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Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach

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ABSTRACT

The mechanisms involved in thyroid homeostasis are complex, and perfluoroalkyl substances (PFASs) have been indicated to interfere at several levels in this endocrine system. Disruption of the maternal thyroid homeostasis during early pregnancy is of particular concern, where subclinical changes in maternal thyroid hormones (THs) may affect embryonic and foetal development.

The present study investigated associations between THs, thyroid binding proteins (TH-BPs) and PFAS concentrations in pregnant women from Northern Norway.

Women participating in The Northern Norway Mother-and-Child contaminant Cohort Study (MISA) donated a blood sample at three visits related to their pregnancy and postpartum period (during the second trimester, 3 days and 6 weeks after delivery) in the period 2007–2009. Participants were assigned to quartiles according to PFAS concentrations during the second trimester and mixed effects linear models were used to investigate potential associations between PFASs and repeated measurements of THs, TH-BPs, thyroxin binding capacity and thyroid peroxidase antibodies (anti-TPOs).

Women within the highest perfluorooctane sulfonate (PFOS) quartile had 24% higher mean concentrations of thyroid stimulating hormone (TSH) compared to the first quartile at all sampling points. Women within the highest quartiles of perfluorodecanoate (PFDA) had 4% lower mean concentrations of triiodothyronine (T3) and women within the highest quartile of perfluoroundecanoate (PFUnDA) had 3% lower mean concentrations of free triiodothyronine (FT3). Further, the difference in concentrations and the changes between three time points were the same for the PFAS quartiles. Thyroxin binding capacity was associated with all the THs and TH-BPs, and was selected as a holistic adjustment for individual changes in TH homeostasis during pregnancy. Finally, adjusting for maternal iodine status did not influence the model predictions.

Findings in the present study suggest modifications of TH homeostasis by PFASs in a background exposed maternal population. The variation in levels of THs between PFAS quartiles was within normal reference ranges and may not be of clinical significance in the pregnant woman. However, subtle individual changes in maternal THs may have significant consequences for foetal health.

1. Introduction

Thyroid hormones (THs) like thyroid stimulating hormone (TSH), thyroxin (T4) and triiodothyronine (T3), are involved in numerous physiological processes e.g. regulation of metabolism, bone remodelling, cardiac function and mental status in the adult. For the embryo and foetus, THs are crucial in all developmental stages. The onset of foetal thyroid function is at approximately 20 weeks of gestation, and thus prior to this, maternal T4 is the sole source of TH to the developing foetal brain (Morreale De et al., 2004). In adults, THs are produced in the thyroid gland and transported to peripheral target tissues aided by thyroid

Abbreviations: Anti-TPOs, anti-thyroid peroxidase antibodies; HTP, hypothalamic pituitary; LOD, limit of detection; MISA, The Northern Norway Mother-and-Child contaminant Cohort Study; PFASs, poly- and perfluoroalkyl substances; PFDA, perfluorodecanoate; PFDoDA, perfluorododecanoate; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoate; PLS, partial least square; T3, triiodothyronine; FT3, free triiodothyronine; T4, thyroxin; FT4, free thyroxin; T-uptake, thyroxin binding capacity; TBG, thyroid binding globulin; TH, thyroid hormone; TH-BP, thyroid hormone binding protein; TSH, thyroid stimulating hormone; TTR, transthyretin; UHPLC–MS/MS, ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry

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hormone binding proteins (TH-BPs) e.g. thyroid binding globulin (TBG), transthyretin (TTR), and albumin. The thyroid function is regulated by negative feedback mechanisms, in which TSH stimulates the thyroid to synthesize T4 which is further converted to T3. TSH is in turn regulated by the hypothalamus as well as by the levels of circulating T3 and T4. In healthy individuals, serum levels of THs are maintained relatively stable with individuals having his or her specific set point (Feldt-Rasmussen et al., 1980).

During the first two trimesters of pregnancy, marked changes are seen in the maternal hypothalamic pituitary (HTP) thyroid axis to increase the availability of THs. In short, these changes lead to a two- to three-fold increase in TBG production and a subsequent decrease in levels of free thyroxin (FT4) and free triiodothyronine (FT3) followed by an increased production of T3 and T4. The increase in T3 and T4 is less than the increase in TBG, resulting in a decreased T4/TBG ratio, creating a state of relative hypothyroxinemia. Hence, these adaptations mimic hyperthyroidism, but thyroid function per se does not change during pregnancy. There is uncertainty regarding reference ranges for thyroid tests during pregnancy as pregnancy-induced changes in thyroid physiology affects laboratory interpretation and presently no universally accepted reference ranges exist (Fitzpatrick and Russell, 2010). Changes in individual TH levels throughout pregnancy varies by gestational age, number of foetuses and study population, but generally, the woman achieves a new steady state in HTP function at the end of 2nd trimester which is maintained until delivery. After delivery, the alterations in thyroid processes are gradually reversed over 4–6 weeks (Blackburn, 2013).

Endocrine systems like the thyroid are susceptible to disruption by naturally-occurring and man-made compounds, possibly by affecting the hormone homeostasis through carrier proteins and receptors. One group of potential endocrine disrupting chemicals are poly- and perfluoroalkyl substances (PFASs). PFASs are persistent substances that have been directly emitted to the environment, intentionally or as by-products, during their production and use (Prevedouros et al., 2006). Diet is currently suspected to be the major on-going exposure pathway of PFASs for humans (Fromme et al., 2009; Haug et al., 2011a; Vestergren and Cousins, 2009). In addition, these chemicals are passed to humans through air, house dust, drinking water and water based beverages (Eschauzier et al., 2013; Haug et al., 2011a,b; Ullah et al., 2011).

Scientific and public concern regarding PFASs, are their potential to perturb maternal hormonal homeostasis and subsequently affect pregnancy outcome by increasing the risk of spontaneous abortions, placental disruptions, foetal distress, malformations, prematurity, decreased birth weight, and hypertension (Boas et al., 2012; Morreale De et al., 2000, 2004; Stahl et al., 2011). Disruption of the maternal thyroid homeostasis during early pregnancy is of particular concern, where subclinical changes in maternal THs may affect embryonic and foetal development (Boas et al., 2012). Compared to the wide population reference ranges for THs, the range of variation within each individual is narrower. Hence, subtle changes in the individual set point of thyroid homeostasis may have significant effects, especially if occurring during critical developmental periods (Feldt-Rasmussen et al., 1980).

T3 and T4 are the only biological molecules which are halogenated (iodine). Similarly, PFASs are halogenated (fluorine) with active sites that resemble those of T3 and T4 (Preau et al., 2014). When assessing effects of PFASs on TH homeostasis, the relevant mechanisms of disruption are; i) disturbance of the overall activity of the thyroid gland by interference with the TH receptors, ii) stimulation or inhibition of enzyme functions which mediates iodine uptake of the thyroid gland in the synthesis of T3 and T4, and iii) competitive displacement of THs on their binding proteins (Boas et al., 2012). Disruption of the thyroid function is often investigated with regard to hypothyroidism with the reporting of TSH concentrations. TSH levels can reflect mild thyroid functional impairment even when T4 and T3 concentrations are within normal ranges but hypothyroxinemia can still occur with normal TSH and T3 concentrations. Hence, in the absence of assessment of the overall thyroid function; the clinical importance of individual TH levels is unclear (Braverman and Utiger, 1986). Therefore, the present study aims to investigate the overall thyroid function in relation to PFAS concentrations by investigating associations between all the THs (TSH, T3, T4, FT3, FT4), thyroxin binding capacity, anti-thyroid peroxidase antibodies (anti-TPOs), thyroid hormone binding proteins (TH-BPs) (TBG, TTR and albumin) at three time points; 2nd trimester of pregnancy, 3 days and 6 weeks after delivery and PFAS concentrations in women from Northern Norway.

2. Materials and methods

2.1. Study participants and collection of blood samples

The selected subjects in the present study represent the 391 women who completed The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) which consists of 515 enrolled pregnant women, recruited from June 2007 to October 2009 (recruitment period; 867 days). All participants answered a detailed questionnaire about diet and lifestyle at enrolment, and donated a blood sample at three visits/time points related to their pregnancy (around gestational week 18, 3 days and 6 weeks after delivery). Detailed information about the study group characteristics, ethical approvals, the food frequency questionnaire (FFQ) and the blood collection procedures have been reported elsewhere (Hansen et al., 2010; Veyhe et al., 2012).

2.2. Chemical analyses

2.2.1. PFAS analyses

Blood samples donated at median gestational week 18 (ranging 10–34) were analysed for a variety of PFASs. A total of 26 PFASs were initially screened for in a sub-group of 50 serum samples. Compounds detected above the limit of detection (LOD) in more than 20% of the samples were further quantified in the remaining serum samples ($N = 391$). Detailed information about the compounds, sample preparation, extraction method, analytical method, reagents and instrumentation has been reported elsewhere (Berg et al., 2014; Hanssen et al., 2013). Briefly, PFASs were determined in serum samples using sonication-facilitated liquid–liquid extraction, activated ENVI-carb clean-up (Powley et al., 2005) and analysed by ultrahigh pressure liquid chromatography triple–quadrupole mass-spectrometry (UHPLC–MS/MS).

Quantification of the compounds was performed by the internal standard addition method with isotope-labelled PFASs (Hanssen et al., 2013). Further details regarding quality control have been reported elsewhere (Berg et al., 2014).

2.2.2. TH and TH-BP analyses

Determination of TH, TH-BP, thyroxin binding capacity and anti-TPO concentrations in non-fasting serum samples from three visits (second trimester, 3 days and 6 weeks after delivery) was performed by laboratory staff at the University Hospital of Northern Norway, Department of Laboratory Medicine. The analyses are routine analyses used in the clinic for diagnostic purposes except for T3, T4 and thyroxin binding capacity. Details on the different methods, instrumentation, analytical variation and reference ranges are provided in Table S1 in the Supplemental material. The laboratory is certified according to ISO 151810 (Norwegian accreditation, 2014) and all reagents, calibrators and equipment were CE-approved. Quality controls are run at three different concentrations every day and additionally the laboratory participates in the LabQuality external quality assessment programme (Labquality Finland, 2014).