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Association between pregnancy exposure to atmospheric pollutants and placental weight

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List of acronyms

AQMS	air quality monitoring stations
CI	confidence interval
DNA	Deoxyribonucleic acid
EDEN	Etudes des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant
FPR	feto-placental weight ratio
Inserm	Institut national de la santé et de la recherché medicale
LMP	last menstrual period
LUR	Land-use regression
NO_2	Nitrogen dioxide
PGH	Placental growth hormone
PM	Particulate matter
PM_{10}	Particulate matter with diameter <10µm
SD	standard deviation
US EPA	United States Environmental Protection Agency
VEGF	vascular endothelial growth factor

1. Introduction

1.1. Adverse health effects of atmospheric pollution

Atmospheric pollutants were defined as particulate or gaseous substances in the atmosphere derived from human activities with potential adverse effect for human health or environment [1]. The United States Environmental Protection Agency (US EPA) mentions ozone, particulate matter (PM), carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and lead as the most common air pollutants [2]. In the past five decades, the level of several air pollutants such as sulphur dioxide has decreased in many urban settings of developed countries, whereas it remained more or less constant for other pollutants such as nitrogen dioxide [3].

Among the main sources of air pollutant emissions are combustion of fossil fuels, more prominently from motor vehicles [4] as well as urban heating and industrial agricultural activities. Nitrogen dioxide is one reasonable marker of traffic-related air pollution for study purposes [5]. Particulate matter with diameter below 10 μm (PM_{10}) is also emitted by mining, agriculture and industrial activities. Particulate matter contains elemental carbon and organic compounds, as well as traces of heavy metals and sulphur [3].

The earliest observed association between air pollution and mortality dates back to the 19th century [6]. In 1952, a smog episode in London has led to the first government regulation on air pollution [7]. Since then, particularly in Europe and North America, the development of research in this area had prompted the governments at country and regional level to issue policies on air pollution.

Focusing specifically on traffic-related air pollution, exposures are assessed with several methods. Exposures are commonly assessed through permanent ambient air or indoor air pollution measurements. Modelling may also be done using spatial analysis to smooth pollution patterns based on data from monitoring campaigns [8]; or simulation of pollutant dispersion from the sources to the environment using dispersion modelling techniques based on data on sources and meteorological parameters. They provide a finer spatial resolution compared to data from permanent monitoring stations. Direct assessment using personal monitoring samplers has also been done. [3]

Traffic-related air pollution have been linked with changes in blood pressure [9], cardiovascular diseases [10], as well as the risk of lung cancer [11], cardiopulmonary-related death [12] and morbidity, and with respiratory illnesses such as asthma and bronchitis, not only in adults but in early childhood as well [13, 14].

1.2. The effect of air pollution on pregnancy outcomes

There is also a growing interest in the association between air pollution and pregnancy outcomes. Prenatal exposure to pollutants has been shown to be associated with birth weight in several studies [8, 15, 16] although the evidence is not yet considered conclusive. One reason is that the possible biological mechanisms whereby atmospheric pollutants could affect fetal growth has not been established [17]. Previous epidemiologic studies on the environmental effects to reproductive health have mainly used fetal growth or birth weight as their main outcomes, defined as a continuous or as a dichotomous variable [18]. Although plausible, placental association with environmental exposures has not been studied in humans.

Fetal growth is associated with various factors, among them maternal and paternal anthropometric measures [19], parity, tobacco smoke exposure and other pregnancy-related complications [20]. Pregnancy outcomes include fetal and infant mortality, low birth weight, intrauterine growth retardation, preterm birth and birth defects [21]. In reproductive epidemiology, it may also include pregnancy complications such as preeclampsia [22]. Birth weight is one of the most used indicators [23], adjusted for gestational duration to focus on fetal growth as the outcome of interest [23, 24]. Its clinical importance is considered because studies have shown that lower birth weight for gestational age may be predictive for a higher risk of chronic diseases and mortality later in life [25, 26].

Pregnancy can be considered via a three-compartment model including the mother, the fetus, and the placenta [27]. The placenta, which develops during implantation of the blastocyst (fertilized ovum) in the uterus, mediates circulation exchange between the mother and the fetus in such a way that avoids the mixing of maternal and fetal blood [28]. The placenta also plays a role in nutritional transport and metabolism [29]. The placental functions are aimed at achieving homeostasis in intrauterine environment to support growth and development of the fetus [30].

Placental weight at birth is correlated with infant's birth weight [31]. In the second half of pregnancy, placental growth tend to slow down to the point that restricts fetal growth (although not clearly shown on the curve in Figure 2), which may explain the flattening of fetal growth curve towards birth [30]. This process may start earlier in adverse circumstances and it may prompt fetal growth restriction, which manifests as lower birth weight for gestational age [30].

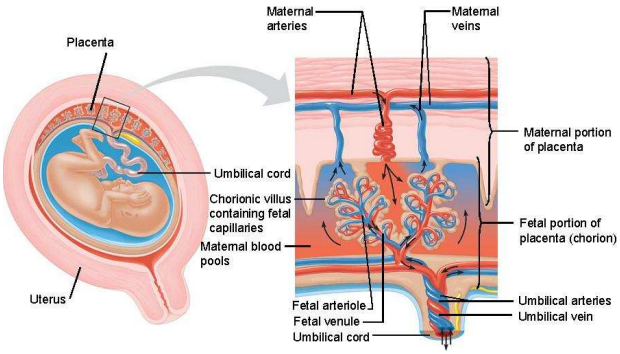

Figure 1: Morphology of placenta [32]

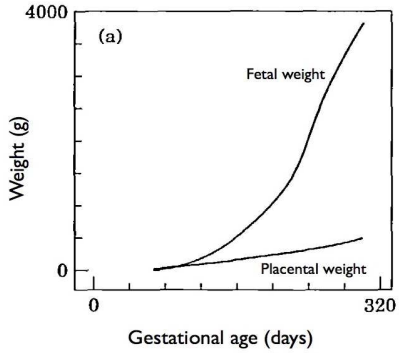

Figure 2: Placental and fetal growth curves [30]

Some studies measure placental growth by the dimensions of the chorionic plate and the third dimension of its thickness, which are represented by the gross weight of the placenta [33]. Placental thickness is a proxy to placental functional efficiency [34], and another study suggested that it may inversely correlated with birth weight [24].

The ratio of fetal weight to placental weight implies the interdependent growth between the fetus and the placenta, which changes throughout pregnancy. A very low feto-placental weight ratio (FPR) at birth is suggestive of a low fetal weight relative to placental weight, which may be due to fetal growth restrictions, and on the other hand, a very high ratio suggests a small placenta, which may as well have a negative influence on fetal growth [35].

Studies have shown that maternal smoking disrupts placental function [36] and fetal growth [37], possibly owing to the fact that carbon monoxide and other pollutants are able to cross the placental barrier and reduce oxygen or nutrient supply to the fetus [38]. As shown from experimental studies in mice, it is plausible that the effects of air pollution on the fetus are mediated through the placenta [39].

There is scarce evidence that explores the potential role of the placenta. Our study will use placental weight and feto-placental weight ratio alongside birth weight as pregnancy outcomes, taking into account similar confounding factors (*e.g.*, maternal smoking, gestational age, and socioeconomic factors) [40].

1.3. Aims and objectives

This study aims to investigate the association between pregnancy exposure to NO_2 and PM_{10} with placental weight and FPR. We will also analyze the association with birth weight to be compared with the effects on both placental weight and feto-placental weight ratio. For comparison purpose, we will also study the possible impact of maternal smoking on the same birth outcomes. The results are expected to add understanding on the potential role of placenta in the mechanism of environmental effects on fetal growth.

2. Population and methodology

2.1. Setting and study population

Our study is based on a part of EDEN cohort. EDEN (Etudes des Déterminants pré et postnatals précoces du développement et de la santé de l'ENfant) is a mother-child cohort on pre- and early postnatal determinants of fetus and infant development and health [41]. The study population was recruited at the maternity wards of two University hospitals in Poitiers and Nancy, France [42] before 28 weeks of pregnancy (calculated from the last menstrual period) between February 2003 and January 2006. The women who carried multiple fetuses, or known to have diabetes, not understanding French, planning to give birth outside the university hospital or to move out from the study region within 3 years of recruitment were not eligible [16].

Data were obtained and analyzed at Inserm (Institut national de la santé et de la recherché medicale) research team in "Environmental Epidemiology Applied to Reproduction and Respiratory Health (E2R2H)," U823, at Institut Albert Bonniot, Grenoble. This study was classified as 'low risk' by The University of Sheffield Ethics Committee because it uses already existing data ('secondary data').

The study population is restricted to all single live births from women enrolled in the cohort with complete data for birth weight, placental weight, parity, maternal education, maternal height and pre-pregnancy weight, and smoking status.

2.2. Assessment of exposures

The exposures of interest are the level of NO_2 and PM_{10} in the ambient air at both cities at each trimester of pregnancy and throughout pregnancy. Data on exposure to NO_2 and PM_{10} were estimated using ADMS-Urban dispersion models [8]. The entry data of the dispersion model included spatial factors such as localization and characteristics of traffic, industrial and urban heating sources, emission strength, hourly meteorology parameters, as well as data from specific permanent monitoring stations to estimate the background (long range) air pollution level [43, 44]. These models were implemented by Airlor and Atmo Poitou-Charentes air quality monitoring networks, in collaboration with Inserm U823 team of environmental epidemiology prior to the trainee period.

Maternal pregnancy exposure was estimated as an average of the hourly estimates from the dispersion model at the home address during specific time windows (pregnancy trimesters or whole pregnancy). In the case when the woman changed home address during pregnancy, exposure was estimated as the average exposures at each home address during the relevant period. Some women had missing information on the daily exposure estimate and thus contributed less than 100% to the average for the corresponding time in pregnancy.

Based on usual practice, the women whose average exposure level estimate consisted of <75% daily values were excluded from the study.

The date of conception was estimated based on last menstrual period (LMP) or ultrasound-based estimate assessed by the obstetrician for those whose LMP date were missing or gave gestational duration of >44 weeks [16]. To obtain an estimate of the whole pregnancy exposure for each woman, we defined gestational age by subtracting the date of delivery with the date of conception. When estimating the trimester-specific exposure average, we defined the first trimester as date of conception to day 91, second trimester as day 92-183, and third trimester as day 184 to delivery. For observations with date of delivery before day 184 (n = 19), there were no third trimester exposure data.

2.3. Assessment of outcome

The outcomes of interest were placental weight, feto-placental weight ratio (FPR), and birth weight. Birth weight and placental weight data in the cohort were obtained from maternity records at birth. Placental weight is correlated with birth weight [45].

2.4. Adjustment factors

This study is an effort to document to which degree air pollution exposure influences pregnancy outcomes. The observational nature of our study makes it prone to confounding. Since the observation is done on the pregnancy outcomes along the different levels of air pollution exposure, we need to take into account relevant factors that potentially confound the association [46]. The effect sizes of air pollution exposure on placental weight and birth weight are possibly small, and therefore the issue of confounders is relevant [47]. The potential confounders for this study were defined as the risk factors of adverse pregnancy outcomes that may be associated with air pollution levels without being a consequence of either exposure or of the outcome of interest [47].

In other words, the conventional criteria from Rothman and Greenland have been used [48]:

(1) A confounder must have an association with the exposure of interest;
(2) A confounder must be a “risk factor” for the outcome (i.e., it must predict who will develop disease), although not necessarily a cause of the outcome;
(3) The confounding factor must not be affected by the exposure or the outcome.

We based on the literature and performed some statistical analysis to assess if the above criteria were fulfilled for each potential confounder, which will be explained below.

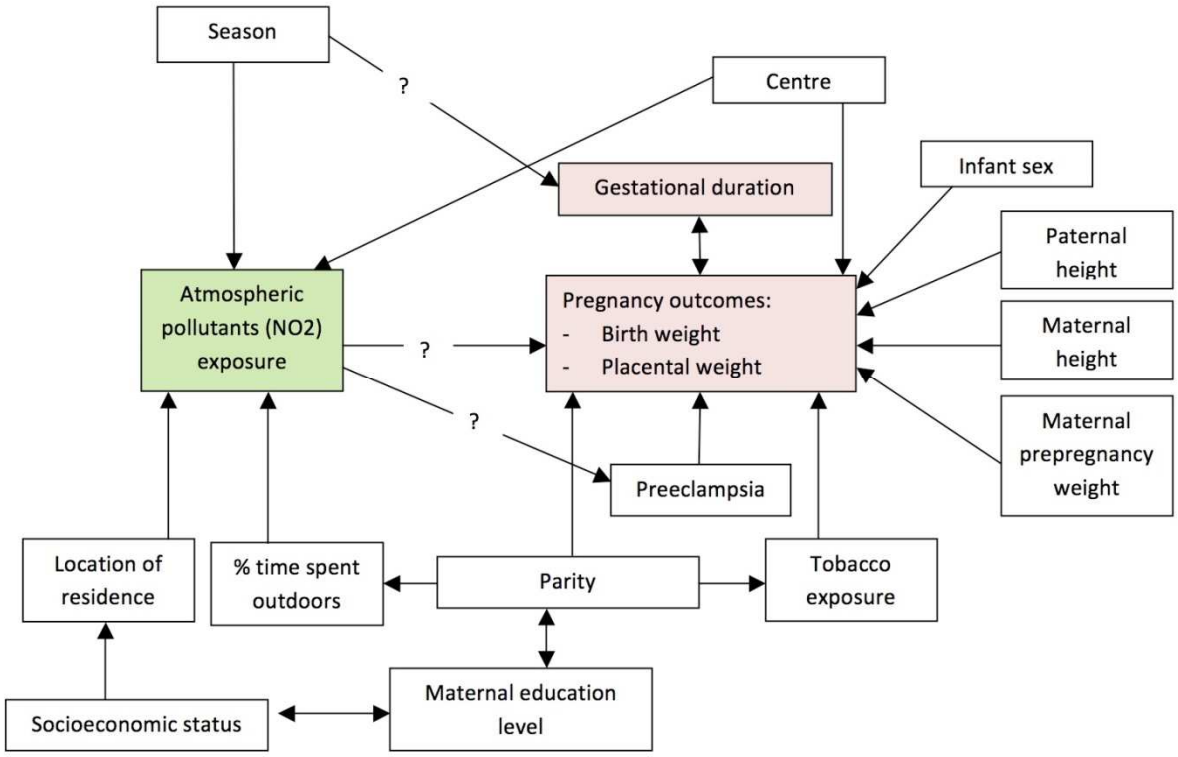

Figure 3: Diagram summarizing the causal pathways hypothesized in selecting the adjustment factors

A causal diagram [48] has been used to help summarizing the hypothesized relations between the considered factors (Figure 3), although we did not further use the formalism of directed acyclic graphs.

This study used the coding of potential confounders determined in previous studies of the EDEN datasets in which birth weight was the outcome [16].

2.4.1. Adjustment for gestational duration

As shown in Figure 3, gestational duration is viewed as an integral part of the measure of fetal and placental growth. That is, the outcome of interest is not placental weight *per se* but gestational age-adjusted placental weight, in order to study if air pollution entailed a change in placental weight compared to infants born at the same gestational age.

In the adjustment, the linear and quadratic terms of gestational duration was used, as analyzed in other studies on EDEN cohort [16].

2.4.2. Other adjustment factors

The maternal characteristics adjusted for were maternal height and pre-pregnancy weight, parity and maternal education level. Maternal height and pre-pregnancy weight are known to influence fetal growth [20]. Maternal height was coded as a continuous variable and maternal pre-pregnancy weight was coded using a broken stick model with a knot at 60 kg

[49] due to different trajectories of fetal birth weight between women weighing less and more than 60 kg. Although paternal genotypic characteristics may also influence fetal growth [50], paternal height was not included in the analysis because there were many missing data.

Parity was divided in three categories: nulliparity, para 1, and para 2 or more. Initial analysis on the data showed that first-born infants (44.4% of the population) had a lower average weight compared to second born or more (3218 *vs.* 3328 g; *t*-test, p<0.01). Risks of adverse pregnancy outcomes are higher in nullipara and multipara (of parity >3) [51]. Moreover, women with more children may have a different activity pattern and location of residence, hence a different air pollution exposure than nulliparous women.

Maternal education level is used as a proxy to socioeconomic status, although it may not explain the whole construct [47]. In several studies, maternal education and socioeconomic status are shown to potentially affect the association between exposure to air pollution with infant mortality [52], birth weight and parity [53, 54]. In the EDEN cohort, maternal education level is represented by the age of the woman at the end of education and coded as a categorical variable. In the initial analysis, we found that women with who finished school at the age of $\geq$21 years (63% of the population) were more likely to deliver infants with higher birth weight (3297 *vs.* 3245 g; *t*-test, p=0.03). Socio-economic status may also be associated with the area of residence, and hence with air pollution exposure.

We also adjusted for centre. Missing placental weight data was more frequent in Nancy than in Poitiers (see Table 1). Other factors that may influence pregnancy outcomes may also differ in the two cities.

Tobacco smoking has been known to interfere with pregnancy outcomes [37, 55]. Trimester-specific data on tobacco smoking were collected from self-reports. Tobacco smoking was considered as continuous, categorical and binary (smoker/non-smoker) variables. In the initial analysis, smoking was not clearly associated with placental weight, but has a stronger association with FPR (*p*-values for continuous, categorical and binary variables were <0.05; tables in Appendix 1). In final models, maternal second-trimester smoking exposure as a continuous variable was included in the regression model.

Season is clearly associated with air pollution levels [56] and may have an influence on birth weight. Seasons with lower temperature during mid-pregnancy is suggested to be associated with lower birth weight [57]. (Note that this study did not adjust for air pollution level). Another study on air pollution level and birth weight found that infants conceived in winter have lower birth weight [18]. Studies restricting women within a certain gestational age (*e.g.*, 40 gestational weeks) commonly uses season of delivery as an adjustment factor [58]. In this study we include all pregnancies without restricting to a certain gestational age, and we coded season of conception as categorical variable divided in quarters (January-March, April-June, July-September and October-December).

Preeclampsia is elevated blood pressure accompanied by proteinuria after the 20th week of pregnancy [59]. The only known effective treatment so far is to deliver the fetus, and hence it becomes one of the main reasons for elective premature delivery [43], which relates to lower birth weight and placental weight. It has been suggested that exposure to air pollution may be associated with an increased risk of preeclampsia [60]. In this study, preeclampsia was hypothesized as a mediator (intermediate factor) in the association between air pollutants exposure and placental weight (Figure 2.1) and as such we decided not to adjust for it.

2.5. Statistical analysis

Linear regression models (Stata SE version 10.1; StataCorp., College Station, TX, USA) have been used to investigate the association between levels of NO_2 and PM_{10} and measurement of birth weight and placental weight. NO_2 and PM_{10} exposures were considered either as continuous variables (in which case we reported the change of placental weight for every 10-μg/m^3 increase of NO_2 or PM_{10}), or in categories, using tertiles defined on the sample population. For categorical variables, linear trend tests were performed with values corresponded to the tertile-specific median NO_2 and PM_{10} levels.

2.6. Sensitivity analyses

To assess the potential for selection bias due to missing values of placental weight, we repeated analyses of air pollution effects on birth weight for all participants, including those with missing placental weight. Analyses were repeated excluding one infant with extreme value of placental weight. Analyses were also repeated, stratified (instead of adjusted) on center.

3. Results

3.1. Study population

The EDEN cohort included 2002 pregnant women, 969 (48.4%) in Poitiers and 1033 (51.6%) in Nancy. Information on birth weight were available for 1893 singleton live births, but placental weight information were missing for 472 (24.9%) of them; 414 of which are from Nancy (43% of all subjects with birth weight information in Nancy). We analyzed the difference in characteristics between subjects with and without placental weight information (Table 1) and performed a similar analysis only for the population in Nancy (Table 2) which yielded similar results.

Table 1: Characteristics of Eden cohort mother-child pairs with placental weight data and of pairs with missing information on placental weight. The comparison is restricted to n=1893 mother-child pairs with birth weight information.

Variables	Total (%)	Non-missing placental weight (N=1421) No.(%)	Missing placental weight (N=472) No.(%)	*p*-value
Centre				0.00[q]
Poitiers	930 (100)	872 (93.8)	58 (6.24)	
Nancy	963 (100)	549 (57.0)	414 (43.0)	
Maternal age at end of education (years)				0.01[b]
≤16	102 (100)	80 (78.4)	22 (21.6)	
17-18	245 (100)	188 (76.7)	57 (23.3)	
19-20	346 (100)	281 (81.2)	65 (18.8)	
≥21	1190 (100)	863 (72.5)	327 (27.5)	
Missing	10 (100)	9 (90.0)	1 (10.0)	
Birth order				0.98[b]
First birth	840 (100)	630 (75.0)	210 (25.0)	
Second birth	694 (100)	522 (75.2)	172 (24.8)	
Third birth or more	356 (100)	266 (74.7)	90 (25.3)	
Missing	3 (100)	3 (100)	-	
Maternal smoking trimester 2 (cig/day)				0.18[b]
0	1551 (100)	1151 (74.2)	400 (25.8)	
1-9	229 (100)	183 (79.9)	46 (20.1)	
10+	94 (100)	70 (74.5)	24 (25.5)	
Missing	19 (100)	17 (89.5)	2 (10.5)	
Infant sex				0.55[a]
Male	997 (100)	754 (75.6)	243 (24.4)	
Female	896 (100)	667 (74.4)	229 (25.6)	
Mode of delivery				0.00[b]
Normal	1397 (100)	1077 (77.1)	320 (22.9)	
Assisted	195 (100)	109 (55.9)	86 (44.1)	
Cesarean section	299 (100)	234 (78.3)	65 (21.7)	
Missing	2 (100)	1 (50.0)	1 (50.0)	
Gestational duration (categorical)				0.35
<37 weeks	107 (100)	80 (74.8)	27 (25.2)	
37-38 weeks	384 (100)	295 (76.8)	89 (23.2)	
39-40 weeks	976 (100)	740 (75.8)	236 (24.2)	
41+ weeks	426 (100)	306 (71.8)	120 (28.2)	
		Mean (SD)	**Mean (SD)**	
Maternal age at conception (years)		29.2 (4.87)	29.5 (4.92)	0.25[a]
Birth weight (g)		3284 (520)	3261 (489)	0.21[c]
Gestational duration (weeks)		39.7 (1.79)	39.7 (1.76)	0.31

[a]*p*-Value of t-test. [b]*p*-Value of chi-square test. [c]*p*-Value of Wilcoxon rank test. All tests were performed excluding the category corresponding to missing values.

We found no significant difference between maternal age at pregnancy, maternal age at the end of education, parity, maternal smoking, infant sex, birth weight and gestational duration (*t*-test, Chi-square test or Wilcoxon rank test; all *p*-values >0.005 between observation with and without information on placental weight). The proportion of infants born through assisted delivery (forceps or vacuum extraction) differed between those with and without placental weight information (Chi-square test, *p*-value for both centres <0.005). In Nancy, 79% of children born through assisted delivery had no information on placental weight. We decided to exclude the 195 infants born with assisted delivery in Nancy and Poitiers from the analyses.

Table 2: Characteristics of Eden cohort mother-child pairs in Nancy with placental weight data and of pairs with missing information on placental weight. The comparison is restricted to n=963 mother-child pairs with birth weight information enrolled in Nancy.

Variable	Total N (%)	Placental weight data in Nancy		*p*-value
		Non-missing (N=549) N (%)	Missing (N=414) N (%)	
Maternal age at end of education (years)				0.05[a]
≤16	50 (100)	29 (58.0)	21 (42.0)	
17-18	98 (100)	50 (51.0)	48 (49.0)	
19-20	165 (100)	109 (19.9)	56 (13.5)	
≥21	646 (100)	358 (55.4)	288 (44.6)	
Missing	4 (100)	3 (75.0)	1 (25.0)	
Birth order				0.37[a]
First birth	403 (100)	222 (55.1)	181 (44.9)	
Second birth	380 (100)	227 (59.7)	153 (40.3)	
Third birth or more	179 (100)	99 (55.3)	80 (44.7)	
Missing	1 (100)	1 (100)	-	
Mode of delivery				0.00[a]
Normal	683 (100)	403 (59.0)	280 (41.0)	
Assisted	96 (100)	20 (20.8)	76 (79.2)	
Cesarean section	183 (100)	126 (68.9)	57 (31.1)	
Missing	1 (100)	-	1 (100)	
Maternal smoking trim 2 (cig/day)				0.81[a]
0	816 (100)	463 (56.7)	353 (43.3)	
1-9	93 (100)	56 (60.2)	37 (39.8)	
10+	50 (100)	28 (56.0)	22 (44.0)	
Missing	4 (100)	2 (50.0)	2 (50.0)	
Infant sex				0.07[a]
Male	465 (100)	251 (54.0)	214 (46.0)	
Female	498 (100)	298 (59.8)	200 (40.2)	
Gestational duration (categorical)				0.03
<37 weeks	54 (100)	32 (59.3)	22 (40.7)	
37-38 weeks	211 (100)	131 (62.1)	80 (37.9)	
39-40 weeks	493 (100)	287 (58.2)	206 (41.8)	
41+ weeks	205 (100)	99 (48.3)	106 (51.7)	
		Mean (SD)	**Mean (SD)**	
Maternal age at conception (years)		29.2 (4.89)	29.5 (4.87)	0.40[b]
Birth weight (g)		3260 (504)	3269 (482)	0.98[b]
Gestational duration (weeks)		39.6 (1.67)	39.8 (1.72)	0.01[b]

[a]*p*-Value of Wilcoxon rank test. [b]*p*-Value of chi-square test. All tests were performed excluding the category corresponding to missing values.

Table 3: Characteristics of study population compared with EDEN cohort

		EDEN Cohort (N = 2002)		Study sample* (N = 810)
Maternal characteristics	N	Mean (SD)	N	Mean (SD)
Age (years)	1905	29.3 (4.9)	810	29.0 (5.0)
Height (cm)	1899	163 (6.2)	810	164 (6.2)
Pre-pregnancy weight (kg)	1910	62.2 (12.8)	810	61.7 (12.8)
Maternal age at end of education		N (%)		N (%)
≤16		107 (5.3)		44 (5.4)
17-18		250 (12.5)		109 (13.5)
19-20		350 (17.5)		156 (19.3)
≥21		1208 (60.3)		501 (61.9)
Missing		87 (4.4)		-
Parity		N (%)		N (%)
0		848 (42.4)		360 (44.4)
1		698 (34.9)		300 (37.0)
2+		357 (17.8)		150 (18.5)
Missing		99 (5.0)		-
Smoking in 2nd trimester (cig/day)		N (%)		N (%)
0		1583 (79.1)		666 (82.2)
1-9		240 (12.0)		109 (13.5)
10+		97 (4.9)		35 (4.3)
Missing		82 (4.10)		-
Pregnancy and infant characteristics	N	Mean (SD)	N	Mean (SD)
Birth weight (g)	1893	3279 (513)	810	3289 (523)
Placental weight (g)	1424	539 (123)	810	536 (125)
FPR (feto-placental weight ratio)	1421	6.26 (1.2)	810	6.32 (1.2)
Gestational duration (weeks)	1905	39.7 (1.8)	810	39.7 (1.8)
Infant sex		N (%)		N (%)
Male		1000 (50.0)		418 (51.6)
Female		903 (45.1)		392 (48.4)
Missing		99 (4.95)		-

*Excluding subjects with missing information on placental weight, birth weight, parity, maternal education, maternal height and weight, smoking and mode of delivery as well as those who gave birth by assisted delivery. Also excluding those without exposure level information or with more than 25% daily exposure values missing.

Of the 1311 infants with complete data for birth weight and placental weight (and not being born with assisted delivery), 41 were missing 1 or more maternal characteristic considered as adjustment factors. Of the remaining pairs of mother and infant, 810 lived within the area of dispersion modelling (see Figure 4) and this represents the study population. The characteristics of the study population are described in Table 3. Mean birth weight for the study population was 3289 g (standard deviation, SD: 523), while the mean placental weight was 536 g (SD: 125) and mean FPR 6.32 (SD: 1.20). In those excluded from the study, mean birth weight was 3271 g (SD: 505), mean placental weight was 544 g (SD: 121) and mean FPR 6.18 (SD: 3.09). In all population, birth weight were correlated with placental weight (r=0.6).

	Total	Poitiers	Nancy
EDEN	2002	969	1033
Study population	810	454	356

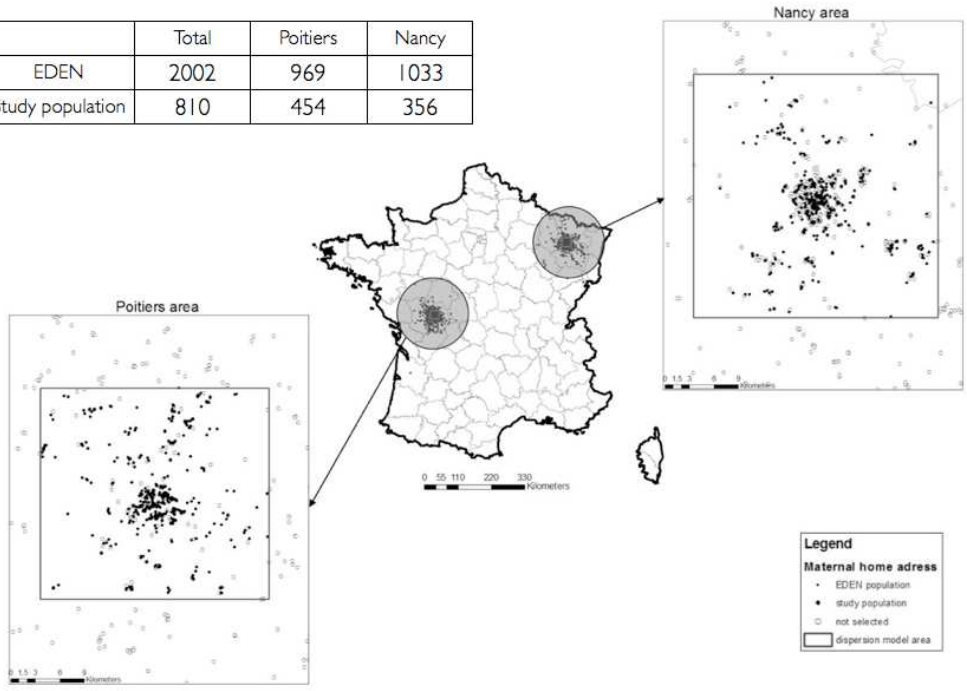

Figure 4 : Map of EDEN cohort based on home address. The square in the inlets represents the area included in the dispersion modelling. The black dots in the inlet represent our study population.

3.2. Air pollution exposure

Of the 810 women with exposure information, there were 801, 796, 788 and 789 women with available exposure value in the first, second and third trimesters and the whole pregnancy, respectively (Table 4). The mean levels of NO_2 and PM_{10} were higher in Nancy compared to Poitiers; for the whole pregnancy exposure window, mean level of NO_2 was 25.0 µg/m^3 in Nancy and 16.1 µg/m^3 in Poitiers (*t*-test, *p*-value <0.005) and mean level of PM_{10} was 23.3 µg/m^3 in Nancy and 16.2 µg/m^3 in Poitiers (*t*-test, *p*-value <0.005).

Table 4: Mean Pregnancy and Trimester-specific exposures to NO_2 and PM_{10} as estimated by ADMS dispersion model

	N*	Mean (SD)	Percentile						
			5th	25th	33th	50th	67th	75th	95th
NO_2, µg/m^3									
First trimester	801	20.1 (8.41)	9.74	13.7	15.0	18.2	22.5	24.7	35.9
Second trimester	796	20.3 (8.84)	9.43	13.7	15.1	18.0	22.4	25.2	37.6
Third trimester	788	19.5 (8.83)	8.72	12.9	13.9	17.1	22.2	24.7	37.1
Pregnancy	789	20.0 (7.81)	11.0	13.9	15.3	18.2	21.6	24.9	34.7
PM_{10}, µg/m^3									
First trimester	801	19.5 (4.64)	13.7	15.9	16.4	18.6	21.3	22.7	27.2
Second trimester	796	19.4 (4.35)	13.9	16.1	16.7	18.6	21.1	22.1	26.9
Third trimester	788	19.1 (4.55)	13.5	15.7	16.3	18.1	20.8	22.3	27.3
Pregnancy	789	19.4 (4.24)	14.7	16.1	16.4	17.3	21.2	22.3	26.3

*Excluding subjects with missing information on placental weight, birth weight, parity, maternal education, maternal height and weight, smoking and mode of delivery as well as those who gave birth by assisted delivery. Also excluding those without exposure level information or with more than 25% of daily exposure values missing.

3.3. Exposures to NO_2 and placental and birth weights

In unadjusted analyses, infants with exposure to NO_2 levels at the highest tertile in the first trimester had a placental weight on average 20 g lower (95% confidence interval, CI, -42 to.27), a birth weight 102 g lower (95%CI, -190 to -13) and FPR 0.04 higher (95%CI, -.17 to .24) than infants whose exposure was in the lowest tertile (Table 5). After adjustment for gestational duration, infant sex, center, maternal height and pre-pregnancy weight, maternal age at the end of education, parity, smoking and season of conception, these changes were -18 g for placental weight (95%CI, -39 to 4; linear trend test, p = 0.06), -37 g (95%CI, -109 to 34; linear trend test, p = 0.39) for birth weight, and +0.15 for FPR (95%CI, -.06 to.36; linear trend test, p = 0.06) (Table 6).

In the continuous coding, the strongest effect of NO_2 on placental weight before adjustment was in the whole pregnancy, with a decrease of 11 g (95%CI, -23 to -.15) and a corresponding decrease of birth weight by 28 g (95%CI, -74 to 20) and increase of FPR by 0.11 (95%CI, .01 to .22) for a 10 µg/m^3 increase of NO_2 (Table 5). After adjustment, a 10 µg/m^3 increase of NO_2 in the whole pregnancy corresponded with a placental weight average decrease by 9 g (95%CI, -20 to 2), a birth weight average decrease by 6 g (95%CI, -43 to 30) and FPR average increase by 0.13 (95%CI, .02 to .23) (Table 6). In Table 6 we also observed that the adjusted decrease of placental weight due to a 10 µg/m^3 increase of NO_2 is highest (in absolute value) in the third trimester, with an average decrease of 13 g (95%CI, -23.9 to -2.78), similar with the birth weight decrease (95%CI, -48 to 22) while the FPR increased by 0.17 (95%CI, .07 to .27).

Table 5: Unadjusted mean and SD of placental weight, birth weight, and feto-placental weight ratio (FPR) by exposures to NO_2 among study sample

NO_2 ($\mu g/m^3$)	N	Placental weight (g)		Birth weight (g)		FPR	
		β* (g)	95% CI	β* (g)	95% CI	β* (g)	95% CI
Trimester 1							
<15.0	267	Referent	-	Referent	-	Referent	-
15.0 – 22.4	267	2.97	-18.2 to 24.1	-66.4	-155 to 22.5	-.186	-.388 to .016
>22.4	267	-20.9	-42.0 to .270	-102	-190 to -12.7	.036	-.166 to .238
Continuous coding**	801	-10.1	-20.3 to .219	-50.3	-93.5 to -7.22	.026	-.073 to .124
Trimester 2							
<15.1	266	Referent	-	Referent	-	Referent	-
15.1 – 22.4	265	2.52	-18.7 to 23.8	32.5	-56.9 to 122	.060	-.143 to .263
>22.4	265	-2.67	-23.9 to 18.6	15.7	-73.7 to 105	.118	-.084 to .321
Continuous coding**	796	-7.8	-17.6 to 1.98	-11.5	-52.8 to 29.8	.107	.013 to 201
Trimester 3							
<14.0	263	Referent	-	Referent	-	Referent	
14.0 – 22.2	263	3.88	-17.4 to 25.1	42.7	-45.4 to 131	.025	-.178 to .228
>22.2	262	-4.06	-25.3 to 17.2	55.0	-33.2 to 143	.202	-.001 to .405
Continuous coding**	788	-10.1	-19.9 to -.267	-3.11	-43.9 to 37.7	.151	.057 to .245
Pregnancy							
<15.4	263	Referent	-	Referent	-	Referent	-
15.4 – 21.5	263	-2.89	-24.3 to 18.5	-16.4	-106 to 73.3	-.019	-.223 to .185
>21.5	263	-7.24	-28.6 to 14.2	-44.8	-135 to 44.9	.031	-.173 to .235
Continuous coding**	789	-11.3	-22.5 to -.146	-27.5	-74.4 to 19.5	.113	.006 to .219

*Excluding subjects with missing information on placental weight, birth weight, parity, maternal education, maternal height and weight, smoking, mode of delivery as well as those who gave birth by assisted delivery and missing information on exposure or less than 75% daily value of exposure **Parameters are reported for an increase by 10-$\mu g/m^3$ of NO_2

Table 6: Adjusted association between NO_2 levels and placental weight, birth weight and feto-placental weight ratio in all trimesters of pregnancy and the whole pregnancy

		Placental weight			Birth weight			Feto-placental weight ratio (FPR)		
NO_2 exposure window (µg/m^3)	N	β* (g)	95% CI	*p*-trend	β* (g)	95% CI	*p*-trend	β*	95% CI	*p*-trend
Trimester 1										
<15.0	267	Referent	-		Referent	-		Referent	-	
15.0 – 22.4	267	6.23	-14.6 to 27.1	.055	-42.3	-112 to 27.4	.389	-.171	-.376 to .034	.060
>22.4	267	-17.8	-39.3 to 3.66		-37.4	-109 to 34.4		.149	-.062 to .359	
Continuous coding**	801	-7.08	-17.4 to 3.25		-7.89	-42.3 to 26.5		.085	-.016 to .187	
Trimester 2										
<15.1	266	Referent	-		Referent	-		Referent	-	
15.1 – 22.4	265	6.41	-15.4 to 28.2	.937	42.2	-30.5 to 115	.705	.016	-.199 to .230	.711
>22.4	265	2.72	-20.2 to 25.6		25.4	-50.9 to 102		.041	-.184 to .267	
Continuous coding**	796	-5.20	-15.5 to 5.14		.027	-34.5 to 34.5		.093	-.009 to .194	
Trimester 3										
<14.0	263	Referent	-		Referent	-		Referent	-	
14.0 – 22.2	263	-.458	-22.2 to 21.3	.275	23.5	-48.8 to 95.8	.843	.017	-.196 to .230	.070
>22.2	262	-11.3	-34.8 to 12.1		14.3	-63.6 to 92.2		.187	-.042 to .417	
Continuous coding**	788	-13.3	-23.9 to -2.78		-12.9	-48.1 to 22.3		.168	.066 to .272	
Pregnancy										
<15.4	263	Referent	-		Referent	-		Referent	-	
15.4 – 21.5	263	.131	-20.5 to 20.8	.530	20.5	-48.0 to 88.9	.324	.017	-.186 to .219	.703
>21.5	263	-6.09	-27.0 to 14.9		-27.9	-97.4 to 41.7		.040	-.166 to .246	
Continuous coding**	789	-9.00	-20.0 to 1.98		-6.40	-42.9 to 30.1		.125	.017 to .232	

*NO_2 estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period.
**Parameters are reported for an increase by 10-µg/m^3 of NO_2

3.4. Exposures to PM_{10} and placental and birth weights

The adjusted analyses (Table 8) showed that the change of placental weight associated with exposure to PM_{10} (continuous coding) was highest for the whole pregnancy exposure; a 10 $\mu g/m^3$ increase of PM_{10} corresponded with a placental weight decrease of 24 g (95% CI, -44 to -4), while the birth weight decreased by 33 g (95%CI, -100 to 34) and the FPR increased by 0.29 (95%CI, .09 to .48).

Similar with the findings on NO_2 exposure, the strongest adjusted effect of PM_{10} exposure on placental weight was observed for the third trimester exposure window, with a decrease of 27 g (95%CI, -46 to -7) for a 10 $\mu g/m^3$ increase of PM_{10}. This exposure window corresponded with a birth weight decrease of 42 g (95%CI, -121 to 37) and FPR increase of 0.29 (95%CI, .11 to .47) (Table 8). In the same window of exposure, infants who were exposed to PM_{10} in the highest tertile had a decrease of placental weight by 19 g (95%CI, -40 to 3; linear trend test, p = 0.03), a decrease of birth weight by 12 g (95%CI, -101 to 41; linear trend test, p = 0.23) and an increase of FPR by 0.21 (95%CI, .00 to .42; linear trend test, p = 0.02) compared to those who were exposed by PM_{10} in the lowest tertile (Table 8).

Table 7: Unadjusted mean and SD of placental weight, birth weight, and feto-placental weight ratio (FPR) by exposures to PM_{10} among study sample

		Placental weight		Birth weight		Feto-placental weight ratio (FPR)	
PM_{10} exposure window ($\mu g/m^3$)	N	β* (g)	95% CI	β* (g)	95% CI	β*	95% CI
Trimester 1							
<16.4	267	Referent	-	Referent	-	Referent	-
16.4 – 21.2	267	1.23	-20.0 to 22.4	-47.8	-137 to 41.3	-.081	-.283 to .121
>21.2	267	-12.9	-34.1 to 8.30	-39.8	-129 to 49.2	.151	-.051 to .353
Continuous coding**	801	-25.1	-43.7 to -6.49	-66.3	-145 to 11.9	.236	.058 to .414
Trimester 2							
<16.7	266	Referent	-	Referent	-	Referent	-
16.7 – 21.1	265	6.88	-14.3 to 28.1	-5.75	-95.1 to 83.6	-.071	-.274 to .131
>21.1	265	-14.6	-35.8 to 6.57	-38.7	-128 to 50.6	.152	-.051 to .354
Continuous coding**	796	-24.0	-43.9 to -4.13	-75.9	-160 to 7.93	.198	.008 to .388
Trimester 3							
<16.3	263	Referent	-	Referent	-	Referent	-
16.3 – 20.7	263	9.24	-11.9 to 30.4	59.6	-28.5 to 148	.024	-.179 to .227
>20.7	262	-17.8	-39.0 to 3.33	-11.9	-100 to 76.2	.246	.043 to .449
Continuous coding**	788	-26.4	-45.4 to -7.42	-42.0	-121 to 37.1	.291	.109 to .473
Pregnancy							
<16.4	263	Referent	-	Referent	-	Referent	-
16.4 – 21.2	263	.688	-20.6 to 22.0	-44.4	-134 to 45.3	-.115	-.318 to .089
>21.2	263	-20.5	-41.8 to .847	-74.3	-164 to 15.4	.137	-.067 to .340
Continuous coding**	789	-29.2	-49.7 to -8.71	-76.8	-163 to 9.46	.267	.071 to .462

*Excluding subjects with missing information on placental weight, birth weight, parity, maternal education, maternal height and weight, smoking, mode of delivery as well as those who gave birth by assisted delivery and missing information on exposure or less than 75% daily value of exposure **Parameters are reported for an increase by 10-$\mu g/m^3$ of PM_{10}

Table 8: Adjusted association between PM_{10} and placental weight and feto-placental weight ratio in all trimesters of pregnancy and the whole pregnancy

		Placental weight			Birth weight			Feto-placental weight ratio (FPR)		
PM_{10} exposure window (µg/m^3)	N	β* (g)	95% CI	*p*-trend	β* (g)	95% CI	*p*-trend	β*	95% CI	*p*-trend
Trimester 1										
<16.4	267	Referent	-		Referent	-		Referent	-	
16.4 – 21.2	267	-3.98	-24.6 to 16.6	.076	-41.5	-110 to 27.3	.277	.006	-.196 to .209	.038
>21.2	267	-17.9	-38.4 to 2.47		-40.9	-109 to 27.1		.202	.001 to .402	
Continuous coding**	801	-21.8	-39.6 to -3.90		-24.2	-83.9 to 35.5		.276	.101 to .451	
Trimester 2										
<16.7	266	Referent	-		Referent	-		Referent	-	
16.7 – 21.1	265	8.82	-11.8 to 29.5	.138	3.06	-65.9 to 72.0	.416	-.083	-.285 to .120	.085
>21.1	265	-14.1	-34.7 to 6.53		-27.0	-95.8 to 41.9		.162	-.040 to .365	
Continuous coding**	796	-17.3	-36.9 to 2.28		-25.6	-90.9 to 39.7		.213	.021 to .405	
Trimester 3										
<16.3	263	Referent	-		Referent	-		Referent	-	
16.3 – 20.7	263	11.5	-9.60 to 32.5	.029	39.1	-31.1 to 109	.234	-.055	-.262 to .152	.022
>20.7	262	-18.6	-39.8 to 2.58		-29.8	-101 to 41.0		.207	-.001 to .415	
Continuous coding**	788	-26.5	-45.7 to -7.39		-39.3	-103 to 24.6		.281	.094 to .469	
Pregnancy										
<16.4	263	Referent	-		Referent	-		Referent	-	
16.4 – 21.2	263	7.83	-12.5 to 28.2	.032	10.9	-57.0 to 78.7	.190	-.091	-.291 to .108	.030
>21.2	263	-17.3	-38.1 to 3.54		-36.8	-106 to 32.5		.166	-.038 to .370	
Continuous coding**	789	-24.3	-44.4 to -4.30		-32.8	-99.6 to 33.9		.287	.091 to .483	

*PM_{10} estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period.
**Parameters are reported for an increase by 10-µg/m^3 of PM_{10}

3.5. Cigarette smoking and placental and birth weights

Figure 5 shows the weight changes of the placenta and the fetus at birth due to exposure of tobacco smoke, one of the adjustment factors used in the regression model. The weight changes reported here were for the same population as for the analysis on atmospheric pollutants. An increase of exposure to tobacco smoking by 1 cigarette/day corresponded with a birth weight decrease of 24 g at all trimesters as well as the whole pregnancy (*p*-value <0.01). The corresponding placental weight decrements were much lower, with a 2 g decrease at all trimesters (*p*-values 0.2) (Figure 5).

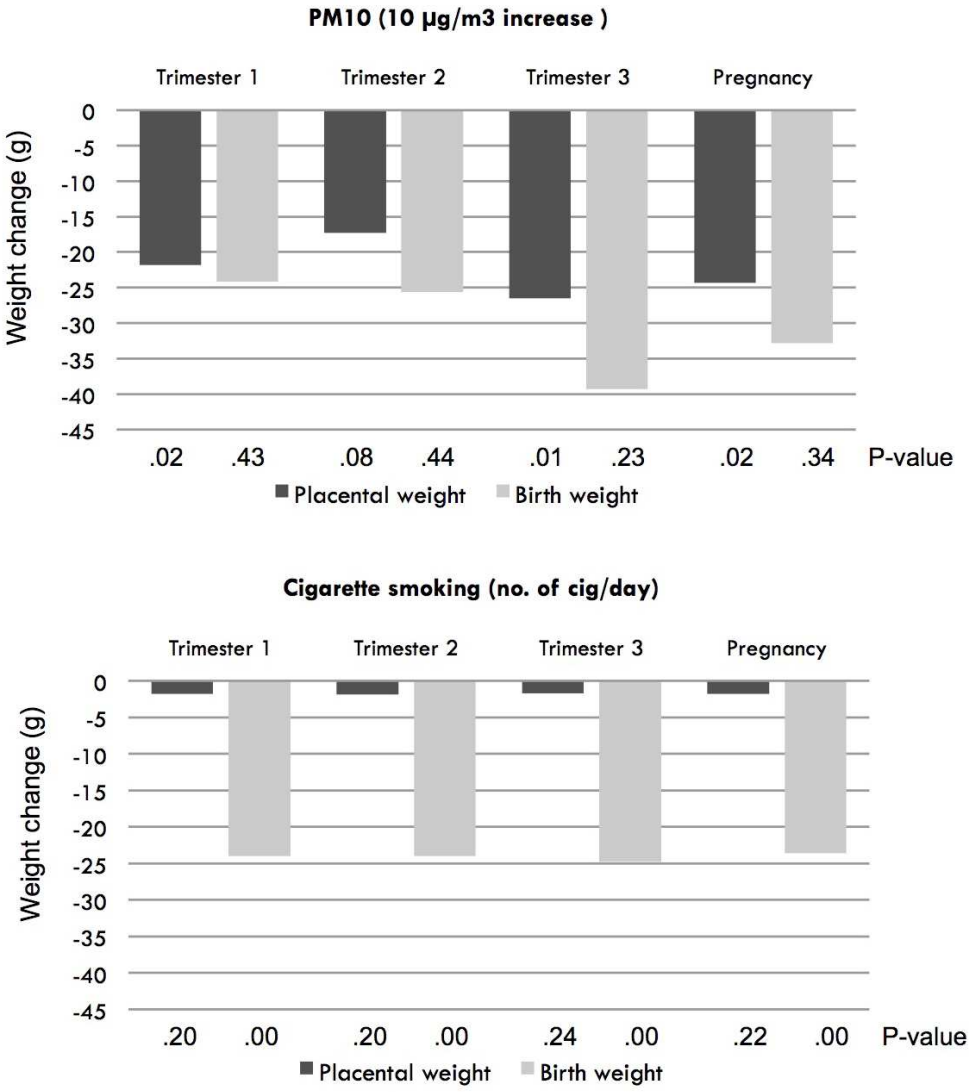

Figure 5: Adjusted effects of PM_{10} (increase by 10 µg/m^3) and maternal cigarette smoking (for an increase by 1 cig/day) on placental and fetal weight (n = 801)

3.6. Sensitivity analyses

In order to discuss possible selection bias due to missing data on placental weight, we repeated the analyses of associations between NO_2 and PM_{10} with birth weight including newborns with missing information on placental weight adjusted for the same adjustment factors, the estimated birth weight change for exposure to NO_2 in the first trimester corresponded to a decrease of 40 g and 22 g in the intermediate and highest tertiles, respectively, compared to the lowest tertile (Table 9). Overall, although associations with NO_2 were quite similar in both populations, the impact of NO_2 exposure tended to be stronger when observations with missing placental weight were excluded.

We observed a similar pattern of associations between birth weight and exposure to PM_{10} when including newborns with missing information on placental weight (Table 10). In the highest tertiles of exposure to PM_{10}, the trimester-specific birth weight decrease were 62 g in the first (95%CI -117 to -8), by 41 g in the second (95%CI -96 to 5) and by 56 g in the third trimester (95%CI -111 to 0.0) while in the whole pregnancy the decrease was 35 g (95%CI -90 to 14) when observations with missing placental weight were included. Overall, therefore, the selected population (with available placental weight information) did not seem to be more sensitive to PM_{10} exposure (at least in terms of effects on birth weight) than the whole EDEN population.

Table 9: Adjusted association between NO_2 and birth weight, for the whole EDEN population with exposure data, and restricted to those with placental weight data

		Birth weight, total			Birth weight, excluding missing placental weight	
NO_2 exposure window (µg/m^3)	N	β* (g)	95% CI	N	β* (g)	95% CI
Trimester 1						
1st tertile	354	Referent		267	Referent	-
2nd tertile	354	-39.5	-94.2 to 15.2	267	-42.3	-112 to 27.4
3rd tertile	354	-21.8	-78.1 to 34.4	267	-37.4	-109 to 34.4
Continuous coding**	1062	-7.23	-34.2 to 19.7	801	-7.89	-42.3 to 26.5
Trimester 2						
1st tertile	352	Referent		266	Referent	-
2nd tertile	352	21.7	-35.1 to 78.5	265	42.2	-30.5 to 115
3rd tertile	352	15.5	-44.4 to 75.3	265	25.4	-50.9 to 102
Continuous coding**	1056	.861	-25.8 to 27.5	796	.027	-34.5 to 34.5
Trimester 3						
1st tertile	347	Referent		263	Referent	-
2nd tertile	347	20.4	-36.7 to 77.4	263	23.5	-48.8 to 95.8
3rd tertile	347	-.684	-62.6 to 61.2	262	14.3	-63.6 to 92.2
Continuous coding**	1041	-7.59	-35.0 to 19.8	788	-12.9	-48.1 to 22.3
Pregnancy						
1st tertile	349	Referent		263	Referent	-
2nd tertile	348	26.8	-27.5 to 81.0	263	20.5	-48.0 to 88.9
3rd tertile	348	-3.10	-58.3 to 52.1	263	-27.9	-97.4 to 41.7
Continuous coding**	1045	-3.42	-31.6 to 24.7	789	-6.40	-42.9 to 30.1

*NO_2 estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period.
**Parameters are reported for an increase by 10-µg/m^3 of NO_2

Table 10: Adjusted association between PM_{10} and birth weight, for the whole EDEN population with exposure data, and restricted to those with placental weight data

		Birth weight, total			Birth weight, excluding missing placental weight	
PM_{10} exposure window (µg/m^3)	N	β* (g)	95% CI	N	β* (g)	95% CI
Trimester 1						
<16.4	354	Referent		267	Referent	-
16.4 – 21.2	354	-31.2	-85.2 to 22.9	267	-41.5	-110 to 27.3
>21.2	354	-62.3	-117 to -7.96	267	-40.9	-109 to 27.1
Continuous coding**	1062	-41.1	-88.0 to 5.87	801	-24.2	-83.9 to 35.5
Trimester 2						
<16.7	352	Referent		266	Referent	-
16.7 – 21.1	352	-56.1	-110 to -1.73	265	3.06	-65.9 to 72.0
>21.1	352	-40.9	-95.8 to 14.0	265	-27.0	-95.8 to 41.9
Continuous coding**	1056	-27.0	-77.6 to 23.6	796	-25.6	-90.9 to 39.7
Trimester 3						
<16.3	347	Referent		263	Referent	-
16.3 – 20.7	347	18.1	-37.1 to 73.3	263	39.1	-31.1 to 109
>20.7	347	-55.6	-111 to -.005	262	-29.8	-101 to 41.0
Continuous coding**	1041	-45.5	-96.5 to 5.46	788	-39.3	-103 to 24.6
Pregnancy						
<16.4	349	Referent		263	Referent	-
16.4 – 21.2	348	8.14	-45.7 to 62.0	263	10.9	-57.0 to 78.7
>21.2	348	-34.7	-89.5 to 20.0	263	-36.8	-106 to 32.5
Continuous coding**	1045	-38.2	-90.5 to 14.1	789	-32.8	-99.6 to 33.9

*PM_{10} estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period.
**Parameters are reported for an increase by 10-µg/m^3 of PM_{10}

The associations with NO_2 as well as PM_{10} remained similar when analyses were repeated excluding one infant with an extreme value of placental weight (1600 g); the birth weight of this newborn was 5260 g (data in tables of Appendix 2).

Analyses were repeated for the continuous values for a 10 µg/m^3 increase of NO_2 and PM_{10} stratified by centre (Table 11). A part for PM_{10} third trimester exposure where parameters quantifying the association between exposure and placental weight were negative in both cities, deleterious associations with placental weight were only observed in Nancy, where air pollution exposure is higher compared to Poitiers.

Table 11: Adjusted association between NO2 and PM_{10} (10 µg/m^3 increase) and placental weight, birth weight and FPR, stratified by centre

Exposure window*	Poitiers			Nancy		
	N	β** (g)	95% CI	N	β** (g)	95% CI
NO_2						
Placental weight						
Trimester 1	446	10.1	-9.70 to 29.9	355	-10.8	-26.5 to 4.81
Trimester 2	442	20.9	-.456 to 42.3	354	-10.3	-25.8 to 5.27
Trimester 3	433	15.9	-7.22 to 38.9	355	-23.2	-38.8 to -7.53
Pregnancy	435	17.8	-5.61 to 41.3	354	-15.3	-31.8 to 1.18
Birth weight						
Trimester 1	446	-10.4	-78.2 to 57.4	355	1.64	-49.6 to 52.9
Trimester 2	442	34.8	-38.5 to 108	354	2.53	-48.5 to 53.5
Trimester 3	433	69.6	-8.70 to 148	355	-36.1	-87.9 to 15.7
Pregnancy	435	33.0	-46.7 to 113	354	-8.07	-62.3 to 46.2
FPR						
Trimester 1	446	-.180	-.366 to .067	355	.157	-.002 to .316
Trimester 2	442	-.222	-.424 to -.020	354	.181	.024 to .338
Trimester 3	433	-.117	-.335 to .101	355	.277	.119 to .434
Pregnancy	435	-.204	-.425 to .017	354	.223	.056 to .389
PM_{10}						
Placental weight						
Trimester 1	446	9.72	-58.7 to 78.1	355	-33.9	-69.7 to 1.89
Trimester 2	442	71.9	1.07 to 143	354	-33.3	-70.5 to 4.03
Trimester 3	433	-15.1	-85.3 to 55.1	355	-42.8	-78.3 to -7.42
Pregnancy	435	62.8	-53.6 to 179	354	-44.6	-84.8 to -4.31
Birth weight						
Trimester 1	446	-146	-380 to 87.1	355	8.40	-109 to 126
Trimester 2	442	-2.28	-245 to 241	354	-6.94	-130 to 116
Trimester 3	433	94.1	-144 to 332	355	-75.3	-192 to 41.7
Pregnancy	435	-47.5	-443 to 348	354	-22.0	-155 to 111
FPR						
Trimester 1	446	-.401	-1.05 to .244	355	.476	.113 to .839
Trimester 2	442	-.803	-1.47 to -.134	354	.422	.044 to .800
Trimester 3	433	.190	-.473 to .853	355	.414	.055 to .774
Pregnancy	435	-.964	-2.06 to .131	354	.553	.145 to .960

*Parameters are reported for an increase by 10-µg/m^3 of NO_2 and PM_{10} **NO_2 and PM_{10} estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60 kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period.

4. Discussion

In a mother-child cohort recruited during pregnancy, air pollution (PM_{10}) exposure was associated with placental weight and birth weight decrease. The placental weight and birth weight decrease had approximately the same absolute amplitude (20 g for a 10 $\mu g/m^3$ increase of PM_{10}). Given the much lower weight of the placenta, this suggests that the placenta is in terms of weight relatively more affected than the fetus by air pollution. Accordingly, the feto-placental weight ratio increased with air pollution exposure.

To our knowledge, this study is one of the first to use placental weight as an outcome of interest for exposure in relation to air pollutants. For this reason, our results can only be compared to experimental studies in animals. Human placenta is quite different from that of rodents; thus comparison should be done cautiously.

There are few animal studies using placental measures in relation to environmental exposures. In one study in mice, exposure to traffic-related air pollution were associated with a decrease of maternal circulatory volume and an increase of fetal circulatory space area in the placenta, as well as decreases in fetal weight [39]. Another mouse study found placental structural and functional disruption due to *intravenous* injected engineered nanoparticles (silica and titanium dioxide) [61]. These studies, albeit showing an adverse effect of environmental pollutants to the placenta, did not study the changes of placental weight.

The assessment of air pollution exposure effects on placental weight and feto-placental weight ratio is the main originality of our study. We also used dispersion models to estimate air pollution exposure, which has so far seldom been done in epidemiological studies of atmospheric pollutants on pregnancy outcomes [62]. The main limitation of this study is generalisability due to the many missing information of placental weight in the cohort in Nancy centre, even though the sensitivity analyses showed no evidence for selection bias for PM_{10} effects on pregnancy outcomes. The other limitation is potential exposure misclassification due to the use of home address to assess air pollution exposure levels, which does not take into account the time spent outside of residence. We took into account changes of home address during pregnancy, which has seldom been done in previous studies.

4.1. Placental weight and smoking

Although our focus was on the placental weight and birth weight changes in association with exposure to PM_{10}, we also reported the changes associated with smoking. One study found that intrauterine exposure to tobacco smoke disrupts placental biological functions, including placental development by inhibiting cytotrophoblast proliferation [63]. Another study investigating the association between placental growth hormone (PGH) and maternal smoking found that in the binary coding (smokers/non-smokers) the average

placental weight was somewhat lower among smoking mothers (average placental weight ± SD: 520 g ± 80) than non-smoking mothers (570 g ± 80); those are non-adjusted values [64]. Overall, very few epidemiologic studies described smoking effects on placental weight. Our results yielded that compared with the changes of placental weight and birth weight associated with PM_{10} exposure, which are of somewhat similar amplitude; smoking has a much larger effect on birth weight than placental weight.

4.2. Possible implications

Several studies found that suboptimal weight at birth was associated with diseases later in life, such as cardiovascular diseases and cardiovascular mortality [25, 65]. In a cohort study in Norway, of people born between 1934 and 1959, a bigger placental weight relative to birth weight was associated with increased cardiovascular mortality [66]. A heavier placenta may be due to trophoblast thickening, which in turn will decrease its circulatory efficiency [67] and may have an adverse effect on the fetal cardiovascular function.

Another study showed that higher placental weight was associated with some diseases such as hydrops fetalis, amniotic fluid infection, maternal diabetes, and maternal anemia; whereas lower placental weight may be associated with uteroplacental hypoperfusion due to preeclampsia or karyotype abnormality [68].

Fetal weight at birth is a reflection of intrauterine growth [69]. Atmospheric pollutants may have an effect at different time points in pregnancy; ultrasonographic study showed that exposures to ambient air pollution at early pregnancy slowed down fetal growth [70]. In our study, the exposures to PM_{10} in the first and third trimesters of pregnancy, and exposure to NO_2 in the third trimester, were significantly associated with a decrease in placental weight, whereas the association with birth weight change was not statistically significant. This may imply that placental weight change is a more sensitive indicator than birth weight in association with exposure to certain air pollutants. However, our sample size was limited so that the uncertainties in the effect estimates on birth weight and placental weight were large.

4.3. Possible biological mechanisms

There are few information on the basic mechanisms of placental insufficiency and its relationship with placental growth and fetal growth [71]. Vascular structure is the main gross anatomy of the placenta, due to its main function as a communication site between fetal and maternal blood circulation [67]. Several studies investigated the circulatory role of the placenta in fetal growth restriction [71] and adverse cardiovascular function [66]. A study using umbilical arterial Doppler velocimetry found that severe fetal growth restriction associated with abnormal umbilical arterial Doppler velocimetry were associated with a steeper relationship between lower placental weights and decreases in fetal weights,

whereas lower placental weight in normal pregnancy did not show a significant decrease in fetal weights [27]. A recent study showed a potential association between air pollution and maternal cardiovascular health during pregnancy [72], which may interfere with placental growth and function.

Placental weight alone may not explain the whole placental functional efficiency. Another study had explored the role of other placental measurements at birth such as the shape, diameters, or disk thickness as potential useful indicators of placental sufficiency [31], but no study described their association with environmental factors.

The larger and smaller placental diameters have higher correlation with placental weight compared with other placental measures (disc shape, distance from cord insertion to nearest margin, placental disc thickness and umbilical cord length) [31]; placental weight decreases with an increase of placental ellipsivity (ratio of larger and smaller diameters) [35]. Placental diameters represent the perfusion surface area of the placenta [35], and a reduction in placental weight is likely due to the interplay of substances in air pollution that disrupts the perfusion. A toxicity study showed that the placenta may respond to environmental pollutants by releasing xenobiotic-metabolizing enzymes [73], although its effects on placental growth are still unclear. Air pollution had been shown to affect placental circulatory volume in mice; *i.e.* reduced maternal blood space volume and diameter in the placenta of pregnant mice exposed to ambient air pollution (non-filtered air) accompanied by a decrease in trophoblast thickness [39]. If exposure to air pollution caused a similar maternal blood flow disruption in humans, one may expect to see a decrease in placental weight.

Studies on intrauterine stress conclude that there may be a different level of resilience to gestational stressors between male and female infants. Female infants are more responsive to chorionic disk thickness changes, and it was hypothesized that hormones may play a bigger role in male fetal growth compared to female [24]. A study on mice suggested that exposure to air pollution may interfere with sex ratio [74]. Hence, analyses stratified on infant sex are worth considering, which we intend to do in the future.

4.4. Study population

A selection of study participants with different exposure and outcome values than in the eligible population may distort the association between exposure and outcome [75]. In this study, the number of missing information on placental weight within Nancy population of the EDEN cohort was high, raising a potential for selection bias. Adjustments for a number of potential confounders have been attempted to reduce the possibility of biased result [76], although adjustment may not be fully efficient at correcting selection bias, in particular if selection is due to unidentified factors. Further, on the sensitivity analyses we repeated the

analyses for the association between NO_2 and PM_{10} and birth weight, including the observations with missing placental weight (Table 9 and Table 10). Qualitatively we found that our estimate of the association between NO_2 and PM_{10} exposure and placental weight in our study was not strongly affected by selection bias. More precisely, in our study the subjects with non-missing placental weight were not specific in terms of association between NO_2 and PM_{10} and birth weight; therefore, we hypothesize that this was also the case for the association between exposures and placental weight. However, our analyses stratified by center indicated that associations possibly differed by center; this difference is difficult to attribute to a specific origin, but we cannot exclude the existence of selection bias explaining the association observed in Nancy area.

4.5. Exposure assessment

Different modelling techniques have been used to estimate air pollution levels in reproductive epidemiology [8, 77]. The main techniques are deterministic (physical) modelling based on the physical, chemical, and/or biological mechanisms known of the associations; and stochastic (statistical) modelling, also called the land use regression (LUR) model [78]. These models are not exclusive and may be used in conjunction to optimize exposure indices [78].

For epidemiologic studies of air pollution and reproductive health in particular, spatial and temporal resolution as well as the ability to take into account time-activity patterns are important features of an exposure model [79]. The women's home address during pregnancy and the timing of relevant periods of pregnancy need to be taken into account [80].

Dispersion method rely on the principles of physical and chemical processes regulating the spatial and temporal variability of a pollutant including the description of sources and meteorological conditions [81]. The dispersion modelling in EDEN cohort used ADMS-URBAN that took into account the meteorological information, temporal factors of emission and background levels of atmospheric pollution in the estimation, combined with the residential address of pregnant women [82]. Separate validation analyses of the exposure model showed that errors on exposures were smaller in a longer period of observation (the whole pregnancy) compared to shorter periods (one trimester) [82]. One comparison study found that when using tertile categorization of the air pollution data, dispersion modelling and LUR yielded similar estimation of association between air pollution and health effects [77]. However, our exposure model did not take into account time-activity patterns and residential mobility during pregnancy, which may influence exposure classification [18].

5. Conclusion

We found an association between maternal exposures to NO_2 and PM_{10} during pregnancy and placental weight and feto-placental weight ratio. Associations were mostly driven by Nancy area, where exposure levels were higher but where some potential for selection bias also existed. Associations were stronger for first trimester and whole pregnancy exposure windows. The association with placental weight were of similar amplitude than the association with birth weight, which, due to a much larger weight of the fetus, may imply a greater sensitivity of the placenta to air pollution. NO_2 is a marker of traffic-related air pollutants and PM_{10} consists of many substances, hence the observed association might be attributable not solely to NO_2 and PM_{10} but rather to a mixture of atmospheric pollutants. We also found that the effect of air pollution on the placenta and the fetus is different than the effects of active cigarette smoking, which suggests that placental response to various chemical substances might be different.

The finding of association between air pollution with placental weight is a first step forward in understanding how environmental pollutants affect the feto-placental unit. Nevertheless, there are still gaps in the knowledge on the mechanisms whereby air pollution affects the placenta. Future research may need to focus on investigating the effects of air pollution on other (finer) placental measures. As in other studies of air pollution and pregnancy outcomes, time-activity patterns during pregnancy should be taken into account to improve exposure classification.

Air pollution is something that a large portion, if not all, of the population are exposed to. Even if air pollution had a small impact on birth weight at the individual level, this widespread exposure might imply a large health burden at the population level. Regulations of pollution level should take into account the effects on early life, besides health benefits such as avoiding premature mortality due to environmental pollution-related diseases.

6. References

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7. Appendices

Appendix 1

Table 1.1 The association between smoking and placental weight

Smoking	N	Feto-placental weight ratio (FPR)		
		β*	95% CI	P-value
First trimester				
Continuous	1380	.455	-.857 to 1.767	.496
Binary (smokers vs non-smokers)	1380	4.34	-9.61 to 18.3	.542
Categorical	1375			.531
0		Referent	-	-
1-9		1.69	-15.2 to 18.6	.844
10+		6.57	-13.5 to 26.6	.520
Second trimester				
Continuous	1380	.154	-2.15 to 1.84	.879
Binary (smokers vs non-smokers)	1380	1.01	-15.1 to 17.1	.902
Categorical	1370			.902
0		Referent	-	-
1-9		1.62	-16.8 to 20.0	.863
10+		.506	-28.1 to 29.1	.972
Third trimester				
Continuous	1380	.199	-1.58 to 1.98	.826
Binary (smokers vs non-smokers)	1380	6.17	-10.0 to 22.4	.455
Categorical	1348			.826
0		Referent	-	-
1-9		5.36	-13.7 to 24.5	.582
10+		-1.58	-30.6 to 27.4	.915

*Adjusted for gestational duration (continuous and quadratic terms), infant sex, parity (categorical), maternal age at end of education (categorical), maternal height, and maternal pre-pregnancy weight as a broken stick model with a knot at 60kg

Table 1.2 The association between smoking and FPR

Smoking	N	Feto-placental weight ratio (FPR)		
		β*	95% CI	P-value
First trimester				
Continuous	1377	.017	-.030 to -4.32x10^{-3}	.009
Binary (smokers vs non-smokers)	1377	-.215	-.353 to -.077	.002
Categorical	1372			.002
0		Referent	-	-
1-9		-.159	-.325 to 7.67x10^{-3}	.062
10+		-.282	-.478 to -.085	.005
Second trimester				
Continuous	1377	-.035	-.055 to -.016	.000
Binary (smokers vs non-smokers)	1377	-.284	-.442 to -.125	.000
Categorical	1367			.000
0		Referent	-	-
1-9		-.203	-.383 to -.022	.028
10+		-.507	-.788 to -.227	.000
Third trimester				
Continuous	1377	-.026	-.043 to -7.92x10^{-3}	.004
Binary (smokers vs non-smokers)	1377	-.304	-.463 to -.144	.000
Categorical	1348			.000
0		Referent	-	-
1-9		-.254	-.442 to -.065	.008
10+		-.410	-.697 to -.124	.005

*Adjusted for gestational duration (continuous and quadratic terms), infant sex, parity (categorical), maternal age at end of education (categorical), maternal height, and maternal pre-pregnancy weight as a broken stick model with a knot at 60kg

Appendix 2

Table 2.1 Adjusted association between an increase of NO_2 and placental weight, birth weight and feto-placental weight ratio excluding observation with extreme value of placental weight

		Placental weight		Birth weight		Feto-placental weight ratio (FPR)	
NO_2 exposure window (µg/m^3)	N	β* (g)	95% CI	β* (g)	95% CI	β*	95% CI
Trimester 1							
<15.0	267	Referent	-	Referent	-	Referent	-
15.0 – 22.4	267	8.46	-11.4 to 28.3	-36.7	-106 to 32.2	-.167	-.371 to .037
>22.4	266	-14.8	-35.2 to 5.65	-31.3	-102 to 39.8	.145	-.066 to .355
Continuous coding**	800	-5.96	-15.8 to 3.86	-6.09	-40.1 to 28.0	.082	-.019 to .183
Trimester 2							
<15.1	265	Referent	-	Referent	-	Referent	-
15.1 – 22.4	265	7.89	-12.8 to 28.6	44.6	-27.2 to 116	.011	-.202 to .225
>22.4	265	4.35	-17.4 to 26.1	28.1	-47.4 to 104	.036	-.188 to .261
Continuous coding**	795	-4.27	-14.1 to 5.58	1.54	-32.6 to 35.7	.090	-.011 to .191
Trimester 3							
<14.0	263	Referent	-	Referent	-	Referent	-
14.0 – 22.2	262	2.65	-18.0 to 23.3	26.7	-44.7 to 98.1	.009	-.203 to .221
>22.2	262	-8.24	-30.5 to 14.0	18.1	-58.8 to 95.0	.179	-.049 to .407
Continuous coding**	787	-11.7	-21.8 to -1.69	-10.3	-45.1 to 24.6	.164	.061 to .267
Pregnancy							
<15.4	263	Referent	-	Referent	-	Referent	-
15.4 – 21.5	263	4.21	-15.4 to 23.8	27.8	-39.8 to 95.5	-.003	-.204 to .198
>21.5	262	-3.49	-23.4 to 16.4	-23.1	-92.0 to 45.7	.032	-.173 to .237
Continuous coding**	788	-7.68	-18.1 to 2.76	-4.24	-40.4 to 31.9	.121	.014 to .228

*NO_2 estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period. **A 10-µg/m^3 increase of NO_2 is used for the continuous coding

Table 2.2 Adjusted association between an increase of PM_{10} with placental weight and feto-placental weight ratio in all trimesters of pregnancy and the whole pregnancy excluding infant with placental weight 1600 g

		Placental weight		Birth weight		Feto-placental weight ratio (FPR)	
PM_{10} exposure window (µg/m^3)	N	β* (g)	95% CI	β* (g)	95% CI	β*	95% CI
Trimester 1							
<16.4	267	Referent	-	Referent	-	Referent	-
16.4 – 21.2	267	-7.92	-27.5 to 11.7	-47.7	-116 to 20.2	.026	-.176 to .227
>21.2	266	-17.2	-36.6 to 2.22	-39.8	-107 to 27.5	.194	.006 to .394
Continuous coding**	800	-21.1	-38.1 to -4.10	-23.1	-82.2 to 36.0	.274	.099 to .449
Trimester 2							
<16.7	265	Referent	-	Referent	-	Referent	-
16.7 – 21.1	265	12.3	-7.28 to 31.9	8.75	-59.5 to 77.0	-.093	-.295 to .109
>21.1	265	-11.1	-30.7 to 8.48	-22.1	-90.2 to 46.0	.153	-.049 to .355
Continuous coding**	795	-15.5	-34.1 to 3.16	-22.6	-87.2 to 42.0	.207	.016 to .399
Trimester 3							
<16.3	263	Referent	-	Referent	-	Referent	-
16.3 – 20.7	262	14.5	-5.44 to 34.5	38.6	-30.8 to 108	-.075	-.280 to .131
>20.7	262	-15.8	-35.9 to 4.35	-28.1	-97.9 to 41.8	.193	-.014 to .400
Continuous coding**	787	-22.8	-41.0 to -4.59	-33.1	-96.4 to 30.1	.270	.083 to .457
Pregnancy							
<16.4	263	Referent	-	Referent	-	Referent	-
16.4 – 21.2	263	11.1	-8.22 to 30.5	15.3	-51.8 to 82.4	-.101	-.300 to .097
>21.2	262	-14.4	-34.2 to 5.33	-34.4	-103 to 34.2	.132	-.047 to .360
Continuous coding**	788	-22.1	-41.2 to -3.09	-29.2	-95.2 to 36.8	.280	.085 to .476

*PM_{10} estimated effect adjusted for gestational duration (linear and quadratic terms), infant sex, center (Poitiers or Nancy), maternal characteristics (height, pre-pregnancy weight as a broken stick model with a knot at 60kg [49], maternal age at the end of education, parity), smoking at second trimester and season of last menstrual period. **A 10-µg/m^3 increase of PM_{10} is used for the continuous coding

Abstract

Association between pregnancy exposure to atmospheric pollutants and placental weight

BACKGROUND: Epidemiologic studies suggest an association between air pollution exposure and fetal growth. The possible biological mechanisms of this effect have little been studied. Animal studies suggested an impact of atmospheric pollutants on placental function, a mechanism so far not considered in humans.

OBJECTIVES: Investigating the association of exposure to nitrogen dioxide (NO_2) and particulate matter with diameter <10 µm (PM_{10}) during pregnancy with placental weight, birth weight and the feto-placental weight ratio (FPR).

METHODS: Among women and infants from the EDEN (Etudes des Déterminants pré et postnatals précoces du développement et de la santé de l'ENfant) mother-child cohort conducted in Poitiers and Nancy, France, NO_2 and PM_{10} levels were assessed using ADMS-Urban dispersion model. We applied multiple linear regressions to characterize the association between levels of NO_2 and PM_{10} with placental weight, birth weight, and FPR, after adjustment for maternal smoking, education, and other potential confounders.

RESULTS: A 10 µg/m^3 increase of PM_{10} in the first trimester of pregnancy was associated with a decrease of placental weight by 22 g (95% confidence interval, CI, -40 to -4), a decrease of birth weight by 24 g (95%CI -84 to 36) and an increase of FPR by .28 (95%CI .1 to .45). Associations were similar for second and third trimester exposure windows, as well as in the whole pregnancy. In NO2, we observed a similar pattern of associations even though the associations were weaker.

CONCLUSION: Exposures to PM_{10} and, to a lesser extent, to NO_2, were associated with decreases of placental weight and birth weight as well as increases in FPR. Associations were stronger for first trimester and whole pregnancy exposure windows. Placental weight and birth weight decrements associated with air pollution exposure had the same amplitude, which, given the much lower weight of the placenta, suggests that the placenta is in terms of weight relatively more affected than the fetus by air pollution. This study is to our knowledge the first to suggest an effect of air pollution on placental weight in humans.

KEYWORDS: air pollution, NO_2, PM_{10}, placental weight, pregnancy, feto-placental weight ratio, fetal growth

Résumé

Exposition intra-utérine aux polluants atmosphériques et poids placentaire

CONTEXTE : Des études épidémiologiques ont indiqué une association entre l'exposition à la pollution atmosphérique durant la grossesse et la croissance fœtale. Les mécanismes biologiques qui pourraient expliquer un tel effet ont été peu étudiés. Un petit nombre d'expérimentations animales ont rapporté un impact des polluants atmosphériques sur la fonction placentaire, mais un tel mécanisme n'a pas été examiné chez humain jusqu'à présent.

OBJECTIFS : Cette étude vise à examiner l'association entre l'exposition au dioxyde d'azote (NO_2) et aux particules en suspension qui ont <10 µm de diamètre (PM_{10}) pendant la grossesse et le poids du placenta, le poids du nouveau-né et le ratio fœtal-placentaire (rapport poids fœtal/poids placentaire, ou FPR).

METHODES : Parmi les femmes et les nourrissons de la cohorte mère-enfant EDEN qui a été menée à Poitiers et Nancy (France), les niveaux de NO_2 et PM_{10} ont été estimés au moyen du modèle de dispersion ADMS-Urban. On a appliqué une régression linéaire multiple pour caractériser l'association entre les niveaux de NO_2 ou PM_{10} et le poids du placenta, le poids du naissance et FPR, après ajustement sur le tabagisme maternel, le niveau de l'étude et les autres facteurs de confusion potentiels.

RESULTATS : Une augmentation de 10 µg/m^3 du niveau de PM_{10} durant le premier trimestre de la grossesse était associé à une baisse de poids du placenta de 22 g (intervalle de confiance, IC, à 95% -40 à -4 g), à une baisse de poids du nouveau-né de 24 g (IC 95% -84 à 36) et à une augmentation du FPR de 0,28 (IC 95% 0,10 à 0,45). Les associations étaient similaires avec l'exposition durant le deuxième et le troisième trimestre, ainsi que durant la grossesse entière. Pour le NO_2, l'association la plus nette était observée avec l'exposition durant le troisième trimestre de grossesse.

CONCLUSION : Les expositions aux NO_2 et PM_{10} étaient associées à une diminution du poids du placenta et du poids du nouveau-né, et à une augmentation du FPR. Les baisses de poids du placenta et du poids de la naissance associées à l'exposition avaient la même amplitude, ce qui, étant donné que le poids du placenta est beaucoup plus faible, semble indiquer que le placenta, en matière du poids, est relativement plus affecté par la pollution de l'air que le fœtus. Cette étude, à notre connaissance, est la première à suggérer un effet de la pollution de l'air sur le poids du placenta chez l'humain, ce qui constitue un mécanisme par lequel la pollution pourrait altérer la croissance fœtale.

MOT-CLEF: pollution a la l'air, NO_2, PM_{10}, poids du placenta, grossesse, ratio fœtaux-placentaire, croissance fœtale

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10 March 2011

Annisa Rahmalia
ScHARR

Dear Annisa

Analysis of association between nitrogen dioxide exposure and pregnancy outcomes using an exposure model with a final spatial resolution

I am pleased to inform you your supervisor has reviewed your project and classed it as 'low risk' so you can proceed with your research. The research must be conducted within the requirements of the hosting/employing organisation or the organisation where the research is being undertaken.

I have received a hard copy of your student declaration together with your Supervisor's confirmation for research that does not involve human participants and that you will be undertaking research which involves analysis of already existing data ('secondary data').

Yours sincerely

Cheryl Oliver
Ethics Committee Administrator

Cc: Dr Remy Slama