than area-based averages, using methods based on geographic information systems (GIS). This better takes into account the spatial variation in air pollutant concentrations in urban areas. However, these studies were not always able to consider the temporal contrasts in air pollution concentrations. Together with a number of recent studies used temporally adjusted land-use regression models or dispersion models to assess exposure [8, 10, 12, 76-78], we were able to consider finer spatial and temporal contrasts in exposure, by using a combination of dispersion modelling and continuous monitoring. Misclassification of air pollution exposure could arise when exposure is based solely on the home address at time of the outcome measurement [79]. This potential misclassification would be greater in studies that encompass small geographic areas, such as our study [80]. We collected information on residential mobility during pregnancy and were thus able to account for the different residential addresses when assigning exposures.

Despite the refined modelling techniques that enabled us to estimate detailed exposures at the different home addresses and the consideration of residential mobility, we should still acknowledge the potential for misclassification of exposure. Exposure levels were only estimated at the home address, whereas pregnant women do not spend all of their time at home. Ideally, other micro-environments (i.e., indoor, occupational, or commuting) should be taken into account, which would improve the ability of the exposure assessment approach to represent true exposures [81-83]. Previous studies that allowed for time-activity patterns of pregnant women observed stronger associations for air pollution exposure estimates with pregnancy-related outcomes, suggesting reduced exposure misclassification [84, 85]. It has been shown that using residence-only based exposures instead of mobility-based exposures could result in an underestimation of the effect estimates [86]. Unfortunately, in our studies no information was available on other sources of air pollution or time-activity patterns of the women. This should be considered when interpreting the results. Non-differential misclassification would occur if all participants have the same probability of being misclassified, whereas differential misclassification refers to the situation in which the probability of being misclassified differs across groups of study subjects [69]. We cannot exclude the possibility that misclassification of air pollution exposure, if any, differed between groups of women (e.g., high- and low-educated women, women with or without paid employment). Whether and in which direction this possible misclassification has affected our effect estimates is unknown. Nevertheless, as pregnant women are likely to spend more time at home than non-pregnant individuals, especially in the last stage of pregnancy [87], the extent of the possible misclassification may be less than in non-pregnant individuals.

Confounding

A confounding variable is independently associated with both the exposure and the outcome of interest, and is no intermediate in the pathway from exposure to outcome. A confounding variable may lead to a biased effect estimate, as the effect of the confounder is mistaken for or mixed with the actual effect of the exposure [55]. For example, maternal age is a confounder in the association of air pollution exposure with infant birth weight, if maternal age is related to both the levels of air pollution exposure and to infant birth weight. Air pollution levels can not affect maternal age, therefore maternal age is no intermediate in the pathway from air pollution exposure to birth weight. If maternal age would not be adjusted for in the association of air pollution exposure with birth weight, this would lead to a distorted estimate for this association. Within the Generation R study, we have collected information on many potential confounding variables, including maternal demographic and lifestyle characteristics, noise exposure, and meteorological variables. In Chapter 2, we showed that air pollution exposure levels may be differentially distributed according to some of these characteristics. This underlines the importance to adjust for these potential confounding variables in our studies. In general, adjusting for road traffic noise exposure resulted in somewhat stronger effect estimates for the associations of air pollution exposure with pregnancy-related outcomes. In the associations of air pollution exposure with CRP levels and maternal blood pressure, additional adjustment for meteorological variables on the day of the outcome measurement did not influence the results. However, in some of the analyses, adjustment for season resulted in slightly stronger effect estimates for air pollution, suggesting that seasonal influences (including meteorological and behavioural factors that correlate with season) may influence the associations of air pollution exposure with pregnancy complications. Residual confounding due to variables that were not measured or incorrectly measured is still possible. For example, we had no (detailed) information on quality of housing, which may confound the associations of air pollution exposure with pregnancy outcomes. In addition, we used maternal educational level as an indicator of maternal socioeconomic status. This may be one of the most important factors determining individual socioeconomic status, however, it may not adequately reflect the overall socioeconomic environment of the mother, which is also related to income level, occupational class, partner's educational level, and neighbourhood socioeconomic characteristics. However, exploratory analyses showed that additional adjustment for average neighbourhood income did not influence any of our effect estimates, suggesting that it did not act as a confounder in the associations of air pollution exposure with pregnancy complications. Nevertheless, future studies would benefit from exploring the influence of other socioeconomic indicators.

External validity

The external validity or generalizability refers to the extent to which the observed associations can be generalized to the source population or to other populations and conditions. First, the selection towards a more healthy population affects the generalizability

of our results, because the women who participated may not be representative of the population from which they were recruited. Therefore, generalization of our findings to other populations of pregnant women should occur with caution. In addition, pregnant women may differ from the general population of non-pregnant individuals in terms of susceptibility to air pollution and physiological adaptations related to the pregnancy. As a results, health affects from air pollution exposure in pregnant women can not be translated to the general population. Furthermore, the generalizability may depend on the exposure variable [67]. In populations with different air pollution exposure levels, the associations of air pollution exposure with pregnancy outcomes may be different, because the associations may not be linear across all exposure levels and may depend strongly on the specific composition of the air pollution mixture.

Study participants lived in the northern part of Rotterdam, an urban area of approximately 150 km^2. As a consequence, the spatial variation in exposure levels was relatively small, which may have limited our ability to detect associations of air pollution exposure with maternal and fetal health outcomes. Studies conducted in populations with a larger exposure variability may have more power to detect associations [67]. Nevertheless, in our population of pregnant women, we already observed substantial spatial and temporal variation in exposure levels, depending on the specific averaging period of exposure.

FUTURE RESEARCH

Many recommendations for future research can be proposed. Future studies on air pollution and pregnancy-related outcomes could benefit from the prospective cohort design, as detailed information on exposures, possible confounding factors, and various outcomes can be (prospectively) collected. In selecting the study population, researchers could aim to select individuals with a wider range of exposure levels, which enhances the ability to detect associations of air pollution exposure with health outcomes. Preferably, information on time-activity patterns of the pregnant women should be collected at various moments in pregnancy. This would enable researchers to improve the exposure assessment by including information on activity patterns and levels of pollutants in different micro-environments (i.e., indoor, commuting, work). Additionally, personal monitoring of air pollution exposure could be performed in a sample of the participants, in order to evaluate the performance of the modelling approach in estimating exposure. It would be valuable to collect information on many potential confounding factors, including various socioeconomic indicators, house characteristics, and ventilation habits. Furthermore, repeated measurements of different outcomes (e.g., CRP levels, markers of placental function and growth) could give insight into longitudinal changes of these variables during pregnancy in relation to air pollution.

In general, longitudinal and time-series studies on additional physiological measures in pregnant women may provide further information on the mechanisms by which air pollution could affect pregnancy. These may include markers of blood coagulability, thrombosis, oxidative damage, heart rate, heart rate variability, subclinical atherosclerosis, endothelium-dependent vasodilation, and systemic, pulmonary or placental inflammation. Although many of these endpoints have been studied in non-pregnant individuals, results are heterogeneous, and the studies should be performed in pregnant women as well. Moreover, follow-up studies are needed to examine the consequences of air pollution exposure in infancy and adulthood. These studies could investigate the impact of both prenatal and postnatal exposure on a variety of outcomes including growth, respiratory symptoms, cardiac function, cognitive function, and neurological and behavioral effects. Furthermore, it is of interest to study other reproductive outcomes such as maternal and paternal fertility, menstrual cycle characteristics, time to pregnancy, implantation rate, placental morphology, abortions, congenital malformations, and secondary sex ratio [6]. Some of these measures have been investigated in relation to air pollution in human or animal studies [50, 88-90], but thus far data is limited.

To advance in this field, future studies could also attempt to identify the characteristics of women who are most susceptible to the adverse effects of air pollution. In the general population, individuals with pre-existing respiratory or cardiovascular conditions, elderly individuals, and children are considered to be more vulnerable to air pollution. Possible, pregnant women with chronic conditions including asthma, hypertension, and diabetes may have different responses to air pollution than women without these conditions [3]. In addition, individual and neighbourhood socioeconomic status might modify the associations of air pollution exposure with pregnancy outcomes [91, 92]. Furthermore, studies have not yet identified specific critical windows of exposure to air pollution. Animal or human studies may provide further information on this topic.

More knowledge is needed on the effects of the composition of the air pollution mixture, and more specifically of particulate matter. It is plausible that a specific compound or a specific combination of pollutants is especially harmful [20]. Toxicological studies should attempt to disentangle the effects of different pollutants. Furthermore, future studies could investigate potential biomarkers of air pollution exposure (markers reflecting the adsorbed dose) and effects [2, 6].

An area of research that is relatively unexplored is the influence of gene-environment interactions. Certain gene polymorphisms may affect susceptibility to air pollution, by influencing the metabolism of harmful compounds. A limited number of studies have explored gene polymorphisms in relation to birth weight and indicated that polymorphisms of the maternal CYP1A1 gene and infant GSTP1 gene, both involved in detoxification pathways, modified the association of particulate matter exposure with birth weight [93, 94]. Also, the association of particulate matter with the risk of preterm delivery was found to be related to polymorphisms in the maternal GSTM1 gene [95]. Genome wide association studies in large populations, including the Generation R Study,

may provide further information on genes that influence the impact of air pollution. In addition, future studies should examine whether air pollution induces epigenetic changes that play a role in the development of pregnancy complications or later health problems.

Finally, an international collaboration has been established that has organized a number of workshops to discuss the relatively new field of research on air pollution and pregnancy outcomes. This International Collaboration on Air Pollution and Pregnancy Outcome (ICAPPO) aims to investigate how differences in research design and methods contribute to variations in findings [96]. Researchers of the different collaborating centers have attempted to apply a standardized study protocol to analyze existing datasets [96, 97]. This enables exploration of the impact of center characteristics and the applied exposure assessment methods on the observed associations. Preliminary results indicate that it seems plausible to synthesize the effect estimates derived from studies with different designs, which would be important for incorporation of research evidence into policy. This is corroborated by a recent review that performed a meta-analysis to quantify the associations of maternal particulate matter exposure with the risks of low birth weight and preterm birth [98]. Further efforts to reconcile inconsistencies between studies and synthesize results should be encouraged. Similar international projects such as the European Study of Cohorts for Air Pollution Effects (ESCAPE) and Transport related Air Pollution and Health impacts – Integrated Methodologies for Assessing Particulate Matter (TRANSPHORM) also aim to investigate the effects of air pollution exposure on human health, by applying standardized exposure estimation approaches in existing cohort studies. These projects will provide more knowledge on the effects of different pollutants on various health outcomes [99, 100].

CONCLUSION

The studies presented in this thesis demonstrate the possible impact of air pollution exposure during pregnancy on various aspects of maternal and fetal health, including fetal growth, inflammatory markers, preterm birth, maternal blood pressure, and gestational hypertension. This indicates that pregnant women and their unborn children may have an increased susceptibility to the adverse effects of air pollution, which should be considered when setting air quality standards. Future studies are needed to confirm our findings and to explore the underlying mechanisms. If the results of future studies point in the same direction, further (inter)national efforts will be needed to reduce air pollution concentrations and to increase awareness of the harmful effects of air pollution.

REFERENCES

1. Bonzini M, Carugno M, Grillo P, Mensi C, Bertazzi PA, Pesatori AC. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. *Med Lav.* 2010; 101(5):341-63.
2. Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. *Basic Clin Pharmacol Toxicol.* 2008; 102(2):182-90.
3. Shah PS, Balkhair T, Knowledge Synthesis Group on Determinants of Preterm and LBW births. Air pollution and birth outcomes: a systematic review. *Environ Int.* 2011; 37(2):498-516.
4. Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. *Epidemiology.* 2004; 15(1):36-45.
5. Lacasana M, Esplugues A, Ballester F. Exposure to ambient air pollution and prenatal and early childhood health effects. *Eur J Epidemiol.* 2005; 20(2):183-199.
6. Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. Meeting report: atmospheric pollution and human reproduction. *Environ Health Perspect.* 2008; 116(6):791-8.
7. Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. Methodological issues in studies of air pollution and reproductive health. *Environ Res.* 2009; 109(3):311-20.
8. Aguilera I, Garcia-Esteban R, Iniguez C, Nieuwenhuijsen MJ, Rodriguez A, Paez M, et al. Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. *Environ Health Perspect.* 2010; 118(5):705-11.
9. Hansen CA, Barnett AG, Pritchard G. The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. *Environ Health Perspect.* 2008; 116(3):362-9.
10. Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A, et al. Maternal personal exposure to airborne benzene and intrauterine growth. *Environ Health Perspect.* 2009; 117(8):1313-21.
11. Rudra CB, Williams MA, Sheppard L, Koenig JQ, Schiff MA. Ambient carbon monoxide and fine particulate matter in relation to preeclampsia and preterm delivery in western Washington State. *Environ Health Perspect.* 2011; 119(6):886-92.
12. Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B. Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. *Environ Health Perspect.* 2009; 117(11):1773-9.
13. Wu J, Wilhelm M, Chung J, Ritz B. Comparing exposure assessment methods for traffic-related air pollution in an adverse pregnancy outcome study. *Environ Res.* 2011; 111(5):685-92.
14. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007; 22(12):917-23.
15. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25(11):823-41.
16. Jaye DL, Waites KB. Clinical applications of C-reactive protein in pediatrics. *Pediatr Infect Dis J.* 1997; 16(8):735-46; quiz 746-7.
17. Raio L, Ghezzi F, Mueller MD, McDougall J, Malek A. Evidence of fetal C-reactive protein urinary excretion in early gestation. *Obstet Gynecol.* 2003; 101(5 Pt 2):1062-3.
18. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. *Environ Health Perspect.* 2006; 114(11):1636-1642.
19. Auchincloss AH, Diez Roux AV, Dvonch JT, Brown PL, Barr RG, Daviglus ML, et al. Associations between recent exposure to ambient fine particulate matter and blood pressure in the Multi-ethnic Study of Atherosclerosis (MESA). *Environ Health Perspect.* 2008; 116(4):486-91.
20. Brook RD, Rajagopalan S, Pope CA, 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation.* 2010; 121(21):2331-78.
21. Choi JH, Xu QS, Park SY, Kim JH, Hwang SS, Lee KH, et al. Seasonal variation of effect of air pollution on blood pressure. *J Epidemiol Community Health.* 2007; 61(4):314-8.

22. Chuang KJ, Yan YH, Cheng TJ. Effect of air pollution on blood pressure, blood lipids, and blood sugar: a population-based approach. *J Occup Environ Med.* 2010; 52(3):258-62.
23. World Health Organization: Health aspects of air pollution with particulate matter, ozone and nitrogen dioxide. 2003. Available: http://www.euro.who.int/__data/assets/pdf_file/0005/112199/E79097.pdf
24. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation.* 2004; 109(21):2655-71.
25. World Health Organization: Health effects of transport-related air pollution. 2005. Available: http://www.euro.who.int/__data/assets/pdf_file/0006/74715/E86650.pdf
26. Pope CA, 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. *J Air Waste Manag Assoc.* 2006; 56(6):709-42.
27. Anderson HR, Bremner SA, Atkinson RW, Harrison RM, Walters S. Particulate matter and daily mortality and hospital admissions in the west midlands conurbation of the United Kingdom: associations with fine and coarse particles, black smoke and sulphate. *Occup Environ Med.* 2001; 58(8):504-10.
28. Brunekreef B, Forsberg B. Epidemiological evidence of effects of coarse airborne particles on health. *Eur Respir J.* 2005; 26(2):309-18.
29. Hesterberg TW, Bunn WB, McClellan RO, Hamade AK, Long CM, Valberg PA. Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: evidence for NO2 no-effect levels. *Crit Rev Toxicol.* 2009; 39(9):743-81.
30. Latza U, Gerdes S, Baur X. Effects of nitrogen dioxide on human health: systematic review of experimental and epidemiological studies conducted between 2002 and 2006. *Int J Hyg Environ Health.* 2009; 212(3):271-87.
31. DCMR Environmental Protection Agency: Air in Numbers 2004. Air quality in the Rijnmond area [in Dutch]. 2005. Available: http://www.dcmr.nl/binaries/publicatie/2005/LUC/luchtincijfers2004.pdf
32. PBL Netherlands Environmental Assessment Agency: Composition and origin of particulate matter in the Netherlands. Results from the Dutch Research Programme on Particulate Matter (BOP). 2010. Available: http://www.pbl.nl/sites/default/files/cms/publicaties/500099007.pdf
33. Alfaro-Moreno E, Nawrot TS, Nemmar A, Nemery B. Particulate matter in the environment: pulmonary and cardiovascular effects. *Curr Opin Pulm Med.* 2007; 13(2):98-106.
34. Laumbach RJ, Kipen HM. Acute effects of motor vehicle traffic-related air pollution exposures on measures of oxidative stress in human airways. *Ann NY Acad Sci.* 2010; 1203:107-12.
35. Romieu I, Castro-Giner F, Kunzli N, Sunyer J. Air pollution, oxidative stress and dietary supplementation: a review. *Eur Respir J.* 2008; 31(1):179-97.
36. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation.* 2010; 121(25):2755-65.
37. Grahame TJ, Schlesinger RB. Cardiovascular health and particulate vehicular emissions: a critical evaluation of the evidence. *Air Qual Atmos Health.* 2010; 3(1):3-27.
38. Alexis NE, Lay JC, Zeman K, Bennett WE, Peden DB, Soukup JM, et al. Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers. *J Allergy Clin Immunol.* 2006; 117(6):1396-403.
39. Thornton CA. Immunology of pregnancy. *Proc Nutr Soc.* 2010; 69(3):357-65.
40. von Versen-Hoeynck FM, Powers RW. Maternal-fetal metabolism in normal pregnancy and preeclampsia. *Front Biosci.* 2007; 12:2457-70.
41. Yoder SR, Thornburg LL, Bisognano JD. Hypertension in pregnancy and women of childbearing age. *Am J Med.* 2009; 122(10):890-5.
42. World Health Organization: Health aspects of air pollution. Results from the WHO project "Systematic review of health aspects of air pollution in Europe". 2004. Available: http://www.euro.who.int/__data/assets/pdf_file/0003/74730/E83080.pdf
43. Selevan SG, Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. *Environ Health Perspect.* 2000; 108 Suppl 3:451-5.
44. Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition--a review. *Placenta.* 2003; 24 Suppl A:S33-46.

45. Tabacova S, Baird DD, Balabaeva L. Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. *Arch Environ Health.* 1998; 53(3):214-21.
46. Dejmek J, Solansky I, Benes I, Lenicek J, Sram RJ. The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect.* 2000; 108(12):1159-1164.
47. Perera FP, Jedrychowski W, Rauh V, Whyatt RM. Molecular epidemiologic research on the effects of environmental pollutants on the fetus. *Environ Health Perspect.* 1999; 107 Suppl 3:451-60.
48. Perera FP, Whyatt RM, Jedrychowski W, Rauh V, Manchester D, Santella RM, et al. Recent developments in molecular epidemiology: A study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. *Am J Epidemiol.* 1998; 147(3):309-14.
49. Rocha e Silva IR, Lichtenfels AJ, Amador Pereira LA, Saldiva PH. Effects of ambient levels of air pollution generated by traffic on birth and placental weights in mice. *Fertil Steril.* 2008; 90(5):1921-4.
50. Veras MM, Damaceno-Rodrigues NR, Caldini EG, Maciel Ribeiro AA, Mayhew TM, Saldiva PH, et al. Particulate urban air pollution affects the functional morphology of mouse placenta. *Biol Reprod.* 2008; 79(3):578-84.
51. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA methylation changes after exposure to traffic particles. *Am J Respir Crit Care Med.* 2009; 179(7):572-8.
52. Edwards TM, Myers JP. Environmental exposures and gene regulation in disease etiology. *Environ Health Perspect.* 2007; 115(9):1264-70.
53. Jardim MJ. microRNAs: Implications for air pollution research. *Mutat Res.* 2011; 717(1-2):38-45.
54. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet.* 2007; 8(4):253-62.
55. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*, 3rd edition. 2008. Philadelphia, PA: Lippincott Williams & Wilkins.
56. Gouveia N, Bremner SA, Novaes HM. Association between ambient air pollution and birth weight in Sao Paulo, Brazil. *J Epidemiol Community Health.* 2004; 58(1):11-7.
57. Pereira G, Nassar N, Bower C, Weinstein P, Cook A. Residential exposure to traffic emissions and adverse pregnancy outcomes *S.A.P.I.E.N.S.* 2010. Online: http://sapiens.revues.org/966
58. EURO-PERISTAT Project: European Perinatal Health Report. 2004. Available: http://www.europeristat.com/publications/european-perinatal-health-report.shtml
59. The Netherlands Perinatal Registry: 10 years of The Netherlands Perinatal Registry, an overview [10 jaar Perinatale Registratie Nederland, de grote lijnen]. 2011. Available: http://www.perinatreg.nl/uploads/173/123/10_jaar_Perinatale_Zorg_in_Nederland_de_grote_lijnen.PDF
60. Barker DJ. Fetal origins of coronary heart disease. *BMJ.* 1995; 311(6998):171-4.
61. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation.* 1996; 94(12):3246-50.
62. McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med.* 1999; 340(16):1234-8.
63. Wilcox AJ, Skjaerven R. Birth weight and perinatal mortality: the effect of gestational age. *Am J Public Health.* 1992; 82(3):378-82.
64. Schutte JM, Schuitemaker NW, van Roosmalen J, Steegers EA, Dutch Maternal Mortality C. Substandard care in maternal mortality due to hypertensive disease in pregnancy in the Netherlands. *BJOG.* 2008; 115(6):732-6.
65. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet.* 2010; 376(9741):631-44.
66. Mann CJ. Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emerg Med J.* 2003; 20(1):54-60.
67. Szklo M. Population-based cohort studies. *Epidemiol Rev.* 1998; 20(1):81-90.
68. Hill AB. The Environment and Disease: Association or Causation? *Proc R Soc Med.* 1965; 58:295-300.
69. Rothman KJ. *Epidemiology. An introduction*. 2002. New York, NY: Oxford University Press Inc.
70. Carlson MD, Morrison RS. Study design, precision, and validity in observational studies. *J Palliat Med.* 2009; 12(1):77-82.
71. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? *Epidemiology.* 2006; 17(4):413-8.

72. Nieuwenhuijsen M, Paustenbach D, Duarte-Davidson R. New developments in exposure assessment: the impact on the practice of health risk assessment and epidemiological studies. *Environ Int.* 2006; 32(8):996-1009.
73. Sagiv SK, Mendola P, Loomis D, Herring AH, Neas LM, Savitz DA, et al. A time-series analysis of air pollution and preterm birth in Pennsylvania, 1997-2001. *Environ Health Perspect.* 2005; 113(5):602-606.
74. Fischer PH, Hoek G, van Reeuwijk H, Briggs DJ, Lebret E, van Wijnen JH, et al. Traffic-related differences in outdoor and indoor concentrations of particles and volatile organic compounds in Amsterdam. *Atmos Environ.* 2000; 34(22):3713-3722.
75. Lewne M, Cyrys J, Meliefste K, Hoek G, Brauer M, Fischer P, et al. Spatial variation in nitrogen dioxide in three European areas. *Sci Total Environ.* 2004; 332(1-3):217-30.
76. Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A, et al. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. *Environ Health.* 2010; 9(6).
77. Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C. A cohort study of traffic-related air pollution impacts on birth outcomes. *Environ Health Perspect.* 2008; 116(5):680-6.
78. Gehring U, Wijga AH, Fischer P, de Jongste JC, Kerkhof M, Koppelman GH, et al. Traffic-related air pollution, preterm birth and term birth weight in the PIAMA birth cohort study. *Environ Res.* 2011; 111(1):125-35.
79. Fell DB, Dodds L, King WD. Residential mobility during pregnancy. *Paediatr Perinat Epidemiol.* 2004; 18(6):408-14.
80. Chen L, Bell EM, Caton AR, Druschel CM, Lin S. Residential mobility during pregnancy and the potential for ambient air pollution exposure misclassification. *Environ Res.* 2010; 110(2):162-8.
81. Nethery E, Leckie SE, Teschke K, Brauer M. From measures to models: an evaluation of air pollution exposure assessment for epidemiological studies of pregnant women. *Occup Environ Med.* 2008; 65(9):579-86.
82. Valero N, Aguilera I, Llop S, Esplugues A, de Nazelle A, Ballester F, et al. Concentrations and determinants of outdoor, indoor and personal nitrogen dioxide in pregnant women from two Spanish birth cohorts. *Environ Int.* 2009; 35(8):1196-201.
83. Dons E, Int Panis L, Van Poppel M, Theunis J, Willems H, Torfs R, et al. Impact of time-activity patterns on personal exposure to black carbon. *Atmos Environ.* 2011; 45(21):3594-3602.
84. Aguilera I, Guxens M, Garcia-Esteban R, Corbella T, Nieuwenhuijsen MJ, Foradada CM, et al. Association between GIS-based exposure to urban air pollution during pregnancy and birth weight in the INMA Sabadell Cohort. *Environ Health Perspect.* 2009; 117(8):1322-7.
85. Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JK. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. *Am J Epidemiol.* 2007; 166(9):1045-52.
86. Setton E, Marshall JD, Brauer M, Lundquist KR, Hystad P, Keller P, et al. The impact of daily mobility on exposure to traffic-related air pollution and health effect estimates. *J Expo Sci Environ Epidemiol.* 2011; 21(1):42-8.
87. Nethery E, Brauer M, Janssen P. Time-activity patterns of pregnant women and changes during the course of pregnancy. *J Expo Sci Environ Epidemiol.* 2009; 19(3):317-24.
88. Jurewicz J, Hanke W, Radwan M, Bonde JP. Environmental factors and semen quality. *Int J Occup Med Environ Health.* 2009; 22(4):305-29.
89. Mohallem SV, de Araujo Lobo DJ, Pesquero CR, Assuncao JV, de Andre PA, Saldiva PH, et al. Decreased fertility in mice exposed to environmental air pollution in the city of Sao Paulo. *Environ Res.* 2005; 98(2):196-202.
90. Veras MM, Damaceno-Rodrigues NR, Guimaraes Silva RM, Scoriza JN, Saldiva PH, Caldini EG, et al. Chronic exposure to fine particulate matter emitted by traffic affects reproductive and fetal outcomes in mice. *Environ Res.* 2009; 109(5):536-43.
91. Genereux M, Auger N, Goneau M, Daniel M. Neighbourhood socioeconomic status, maternal education and adverse birth outcomes among mothers living near highways. *J Epidemiol Community Health.* 2008; 62(8):695-700.
92. Ponce NA, Hoggatt KJ, Wilhelm M, Ritz B. Preterm birth: the interaction of traffic-related air pollution with economic hardship in Los Angeles neighborhoods. *Am J Epidemiol.* 2005; 162(2):140-8.

93. Suh YJ, Kim BM, Park BH, Park H, Kim YJ, Kim H, et al. Cytochrome P450IA1 polymorphisms along with PM(10) exposure contribute to the risk of birth weight reduction. *Reprod Toxicol.* 2007; 24(3-4):281-8.
94. Slama R, Grabsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S, et al. Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. *Reprod Toxicol.* 2010; 30(4):600-12.
95. Suh YJ, Ha EH, Park H, Kim YJ, Kim H, Hong YC. GSTM1 polymorphism along with PM10 exposure contributes to the risk of preterm delivery. *Mutat Res.* 2008; 656(1-2):62-7.
96. Woodruff TJ, Parker JD, Adams K, Bell ML, Gehring U, Glinianaia S, et al. International Collaboration on Air Pollution and Pregnancy Outcomes (ICAPPO). *Int J Environ Res Public Health.* 2010; 7(6):2638-52.
97. Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML, et al. The International Collaboration on Air Pollution and Pregnancy Outcomes: initial results. *Environ Health Perspect.* 2011; 119(7):1023-8.
98. Sapkota A, Chelikowsky A, Nachman K, Cohen A, Ritz B. Exposure to particulate matter and adverse birth outcomes: a comprehensive review and meta-analysis. *Air Qual Atmos Health.* 2010; doi: 10.1007/s11869-010-0106-3.
99. ESCAPE - European Study of Cohorts for Air Pollution Effects. Available: http://www.escapeproject.eu/index.php
100. TRANSPHORM: Transport related Air Pollution and Health impacts – Integrated Methodologies for Assessing Particulate Matter. Available: http://www.transphorm.eu

Chapter 5

Summary/ Samenvatting

SUMMARY

In the last decades, accumulating evidence has linked air pollution exposure to various adverse health effects, including cardiovascular and respiratory disease. Pregnant women and their unborn children may be more susceptible to the adverse effects of air pollution. Many studies have observed associations of air pollution exposure during pregnancy with neonatal complications, but thus far, results are inconsistent. Furthermore, not much is known about the underlying mechanisms through which air pollution exposure affects pregnancy. As described in **Chapter 1**, the main objectives of this thesis were to examine the associations of air pollution exposure during pregnancy with the risks of maternal and neonatal complications, and to examine the mechanisms that underlie these associations. To address these aims, we have evaluated the effects of air pollution on inflammatory markers, placental function, fetal growth, maternal blood pressure, neonatal complications, and maternal complications. Maternal complications included gestational hypertension, preeclampsia, and gestational diabetes. Neonatal complications included adverse birth outcomes such as low birth weight, preterm birth, and small for gestational age at birth.

All studies were embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, the Netherlands. In total, 8880 pregnant women with a delivery date between April 2002 and January 2006 were enrolled in the study. Information about sociodemographic characteristics, lifestyle factors, and pregnancy outcomes was obtained from questionnaires, physical assessments, ultrasound examinations, biological samples, and medical records.

Chapter 2 describes the methodology of the air pollution exposure assessment for participants of the Generation R Study. Individual exposures to PM_{10} and NO_2 were assessed at the home addresses, using a combination of geographic information system based dispersion modelling techniques and continuous monitoring data. This approach takes into account the spatial and temporal variation in air pollutants. We derived air pollution exposure averages for different prenatal periods. These exposure estimates have been applied in the studies presented in this thesis focused on the effects of air pollution exposure on maternal and fetal health.

Chapter 3 presents different studies that examined the associations of air pollution exposure during pregnancy with maternal and neonatal outcomes. In **Chapter 3.1** we showed that short-term exposure to higher PM_{10} and NO_2 exposure levels was associated with elevated maternal C-reactive protein (CRP) levels in the first trimester of pregnancy, suggesting an inflammatory response. Higher long-term average PM_{10} and NO_2 exposure levels were associated with elevated CRP levels in fetal cord blood. These findings indicate that air pollution exposure may promote inflammatory processes in mother and fetus. In **Chapter 3.2** we hypothesized that air pollution exposure may lead to suboptimal placental growth and function, which could eventually result in maternal and fetal complications. We evaluated the associations of air pollution exposure with various markers of placental

growth and function, including angiogenic growth factors (which are involved in the growth of new blood vessels and the formation of the placenta), vascular resistance in the placenta (which reflects the blood flow) and placenta weight. We observed that higher PM_{10} and NO_2 exposure levels were associated with changes in the concentrations of angiogenic growth factors (sFlt-1 and PlGF) in fetal cord blood, reflecting an anti-angiogenic state that may have limited the placental vascular development. This pattern was not observed for maternal sFlt-1 and PlGF levels in first and second trimester of pregnancy. We showed that air pollution exposure was not associated with placental vascular resistance. Furthermore, we observed associations of PM_{10} and NO_2 exposure with lower placental weight, but these associations were not consistent for the different exposure periods. Although these results suggest that air pollution exposure may influence placental growth and function, the evidence is insufficient to identify this pathway as playing a major role in the associations of air pollution with pregnancy complications. **Chapter 3.3** showed that higher PM_{10} and NO_2 exposure levels were associated with increased systolic blood pressure levels in the different trimesters of pregnancy. No consistent associations were observed with diastolic blood pressure levels. Also, higher PM_{10} exposure was associated with an increased risk of gestational hypertension, but not with the risk of preeclampsia. These findings indicate that air pollution exposure may affect cardiovascular health in pregnant women. In **Chapter 3.4** we evaluated whether air pollution exposure was associated with fetal growth characteristics and the risks of neonatal complications. We observed that maternal exposure to higher PM_{10} and NO_2 levels was inversely associated with third trimester fetal head circumference and birth weight, and that higher NO_2 levels were inversely associated with second and third trimester fetal femur length. However, air pollution exposure was not consistently associated with neonatal head circumference or length. Furthermore, higher PM_{10} exposure was associated with increased risks of preterm birth and small size for gestational age at birth. **Chapter 3.5** describes the associations of residential proximity to traffic, as an indicator of long-term exposure to traffic pollutants, with the risks of neonatal complications and gestational hypertensive complications. Although we previously showed that higher PM_{10} and NO_2 exposure levels were associated with increased risks of gestational hypertension, preterm birth, and small size for gestational age at birth, we observed no consistent associations of residential proximity to traffic with birth weight, neonatal complications, or gestational hypertensive complications. This suggests that our exposure estimates based on dispersion modelling techniques (applied in Chapters 3.1 to 3.4) may better capture both the spatial and temporal variation in air pollution exposure.

Finally, **Chapter 4** summarizes the main findings of the studies in this thesis and discusses the interpretation of the findings and the methodological considerations. Also, suggestions for future research are proposed.

In conclusion, the studies described in this thesis demonstrate that air pollution exposure during pregnancy may adversely affect various aspects of maternal and fetal health, including fetal growth, inflammatory markers, maternal blood pressure, and the

risks of gestational hypertension and preterm birth. This indicates that pregnant women and their unborn children may be more susceptible to the impact of air pollution. Future studies are needed to confirm our findings and to (further) elucidate the underlying mechanisms. Results of future studies and our studies may lead to an increased awareness of the harmful effects of air pollution in the population in general, and in pregnant women and their unborn children in particular.

SAMENVATTING

In de laatste decennia hebben verschillende studies aangetoond dat blootstelling aan luchtverontreiniging samenhangt met diverse ongunstige gezondheidseffecten, waaronder hart- en vaatziekten en luchtwegaandoeningen. Mogelijk zijn zwangere vrouwen en hun ongeboren kinderen gevoeliger voor de nadelige effecten van luchtverontreiniging. Diverse studies hebben een verband beschreven tussen blootstelling aan luchtverontreiniging tijdens de zwangerschap en ongunstige geboorte-uitkomsten, maar tot nu toe zijn de resultaten inconsistent. Bovendien is er weinig bekend over de onderliggende mechanismen die een rol kunnen spelen bij de effecten van luchtverontreiniging op de zwangerschap. Zoals beschreven in **Hoofdstuk 1** waren de belangrijkste doelstellingen van de studies in dit proefschrift om de verbanden te onderzoeken tussen blootstelling aan luchtverontreiniging tijdens de zwangerschap en maternale en neonatale gezondheid, en om de onderliggende mechanismen van deze verbanden te bestuderen. Hierbij hebben we specifiek gekeken naar ontstekingsmarkers, placentafunctie, foetale groei, bloeddruk van de moeder, neonatale complicaties en maternale complicaties. Met maternale complicaties worden zwangerschapshypertensie (hoge bloeddruk in de zwangerschap), pre-eclampsie (zwangerschapsvergiftiging) en zwangerschapsdiabetes (zwangerschapssuiker) bedoeld. Met neonatale complicaties worden nadelige geboorte-uitkomsten bedoeld zoals een laag geboortegewicht, vroeggeboorte en een te klein geboren kind ten opzichte van de zwangerschapsduur.

Alle studies werden uitgevoerd binnen het Generation R onderzoek, een populatie-gebaseerde prospectieve cohortstudie vanaf de vroege zwangerschap in Rotterdam. In totaal namen 8880 zwangere vrouwen met een bevallingsdatum tussen april 2002 en januari 2006 deel aan het onderzoek. Informatie over sociaal-demografische kenmerken, leefstijlfactoren en zwangerschapsuitkomsten werd verzameld door middel van vragenlijsten, lichamelijke onderzoeken, echometingen, bloedbepalingen en medische dossiers.

Hoofdstuk 2 beschrijft de methodologie die gebruikt is om de blootstelling aan lucht-verontreiniging te bepalen voor de deelnemers van het Generation R onderzoek. De individuele blootstelling aan PM_{10} (fijnstof) en NO_2 (stikstofdioxide) concentraties werd bepaald op het woonadres met behulp van dispersiemodellering, gebaseerd op een geografisch informatie-systeem, in combinatie met continue meetgegevens. Deze methode houdt rekening met de ruimtelijke variatie en de tijdsvariatie in luchtverontreinigende stoffen. We hebben de gemiddelde blootstelling aan luchtverontreiniging berekend voor verschillende prenatale periodes. Vervolgens zijn deze gemiddelde blootstellingen toegepast in de verschillende studies in dit proefschrift gericht op de effecten van luchtverontreiniging op de gezondheid van moeder en kind.

In **Hoofdstuk 3** worden verschillende studies beschreven waarin het verband is be-studeerd tussen blootstelling aan luchtverontreiniging tijdens de zwangerschap en maternale en neonatale uitkomsten. In **Hoofdstuk 3.1** toonden we aan dat kortdurende

blootstelling aan hogere PM_{10} en NO_2 concentraties samenhing met een hogere concentratie C-reactieve proteïne (CRP) bij de moeder in het eerste trimester van de zwangerschap, wat wijst op een ontstekingsreactie. Langdurige blootstelling aan hoge gemiddelde PM_{10} en NO_2 concentraties was gerelateerd aan hogere foetale CRP concentraties in navelstrengbloed. Deze resultaten suggereren dat blootstelling aan luchtverontreiniging kan leiden tot ontstekingsprocessen in de moeder en foetus. In **Hoofdstuk 3.2** hebben we de hypothese getest dat blootstelling aan luchtverontreiniging kan leiden tot een suboptimale placentagroei en -functie, wat uiteindelijk kan resulteren in complicaties bij de moeder en foetus. We hebben gekeken naar het verband tussen blootstelling aan luchtverontreiniging en diverse markers van placentagroei en -functie, zoals angiogene groeifactoren (die betrokken zijn bij de nieuwvorming van bloedvaten en de vorming van de placenta), weerstanden van de bloedvaten in de placenta (die de bloedstroom reflecteren) en het placentagewicht. We vonden dat een hogere blootstelling aan PM_{10} en NO_2 concentraties gerelateerd was aan veranderde concentraties angiogene groeifactoren (sFlt-1 en PlGF) in foetaal navelstrengbloed, duidend op een anti-angiogene staat, wat mogelijk een remmende werking had op de ontwikkeling van de placenta. Dit patroon werd niet waargenomen voor sFlt-1 en PlGF concentraties bij de moeder in het eerste en het tweede trimester van de zwangerschap. Blootstelling aan luchtverontreiniging hing niet samen met de vaatweerstanden in de placenta. Daarnaast hebben we laten zien dat hogere PM_{10} en NO_2 blootstelling geassocieerd is met een lager placentagewicht, maar dit verband was niet consistent voor de verschillende middelingperiodes voor luchtverontreiniging. Deze bevindingen suggereren dat blootstelling aan luchtverontreiniging invloed kan hebben op placentagroei en -functie, echter het bewijs is onvoldoende om te concluderen dat dit mechanisme een belangrijke rol speelt in de relatie tussen luchtverontreiniging en zwangerschapscomplicaties. In **Hoofdstuk 3.3** toonden we aan dat blootstelling aan hogere PM_{10} en NO_2 concentraties samenhing met een hogere systolische bloeddruk in de verschillende trimesters van de zwangerschap. Er werden geen consistente effecten gevonden op de diastolische bloeddruk. Daarnaast hadden vrouwen die aan hogere PM_{10} concentraties waren blootgesteld een hoger risico op zwangerschapshypertensie, maar geen hoger risico op pre-eclampsie. Deze bevindingen suggereren dat luchtverontreiniging invloed kan hebben op de hart- en vaatfunctie van zwangere vrouwen. In **Hoofdstuk 3.4** hebben we onderzocht of blootstelling aan luchtverontreiniging gerelateerd was aan foetale groeipatronen en het risico op neonatale complicaties. Kinderen van moeders die waren blootgesteld aan hoge PM_{10} en NO_2 concentraties hadden een kleinere foetale hoofdomtrek in het derde trimester en een lager geboortegewicht. Tevens was blootstelling aan hoge NO_2 concentraties gerelateerd aan een kleinere foetale beenlengte in het tweede en het derde trimester. Blootstelling aan luchtverontreiniging was echter niet consistent geassocieerd met de hoofdomtrek of de lengte van het kind na de geboorte. Verder hing een hogere PM_{10} blootstelling samen met hogere risico's op vroeggeboorte en een te klein geboren kind ten opzichte van de zwangerschapsduur. **Hoofdstuk 3.5** beschrijft de relatie tussen wonen nabij verkeer, als

indicator voor lange-termijn blootstelling aan verkeersgerelateerde luchtverontreiniging, en de risico's op maternale en neonatale complicaties. Hoewel we eerder lieten zien dat blootstelling aan hoge PM_{10} en NO_2 concentraties geassocieerd was met een hoger risico op zwangerschapshypertensie, vroeggeboorte en een te klein geboren kind ten opzichte van de zwangerschapsduur, vonden we geen consistente verbanden tussen wonen nabij verkeer en geboortegewicht, neonatale complicaties, of maternale complicaties. Dit suggereert dat onze blootstellingschattingen gebaseerd op dispersiemodellering (gebruikt in Hoofdstuk 3.1-3.4) beter rekening houden met zowel de ruimtelijke variatie als de tijdsvariatie in luchtverontreiniging.

Tot slot geeft **Hoofdstuk 4** een overzicht van de belangrijkste bevindingen van de studies in dit proefschrift en worden de interpretatie van de bevindingen en de methodologische overwegingen besproken. Ook worden suggesties voor toekomstig onderzoek gedaan.

Concluderend, de studies in dit proefschrift tonen aan dat blootstelling aan luchtverontreiniging tijdens de zwangerschap een negatieve invloed kan hebben op verschillende aspecten van maternale en foetale gezondheid, waaronder foetale groei, ontstekingsmarkers, maternale bloeddruk, en de risico's op zwangerschapshypertensie en vroeggeboorte. Dit suggereert dat zwangere vrouwen en hun ongeboren kinderen gevoeliger kunnen zijn voor de effecten van luchtverontreiniging. Toekomstige studies zijn nodig om onze bevindingen te bevestigen en om de onderliggende mechanismen (verder) te verduidelijken. De resultaten van onze studies en toekomstige studies kunnen leiden tot een verhoogd bewustzijn van de schadelijke effecten van luchtverontreiniging voor de bevolking in het algemeen en voor zwangere vrouwen en hun ongeboren kinderen in het bijzonder.

Chapter 6

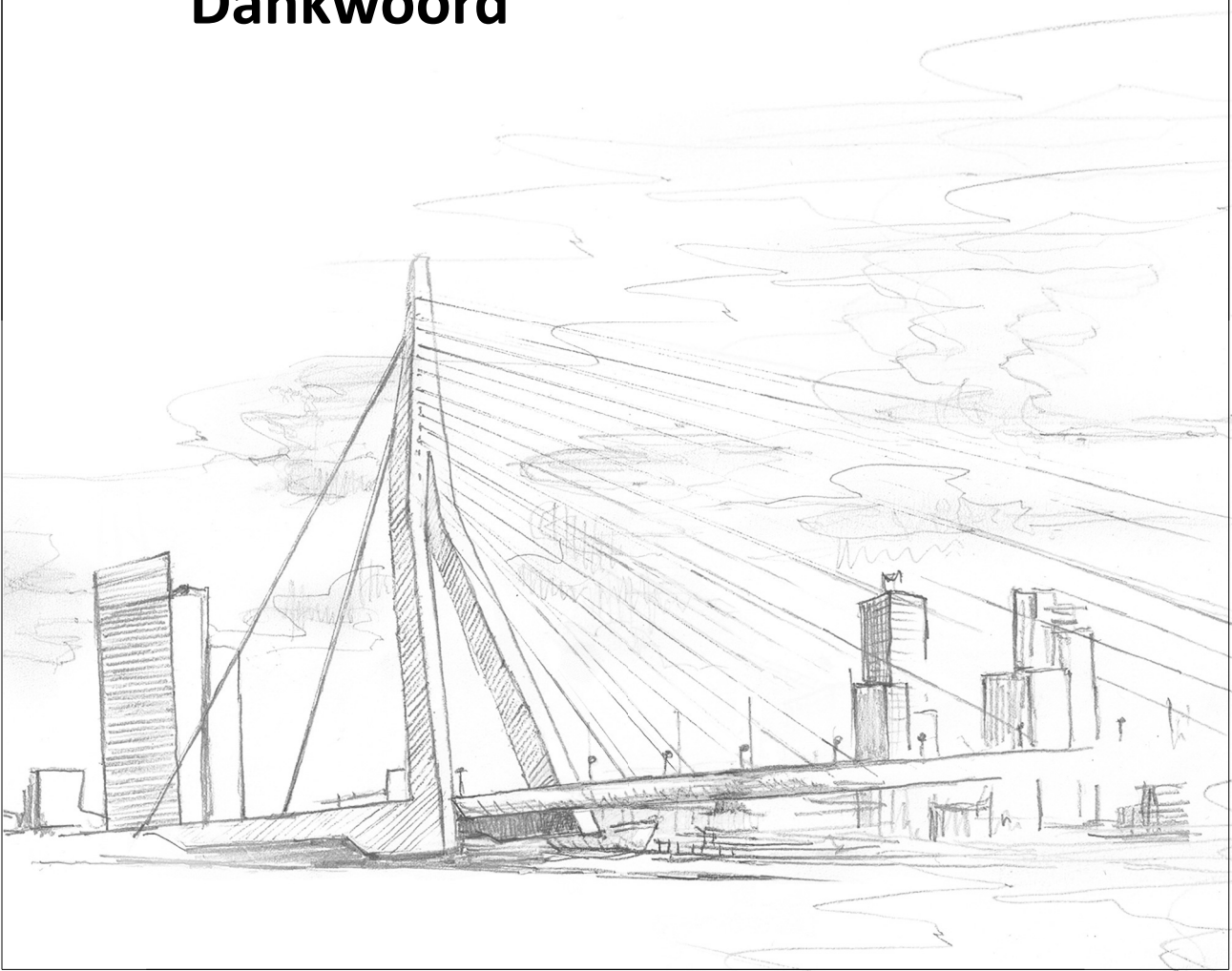
Authors and affiliations

Publication list

About the author

PhD portfolio

Dankwoord



AUTHORS AND AFFILIATIONS

In order of appearance in this thesis

Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands
Albert Hofman, Vincent WV Jaddoe, Sten P Willemsen

The Generation R Study Group, Erasmus Medical Center, Rotterdam, the Netherlands
Albert Hofman, Vincent WV Jaddoe

Department of Paediatrics, Erasmus Medical Center, Rotterdam, The Netherlands
Vincent WV Jaddoe

Urban Environment and Safety, TNO, Utrecht, the Netherlands
Frank H Pierik, Sjoerd W van Ratingen, Peter YJ Zandveld, Ernst W Meijer, Henk ME Miedema, Yvonne de Kluizenaar

Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam, The Netherlands
Jan Lindemans, Henk Russcher

Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, The Netherlands
Eric AP Steegers

Department of Public Health, Erasmus Medical Center, Rotterdam, The Netherlands
Johan P Mackenbach

Department of Biostatistics, Medical Center, Rotterdam, The Netherlands
Sten P Willemsen

PUBLICATION LIST

van den Hooven EH, Jaddoe VWV, de Kluizenaar Y, Hofman A, Mackenbach JP, Steegers EAP, Miedema HME, Pierik FH. Residential traffic exposure and pregnancy-related outcomes in mother and child: A prospective birth cohort study. *Environ Health 2009; 8:59*

van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, Mackenbach JP, Steegers EAP, Miedema HME, Jaddoe VWV. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy. The Generation R Study. *Hypertension 2011; 57:406-412*

Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML, Bonzini M, Brauer M, Darrow L, Gehring U, Gouveia N, Grillo P, Ha E, **van den Hooven EH**, Jalaludin B, Jesdale BM, Lepeule J, Morello-Frosch R, Morgan GG, Slama R, Pierik FH, Pesatori AC, Sathyanarayana S, Seo J, Strickland M, Tamburic L, Woodruff TJ. The International Collaboration on Air Pollution and Pregnancy Outcomes: initial results. *Environ Health Perspect 2011; 119:1023-8.*

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, Zandveld PYJ, Mackenbach JP, Steegers EAP, Miedema HME, Jaddoe VWV. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. *Environ Health Perspect 2012; 120:150-156*

van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, Lindemans J, Russcher H, Steegers EAP, Miedema HME, Jaddoe VWV.
Air pollution exposure during pregnancy and maternal and fetal C-reactive protein levels. The Generation R Study. *Environ Health Perspect 2012; doi: 10.1289/ehp.1104345*

van den Hooven EH, Pierik FH, van Ratingen SW, Zandveld PYJ, Meijer EW, Hofman A, Miedema HME, Jaddoe VWV, de Kluizenaar Y. Air pollution exposure estimation using dispersion modelling and continuous monitoring data in the Generation R Study. *Environ Health 2012; 11:9*

van den Hooven EH, Pierik FH, de Kluizenaar Y, Hofman A, van Ratingen SW, Zandveld PYJ, Russcher H, Lindemans J, Miedema HME, Steegers EAP, Jaddoe VWV. Air pollution exposure and markers of placental growth and function. The Generation R Study. *Submitted for publication*

ABOUT THE AUTHOR

Edith van den Hooven was born on October 10th 1984 in Zwolle, the Netherlands. In 2002 she completed secondary school at the Etty Hillesum Lyceum in Deventer. In the same year she started her study Health Sciences at Maastricht University. During her Bachelor, she studied for three months at the Institute of Physical Education and Sport of the University of Malta. In 2005, she obtained her Bachelor's degree. She continued with the Master Physical Activity and Health, specialization Metabolism and Nutrition, and obtained her Master of Science degree in 2006. Afterwards, she studied European Studies on Society, Science and Technology at Maastricht University. As part of this program, she spent a semester in Linköping, Sweden, where she followed courses on Medical Technologies of Sex and Gender at the University of Linköping. She obtained her Master of Arts degree in 2007. Research on human lifestyle and health continued to fascinate her, and in 2008, she started her PhD project at the Generation R Study Group and the Department of Epidemiology at the Erasmus Medical Center Rotterdam, in close collaboration with TNO Urban Environment and Safety in Delft. The results of this PhD project are presented in this thesis. During her project, she followed the Master of Epidemiology at the Netherlands Institute for Health Sciences, for which she obtained her degree in 2011. In the beginning of 2011, she travelled for three months in South America. From February 2012 onwards, she will work as a postdoctoral researcher at the Department of Epidemiology at the Erasmus Medical Center Rotterdam.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Edith H. van den Hooven
Erasmus MC Department: Epidemiology
Research School: NIHES
PhD period: April 2008 - January 2012
Promotors: Prof.dr. A. Hofman, Prof.dr. E.A.P. Steegers
Copromotors: Dr. V.W.V. Jaddoe, Dr. F.H. Pierik

PhD training and teaching	Year	Workload (ECTS)
General courses		
Biomedical English Writing and Communication Information	2008	4.0
Specific courses: Master of Science in Epidemiology (NIHES)		
Study Design	2009	4.3
Classical Methods for Data-analysis	2008	5.7
Clinical Epidemiology	2010	5.7
Methodologic Topics in Epidemiologic Research	2010	1.4
Modern Statistical Methods	2008	4.3
Principles of Research in Medicine and Epidemiology	2008	0.7
Methods of Public Health Research	2008	0.7
Health Economics	2008	0.7
Conceptual Foundation of Epidemiologic Study Design	2009	0.7
Cohort Studies	2009	0.7
Case-control studies	2008	0.7
Introduction to Public Health	2008	0.7
Principles of Genetic Epidemiology	2009	0.7
Primary and Secondary Prevention Research	2008	0.7
Introduction to Decision-making in Medicine	2009	0.7
Demography of Ageing	2010	0.7
Social Epidemiology	2010	0.7
Courses for the Quantitative Researcher	2009	1.4
Repeated Measurements in Clinical Studies	2009	1.4
Missing values in Clinical Research	2009	0.7
Analysis of Time-varying Exposures	2009	0.7
Ethnicity, Health and Health Care	2010	1.1

Attended seminars, symposia and conferences

Generation R research meetings	2008-2010	1.0
Seminars at the Department of Epidemiology	2009-2011	1.0
Amsterdam Born Children and their Development (ABCD) Symposium, Amsterdam, the Netherlands	2008	0.3
Conference of the International Society of Environmental Epidemiology, (ISEE), Pasadena, United States	2008	1.0
Generation R Symposium: Imaging and brain development Rotterdam, the Netherlands	2008	0.3
Generation R Symposium: Epidemiology of childhood asthma Rotterdam, the Netherlands	2009	0.3
Generation R Symposium: Genetics in child cohort studies Rotterdam, the Netherlands	2010	0.3
Bessensap, Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), Den Haag, the Netherlands	2010	0.3
Meeting of Environmental Health Risks in European Birth Cohorts (ENRIECO), Barcelona, Spain	2009	0.6
Meeting of Environmental Health Risks in European Birth Cohorts (ENRIECO), Utrecht, the Netherlands	2010	0.6

(Inter)national conference presentations

Generation R research meeting	2009	0.5
Intern research day of the Netherlands Organisation of Applied Scientific Research (TNO), Utrecht, the Netherlands – poster presentation	2009	0.5
Conference of the International Society of Environmental Epidemiology, (ISEE), Dublin, Ireland – oral presentation	2009	2.0
Symposium Werkgroep Epidemiologen Onderzoek Nederland (WEON), Nijmegen, the Netherlands – poster presentation	2010	1.0
Intern Research Day of the Netherlands Organisation of Applied Scientific Research (TNO) – oral presentation	2010	0.5
Conference of Epidemiological Longitudinal Studies in Europe (CELSE), Paphos, Cyprus – two oral presentations	2010	2.0
Conference of the International Society of Environmental Epidemiology, (ISEE), Barcelona, Spain – oral presentation	2011	2.0

Other skills

Review paper for Environmental Research	2010	
Review paper for Polish Journal of Environmental Studies	2011	
Review paper for American Journal of Epidemiology	2011	
Review paper for Environmental Research	2011	

Teaching

Supervising Master's Thesis "Associations of maternal haemoglobin levels with blood pressure and the risks of hypertensive complications during pregnancy", Siham Yassine, MSc	2011	0.5

DANKWOORD

Tot slot is er een aantal mensen die ik graag wil bedanken voor hun bijdrage aan dit proefschrift. Als eerste dank aan alle proefpersonen voor deelname aan het Generation R onderzoek, het invullen van de vragenlijsten en de bezoekjes aan onze onderzoekscentra. Dit heeft een grote hoeveelheid aan gegevens opgeleverd, waardoor veel interessante onderzoeksvragen beantwoord kunnen worden.

Dank ook aan mijn promotoren, professor Hofman en professor Steegers. Prof.dr. A. Hofman, beste Bert, bedankt voor de begeleiding, de inspirerende colleges bij de Nihes en de interessante discussies bij de Generation R research meetings en de Epidemiologie seminars. Uw enthousiasme voor onderzoek en epidemiologie is erg aanstekelijk. Prof. dr. E. Steegers, beste Eric, bedankt voor het duidelijke commentaar op de artikelen en het meedenken over de 'grotere lijn' van onze papers tijdens de Groei & Ontwikkeling overleggen, waarbij uw uitgebreide kennis over de zwangerschap een zeer waardevolle bijdrage was.

Vincent en Frank, mijn copromotoren. In het begin was het even wennen om met twee begeleiders te werken, die (soms) elk een andere mening hebben. Gelukkig konden we daar een manier in vinden. Ik heb de samenwerking met jullie beiden als prettig ervaren. Vincent, ik ken weinig mensen die zo efficiënt en hard kunnen werken als jij. Dat dit af en toe leidt tot het vergeten van afspraken of het 'kwijtraken' van e-mails is hier waarschijnlijk inherent aan. Ik heb veel gehad aan jouw oog voor de betekenis van onderzoeksresultaten en de talloze ideeën voor 'spannende' nieuwe papers. Jouw snelle reacties – op de meest uiteenlopende tijdstippen – en kritische commentaar hebben de artikelen ongetwijfeld verbeterd. Ik heb veel van je geleerd, bedankt daarvoor. Frank, bedankt voor het vertrouwen dat je me hebt gegeven, het feit dat je altijd de tijd nam om te overleggen, en voor het delen van je kennis en inzichten. Je bent vanaf het begin erg betrokken geweest, informeerde regelmatig of ik als nieuwkomer in Rotterdam m'n draai al had gevonden en gaf me tips over restaurantjes en festivals. Mede hierdoor heb ik Rotterdam snel leren kennen. Ook in dit rijtje wil ik graag Yvonne noemen, mijn 'derde' begeleider. Yvonne, jouw gedrevenheid om het naadje van de kous te weten heeft geleid tot veel leerzame overleggen. Je grondige, kritische blik zorgde er soms voor dat ik even met m'n handen in het haar zat, maar ik ben je er erg dankbaar voor. Je hebt me gestimuleerd om goed na te denken over de interpretatie van onderzoeksresultaten en mogelijk onderliggende mechanismen. Je hebt me veel geleerd over air pollution (modellering) en nam altijd de tijd om samen dingen door te nemen. Bedankt voor de interesse die je altijd hebt getoond, de gezellige gesprekken, en de motiverende woorden die me vaak deden realiseren dat het mooi was wat we aan het doen waren.

Prof.dr. Lex Burdorf en Prof.dr. Irwin Reiss, hartelijk dank voor de deelname in mijn leescommissie en de beoordeling van mijn manuscript. Prof.dr. Jordi Sunyer, thank you for reviewing my thesis and your valuable comments to improve it. I am grateful that you participate in my PhD committee. Rémy Slama, thanks for our interesting discussions

during conferences and for your participation in the PhD committee. Prof.dr. Johan de Jongste, bedankt voor het lezen van mijn proefschrift en het plaatsnemen in de commissie.

Alle co-auteurs, bedankt voor de bijdrage aan de papers. Ernst Meijer, Peter Zandveld en Sjoerd van Ratingen, bedankt voor het meedenken over de beschrijving van de air pollution modellering methode. Het is voor epidemiologen niet altijd makkelijk om de details van dit soort berekeningen te volgen, en het was dan ook een uitdaging om dit op een begrijpelijke wijze te noteren. Jullie hebben me hier vele keren bij geholpen. Sjoerd, ik heb regelmatig met je aan de telefoon gezeten, bedankt dat je altijd de tijd nam om dingen door te nemen. Henk Miedema, een aantal jaar geleden heb jij mede mogelijk gemaakt dat dit onderzoek kon starten. Mijn dank hiervoor. Tijdens het onderzoek was je voornamelijk aan de zijlijn betrokken, en onze overleggen stimuleerden me om na te denken over de implicaties van de bevindingen en de grotere lijn van het onderzoek. Sten Willemsen, veel dank voor de hulp bij het modelleren van de foetale groeicurves. Het ging minder voorspoedig dan we verwacht hadden, maar uiteindelijk heeft het tot een mooi resultaat geleid. Professor Johan Mackenbach, Professor Jan Lindemans en Henk Russcher, bedankt voor jullie bijdrage aan de artikelen.

Dan alle collega's met wie ik de afgelopen vier jaar een fijne tijd heb doorgebracht. De TNO collega's in Delft: Frank, Yvonne, Sabine, Reinier, Henk Vos, Hilde, Jamie, Jeroen, Corrie, Marga, Han, Erik, Ralph, Walter, Dennis, Henk Verhagen, Michan, Ton en Henk Pollinder. Bedankt voor het hartelijke welkom in het begin, de gezellige praatjes en de interesse in de voortgang van het onderzoek. De vele collega's in Utrecht, waaronder Menno, Jan, Dick, Ernst, Peter en Sjoerd, bedankt voor de input bij overleggen en de hulp bij specifieke vragen over luchtverontreiniging. Hilde en Jamie, met jullie heb ik een tijd als 'directe' buren gewerkt, wat ervoor zorgde dat we (naast werken) veel gezellige gesprekken hebben gehad. Henk Vos, bedankt voor de vele databestanden die je voor ons gekoppeld hebt, wat soms gepaard ging met flink wat frustratie vanwege missende postcodes, onjuiste conceptiedatums of andere foutmeldingen… Bedankt ook voor de uitleg over het koppelen van adresbestanden aan het begin van het onderzoek. Reinier, Han, Ton en Henk Pollinder, bedankt voor de berekeningen die jullie hebben uitgevoerd en het aanleveren van de mooie kaartjes en figuren.

De Generation R promovendi: Agnes, Akhgar, Alette, Andrea, Anne, Anne, Ank, Ankie, Annemarie, Anne-Marie, Busra, Celine, Claudia, Dennis, Denise, Ehsan, Esther, Eszter, Fleur, Hannan, Ilse, Jens, Jessica, Jolien, Jolien, Joost, Layla, Lenie, Lindsay, Maartje, Maartje, Marieke, Marina, Marlies, Michelle, Nathalie, Nicole, Nienke, Nina, Noor, Pauline, Rachel, Ralf, Rianne, Rob, Rolieke, Romy, Sabine, Sandra, Sarah, Selma, Tamara, en Mónica (geen promovendus meer, maar toch in dit rijtje). Veel gezelligheid tijdens en na het werk met de vele keren koffie drinken, borrelen, etentjes, en WK kijken. Busra en Dennis, kamergenoten in de eerste tijd. Dennis, je episodes van koortsachtig geconcentreerd werken afgewisseld met enthousiaste kreten maakten je tot een inspirerende kamergenoot. Busra, bedankt voor de gezellige sfeer en de praatjes. Jammer dat je net weer terugkomt nu ik wegga, maar we blijven elkaar vast tegenkomen. Rachel,

Denise en Romy, met jullie werd de kamer opeens een stuk 'huiselijker', met plantjes, een Senseo en een altijd gevulde snoeppot. Denise en Romy, op het laatst was er niet altijd tijd om lang te kletsen, maar het was fijn om altijd even een praatje te kunnen maken over leuke, frustrerende of bijzondere dingen. Het was fijn om jullie als kamergenoten te hebben! Claudia, Rolieke, Layla, Jessica, en Ank, veel gekletst en gelachen tijdens de koffiepauzes en etentjes. Wat mij betreft blijven we dat volhouden, ook al werken we binnenkort allemaal op een andere plek. Rob, Layla, Denise, Romy en Agnes, het was leuk om samen naar Cyprus te gaan! Ik moet nog steeds grinniken als ik denk aan ons Romeinse hotel en 'Rob met z'n vijf vrouwen'. Layla en Jessica, vanaf het begin af aan zocht ik jullie vaak op voor een praatje over werkgerelateerde of niet-werkgerelateerde dingen. Wie weet zullen we in de toekomst weer samenwerken. Ik ben blij dat jullie als paranimfen naast me staan!

Uiteraard ook Patricia, Rose, Alwin, Claudia, Ronald, Elise, Karien, Natalia, Marian, Sabah, Rukiye en alle focusdames: bedankt voor het uitvoeren van alle metingen op het onderzoekscentrum, de secretariële en logistieke taken, de computerondersteuning, de communicatie-gerelateerde zaken, en alles rondom het datamanagement. Jullie aanwezigheid zorgt ervoor dat Generation R zo goed draait!

Bedankt aan alle familie, schoonfamilie en vrienden voor de interesse in het onderzoek en natuurlijk de welkome afleiding in de vorm van etentjes, borrels, stapavonden, sauna- of bioscoopbezoekjes en weekendjes weg. Elke, Linda, Merel en Tineke, wat mij betreft blijven we elk jaar op vakantie gaan! Alle meiden uit Deventer: ook al komt het niet meer elk jaar van een kerstdiner, de borrels en weekendjes Bergen aan Zee zijn een goed alternatief. Lieve pap en mam, bedankt voor het feit dat jullie altijd achter me hebben gestaan en me hebben gestimuleerd om mijzelf te ontwikkelen. Ik kan altijd met m'n verhalen bij jullie terecht, of het nou gaat over werk ("hoe zat het nou ook alweer met dat 4e artikel?") of andere zaken. Iris en Marieke, lieve zus en zusje, soms een beetje druk ;), maar bovenal ben ik blij dat we gezellig kunnen kletsen en lachen. Lieve Derk, bedankt voor je steun en vertrouwen en het geduld dat je met me hebt (gehad). Bij alle keuzes die ik heb moeten maken, stond je achter me, zonder daarbij aan je eigen belang te denken. Ik ben blij dat we niet meer zo ver uit elkaar wonen, samen is toch een stuk gezelliger.