Maternal exposure to air pollutants, PCSK9 levels, and fetal growth – an Italian cohort

Chiara Macchi[1], Simona Iodice[2], Nicola Persico[1], Luca Ferrari[1], Laura Cantone[2], B Ischia[2], Maria Francesca Greco[2], Elena Dozio[2], Alberto Corsini[2], Cesare Sirtori[2], Valentina Bollati[1], and Massimiliano Ruscica[2]

[1]University of Milan
[2]Affiliation not available

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Abstract

Objective. Exposure to airborne pollutants during pregnancy appears to be associated with uterine growth restriction and adverse neonatal outcome. Proprotein convertase subtilisin/kexin type (PCSK9) is a key modulator of low-density lipoprotein (LDL) metabolism, and increases following short term particulate matter (PM10) exposure. Because maternal cholesterol is required for fetal growth, PCSK9 levels could be used to evaluate the potential impact of airborne pollutants on fetal growth. Design. A cohort of 134 healthy women during early pregnancy (11–12 weeks of gestational age) was studied. Results. A significant association was found between circulating PCSK9 levels and three tested air pollutants (PM10, PM2.5, nitric oxide (NO2)). Of importance, gestational age at birth was reduced by approximately 1 week for each 100 ng/mL rise in circulating PCSK9 levels. This effect became more significant at the highest quartile of PM2.5 (with a 1.8 week advance in delivery date for every 100 ng/mL rise in circulating PCSK9). This finding was supported by a significant elevation of the odds ratio for urgent cesarean delivery for each 100 ng/mL rise in PCSK9 (2.99, 95% CI, 1.22–6.57), with similar trends being obtained for PM10 and NO2. Conclusions. The association between exposure to air pollutants during pregnancy and elevation in PCSK9 advances our understanding of the unforeseen influences of environmental exposure in terms of pregnancy associated disorders.

1. Introduction

The developmental origins of the health and disease hypothesis state that exposure during the intrauterine period of life modulates the risk of disease later in life. In particular, low birth weight has been associated to an increased risk of hypertension, diabetes, and cardiovascular disease (1). Therefore, investigations on how environmental exposure, such as air pollution, affects fetal growth and the duration of pregnancy represent a crucial step in defining pathways that link prenatal exposure, intrauterine stress, and future outcomes.

The possible link between exposure to air pollutants and fetal growth has been investigated by a growing number of studies (2, 3). The effects of air pollutants, particularly fine particulate matter (PM) and nitrogen dioxide (NO_2), mostly contribute towards restricting in utero growth (4-10) or adverse neonatal outcomes, such as reduced birthweight and prematurity (11-17). Although many methodological issues exist, such as small sample size and the large variety of approaches used to determine pollutant exposure, collectively these studies support the association between increased maternal exposure and reduced fetal growth.

A key requirement of fetal growth is the maternal supply of cholesterol, the uptake of which is largely mediated by the LDL receptor (LDL-R) which is expressed abundantly in the placenta but expressed at low levels, if at all, in the yolk sac (18). However, the mechanisms by which placental endothelial cells transport cholesterol to the fetal microcirculation, the regulation of efflux, and their ability to deliver substantial quantities of cholesterol are still unknown (19).

Proprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as a major modulator of LDL metabolism, as well as being a marker of cardiovascular risk. We previously reported increased levels of PCSK9 after short term PM exposure, with this phenomenon being particularly significant in individuals with a lower inflammatory burden,based on an assessment of plasma interferon levels (20). These individuals represent hypersusceptible subjects, who are more sensitive to the damaging effects of exposure to environmental PM.

PCSK9 was initially discovered as an anti-apoptotic mediator in the brain (21). PCSK9 levels in pregnant women might provide important information on the potential use of the currently available PCSK9 monoclonal antibodies in these subjects. Reduced PCSK9 levels in rat embryos was associated with the occurrence of neural tube defects (22), indicating the sensitivity of this biomarker in pregnancy. Consequently, it was hypothesized that the potential reduction of neuronal inflammation and amyloid ?-aggregation is antagonized by PCSK9 (23).

The present study aimed to characterize how air pollutants (*i.e.,*particulate matter, PM_{10} and $PM_{2.5}$) and NO_2 influence the PCSK9 levels of Italian women during early pregnancy (11–12 weeks of gestational age). Within this framework, we also investigated how these changes were correlated with fetal growth (i.e. birth weight and gestational age at birth). Pregnant women are of particular interest because they have generally healthy life habits, particularly in the Western world. In this region of the world, the decline in the number of pregnancies has led to the greater care of child-bearing women, who are encouraged to follow a healthy diet and maintain physical exercise to not gain excess weight (24). This population, thus, provides a unique opportunity to evaluate the damage exerted by ambient pollution on both women and their fetuses.

2. Patients and Methods

Study design and participants. We recruited 134 healthy pregnant women at the "Clinica Mangiagalli", Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. The women were randomly selected from individuals who were attending prenatal healthcare clinics during the 11–12th week of pregnancy. Exclusion criteria included a history of illicit drug use, diabetes, hypertension, previous pregnancy with pre-eclampsia/eclampsia or gestational hypertension, and current use of acetylsalicylic acid or low-molecular-weight heparin. Information about demographics and lifestyle characteristics of the mother, such as smoking habits and alcohol consumption, were collected. An informed consent form was signed by all participants and the study was approved by the ethics committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (approval number 681/2017).

Clinical and laboratory measurements. Body weight and height were determined on a standard scale. Body mass index (BMI) was expressed as Kg/m^2. Systolic and diastolic blood pressure (SBP and DBP, respectively) were taken on the left arm using a mercury sphygmomanometer (mean of two measurements taken after 5 min of rest). Plasma lipids/lipoproteins and glucose were measured by certified enzymatic techniques on a Roche c311 autoanalyzer. Lipoprotein (a) [Lp(a)] levels were measured by immunoturbidimetry on a Roche c311 autoanalyzer. Standard evaluations for early pregnancy in Italy are serum pregnancy-associated plasma protein-A (PAPP-A), α-fetoprotein, and human chorionic gonadotropin (hCG). These parameters were measured at 11–12 weeks of gestation. Gestational age was calculated from the last menstrual period, and was verified by ultrasound parameters. In particular, fetal crown-rump length was used to estimate gestational age, and women were included if this parameter ranged between 45 and 84 mm.

Enzyme-linked immunosorbent assay (ELISA). Plasma PCSK9 concentrations were measured by a commercial ELISA kit (R&D Systems, MN). All patients fasted overnight and had blood sampled at around 09:00, thus minimizing any possible confounding effects of circadian variation in PCSK9 levels. In brief, samples were diluted 1:20 and incubated onto a microplate pre-coated with a monoclonal human PCSK9-specific antibody. Sample concentrations were obtained by a four-parameter logistic curve-fit, with a minimum detectable PCSK9 concentration of 0.219 ng/mL (25). Intra- and inter-assay CVs were 3.8% and 6.2%, respectively.

Air pollutant assessments. Daily air pollutant (PM_{10}, $PM_{2.5}$, and $NO_{2,}$) concentrations were derived from

the archives of the Regional Environmental Protection Agency (ARPA Lombardy). This organization collects data at a regional scale using the FARM (Flexible Air quality Regional Model) chemical-physical model of air quality (26). This model is a three-dimensional Eulerian model that simulates the dispersion and chemical reactions of atmospheric pollutants. The estimated levels of daily PM_{10}, $PM_{2.5}$, and NO_2concentrations were assigned to each subject for the day of evaluation and 14 days before blood was sampled. We also calculated the average exposure from the first week before the clinical visit and 12 weeks earlier (*i.e.,* weeks 0–1 being the mean over the first week of exposure and weeks 0–12 being the mean over the 12 weeks before the visit. All participants were assigned pollutant levels that were estimated in the Municipality of Milano, as 93% of the women lived or worked there.

Statistical analysis. Descriptive statistics were performed on all variables. Continuous variables were expressed as the mean ± standard deviation (SD) or as the median with first-, and third-quartile (Q1–Q3), as appropriate. Categorical data were reported as frequencies with percentages. Descriptions of each exposure variable were given by the means of box-plots, describing pollutants at each averaged time window. We applied univariate and multivariable linear regression models to evaluate the relationship between pollutant exposure (for each averaged one-week period from week 0–1 to week 0–12) and circulating PCSK9 levels. Each model was tested for normality and linearity. All potential confounders were included in the multivariate model after verifying the presence of an association in a univariate model. Best model selection was based on the minimization of the Akaike information criterion and maximization of the explained variance of the model. The final models were adjusted for low-density lipoprotein cholesterol (LDL-C), interleukine (IL)-6, fibrinogen, season, BMI, and smoking habit. Estimated effects are reported as β and standard error (SE) associated with an increase of 1 unit in each pollutant.

We examined the association between PCSK9 and the variables measured on the newborn (gestational age at birth, weight, length, cranial circumference, APGAR score), after adjusting each model for the pollutants most associated with PCSK9 levels in the multivariate analysis. Each model was also adjusted for birth mode (urgent caesarean, elective caesarean, and spontaneous delivery) and for the interaction between pollutant and PCSK9 concentrations. Using a univariate logistic regression, we evaluated the odds ratio of urgent cesarean delivery associated with a 100 mg/dL increase in PCSK9.

We calculated the q-FDR values using the multiple comparison method based on Benjamini-Hochberg False Discovery Rate (FDR), which takes the high number of comparisons into account, with a threshold of 0.10 to detect significance.

A sensitivity analysis was performed using the residential address for pollutant imputation, with no relevant changes being made to the results (data not shown). Statistical analyses were performed with SAS software, version 9.4.

3. Results

Study population **.** The study population included 134 pregnant women (age 33 ± 4 years). BMI was 22.6 ± 4.2 kg/m^2. Pregnancy associated endocrine factors, such as placental growth factor (PLGF) and pregnancy associated plasma protein A (PAPP-A), were in the normal range, as was hCG (Table 1). Signs of diabetes and dyslipidemias were not found. Specifically, median glucose was 86.6 ± 14.8 mg/dL. LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C), HDL-C, and triglycerides (TG) were also within the normal range (97.4 ± 22.1 mg/dL, 117.2 ± 25 mg/dL, 65.5 mg/dL and 99 ± 38.3 mg/dL, respectively). Levels of Lp(a) were 13.7 ± 19 mg/dL (Table 1). Circulating levels of plasma PCSK9 were normally distributed, with a mean of 193.7 ± 54.2 ng/mL (Figure 1). These levels were lower than those previously described by us for non-pregnant women recruited in the same geographical area (27). None of these healthy women were on any drug treatment. The estimated level of exposure to $PM_{10,}$ $PM_{2.5,}$ and NO_2, from the first week before the visit (week 0–1) and 12 weeks previously (week 0–12), are depicted in Figure 2. The similarities in pollutant concentrations across the weeks of exposure were clearly observed. Mean PM_{10} and NO_2concentrations remained beneath the annual regional air-quality standards of 40 $\mu g/m^3$. Mean $PM_{2.5}$concentrations were slightly higher than annual limits, which are set at 25 micrograms per $\mu g/m^3$.

Mean fetal crown-rump length at the time of exposure assessment was 62.4±5.2 mm. Nuchal translucency thickness, which is an ultrasound marker for chromosomal and structural abnormalities, was within the normal range in all cases, as well as fetal heart rate and ductus venosus blood flow (Table 1). All pregnancies ended with the live birth of a phenotypically normal neonate, at a mean gestational age of 38.7 ? 1.4 weeks. Neonatal biometric parameters are presented in Table 1.

Univariate data analysis. None of the three air pollutants was associated to circulating PCSK9 levels in the univariate analysis (Supplemental Table 1). As expected, the main lipid parameters related to CV risk, LDL-C (β = 0.605, SE = 0.235, p = 0.011) and non-HDL-C (β = 0.425, SE = 0.210, p = 0.045) were positively associated with PCSK9. Inflammatory markers, which are linked to the initiation and progression of atherosclerosis, were also evaluated. PCSK9 levels were positively associated with the IL-6 (β = 3.447, SE = 1.711, p = 0.046) and fibrinogen (β = 0.170, SE = 0.083, p = 0.043) levels. No relationship was obtained with the high-sensitivity C-reactive protein and with the adhesion molecules, ICAM and VCAM. Newborn features, *e.g.,*crown-rump length, fetal heart rate, and cranial circumference were not associated with PCSK9 levels (Supplemental Table 1).

Multivariable data analyses. To define the exposure window to air pollutants that was most effective in modifying PCSK9, we investigated how different time lags were associated with PCSK9 levels.

We observed a positive significant effect of PM_{10}exposure for all time lags. The effect on PCSK9 levels was maximal, as we considered the mean of the entire gestational period, *i.e.,*0–12 weeks (Table 2). In particular, for each 1-$\mu g/m^3$increase in PM_{10} concentration, we observed a significant increase in PCSK9 levels (β = 1.903, SE = 0.733, p = 0.011). This effect was also confirmed for NO_2 exposure, as every unit increase in NO_2 led to a 2.265 ng/mL rise in PCSK9 levels (β = 2.265, SE = 1.002, p = 0.026). For $PM_{2.5}$ exposure, a significant positive association was only detected with the 0–6 and 0–10-week time lags.

Association of PCSK9 concentrations with gestational age for different levels of exposure to pollutants. No association was found between PCSK9 measured at the first trimester of pregnancy and features of newborns at birth, such as weight and length at birth, cranial circumference at birth, and APGAR score (Supplemental table 1). Nevertheless, when the interaction between PCSK9 concentrations and gestational age at birth was taken into account, we observed a strong modifying effect of air pollutants, particularly $PM_{2.5}$. For different $PM_{2.5}$ levels (15 mg/m^3, 24 mg/m^3, 42 mg/m^3, and 55 mg/m^3, respectively; 25°, 50°, 75°, and 95° percentile), the association was significant at the highest $PM_{2.5}$ concentrations (*i.e.,* those in the 75^{th} and 95^{th} percentiles; Figure 3). For example, at a $PM_{2.5}$ concentrations of 42 mg/m^3, we observed an advance in delivery date of approximately 1 week every 100 ng/mL rise in circulating levels of PCSK9 (β = -0.810, SE = 0.332, p = 0.0164). The steepness of the association was more evident when the highest quartile of $PM_{2.5}$ was considered (*i.e.,* delivery advanced by 1.28 weeks for every 100 ng/mL change in PCSK9 levels; β = -1.282, SE = 0.498, p = 0.012). Overall, at fixed $PM_{2.5}$ concentrations of 42 mg/m^3 and 55 mg/m^3, for every 100 ng/mL increment in PCSK9, the gestational age decreased by 2.3% to 3.7% weeks. This finding was also supported by the observation that the odds ratio (OR) of urgent cesarean delivery associated with a 100 mg/dL rise in PCSK9 was 2.99 (95% CI 1.22–6.57). A similar trend was found when PM_{10} and NO_2 were considered (Supplemental table 3).

4. Discussion

The present study is the first to describe the levels of PCSK9 circulating in women during early pregnancy (first trimester; 134 women), demonstrating how air pollutants impact PCSK9 and its association with birth weight and gestational age at birth. In this study population, mean PCSK9 levels were lower than those previously described in non-pregnant women (27, 28). We observed an association between exposure to PM (in particular PM_{10}) and NO_2 and increased levels of PCSK9. PCSK9 levels were also associated to a decrease of gestational age at delivery and to an increased probability of urgent c-sections at delivery.

A previous study evaluated PCSK9 levels in pregnancy characterized by intrauterine growth restriction (IUGR), with lipid/lipoprotein levels being available for 70 patients *vs* 102 controls during late gestation. This previous study confirmed that the immunological expression of PCSK9 could be detected in the placenta,

as well as in fetal and maternal plasma. Plasma PCSK9 levels were at a higher range to those reported in the present study (29). PCSK9 levels were also reported in a study with a small sample size that compared normal pregnant women (n = 6), diabetics (n = 6), and overweight/obese women that were diabetic (n = 10) at term (30). The latter two groups had significantly reduced PCSK9 levels, with raised LDL receptor activity and reduced LDL-C levels. The authors attributed these findings to the maternal inflammatory status with raised placental cytokines, which is widely demonstrated in diabetic pregnancies (31). However, PM_{10} and $PM_{2.5}$ exposure has been associated with acute placental inflammation; thus, these pollutants might contribute to adverse pregnancy outcomes (32, 33). A clear mediator of these effects has not been reported yet. Inflammation and dyslipidemia early in pregnancy are independently associated with an increased risk of pre-term birth. However, this risk might be elevated when both conditions are present before 21 weeks of gestation (34). Recently, the ABCD (Amsterdam Born Children and Their Development) study showed that atherogenic lipid profiles during the first trimester confer an increased risk of adverse pregnancy outcomes, including maternal morbidity, mortality, and preterm delivery. TG levels, but not total cholesterol levels, during the first third of pregnancy were independently and positively associated with adverse pregnancy outcomes for both mother and newborn (35). Thus, considering that none of our child-bearing women were either dyslipidemic or pathologically inflamed, the present report presents a highly novel finding on the association between plasma PCKS9 elevation and time of delivery. Overall, the time of delivery was estimated to advance by between 0.8 and 1.8 weeks for every 100 ng/mL increment in PCSK9 levels. Moreover, the observation that the risk of experiencing an urgent cesarean section is significantly higher (OR = 2.99) in women with higher levels of PCSK9 indicates a general condition that is not perceived during pregnancy, but is related to peripartum risk.

Relative to circulating PCSK9 levels, the present report appears to provide results that are in line with the physiological rise of estradiol (E2) levels during pregnancy. In other words, maternal E2 levels progressively rise from 367 pM (luteal phase) to between 11,000 and 37,000 pM at the end of pregnancy (36). At approximately nine weeks of gestation, a hormonal ovary-to-placenta shift occurs, resulting in direct E2 placental production (36). Retrospective studies reported that, compared to pre-menopausal women, post-menopausal women with low endogenous estrogens have higher PCSK9 levels (27, 37, 38). Conversely, after *in vitro* fertilization, the stimulation of endogenous estrogens markedly reduces PCSK9 levels (38).

The lack of an association between PCSK9 levels and newborn features (*e.g.,* crown-rump length, nuchal translucency, fetal heart rate, and ductus venosus pulsatility index) might be of interest in the still questioned field of lipid management during pregnancy. This phenomenon is particularly relevant in the debate on whether implications exist for both the mother and unborn child. Also, knowledge remains limited on the safety of newer agents, such as PCSK9-inhibitory therapy with evolocumab or alirocumab. Of note, PCSK9 was initially identified as NARC-1 (neural apoptosis-regulated convertase-1), which was implicated in the differentiation of cortical neurons (39), and has been recently described as a possible modulator of brain cholesterol homeostasis (21). Studies evaluating the safety of the PCSK9 inhibitor evolocumab confirmed placental transfer to the infant (40); however, at present information remains unclear regarding its safety in pregnancy and lactation. This issue might be particularly pertinent for women with severe forms of familial hypercholesterolemia (FH), where this treatment would be required (41). Guidance on the care of familial hypercholesterolemia currently recommends that the use of statin/ezetimibe/niacin is stopped at least four weeks (preferably 12 weeks) before conception, and should not be used during pregnancy or lactation (42). The ongoing NCT 02957604 trial is enrolling 375 pregnant women with hypercholesterolemia, with and without atherosclerotic CV disease, who will be exposed to evolocumab for the whole pregnancy. These women will be followed to delivery or abortion and breastfeeding, if feasible. In addition, infants will be followed for five years post-partum.

CV risk factors in pregnancy have been evaluated by many investigators, indicating that in a large number of pregnancies are associated with preexisting CV risk factors, (34, 43-45). Thus, in addition to evaluating lipoprotein cholesterol concentrations, it was of interest to evaluate a key regulator of cholesterolemia (*i.e.,* PCSK9 levels) in the current study. This protein fosters the catabolism of the LDL receptor, thus increasing cholesterolemia (35). The highly significant correlation between PCSK9 levels and LDL-cholesterolemia is of

interest, since lipid levels during early pregnancy could be used to identify women at risk for hypertension and future CVD (34). A large meta-analysis by Sun et al. (46) reported a significant association between $PM_{2.5}$ (not PM_{10}) exposure and hypertension development, albeit to a modest degree. Ambient exposure appeared to be more strongly connected to the development of hypertension during the first and third trimesters. In our cohort, PCSK9 was not correlated with hypertension.

Moreover, complications occurring during pregnancy indicate a future increased risk for atherosclerotic disease (47). Epidemiologic data consistently showed the early onset cardiovascular disease in women who experienced pregnancy loss, preterm pregnancy, or pregnancy complicated by intrauterine growth restrictions. These phenomena possibly arise as a result of metabolic, endothelial, and inflammatory changes during complicated pregnancies. Based on these epidemiological observations, the American Heart Association recognizes pregnancy complications as independent risk factors for future CVD.

PM inhalation is an established trigger of CV events (48). Such events might occur within hours or days of exposure. Short-term exposure to PM pollution contributes to acute CV morbidity and mortality. In particular, long-term exposure to elevated PM levels is associated with a reduced life expectancy (49). A previous report on an obese population showed that 12- and six-month exposure to PM_{10} is associated with a significant rise in circulating PCSK9 levels, which are positively associated with the Framingham Risk Score (20). The results of the current study on pregnant women support the finding that ambient pollutants (*i.e.* PM_{10}, $PM_{2.5}$, and NO_2) are associated with raised PCSK9 levels. Consequently, the current study provides a further insight into the potential association of CV risk variables with pollutants. A meta-analysis of 21.09 million participants showed that each 10 $\mu g/m^3$ rise in $PM_{2.5}$ corresponds to an increased relative risk (RR) of total CVD events (RR 1.12, 95% CI 1.05–1.19), CVD incidence (RR 1.12, 95% CI 1.05–1.19), and CVD mortality (RR 1.11, 95% CI 1.08–1.14). A more robust association was obtained with NO_2, whereby every 10 $\mu g/m^3$ increase in NO_2 led to a higher risk of total CVD events (RR 1.36, 95% CI 1.09–1.64) and CVD mortality (RR 1.46, 95% CI 1.13–1.79) (50).

Conclusions

This study demonstrated that, in healthy pregnant women, PCSK9 levels are associated to the outcomes of pregnancy occurring at delivery (approximately 28–30 weeks after PCSK9 was quantified). These findings are of special interest because they suggest that PCSK9 regulation is modified early (possibly induced by air pollutants), which might have consequences on the entire pregnancy. Therefore, the results of this study advance current knowledge on how PCSK9 contributes to pregnancy, and how it is associated with a number of variables potentially linked to pregnancy diseases.

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Contribution to authorship: VB, MR, NP conceived the study and wrote the manuscript; CM measured PCSK9 and all the biochemical variables and wrote the manuscript; SI performed all the statistical analyses; AC and CS critically edited the manuscript; LF, LC, BI, MFG, ED collected all the data and helped in biochemical analyses.

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Figure legends.

Figure 1 . *Distribution of fasting plasma concentrations of PCSK9 in 134 pregnant women* . Levels of PCSK9 were measured using a sandwich ELISA in plasma samples obtained after women fasted overnight; see Subjects and Methods for details.

Figure 2. *Box plot showing exposure to PM_{10}, $PM_{2.5}$, and NO_2.*Exposure was evaluated as the mean from one week before the visit (week 0–1) and 12 weeks earlier (week 0–12). PM_{10}, $PM_{2.5}$, and NO_2 were averaged over mean daily concentrations.

Figure 3 . *Interaction effect of $PM_{2.5}$ and PCSK9 levels on gestational age* . Strength of association between PCSK9 and gestational age at four selected levels of PM2.5 (25°, 50°, 75°, and 95° percentile). Estimates were calculated from multivariate models adjusted for birth mode (spontaneous, urgent, and elective cesarean) and interaction between PCSK9 and PM2.5 at 0–10 weeks; the P-value for the interaction term was = 0.0258. Adjusted β regression coefficients are reported for 1 mg/m^3 increase in PCSK9 concentration.

Table 1. Clinical characteristics of the pregnant women and newborns

Characteristic	**Value**
Age, year	33.0 ± 4.0
Anthropometric and biochemical features	
BMI, Kg/m^2	22.6 ± 4.2
Categorical BMI	
Underweight (BMI < 18.5)	16 ± 11.9
Normal (18.5 [?] BMI < 25)	87 ± 64.9
Overweight (BMI > =25)	31 ± 23.1
Glucose, mg/dL	86.6 ± 14.8
TC, mg/dL	182.7 ± 30.3
LDL, mg/dL	97.4 ± 22.1
Lipoprotein a, mg/dL	13.7 ± 19.0
non-HDL-C, mg/dL	117.2 ± 25.0
HDL, mg/dL	65.5 ± 13.9
TG, mg/dL	99.0 ± 38.3
PCSK9, ng/mL	193.7 ± 54.2
ICAM, pg/mL	349373 ± 66161
VCAM, pg/mL	769251 ± 190723
CRP mg/dL	2.56 (1.64, 4.51)
Fibrinogen, mg/dL	146.8 ± 56.1
IL-6, pg/mL	1.7 (1.2, 2.1)
Smoking habits	
Never smoked	101 ± 75.4
Stopped during pregnancy	14 ± 10.5
Smoker	19 ± 14.2
Features related to pregnancy	
Parity	
Nulliparity	84 ± 62.7
Multiparity	50 ± 37.3
Pregnancy associated endocrine factors	
PAPPA, IU/L	1.3 ± 0.7
PLGF, pg/mL	33.4 ± 13.6
hCG, IU/L	53.7 ± 41.3
PAPPA MoM	1.2 (0.8, 1.6)
hCG MoM	0.9 (0.6, 1.5)
PLGF MoM	1.2 (0.8, 1.4)
Birth delivery mode	
Spontaneous	80 (70.2%)
Urgent cesarean	16 (14.0%)
Elective cesarean	18 (15.8%)
Gestational age at birth	38.7 ± 1.4
Fetal parameters	
Crown-rump length	62.4 ± 5.2
Nuchal translucency thickness	1.9 ± 0.4

Characteristic	Value
Fetal heart rate	160.4 ± 6.1
Ductus venosus pulsatility index	1 ± 0.1
Mena blood pressure, mmHg	85.8 ± 7.1
Neonatal parameters	
Weight (gr)	3239.8 ± 467.1
Length (cm)	49.9 ± 2
Cranial circumference (cm)	34.2 ± 1.6
APGAR score	9.8 ± 0.6

BMI, body mass index; CRP, c-reactive protein; hCG, human chorionic gonadotropin; HDL-C, high-density lipoprotein cholesterol; ICAM, Intercellular Adhesion Molecule 1, LDL-C, low-density lipoprotein cholesterol; PAPP-A, pregnancy-associated plasma protein-A; PLGF, placental growth factor; PCSK9, proprotein convertase/subtilisin kexin type 9; TC, total cholesterol; TG, triglycerides; VCAM, vascular cell adhesion molecule 1; MoM, multiple of median. For normal distributions, continuous values are expressed as mean ± standard deviation. When not normally distributed, values are expressed as medians (Q1, Q3). Categorical values are expressed as frequencies with percentages.

Table 2. Multivariate analyses reporting the associations among exposure to PM_{10}, $PM_{2.5}$, NO_2, and PCSK9 (ng/mL) levels.

Average exposure	**PM_{10}**	**PM_{10}**	**PM_{10}**	**$PM_{2.5}$**	**$PM_{2.5}$**	**$PM_{2.5}$**	**NO_2**	**NO_2**	**NO_2**
	β	SE	P-value	β	SE	P-value	β	SE	P-value
0–1 week	0.398	0.481	0.410	0.361	0.503	0.474	0.543	0.801	0.500
0–2 weeks	1.253	0.581	**0.034**	0.978	0.513	0.060	1.909	0.898	**0.036**
0–3 weeks	1.892	0.639	**0.004**	0.92	0.469	0.053	2.050	0.868	**0.020**
0–4 weeks	1.766	0.710	**0.015**	0.217	0.562	0.700	2.173	0.962	**0.026**
0–5 weeks	1.585	0.700	**0.026**	0.467	0.508	0.360	2.206	0.979	**0.027**
0–6 weeks	1.609	0.636	**0.013**	1.016	0.390	**0.011**	2.221	0.888	**0.014**
0–7 weeks	1.554	0.633	**0.016**	0.523	0.449	0.248	2.176	0.854	**0.013**
0–8 weeks	1.490	0.668	**0.028**	-0.157	0.524	0.765	2.038	0.868	**0.021**
0–9 weeks	1.512	0.675	**0.028**	0.584	0.528	0.271	2.022	0.896	**0.026**
0–10 weeks	1.695	0.672	**0.013**	1.156	0.492	**0.021**	2.197	0.926	**0.020**
0–11 weeks	1.857	0.695	**0.009**	0.866	0.482	0.076	2.333	0.943	**0.015**
0–12 weeks	1.903	0.733	**0.011**	-0.088	0.599	0.884	2.265	1.002	**0.026**

Exposure is evaluated as the average from the first week before the visit (0–1 weeks) and 12 weeks earlier (0–12). Significant P-values (P [?] 0.05) are reported in bold when the p-FDR is <0.10. β regression coefficients are reported for 1 $\mu g/m^3$increase in the concentration of each pollutant. Estimates were calculated from multivariate models adjusted for LDL-C (Low-Density Lipoprotein Cholesterol), IL-6 (interleukine-6), fibrinogen, season, BMI (body mass index), and smoking habits. PM, particulate matter; NO_2, nitrogen dioxide

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