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# Fetal Thyroid Function, Birth Weight, and *in Utero* Exposure to Fine Particle Air Pollution: A Birth Cohort Study

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# ABSTRACT

**Background:** Thyroid hormones are critical for fetal development and growth. Whether prenatal exposure to fine particle air pollution ( $PM_{2.5}$ ) affects fetal thyroid function and what the impact is on birth weight in normal healthy pregnancies is not studied yet.

**Objectives:** To study the impact of third trimester  $PM_{2.5}$  exposure on fetal and maternal thyroid hormones and their mediating role on birth weight.

**Methods:** We measured the levels of free thyroid hormones (FT<sub>3</sub>, FT<sub>4</sub>) and thyroid stimulating hormone (TSH) in cord blood (n = 499) and maternal blood (n = 431) collected after delivery from mother-child pairs enrolled between February 2010 and June 2014 in the ENVIR*ON*AGE birth cohort with catchment area in the province of Limburg, Belgium.

**Results:** An interquartile range (IQR) increment (+8.2  $\mu$ g/m<sup>3</sup>) in third trimester PM<sub>2.5</sub> exposure was inversely associated with cord blood TSH levels (-11.6%; 95% CI: -21.8, -0.1) and the FT<sub>4</sub>/FT<sub>3</sub> ratio (-62.7%; -91.6, -33.8). A 10<sup>th</sup>-90<sup>th</sup> percentile decrease in cord blood FT<sub>4</sub> levels was associated with a 56 g decrease in mean birth weight (95% CI: -90, -23). Assuming causality, we estimated that cord blood FT<sub>4</sub> mediated 21% (-19 g; 95%CI: -37, -1) of the estimated effect of an IQR increment in third trimester PM<sub>2.5</sub> exposure on birth weight. Third trimester PM<sub>2.5</sub> exposure was inversely but not significantly associated with maternal blood FT<sub>4</sub> levels collected one day after delivery (-4.0%, 95% CI: -8.0, 0.2 for an IQR increment in third trimester PM<sub>2.5</sub>).

**Conclusions:** In our study population of normal healthy pregnancies, third trimester exposure to  $PM_{2.5}$  air pollution was associated with differences in fetal thyroid hormone levels that may contribute to reduced birth weight. Additional research is needed to confirm our findings in other populations and to evaluate potential consequences later in life.

## **INTRODUCTION**

During prenatal life, thyroid hormones are critical for fetal growth and development, especially neurodevelopment (Burrow et al. 1994; Morreale de Escobar et al. 2004). Unbalanced thyroid function influences pregnancy outcomes and adversely affects the fetus. In particular, both maternal hypo- and hyperthyroidism are associated with increased risk of low birth weight (Blazer et al. 2003; Millar et al. 1994), while other studies also suggest an important role of fetal thyroid function in regulating fetal growth (Medici et al. 2013; Shields et al. 2011).

Thyroxine ( $T_4$ ), the major form of thyroid hormone, and triiodothyronine ( $T_3$ ), the active form, are controlled by thyroid-stimulating hormone (TSH) and released by the thyroid gland. Bound to plasma proteins, these thyroid hormones are transported throughout the body and diffuse from maternal blood across the placenta to reach the fetus (Calvo et al. 2002). However, it is the unbound, free fraction of these hormones ( $FT_4$  and  $FT_3$ ) that are taken up by different cell types to regulate their functioning (Hennemann et al. 2001). From the second trimester of gestation onwards, the fetal thyroid gland becomes functional and the fetus is able to produce its own supply of thyroid hormones in addition to the maternal supply (Morreale de Escobar et al. 2004).

Findings from previous studies suggest that airborne persistent organic pollutants (Abdelouahab et al. 2013; Baccarelli et al. 2008; Maervoet et al. 2007), cadmium (Iijima et al. 2007), and exposure to active and passive cigarette smoke (Soldin et al. 2009) may affect thyroid hormone regulation and function in neonates and adults, however epidemiological studies on the impact of exposure to particulate matter (PM) air pollution on thyroid hormones are lacking. In large areas of the world, PM air pollution is an omnipresent environmental risk factor of public health concern, especially the fine particles with an aerodynamic diameter  $\leq 2.5 \ \mu m (PM_{2.5})$ . Exposure to ambient  $PM_{2.5}$  pollution during pregnancy has been found to be significantly associated with increased risk of low birth weight at term in mother-child cohorts

of 12 European countries (Pedersen et al. 2013) and preterm birth (20-36 weeks of gestation) in a very large cohort of singleton pregnancies from three states of the USA (Rappazzo et al. 2014).

In spite of the well-established link between  $PM_{2.5}$  air pollution and adverse gestational outcome (Pedersen et al. 2013), the role of fetal thyroid function in this association has never been investigated. Therefore, we hypothesized that airborne  $PM_{2.5}$  exposure during gestation affects fetal thyroid hormone function in normal healthy pregnancies and contributes to reduced birth weight. We tested this hypothesis in the framework of a mother-child cohort by studying the impact of third trimester  $PM_{2.5}$  exposure on fetal and maternal thyroid hormone function, as reflected by the levels of  $FT_3$ ,  $FT_4$ , and TSH in cord blood and maternal blood, and their mediating role on birth weight.

## **METHODS**

## **Study population**

From February 2010 to June 2014, we recruited 640 mother-child pairs after delivery at the East-Limburg Hospital in Genk (Belgium). They were enrolled in the on-going ENVIRONAGE birth cohort study (ENVIRonmental influence *ON* early AGEing) following procedures previously approved by the Ethical Committee of Hasselt University and the East-Limburg Hospital (Janssen et al. 2012). The study was conducted according to the principles outlined in the Helsinki Declaration for investigation of human subjects. The participation rate of eligible mothers in the birth cohort (mothers able to fill out a Dutch language questionnaire and those without planned Caesarean section) was 61% and enrolment was spread equally over all seasons of the year. Midwives recorded the reason of nonparticipation. The main reasons (in descending importance) were failure to ask for participation, communication problems, or complications during labor. Participating mothers provided written informed consent when

they arrived at the hospital for delivery. They completed study questionnaires in the postdelivery ward to provide detailed information on maternal age, pre-pregnancy body mass index (BMI), maternal education, occupation, self-reported smoking status, alcohol consumption, place of residence, use of medication, parity, and newborn's ethnicity. Former-smokers were defined as those who had quit smoking before pregnancy. Smokers continued smoking during pregnancy. Based on the native country of the newborn's grandparents we classified his/her ethnicity as European-Caucasian when two or more grandparents were European, or non-European when at least three grandparents were of non-European origin. We asked the mothers whether they consumed alcohol during pregnancy. Maternal education was coded as "low" (no diploma or primary school), "middle" (high school) or "high" (college or university degree). After birth, we collected perinatal parameters from the medical files such as birth date, gestational age, newborn's sex, birth weight and length, length of labor, Apgar score, pH of arterial cord blood, and ultrasonographic data.

The main analysis of our investigation was conducted in a sub-cohort of the 640 singleton pregnancies in the ENVIR*ON*AGE birth cohort, after excluding 16 mothers with thyroid gland complications, including hyper- or hypothyroidism, 79 mothers from which we had no complete set of cord blood thyroid hormone values, 28 Caesarean sections, and 18 preterm births (< 37 weeks), leaving 499 mother-child pairs for the main analysis (Figure 1). Additionally, maternal blood could not be collected from 68 mothers resulting in a study population of 431 for the maternal thyroid hormone analysis (mother group) (Figure 1). Our study population was generally similar to all births in Flanders [data obtained from the Study Centre for Perinatal Epidemiology (SPE)] as to maternal age, education, parity, sex, ethnicity, and birth weight (Supplemental Material, Table S1) (Cox et al. 2013).

## Ambient exposure assessment

For each mother's residential address, we interpolated the regional background  $PM_{2.5}$  (µg/m<sup>3</sup>) using a spatial temporal interpolation method (Kriging method) (Janssen et al. 2008) that uses pollution data collected by the official fixed-site monitoring network and land-cover data obtained from satellite images (CORINE land-cover data set) in combination with a dispersion model (Lefebvre et al. 2011). This model chain provides daily  $PM_{2.5}$  values using data from the Belgian telemetric air quality network, and point and line sources which is then interpolated in a high resolution receptor grid. In the Flemish region of Belgium, the interpolation tool explained more than 80% of the temporal and spatial variability (Maiheu et al. 2012). We defined the third trimester of pregnancy as from week 27 to delivery and calculated the mean  $PM_{2.5}$  values for this trimester. The date of conception was estimated on the basis of the first day of the mother's last menstrual period, combined with the first ultrasound exam. Complete information for the residential address during pregnancy was obtained by questionnaire and checked with hospital records. For those who moved during pregnancy (n = 54; 10.8%), we calculated the third trimester exposure window allowing for the changes in address during this period.

Mean daily temperatures and relative humidity for the study region were provided by the Royal Meteorological Institute (Brussels, Belgium). We calculated the third trimester apparent temperature by using the following formula (Kalkstein and Valimont 1986; Steadman 1979): –  $2.653 + (0.994 \times Ta) + (0.0153 \times Td^2)$ , where Ta is air temperature and Td is dewpoint temperature (degrees Celsius).

## Blood collection and thyroid hormone measurements

Umbilical cord and maternal blood samples (8 mL each) were collected in plastic BD Vacutainer® Lithium Heparin Tubes (BD, Franklin Lakes, NJ, USA) immediately after delivery and one day after delivery respectively. The samples were centrifuged (3200 rpm for 15 min) to retrieve plasma which was instantly frozen at  $-80^{\circ}$ C. The plasma levels of FT<sub>4</sub> (pmol/L), FT<sub>3</sub> (pmol/L), and TSH (mU/L) were measured with an electro-chemiluminescence immunoassay using the Modular E170 automatic analyzer (Roche, Basel, Switzerland) at the clinical lab of East-Limburg Hospital.

## Statistical analysis

For database management and statistical analysis, we used the SAS software program (version 9.2; SAS Institute Inc., Cary, NC, USA). Thyroid hormone levels were log<sub>10</sub>-transformed to improve the normality of the distributions and described by geometric mean and 10<sup>th</sup>-90<sup>th</sup> percentile. The ratio FT<sub>4</sub>/FT<sub>3</sub> was calculated using untransformed values and had a normal distribution. Pearson correlation coefficients were calculated between the different thyroid hormone levels in blood (FT<sub>3</sub>, FT<sub>4</sub>, and TSH) and between thyroid hormone levels and birth weight. We performed multiple linear regressions to assess the associations between newborn or maternal thyroid hormones and PM<sub>2.5</sub> exposure during 3<sup>rd</sup> trimester of gestation, and between newborn or maternal thyroid hormones and birth weight. Exposures to PM<sub>2.5</sub> were fitted as linear variables in the models and effect estimates on thyroid hormones were calculated for an interquartile range (IQR) increment in PM2.5. The effect estimates of cord blood FT<sub>4</sub> on birth weight was calculated for a 10<sup>th</sup>-90<sup>th</sup> percentile decrease in FT<sub>4</sub>, which corresponds to a 11% difference in FT<sub>4</sub>. All cord blood models were adjusted for sex, gestational age (weeks), season of delivery (Winter  $[21^{st} \text{ of } Dec - 20^{th} \text{ of } Mar] / \text{Spring } [21^{st} \text{ of }$ Mar  $-20^{\text{th}}$  of Jun] / Summer [21<sup>st</sup> of Jun  $-20^{\text{th}}$  of Sep] / Autumn [21<sup>st</sup> of Sep  $-20^{\text{th}}$  of Dec]), Appar score (< 9 / 9 / 10), maternal age (years), pre-pregnancy BMI (kg/m<sup>2</sup>), smoking status (never-smoker / cessation before pregnancy / smoker), parity  $(1 / 2 / \ge 3)$ , ethnicity (European-Caucasian, yes or no), maternal education (low / middle / high), and third trimester apparent temperature (°C), while all models for maternal blood were adjusted for the same covariates except newborn's sex and Apgar score. In an additional analysis, we adjusted the cord blood models for maternal thyroid hormones. The Shapiro-Wilk statistic and Q-Q plots of the residuals were used to test the assumptions of model linearity.

We used mediation analysis to investigate potential associations that may underlie the relation between the exposure variable ( $PM_{2.5}$ ) and the continuous outcome variable (birth weight, g) by examining how they relate to a third variable, the mediator (cord blood FT<sub>4</sub> levels) (Valeri and Vanderweele 2013). We accomplished this by decomposing the total effect into a direct effect (DE: exposure effect on outcome at a fixed level of the mediator) and an indirect effect (IE: exposure effect on outcome that operates through the mediator). Mediation analysis is based on several assumptions: all associations are causal, with no uncontrolled confounding of associations between the exposure and mediator, the exposure and the outcome, or the mediator and the outcome; no measured mediator (Valeri and Vanderweele 2013).

#### Sensitivity analysis

Tobacco smoke exposure, a form of personalized airborne PM exposure, has been shown to influence maternal and fetal thyroid function through changes in thyroid hormone levels (Männistö et al. 2012; McDonald et al. 2008; Shields et al. 2009; Soldin et al. 2009). In a sensitivity analysis (Supplemental Material), we performed linear regression analysis to examine the associations between newborn or maternal thyroid hormones and smoking, adjusting for the same co-variables as mentioned above except smoking. Additionally, we repeated the analysis between cord blood thyroid hormones and PM<sub>2.5</sub> exposure while excluding smokers.

Thyroid hormones may also show seasonal variations linked to changes in temperature (Reed 1995). To account for possible seasonality differences between subjects, we calculated for each subject an exposure window covering a 1-year period, i.e. 365 days calculated backwards from the date of delivery.

Furthermore, it is known that cord blood thyroid hormone levels are influenced by different external factors. We explored whether covariates such as cord plasma estradiol (Lv et al. 2014), passive smoking (Soldin et al. 2009), alcohol consumption (Herbstman et al. 2008), pH of arterial cord blood (Chan et al. 2001), or length of labor (Parate et al. 2010), known for their interference with thyroid hormones, may alter the association between cord blood thyroid hormones and  $3^{rd}$  trimester PM<sub>2.5</sub> exposure.

## RESULTS

## **Demographics of participants**

Table 1 shows demographic characteristics and perinatal traits of the mother-child group (n = 499). Mean (10<sup>th</sup>-90<sup>th</sup> percentile) maternal age was 29.1 years (23-35) and mean pre-pregnancy BMI was 23.9 (19.6-29.8) kg/m<sup>2</sup>. Most women never smoked (n = 316), 113 stopped smoking before pregnancy, and 70 mothers reported to continue with smoking during pregnancy (on average 8.6 cigarettes/day). More than 80% of the mothers reported no consumption of alcoholic beverages during pregnancy. The newborns, among them 254 girls (50.8%), had a mean gestational age of 39.4 weeks (38-41) and comprised 275 primiparous and 170 secundiparous newborns. About 90% of the newborns were Europeans of Caucasian ethnicity. The mean birth weight of the newborns was 3446 (2915-3990) g. Five minutes after delivery, more than 90% of the newborns had an Apgar score  $\geq 9$ .

## Thyroid hormone levels in cord blood and maternal blood

The geometric means of thyroid hormone levels in cord blood (n = 499) were 10.3 mU/L for TSH, 2.5 pmol/L for FT<sub>3</sub>, and 15.7 pmol/L for FT<sub>4</sub>, while in maternal blood (n = 431) it was 2.1 mU/L, 4.2 pmol/L, and 12.5 pmol/L respectively (Table 1). A positive correlation was observed between FT<sub>3</sub> and FT<sub>4</sub> (cord blood: r = 0.30; p < 0.0001, maternal blood: r = 0.27; p < 0.0001) and between FT<sub>3</sub> and TSH (cord blood: r = 0.11; p = 0.01, maternal blood: r = 0.19; p < 0.0001). Maternal FT<sub>4</sub> levels were positively correlated with cord blood FT<sub>4</sub> levels (r = 0.21, p < 0.0001), whereas an inverse correlation was observed with cord blood FT<sub>3</sub> levels (r = -0.11, p = 0.01). Compared with maternal values, the measured cord blood FT<sub>3</sub> levels were approximately two-fold lower and the TSH levels much higher. The thyroid hormone concentrations in cord blood were similar to values published by others (Abdelouahab et al. 2013).

## **Ambient exposure levels**

Average ( $25^{\text{th}}$ - $75^{\text{th}}$  percentile) PM<sub>2.5</sub> exposure and apparent temperature for the third gestational trimester were respectively 16.0 µg/m<sup>3</sup> (11.6-19.8) and 8.7°C (3.2-14.7). Mean levels of both parameters were similar throughout the trimesters of pregnancy (data not shown).

## Thyroid hormones and PM<sub>2.5</sub> exposure during gestation

In cord blood (n = 499), TSH levels and FT<sub>4</sub>/FT<sub>3</sub> ratios correlated inversely with PM<sub>2.5</sub> exposure during the third trimester of pregnancy (Figure 2). After adjustment for sex, gestational age, season of delivery, Apgar score, maternal age, pre-pregnancy BMI, smoking status, parity, ethnicity, maternal education, and apparent temperature, an IQR increment (+8.2  $\mu$ g/m<sup>3</sup>) in PM<sub>2.5</sub> exposure during the third trimester was associated with a lowering of 11.6% (95% CI: -21.8, -0.1; p < 0.05) in cord blood TSH levels (Figure 3, panel A) and a lowering of 62.7% (95% CI: -91.6, -33.8; p < 0.0001) in cord blood FT<sub>4</sub>/FT<sub>3</sub> ratio (Figure 3, Panel A).

Considering the FT<sub>4</sub> and FT<sub>3</sub> levels in cord blood separately (Figure 3, panel B), we observed opposite associations for an IQR increment of PM<sub>2.5</sub> exposure on these two hormones during the third trimester (FT<sub>4</sub>, -3.7%; 95% CI: -6.4, -0.9; p = 0.009, and FT<sub>3</sub>, +6.4%; 95% CI: 1.8, 11.1; p = 0.006). Additional adjustment for maternal thyroid hormones in the cord blood models did not alter our findings for cord blood (data not shown).

In maternal blood (n = 431), TSH and FT<sub>4</sub> levels correlated inversely with third trimester PM<sub>2.5</sub> exposure (r = -0.10, p = 0.04 and r = -0.13, p = 0.005 respectively). After adjustment for gestational age, season of delivery, maternal age, pre-pregnancy BMI, smoking status, parity, ethnicity, maternal education, and apparent temperature, only maternal FT<sub>4</sub> levels were inversely but not significantly associated with an IQR increment in third trimester PM<sub>2.5</sub> exposure (-4.0%; 95% CI: -8.0, 0.2; p = 0.06), similar to that with cord blood FT<sub>4</sub> (Figure 3, Panel B). Neither TSH nor the FT<sub>4</sub>/FT<sub>3</sub> ratio in maternal blood showed a significant difference with an IQR increment in third trimester PM<sub>2.5</sub> exposure.

## Thyroid hormones and birth weight

After adjustment for gestational age and sex, neither FT<sub>3</sub> nor TSH levels in maternal or cord blood were associated with birth weight ( $p \ge 0.47$ ). Though, a 10<sup>th</sup>-90<sup>th</sup> percentile decrease (11%) in cord blood FT<sub>4</sub> (log<sub>10</sub> values) was associated with a lowering in birth weight of 71 g (95% CI: -103, -38; p < 0.0001). After additional adjustment for maternal age, pre-pregnancy BMI, smoking status, parity, season of delivery, Apgar score, ethnicity, maternal education, and apparent temperature, the association for the cord blood model remained significant (-56 g; 95% CI: -90, -23; p = 0.001). In contrast, a 10<sup>th</sup>-90<sup>th</sup> percentile decrease (15%) in maternal FT<sub>4</sub> was positively associated with birth weight (+45 g; 95% CI: 6, 83; p = 0.02), though the association was weaker and no longer significant after full adjustment (+31 g; 95% CI: -7, 69, p = 0.11).

We performed mediation analysis to estimate the proportion of the PM<sub>2.5</sub> exposure effect on birth weight as mediated by cord blood FT<sub>4</sub>. Although we did not observe a significant association between third trimester PM<sub>2.5</sub> exposure and birth weight (p = 0.70), there is consensus among statisticians that the relationship between exposure (e.g. PM<sub>2.5</sub>) and outcome (e.g. birth weight) does not need to be statistically significant for a variable (e.g. FT<sub>4</sub>) to be a mediator (Valeri and Vanderweele 2013). Assuming causality, adjusted estimates of the proportion of mediation suggest that cord blood FT<sub>4</sub> levels explained 21% (indirect effect: -19 g; 95% CI: -37, -1; p = 0.03) of the association between the third trimester PM<sub>2.5</sub> exposure and birth weight (Figure 4). Since maternal thyroid hormones did not meet the assumptions for mediation (no association between maternal FT<sub>4</sub> and birth weight), we did not perform a mediation analysis.

## Sensitivity analysis

After adjustment for newborn's sex, gestational age, season of delivery, Apgar score, maternal age, pre-pregnancy BMI, parity, ethnicity, and apparent temperature, cord blood TSH levels were lower in mothers who continued smoking during pregnancy (-18.7%; 95% CI: -29.1, -6.7%; p = 0.003) and also in those who stopped smoking before pregnancy (-10.3%; 95% CI: -19.6, 0.1; p = 0.05) in comparison with never-smoking mothers (Supplemental Material, Table S2). When excluding never-smokers, we observed as expected an inverse association between smoking years and TSH levels in cord blood (r = -0.21; p = 0.004; n = 173). We did not find differences in cord blood FT<sub>4</sub> levels between smokers and former smokers compared with non-smokers. Newborns from women who stopped smoking before pregnancy had slightly lower FT<sub>3</sub> cord blood levels (-3.7%; 95% CI: -7.5, 0.1; p = 0.06) but, surprisingly, those who continued smoking had higher levels of cord blood FT<sub>3</sub> (+3.7%; 95% CI: -1.3, 9.0; p = 0.15) compared with non-smokers. We did not find an association between maternal thyroid

hormones and smoking status during pregnancy ( $p \ge 0.31$ ). The associations between cord blood thyroid hormones and PM<sub>2.5</sub> exposures did not alter while excluding women who smoked during pregnancy (data not shown).

The analysis to account for seasonality differences invariably showed for an IQR increment (+3.7  $\mu$ g/m<sup>3</sup>) of PM<sub>2.5</sub> during a 1-year period inverse associations with cord blood TSH levels (-8.8%; 95% CI: -14.8, -2.4; *p* = 0.008), the FT<sub>4</sub>/FT<sub>3</sub> ratio (-32.7%; 95% CI: -48.8, -16.6; *p* < 0.0001), the FT<sub>4</sub> levels (-1.7%; 95% CI: -3.2, -0.1; *p* = 0.03), and a positive association with FT<sub>3</sub> levels (+3.5%; 95% CI: 1.0, 6.0; *p* = 0.006), corroborating the associations found for the third trimester of pregnancy (Figure 3).

Additional adjustments for cord blood plasma estradiol (n = 498), passive indoor tobacco smoke exposure (n = 486), alcohol consumption (n = 485), or pH of arterial cord blood (indicator of hypoxemia) (n = 431), did not alter the associations between PM<sub>2.5</sub> exposure and FT<sub>3</sub> and FT<sub>4</sub> thyroid hormones shown in the main analysis (Supplementary Material, Table S3). Length of labor may influence thyroid hormone levels possibly due to the high energy demand during labor (Parate et al. 2010). Adjusting the main models for length of labor (n = 427) also did not alter the reported associations except that the association between cord blood TSH levels and third trimester PM<sub>2.5</sub> exposure was not significant anymore (Supplemental Material, Figure S1).

## DISCUSSION

To the best of our knowledge, our study is the first to show associations between airborne  $PM_{2.5}$  exposure and cord blood thyroid hormones. A key finding is that  $PM_{2.5}$  exposure during the third trimester of gestation is inversely associated with TSH levels and the  $FT_4/FT_3$  ratio in cord blood, but not with thyroid hormones in maternal blood. The  $FT_4/FT_3$  ratio in cord blood is a useful indicator of how effectively the body is able to convert  $T_4$  into  $T_3$  (Yoshimura Noh

et al. 2005). In addition, results of the mediation analysis suggested that cord blood  $FT_4$  is a partial mediator of the association between last-trimester pregnancy  $PM_{2.5}$  exposure and birth weight, assuming the underlying causal assumptions of mediation analysis are valid. Our findings highlight the potential influence early life environmental exposure to  $PM_{2.5}$  on fetal thyroid function and fetal growth. In addition, our results remained robust in multiple sensitivity analyses comprising maternal tobacco smoking, passive indoor smoking, seasonal variations, alcohol consumption, fetal hypoxemia, maternal estrogen levels, and length of labor.

During pregnancy, thyroid hormones regulate metabolism, stimulate differentiation and growth of the fetus, and influence neurocognitive development (Burrow et al. 1994; Morreale de Escobar et al. 2004). Despite the fact that the fetus starts secreting small amounts of thyroid hormone from mid-gestation onwards (Thorpe-Beeston et al. 1991), the mother already supplies thyroid hormones to the fetal circulation from the first trimester without compromising her own supply (Vulsma et al. 1989). The rise of maternal thyroid hormones in the first trimester of pregnancy is considered critical to ensure normal (neurological) development (Morreale de Escobar et al. 2004). Maternal T<sub>4</sub> and T<sub>3</sub> diffuse across the placenta to reach concentrations in the fetus which are in the same range as those in adult tissues (Calvo et al. 2002). Fetal T<sub>3</sub> is generated locally from T<sub>4</sub> by type-2 deiodinase, has a high affinity for nuclear binding sites in the placenta, and stimulates the production of factors that control trophoblast growth and development (Maruo et al. 1991). This suggests that thyroid hormones play an important role in normal placentation and development of the fetus. (Shields et al. 2011) showed in women with normal healthy pregnancies that placental weight was positively associated with cord blood FT<sub>4</sub> levels and inferred that thyroid hormones may influence fetal growth indirectly by affecting placental growth. These authors found that lower FT<sub>4</sub> levels in cord blood were associated with reduced birth weight and their results are corroborated by our study and two studies from the Netherlands (Korevaar et al. 2015; Medici et al. 2013).

Moreover, our study estimated that during the last trimester of pregnancy the estimated effect of PM<sub>2.5</sub> exposure on birth weight was for 21% (on average -19 g) mediated by cord blood FT<sub>4</sub> levels, assuming that all unverifiable assumptions of mediation analysis are valid. As in all observational studies, these estimates should be interpreted with caution because the underlying assumptions of causality between each pair of factors in the mediation analysis cannot be verified. Nevertheless, this finding suggests that the last trimester of pregnancy, when the fetus significantly increases in size, is an important window of susceptibility to PM<sub>2.5</sub> exposure. (Shields et al. 2011), (Medici et al. 2013), (León et al. 2015), as well as our study, report an inverse association between maternal FT<sub>4</sub> and birth weight, which is opposite to cord blood FT<sub>4</sub>. In a study of pregnant women without history of thyroid dysfunction, it has been shown that lower concentrations of FT<sub>4</sub> in maternal blood were related with increased placental growth (Bassols et al. 2011). These observations together suggest a functional discrepancy for FT<sub>4</sub> between maternal and fetal blood, especially with regard to fetal growth. In our study, we observed an inverse but no significant association (p = 0.06) between maternal FT<sub>4</sub> and third trimester PM<sub>2.5</sub> exposure in accordance with our findings in cord blood.

Contrary to maternal  $T_4$  and  $T_3$ , perfusion experiments with TSH on human term placentas have shown that TSH crosses placental tissue and fetal membranes only sparingly (Bajoria and Fisk 1998). Hence, our finding of an inverse association between cord blood TSH levels and PM<sub>2.5</sub> exposure during pregnancy suggests a potential effect of PM<sub>2.5</sub> on fetal thyroid function. Experimental studies showed that PM exposure in healthy rats modulates the hypothalamic-pituitary-thyroid axis and leads to increases in markers of glucocorticoid activity (Thomson et al. 2013), which are known to suppress TSH release (Wilber and Utiger 1969). In the context of anti-inflammatory actions of glucocorticoids, it is interesting to note previous findings from our birth cohort which suggest that ambient PM<sub>2.5</sub> exposure mayinduce a systemic oxidative stress response (Janssen et al. 2012) and increased placental protein-bound 3-nitrotyrosine (Saenen et al. 2016).

The FT<sub>4</sub>/FT<sub>3</sub> ratio in cord blood, a useful indicator of how effectively the body is able to convert  $T_4$  into  $T_3$  (Bassols et al. 2011), was inversely associated with  $PM_{2.5}$  during pregnancy. This finding could be explained by the fact that placental type 2 deiodinase activity increases when the availability of T<sub>4</sub> decreases, thus representing a potential homeostatic mechanism for maintaining T<sub>3</sub> production when T<sub>4</sub> concentrations are reduced (Glinoer 2004). In a population of 4837 euthyroid Finnish mothers, they observed that mothers who smoked before, or continued smoking during first trimester of pregnancy, had reduced blood levels of FT<sub>4</sub> and increased levels of FT<sub>3</sub> compared with non-smokers (Männistö et al. 2012). Constituents of tobacco smoke may stimulate the conversion of T<sub>4</sub> to T<sub>3</sub> in tissues by boosting type 2 deiodinase activity, as shown in cultured rat brain glial cells (Gondou et al. 1999). Low levels of TSH and FT<sub>4</sub> are suggestive of central hypothyroidism (defect of thyroid hormone production due to insufficient stimulation by TSH of an otherwise normal thyroid gland) (Persani 2012). Recently, it has been shown that intrauterine exposure to insufficient maternal thyroid hormone levels, characterized by low levels of FT<sub>4</sub> coexisting with reference TSH levels, was associated with higher scores for attention deficit hyperactivity disorder (ADHD) symptoms in 127 children at 8 years of age of a population-based birth cohort in the Netherlands (Modesto et al. 2015). Additional research is needed to confirm our findings in other populations and to evaluate potential consequences later in life.

Our study has some limitations. Firstly, thyroid hormones are responsive to environmental temperature (Reed 1995) and show a seasonal pattern with lower values in the cold period compared to the warm period of the year. Nevertheless, our results were robust for both seasonality differences between subjects as well as adjustment for third trimester apparent temperature. Secondly, iodine is required for the synthesis of thyroid hormones, but we did not

have information on iodine levels in maternal or cord blood. However, we excluded *a priori* clinically confirmed cases of hypo- and hyperthyroidism. Lastly, we acknowledge the fact that we cannot fully exclude residual or unmeasured confounding by other factors such as noise, polychlorinated biphenyls, heavy metals, or pesticides that could be associated with both ambient air pollution and thyroid function. Overall, the characteristics of the ENVIR*ON*AGE birth cohort were generally similar compared with all births in Flanders, except that we excluded Caesarean sections and preterm births, so our findings might be generalizable to the gestational segment of the population at large. (Supplemental Material, Table S1). We used a standardized fine-scale exposure model for the estimation of residential fine particle air pollution levels of the pregnant mothers (on average 16.0  $\mu$ g/m<sup>3</sup>) which are comparable with other European and USA cohorts with mean PM<sub>2.5</sub> exposure values amounting to 18.5 (Pedersen et al. 2013) and 14.5  $\mu$ g/m<sup>3</sup> (Rappazzo et al. 2014) respectively.

## CONCLUSION

Our epidemiological finding of differences in fetal thyroid function in association with  $PM_{2.5}$  exposure is in line with the known effects of cigarette smoking on thyroid function during pregnancy (Männistö et al. 2012). Although confirmation in other study populations is needed, our findings suggest that cord blood FT<sub>4</sub> may play a mediating role between PM<sub>2.5</sub> exposure and birth weight during late pregnancy. The potential mechanisms and possible later-in-life adverse consequences are far from elucidated. Our findings are of critical public health importance because of the ubiquity of fine PM air pollution and the possibility of long-term health consequences of early-life alterations in thyroid function. Therefore, to promote a healthier living environment for children our findings support a down-revision of the current EU air pollution limit for PM<sub>2.5</sub> of 25 µg/m<sup>3</sup> (annual average threshold) in the direction of the WHO recommended limit of 10 µg/m<sup>3</sup> (annual average).

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Characteristic	Numbers (%) or means (10 <sup>th</sup> -90 <sup>th</sup> percentiles)		
Mothers			
Age, y	29.1 (23-35)		
Pre-pregnancy BMI, $kg/m^2$	23.9 (19.6-29.8)		
Education <sup>a</sup>			
Low	61 (12.3%)		
Middle	182 (36.5%)		
High	256 (51.2%)		
Self-reported smoking status			
Never-smoker	316 (63.2%)		
Cessation before pregnancy	113 (22.7%)		
Smoker during pregnancy	70 (14.1%)		
Self-reported passive indoor smoking $(n = 486)$	43 (8.8%)		
Alcohol consumption ( $n = 485$ )			
None	398 (82.1%)		
Occasionally	87 (17.9%)		
Parity			
1	275 (55.0%)		
2	170 (34.1%)		
$\geq$ 3	54 (10.9%)		
Newborns			
Sex			
Female	254 (50.8%)		
European-Caucasian ethnicity <sup>b</sup>	435 (87.2%)		
Gestational age, w	39.4 (38-41)		
Season of delivery			
Winter (Dec - Mar)	142 (28.5%)		
Spring (Mar - Jun)	113 (22.7%)		
Summer (Jun - Sep)	107 (21.4%)		
Autumn (Sep - Dec)	137 (27.4%)		
Apgar score 5 min after birth			
7 or 8	39 (7.8%)		
9	140 (28.1%)		
10	320 (64.1%)		
pH of arterial cord blood ( $n = 431$ )	7.2 (7.2-7.3)		
Birth weight, g	3446 (2915-3990)		
Minutes of labor $(n = 427)$	27.3 (8-54)		
Cord blood thyroid hormones			
TSH, mU/L	10.3 (5.5-22.3)		
FT <sub>3</sub> , pmol/L	2.5 (2.0-3.2)		
FT <sub>4</sub> , pmol/L	15.7 (13.5-18.5)		
Ratio $FT_4/FT_3$	6.4 (5.0-8.0)		

Table 1.	Characteristics an	d thyroid hormor	ne levels of the	mother-child pairs	(n = 499).
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# Maternal thyroid hormones $(n = 431)^{c}$

TSH, mU/L	2.1 (1.1-4.0)
FT <sub>3</sub> , pmol/L	4.2 (3.4-5.1)
FT <sub>4</sub> , pmol/L	12.5 (10.0-15.2)
Ratio FT <sub>4</sub> /FT <sub>3</sub>	3.0 (2.4-3.8)

For TSH, FT<sub>3</sub>, and FT<sub>4</sub> levels the geometric mean ( $10^{\text{th}}$ - $90^{\text{th}}$  percentiles) is given.

<sup>a</sup> Mother's education: low (no high school diploma), middle (high school diploma), high (college or university diploma).

<sup>b</sup> Based on the native country of the newborn's grandparents. European-Caucasian when two or more grandparents were European, or non-European when at least three grandparents were of non-European origin. <sup>°</sup> Total group *minus* mothers from whom blood samples were not available.

## **FIGURE LEGENDS**

**Figure 1.** Flowchart depicting the selection procedure of study participants from the ENVIR*ON*AGE birth cohort, Limburg, Belgium

**Figure 2.** Unadjusted correlation between third gestational trimester  $PM_{2.5}$  exposure ( $\mu g/m^3$ ) and cord blood TSH (mU/L,  $log_{10}$ ) levels (left) and the  $FT_4/FT_3$  ratio (right).

**Figure 3.** Difference in cord (•) and maternal (•) blood thyroid hormones in association with third gestational trimester PM<sub>2.5</sub>. The estimated relative difference in percentage (95% CI) is calculated for an IQR increment (+8.2  $\mu$ g/m<sup>3</sup>) in third trimester PM<sub>2.5</sub> exposure . Panel A displays the difference in TSH (left) and the difference in FT<sub>4</sub>/FT<sub>3</sub> ratio (right). Panel B displays the difference in FT<sub>4</sub> (left) and FT<sub>3</sub> (right). The cord blood models were adjusted for sex, gestational age, season of delivery, Apgar score, maternal age, pre-pregnancy BMI, smoking status, parity, ethnicity, maternal education, and third trimester apparent temperature, whereas for maternal blood sex and Apgar score were excluded. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

**Figure 4.** Estimated proportion of the association between  $PM_{2.5}$  exposure and birth weight (g) mediated by cord blood FT<sub>4</sub> levels. The figure displays cord blood FT<sub>4</sub> levels as mediator, the estimate of the indirect effect (IE), the estimate of the direct effect (DE), and the proportion of mediation (IE/DE+IE). The estimated effect is calculated for an IQR increment (+8.2 µg/m<sup>3</sup>) in  $PM_{2.5}$  exposure during the third trimester of pregnancy. The mediation model was adjusted for sex, gestational age, season of delivery, Apgar score, maternal age, pre-pregnancy BMI, smoking status, parity, ethnicity, maternal education, and third trimester apparent temperature.

## Figure 1.



Figure 2.



Figure 3.



Figure 4.

