Association of Exposure to Ambient Air Pollution and Thyroid Function During Pregnancy

Akhgar Ghassabian, MD[1]; Livia Pierotti, MSc[2,3,4]; Mikel Basterrechea, PhD[3,5,6]; Leda Chatzi, MD[7,8]; Marisa Estarlich, PhD[3,9]; Ana Fernández-Somoano, PhD[3,10]; Abby F Fleisch, MD[11,12]; Diane R Gold, MD[13,14]; Jordi Julvez, PhD[2,3,4,15]; Polyxeni Karakosta, MD[7,16]; Aitana Lertxundi, PhD[3,5,17]; Maria-Jose Lopez-Espinosa, PhD[3,9]; Tessa A Mulder, BSc(Med)[18,19]; Tim IM Korevaar, MD[18], Emily Oken, MD[20,21]; Robin P Peeters, MD[18]; Sheryl Rifas-Shiman, MPH[20]; Euripides Stephanou, PhD[22]; Adonina Tardón, MD[3,10]; Henning Tiemeier, MD[19,23]; Martine Vrijheid, PhD[2,3,4]; Tanja GM Vrijkotte, PhD[24]; Jordi Sunyer, MD[2,3,4,15]; Mònica Guxens, MD[2,3,4,19]

[1]Departments of Pediatrics, Environmental Medicine, and Population Health, New York University School of Medicine, New York, NY, USA

[2]ISGlobal, Barcelona, Spain

[3]Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain

[4]Universitat Pompeu Fabra (UPF), Barcelona, Spain

[5]BIODONOSTIA Health Research Institute, San Sebastian, Spain;

[6]Public Health Division of Gipuzkoa, Basque Government, San Sebastian, Spain

[7]Department of Social Medicine, University of Crete, Heraklion, Greece

[8]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

[9]Epidemiology and Environmental Health Joint Research Unit, FISABIO-Universitat Jaume I-Universitat de València, Valencia, Spain

[10]IUOPA-Departamento de Medicina, University of Oviedo, Oviedo, Spain

[11]Department of Pediatric Endocrinology and Diabetes, Maine Medical Center, Portland, ME, USA

[12]Center for Outcomes Research and Evaluation, Maine Medical Center Research Institute, Portland, ME, USA

[13]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

[14]Department of Environmental Health, Harvard TH Chan School of Public Health, Boston, MA, USA

[15]Hospital del Mar Research Institute, Barcelona, Spain

[16]Clinical Microbiology Laboratory, Attikon University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.

[17]Department of Public Health and Preventive Medicine, University of Basque Country, Bilbao, Spain

[18]Academic Center for Thyroid Diseases, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

[19]Department of Child and Adolescent Psychiatry, Erasmus Medical Center — Sophia Children's Hospital, Rotterdam, the Netherlands

[20]Division of Chronic Disease Research Across the Lifecourse, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA

[21]Department of Nutrition, Harvard TH Chan School of Public Health, Boston, MA, USA

[22]Department of Chemistry, University of Crete, Heraklion, Greece

[23]Department of Social and Behavioral Sciences and Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, MA, USA

[24]Department of Public Health, Amsterdam Public Health Research Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

Correspondence to: Mònica Guxens MD, ISGlobal, Barcelona Institute for Global Health - Campus MAR Barcelona Biomedical Research Park (PRBB) (office 190), Doctor Aiguader, 88, 08003 Barcelona, Spain; Tel. +34 93 214 7330 | Fax +34 93 214 7302; email: monica.guxens@isglobal.org

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Key points

Question Is the first trimester exposure to ambient air pollution associated with thyroid function across pregnancy?

Findings In 9931 pregnant women in four European cohorts and one in the US, an increase of 5 $\mu g/m^3$ in exposure to particulate matter with an aerodynamic diameter ≤ 2.5 µm ($PM_{2.5}$) was associated with 20% higher odds of hypothyroxinemia (defined as low free thyroxine despite normal thyroid stimulating hormone) in pregnant women.

Meaning This study raises the possibility that exposure to particulate matter might disrupt thyroid function in pregnant women.

Abstract

Importance: Air pollutants interact with estrogen nuclear receptors, but their impact on thyroid signaling is less clear. Thyroid function is of particular importance in pregnant women due to the role of thyroid for fetal brain development.

Objective: To determine the short-term association between air pollution exposure in the first trimester and thyroid function across pregnancy.

Design and setting: Population-based birth cohorts. Statistical analyses were conducted from January-2018 to April-2019.

Participants: Pregnant women from four European and one US cohorts with data on air pollution exposure and thyroid function during pregnancy. Recruitment periods were during 2003-2004 for ABCD; 2002-2006 for Generation R; 2003-2008 for INMA; 2007 for Rhea; and 1999-2002 for Project Viva.

Main outcomes and measures: We estimated residential air pollution concentrations (i.e., nitrogen oxide [NO] and particulate matter [PM]), during the first trimester of pregnancy using land-use regression and satellite-derived aerosol optical depth models. We measured free thyroxine (T_4), thyroid stimulating hormone (TSH), and thyroid peroxidase antibodies (TPOAb) across gestation (median~13 weeks). We defined hypothyroxinemia as free T_4 below the 5th percentile of cohort distribution with normal TSH following guidelines.

Results: Among 9931 participants, mean age was 31.2 years (SD=4.8), 50% had high educational levels, 57% were nulliparous, 4.2% had hypothyroxinemia, and 6.7% were TPOAb positive. Concentrations of NO_2 and particulate matter with an aerodynamic diameter≤2.5 μm ($PM_{2.5}$) were lower and had less variation in Project Viva than European cohorts. We found no associations between NO and thyroid function. Higher exposures to $PM_{2.5}$ were associated with higher odds of hypothyroxinemia in pregnant women (OR per Δ5 μg/m^3=1.21, 95%CI: 1.00-1.47). PM_{10} exposure was associated with higher odds of hypothyroxinemia, but the coefficient was smaller and imprecise (OR per Δ10 μg/m^3 of PM_{10}=1.18, 95%CI: 0.93-1.48). $PM_{2.5-10}$ and $PM_{2.5}$ absorbance were not associated with hypothyroxinemia. There was a substantial heterogeneity among cohorts with respect to TPOAb ($P_{heterogeneity}$<0.001), showing associations of NO and PM with thyroid autoimmunity only in Generation R.

Conclusions and Relevance: Associations between $PM_{2.5}$ exposures and thyroid function during pregnancy are of global health importance because air pollution exposure is widespread and hypothyroxinemia may adversely influence offspring brain development.

Key words environmental pollutants; nitrogen dioxide; particulate matter; hypothyroxinemia; pregnant women; longitudinal studies

Introduction

Exposure to ambient air pollution is one of the leading contributors to the burden of disease globally. Exposure to air pollutants such as nitrogen dioxide (NO_2), nitrogen oxides (NO_x), particulate matter (PM), and polycyclic aromatic hydrocarbons (PAH) during pregnancy are associated with brain structural abnormalities, impaired executive function, learning disabilities, and behavioral problems in the offspring.[1-4] Among underlying factors are oxidative stress, neuroinflammation, or disruption of hypothalamus-pituitary-adrenal axis.[5,6] Endocrine disruption might be among other mechanisms; for example PAH induce activation of the estrogen receptor gene and both PAH and particulate matter can interfere with nuclear receptors such as estrogen receptor signaling.[7,8] Experimental and epidemiological studies also show associations between PAH exposure and thyroid function.[9,10] Nonetheless, evidence is limited on whether exposure to air pollution might also disrupt thyroid signaling and thyroid function.

Thyroid function is of particular importance in pregnant women due to the critical role of thyroid hormones for fetal brain development. Because the fetal thyroid gland achieves its full function only from mid-gestation onwards, undetected or inadequately treated thyroid insufficiency in pregnant women adversely influence growth and development of the offspring, even in the absence of neonatal hypothyroidism.[11] Recent evidence suggests that mild thyroid insufficiency, i.e., hypothyroxinemia, defined as low free thyroxine (T_4) with normal thyroid stimulating hormone (TSH) concentrations, during pregnancy may also contribute to impaired cognition and neurodevelopmental disorders in the offspring.[12-14] While inadequate iodine intake is a common cause of thyroid insufficiency worldwide, exposure to environmental contaminants are increasingly considered important.[15] A handful of studies to date have examined the influence of exposure to ambient and traffic-related air pollution on thyroid function in pregnant women. One study in 499 mother-child pairs in Belgium examined associations between the 3rd trimester fine particles with an aerodynamic diameter ≤ 2.5 µm ($PM_{2.5}$) and maternal and fetal thyroid function.[16] This study found inverse associations of $PM_{2.5}$ exposure with fetal cord blood free T_4, free triiodothyronine (T_3), and TSH and as well as maternal free T_4. In 8077 pregnant women in Shanghai, exposures to higher concentrations of $PM_{2.5}$ but not NO_2 in the first and second trimesters were associated with hypothyroxinemia in mid-gestation.[17] A study in California (n=2050) showed that newborns who were prenatally exposed to $PM_{2.5}$ and particles with an aerodynamic diameter ≤ 10 µm (PM_{10}) had higher concentrations of total T_4 measured in heel-stick blood spots.[18] A potential association between air pollution exposure and thyroid function during gestation might further clarify underlying mechanisms of hypothyroxinemia in pregnant women. Accordingly, in five birth cohorts (four in Europe and one from the US), we examined whether measures of air pollutants, i.e., NO_2, NO_x and PM, averaged in the first trimester were associated with thyroid function and thyroid autoimmunity across pregnancy. We primarily investigated the short-term associations of air pollutants with mild thyroid hormone insufficiency, i.e., hypothyroxinemia and high TSH, because of the

emerging evidence suggesting their implications for fetal development.[12-14,19] We also examined thyroid peroxidase antibody (TPOAb) positivity on the basis of evidence suggesting the association between air pollution exposure and inflammation[20] and our earlier findings on the association between TPOAb positivity in pregnancy and maternal and child health outcomes.[21-23] For each woman, thyroid assessments were performed once throughout pregnancy, while the majority of the measures were in the first half of pregnancy (gestational age <17 weeks). We specifically focused on air pollution exposure during the first trimester to ensure that air pollution exposure preceded thyroid function measurement.

Methods

Participants

This analysis used data from five birth cohorts with prenatal recruitment, including the Amsterdam Born Children and their Development (ABCD, Amsterdam, the Netherlands; n=3867),[24] the Generation R Study (Rotterdam, the Netherlands; n=2605),[25] Infancia y Medio Ambiente (INMA, including the regions of Sabadell, Gipuzkoa, Valencia, and Asturias in Spain; n=2239),[26] Rhea (Island of Crete, Greece; n=483),[27] and Project Viva (eastern Massachusetts, US; n=737)[28] (total n=9931). Recruitment periods for the cohorts were as following: ABCD: 2003-2004; Generation R: 2002-2006; INMA: 2003-2008; Rhea: during 2007; and Project Viva: 1999-2002.

Iodine status of participants varied across cohorts. Median urinary iodine concentrations of pregnant women in Generation R and Rhea were optimal according to the World Health Organization references.[29,30] In contrast, mild to moderate iodine insufficiency was observed in INMA (except for Gipuzkoa).[31] Urinary iodine concentrations were not measured in ABCD and Project Viva. However, reports from pregnant women in Massachusetts show optimal iodine status[32] and iodine status of participants in Amsterdam and Rotterdam are expected to be comparable. There was no pattern suggesting associations between iodine status of cohorts and air pollution exposure.

In each cohort, we included pregnant women had data on both first trimester air pollution exposure and gestational thyroid function. We excluded women with twin pregnancies, women with a history of thyroid disease, and those who reported taking medication affecting thyroid function. There were no data available on medication use in INMA. We did not exclude women who were positive for thyroid peroxidase antibodies (TPOAb) because conditioning on autoimmune processes, an intermediate factor, might introduce bias in the analysis. Ethical approval was obtained from the local authorized Institutional Review Boards. All participants provided written informed consent. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology guideline for cohort studies.

Measurements

In ABCD, Generation R, INMA, and Rhea, three two-week periods of air pollution monitoring campaigns were performed within one year between January 2009 and April 2011. Air pollution concentrations [i.e., NO_2, NO_x, $PM_{2.5}$ absorbance

(determined as the reflectance of $PM_{2.5}$ filters), $PM_{2.5}$, PM_{10}, and PM with aerodynamic diameters of 2.5–10 µm ($PM_{2.5-10}$)] at the participants' home addresses were estimated on a daily basis for the whole period of pregnancy using land-use regression following a standardized procedure.[33,34] Among the INMA regions, data on PM were only available in Sabadell and in Rhea, only PM_{10}, $PM_{2.5}$, and $PM_{2.5-10}$ concentrations were estimated. Land-use regression models were developed for each pollutant metric using all measurement sites. We used a back-extrapolation procedure to estimate exposure concentrations for each woman averaged across the first trimester at the participant's home address using daily concentrations from routine background monitoring network sites. LUR models explained a large fraction of the spatial variance in measured annual average air pollutant concentrations.[33,34] In Project Viva, validated prediction models were used to obtain spatially and temporally resolved estimates of daily $PM_{2.5}$ exposure at each participant's residential address following a method described elsewhere.[35] Briefly, this method combines the satellite aerosol optical depth (AOD) data at the 10 km×10 km spatial grid with the spatiotemporal land use regression models based on monitored ground $PM_{2.5}$ measurements. Satellite remote sensing provides an important tool for monitoring aerosols where surface monitors are not available. For measurement of NO_2, we calculated hourly ambient concentrations of NO_2 by averaging data from the Massachusetts Department of Environmental Protection's Greater Boston monitoring sites (http://public.dep.state.ma.us/MassAir/), then calculated daily and the first trimester NO_2 exposure. In ABCD, Generation R, INMA, and Project Viva, if more than one address was collected during the first trimester, we calculated the weighted average concentrations of all addresses according to the time spent at each address.

Serum free T_4 and TSH concentrations were measured at the median gestational age of 13 weeks in ABCD (range: 5-37 weeks), Generation R (range: 6-18 weeks), INMA (range: 7-33 weeks) and Rhea (range: 6-27 weeks). In Project Viva, TSH, total T_4, and T_3 resin uptake were measured at median gestational age of 9.6 weeks (range: 6-21 weeks) allowing calculation of the free T_4 index, an estimate of circulating free T_4 levels from total T_4 × T_3 uptake. Cohorts used different assays for measurements of thyroid hormones and antibody (eTable 1 in the Supplement).

To define hypothyroxinemia, we followed the recommendation of the American Thyroid Association guideline, which describes hypothyroxinemia as free T_4 concentrations in the lower 2.5^{th}–5^{th} percentile of the population despite normal TSH.[36] Following this guideline, we calculated population-specific cut-offs in individuals without a history of thyroid disease or thyroid medication use and those who are negative for TPOAb. In INMA, TPOAb was not measured and, thus, no exclusion was made based on TPOAb positivity. We also defined high TSH as concentrations higher than 95^{th} percentile of the cohort (population-specific cut-off, corresponding cut-offs in each cohort in eTable 2). We also tested alternative cut-offs of 2.5^{th} percentile for free T_4 as well as the cut-off of 0.03-2.5 mIU/L for normal TSH to examine whether any

observed associations were independent of cut-off choice. We used the laboratory recommended cut-offs for TPOAb positivity (eTable 1). For details on Measurements of Covariates, see Supplement.

Statistical analysis

We used Spearman correlations to examine the relationship between concentrations of air pollutants. We performed logistic regression models to assess the short-term association of air pollutants (averaged in the first trimester) with hypothyroxinemia, high TSH, and TPOAb positivity in pregnant women (across pregnancy). First, we explored any indication of non-linearity in the associations between air pollutants and thyroid parameters using generalized additive models. Results confirmed the linearity of associations and indicated no threshold effect. Next, associations of air pollutants with hypothyroxinemia, high TSH, and TPOAb positivity were examined in each cohort. Cohort-specific effect estimates from regression models were then combined using random-effects meta-analysis after exploring the heterogeneity in the estimates among cohorts. We assessed heterogeneity in the estimates using the Q test and the I^2 statistic. Similar to previous studies, we reported the odds ratios (OR) of hypothyroxinemia, high TSH, and TPOAb positivity per 10 $\mu g/m^3$ change in NO_2 and PM_{10}, 20 $\mu g/m^3$ for NO_X, 5 $\mu g/m^3$ for $PM_{2.5}$ and $PM_{2.5-10}$, and $10^{-5} m^{-1}$ for $PM_{2.5}$ absorbance based on the distribution of pollutants.[17]

Selection of confounders was *a priori* and based on the direct acyclic graph of the study question and factors associated with air pollution exposure and thyroid function.[37-39] We did not adjust for season because no evidence exists on the relationship between season of pregnancy and free T4 or TSH levels (despite associations with air pollution).[40,41] Models included information on maternal age at enrollment, education, country of birth, smoking and alcohol intake during pregnancy, parity, pre-pregnancy BMI, gestational age at thyroid measurement, social class, and marital status. We adjusted the analyses in INMA for four regions.

Pregnant women who were included in the analysis were different from those excluded because of missing data on exposure and outcome (eTable 3). To address the selective non-response arising from these differences, we used inverse probability weighting. Briefly, we used the available information for eligible women (eTable 4) to predict the probability of participation in the study, and used the inverse of those probabilities as weights in the analyses so the results would be representative of the full cohort.

Among participants with exposure and outcome data, information on covariates was missing in <10% of participants, except for pregnancy smoking (10% in Generation R), alcohol intake (15% in Rhea), social class (16% in ABCD, 25% in Generation R, and 30% in Rhea), and pre-pregnancy BMI (17% in Generation R). We addressed missing data in covariates by imputing data using STATA ice command for chained equations imputation. We created 25 datasets with

complete observations in which analyses were performed using standard combination rules for multiple imputations (eTable 5).

In a sensitivity analysis, we ran the meta-analysis for hypothyroxinemia excluding Project Viva for which a measure of free T_4 index was available instead of free T_4. Additionally, in Project Viva, NO_2 was measured using central monitors; therefore, we also reran the NO_2 meta-analysis excluding Project Viva.

All analyses were performed in STATA 14.0 (Stata Corporation, College Station, Texas) between January 2018 and April 2019.

Results

Table 1 summarizes participants' characteristics. Overall, the mean age of participants at enrollment was 31.2 years (SD=4.8), 50% had high educational levels, 57% were nulliparous, and only 6% were single. From 9931 women included in the analysis, 80% did not smoke during pregnancy and 70% reported alcohol intake in pregnancy. A larger proportion of participants in Project Viva had a 'high social class' as compared to women in other cohorts. Women in INMA and Rhea had lower educational levels compared to women in other cohorts. 13.9% of women were positive for TPOAb in Project Viva, which was modestly higher than ABCD (5.5%), Generation R (6.0%), and Rhea (9.3%). Median (IQR) concentrations of $PM_{2.5}$ were between 11.5 $\mu g/m^3$ (1.6) in Project Viva and 20.6 $\mu g/m^3$ (4.5) in ABCD (eTable 6, Figure 1 and eFigure 1). These numbers were 21.6 $\mu g/m^3$ (2.6) and 41.6 $\mu g/m^3$ (14.7) for NO_2 in Project Viva and ABCD. Concentrations of NO_2 and $PM_{2.5}$ were lower and had less variation in Project Viva as compared to four European cohorts. eTable 7 shows the correlation between air pollutants in each cohort, reflecting high correlations between NO_2 and NO_x and among PM concentrations.

The short-term associations of air pollutants (concentrations averaged in the first trimester) with hypothyroxinemia and high TSH across pregnancy are presented in Table 2 and Figure 2 (unadjusted analyses in eTable 8; complete cases analysis without imputation of covariates in eTable 9). We found no associations between NO_2 and NO_x concentrations and hypothyroxinemia during pregnancy in the meta-analysis of estimates in cohorts with available data. When we ran the meta-analysis excluding Project Viva, results remained unchanged (data not shown). NO_2 and NO_x exposures were not associated with high TSH during pregnancy.

Women with higher exposures to $PM_{2.5}$ in the first trimester had higher odds of hypothyroxinemia during pregnancy (Table 2 and Figure 3, unadjusted analyses in eTable 8, complete case analysis in eTable 9). Exposure to PM_{10} was associated with higher odds of hypothyroxinemia but the coefficient was smaller and imprecise. When we used the free T_4 cut-off of 2.5^{th} percentile to define hypothyroxinemia, the results did not change (data not shown). The associations between PM exposures and high TSH (concentrations >95^{th} percentile) during pregnancy were not significant (Table 2); but when we

used the clinical cut-off of 0.03-2.5 mIU/l for normal TSH, we found significant associations between exposures to $PM_{2.5}$ and PM_{10} and high TSH (OR per Δ 5 $\mu g/m^3$ of $PM_{2.5}$ =1.23, 95%CI: 1.09-1.39 and OR per Δ 10 $\mu g/m^3$ of PM_{10} =1.24, 95%CI: 1.02-1.51). $PM_{2.5-10}$ and $PM_{2.5}$ absorbance were not associated with hypothyroxinemia or high TSH, with effect estimates close to null (Table 2).Examination of the association between air pollution exposure and TPOAb positivity showed large heterogeneity among cohorts (p values for heterogeneity < 0.001 for NO_2, PM_{10}, $PM_{2.5}$ and $PM_{2.5-10}$, and 0.01 for NO_x). Therefore, we only performed cohort-specific analysis for TPOAb. In Generation R, higher concentrations of air pollutants were associated with TPOAb positivity (e.g., OR per Δ10 $\mu g/m^3$ of NO_2=1.22, 95%CI: 1.11-1.34; OR per Δ5 $\mu g/m^3$ of $PM_{2.5}$=1.76, 95%CI: 1.51-2.04; and OR per Δ10 $\mu g/m^3$ of PM_{10}=1.96, 95%CI: 1.64-2.35). There was no association between air pollution exposure and TPOAb positivity in other cohorts with available data.

Discussion

In a large sample from five cohorts in Europe and the US, we found that first trimester exposures to $PM_{2.5}$ were associated with mild thyroid dysfunction across pregnancy. NO_x and NO_2 exposures were not associated with hypothyroxinemia or high TSH during pregnancy. In Generation R, we observed that pregnant women with higher exposures to NO and PM were more likely to be TPOAb positive.

Studies have shown associations between PAH exposure and thyroid dysfunction in non-pregnant populations,[42] and cigarette smoking and thyroid dysfunction in pregnant women.[39] Three observational studies have specifically examined the impact of air pollutants on thyroid function of pregnant women and their neonates.[16-18] Howe et al. showed that prenatal exposures to $PM_{2.5}$ and PM_{10} but not NO_2 and ozone were associated with higher neonatal total T4.[18] Zhao et al. reported positive associations between residential $PM_{2.5}$ concentrations and maternal hypothyroxinemia during mid-gestation.[17] Janssen et al. found that the third trimester $PM_{2.5}$ exposure was negatively associated with free T_4 in maternal serum.[16] Our results extend these observations and show that the association between $PM_{2.5}$ concentrations and hypothyroxinemia is present in the first trimester, the period when the fetus is the most sensitive to maternal thyroid dysfunction. In addition, we found no association between NO_2 and NO_x exposures and thyroid function in pregnancy, similar to the previous study in China. Consistent findings on null associations between NO_2 and thyroid function –and our first report on null associations of NO_x and thyroid function– in combination with observed associations between PM and thyroid function suggest that the impact of air pollutants on thyroid function may be mostly related to PM. Importantly, our findings also confirms that the associations between air pollution exposure and thyroid dysfunction in pregnant women is present with concentrations of pollutants at levels much lower that the study in China,[17] as also shown in Belgium and Calidfornia.[16,18]

While our findings indicate a short-term relationship between PM exposure and thyroid function, mechanisms of this association are not fully understood and need further investigation. Even though speculative, direct interference in the intracellular action of deiodinase enzymes and induction of oxidative stress and inflammation are among the short-term mechanisms.[5,43]Autoimmune processes might act on thyroid function in a longer period. In women of reproductive age, autoimmunity is a common cause of thyroid dysfunction in iodine-sufficient areas. Earlier studies in pregnant women have shown that prolonged lead exposure is associated with TPOAb positivity and subsequently low thyroid function.[44] We found that exposure to PM during early pregnancy was associated with higher odds of thyroid autoimmunity in Generation R, an iodine sufficient cohort in the Netherlands. The observed associations between PM exposures and low thyroid function and the null association with NO_2, NO_x, and $PM_{2.5}$ absorbance, a measure of black carbon, suggest that the composition of PM rather than the general markers of traffic pollution may be responsible for thyroid disruption. One hypothesis –supported by *in vitro* studies[45]– is that thyroid toxicity of PM exposure is due to the effect of PAH but we cannot rule out the impact of other components such as trace elements.

Cohorts varied with respect to the concentrations of air pollution exposure. For example, ABCD and Generation R had higher concentrations of $PM_{2.5}$ exposure compared to other cohorts. Exposure to concentrations of NO and PM measures in European cohort were positive and moderate to strong; but there was a negative and small correlation between NO_2 and $PM_{2.5}$ in Project Viva. These differences can be explained by varying sources for exposure to pollutants in different regions as well as different exposure assessment methods in European cohorts and in Project Viva. Nonetheless, examination of the heterogeneity in estimates for analysis of NO_2 and $PM_{2.5}$ with thyroid function confirmed that estimates across cohorts could be combined in the meta-analysis. There was an inverse but imprecise association between $PM_{2.5}$ and hypothyroxinemia in Project Viva, the smallest cohort with lowest concentrations of $PM_{2.5}$, suggesting that the association might be present at a threshold of exposure. Nonetheless, results of analyses using generalized additive models to create the soothing curve spline confirmed no threshold effect of pollutants. With regard to TPOAb positivity, appropriate testing showed large heterogeneity among cohorts. In particular, a larger number of women were positive for TPOAb in Project Viva compared to other cohorts, potentially explained by natural variation, use of different assay, slightly higher mean age in Project Viva at the time of assessment, and earlier measurement during pregnancy. Also, the association between air pollution exposure and TPOAb positivity was only present in Generation R. Whether iodine status of the cohort population or other characteristics might explain the differences on how air pollution exposure influence thyroid autoimmunity, further investigation is needed.

Limitation

This study has several strengths including a large number of participants from regions with different iodine status and diverse sociodemographic characteristics in Europe and the US. Nonetheless, we faced important limitations. We had only measures of TSH, free T_4, and TPOAb during pregnancy (mostly in the first half of pregnancy) and measurements were performed using different assays across cohorts. While the absolute concentrations of TSH and free T_4 can vary between assays, we defined the outcomes by population and assay-specific cut-offs to overcome any issues related to the interchangeability of absolute concentrations assay results. We adjusted the models for history of smoking in pregnancy, but we did not have data on secondhand tobacco smoke exposure in pregnant women. We relied on residential addresses to estimate air pollution exposure, without consideration for within-individual spatial variation in exposure. Furthermore, estimation of air pollution exposure in European cohorts did not account for temporal variation across the first trimester and for NO_2 in Project Viva for spatial variation between individuals. Another limitation concerning the European cohorts is that air pollution measurements were done some years after the pregnancy periods and we used routine monitoring data to back-extrapolate the concentrations to the exact first trimester of pregnancy. We therefore assumed that the spatial distribution of the sources and predictors of air pollution levels remain stable over time, as previous research showed.[47] We did not have history of addresses in pregnant women and thus could not examine the association between air pollution exposure prior to pregnancy and thyroid function. Subsequently, no conclusion on the critical window of exposure or accumulation of risk can be drawn from this analysis.

Conclusion:

The associations between $PM_{2.5}$ exposures and thyroid function in pregnant women are of global health importance because air pollution exposure is widespread and hypothyroxinemia may adversely influence offspring brain development.

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Authors' contributions

Monica Guxens had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

AG and MG conceived the study, designed the analytical plan, and drafted the manuscript. LP performed the statistical analysis. MB, ME, AFS, JJ, AL, MJLE, AT, MV, and JS are the principal investigators of INMA four sub-cohorts and were responsible for the cohort data preparation. LC, PK, and ES are the principal investigators of Rhea and were responsible for the cohort data preparation. EO is the principal investigator and AFF and DRG are co-investigators in the Project Viva. SR is the Project Viva data analyst and was responsible for the cohort data preparation. RP and HT are the principal investigators of Generation R and helped in the interpretation and collection of the thyroid data. TAM and TIMK were responsible for Generation R data preparation. TGMV is the principal investigator of ABCD cohort and was responsible for the cohort data preparation. All authors contributed to interpretation of the results and critically reviewed and revised the manuscript. All authors have read and approved the final version.

Conflict of interest statements

No conflict of interest to declare.

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The funders of the study had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Figure 1 Title Distribution of air pollutants averaged across the first trimester of pregnancy in five birth cohorts

Figure 1 Legend

NO_2: Nitrogen dioxide; NO_2: Nitrogen dioxide; $PM_{2.5}$: Particulate matter less than 2.5µm; PM_{10}: Particulate matter less than 10µm

Data on PM were only available in Sabadell sub-cohort of INMA.

Figure 2 Title Exposure to NO_2 and NO_x in the first trimester and thyroid function during pregnancy: results from five birth cohorts

Figure 2 Legend:

CI: Confidence interval; OR: Odds ratio; NO_2: Nitrogen dioxide; NO_2: Nitrogen dioxide; P: P value of heterogeneity; T4: Thyroxine; TSH: Thyroid stimulating hormone.

Odds ratios (95%CI) are shown for the associations between NO_2 exposure and hypothyroxinemia (a), NO_x exposure and hypothyroxinemia (b), NO_2 exposure and high TSH (c), and NO_x exposure and high TSH (d),estimated using random-effects meta-analysis by cohort (ABCD, Generation R, INMA, Rhea, and Project Viva).

We defined hypothyroxinemia as free T_4 below the 5^{th} percentile of cohort distribution despite normal TSH. High TSH was defined as values > 95^{th} percentile. I^2 refers to the percentage of the total variability due to between-areas heterogeneity. P value of heterogeneity was estimated using the Cochran's Q test. Models were adjusted for pregnant maternal age at enrollment, educational level, country of birth, gestational age at thyroid measurement, history of smoking, alcohol intake during pregnancy, socioeconomic status, marital status, parity, and pre-pregnancy body mass index. In addition, analysis in INMA was adjusted for regions (Sabadell, Gipuzkoa, Valencia, and Asturias).

Figure 3 Title: Exposure to $PM_{2.5}$ and PM_{10} in the first trimester and thyroid function during pregnancy: results from five birth cohorts

Figure 3 Legend:

CI: Confidence interval; OR: Odds ratio; P: P value of heterogeneity; $PM_{2.5}$: Particulate matter less than 2.5µm; PM_{10}: Particulate matter less than 10µm; T4: Thyroxine; TSH: Thyroid stimulating hormone.

Odds ratios (95%CI) are shown for the association between $PM_{2.5}$ exposure and hypothyroxinemia (a), PM_{10} exposure and hypothyroxinemia (b), $PM_{2.5}$ exposure and high TSH (c), and PM_{10} exposure and high TSH (d), estimated using random-effects meta-analysis by cohort (ABCD, Generation R, INMA, Rhea, and Project Viva). We defined hypothyroxinemia as free T_4 below the 5^{th} percentile of cohort distribution despite normal TSH. High TSH was defined as values > 95^{th} percentile. I^2 refers to the percentage of the total variability due to between-areas heterogeneity. P value of heterogeneity was estimated using the Cochran's Q test. Models were adjusted for pregnant maternal age at enrollment, educational level, country of birth, gestational age at thyroid measurement, history of smoking, alcohol intake during pregnancy, socioeconomic status, marital status, parity, and pre-pregnancy body mass index. Data on PM was only available in Sabadell region of INMA.

Table 1 Participants' characteristics (n=9931)

	Cohorts					
	ABCD (NL) n = 3867	**Generation R (NL) n = 2605**	**INMA (ES) n = 2239**	**Rhea (GR) n = 483**	**Project Viva (US) n = 737**	**Total n=9931**
Age at enrollment, y	33.6 (3.9)	30.8 (4.7)	31.4 (4.2)	29.3 (4.9)	32.5 (4.7)	31.2 (4.8)
Educational levels, %						
Elementary	17.8	17.2	24.4	20.1	1.2	18.0
Secondary	27.6	30.4	41.2	53.0	24.3	32.4
Higher	54.6	52.4	34.4	26.9	74.5	49.6
Nulliparous, %	60.5	60.7	56.6	40.4	49.7	56.7
Country of birth, foreign, %	31.3	42.8	8.4	8.9	16.2	27.0
Marital status, single, %	7.1	10.4	1.7	12.8	5.4	6.0
History of smoking, never, %	93.8	75.5	68.2	66.7	69.5	79.1
Alcohol intake in pregnancy, %	24.1	46.7	9.6	25.1	73.6	69.6
Social class, low, %	12.0	3.9	53.1	8.3	1.4	20.0
Pre-pregnancy BMI, kg/m^2	21.9 (3.5)	22.6 (4.4)	22.5 (4.4)	23.4 (5.3)	23.5 (5.3)	23.4 (4.2)
Thyroid function in pregnancy						
Free T_4, pmol/L	9.5 (8.6-10.4)	14.8 (13.2-16.6)	10.4 (9.5-11.4)	15.1 (13.9-16.6)	2.1 (1.9-2.3)[a]	10.7 (9.4-13.5)[b]
TSH, mIU/L	1.2 (0.8-1.7)	1.7 (1.0-2.5)	1.3 (0.8-1.8)	1.1 (0.7-1.6)	1.2 (0.7-1.9)	1.3 (0.8-1.9)
Hypothyroxinemia, %	4.1	4.4	4.2	4.8	3.2	4.2
TPOAb positive, %	5.5	6.0	NA	9.3	13.9	6.7
Gestational age at thyroid measurement, weeks	13.0 (11.9-14.0)	13.1 (12.1-16.8)	13.0 (12.3-14.0)	13.0 (12.0-15.0)	9.6 (8.7-10.7)	12.9 (11.9-12.3)

BMI: Body mass index; NA: Not available; T_4: Thyroxine; TSH: Thyroid stimulating hormone; TPOAb: Thyroid peroxidase antibodies.

Numbers are mean (SD) for continuous variables with normal distribution (age, BMI), median (25^{th} and 75^{th} percentile) for continuous variables with skewed distribution (free T_4, TSH, and gestational age at thyroid measurement), and percentages for categorical variables.

We defined hypothyroxinemia as free T_4 below the 5^{th} percentile of cohort distribution despite normal TSH.

[a] Free T_4 index (no unit) in Project Viva, calculated from total T_4 × triiodothyronine resin uptake

[b] Excluding Project Viva

Table 2 Associations between exposure to ambient air pollutants in the first trimester and thyroid function during pregnancy: results from five birth cohorts.

	Hypothyroxinemia				High TSH			
	N[a]	OR (95%CI)	P_h	I^2	N[a]	OR (95%CI)	P_h	I^2
NO_2 (per Δ10 µg/m^3)	4	0.96 (0.82-1.12)[b]	0.16	41.25	4	1.02 (0.94-1.12)	0.83	0.00
NO_X (per Δ 20 µg/m^3)	3	0.95 (0.87-1.03)	0.60	0.00	3	0.99 (0.93-1.06)	0.80	0.00
$PM_{2.5}$ (per Δ 5 µg/m^3)	5	1.21 (1.00-1.47)[c]	0.37	6.65	5	1.14 (0.88-1.48)	0.12	45.58
PM_{10} (per Δ 10 µg/m^3)	4	1.18 (0.93-1.48)	0.33	13.02	4	1.17 (0.87-1.58)	0.09	53.72
$PM_{2.5-10}$ (per Δ 5 µg/m^3)	4	1.05 (0.76-1.45)	0.17	40.63	4	1.18 (0.88-1.57)	0.12	47.79
$PM_{2.5}$ absorbance (per Δ 10^{-5}m^{-1})	4	1.05 (0.88-1.26)	0.40	0.00	4	1.10 (0.95-1.26)	0.84	0.00

CI: Confidence interval; OR: Odds ratio; NO_2: Nitrogen dioxide; NO_x: Nitrogen oxides; P_h: P value of heterogeneity; PM_{10}: Particulate matter less than 10µm; $PM_{2.5}$: Particulate matter less than 2.5µm; PM_{coarse}: Particulate matter between 2.5 and 10µm; $PM_{2.5}$ absorbance: Reflectance of $PM_{2.5}$ filters; T_4: Thyroxine; TPOAb: Thyroid peroxidase antibodies; TSH: Thyroid stimulating hormone.

Odds ratios (95%CI) were estimated using random-effects meta-analysis by cohort (ABCD, Generation R, INMA, Rhea, and Project Viva).

The median gestational age at thyroid measurement was at 13 weeks in all cohorts, except for Project Viva in which measurement was at median gestational age of 10 weeks.

We defined hypothyroxinemia as free T_4 below the 5th percentile of cohort distribution despite normal TSH. High TSH was defined as values > 95th percentile.

I^2 refers to the percentage of the total variability due to between-areas heterogeneity. P value of heterogeneity was estimated using the Cochran's Q test.

Models were adjusted for pregnant maternal age at enrollment, educational level, country of birth, gestational age at thyroid measurement, history of smoking, alcohol intake during pregnancy, socioeconomic status, marital status, parity, and pre-pregnancy body mass index. In addition, analysis in INMA was adjusted for regions (Sabadell, Gipuzkoa, Valencia, and Asturias).

[a] Number of cohorts included in the meta-analysis. Data on PM was only available in Sabadell region of INMA.

[b] OR excluding participants of Project Viva: per Δ10 µg/m^3 of NO_2=0.97 (95%CI: 0.87-1.08)

[c] OR excluding participants of Project Viva: per Δ 5 µg/m^3 of $PM_{2.5}$=1.23 (95%CI: 1.04-1.47)