Environmental Research 197 (2021) 111132

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Association between prenatal exposure to air pollutants and newborn thyroxine (T4) levels

Amaia Irizar [a,b,c,*], Arantxa Txintxurreta [d], Amaia Molinuevo [a], Alba Jimeno-Romero [c], Asier Anabitarte [a,c], Jon Iñaki Álvarez [e], María Dolores Martínez [a,f], Loreto Santa-Marina [a,b,d], Jesús Ibarluzea [a,b,c,d], Aitana Lertxundi [a,b,c]

[a] Biodonostia Health Research Institute, Group of Environmental Epidemiology and Child Development, 20014, San Sebastian, Spain
[b] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, C/Monforte de Lemos 3-5, 28029, Madrid, Spain
[c] Department of Preventive Medicine and Public Health, Faculty of Medicine, University of the Basque Country (UPV/EHU), 48940, Leioa, Spain
[d] Ministry of Health of the Basque Government, SubDirectorate for Public Health and Addictions of Gipuzkoa, 20013, San Sebastián, Spain
[e] Laboratory of Public Health of Department of Health of the Basque Government, Government of the Basque Country, Donostia, Spain
[f] Department of Economic Development, Sustainability and Environment, Territorial Delegation of Industria, Administration of Gipuzkoa, Government of the Basque Country, San Sebastian, Spain

ARTICLE INFO

Keywords:
Prenatal exposure
$PM_{2.5}$
NO_2
TT4 at birth
Sensitivity windows

ABSTRACT

Background: Thyroid hormones play a key role in fetal and child development. Recent studies have linked prenatal exposure to atmospheric contaminants with changes in thyroid hormone levels in newborns, but the data from the few studies that have explored this issue are inconclusive. The present study aims to assess the association of total thyroxine (TT4) levels in newborns with weekly prenatal exposure to PM2.5 and NO2 and to identify sensitivity windows to exposure to air pollution in different developmental stages. **Methods**: This prospective cohort study included mother-child pairs from the INMA-Gipuzkoa project. Specifically, 463 mother-child pairs with data on PM2.5 and NO2 exposure during pregnancy and TT4 levels at birth were included. PM2.5 and NO2 levels were measured by high-volume aerosol samplers and passive samplers respectively during the women's pregnancies. TT4 levels were measured in heel-prick blood samples from infants. Data on maternal and infant covariates were gathered through questionnaires administered in the first and third trimesters of pregnancy and review of clinical records. Potential associations of $PM_{2.5}$ and NO2 with TT4 levels over the entire pregnancy was assessed by linear regression models and DLMs were used to identify susceptibility windows. **Results**: The exposure of pregnant women to $PM_{2.5}$ during pregnancy was positively associated with infant TT4 level at birth (β [95% CI] = 0.198 [0.091, 0.305]. DLMs identified three different sensitivity windows, one in the periconceptional period with a negative association between $PM_{2.5}$ exposure and TT4 levels at birth, and a second (weeks 12–17) and a third one (weeks 31–37) with a positive association. In addition, the later the exposure, the stronger the association. In contrast, no association was observed between NO_2 exposure and TT4 levels. **Conclusions**: The results indicate that prenatal exposure to $PM_{2.5}$ could lead to a thyroid function impairment in newborns.

1. Introduction

Thyroid function is of vital importance at various stages of life. During fetal development, it plays a critical role particularly in neurological development (Trumpffet al., 2015), (Patel et al., 2011). Congenital hypothyroidism in newborns has been linked to immaturity of liver and bone, causing hyperbilirubinemia and delayed skeletal maturation, and a risk of permanent neurodevelopmental problems/outcomes (Segni, 2017).

Levels of thyroid hormones (thyroxine [T4] and triiodothyronine [T3]) in the fetus depend on maternal supply in the first trimester of pregnancy, since the fetal thyroid gland is not able to secrete its own hormones (Burrow et al., 1994). After the 12th week of gestation, the supply of thyroid hormones from the mother gradually decreases in importance, until the thyroid gland of the fetus becomes fully functional during the second trimester of pregnancy (Burrow et al., 1994), (Fisher,

* Corresponding author. Faculty of Medicine and Nursery of the University of the Basque Country, Barrio Sarriena s/n, 480940, Leioa, Spain.
E-mail address: amaia.irizar@ehu.eus (A. Irizar).

https://doi.org/10.1016/j.envres.2021.111132
Received 29 October 2020; Received in revised form 2 April 2021; Accepted 3 April 2021
Available online 9 April 2021
0013-9351/

2008). The main thyroid hormone in the bloodstream is T4, in a T4:T3 ratio of 14:1 (Pilo et al., 1990), and around 99.97% of T4 is protein-bound, mainly to thyroxine-binding globulin (TBG), but also to albumin and transthyretin (Leung, 2012).

Thyroid dysfunction during pregnancy can be caused by various factors, including iodine deficiency, autoimmune processes and exposure to environmental contaminants. Several studies have observed that endocrine-disrupting chemicals (EDCs) such as polychlorinated biphenyls (N et al., 2013), polybrominated diphenyl ethers, phthalates and bisphenol A (Aung et al., 2017), (Minatoyaet al., 2017) and polyfluoroalkyl substances PFAS (Fraseret al., 2012), (Berget al., 2015) affect thyroid function during pregnancy.

EDCs are generated in a range of human activities, from fuel combustion, power plants, and industrial processes, to cigarette smoking. Since these activities are also important sources of fine particulate matter (PM), EDCs often appear bound or adhered to PM, and hence, PM is classified as an endocrine disruptor (Darbre, 2018). They are also present in several personal care products, containing volatile solvents or added fragrances, and aerosol sprays, including household cleaners and pesticides.

Mechanisms of action by which EDCs may disrupt thyroid function disruption include: disruption of hormone synthesis, competition with hormones as ligands for a binding site of a receptor, and alteration of hormone transport and metabolism or excretion of hormones (Darbre). Additionally, the effect of PM could be related to the oxidative stress caused by the particles, suspended PM with a diameter of $\leq$2.5 μm ($PM_{2.5}$) potentially promoting oxidative stress through the direct generation of reactive oxygen species (ROS) (Castañedaet al., 2018). In fact, increased ROS levels have been detected in goitrous and involuting thyroid glands (JF et al., 1998)– (Mahmoud et al., 1986).

Recent epidemiological cohort studies have observed an association between $PM_{2.5}$ exposure and thyroid function impairment in pregnant women and in newborns. A large study, published in 2019 including 9931 pregnant women, observed a positive association between $PM_{2.5}$ exposure and hypothyroxinemia in the first trimester of pregnancy (Ghassabian et al., 2019). Likewise, another study on $PM_{2.5}$ exposure during the second and third trimesters of pregnancy showed increased odds of hypothyroxinemia in pregnant women (Zhaoet al., 2019). These two studies also investigated the effect of prenatal NO_2 exposure on maternal T4 levels, but the results were inconsistent, the first study finding no association while the second described a negative relationship (Ghassabian et al., 2019). Considering that maternal first trimester T4 level is associated with T4 levels in newborns (Korevaar et al., 2015), maternal hormone levels could play a role in the relationship between $PM_{2.5}$ exposure and newborn T4 levels.

Nevertheless, to our knowledge, just two studies have been published focused on prenatal $PM_{2.5}$ exposure and its effects on T4 levels in newborns (Janssen et al., 2017), (Howe et al., 2018), and they yielded contradictory results. The differences in the association observed between $PM_{2.5}$ and thyroxine in these two studies might be a consequence of the different outcome variables considered, cord blood free T4 levels in Janssen et al. (2017) and total T4 (TT4) levels in heel-stick blood spot in Howe et al. (2018). Regarding NO_2, a single study has investigated NO_2 and thyroid levels at birth (Howe et al., 2018), without observing any significant association.

The present study aimed to assess the association of total T4 levels at birth with weekly prenatal exposure to $PM_{2.5}$ and NO_2 and to identify the period most sensitive to atmospheric pollution.

2. Methods

2.1. Study area

The study area, with a total surface area of 519 km^2, is located in the province of Gipuzkoa, the Basque Country (north of Spain), and includes three valleys (Goierri- High Urola, Urola Medium and Alto Oria) (Fig. 1). The population of around 88000 is distributed across 25 small localities. The roads crossing the area carry a moderate amount of traffic (10000–40000 cars a day). The iron and steel industry accounts for a high proportion of the economic activity in the area, and 11 companies listed on the Spanish Register of Emissions and Pollutant Sources (PRTR-España, in line with E-PRTR regulations) ("España | Registro E, 2020) had facilities in the study area at the time of pregnancy and birth follow-up.

2.2. Study population

The present study is part of the INMA (from *INfancia y Medio Ambiente* in Spanish, meaning Childhood and the Environment) project, a prospective birth cohort study conducted in several geographical areas of Spain. The study population was composed of 638 mother and child pairs from the INMA- Gipuzkoa cohort. The recruitment of pregnant women was performed in 2006–2008 at their first ultrasound scan, and the inclusion criteria were being older than 16 years, as well as having a

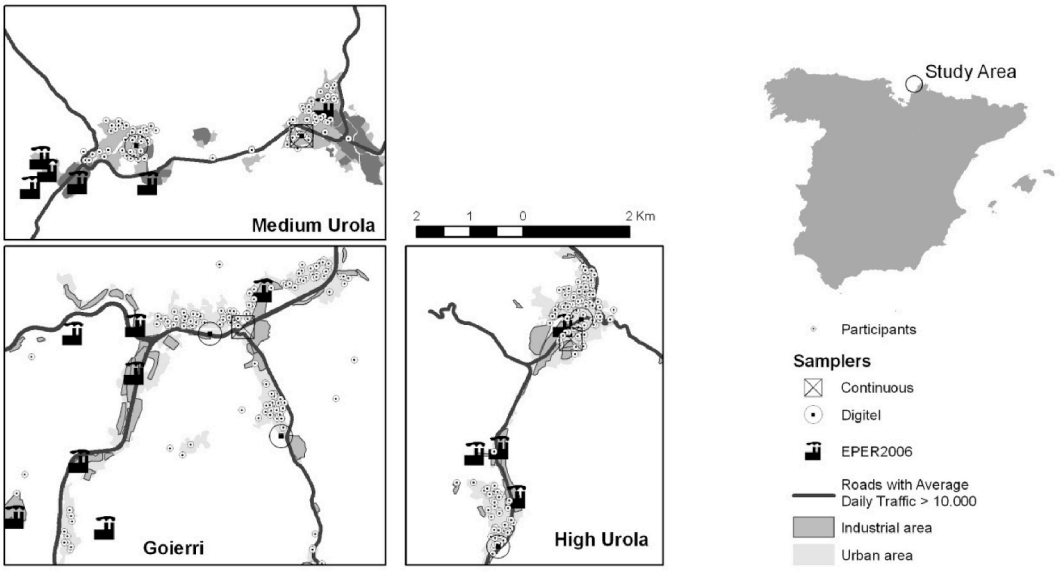

Fig. 1. Study site and location of air samplers, industries and urban areas.

natural pregnancy and no communication problems. After recruitment, follow-up was performed once more during pregnancy, in the third trimester (week 32), and at birth. The study was approved by the Ethics Committee of Donostia University Hospital (Gipuzkoa) and all mothers gave written informed consent before inclusion.

The study sample (mother-child pairs) for the present study was selected according to the following criteria: term pregnancies of mothers with no diagnosis of thyroid diseases, and with data available on $PM_{2.5}$ or NO_2 levels during pregnancy and newborn TT4 levels. Information about thyroid disease diagnosis was reported by the participants in a questionnaire.

2.3. Environmental characteristics

2.3.1. Prenatal $PM_{2.5}$ exposure

Daily $PM_{2.5}$ levels were measured, using three high volume aerosol samplers (Digitel DHA-80), during the entire study period. The methodology has been detailed elsewhere (Lertxundi et al., 2010). Briefly, one sampler was placed at a fixed site in Alto Oria valley while the other two were rotated around various sites in the other two valleys. In all cases, the samplers were placed to avoid direct exposure to traffic or industry. Further, we had access to daily data on $PM_{2.5}$ recorded by fixed devices in each of the valleys, which are part of the Air Quality Network of the Government of the Basque Country. To compensate for the information missing due to the rotation of the devices, we used the multiple imputation procedure developed by Li et al. (2014) (Li et al., 2014), (Lertxundiet al., 2015). Each woman was assigned the mean $PM_{2.5}$ concentration as measured by the sampler in her own locality or the closest one (maximum of 4 km) in each trimester of pregnancy and over the entire pregnancy period. For women that moved to a new residence during pregnancy, the imputation of air exposure was performed accounting for the time lived in each place.

2.3.2. Prenatal NO_2 exposure

Data on NO_2 levels were obtained from radial passive samplers (Radiello®; Fundazione Salvatore Maugeri, Padua, Italia), distributed across the study area according to geographical criteria (Estarlichet al., 2011). The method has been described elsewhere (Esplugueset al., 2007). Briefly, the samplers were placed at the same site three times during pregnancy, and each time they took readings for 2 weeks. We used land-use regression models to estimate NO_2 levels corresponding to the place of residence of participants. Data collected by air samplers from the Air Quality Network of the Government of the Basque Country were used to estimate NO_2 levels for each trimester and the entire pregnancy. In this way, we obtained spatiotemporal data on NO_2 exposure. As mentioned in section 2.3.1, moving to a new residence was taking into account for NO_2 exposure imputation by considering the time lived in each place.

2.4. Newborns' T4 levels

Blood samples were taken from newborns within 48 h after birth by heel prick and collected on filter paper, in accordance with the protocol of the Basque neonatal screening program. To quantify TT4, T4 B065-112 kits (Wallac Oy, Turku, Finland) were used. The central unit responsible for the neonatal screening program is the Clinical Chemistry Unit of the reference public health laboratory which is ISO 15189 accredited by the Spanish National Accreditation Board (ENAC). The cutoff value for TT4 levels from the public health laboratory were 6–20 μg/dL for infants with birth weights >2500 g and 5–20 μg/dL for those with lower birth weights.

2.5. Covariates

Information on sociodemographic and lifestyle habits of pregnant women was collected through paper and pencil questionnaires administered in the first and second trimesters of pregnancy. Specifically, data were recorded on: maternal age, pre-pregnancy weight (kg), height (m), level of education (primary or less, secondary or tertiary), iodine supplementation status (whether the mother took iodine-containing supplements in the first two trimesters of pregnancy) and complications during pregnancy (fever, urine infection, high blood pressure, loss of amniotic fluid or blood or contractions). Pre-pregnancy BMI was calculated as the weight (kg) divided by the square of the height (m), and was classified in 4 categories: underweight (BMI: <18.5), normal (BMI: 18.5–24.9), overweight (BMI: 25–29.9) and obese (BMI: >30).

Further, the following data were retrieved from clinical records: the infant's sex, birth weight, gestational age (weeks), type of delivery and date of birth. The type of delivery was divided into three categories: non-instrumental vaginal delivery, instrumental vaginal or cesarean delivery. Season of birth was calculated from the date of birth.

2.6. Statistical analysis

The main characteristics of the study population were described using percentages for qualitative variables and mean and standard deviation (SD) for quantitative variables. In order to decide a priori which potential confounding or predictor variables should be included in our models, we drew directed acyclic graphs (DAGs) based on current knowledge from the scientific literature (Fig. 1, supplementary material). Results identified two minimally sufficient adjustment sets containing the following variables: season of birth and education level. Iodine supplementation was not included in the models since most pregnant women (97.2%) reported taking iodine supplements. Maternal hormone levels are a mediator in the relationship between $PM_{2.5}$ exposure and infant TT4 level at birth, since free T4 levels in newborns are associated with maternal free T4 levels (Korevaar et al., 2016) and maternal free T4 levels are associated with $PM_{2.5}$(Ghassabian et al., 2019). Therefore, this variable was not included in the models.

In a first step, linear regression models were used to analyze the association of average pregnancy $PM_{2.5}$ and NO_2 exposure with newborn T4 level. Based on the aforementioned minimally-adjusted regression models, several different models were constructed including the variables that changed the effect estimate in more than 10%. Then, the model with the best fit according to the Akaike information criterion (AIC). Thus, the final models were adjusted for education level, season of birth, child age and type of delivery. In addition, Cook's distance was estimated to detect influential points. Generalized additive models (GAMs) were fitted to check the linearity of the relationships of newborn T4 level with $PM_{2.5}$ and NO_2 using natural cubic splines with one internal knot. The association of both pollutants and TT4 levels at birth was also explored with a multipollutant regression model.

In a second step, linear regression models with weekly $PM_{2.5}$ and NO_2 levels were calculated, and subsequently, distributed lag models (DLMs) were built to account for current and previous exposure effects on TT4 levels in newborns and to detect sensitivity windows. The DLM analysis included weeks 1–37 of pregnancy (at term), since 4.5% of women gave birth between week 37 and week 40 of pregnancy and our priority was to include all mother-child pairs in the analysis. Following the procedure by Howe et al. (2018) natural cubic spline DLMs with 3 df (ie, 1 knot placed at the 10th week of pregnancy) were initially performed, coinciding with the onset of maternal circulation to placenta. In an additional step, a second knot in week 25 of pregnancy (coinciding with the peak of maternal T4 levels) was added and natural cubic spline DLMs with 4 df were performed in order to identify susceptibility windows related to the origin of T4 production. The DLMs were compared using the Akaike information criterion (AIC). The analysis was performed using the dlnm package in R version 3.6.1.

A sensitivity analysis was carried out to analyze differences in the study variables between the participants included in the study and the excluded population.

3. Results

From the 638 mother-child pairs initially recruited, 467 had information about maternal $PM_{2.5}$ and NO_2 exposure during pregnancy and TT4 levels in newborns. Three pairs were excluded due to maternal thyroid disease, and one other pair due to the corresponding values being classified as influential according to the Cook distance estimated from the regression models. In total, 463 mother and child pairs were included in the analysis (Fig. 2).

The mean age and pre-pregnancy BMI of mothers were 31.5 years and 22.96 kg/m2, respectively, 49.9% of them had a university degree and 25.27% reported complications during pregnancy (Table 1). Regarding child characteristics, 50.1% were female, 71.1% were born by non-instrumental vaginal delivery and the mean gestational age was 39.9 weeks (Table 1). Concerning season of birth, 28.5%, 17.3%, 29.8% and 24.4% of the births were in the winter, spring, summer and autumn, respectively (Table 1). The mean (SD) age of newborns at the time of the heel-prick test was 62.8 h (11.7), and the mean (SD) TT4 levels were 13.8 (3.32) μg/dl. According to the sensitivity analysis, the newborns from the study population had a longer gestational age compared with the excluded population (mean (SD) of 39.9 (1.17) weeks vs. 39.0 (2.17) weeks, respectively, p-value <0.001) and had a lower exposure to NO2 during pregnancy (18.5 (6.12) μg/m3 vs. 16.3 (4.99) μg/m3, respectively, p-value <0.001) (Table 1, Supplementary material).

Concerning the exposure of pregnant women to atmospheric pollutants, the mean (standard deviations [SD]) levels of $PM_{2.5}$ and NO_2 during pregnancy were 16.9 (2.44) $\mu g/m^3$ and 18.5 (6.12) $\mu g/m^3$ respectively (Table 1). For $PM_{2.5}$, the highest levels were measured in the first trimester of pregnancy and the lowest in the third trimester. In the case of NO_2, the highest levels were detected in the second trimester and the lowest in the third trimester.

The potential associations between TT4 levels at birth and atmospheric pollution were investigated by multivariate linear regression models. First, models were built accounting for individual exposure to $PM_{2.5}$ and NO_2 in the entire pregnancy period. The minimally-adjusted models included education level and season of birth, while the fully-adjusted models also considered child age at the time of the heel-prick test and type of delivery. Infant TT4 levels at birth were found to be positively associated with $PM_{2.5}$ exposure during pregnancy, with a β (95%CI) of 0.206 (0.083, 0.329) (p = 0.001), but not significantly associated with NO_2 exposure (Table 2). The multipollutant model including both contaminants showed similar results, with a significant association of $PM_{2.5}$ exposure with a β (95%CI) of 0.195 (0.07, 0.32), and no association of NO_2 and TT4 levels at birth (Table 2).

Subsequently, the association analysis was performed by weeks of pregnancy (Table 2, supplementary material). According to the results, newborns' TT4 levels were positively associated with $PM_{2.5}$ exposure mostly after week 27 of pregnancy. In contrast, the association of infant TT4 level at birth with NO_2 exposure remained non-significant for almost all pregnancy weeks.

Oppositely, the DLMs (Fig. 3 and 3D image in Fig. 2, supplementary material), which account for both present and past exposures, showed a significant negative association of $PM_{2.5}$ exposure and TT4 levels at birth in the first 4 weeks of pregnancy with effect estimates (β (95% CI)) between −0.62 (−1.18, −0.05) and −1.25 (−2.19, −0.31), and a positive association in weeks 12–17 (range of β (95% CI) between 0.45 (0.04, 0.87) and 0.55 (0.07, 1.03)) and in weeks 31–37 (range of β (95% CI) between 0.4 (0, 0.8) and 1.39 (0.14, 2.64)) (Table 3, supplementary material). In the rest of pregnancy weeks the association remained non-significant. Regarding NO_2, no significant associations were observed in any of the studied weeks of pregnancy (Fig. 3).

The associations of confounding variables with infant TT4 levels are reported in the supplementary material (Table 4, supplementary

Table 1
Characteristics of the study sample.

mean (sd)/N (%)	N = 463	Missing values
Mother		
Age (years)	31.5 (3.61)	0 (0%)
Education level		2 (0.43%)
Primary	65 (14.0%)	
Secondary	165 (35.6%)	
University	231 (49.9%)	
Iodine supplementation during pregnancy		0 (0%)
No	13 (2.81%)	
Yes	450 (97.2%)	
Complications during pregnancy		7 (1.51%)
No	339 (73.22%)	
Yes	117 (25.27%)	
Pre-pregnancy BMI (kg/m2)	22.96 (3.70)	1 (0.22%)
Child		
TT4 (μg/dl)	13.8 (3.31)	0 (0%)
Sex		0 (0%)
Female	232 (50.1%)	
Male	231 (49.9%)	
Age at the time of heel-prick test (hours)	62.6 (10.6)	
Season of birth		0 (0%)
Winter	132 (28.5%)	
Autumn	80 (17.3%)	
Spring	138 (29.8%)	
Summer	113 (24.4%)	
Gestational age (weeks)	39.9 (1.17)	0 (0%)
Type of delivery		0 (0%)
Non-instrumental vaginal	329 (71.1%)	
Cesarean	52 (11.2%)	
Instrumental vaginal	82 (17.7%)	
Environmental pollutants		
$PM_{2.5}$ ($\mu g/m^3$) during pregnancy	16.9 (2.44)	0 (0%)
NO_2 ($\mu g/m^3$) during pregnancy	18.5 (6.12)	0 (0%)

*Qualitative variables are expressed as absolute frequencies and percentages and quantitative variables as means and standard deviations.

Table 2
Linear regression models for the association between infant TT4 level at birth and $PM_{2.5}$, NO_2 and both contaminant exposure (N = 463). Significant associations (p < 0.05) in bold.

	aβ (95% CI)	*p value*	Adjusted R^2	AIC
Single pollutant model				
$PM_{2.5}$ ($\mu g/m^3$)	**0.206 (0.083, 0.329)**	***0.001***	0.069	2386.4
NO_2 ($\mu g/m^3$)	0.037 (−0.012, 0.086)	*0.135*	0.051	2395.0
Multipollutant model			0,069	2387,5
$PM_{2.5}$ ($\mu g/m^3$)	**0.195 (0.07, 0.32)**	***0,002***		
NO_2 ($\mu g/m^3$)	0.024 (−0.025, 0.073)	*0,346*		

Adjusted for education level, season of birth, child age (hours) and type of delivery.

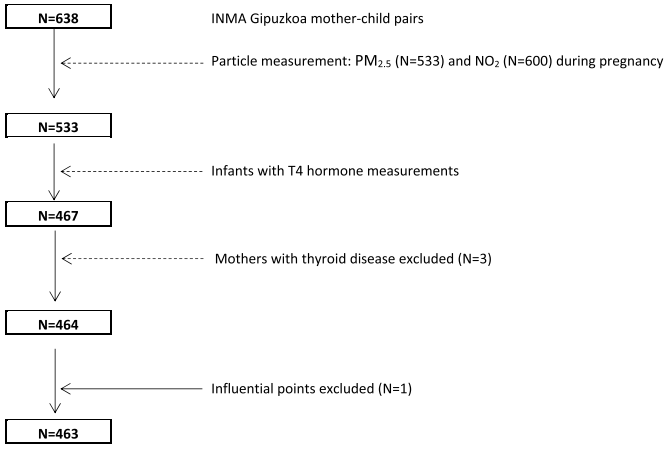

Fig. 2. Flowchart of the study sample.

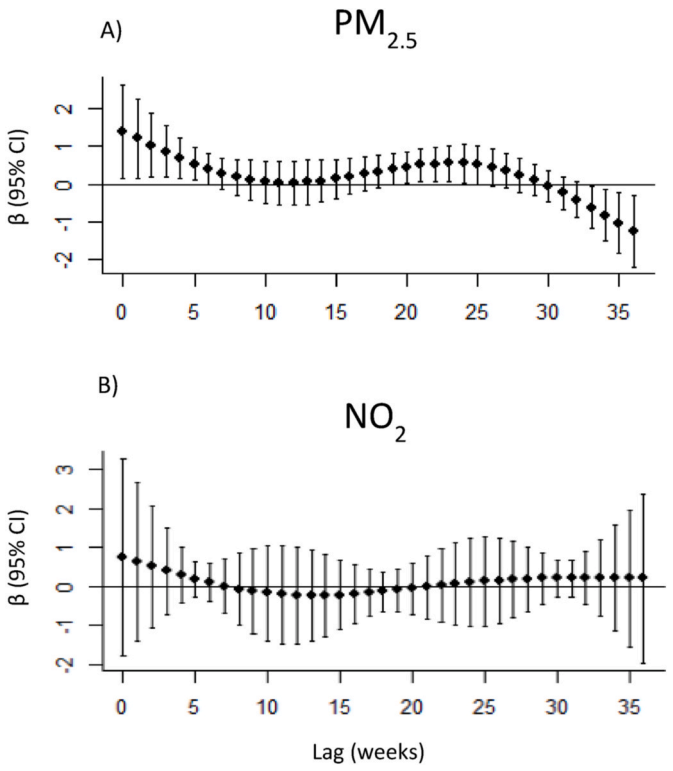

Fig. 3. DLMs of the association between A) $PM_{2.5}$ and B) NO_2 and infant TT4 levels at birth. B values represent the effect size of the exposure to 38 μg/m^3 of $PM_{2.5}$ and 72 μg/m^3 of NO_2, the highest values of each pollutant detected during the study period. The starting point of the analysis is week 37, and the lag represents the number of weeks backwards.

material). Season of birth was significantly associated with TT4 levels at birth in both $PM_{2.5}$ and NO_2 models. Infants born in the winter had significantly higher TT4 levels than those born in the spring or summer. Child age at heel-prick test was negatively associated with TT4 levels at birth in all the models. Regarding the type of delivery, infants born by instrumental delivery had significantly higher levels of TT4 than those born by non-instrumental vaginal delivery in all the models. In contrast, education level of the mother was not significantly associated with infant TT4 levels at birth in any of the models.

4. Discussion

This study is so far the first conducted in a European population linking air pollution during pregnancy and thyroid function in newborns as reflected in hormone levels measured in heel-stick blood samples, adding to a single previous study on the effects of prenatal $PM_{2.5}$ exposure on TT4 levels in newborns (Howe et al., 2018). Our findings suggest that exposure to $PM_{2.5}$ during pregnancy is positively associated with TT4 levels in newborns. According to our data, TT4 levels in infants' blood collected 3 days after birth increase by 0.206 μg/dl for each 1 μg/m^3 increase in exposure to $PM_{2.5}$ during pregnancy. Regarding NO_2, no association has been found between exposure during pregnancy and TT4 levels at birth.

The only previous study to date exploring the association between $PM_{2.5}$ exposure during pregnancy and TT4 levels in newborns yielded similar results (Howe et al., 2018). The authors observed a positive association of similar magnitude between TT4 levels and prenatal $PM_{2.5}$ exposure and no association with NO_2 exposure. In contrast, another study observed the opposite, namely, an inverse association between $PM_{2.5}$ exposure and free T4 levels measured in cord blood at birth (Janssen et al., 2017). Although these results might seem inconsistent, it is plausible to observe low free T4 (FT4) in cord blood and high TT4 in heel-stick blood samples, as thoroughly explained by Howe et al. (2018). Briefly, they hypothesized that $PM_{2.5}$ exposure increases the production of serum transport proteins such as the TBG in the fetus, decreasing the amount of free T4 in blood. Since the heel-stick test quantifies TT4 concentrations (FT4 and protein-bound T4), it would be possible for both TT4 levels and TBG to increase due to exposure to $PM_{2.5}$, and to detect a decrease in free T4 levels due to the increase in the protein-bound T4.

The DLMs have identified various vulnerability windows of $PM_{2.5}$ exposure: the first 4 weeks of pregnancy (periconceptional period), weeks 12–17 of pregnancy (end of the first trimester and beginning of the second trimester of pregnancy) and week 31–37 of pregnancy (third trimester). Previous reviews and meta-analyses studying temporal vulnerability windows to $PM_{2.5}$ exposure related to preterm birth, have also reported first trimester, third trimester or last month of pregnancy as a possible critical window of exposure (Bonzini et al., 2010)– (Stieb et al., 2012).

The first sensitivity window was located in the periconceptional period, which is defined as the period that covers the 14 weeks prior the conception and 10 weeks after conception (Steegers-Theunissen et al., 2013), or according to the 'Developmental Origins of Health and Disease' (DOHaD) concept, the period covering the meiotic maturation of oocytes, differentiation of spermatozoa, fertilisation and resumption of mitotic cell cycles in the zygote (what includes the transition from parental to embryonic genomes and the onset of morphogenesis up to implantation) (Fleminget al., 2018). In addition, the onset of maternal circulation to placenta does not occur until the 10th week of pregnancy, although at the 8th week maternal plasma may cross the intercellular spaces until reaching the fetus (Burton et al., 2001). Hence, maternal $PM_{2.5}$ transfer to the fetus would not be possible in this period. Therefore, our data suggest that $PM_{2.5}$ exposure could be related with key changes, such as epigenetic modifications of DNA that could occur in the oocyte during the ovarian follicular development and which could increase the sensitivity of the ovum to external influences (Steegers--Theunissen et al., 2013), or during the mentioned fertilization, genome transition from parental to embryonic, and early morphogenesis and implantation. Accordingly, a recent study has identified a sensitivity window to $PM_{2.5}$ exposure in the first weeks of pregnancy related with cord blood methylation using DLNMs (Wanget al., 2020).

Our results indicate that the exposure to $PM_{2.5}$ in the periconceptional period would decrease TT4 levels in newborns. However, the trend of the association between $PM_{2.5}$ and TT4 shifts to positive coinciding with the onset of maternal circulation to placenta. In fact, the positive association of maternal exposure to $PM_{2.5}$ and TT4 levels at birth is significant between the 12th and the 17th weeks, what could suggest that maternal thyroid function could partially mediate the relationship between $PM_{2.5}$ and TT4 levels at birth. Considering that in early pregnancy, until around week 12, fetal T4 supply depends completely on the mother, and that fetal thyroid gland is immature until weeks 18–20, the observation of a significant association between TT4 levels and $PM_{2.5}$ exposure indicates that maternal TBG may play an important role in the process. According to the aforementioned hypothesis of Howe et al. (2018), $PM_{2.5}$ exposure may increase maternal TBG. As T4 can only be transported through the placenta bound to a transporter, T4 transport to the fetus would increase, as would fetal TT4. This hypothesis is also supported by a more recent study, which showed that $PM_{2.5}$ exposure during the first trimester was negatively associated with FT4 levels in the same period (Ghassabian et al., 2019), and this could be explained by an increase in maternal TGB. The inclusion of TGB measurements in future studies would be necessary to elucidate the mechanisms of action of $PM_{2.5}$ in maternal and newborn thyroid function.

Our data indicate that TT4 at birth is also positively associated with $PM_{2.5}$ exposure in the third trimester (weeks 31–37). Moreover, the later the exposure, the stronger the association (β (95%CI): gradual increase from 0.4 (0, 0.8) in week 31, to 1.39 (0.14, 2.64) in week 37). Although

we could not detect any significant association between weeks 18–30, the increase of the association is consistent with the gradual increase in the secretion of fetal T4 (Burrow et al., 1994) and TGB (Blackburn, 2007) from week 12 onwards until it plateaus at week 36. This suggests that once the fetal thyroid gland starts to produce T4, from the second trimester onwards, the influence of $PM_{2.5}$ exposure on TT4 at birth is the result of a joint effect on maternal and fetal TGB levels. These results do not, however, agree with those of Howe et al. (2018), who only detected a significant association between TT4 levels at birth and $PM_{2.5}$ exposure in the second trimester, coinciding with the peak of maternal T4 levels. Therefore, they suggest that maternal thyroid function could be a full mediator of the association between $PM_{2.5}$ and newborn's TT4 levels. Nevertheless, since $PM_{2.5}$ could have crossed the placental barrier (Bovéet al., 2019), we would expect to observe a similar effect of $PM_{2.5}$ in mothers and fetuses, and this would be consistent with our results. In this context, more studies are needed to test this hypothesis and to understand the underlying process.

Regarding the role of confounding variables, TT4 levels were negatively associated with age at heel-prick test. Previous studies observed a decline in TT4 levels from the first day to 4–7 days after birth (RH et al., 1974) and in FT4 after the first 24 h of life (MSet al., 2018). Delivery type is also an important variable that affects hormone levels at birth. In this study, we observed that instrumental vaginal delivery, which is more stressful for the fetus than non-instrumental vaginal delivery, was positively associated with thyroxine levels, in accordance with previous studies (Eet al., 2000)– (SG et al., 2016). Seasonality seems to play a role in birth outcomes (Qet al., 2019), and in TT4 levels at birth in particular. Previous studies have observed higher T4 levels in newborns born in winter (Trumpffet al., 2015), (Janssen et al., 2017), and our findings are consistent with this pattern.

The molecular mechanisms of action underlying the association of TT4 levels in newborns with $PM_{2.5}$ exposure are unclear. On the one hand, $PM_{2.5}$ present in air samples from the present study were composed of different metals, including cadmium, lead, manganese, mercury, arsenic and zinc (Lertxundi et al., 2010), which have been described to have endocrine-disrupting properties (De Coster and van Larebeke, 2012), (Iavicoli et al., 2009). On the other, polycyclic aromatic hydrocarbons have also been found to be adhered to $PM_{2.5}$ in air collected in the study area (data not shown) and their metabolites in urine have been associated with thyroid function impairment in children and adolescents (Kelishadiet al., 2018). Lastly, $PM_{2.5}$ could contain many other EDCs which have not been identified, like polychlorinated biphenyls or polyfluoroalkyl substances, due to the diverse industry located in the study area.

Nevertheless, one of the limitations of this study is the lack of information on TBG levels. This information would be crucial to explain the mechanism through which $PM_{2.5}$ influences infant TT4 levels at birth. In addition, the sample size is not as large as in the previous study by Howe et al. (2018), although the results obtained were significant and consistent with previous research and the study hypothesis.

We are unable to exclude residual confounding in our study. The regression models were able to explain around 6% of the variability, and unmeasured confounders might have increased the bias in exposure effect estimates. Nonetheless, covariates were selected based on data available in the literature, and we believe we have considered all the confounders that are likely to have generated a significant change in the effect size.

The main strength of the study was the availability of weekly data of maternal $PM_{2.5}$ exposure and the possibility of conducting a DLMs analysis to detect windows of vulnerability throughout pregnancy.

In conclusion, the present study suggests that $PM_{2.5}$ exposure during pregnancy is associated with infant TT4 levels at birth. The association was negative in the periconceptional period, suggesting that $PM_{2.5}$ exposure could have affected the oocyte development, or the events during the fertilization or implementation. However, after the onset of the maternal circulation to placenta, the association turned to positive, and the strongest effect of $PM_{2.5}$ exposure was observed in the last weeks of pregnancy, which could indicate that $PM_{2.5}$ affects both maternal and fetal thyroid function. However, further research is needed to confirm this hypothesis, since the association of $PM_{2.5}$ exposure and maternal thyroid function was not assessed in the present study.

Observations and funding

This work is part of the INMA Project (INfancia y Medio Ambiente), which has received funding from the following organizations: Carlos III Health Institute (PI06/0867), Department of Health of the Government of the Basque Country (2005111093), Gipuzkoa Regional Government (DFG06/002) and the local councils of the study areas (Zumarraga, Urretxu, Legazpi, Azkoitia, Azpeitia and Beasain).

Author's contributions

Amaia Irizar: Conceptualization, investigation, data curation, writing - original draft, writing - Review and editing. **Arantxa Txintxurreta**: Conceptualization, investigation, writing - original draft, writing **Amaia Molinuevo**: data curation, formal analysis, writing - original draft and writing - review and editing. **Alba Jimeno-Romero**: methodology and writing - original draft. **Asier Anabitarte**: investigation and writing – Review and editing. **Jon Iñaki Álvarez**: Data collection, formal analysis and writing - original draft. **María Dolores Martínez**: Data collection, formal analysis and writing - original draft. **Loreto Santa Marina:** conceptualization, supervision, funding acquisition and writing - original draft. **Jesús Ibarluzea**: Conceptualization, funding acquisition and writing - original draft, **Aitana Lertxundi**: Conceptualization, Project administration, funding acquisition, supervision, writing - original draft, writing - Review and editing.

Ethics committee approval

The study was approved by the Ethics Committee of Donostia University Hospital (Gipuzkoa) and all mothers gave written informed consent before inclusion.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111132.

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