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# Association between trimester-specific prenatal air pollution exposure and placental weight of twins

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A R T I C L E I N F O

*Keywords:*
Air pollution
Particulate matter
Prenatal exposure
Placental weight
Trimester
Twin

A B S T R A C T

*Introduction:* This study investigates the association between maternal exposure to particulate matter ($PM_{10}$) and nitric dioxide ($NO_2$) during the first, second and third trimester and placental weight and birth weight/placental weight (BW/PW) ratio in twins at birth.

*Methods:* Cross-sectional data of 3340 twins from the East Flanders Prospective Twin Survey was used. Air pollutant exposure was estimated via spatial temporal interpolation. Univariable and multivariable mixed model analyses with a random intercept to account for the relatedness of newborns were conducted for twins with separate placentas. Twin pairs with one placental mass were studied with linear and logistic regression.

*Results:* In the third trimester, for each 10 $\mu m/m^3$ increase in $PM_{10}$ or $NO_2$ placental weight decreased −19.7 g (95%-C.I. −35.1; −4.3) and −17.7 g (95%-C.I. −30.4; −0.5) respectively, in moderate to late preterm twins with separate placentas. Consequently, BW/PW ratio increased with higher air pollution exposure. $PM_{10}$ exposure in the last week of pregnancy was associated with a higher odds ratio (OR) of 1.20 (95%-C.I. 1.00; 1.44) for a "small for gestational age placenta" (placental weight <10th percentile). Conversely, first trimester air pollutant exposure was associated with lower ORs of 0.55 (95%-C.I. 0.35; 0.88) and 0.60 (95%-C.I. 0.42; 0.84).

*Discussion:* The association of $PM_{10}$ and $NO_2$ on placental weight is trimester-specific, differs for twins with one versus two placentas and is most pronounced in moderate to late preterm twins. Longitudinal studies are needed to better understand the relationship between air pollutant exposure and placental weight evolution across different trimesters.

## 1. Introduction

Fetal development is vulnerable to environmental stressors such as ambient air pollution due to the high level of cell proliferation and organogenesis [1]. Evidence suggests that prenatal exposure to air pollution is associated with lower birth weight in full-term singletons [2–4]. So far, there is limited evidence about the association between air pollution and placental weight, despite its function being an important factor in the development of the unborn child. Previous studies indicate that higher prenatal exposure to $PM_{10}$ and $NO_2$ is associated with a decrease in placental weight [5,6]. Furthermore, a study using distance to roads as an indicator of traffic-related air pollution found a significant reduction in placental weight [7]. Moreover, a study in mice exposed to prenatal air pollution showed a significant reduction in placental

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weights compared to non-exposed mice [8].

The birth weight/placental weight (BW/PW) ratio is used as an approximate measure for placental efficiency, representing the grams of newborn produced per gram of placenta [9]. Few studies have investigated the BW/PW ratio in relation to air pollutants and have yielded inconsistent results. Some studies found no association [5,10], while one study reported a significant reduction in the BW/PW ratio [7].

Twins are an interesting study population as they have a higher risk of preterm birth and low birth weight compared to singletons [11]. The restricted capabilities of the uterine environment to nurture more than one fetus at a time might be an underlying mechanism [12,13]. In contrast to singletons, the fastest intrauterine growth for twins is achieved earlier in gestation, the duration of an "ideal twin pregnancy" is between 35 and 38 weeks [14]. In addition, twins show a dip in their growth chart after week 40, which is mainly due to the monozygotic-monochorionic (MZ-MC) twins [15], indicating that placentation is crucial. Twins with one placental mass have lower birth weights compared to twins with two separate placentas [15]. In MZ twin pregnancies, if splitting occurs before day two, two placentas (dichorionic) are formed, which can fuse to one placental mass. If splitting occurs after day two, one placenta (MC) is formed. Dizygotic (DZ) twin pregnancies always result in two placentas, which can fuse [16].

Intrauterine crowding with limited placental capacity could make the twin placenta more susceptible to environmental influences. Prenatal exposure to $PM_{10}$ and $NO_2$ is associated with a higher risk of small for gestational age and low birth weight in moderate to late preterm born twins during the last trimester [17]. Moreover, air pollution exposure is associated with discordant birth weight of twins [17]. An adverse intra uterine environment, which favours an asymmetrical growth of the twins might be an explanation [18]. However, trimester-specific effects of $PM_{10}$ and $NO_2$ on placental weight are not known.

The aim of this study is therefore to investigate the association between maternal exposure to $PM_{10}$ and $NO_2$ during the first, second and third trimester and placental weight and BW/PW ratio in twins.

## 2. Methods

### 2.1. Subjects

The East Flanders Prospective Twin Survey (EFPTS) is a prospective population-based register that includes all twins or higher order multiple births born in the Belgian Province of East Flanders [19]. Since 1964, over 10.000 twin pairs who met the World Health Organization criteria for live born infants (birth weight ≥500 g) have been enrolled. Regional background levels of $PM_{10}$ and $NO_2$ were available starting from 2001. For this study, the addresses of 5190 twin pairs born between 2001 and 2013 were geocoded, using the same data as a previous study by Bijnens et al. [17]. Twin pairs with stillbirths, malformation of one or both twins, and missing data on covariates were excluded. Additionally, twin pairs with missing placental weight of one or both twins (n = 641 pairs) and missing information on umbilical cord insertion (n = 29 pairs) were also excluded, resulting in a study population of 1670 pairs (3340 twins)

### 2.2. Data collection

Methods of data collection have been previously described in detail [15,19–22]. In brief, a comprehensive set of obstetric and perinatal data were recorded, including year of birth, gestational age, maternal age, birth order, parity, sex and neonatal survival. Placentas were kept at constant temperature around 4 °C until a trained midwife examined them within 24 h after delivery according to a standardized protocol. Chorionicity, number of placentas, placental weight and insertion of the umbilical cord were determined. The umbilical cord insertion was categorized as central (central and eccentric) or peripheral (paramarginal, marginal, on the surrounding and on the dividing membrane). Zygosity and chorionicity were determined with an accuracy of 99 % [19]. The total weight of the placental mass (without membranes and

**Table 1**
Baseline characteristics of the 1670 twin pairs (3340 twins) of EFPTS.

| | Overall 1670 pairs 3340 twins | One placental mass[a] 1052 pairs 2104 twins | Separate placentas 618 pairs 1236 twins | p-value[b] |
|---|---|---|---|---|
| Zygosity and Chorionicity | | | | <0.001 |
| DZDC | 2224 (66.6 %) | 1188 (56.5 %) | 1036 (83.8 %) | |
| MZDC | 344 (10.3 %) | 144 (6.8 %) | 200 (16.2 %) | |
| MZMC | 772 (23.1 %) | 772 (36.7 %) | 0 | |
| Birth weight (gram) | 2418 ± 525 | 2364 ± 523 | 2512 ± 515 | <0.001 |
| Total placental weight (gram) | 725 ± 173 | 705 ± 165 | 759 ± 180 | <0.001 |
| Separate placenta weight | | | 379 ± 99 | |
| BW/PW ratio | 6.7 (6–7.5) | 6.7(5.9–7.6) | 6.7 (5.9–7.6) | 0.28 |
| Umbilical cord insertion (=peripheral) | 910 (27.2 %) | 654 (31.1 %) | 256 (20.7 %) | <0.001 |
| Twin sex (=female) | 1694 (50.7 %) | 1108 (52.7 %) | 586 (47.2 %) | 0.004 |
| Sex of twin pair | | | | 0.008 |
| 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 %) | |
| Gestational age (weeks) | 36 (35–37) | 36 (34–37) | 36 (35–37) | <0.001 |
| Preterm | | | | <0.001 |
| <32 weeks | 222 (6.6 %) | 160 (7.6 %) | 62 (5.0 %) | |
| ≥32 and ≤ 36 weeks | 1686 (50.5 %) | 1104 (52.5 %) | 582 (47.1 %) | |
| >36 weeks | 1432 (42.9 %) | 840 (39.9 %) | 592(47.9 %) | |
| Parity (= primipara or higher) | 1594 (47.7 %) | 968(46.0 %) | 626 (50.6 %) | 0.01 |
| Age of mother (years) | 30.21 ± 4.63 | 30.04 ± 4.58 | 30.50 ± 4.70 | 0.01 |
| Neighbourhood income. euros | 19898 ± 3191 | 19915 ± 3180 | 19869 ± 3212 | 0.69 |
| Birth season | | | | 0.48 |
| Fall | 810 (24.3 %) | 494 (23.5 %) | 316(25.6 %) | |
| Spring | 830 (24.9 %) | 528 (25.1 %) | 302 (24.4 %) | |
| Summer | 842 (25.2 %) | 528 (25.1 %) | 314 (25.4 %) | |
| Winter | 858 (25.7 %) | 554 (26.3 %) | 302(24.6 %) | |

Legend: For continuous variables and with a normal distribution data is given as means and standard deviations and for non-normally distributed data as median and interquartile range. For categorical variables data is given as frequencies and percentages.DZ = dizygotic, MZ = monozygotic, DC dichorionic, MC monochorionic.

[a] 2 fused placentas (MZDC or DZDC) or one placenta (MZMC twins).

[b] for continuous variables with a normal distribution ANOVA-, with a non-normal distribution Kruskal Wallis test- and for categorical variables chi square test was performed.

**Table 2**
Maternal exposure characteristics.

| *Maternal exposure characteristics* | Overall 1670 pairs 3340 twins | One placental mass[a] 1052 pairs 2104 twins | Separate placentas 618 pairs 1236 twins | p-value[b] |
|---|---|---|---|---|
| $PM_{10}$ μg/m$^3$ | | | | |
| Trimester 1 | 30.8 ± 6.0 | 30.7 ± 6.0 | 31.0 ± 6.2 | 0.26 |
| Trimester 2 | 30.7 ± 6.2 | 30.7 ± 6.2 | 30.7 ± 6.3 | 0.95 |
| Trimester 3 | 30.2 ± 7.2 | 30.2 ± 7.4 | 30.2 ± 6.8 | 0.75 |
| Last month | 29.7 (23.8–35.3) | 29.9 (23.8–35.5) | 29.3 (23.9–34.9) | 0.64 |
| Last week | 27.6 (21.8–36.5) | 27.4 (21.5–36.6) | 27.4 (21.5–36.6) | 0.44 |
| Whole pregnancy | 30.7 ± 4.7 | 30.6 ± 4.6 | 30.7 ± 4.7 | 0.55 |
| $NO_2$ μg/m$^3$ | | | | |
| Trimester 1 | 24.5 ± 7.2 | 24.6 ± 7.2 | 24.4 ± 7.2 | 0.44 |
| Trimester 2 | 24.6 ± 7.3 | 24.7 ± 7.2 | 24.3 ± 7.3 | 0.1 |
| Trimester 3 | 24.3 ± 7.8 | 24.4 ± 7.8) | 24.2 ± 7.8 | 0.41 |
| Last month | 23.7 (18.3–29.3) | 23.9 (18.5–29.3) | 23.4 (18.2–29.4) | 0.27 |
| Last week | 23.1 (17.3–30) | 23.19 (17.6–30.22) | 23.1 (16.8–29.7) | 0.52 |
| Whole pregnancy | 24.5 ± 5.9 | 24.6 ± 7.2 | 24.3 ± 6 | 0.16 |

Legend: For continuous variables and with a normal distribution data is given as means and standard deviations and for non-normally distributed data as median and interquartile range.
[a] 2 fused placentas (MZDC or DZDC) or one placenta (MZMC twins).
[b] for continuous variables with a normal distribution ANOVA-, with a non-normal distribution Kruskal Wallis test was performed.

umbilical cord) was recorded and if two separate placentas were present, the individual placental weight was also noted.

Informed consent was obtained from the mothers at birth and ethical approval, including the registry data from 2001 to 2013, was given by Ghent University Hospital (METC: BC-04342) on the June 25, 2020.

### 2.3. Outcome

The outcome parameters included placental weight and BW/PW ratio. Two placenta groups were distinguished: (1) in case of one placental mass, the BW/PW ratio was calculated by dividing the combined birthweight of both twins by the total placental weight; (2) in cases of two separate placentas, placental weight and the BW/PW ratio were calculated for each twin individually. Additionally, if two separate placentas were present, the intrapair difference in placental weight and BW/PW ratio was calculated. Similar to the internationally accepted cut-off value for small for gestational age (SGA) for birth weight, a placental weight or BW/PW Ratio below the 10th percentile of this study sample is defined as an "SGA-placenta" or "SGA-BW/PW ratio" and was calculated for the two placenta groups separately.

**Table 3**
Change in placental weight (gram) in association with a 10 μg/m$^3$ increment in $PM_{10}$ or $NO_2$ exposure.

| Exposure | Total | | Term | | Moderate to late preterm | |
|---|---|---|---|---|---|---|
| | Beta[a] | 95 % CI | Beta[a] | 95 % CI | Beta[a] | 95 % CI |
| *Separate placentas* | | | | | | |
| **$PM_{10}$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 7.6 | −4.7-19.9 | 18.6 | −16.6-20.3 | 10.5 | −6.8-27.8 |
| Trimester 2 | 6.5 | −5.6-18.7 | 15.8 | −1.47-33.0 | −5.6 | −24.2-12.9 |
| Trimester 3 | −10.0 | −20.5-0.6 | −0.9 | −16.8-15.0 | −19.7 | −35.1 to −4.3[b] |
| Last Month | −7.9 | −16.9-1.0 | −2.9 | −15.6-9.9 | −16.8 | −30.3 to −3.3[b] |
| Last Week | −6.2 | −12.4-0.1 | −3.6 | −12.2-5.0 | −10.5 | −20.3 to −0.7[b] |
| Whole pregnancy | 5.4 | −13.2-2.4 | 14.2 | −12.5-40.8 | −0.93 | −37.3-18.7 |
| **$NO_2$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 3.6 | −5.6-12.9 | 6.8 | −6.3-19.9 | −0.37 | −14.2-13.5 |
| Trimester 2 | −2.6 | −11.7-6.5 | 7.5 | −5.7-20.6 | −13.8 | −27.2 to −0.5[b] |
| Trimester 3 | −10.5 | −18.9 to −2.1[b] | −4.15 | −16.2-7.9 | −17.7 | −30.4 to −0.5[b] |
| Last Month | −9.4 | −17.5 to −1.2[b] | −4.1 | −15.3-7.1 | −17.9 | −30.7 to −5.1[b] |
| Last Week | −8.0 | −14.9 to −1.0[b] | −4.6 | −14.2-5.0 | −12.6 | −23.5 to −1.7[b] |
| Whole pregnancy | −3.8 | −15.0-7.3 | 5.4 | −10.6-21.4 | −15.5 | −32.4-1.3 |
| *One placental mass* | | | | | | |
| **$PM_{10}$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 5.4 | −11.9-22.8 | −5.9 | −37.3-25.6 | 11.0 | −11.0-32.9 |
| Trimester 2 | 0.9 | −15.5-17.3 | −4.8 | −34.1-24.5 | 3.1 | −18.0-24.2 |
| Trimester 3 | −2.8 | −16.5-10.9 | 7.8 | −18.2-33.7 | −7.4 | −24.3-9.6 |
| Last Month | −1.9 | −13.7-9.9 | 2.1 | −19.4-23.7 | −5.3 | −20.0-9.2 |
| Last Week | −3.0 | −10.9-4.8 | −2.5 | −16.1-11.0 | −1.3 | −11.2-8.7 |
| Whole pregnancy | 4.3 | −21.5-30.1 | −2.4 | −50.6-45.8 | 5.1 | −27.5-37.6 |
| **$NO_2$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 5.1 | −7.3-17.6 | −0.3 | −21.2-20.6 | 8.0 | −8.6- 24.5 |
| Trimester 2 | 2.0 | −10.5-14.5 | −0.4 | −22.3-21.5 | 0.19 | −15.9-16.3 |
| Trimester 3 | −2.0 | −13.8-9.8 | 1.1 | −20.2-22.5 | −4.31 | −19.2-10.6 |
| Last Month | −0.2 | −11.4-11.0 | 0.7 | −19.5-20.9 | −3.3 | −17.3-10.7 |
| Last Week | −2.2 | −11.6-7.2 | −0.6 | −16.5-15.3 | −2.9 | −15.3-9.5 |
| Whole pregnancy | 4.1 | −11.5-19.6 | 0.1 | −26.9-27.2 | 2.7 | −17.5-22.8 |

Legend.
[a] adjusted for birth year, gestational age and parity.
[b] $p < 0.05$.

### 2.4. Exposure

Between 2001 and 2013 exposure to particulate matter with a diameter of less than 10 μm ($PM_{10}$) and to nitrogen dioxide ($NO_2$) was estimated at the maternal home addresses using a combination of monitoring stations (n = 19 for $PM_{10}$ and n = 44 for $NO_2$) and a spatial temporal interpolation method (Kriging). The method utilizes land cover data from satellite images (Corine land cover data) and provides interpolated data for daily exposure to air pollutants in areas of 4x4 $km^2$. Mean exposures for the whole pregnancy, each trimester, last month and last week were calculated. Levels of $PM_{2.5}$ were only available after 2006. In addition, in 2010 a new monitoring station was installed in East Flanders (Ghent) leading to higher concentrations of $PM_{2.5}$ after 2010, making these exposure data less reliable. Therefore, we decided not to include the $PM_{2.5}$ data in our main analysis.

### 2.5. Covariates

The following covariates were selected a priori: gestational age, twin's birth year, maternal age, sex, parity (primiparity vs. multiparity), socioeconomic status, zygosity-chorionicity group, umbilical cord insertion (central vs. peripheral) and birth season (winter, spring, summer, fall). Gestational age was based on the last menstruation or a first trimester ultrasound and was calculated as the number of completed weeks of pregnancy. Socioeconomic status (SES) was approximated by the median income of the neighbourhood as described before [17].

### 2.6. Statistical analysis

Data management and statistical analysis were performed using IBM SPSS Statistics version 26 (S.B.), SAS version 9.4 software package (SAS Institute Inc., Cary, NC, USA) (M.G) and R version 4.3.0 (A.Z.). All reported p-values are two-sided and considered statistically significant when $p < 0.05$ unless stated otherwise. Distribution of all variables was inspected. Analysis of variance (ANOVA) was performed for continuous variables with a normal distribution and the Kruskal-Wallis Test was used for variables that were not normally distributed. Chi-Square analysis was conducted for categorical variables.

We stratified the analyses a priori using the international cut-off values for preterm and very preterm birth: term (>36 weeks), moderate to late preterm (32–36 weeks), and very preterm (<32 weeks).

Since determining the actual portion of the placenta belonging to one twin when one placental mass is present is unknown, we adopted a clinically relevant and statistically valid strategy. Dividing the weight of one placental mass into two halves would not accurately reflect the clinical reality, so we analyzed the groups with one placental mass and those with two separate placentas separately. Furthermore, we assessed the interaction between $NO_2$ or $PM_{10}$ and the number of placentas in a linear regression analysis using all twins as pairs (Supplementary Table 1). Given the significance of the interaction term in a part of the analysis and the clinical reasons, stratified analysis was justified.

In case of two separate placentas, linear mixed model analysis was conducted. To account for the relatedness between the twins, a random intercept was added. In case of one placental mass, the twin pair was used as the unit of the analyses and linear regression analysis was performed.

First, univariable analyses were conducted to determine the crude

**Table 4**
Change in BW/PW Ratio in association with a 10 μg/$m^3$ increment in $PM_{10}$ or $NO_2$ exposure.

| Exposure | Total | | Term | | Moderate to late preterm | |
|---|---|---|---|---|---|---|
| | Beta[a] | 95 % CI | Beta[a] | 95 % CI | Beta[a] | 95 % CI |
| *Separate placentas* | | | | | | |
| **$PM_{10}$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | −0.1 | −0.3-0.1 | −0.0 | −0.4-0.3 | −0.1 | −0.4-0.2 |
| Trimester 2 | −0.1 | −0.4-0.1 | −0.3 | −0.7-0.0 | 0.1 | −0.2-0.4 |
| Trimester 3 | 0.1 | 0.1–0.3 | −0.3 | −0.7-0.0 | 0.5 | 0.2–0.7[d] |
| Last Month | 0.1 | −0.1-0.2 | −0.1 | −0.3-0.2 | 0.3 | 0.1–0.5[c] |
| Last Week | 0.1 | −0.0-0.2 | 0.1 | −0.1-0.2 | 0.2 | 0.0–0.3[b] |
| Whole pregnancy | −0.1 | −0.4-0.2 | −0.4 | −0.9-0.1 | 0.3 | −0.2-0.7 |
| **$NO_2$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | −0.1 | −0.2-0.1 | −0.0 | −0.4-0.1 | 0.1 | −0.1-0.3 |
| Trimester 2 | −0.0 | −0.2-0.1 | −0.3 | −0.6–−0.1 | 0.2 | 0.0–0.5[b] |
| Trimester 3 | 0.1 | −0.1-0.3 | −0.2 | −0.4-0.1 | 0.4 | 0.2–0.6[d] |
| Last Month | 0.1 | −0.1-0.2 | −0.1 | −0.4-0.1 | 0.4 | 0.1–0.6[c] |
| Last Week | 0.1 | −0.0-0.2 | 0.0 | −0.2-0.2 | 0.3 | 0.1–0.4[c] |
| Whole pregnancy | 0.0 | −0.2-0.2 | −0.3 | −0.5-0.0 | 0.3 | 0.0–0.5[b] |
| *One placental mass* | | | | | | |
| **$PM_{10}$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | 0.0 | −0.2-0.2 | 0.12 | −0.2-0.5 | −0.0 | −0.3-0.3 |
| Trimester 2 | −0.0 | −0.2-0.2 | 0.0 | −0.3-0.4 | −0.0 | −0.3-0.3 |
| Trimester 3 | 0.1 | −0.1-0.2 | 0.1 | −0.3-0.4 | −0.1 | −0.3-0.2 |
| Last Month | 0.0 | −0.1-0.1 | 0.1 | −0.1-0.3 | −0.1 | −0.2-0.1 |
| Last Week | 0.0 | −0.1-0.1 | 0.1 | −0.1-0.2 | −0.1 | −0.2-0.1 |
| Whole pregnancy | −0.0 | −0.3-0.3 | 0.1 | −0.4-0.6 | −0.1 | −0.5-0.3 |
| **$NO_2$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | −0.1 | −0.2-0.1 | −0.0 | −0.3-0.2 | −0.1 | −0.3-0.1 |
| Trimester 2 | −0.1 | −0.2-0.1 | −0.1 | −0.3-0.2 | −0.1 | −0.3-0.1 |
| Trimester 3 | −0.1 | −0.2- 0.1 | 0.0 | −0.2-0.3 | −0.1 | −0.3-0.1 |
| Last Month | −0.1 | −0.2-0.1 | 0.01 | −0.1-0.3 | −0.1 | −0.3-0.1 |
| Last Week | 0.0 | −0.1-0.1 | 0.01 | −0.1-0.3 | −0.1 | −0.2-0.1 |
| Whole pregnancy | −0.1 | −0.3-0.1 | −0.0 | −0.3-0.2 | −0.1 | −0.4-0.1 |

Legend.
[a] Adjusted for birth year, gestational age, sex of the twin pair and birth season.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.

**Table 5**
Odds Ratio (OR) for a small for gestational age placenta in association with a 10 μg/$m^3$ increment in $PM_{10}$ or $NO_2$ exposure.

| Exposure | Total | | Term | | Moderate to late preterm | |
|---|---|---|---|---|---|---|
| | OR[a] | 95 % CI | OR[a] | 95 % CI | OR[a] | 95 % CI |
| *Separate placentas* | | | | | | |
| **$PM_{10}$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | 1.00 | 0.40–2.50 | 0.93 | 0.28–3.10 | 1.11 | 0.28–4.41 |
| Trimester 2 | 0.88 | 0.36–2.17 | 0.89 | 0.28–2.84 | 1.10 | 0.27–4.43 |
| Trimester 3 | 1.23 | 0.54–2.79 | 0.89 | 0.29–2.78 | 1.81 | 0.51–6.41 |
| Last Month | 1.14 | 0.57–2.29 | 1.00 | 0.39–2.54 | 1.51 | 0.52–4.36 |
| Last Week | 1.15 | 0.71–1.87 | 1.16 | 0.63–2.16 | 1.21 | 0.54–2.71 |
| Whole pregnancy | 0.98 | 0.29–3.27 | 0.83 | 0.18–3.89 | 1.54 | 0.24–9.99 |
| **$NO_2$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | 1.02 | 0.46–2.25 | 0.92 | 0.33–2.59 | 1.30 | 0.39–4.36 |
| Trimester 2 | 1.09 | 0.51–2.36 | 0.97 | 0.35–2.73 | 1.44 | 0.44–4.69 |
| Trimester 3 | 1.13 | 0.44–2.88 | 0.93 | 0.27–3.21 | 1.75 | 0.41–7.52 |
| Last Month | 1.20 | 0.61–2.36 | 0.99 | 0.42–2.36 | 1.74 | 0.56–5.39 |
| Last Week | 1.19 | 0.67–2.11 | 1.06 | 0.51–2.21 | 1.43 | 0.56–3.69 |
| Whole pregnancy | 1.13 | 0.44–2.88 | 0.93 | 0.27–3.21 | 1.75 | 0.41–7.52 |
| *One placental mass* | | | | | | |
| **$PM_{10}$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | 0.55 | 0.35–0.88[b] | 0.57 | 0.26–1.24 | 0.57 | 0.28–1.15 |
| Trimester 2 | 0.69 | 0.45–1.06 | 0.39 | 0.18–0.86[b] | 1.07 | 0.57–2.00 |
| Trimester 3 | 1.14 | 0.82–1.58 | 0.74 | 0.39–1.43 | 1.46 | 0.92–2.33 |
| Last Month | 1.21 | 0.91–1.61 | 0.97 | 0.59–1.60 | 1.51 | 1.01–2.26[b] |
| Last Week | 1.20 | 1.00–1.44[b] | 1.17 | 0.88–1.54 | 1.16 | 0.88–1.53 |
| Whole pregnancy | 0.47 | 0.24–0.92[b] | 0.19 | 0.06–0.69[b] | 0.95 | 0.35–2.56 |
| **$NO_2$, 10 μg/$m^3$** | | | | | | |
| Trimester 1 | 0.60 | 0.42–0.84[c] | 0.62 | 0.36–1.05 | 0.60 | 0.34–1.03 |
| Trimester 2 | 0.81 | 0.59–1.12 | 0.52 | 0.30–0.92[b] | 1.23 | 0.76–1.98 |
| Trimester 3 | 1.14 | 0.86–1.53 | 0.95 | 0.58–1.55 | 1.45 | 0.95–2.21 |
| Last Month | 1.20 | 0.91–1.58 | 1.07 | 0.69–1.68 | 1.48 | 0.99–2.20 |
| Last Week | 1.23 | 0.98–1.55 | 1.19 | 0.85–1.67 | 1.39 | 0.97–1.99 |
| Whole pregnancy | 0.69 | 0.45–1.04 | 0.51 | 0.26–1.02 | 1.01 | 0.55–1.89 |

Legend.
[a] Twins with separate placentas in total were adjusted for sex, no adjustments for preterm or term born twins. Twins with fused placentas in total were adjusted for gestational age, birth year, parity, sex of the pair-term born were adjusted for maternal age, median income, sex of the pair, zygosity/chorionicity, and moderate to late preterm were adjusted for gestational age, birth year and parity.
[b] $p < 0.05$.
[c] $p < 0.005$.

associations between placental weight or BW/PW ratio and early-life environmental exposures. Next, multivariable analyses with backward elimination using the a priori selected covariates (gestational age, birth year, maternal age, sex of the twins, parity, socioeconomic status, zygosity-chorionicity-group, side of umbilical cord insertion and birth season) were carried out separately for every combination of outcome (PW, BW/PW ratio, SGA-placenta, SGA-BW/PW ratio) and group (one placental mass or two placentas). Covariates were included based on a significance level of $p < 0.20$. To investigate the environmental co-effect of $PM_{10}$ and $NO_2$ multicollinearity was assessed with the Variance Inflation Factor (VIF). Next, a multiplicative model, which included an interaction term and an additive model in which one exposure was additionally adjusted for the other exposure were built.

#### 2.6.1. *Within-pair analysis*

To test for asymmetric growth univariable and multivariable linear regression analyses were conducted with the intrapair difference of placental weight or BW/PW ratio as outcome using the same strategy as mentioned above.

#### 2.6.2. *"SGA-placenta" and "SGA-BW/PW ratio"*

As there are no widely used clinical definitions for "SGA-placenta" and "SGA-BW/PW ratio", we used our study sample to estimate cut off values for the 10th percentile. The 10th percentile was calculated per gestational week. A regression line was fitted through these values and the estimated 10th percentile values of the regression line were used as the cut off value for the 10th percentile. A linear model was found to best fit the data, except for the group with two separate placentas, where a model including a quadratic term provided the best fit for "SGA-BW/PW ratio".

## 3. Results

The baseline characteristics of the study population, which consisted of 1670 twin pairs, are presented in Table 1. Twins with one placental mass had significantly lower placental weights, birth weight, shorter gestational age, maternal age, lower proportion of primiparity and had more often a peripheral insertion of the umbilical cord compared to twins with separate placentas. The BW/PW ratio did not differ significantly between both groups (overall BW/PW ratio 6.7 (IQR 6–7.5)). The mean air pollution exposure during the whole pregnancy for all twin pairs was 30.7 ± 4.7 μg/$m^3$ for $PM_{10}$ and 24.5 ± 5.9 μg/$m^3$ for $NO_2$ and did not differ significantly between the two placenta groups (Table 2).

The interaction term of $NO_2$ with number of placentas was significant when analyzing all twins as pairs in the third trimester and last week. For preterm born twins the interaction term was significant in the third trimester and last month in univariable and multivariable analysis (Supplementary Table 1). Given the significance of the interaction term in a part of the analysis and the clinical reasons, we present the results for the two placenta groups separately.

### 3.1. *The stratified association between air pollution and placental weight*

$PM_{10}$ was negatively associated with placental weight in the third trimester, last month and last week in multivariable analysis in twins with separate placentas born moderate to late preterm (Table 3). In the

**Table 6**
Odds Ratio (OR) for a small for gestational age BW/PW ratio in association with a 10 μg/m$^3$ increment in $PM_{10}$ or $NO_2$ exposure.

| Exposure | Total | | Term | | Moderate to late preterm | |
|---|---|---|---|---|---|---|
| | OR[a] | 95 % CI | OR[a] | 95 % CI | OR[a] | 95 % CI |
| *Separate placentas* | | | | | | |
| **$PM_{10}$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 1.53 | 0.52–4.55 | 1.18 | 0.14–9.59 | 1.34 | 0.35–5.16 |
| Trimester 2 | 1.36 | 0.47–3.90 | 1.31 | 0.17–10.36 | 1.21 | 0.32–4.58 |
| Trimester 3 | 0.93 | 0.35–2.47 | 0.82 | 0.10–6.58 | 0.85 | 0.24–3.01 |
| Last Month | 0.95 | 0.40–2.21 | 0.84 | 0.15–4.82 | 0.87 | 0.30–2.57 |
| Last Week | 0.84 | 0.44–1.62 | 0.79 | 0.20–3.09 | 0.78 | 0.33–1.88 |
| Whole pregnancy | 1.59 | 0.38–6.71 | 1.18 | 0.08–18.08 | 1.32 | 0.05–1.06 |
| **$NO_2$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 1.17 | 0.45–3.05 | 1.14 | 0.19–6.90 | 0.93 | 0.27–3.19 |
| Trimester 2 | 0.90 | 0.35–2.27 | 0.94 | 0.15–6.06 | 0.79 | 0.24–2.58 |
| Trimester 3 | 0.72 | 0.29–1.76 | 0.62 | 0.10–4.03 | 0.70 | 0.22–2.22 |
| Last Month | 0.77 | 0.33–1.82 | 0.70 | 0.13–3.79 | 0.73 | 0.23–2.27 |
| Last Week | 0.81 | 0.38–1.71 | 0.76 | 0.18–3.31 | 0.77 | 0.06–1.07 |
| Whole pregnancy | 0.91 | 0.29–2.86 | 0.84 | 0.09–8.14 | 0.73 | 0.16–3.29 |
| *One placental mass* | | | | | | |
| **$PM_{10}$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 1.02 | 0.98–1.05 | 1.02 | 0.96–1.07 | 1.02 | 0.98–1.06 |
| Trimester 2 | 1.04 | 1.00–1.07[b] | 1.05 | 0.99–1.11 | 1.02 | 0.98–1.06 |
| Trimester 3 | 1.01 | 0.98–1.03 | 1.01 | 0.97–1.06 | 1.01 | 0.97–1.04 |
| Last Month | 1.01 | 0.98–1.03 | 1.00 | 0.96–1.04 | 1.01 | 0.98–1.04 |
| Last Week | 1.00 | 0.98–1.01 | 0.99 | 0.96–1.02 | 1.00 | 0.98–1.02 |
| Whole pregnancy | 1.04 | 1.00–1.09 | 1.05 | 0.97–1.13 | 1.03 | 0.97–1.09 |
| **$NO_2$, 10 μg/m$^3$** | | | | | | |
| Trimester 1 | 1.02 | 0.99–1.04 | 1.04 | 1.00–1.09 | 1.00 | 0.96–1.03 |
| Trimester 2 | 1.03 | 1.00–1.06[b] | 1.04 | 0.99–1.09 | 1.01 | 0.97–1.05 |
| Trimester 3 | 1.01 | 0.98–1.03 | 1.00 | 0.96–1.05 | 1.01 | 0.98–1.04 |
| Last Month | 1.01 | 0.98–1.03 | 0.99 | 0.95–1.03 | 1.01 | 0.98–1.04 |
| Last Week | 1.00 | 0.98–1.02 | 0.99 | 0.96–1.03 | 1.00 | 0.98–1.03 |
| Whole pregnancy | 1.03 | 1.00–1.06 | 1.05 | 0.99–1.11 | 1.01 | 0.96–1.05 |

Legend.
[a] Twins with separate placentas in total were adjusted for gestational age and sex, no adjustments for term born twins and moderate to late preterm were adjusted for sex. Twins with fused placentas in total were adjusted for zygosity/chorionicity and sex of the pair, term born were adjusted for median income, zygosity/chorionicity and sex of the pair, and moderate to late preterm were adjusted for sex of the pair.
[b] $p < 0.05$.

third trimester for every 10 μg/m$^3$ increase in $PM_{10}$ placental weight decreased −19.7 g (95 % C.I. −35.1 to −4.3). During the last month and last week of pregnancy, for every 10 μg/m$^3$ increase in $PM_{10}$ placental weight decreased −16.8 g (95 % C.I. −30.3 to −3.3) and −10.5 g (95 % C.I. −20.3 to −0.7) respectively.

Similar results were found for $NO_2$ exposure. In the third trimester for every 10 μg/m$^3$ increase of exposure to $NO_2$ placental weight decreased −10.5 g (95 % C.I. −18.9 to −2.1) in all twins and −17.7 g (95 % C.I. −30.4 to −0.5) in moderate to late preterm twins. During the last month of pregnancy for every 10 μm/m$^3$ increase of $NO_2$ placental weight decreased −9.4 g (95 % C.I. −17.5 to −1.2) in all twins and −17.9 g (95 % C.I. −30.7 to −5.1) in moderate to late preterm twins. Respectively, during the last week of pregnancy for every 10 μm/m$^3$ increase of $NO_2$ placental weight decreased −8 g (95 % C.I. −14.9 to −1.0) in all twins and −12.6 g (95 % C.I. −23.5 to −1.7) in moderate to late preterm. In addition, in twins born moderate to late preterm during the second trimester placental weight decreased −13.8 g (95 % C.I. −27.2 to −0.5) with every 10ug/m$^3$ increase of $NO_2$.

For twins with one placental mass or twins born at term, no association between $PM_{10}$ or $NO_2$ and placental weight was observed (Table 3).

### 3.2. The stratified association between air pollution and BW/PW ratio

$PM_{10}$ was positively associated with BW/PW ratio in the third trimester, last month and last week in multivariable analysis in twins with separate placentas born moderate to late preterm (Table 4). For every 10 μg/m$^3$ increase in $PM_{10}$ the BW/PW ratio increased 0.5 units (95 % C.I. 0.2 to 0.7) during the third trimester; 0.3 units (95 % C.I 0.1 to 0.5) during last month and 0.2 units (95 % C.I. 0.0 to 0.3) during last week respectively. Similar results were found for $NO_2$ exposure. For every 10 μg/m$^3$ increase in $NO_2$ BW/PW ratio increased 0.2 units (95 % C.I. 0.0 to 0.5) during second trimester; 0.4 units (95 % C.I. 0.2 to 0.6) during the third trimester; 0.4 units (95 % C.I. 0.1 to 0.6) during last month; 0.3 units (95 % C.I. 0.1 to 0.4) during last week; 0.3 units (95 % C.I. 0.0 to 0.5) during whole pregnancy.

For twins with one placental mass, no association between $PM_{10}$ or $NO_2$ and BW/PW ratio was observed (Table 4).

### 3.3. The environmental co-effect of $PM_{10}$ and $NO_2$ on placental weight and BW/PW ratio

Multicollinearity between the exposures was not a cause of concern (VIF <10). For placental weight the interaction term of the environmental co-effect of $PM_{10}$ and $NO_2$ in the multiplicative model was in one out of all interactions significant (Supplementary Tables 2 and 3). In the additive model, one significant co-effect of $PM_{10}$ and $NO_2$ was found in twins with separate placentas in the second trimester (Supplementary Tables 2 and 3). For BW/PW ratio only one co-effect was present (Supplementary Tables 4 and 5).

### 3.4. Within-pair analysis

There was no asymmetric growth in twins with separate placentas. No associations between $PM_{10}$ or $NO_2$ and intrapair difference in placental weight or BW/PW ratio were present (Supplementary Tables 6 and 7).

### 3.5. "SGA-placenta" and "SGA- BW/PW ratio"

In the one placental mass group for all twins, $PM_{10}$ exposure during the last week of pregnancy was associated with a higher OR for an "SGA-placenta"; 1.20 (95 % C.I. 1.00 to 1.44) and during the whole pregnancy with a lower OR of 0.47 (95 % C.I. 0.24–0.92) (Table 5).

Analysis of air pollutant exposure in the one placental mass group including all twins during the first trimester revealed a lower OR for an "SGA-placenta". The OR for increased exposure to $PM_{10}$ was 0.55 (95 % C.I. 0.35 to 0.88) and for $NO_2$ 0.60 (95 % C.I. 0.42 to 0.84).

In term born twins, exposure to $PM_{10}$ during the second trimester and the whole pregnancy was associated with a lower OR for an "SGA-placenta"; OR of 0.39 (95 % C.I. 0.18 to 0.86) and 0.19 (95 % C.I. 0.06 to 0.69) respectively. In line with this $NO_2$ in term born twins during the second trimester was associated with an "SGA-placenta" with an OR of 0.52 (95 % C.I. 0.30 to 0.92).

$PM_{10}$ and $NO_2$ were associated with "SGA-BW/PW ratio" in multivariable logistic regression analysis in all twins with one placental mass during the second trimester. For every 10 $\mu g/m^3$ increase in $PM_{10}$ the OR for an "SGA-BW/PW ratio" was 1.04 (95 % C.I. 1.00 to 1.07) and for every 10 $\mu g/m^3$ increase in $NO_2$ the OR was 1.03 (95%C.I. 1.00 to 1.06) (Table 6).

## 4. Discussion

In this study we investigated the association between placental weight and BW/PW ratio and prenatal exposure to air pollution. We observed several associations, which differ (1) between term and moderate to late preterm born twins, (2) per trimester and (3) between the one and two placenta groups.

In the case of separate placentas, higher exposure to $PM_{10}$ and $NO_2$ was associated with a significant reduction in placental weight in moderate to late preterm born twins in the third trimester. Additionally, we observed a higher BW/PW ratio in late gestation in those twins. In the case of one placental mass twins, air pollution exposure during the first trimester was associated with a lower odds ratio (OR $<$ 1) for an "SGA-placenta". While air pollution exposure in the last month and last week of pregnancy was associated with a higher odds ratio (OR $>$ 1) for an "SGA-placenta".

The negative effect of air pollutants on birth weight and premature birth has been shown by several studies [23–25]. In addition, some studies examined the critical time window during pregnancy for birth weight. Air pollution is associated with a lower birth weight in mid and late pregnancy [26] respectively second and third trimester [27]. The effects of maternal $PM_{10}$ exposure on birth weight are higher for moderately preterm (32–36 weeks) than term born neonates [28]. In contrast to birth weight only a few studies have focused on how air pollutants affect the placenta. Exposure to air pollution has been associated with lower placental weight in singletons [5]. The decrease in placental weight described by van den Hooven et al. [5] was −11.8 g for exposure to $PM_{10}$ and -10.7 g for $NO_2$ in the prior two months before date of birth, which would be comparable to our results of the last trimester. Interestingly, the loss in placental weight for $PM_{10}$ was almost twice as high in our study, which could support the hypothesis that the twin placenta is more vulnerable to environmental influences.

In clinical practice and in large birth cohort studies, placental weight can easily be measured or collected and the BW/PW ratio could be used as a proxy of placental efficiency [9]. However, the actual function is not measured and lower placental weight might be associated with altered placental function [13]. In a previous study altered placental function such as higher maternal angiogenetic factors or a higher placental vascular resistance indices, has been shown in association with exposure to $NO_2$ and $PM_{10}$ [5]. There are also observations that exposure to air pollution during pregnancy is associated with nitrosative stress, epigenetic alterations and mitochondrial dysfunction in the placenta [29,30].

Our study complements the findings of Bijnens et al. [17], wherein an increase of 10 $\mu g/m^3$ $PM_{10}$ or $NO_2$ during pregnancy was associated with a decrease in birth weight of twins (−40.2 g and −27.3 g respectively) and highlights the importance of the placenta. In our study, higher exposure to air pollutants resulted in a lower placental weight and a higher BW/PW ratio during the last trimester, indicating the importance of the placenta as the placental weight decreases relatively more than birth weight. Moreover, in this study exposure to air pollutants at the beginning of pregnancy is associated with a lower chance of an "SGA-placenta". We hypothesize that the effect of prenatal exposure to air pollutants on the placental weight changes at different time windows: in the first two trimesters prenatal exposure leads to an overcompensation of growth in placental tissue to compensate for the negative effects of air pollution. This compensatory mechanism is finite and early compensation for the effects of air pollution might eventually result in a lower placental weight and a higher chance for an "SGA--placenta" in the third trimester of pregnancy. However, studies with longitudinal data, estimating placental weight via prenatal ultrasound at different time windows, are needed to confirm our hypothesis.

The associations between air pollution and placental weight and BW/PW ratio were only seen in twins with separate placentas. One reason could be, that one placental mass twins have a lower total placental weight per se [20]. In this study, the placenta weight of twins with one placental mass was 54 g lower than the total placenta mass of twins with separate placentas. Therefore, the effect of air pollutants on placental weight might be not as pronounced as in twins with separate placentas. Moreover, one placental mass twins include the monochorionic twins. As postulated by Machin [16] monochorionic twin pregnancies differ from dichorionic as the monochorionic placenta mimics the structure of a singleton placenta. There could be unknown variables which mask the effect of air pollution on placental weight in monochorionic twins. Moreover, a study showed that zygosity and chorionicity do influence placental weight in twins with one placental mass; the placental growth curve of monozygotic-dichorionic twins is lower than the that of dizygotic-dichorionic and monozygotic-monochorionic ones [20].

The association between air pollution and placental weight was mainly seen in twins between 32 and 36 weeks of gestational age. Placental weight is likely to be most affected during this period, as it is when the placenta experiences the most growth. Our hypothesis is that placental weight gain in a twin pregnancy is restricted after 36 weeks due to intrauterine crowding. However, in the case of two placentas, the smaller placenta can continue to grow after 36 weeks to compensate for the adverse effects of air pollution on placental weight. Consequently, the negative impact of air pollution may not be observable after 36 weeks. As shown by Gielen et al. [20] the placental weight in twins growths with higher gestational age but stabilizes at week 40.

One of the main strengths of this study is the inclusion of relevant confounders related to placentation namely zygosity, chorionicity, number of placentas and side of the umbilical cord insertion, to study the intrauterine environment in depth. Other strengths of this study are the large sample size and its population-based design, minimizing selection bias. The availability of the residential address of the mother instead of area-based data as well the trimester specific availability of $NO_2$ and $PM_{10}$ is an advantage. We also recognize some limitations of the present study. First, measurement of the air pollutants based on the maternal residential address at birth, might not fully reflect exposure to air pollution because no information about time spent in other surroundings was collected and mothers may not have lived at the same address throughout the whole pregnancy. Second, information about maternal smoking status, maternal anthropometric measures, pregnancy complications (hypertensive disorders or gestational diabetes), perinatal infections, diet and drinking behaviour of mothers, which are all known risk factors for adverse perinatal outcomes is lacking [31]. In previous studies, investigating the association of $PM_{10}$ and placental mitochondrial damage and $PM_{2.5}$ and DNA-methylation of placental tissue, BMI appeared not to be a significant covariate and adjustment for smoking status and pregnancy complications, did not change the results [30,32]. Third, we did not adjust for the air pollutants black carbon, as the concentration of black carbon is measured since 2010 in East Flanders and was therefore not available during the study period, and ozone ($O_3$) because of a high seasonal fluctuation [33]. The production of ozone is catalysed by sunlight and $O_3$ concentrations are highest during the spring and summer in Belgium [34]. Fourth, the use of cross-sectional data instead of longitudinal data because growth itself is not measured. Nevertheless, a notable advantage is the use of actual weights instead of estimated weights.

In conclusion this study shows that prenatal exposure to $PM_{10}$ and $NO_2$ has trimester specific effects on placental weight, differs for twins with one versus two placentas and is most pronounced in moderate to late preterm twins. Longitudinal studies are needed to better understand the relationship between air pollutant exposure and placental weight evolution across different trimesters. Finally, the results are relevant for public health because they support the hypothesis that adverse pregnancy outcomes, especially lower placental weight, could be prevented if air pollution would be reduced.

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## CRediT authorship contribution statement

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

## 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.placenta.2024.07.309.

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