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The moderating and mediating role of the placenta in the association between prenatal exposure to air pollutants and birth weight: A twin study

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ABSTRACT

Prenatal exposure to air pollution has been linked to lower birth weight, yet the role of the placenta in this association is often overlooked. This study investigates whether placental characteristics act as moderators or mediators in the association between prenatal exposure to particulate matter (PM_{10}) and nitrogen dioxide (NO_2) and birth weight in twins.

The study included 3340 twins (born 2002–2013) from the East Flanders Prospective Twin Survey. Prenatal exposure was estimated using spatial-temporal interpolation for the whole pregnancy and its trimesters. Moderation (interaction) and mediation (direct, indirect, and total effects) of placental weight and umbilical cord insertion were assessed with analyses stratified a priori based on the presence of one or two placentas. Sensitivity analyses included stratification by prematurity.

Placental weight acted as both a moderator and mediator. Moderation analysis: in twins with low placental weight, birth weight decreased with −93.18 g per 10 μg/m^3 PM_{10} (95% CI: −164.17, −22.19) and −69.28 g per 10 μg/m^3 NO_2 (95% CI: −124.81, −13.76) for the whole pregnancy. Mediation analysis showed positive indirect effects of placental weight indicating an increase of 36.05 g (95% CI: 0.88, 70.61) birth weight per 10 μg/m^3 PM_{10} over the whole pregnancy, particularly in twins born <35 weeks. Direct effects were mainly negative, but not significant. For NO_2, significant negative indirect effects were observed in the third trimester. Moderation was most pronounced in the second half of pregnancy, while both moderation and mediation were more evident in twins with separate placentas. Positive indirect effects prevailed in the first half of pregnancy, while negative indirect effects were observed in the second half. Umbilical cord insertion was neither a moderator nor a mediator.

To conclude, the placenta acts both as moderator and mediator in the association between air pollution and birth weight, highlighting the need to consider these pathways in future research.

1. Introduction

Low birth weight (LBW) is a significant public health concern in Europe with a prevalence ranging from 4.2% to 10.6% of all live births (Euro-Peristat Project, 2018). LBW is not only associated with higher morbidity and mortality in the neonatal period (McIntire et al., 1999) but also with an increased risk for cardiovascular disease in adulthood (Belbasis et al., 2016; Wang et al., 2014) Prenatal exposure to the air

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pollutants particulate matter with a diameter of less than 10 μm (PM_{10}) and nitrogen dioxide (NO_2) is associated with lower birth weight (Bijnens et al., 2016; Li et al., 2020; Uwak et al., 2021; Padula et al., 2012). Air pollution is estimated to be responsible for about 20% of newborn deaths, primarily due to complications related to LBW and prematurity (Health Effects Institute, 2020).

While previous research has established a link between prenatal exposure to air pollution and birth weight, the underlying mechanisms remain unclear. Air pollutants may impair placental function leading to reduced nutrient and oxygen exchange (Simoncic et al., 2020). In three studies, PM_{10} and NO_2 exposure were as well associated with lower placental weight (van den Hooven et al., 2012a; Pesatori et al., 2008; Böhm-González et al., 2024).

Twins provide a unique opportunity to study the prenatal environment, as they might be more vulnerable to the adverse effects of air pollution during pregnancy than singletons, making the impact of environmental factors potentially more pronounced. They are particularly susceptible to LBW (Euro-Peristat Project, 2018) with twins weighing on average about 900 g less at birth than singletons (Gielen et al., 2006). Additionally, while twins share the same mother, their intrauterine environments, influenced by placentation, often differ, allowing for deeper insights into the role of prenatal environmental factors. Against this background, Bijnens et al. (2016) showed that a 10 $\mu g/m^3$ decrease of particulate air pollution may account for a reduction of 40% in small for gestational age in twins born moderate to late preterm. The corresponding estimates for PM_{10} and NO_2 on birth weight showed a reduction of 40.2 g (95%CI: 69.0, 11.3) and 27.3 g (95%CI: 52.9, 1.7) per 10 $\mu g/m^3$, respectively (Bijnens et al., 2016). Furthermore, Böhm-González et al. (2024) found for each 10 $\mu g/m^3$ increase in PM_{10} or NO_2 in the third trimester a decrease in placental weight of −19.7 g (95%CI − 35.1; −4.3) and −17.7 g (95%CI − 30.4; −0.5) respectively, in preterm twins with separate placentas (Böhm-González et al., 2024).

So far, we have investigated the association between ambient air pollution and birth weight or placental weight in twins in separate analysis. However, the complex role of the placenta in the association between air pollution and birth weight was not investigated. Especially in twins the placenta is an important factor to consider. Monozygotic twins can have either one shared placenta (monochorionic) if splitting occurs over 4 days after fertilization, or two separate placentas (dichorionic) if division occurs within 4 days (Bulmer, 1970). Monozygotic-dichorionic and dizygotic twins have two separate placentas, which may fuse into one placental mass during development. Twins with one placental mass -whether shared or fused- have lower birth weights, higher prematurity, higher morbidity and mortality than twins with two separate placentas (De Paepe et al., 2015; Hack et al., 2008). But also, the placental weight is lower in case of one placental mass as compared to the added weight of two separate placentas (Gielen et al., 2006). Additionally, the proportion of peripheral umbilical cord insertion is higher in twins with one placental mass (Gielen et al., 2006), which is associated with a lower birth weight of approximately 150 g compared to central umbilical cord insertion in twins (Loos et al., 2001; Gielen et al., 2007). Abnormal umbilical cord insertion has as well been associated with higher exposure to fine particulate matter with a diameter <2.5 μm ($PM_{2.5}$) during the first trimester (Michikawa et al., 2021). These placental characteristics might therefore partly explain the association between ambient air pollution and birth weight and could explain not finding an association.

Despite the potential importance of placental characteristics in the association between air pollution and birth weight, few studies have investigated their role comprehensively. These roles may include confounding, mediation, or moderation (Supplementary Fig. 1). A confounder is associated with both the exposure and outcome, but not part of the causal pathway, which could bias results if not accounted for. Mediation occurs when a variable is part of the pathway and therewith explaining a part of how the exposure impacts the outcome. In contrast, moderation occurs when a variable modifies the strength or direction of the association, indicating that some groups may be more susceptible to the effects of air pollution than others (Bijnens et al., 2016). In the context of this study, it is more likely that placental characteristics act as mediators than as confounders, as they are more plausibly part of the causal pathway between air pollution and birth weight. Therefore, investigating their potential roles as mediators and moderators is critical for understanding the complex interplay between air pollution, placental characteristics, and birth weight. This study examines the role of the placenta in the association between prenatal exposure to ambient air pollution and birth weight, in twins from the East Flanders Prospective Twin Survey (EFPTS). It investigates the potential moderating and/or mediating role of placental weight and umbilical cord insertion in the relationship between PM_{10} and NO_2 and birth weight.

2. Methods

2.1. Study population

The East Flanders Prospective Twin Survey (EFPTS) is a prospective population-based register of all twins or higher order multiples born in East Flanders, Belgium (Derom et al., 2019). Since 1964 over 10.000 twin pairs with a birth weight of at least 500 g, or gestational age >22 weeks, have been registered. The vast majority (>99%) of twins are of Caucasian origin. In a sub-study of the EFPTS, the mothers' addresses at the time of delivery were geocoded for 5400 live born twins (2700 twin pairs) born between 2002 and 2013. Twins with major congenital malformation (n = 672) were excluded (Loos et al., 2001). Additionally twins with missing data for placental weight (n = 1282 of which 86% were male-female twin pairs), zygosity (n = 2), median household income (n = 4), parity (n = 42) and insertion of umbilical cord (n = 58) were excluded. This led to a total study population of 3340 twins (1670 twin pairs) (Supplementary Fig. 2).

2.2. Data collection

Data collection for the EFPTS has been described in detail before (Gielen et al., 2006; Loos et al., 1998, 2001; Derom et al., 2019). In short, birth weight in grams, birth order of the twin, twin's sex, gestational age, birth date, maternal age in years and parity, were drawn from medical records from the maternity ward. Placental weight, umbilical cord insertion, chorionicity and number of placentas were determined by a trained midwife of the EFPTS within 24 h after delivery according to a standardized procedure (Loos et al., 1998). Until examination placentas were kept at a temperature of about 4 °C. Placentas were weighed without fetal membranes, fresh and unfixed (Loos et al., 1998). The umbilical cord insertion was categorized as central (central and eccentric) or peripheral (paramarginal, marginal, on the surrounding and on the dividing membrane). Zygosity determination involved a stepwise approach considering sex, chorionicity, blood groups, and DNA fingerprints if necessary, achieving a final security level of 99.9% (Derom et al., 2019; Loos et al., 1998). During data collection of this study period, part of the placentas of different-sex twin pairs (dizygotic twin pairs) were not weighed due to policy reasons.

Gestational age was calculated as completed weeks of pregnancy, based on the first day of the last menstrual period or on the first trimester ultrasound. As twins differ in their intrauterine growth from singletons, achieving optimal size earlier than singletons (Antoniou et al., 2011; Gielen et al., 2008a), we decided not to use clinical cut-offs for prematurity derived from singletons. Instead, categories for prematurity were made using the interquartile range (IQR) of the 25th percentile and the 75th percentile (p25-p75) of our dataset as cut-offs for "preterm" (<35 weeks), "term" (35–37 weeks) and "post-term" (>37 weeks). Parity was dichotomized into primiparity and multiparity. Season of conception was derived from birth date and gestational age in weeks and then categorized as spring, summer, fall and winter. Infertility treatment was categorized as: spontaneous, artificial ovulation induction only, assisted

reproductive technology (ART), and unknown mode of conception. As a proxy for socioeconomic status, mean neighborhood household income was measured as described before (Bijnens et al., 2016).

Exposure to ambient air pollutants PM_{10} in $\mu g/m^3$ and NO_2 in $\mu g/m^3$ were estimated using a spatial-temporal interpolation method (Kriging) based on daily measurements of monitoring stations (n = 19 for PM_{10}, n = 44 for NO_2 in 2002, increasing to 36 and 64 respectively in 2013) and land cover data (CORINE data set from European Environmental Agency) (Janssen et al., 2008). This model provided daily concentrations from 2002 to 2013 for 4 × 4 km^2 grids from the Belgian telemetric air quality networks. Individual mean exposure concentrations at the mother's residential address at time of delivery were estimated for specific periods (the overall pregnancy, the trimesters (defined as 1–13 weeks (trimester 1), 14–26 weeks (trimester 2) and 27 weeks to delivery (trimester 3), the last month and the last week of pregnancy). $PM_{2.5}$ exposure data were available only for 764 twin pairs born after 2006. Additionally, a new monitoring station installed in East Flanders (Ghent) in 2010 reported higher $PM_{2.5}$ concentrations after 2010 compared to 2007–2010, reducing the reliability of these data. Therefore, $PM_{2.5}$ was excluded from the analysis.

Informed consent was obtained from the parents at birth for registry data. Ethical approval, including the registry data from 2001 to 2013, was renewed based on current Belgian and European law by Ghent University Hospital (METC: BC-04342) on June 25, 2020.

2.3. Statistical analysis

For statistical analysis software package R (Version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria) was used. Statistical significance level was set a priori at alpha = 0.05, two-sided. Normality was assessed visually using histograms and QQ-Plots. Differences between groups in baseline characteristics were assessed using ANOVA and Chi-squared tests. In case of non-normality, a Kruskal Wallis Test was used. To compare Pearson correlation coefficients a Fisher r to z transformation was used. To test selection bias, baseline characteristics of included twins were compared to twins with missing placental weight (Supplementary Table 1).

2.3.1. Modelling of multivariable regression equations

For moderation (interaction) and mediation analysis the same models were used with birth weight as outcome and the air pollutants PM_{10} or NO_2 as exposure in six separate analyses corresponding to the exposure windows: the whole pregnancy, the three trimesters, the last month, and the last week.

Given the difficulty in determining how much of a single placental mass belongs to each twin, we implemented a clinically relevant and statistically valid approach. Instead of simply dividing the weight of a fused placenta in half, which would not accurately represent the clinical situation, we analyzed cases with one placental mass separately from those with two separate placentas. Twins with one placental mass were analyzed as pairs with added birth weights in a linear regression analysis. Twins with separate placentas were analyzed individually in a mixed model with a random intercept to account for the relatedness of twins in a pair. For umbilical cord insertion as moderator or mediator all twins were analyzed individually in a mixed model approach with a random intercept, adjusted for the presence of one or two placentas, as using the whole population increases power and information about umbilical cord insertion was available for each twin individually.

A multivariable analysis was conducted. For confounder selection twin's sex, parity, maternal age, zygosity, chorionicity, gestational age, infertility treatment, year of birth, season of conception, neighborhood household income and the presence of one or two placentas (in case all twins were analyzed) as well as birth order of the twin were added to the regression models. Then confounders were selected for each combination of air pollutant and twin population (one placental mass, separate placentas, all twins) separately in a stepwise backwards elimination process with significant covariates ($p < .1$) staying in the model. Assumptions for linear regression and mixed models were assessed for all models.

2.3.2. Moderation analysis

First, interaction terms between the air pollutants and placental weight were added to assess moderation. In case of significant interaction terms a stratified analysis was done using tertiles of placental weight to form three groups. Additionally, to visualize interactions, plots were made using the package "interactions" in R (Long, 2019) using the mean, the mean minus the standard deviation, and the mean plus the standard deviation as example values. Moreover, models with an interaction between a categorized placental weight using the tertiles as categories were assessed.

Next, interaction terms for air pollutants and umbilical cord insertion, categorized as central or peripheral insertion, were assessed. In case of significant interaction terms, the analysis was stratified.

2.3.3. Mediation analysis

Usually, the estimated effect of an association is the total effect. In mediation analysis this total effect is split up into a direct and indirect effect (Fig. 1). Mediation analysis for the relationship between air pollutants and birth weight was done for the possible mediators: placental weight and umbilical cord insertion. First, two models were constructed, (1) a model with the possible mediator as dependent variable, the air pollutant and the significant confounders as independent variables and (2) a model with birth weight as dependent variable, the air pollutant, the mediator and the significant confounders as independent variables. Then, indirect, direct and total effects (indirect and direct effects added up) were estimated with the R package "mediation" (Version 4.5.0) (Tingley et al., 2014) using a counterfactual approach. For placental weight 95% confidence intervals (95%CI) of the indirect effect were computed using percentile bootstrapping (N = 1000). For umbilical cord insertion a quasi-Bayesian Monte Carlo method was used to estimate confidence intervals. Mediation was considered to be present when the indirect effect was significant, as advised by Zhao et al. (2010).

2.3.4. Sensitivity analysis

To complement the separate analyses in twins with one placental mass and two separate placentas, all twins were analyzed together as pairs using combined birth weights and total placental weights in a linear regression, adjusted for the presence of one or two placental masses. Furthermore, analyses were done stratified by prematurity, motivated by the results of a previous study by Bijnens et al. (2016) showing different associations between PM_{10} and NO_2 and birth weight for preterm and term born twins.

To investigate the environmental co-effect of PM_{10} and NO_2, models

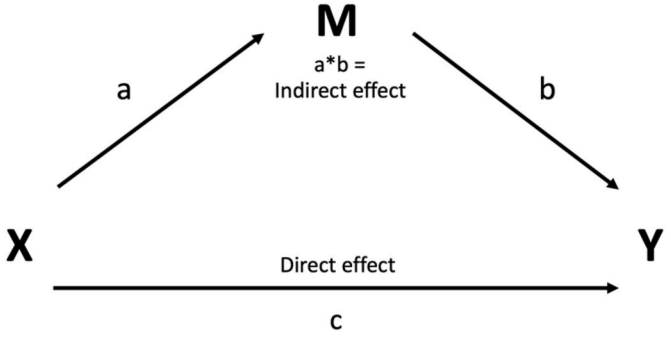

Fig. 1. Mediation triangle.
X = exposure, Y = outcome, M = mediator, a = effect on mediator, b = effect of mediator on outcome.
c = direct effect, a*b = indirect effect of X on Y through the mediator. Total effect = indirect + direct effect.

including both air pollutants were assessed. First, multicollinearity was assessed using the Variance Inflation Factor (VIF). Three models were compared: (1) multiplicative models including a three-way interaction between PM_{10}, NO_2 and the placental characteristic, (2) additive models adjusting for the other air pollutant, (3) single-pollutant models with either PM_{10} or NO_2.

3. Results

3.1. Characteristics of the study population

Table 1 summarizes the baseline characteristics of the 1670 twin pairs included in the study. Among them, 1052 (63%) had one placental mass and 618 (37%) had two separate placentas. Twins with one placental mass had significantly lower birth weight, total placental weight, gestational age, maternal age, and proportion of primiparity. They also had fewer cases of central cord insertion, different-sex pairs, and male twins ($p < .05$). However, the birth weight-to-placental weight ratio (BW/PW ratio), the neighborhood income and the season of conception did not differ significantly between groups. Most twin pairs were dizygotic-dichorionic twins (66,6%), followed by monozygotic-monochorionic (23,1%) and only 10,3% were monozygotic-dichorionic.

The mean air pollution exposure did not differ significantly between twins with one placental mass and those with separate placentas (Table 2).

Excluded twins differed from included twins, with over-representation of different-sex twin pairs and consequently a higher proportion of dizygotic twins, heavier birth weights and longer gestation periods (Supplementary Table 1).

3.2. Moderation analysis

3.2.1. Placental weight: main analysis

For twins with separate placentas, significant interactions were observed between placental weight and PM_{10} exposure during the whole pregnancy, last trimester, and last month, as well as between placental weight and NO_2 exposure for all periods except the last week (Table 3). When stratified for placental weight category, the associations between the air pollutants and birth weight were only significant in twins in the lowest tertile of placental weight, indicating a change of −93.18 g (95% CI: −164.17, −22.19) and −69.28 g (95%CI: −124.81, −13.76) birth weight per increase of 10 $\mu g/m^3$ exposure to PM_{10} and NO_2 during the whole pregnancy respectively (Table 4).

Fig. 2 illustrates the differential effects of PM_{10} and NO_2 exposure over the whole pregnancy on birth weight. Supplementary Table 2 presents the interaction terms for placental weight categories. Supplementary Figs. 3 and 4 show other exposure windows.

In twins with one placental mass, only exposure to NO_2 in the last week of pregnancy showed a significant interaction (Table 3). Analysis stratified by placental weight category showed no significant associations between NO_2 and PM_{10} exposure and birth weight (Supplementary Table 3).

3.2.2. Placental weight: sensitivity analysis

When analyzing all twins as pairs, significant interactions were observed for PM_{10} exposure during the whole pregnancy and for NO_2

Table 1
Baseline characteristics of the study population.

	All twins	Twins with one placental mass	Twins with separate placentas	p
N individual/twin pairs	3340/1670	2104/1052	1236/618	
Birth weight (BW), gram	2418 (525)	2364 (523)	2512 (515)	<0.001
Placental weight(PW), gram	725 (173)	705 (165)	759 (180)	<0.001
individual			379 (99)	
BW/PW Ratio	6.7 [6,7.5]	6.72 [5.9,7.6]	6.7 [5.9,7.6]	0.275
Correlation BW and PW (Pearson)	0.62 [0.58,0.66]	0.62 [0.59,0.66]	0.57 [0.53,0.61]	0.046
Cord insertion (peripheral)	910 (27,2%)	654 (31,1%)	256 (20,7%)	<0.001
Gestational age, weeks	36 [35, 37]	36 [34, 37]	36 [35, 37]	<0.001
Prematurity				<0.001
<35 weeks	764 (22.9)	528 (25.1)	236 (19.1)	
>=35 and ≤ 37 weeks	1920 (57.5)	1178 (56.0)	742 (60.0)	
>37 weeks	656 (19.6)	398 (18.9)	258 (20.9)	
Twin sex (female)	1694 (50,7%)	1108 (52,7%)	586 (47,2%)	0.004
Sex of twin pair				<0.001
Female-female	693 (41,5%)	465 (44,2%)	228 (36,9%)	
Male-female	308 (18,4%)	178 (16,9%)	130 (21,0%)	
Male-male	669 (40,1%)	409 (38,9%)	260 (42,1%)	
Zygosity and Chorionicity				<0.001
DZ DC	2224 (66,6%)	1188 (56,5%)	1036 (83,8%)	
MZ DC	344 (10,3%)	144 (6,8%)	200 (16,2%)	
MZ MC	772 (23,1%)	772 (36,7%)	0	
Conception season				0.221
fall	828 (24.8%)	542 (25.8%)	286 (23.1%)	
spring	868 (26.0%)	544 (25.9%)	324 (26.2%)	
summer	834 (25.0%)	528 (25.1%)	306 (24.8%)	
winter	810 (24.3%)	490 (23.3%)	320 (25.9%)	
Parity (multipara)	1594 (47,7%)	968(46.0%)	626 (50.6%)	0.011
Age of mother, years	30.21 (4.63)	30.04 (4.58)	30.50 (4.70)	0.006
Infertility Treatment				<0.001
Spontaneous	2002 (59.9%)	670 (54.2%)	1332 (63.3%)	
Artificial ovulation induction only	446 (13.4%)	188 (15.2%)	258 (12.3%)	
Assisted reproductive technology	806 (24.1%)	350 (28.3%)	456 (21.7%)	
Unknown	6 (2.6%)	28 (2.3%)	58 (2.8%)	
Neighborhood income, euros	19898 (3191)	19915 (3180)	19869 (3212)	0.687

For continuous variables and with a normal distribution data is given as means and standard deviations (SD) and for non-normally distributed data as median and interquartile range [IQR]. For categorical variables data is given as frequencies and percentages. Differences between groups were assessed using ANOVA and Chi-squared tests. In case of non-normality, a Kruskal Wallis Test was used. Pearson Correlation Coefficients are presented with 95% Confidence Interavals. Comparison of Pearson Correlation Coefficients were done with Fisher's R to Z Transformation. DZ DC = Dizygotic dichorionic, MZ DC = Monozygotic dichorionic, MZ MC = Monozygotic monochorionic.

Table 2
Exposure characteristics.

	All twins	Twins with one placental mass	Twins with separate placentas	p
PM_{10} µg/m^3				
Trimester 1	30.8 (6,0)	30.7(6,0)	31.0 (6,2)	0.260
Trimester 2	30.7 (6,2)	30.7 (6,2)	30.7 (6,3)	0.949
Trimester 3	30.2 (7,2)	30.2 (7,4)	30.2 (6,8)	0.746
Last month	29.7 [23,8-35,3]	29.9 [23,8-35,5]	29.3 [23,9-34,9]	0.635
Last week	27.6 [21,8-36,5]	27.4 [21,5-36,6]	27.4 [21,5-36,6]	0.438
Whole pregnancy	30.7 (4,7)	30.6 (4,6)	30.7 (4,7)	0.552
NO_2 µg/m^3				
Trimester 1	24.5 (7,2)	24.6 (7,2)	24.4 (7,2)	0.444
Trimester 2	24.6 (7,3)	24.7(7,2)	24.3 (7,3)	0.095
Trimester 3	24.3 (7,8)	24.4(7,8)	24.2 (7,8)	0.412
Last month	23.7 [18,3-29,3]	23.9 [18,5-29,3]	23.4 [18,2-29,4]	0.267
Last week	23.1 [17,3-30]	23.19 [17,6-30,22]	23.1 [16,8-29,7]	0.516
Whole pregnancy	24.5 (5,9)	24.6 (7,2)	24.3 (6)	0.162

For continuous variables and with a normal distribution data is given as means and standard deviations (SD) and for non-normally distributed data as median and interquartile range [IQR]. Differences between groups were assessed using ANOVA. In case of non-normality, a Kruskal Wallis Test was used.

exposure during the whole pregnancy and in the second trimester (Supplementary Table 4). When stratified for placental weight group, there was a significant increase of birth weight of 102.21 g (95%CI: 13.53, 190.90) per 10 µg/m^3 PM_{10} in the high placental weight group in the first trimester and a significant decrease of birthweight of −60.10 g (95%CI: −135.75, −2.46) per 10 µg/m^3 NO_2 in the low placental weight group in the second trimester (Supplementary Table 5).

When stratified by prematurity categories based on our cut off values, significant interactions for PM_{10} were mainly present in "preterm" twins with a gestational age under 35 weeks (Supplementary Table 6). For NO_2 significant interactions were present in all three prematurity categories. In contrast to the main analysis, in "preterm" twins we observed three significant interactions for NO_2 in twins with one placental mass (Supplementary Table 6). Supplementary Tables 7, 8 and 9 show further stratification by prematurity and placental weight category.

3.2.3. Umbilical cord insertion: moderation analysis

There were no significant interactions between umbilical cord insertion and air pollution exposure in the main or stratified analysis (Supplementary Table 10).

3.2.4. The environmental co-effect of PM_{10} and NO_2 in moderation analyses

There was no multicollinearity between the exposures (VIF <5). The multiplicative model with a three-way interaction between PM_{10}, NO_2 and placental weight did not improve the associations of the single-pollutant model. In the moderation analysis only one interaction term was significant. Additionally, adding the other air pollutant to the models did not change the results substantially (Supplementary Table 11). For umbilical cord insertion results of moderation analysis remained non-significant in multiplicative and additive models (Supplementary Table 12).

3.3. Mediation analysis

3.3.1. Placental weight: main analysis

Mediation by placental weight was present in twins with separate placentas and in twins with one placental mass. In twins with separate placentas, the exposure to PM_{10} in the first and second trimester showed positive indirect effects (increase of 21.31 g (95% CI: 0.76, 42.21) and 20.13 g (95% CI: 0.12, 40.32) birth weight per increase of 10 µg/m^3 PM_{10}) while direct effects were negative, but not significant. Also, for the exposure to NO_2 in the third trimester, the last month and the last week significant indirect effects were observed. These were negative with a decrease of −19.69 g (95% CI: −36.44, −4.08) birth weight for the third trimester, a decrease of −17.57 g (95% CI: −33.41, −2.58) birth weight for the last month of the pregnancy and −15.07 g (95% CI: −28.53, −2.26) birth weight for the last week of pregnancy per increase of 10 µg/m^3 NO_2 (Table 5).

In twins with one placental mass, placental weight was a mediator in the relationship between PM_{10} and birth weight over the whole

Table 3
Overview of results of moderation (interaction) analysis for placental weight.

Interaction Term	Twins with one placental mass (n = 1052 pairs)			Twins with separate placentas (n = 618 pairs)		
	beta	95% CI	p	beta	95% CI	p
PM_{10}*placental weight						
Whole Pregnancy	0.10	[-0.32, 0.51]	0.639	0.67*	[0.35, 0.98]	0.023
Trimester 1	0.07	[-0.24, 0.38]	0.661	0.31	[0.05, 0.57]	0.314
Trimester 2	0.08	[-0.22, 0.39]	0.603	0.49	[0.23, 0.75]	0.113
Trimester 3	−0.04	[-0.30, 0.21]	0.731	0.47*	[0.23, 0.72]	0.013
Last month	0.01	[-0.21, 0.23]	0.935	0.40*	[0.17, 0.63]	0.015
Last week	−0.02	[-0.18, 0.14]	0.830	0.08	[-0.11, 0.27]	0.625
NO_2*placental weight						
Whole Pregnancy	0.00	[-0.29, 0.30]	0.980	0.07***	[0.04, 0.10]	<0.001
Trimester 1	0.03	[-0.21, 0.26]	0.828	0.03*	[0.01, 0.06]	0.018
Trimester 2	0.04	[-0.22, 0.29]	0.776	0.05***	[0.02, 0.07]	<0.001
Trimester 3	−0.13	[-0.36, 0.11]	0.290	0.05***	[0.02, 0.07]	<0.001
Last month	−0.10	[-0.32, 0.12]	0.361	0.04***	[0.02, 0.06]	0.001
Last week	−0.19*	[-0.38, −0.01]	0.043	0.01	[-0.01, 0.03]	0.414

***p < .001, **p < .01, *p < .05. Interaction terms for twins with one placental mass are derived from a linear regression adjusted for: gestational age, sex of twin pair, chorionicity, conception season and parity. Interaction terms for twins with separate placentas are derived from mixed models with a random intercept adjusted for gestational age, twin's sex, parity, age of the mother and birth order of the twin.

Table 4
Change of birth weight (g) for an increment of 10 μg/m^3 PM_{10} or NO_2 stratified by placental weight groups in twins with separate placentas.

Exposure	Low placental weight (n = 412)			Normal placental weight (n = 412)			High placental weight (n = 412)		
	beta	95% CI	p	beta	95% CI	p	beta	95% CI	p
PM_{10} in μg/m^3									
Whole Pregnancy	−93.18*	[-164.17, −22.19]	0.010	−18.36	[-82.95, 46.23]	0.576	2.71	[-70.12, 75.54]	0.942
Trimester 1	−61.22*	[-116.57, −5.88]	0.030	−38.99	[-86.57, 8.59]	0.108	3.64	[-50.92, 58.20]	0.896
Trimester 2	−41.67	[-95.47, 12.12]	0.128	−0.70	[-48.60, 47.20]	0.977	−8.61	[-62.13, 44.90]	0.752
Trimester 3	−47.18*	[-93.38, −0.98]	0.045	25.43	[-21.84, 72.70]	0.291	14.21	[-37.24, 65.66]	0.587
Last month	−14.69	[-56.39, 27.00]	0.488	19.05	[-18.99, 57.09]	0.325	14.06	[-30.72, 58.85]	0.537
Last week	15.67	[-14.25, 45.60]	0.303	24.18	[-3.70, 52.05]	0.089	2.88	[-29.78, 35.54]	0.862
NO_2 in μg/m^3									
Whole Pregnancy	−69.28*	[-124.81, −13.76]	0.015	−4.59	[-55.66, 46.48]	0.860	15.59	[-41.09, 72.26]	0.589
Trimester 1	−54.75*	[-101.29, −8.21]	0.021	−20.29	[-61.76, 21.18]	0.336	3.83	[-43.95, 51.61]	0.875
Trimester 2	−43.55	[-88.23, 1.14]	0.056	8.92	[-33.08, 50.93]	0.676	11.56	[-35.70, 58.83]	0.631
Trimester 3	−40.47	[-85.46, 4.53]	0.078	9.61	[-29.49, 48.72]	0.629	16.53	[-25.21, 58.27]	0.436
Last month	−29.22	[-73.37, 14.93]	0.194	4.13	[-33.41, 41.67]	0.829	14.46	[-25.48, 54.40]	0.477
Last week	0.30	[-36.16, 36.77]	0.987	21.94	[-10.45, 54.32]	0.184	0.16	[-34.53, 34.85]	0.993

***p < .001, **p < .01, *p < .05 Betas were derived from mixed models with a random intercept adjusted for: gestational age, twin's sex, parity, age of the mother and birth order of the twin.

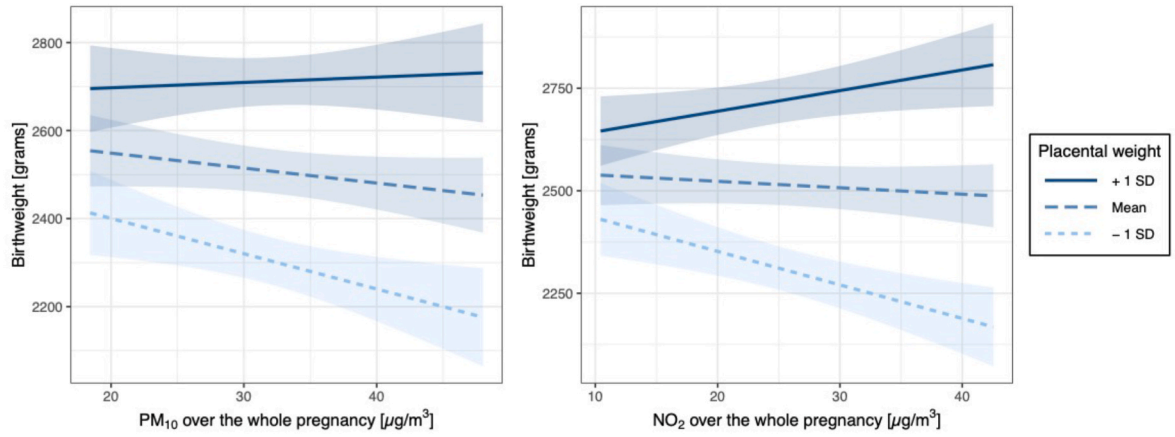

Fig. 2. Interaction plots for PM_{10} and NO_2 over the whole pregnancy in twins with separate placentas.

pregnancy and the first trimester. The indirect effect was for both pregnancy periods positive (increase of 36.05 g (95% CI: 0.88, 70.61) and 33.17 g (95% CI: 3.12, 63.91) birth weight per increase of 10 μg/m^3 PM_{10} over the whole pregnancy and the first trimester, respectively), while the direct effects were not significant and negative for the whole pregnancy and positive for the first trimester (Table 5).

3.3.2. *Placental weight: sensitivity analysis*

In all twins analyzed as pairs, placental weight was a mediator in the association between birth weight and PM_{10} exposure during the whole pregnancy, first and second trimester, with a significant positive indirect effect (Supplementary Table 13).

Stratified by prematurity categories, in twins with one placental mass, mediation by placental weight was observed mainly in twins born before 35 weeks. Significant positive indirect effects were observed for PM_{10} and NO_2 exposure over the whole pregnancy, the first and second trimester (Supplementary Table 14).

In twins with separate placentas, mediation was observed for PM_{10} only in twins born between 35 and 37 weeks and for NO_2 only in twins born before 35 weeks (Supplementary Table 15).

In all twins as pairs, stratified by prematurity, mediation was present in twins born before 35 weeks and twins born between 35 and 37 weeks (Supplementary Table 16).

3.3.3. *Umbilical cord insertion: mediation analysis*

Umbilical cord insertion was no mediator in the relationship between air pollutants and birth weight (Supplementary Table 17). Results remained non-significant when stratified by prematurity. (Supplementary Table 18).

3.3.4. *The environmental co-effect of PM_{10} and NO_2 in mediation analysis*

Mediation of placental weight for PM_{10} exposure in twins with separate placentas became more evident in models adjusted for NO_2 and those incorporating a three-way interaction. For NO_2 exposure mediation shifted slightly towards the beginning of pregnancy (Supplementary Table 19). In twins with one placental mass indirect effects became insignificant in multiplicative and additive models (Supplementary Table 20).

For umbilical cord insertion results of mediation analysis remained non-significant in multiplicative and additive models (Supplementary Table 21).

4. Discussion

We investigated the role of the placenta in the relationship between the air pollutants PM_{10} and NO_2, and birth weight. We identified placental weight as a moderator and mediator for both pollutants in this relationship. Moderation occurred mainly in twins with separate placentas. In these twins, we observed a significant reduction of birth weight with increasing PM_{10} and NO_2 exposure in twins with low placental weight. In general, moderation was more evident in mid-to-late pregnancy. Placental weight acted as a mediator for both pollutants in twins with one placental mass and in twins with separate placentas. In both groups, mediation was mainly seen in twins born <35

Table 5
Mediation analyses for placental weight in twins with one placental mass and twins with separate placentas.

Exposure	Indirect Effect			Direct Effect			Total Effect			Effect on mediator		
	beta	**95% CI**	**p**	**beta**	**95% CI**	**p**	**beta**	**95% CI**	**p**	**beta**	**95% CI**	**p**
Twins with one placental mass												
PM_{10} in µg/m^3												
Whole Pregnancy	36.05*	[0.88, 70.61]	0.048	−13.90	[-77.19, 53.47]	0.738	22.15	[-57.11, 95.42]	0.568	18.96	[-2.03, 39.96]	0.077
Trimester 1	33.17*	[3.12, 63.91]	0.026	15.12	[-40.86, 69.57]	0.652	48.30	[-16.52, 108.23]	0.158	17.50*	[0.03, 34.97]	0.050
Trimester 2	26.60	[-4.17, 57.38]	0.098	−21.01	[-76.31, 37.38]	0.486	5.59	[-59.72, 71.36]	0.848	13.98	[-3.54, 31.50]	0.118
Trimester 3	4.18	[-19.99, 29.42]	0.740	−13.27	[-58.91, 32.04]	0.508	−9.08	[-61.63, 41.81]	0.716	2.20	[-12.14, 16.55]	0.763
Last month	1.73	[-20.72, 25.59]	0.926	−11.05	[-49.89, 30.90]	0.624	−9.33	[-53.26, 35.54]	0.696	0.91	[-11.40, 13.22]	0.885
Last week	−4.10	[-19.16, 10.17]	0.552	−6.86	[-31.62, 19.49]	0.632	−10.96	[-40.09, 22.11]	0.458	−2.16	[-10.11, 5.78]	0.594
NO_2 in µg/m^3												
Whole Pregnancy	15.08	[-16.92, 48.25]	0.332	−40.49	[-96.32, 20.96]	0.168	−25.41	[-89.89, 41.91]	0.450	7.92	[-8.49, 24.34]	0.344
Trimester 1	24.30	[-6.17, 54.26]	0.122	−30.19	[-81.41, 24.81]	0.268	−5.90	[-65.03, 60.02]	0.872	12.75	[-2.60, 28.11]	0.103
Trimester 2	10.66	[-17.75, 41.14]	0.438	−40.38	[-90.83, 12.65]	0.126	−29.71	[-87.80, 29.61]	0.320	5.60	[-9.45, 20.66]	0.465
Trimester 3	−4.84	[-30.02, 22.72]	0.732	−26.98	[-74.81, 18.90]	0.240	−31.82	[-82.45, 19.19]	0.206	−2.55	[-16.53, 11.44]	0.721
Last month	−1.36	[-26.16, 26.73]	0.934	−17.92	[-60.42, 26.00]	0.428	−19.28	[-67.40, 30.94]	0.456	−0.71	[-13.74, 12.31]	0.914
Last week	−5.92	[-26.50, 14.16]	0.616	−3.22	[-38.71, 33.00]	0.874	−9.14	[-45.82, 30.71]	0.684	−3.12	[-13.66, 7.43]	0.562
Twins with separate placentas												
PM_{10} in µg/m^3												
Whole Pregnancy	24.20	[-2.95, 51.85]	0.078	−32.63	[-74.33, 7.43]	0.136	−8.43	[-57.87, 40.65]	0.756	12.50	[-1.49, 26.48]	0.080
Trimester 1	21.31*	[0.76, 42.21]	0.042	−29.17	[-61.08, 1.18]	0.056	−7.85	[-45.73, 29.07]	0.696	10.96*	[0.39, 21.53]	0.042
Trimester 2	20.13*	[0.12, 40.32]	0.050	−16.63	[-48.35, 13.18]	0.316	3.50	[-33.52, 39.67]	0.872	10.38	[-0.07, 20.82]	0.051
Trimester 3	−9.00	[-27.73, 8.78]	0.340	−1.86	[-31.68, 24.63]	0.890	−10.86	[-43.10, 21.29]	0.514	−4.42	[-14.13, 5.29]	0.372
Last month	−7.68	[-23.70, 7.51]	0.344	5.32	[-20.40, 28.70]	0.680	−2.36	[-31.41, 26.65]	0.886	−3.78	[-12.16, 4.61]	0.377
Last week	−9.04	[-20.76, 2.25]	0.154	12.84	[-5.67, 30.14]	0.168	3.80	[-17.10, 24.72]	0.742	−4.50	[-10.58, 1.59]	0.147
NO_2 in µg/m^3												
Whole Pregnancy	−5.12	[-26.81, 15.12]	0.636	−10.47	[-43.99, 20.49]	0.552	−15.60	[-53.73, 21.70]	0.450	−2.44	[-13.53, 8.65]	0.666
Trimester 1	8.68	[-9.00, 26.13]	0.324	−18.94	[-46.61, 6.77]	0.184	−10.25	[-42.37, 21.31]	0.562	4.56	[-4.64, 13.76]	0.331
Trimester 2	−3.56	[-21.36, 13.62]	0.684	−1.85	[-30.10, 24.77]	0.872	−5.41	[-37.63, 25.69]	0.726	−1.65	[-10.74, 7.43]	0.721
Trimester 3	−19.69*	[-36.44, −4.08]	0.016	5.94	[-19.70, 29.18]	0.640	−13.75	[-41.85, 14.28]	0.332	−9.84*	[-18.30, −1.39]	0.022
Last month	−17.57*	[-33.41, −2.58]	0.028	4.62	[-20.18, 27.41]	0.722	−12.95	[-41.05, 15.65]	0.384	−8.81*	[-16.96, −0.65]	0.034
Last week	−15.07*	[-28.53, −2.26]	0.028	10.27	[-10.97, 29.61]	0.334	−4.79	[-28.65, 19.46]	0.720	−7.54*	[-14.47, −0.60]	0.033

***p < .001, **p < .01, *p < .05. For twins with one placental mass linear regression models used for mediation analysis were adjusted for: gestational age, sex in the twin pair, chorionicity, parity, and season of conception. For twins with separate placentas mixed models used for mediation analysis were adjusted for gestational age, newborn's sex, parity, age of the mother and rang of the twin.

weeks and between 35 and 37 weeks. Positive indirect effects were observed in the first half of pregnancy for both PM_{10} and NO_2, while negative indirect effects emerged in the second half for NO_2 only. Overall, both moderation and mediation were more pronounced in twins with separate placentas. Umbilical cord insertion was neither identified as moderator, nor mediator.

Our study's air pollutant levels were above the WHO Air Pollution Guidelines but were comparable to or slightly lower than in other European studies from similar time periods (Simoncic et al., 2020; Dadvand et al., 2013). Despite this, our estimates for the association between PM_{10} and NO_2 and birth weight were substantially larger than those in previous studies. For instance, a recent meta-analysis reported a pooled estimate of −8.65 g (95% CI: −16.83, −0.48) per increase of 10 $\mu g/m^3$ PM_{10} during the whole pregnancy (Uwak et al., 2021). Even the highest estimates in the meta-analysis were −36 g (95%CI: −67, −04) and −34 g (95%CI: −62, −0.6) per 10 $\mu g/m^3$ for PM_{10} and NO_2, respectively, in term born singletons (van den Hooven et al., 2012b). Previously some authors proposed that air pollution affects especially fetuses at risk (Bijnens et al., 2016; Pedersen et al., 2013), which could be an explanation for the higher estimates in our study, particularly in twins with low placental weight and those born prematurely. In agreement with this, previous studies reported stronger associations between air pollution and very low birth weight (Rogers and Dunlop, 2006) and fetal growth restriction in preterm births compared to term births (Pereira et al., 2016; Winckelmans et al., 2015).

In line with this, moderation by placental weight for PM_{10} exposure was most prominent in twins born before 35 weeks of gestation and for NO_2 exposure, it was stronger in twins born before 37 weeks of gestation (including <35 weeks). This moderation was primarily observed in twins with separate placentas. One reason could be the inclusion of monochorionic twins in the group of twins with one placental mass. Monochorionic twins always share one placenta, are at higher risk of prematurity, low birth weight and birth weight discordance due to unequal placenta sharing, raising the possibility that the relative contribution of air pollutants may be diminished or obscured in analyses (Fick et al., 2006; Blickstein, 2018). Furthermore, genetic variation may additionally play a role in modulating placental and fetal growth, although we controlled for zygosity in our analysis. However, for birth weight environmental factors are more important than genetic factors. Heritability estimates (proportion of the variance that is explained by genetic factors) for birth weight decrease during gestation from 38% at 25 weeks to 15% at 42 weeks and at the same time the environment becomes more important (Gielen et al., 2008b). While we did not collect genetic data in our study and therefore cannot directly investigate their role, it would be highly interesting for future research to explore these aspects. Another reason could be the use of combined birth weights in twins with one placental mass, which might have reduced the statistical power to detect interactions.

Our mediation analysis revealed opposing directions of the indirect and direct effects, which highlights the importance of investigating the role of the placenta. For PM_{10} exposure, we observed increased birth weight mediated through increased placental weight in early and mid-pregnancy, while the direct effect indicated a reduced birth weight. Contrary, for NO_2 we observed negative indirect effects at the end of pregnancy, indicating reduced birth weights through decreased placental weights. In line with this, van den Hooven et al. (van den Hooven et al., 2012a) found lower placental weights associated with NO_2 exposure in the last two month of pregnancy. Contrary to our results, they also reported lower placental weights associated with PM_{10} exposure (van den Hooven et al., 2012a). Consistent with this, Rocha et al. (2008) found a decreased placental weight in mice exposed to air pollution during pregnancy, regardless of the period of gestation. On the other hand, smoking during pregnancy was associated with higher placental weights (Mitsuda et al., 2020; Jaitner et al., 2024).

Exposure to particulate matter and NO_2 has been linked to oxidative stress markers in the placenta and umbilical cord blood, as well as reduced placental mitochondrial DNA content, which may impair energy-dependent processes critical for fetal growth (Mitsuda et al., 2020). Additionally, these pollutants are associated with lower levels of placental growth factor (PlGF) and higher levels of its inhibitor, soluble fms-like tyrosine kinase 1 (sFlt), suggesting an anti-angiogenic state (van den Hooven et al., 2012a) as well as increased levels of proinflammatory biomarkers (Fussell et al., 2024). Similar pathways are seen with smoking during pregnancy, which also induces oxidative stress, inflammation, and impaired vascularization in the placenta (Jaitner et al., 2024; Fussell et al., 2024). These alterations through exposure to air pollutants could create a hypoxic environment that could restrict fetal and placental growth (Rocha et al., 2008) as observed in some studies (van den Hooven et al., 2012a; Rocha et al., 2008). However, our findings suggest a compensatory response, wherein early exposure to air pollution stimulates placental growth to support fetal development. Supporting this, animal studies showed increased placental weights in response to hypoxic conditions in rodents (Matheson et al., 2016; Nuzzo et al., 2018; Thompson et al., 2016).

In multipollutant models, the effect estimates of the moderation results did not change substantially. Contrary, mediation of placental weight for PM_{10} exposure in twins with separate placentas became more evident in multipollutant models. For NO_2, mediation shifted toward earlier pregnancy, with significant indirect effects observed in the second and third trimesters and across the whole pregnancy. This could suggest that accounting for the other pollutant might reduce potential confounding, clarifying the association of the air pollutant and birth weight. The combined effects of the two pollutants may also amplify the observed associations, as they may act through overlapping but distinct biological pathways. Conversely, in twins with one placental mass, significant indirect effects in single-pollutant models became insignificant in multipollutant models, yet betas remained similar. This attenuation may reflect shared variability between PM_{10} and NO_2 exposures, potentially diminishing the statistical power to detect associations when both pollutants are included. Alternatively, it could indicate that the single shared placenta buffers against or homogenizes the combined influence of multiple pollutants, reducing the differentiation of effects in this subgroup.

We hypothesize that the association between air pollution and birth weight could vary by gestational time windows. During the first and second trimesters air pollution could stimulate a compensatory placental growth and consequently lead to higher birth weights as placental size is highly correlated with birth weight (Tepla et al., 2022). However, in later pregnancy, the diminished compensatory capacity may potentially be leading to decreased placental weights as seen in other studies (van den Hooven et al., 2012a; Rocha et al., 2008). In line with this, our mediation analysis showed negative indirect effects in the third trimester, last month and last week. In the second half of pregnancy, placental size could play a more important role for the association between air pollution and birth weight, as in our study moderation was mainly seen in the second and third trimester. In line with this, other studies suggest the first trimester to be a sensitive period for placental changes, including altered DNA methylation (Maghbooli et al., 2018) and inflammation as well as endothelial dysfunction (Mozzoni et al., 2022). While studies focusing on critical time windows during pregnancy found associations in mid and late pregnancy (Johnson et al., 2022; Arroyo et al., 2019; Sun et al., 2016). However, longitudinal studies using prenatal ultrasound to estimate placental weight at different time points during pregnancy are needed to confirm this hypothesis.

The main strength of our study lies in the detailed information on zygosity, chorionicity and placental examination, allowing for a comprehensive investigation of the role of the placenta. The large sample from a prospective population-based cohort minimized selection bias and allowed for stratified analyses. We used placental characteristics that easily can be measured in clinical practice and are cost-effective even in large cohorts, though we realize they are only approximate

measures for placental function and efficiency. However, several limitations should be considered. Exposure misclassification may have occurred due to reliance on the mother's residential address at time of delivery without accounting for time spent elsewhere (e.g., vacation, or workplace), outdoors, in traffic or moving houses during pregnancy. This exposure misclassification is likely non-differential, potentially leading to an underestimation of the associations. Two studies investigated the additional benefit of adjustment for moving houses during pregnancy in studies about the relationship between prenatal air pollution exposure and birth weight and found no change in effect sizes (Pereira et al., 2016; Chen et al., 2010). Although we adjusted for many covariates, we lacked data on maternal lifestyle factors such as smoking or drinking habits, diet, anthropometric measures including BMI and pregnancy complications (hypertensive disorders or gestational diabetes) (Kramer, 1987). Neighborhood household income was used as a proxy for socioeconomic status, even though it only partly accounts for lifestyle factors and might not be a valid proxy of socioeconomic status as it neglects individual-level information on education, income and occupation (Ritz and Wilhelm, 2008). Furthermore, we did not adjust for temperature and humidity, even though season of conception was included as a rough proxy for weather conditions. Excluding twins with missing placental weight data may have introduced selection bias, as these were predominantly opposite-sex dizygotic-dichorionic twins.

To the best of our knowledge, this is the first study investigating the role of the placenta in the relationship between air pollution and birth weight in twins. Our results emphasize the importance of considering placental characteristics as potential moderators and mediators, providing insights that might help to explain contradictory findings in previous research. Future research should further explore the role of the placenta and investigate additional placental factors to enhance our understanding of the complex interplay between air pollution, placental function, and birth outcomes.

CRediT authorship contribution statement

Alischa Corinna Ziemendorff: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Simone Teresa Böhm-González:** Writing – review & editing. **Eline Meireson:** Writing – review & editing, Investigation. **Steven Weyers:** Resources, Investigation. **Tim Nawrot:** Resources, Investigation. **Esmée Bijnens:** Writing – review & editing, Resources, Investigation. **Marij Gielen:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.120952.

Data availability

Data will be made available on request.

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