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Thyroid hormones in relation to toxic metal exposure in pregnancy, and potential interactions with iodine and selenium

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ABSTRACT

Background: Several endocrine-disrupting metals may affect thyroid function, but the few available studies of exposure during pregnancy and thyroid hormones are inconclusive.
Objective: To explore if environmental exposure to cadmium (Cd), lead (Pb), and methylmercury (MeHg) impacts thyroid function in pregnancy, and interacts with iodine and selenium status.
Methods: Women in a Swedish birth cohort provided blood and urine samples in early third trimester. Concentrations of erythrocyte Cd, Pb, and Hg (n = 544), urinary Cd and iodine (n = 542) and plasma selenium (n = 548) were measured using inductively coupled plasma-mass spectrometry. Free and total thyroxine (fT4, tT4) and triiodothyronine (fT3, tT3), and thyroid stimulating hormone (TSH), were measured in plasma (n = 548) with electrochemiluminescence immunoassays. Metal-hormone associations were assessed in regression models, and metal mixture effects and metal-nutrient interactions were explored in Bayesian kernel machine regression (BKMR).
Results: In multivariable-adjusted regression models, a doubling of urinary Cd was associated with a mean increase in tT4 of 2.7 nmol/L (95% CI: 0.78, 4.6), and in fT3 and tT3 of 0.06 pmol/L (0.02, 0.10) and 0.09 nmol/L (0.05, 0.13), respectively. A doubling of urinary Cd was associated with a −0.002 (−0.003, −0.001) and −0.03 (−0.05, −0.02) decrease in the fT4:tT4 and fT3:tT3 ratio, respectively. A doubling of erythrocyte Hg (>1 μg/kg) was associated with a decrease in fT3 and tT3 by −0.11 pmol/L (−0.16, −0.05) and −0.11 nmol/L (−0.16, −0.06), respectively, and a −0.013 (−0.02, −0.01) decrease in the fT3:fT4 ratio. BKMR did not indicate any mixture effect of toxic metals or interactions between metals and iodine or selenium in relation to the hormones.
Conclusion: Our findings suggest that exposure to Cd and Hg, at levels globally prevalent through the diet, may affect thyroid function during pregnancy, independently of iodine and selenium levels. Further studies on potential implications for maternal and child health are warranted.

1. Introduction

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), regulate the metabolism, growth, and development of the human body (Mullur et al. 2014). The active form of thyroid hormone is T3, which is mainly generated in peripheral tissue (about 80%; Robbins 1981) by deiodination of the less active T4, the primary hormone produced in the thyroid gland under influence of thyroid-stimulating hormone (TSH). Through a negative feedback loop, reduced levels of T3 and T4 in the circulation stimulates the release of TSH from the pituitary gland, which augments production of T4 and T3. Most of the circulating T4 and T3 are bound to proteins, mainly thyroxine-binding globulin, leaving<1% of these hormones free and biologically active. Pregnancy requires increased levels of thyroid hormones as the fetus relies entirely on

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maternal thyroid hormone production until mid-gestation, when the fetal thyroid starts producing its own hormones (Zimmermann 2009; Obregon et al. 2007). Still, the maternal supply of iodine continues throughout gestation to maintain fetal T4 levels. Both low and elevated thyroid hormones levels in pregnancy may adversely impact fetal development (Korevaar et al. 2016). Even in women with no overt thyroid disease, particularly elevated free T4 levels have been associated with impaired fetal growth and neurodevelopment (Johns et al. 2018; Korevaar et al. 2016; Leon et al. 2015; Medici et al. 2013).

The toxic metals cadmium (Cd), lead (Pb), and methylmercury (MeHg) are commonly present in some of our most valuable food items and are known to affect fetal development, including cognitive function (EFSA 2010, 2012; Liu et al. 2019). All have pro-oxidant and endocrine disruptive properties, and experimental data suggest that exposure to these metals may interfere with thyroid function either by accumulation in the thyroid or by affecting the regulation (Iavicoli et al. 2009). However, epidemiological studies of toxic metal exposure and thyroid function are scarce and have mostly involved occupationally exposed individuals (Ellingsen et al. 2000; Krieg 2016; Rosati et al. 2016) or the general adult population, mainly in the U.S. (Chen et al. 2013; Yorita Christensen 2013; Luo and Hendryx 2014). Associations of multiple metals, including at least one of the toxic metals Cd, Pb and Hg, with thyroid function in pregnant women have been investigated in three recent Chinese studies (Guo et al. 2018; Sun et al. 2019; Wang et al. 2020), one study from Puerto Rico (Rivera-Nunez et al. 2021), and two European studies (Llop et al. 2015; Ursinyova et al. 2012), with mixed results. None of the aforementioned studies explored potential interactions with iodine and selenium status. It has been suggested that exposure to mixtures of thyroid hormone-disrupting toxicants in pregnancy in combination with iodine deficiency may aggravate the impact on fetal development (Demeneix 2020). Iodine is a component of T3 and T4, whereas selenium is an integral part of the deiodinases that activate or inactivate thyroid hormones. Consequently, an adequate intake of iodine and selenium during pregnancy is required for sufficient maternal production of thyroid hormones (Glinoer 1997; Krassas et al. 2010), as well as for the fetal production, initiated in mid-pregnancy (Obregon et al. 2007). Furthermore, selenium is a crucial part of the antioxidant enzymes protecting the thyroid from oxidative damage (Kohrle 2015).

The present study aimed to elucidate the potential impact of low-level exposure, common through the diet, to Cd, Pb, and MeHg on thyroid hormone and TSH levels in pregnant women, and to explore potential interactions with both iodine and selenium.

2. Methods

2.1. Study population

The present study is part of the prospective birth-cohort NICE (Nutritional impact on the Immunological Maturation during Childhood in relation to the Environment) in northern Sweden. As previously described (Barman et al. 2018), the primary aim of the NICE study is to assess how the early-life environment may influence immune maturation and allergy development during early childhood. Secondary outcomes include infant and child anthropometry and neurological development. The cohort was established between 2015 and 2018 in the catchment area of Sunderby Hospital in Norrbotten county in the very north of Sweden. Expecting parents received an information leaflet at their visit to the local maternity clinics, and at the routine ultrasound in gestational week 18–19 parents wishing to participate were given further information and an informed consent to sign at home and send back. Finally, to be included in the study, the parents had to be residents in the southern or eastern part of Norrbotten county, planning to give birth at Sunderby Hospital, and be able to communicate in written and spoken Swedish.

A total of 655 pregnancies were included in the NICE cohort (Supplementary Fig. S1), 18 of which were second pregnancies in already participating families. Only data from the first pregnancy were included in the present study, except for two families where the first child was stillborn (in gestational week 29 and 37, respectively), for which data only from the second pregnancy was included. We also excluded twin births (n = 3) and one mother that withdrew from the study. Of the 633 eligible singleton pregnancies, we additionally excluded women with any notation in their pregnancy hospital records regarding thyroid dysfunction (i.e., hypothyroidism, hyperthyroidism, Graves' disease, removed thyroid) and/or medication with any thyroid interfering drugs (n = 44). Of the remaining women, 548 had donated blood samples from which the plasma fraction was available for assessing thyroid biomarkers (excluding one mother with an extreme TSH concentration of > 20 mIU/L), and the erythrocyte fraction (n = 544) and/or urine samples (n = 542) were available for trace element analyses.

The study was approved by the Regional Ethical Review Board, Umeå, Sweden, and performed in accordance with the Helsinki declaration. The participants were informed that they were free to withdraw from the study at any given time without further explanation.

2.2. Sample collection

At the local maternity health clinics, venous blood and spot urine samples were collected at around gestational week 29 (mean: 29; range: 24–36), as previously described (Barman et al. 2018; Gustin et al. 2020). Blood samples were collected in both 10 mL EDTA tubes (Becton Dickinson, Plymouth, UK), for thyroid biomarkers and selenium analyses, and 6 mL trace element-free Na-heparin tubes (Greiner bio-one, Kremsmünster, Austria), for metal analyses. Spot urine samples (midstream) were collected in urine collection cups and then transferred to 24-mL polyethylene bottles, both containers tested free of trace elements. All samples were stored at 4 °C at the local clinics until transported cold to the hospital laboratory the same or following workday. At the hospital laboratory, blood samples were centrifuged for 5 min at 2400 rpm (Hettich Rotina 420, Hettich Lab Technology, Tuttlingen, Germany), and the erythrocyte and plasma fractions were separated. The plasma fraction was aliquoted to 1.4 mL polypropylene tubes (Micronic, Nordic Biolabs AB, Sweden). All samples were stored at −20 or −80 °C until transported frozen to the Department of Clinical Chemistry at the University Hospital of Malmö, Sweden, for hormone analyses, or to Karolinska Institutet, Sweden, for trace element analyses.

2.3. Thyroid biomarkers

All hormone analyses were performed at the accredited laboratory of the Department of Clinical Chemistry at the University Hospital of Malmö, Sweden. The plasma samples, collected around gestational week 29, were analyzed for free and total T4 (fT4, tT4) and free and total T3 (fT3, tT3) via automated electrochemiluminescence immunoassays (ECLIA) by Roche Cobas (Roche Diagnostics, Solna, Sweden), consisting of a two-step immunometric-competitive technique followed by chemiluminescent emission measurement, according to the producer's instructions. Plasma concentrations of TSH were analyzed using a one-step sandwich method with ECLIA (Roche Cobas, Roche Diagnostics, Solna, Sweden).

2.4. Assessment of toxic metals and trace elements

Metal exposure during pregnancy was assessed by concentrations of Cd, Pb, and total Hg in erythrocytes, and Cd also in urine. The majority of blood Cd and Pb is found in the erythrocytes (Carlson and Friberg 1957; Schultze et al. 2014) and are therefore thought to reflect the exposure of a few months, related to the lifespan of erythrocytes (Shemin and Rittenberg 1946). We also measured the Cd concentration in urine, as this is a recognized long-term biomarker of Cd exposure, due to the accumulation of Cd in the kidneys with a half-life of 10–45 years (Akerstrom et al. 2013; Amzal et al. 2009). Like Cd and Pb, blood MeHg

is mainly localized to the erythrocytes, and > 90% of the erythrocyte Hg consists of MeHg, with small variations between individuals and exposure levels (Berglund et al. 2005). The small fraction of inorganic Hg present in erythrocytes is largely due to demethylation of MeHg (Berglund et al. 2005). The erythrocyte metal concentrations were measured by weight (μg/kg), and urinary Cd by volume (μg/L).

Maternal iodine status was assessed by concentrations in urine (the main route of iodine excretion), which is considered a valid biomarker of iodine status on a population level (Andersson et al., 2007). Selenium status was measured by concentrations in plasma, the most commonly used biomarker (Burk and Hill 2015), reflecting intake over the past week(s) (Neve 1991). The women's plasma concentrations were measured by weight (μg/kg) and then converted to the more commonly used μg/L by multiplying the concentrations with the average density of plasma of 1.026 kg/L (Lentner 1981).

Concentrations of toxic metals and essential trace elements in erythrocytes, urine, and plasma were measured with inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x, Agilent Technologies, Tokyo, Japan), equipped with an octopole reaction system. Cadmium (isotope 111), iodine (isotope 127) and selenium (isotope 78) were measured in helium mode, whereas Pb (isotope 208) and Hg (isotope 202) were measured in no gas mode. Prior to the ICP-MS analyses, erythrocyte and plasma samples were diluted 1:25 in an alkali solution [2% (w/v)1-butanol, 0.05% (w/v) EDTA, 0.05% (w/v) Triton X-100, 1% (w/v) NH_4OH and 20 μg/L internal standard (germanium, rhodium, lutetium, and iridium)] and vortex mixed, then sonicated for five minutes and centrifuged at 1000 rpm for two minutes (MSE centrifuge, Super Minor, MSE (UK) Ltd, London, England) (Lu et al. 2015). The limit of detection (LOD; calculated as 3 times the standard deviation of the blank concentrations) was 0.0037 μg/kg, 0.11 μg/kg, 0.010 μg/kg, and 0.36 μg/L for Cd, Pb, Hg, and selenium, respectively. No sample had a concentration below the LOD for either Cd, Pb, or selenium, but two samples had a concentration below the LOD for Hg, which were replaced by LOD/$\sqrt{2}$. Quality control of the ICP-MS analyses of erythrocyte metals was performed by the inclusion of two commercial reference materials of whole blood, and the analyses of plasma selenium included two commercial reference materials of serum, in each run and obtained values were in good agreement with the reference values (Supplementary Table S1).

Prior to ICP-MS analyses of urinary Cd, the urine samples were diluted 1:10 in 1% nitric acid (67–69% w/w, NORMATOM®, VWR, Butterworth, UK). The LOD was 0.003 μg/L, and no urine sample had a Cd concentration below this limit. Urine samples analyzed for iodine concentrations were diluted 1:10 in 0.1 % ammonium hydroxide solution (NH_4OH; 25 % suprapur, Merck, Darmstadt, Germany) (Rydbeck et al. 2014). The LOD was 1.85 μg/L, and no sample had an iodine concentration below this limit. For the measurements of Cd and iodine, we included two commercial urine reference materials in each run, and there was good agreement between obtained values and recommended reference values (Supplementary Table S1). To compensate the urinary concentrations for the variation in urine dilution, the specific gravity of each urine sample (SG_{sample}) was measured with a digital refractometer (EUROMEX RD712, Clinical Refractometer, Holland), and all urinary concentrations (C) were then adjusted (C_{adj}) to the mean specific gravity (SG_{mean}) of 1.017, using the following formula: $C_{adj} = C \times (SG_{mean}-1)/(SG_{sample}-1)$, as reported previously (Nermell et al. 2008).

2.5. Covariates

Information on maternal age (years), early-pregnancy body mass index [BMI (kg/m^2); calculated based on body weight (kg) and height (cm) recorded at registration at the maternity clinic in the first trimester], parity (number of previous births), education (elementary school, high school, or university), pre-pregnancy smoking (never, sometimes, or daily), pre-pregnancy snuff (non-smoking tobacco) use (never, sometimes, or daily), and pre-pregnancy alcohol consumption (never, sometimes, or daily) was obtained from the hospital records. We also obtained information on early pregnancy use of tobacco and alcohol consumption, but since very few, or none, of the women used tobacco or alcohol during pregnancy, this was not further explored. Since thyroid hormone and TSH levels may fluctuate depending on the season (Mahwi and Abdulateef 2019; Wang et al. 2018), the dates of the collection of the maternal blood samples were classified into four seasons (spring: 1 March-31 May; summer: 1 June-31 August; fall: 1 September-30 November; winter: 1 December-28 or 29 February).

2.6. Statistical analyses

Statistical analyses were performed using the software Stata/IC 15.0 (StataCorp, TX, USA) or R 3.6.2 (R Core Team 2019). P-values and sharpened q-values below 0.05 were considered statistically significant, but we also considered the consistency and robustness of the results.

Bivariate associations between the maternal metal concentrations (erythrocyte concentrations of Cd, Pb and Hg, and urinary Cd), hormones (plasma concentrations of fT4, tT4, fT3, tT3, and TSH), hormone ratios (fT4:tT4, fT3:tT3, and fT3:fT4), and all potential covariates were initially explored with either Spearman rank test (continuous variables), Mann-Whitney *U* test or Kruskal-Wallis test (continuous and categorical variables), or chi-square test (categorical variables).

Model covariates were selected with the aid of a directed acyclic graph (DAG; Supplementary Fig. S2), where the assumed causal relationships between exposures, outcomes, and covariates were visualized. As maternal weight and height were correlated with BMI, only BMI was explored as a covariate in the DAG. Similarly, maternal pre-pregnancy smoking and use of snuff were associated but only smoking was associated with the exposures and outcomes and therefore kept as a covariate. Exposure to Pb has previously been linked to alcohol consumption (Wennberg et al 2017), but Pb and alcohol intake were not associated among the present mothers (nor was alcohol use associated with any of the other exposures or the outcomes), so alcohol intake was not included in the DAG. Gestational week at sampling, and season at sampling, were identified as potential confounders in the DAG, but gestational week at sampling was not associated with any of the measured hormones, and season of sampling only very weakly associated with tT3. Therefore, these were not adjusted for in the main analyses, but were included in the sensitivity analyses (described below). Thus, the main models were adjusted for parity (two groups: '0′ and '>0′), maternal education (two groups: 'lower than university' and 'university'), and maternal pre-pregnancy smoking (two groups: 'never' and 'sometimes or daily'). We then created additional models also adjusted for maternal urinary iodine (μg/L; log_2-transformed) and plasma selenium (μg/L) concentrations.

All the measured metal concentrations were log_2-transformed (due to right-skewness), and linearity of the log_2-transformed concentrations with the maternal hormone concentrations, and hormone ratios, was checked with scatter plots with moving average Lowess curves. These indicated a tendency of non-linear relationships for some of the associations. Therefore, linearity of the metal-hormone associations was statistically tested using generalized additive models (GAMs), with two degrees of freedom and adjusted as the main models, and a p-gain < 0.05 was considered to indicate non-linearity. The associations suggested to be non-linear were erythrocyte and urinary Cd with TSH, and erythrocyte Hg with fT3, tT3, and the fT3:fT4 ratio. These associations were further explored by comparing the Akaike information criterion (AIC; an estimator of relative model quality) between i) a linear model, ii) a model including a quadratic exposure term, and iii) a model including a spline knot for which the position was visually determined by plotting the covariate-adjusted association of the GAM models (Supplementary Fig S3A-E). The model with the lowest AIC (highest quality) was then kept in the main analyses. The quadratic exposure term-model had the lowest AIC for the association of erythrocyte Cd with TSH, while the spline models had the lowest AIC for urinary Cd with TSH (spline knot at

the Cd concentrations of 0.09 μg/L; Supplementary Fig. S3B), and for Hg with fT3, tT3, and fT3:fT4 (spline knots at the Hg concentrations of 1 μg/kg; Fig S3C-E). All other associations between the metals and hormones, and hormone ratios, appeared to be linear and were therefore modelled with linear regression.

Due to the multiple testing (four exposure biomarkers against eight outcomes), we computed sharpened false discovery rate (FDR) q-values for the main regression analyses, using Michael Anderson's code (described in Anderson 2008). The interpretation of the sharpened q-values is similar to that of p-values as they represent the probability of type I error.

In sensitivity analyses, we further explored the associations of the toxic metal concentrations and the hormones without any covariate adjustment (crude model) and by including all potential confounders identified in the DAG (i.e. additionally adjusting for gestational week at sampling and season at sampling; Supplementary Fig. S2), and we also conducted sub-group analyses of only never-smoking women.

For all regression models, missing covariate data was imputed by multiple imputation by chained equations (MICE) in Stata (Royston 2004), which can impute data for categorical variables. Separate imputation models were created for each regression model, including all variables (transformed if transformed in the regression model) that were included in the regression model. For models including gestational week at sampling, maternal early-pregnancy BMI was also included in the imputation model (but not in the regression model), as an auxiliary variable, due to it being associated with missingness of gestational week at sampling. The number of imputed data sets was set to 50 for all models. The proportion of missing observations were 1.1% (n = 6) for maternal education, 1.1% (n = 6) for parity, and 1.5% (n = 8) for maternal pre-pregnancy smoking. In models adjusted also for gestational week at sampling, the proportion of missing data for this variable was 7.3% (n = 40). In models with exposure assessed in erythrocytes and adjusted for urinary iodine, 1.1% (n = 6) of the urinary iodine observations were imputed. We chose to not impute the main exposures (toxic metal concentrations).

Lastly, we explored possible interactions between urinary Cd, erythrocyte Pb, and erythrocyte Hg in relation to the maternal hormones, as well as any potential joint effects of mixed exposure to all three metals, with Bayesian kernel machine regression (BKMR; Bobb et al. 2015) using the R package *bkmr* (Bobb 2017). We conducted the BKMR with centered and scaled metal concentrations (the concentration subtracted by the mean and divided by the standard deviation), applying the option of variable selection and 50 000 iterations by the Markov chain Monte Carlo algorithm. Due to the BKMR being sensitive to outliers, extreme concentrations of urinary Cd (>0.63 μg/L; n = 5), erythrocyte Pb (>69 μg/kg; n = 2) and Hg (>8.3 μg/kg; n = 1) were omitted from the analyses, as well as six women who had not provided both urine and erythrocyte samples. All BKMR analyses were adjusted for the same covariates as our main regression model, defined above. Multiple imputation could not be performed for the BKMR, why women with missing data on parity (n = 6) and pre-pregnancy smoking (n = 7) were additionally excluded, leaving a total of 517 women included (Supplementary Fig. S1). An additional BKMR model was then created to explore potential interactions between the metals and the women's iodine and selenium levels. Like the metals, the urinary iodine and plasma selenium concentrations were centered and scaled and included among the exposure variables in the BKMR. Extreme iodine (>382 μg/L) concentrations were removed in the analyses (n = 10), leaving a final sample of 507 women. In the BKMR, interactions were defined as the difference in the predicted single-exposure effect when the other exposures were fixed at different percentiles (10th, 50th, and 90th percentile) and the results were visualized in plots, where non-parallel dose–response curves would indicate that the single-exposure effect differed depending on the level of the other exposures. Joint exposure effects were assessed in the BKMR as the predicted change in the outcome when all metals were held at particular percentiles, compared to when all metals were held at their median concentration, also visualized in plots.

3. Results

3.1. Background characteristics

General characteristics of the 548 women included in the study are presented in Table 1 (non-imputed data). The mean age was 30 years, with a mean early-pregnancy BMI of 24.4 kg/m^2. Almost half of the women were nulliparous (49%) and 71% had a university education. Few of the women smoked, 6.7% prior to pregnancy and 1.3% in early pregnancy. Most women (75%) reported occasional alcohol consumption prior to pregnancy, while none reported any alcohol consumption in early pregnancy. The median urinary iodine concentration among the women was 113 μg/L (range: 29–512 μg/L), and the median plasma selenium concentration was 67 μg/L (range: 32–130 μg/L).

3.2. Concentrations of cadmium, lead, and mercury in erythrocytes and urine

The median concentrations of erythrocyte Cd, Pb, and Hg among the pregnant women were 0.29 μg/kg, 11 μg/kg, and 1.5 μg/kg, respectively. The median urinary Cd concentration was 0.10 μg/L. Concentrations of the different metals were not strongly correlated (Supplementary Table S2), the strongest correlation was observed between erythrocyte and urinary Cd (r_s: 0.43). Weak positive correlations were observed between erythrocyte Cd and Pb (r_s: 0.16), erythrocyte Pb and Hg (r_s: 0.12), and urinary Cd and erythrocyte Pb (r_s: 0.10). Cd concentrations in erythrocytes were higher ($p < 0.01$) in pre-pregnancy smokers than among never-smoking women (median: 0.55 μg/kg and 0.28 μg/kg, respectively; $p < 0.01$), the same was true for Cd in urine (median: 0.13 μg/L and 0.10 μg/L, respectively, $p < 0.01$). Erythrocyte Hg was slightly higher among women with a university education than among women with either an elementary or high school education

Table 1
Characteristics of the included mothers.

Maternal characteristics	n	Median (5th-95th percentile) or %
Age (y)	547	30 (23–39)
Weight (kg)	531	68 (53–98)
Height (cm)	548	167 (158–178)
BMI (early pregnancy)	532	24.4 (19.4–34.6)
Parity (% nulliparous)	542	49
Education (% university education)	542	71
Pre-pregnancy smoking (% yes)	540	6.7
Gestational week at sample collection (weeks)	508	28.6 (27.3–32.1)
Season at sampling (% spring/summer/fall/winter)	548	30/27/20/23
fT4 (pmol/L)	548	12 (9.6–15)
tT4 (nmol/L)	548	122 (94–154)
fT3 (pmol/L)	548	4.1 (3.4–4.8)
tT3 (nmol/L)	548	2.6 (1.9–3.4)
TSH (mIU/L)	548	1.6 (0.61–3.3)
fT4:tT4 ratio	548	0.10 (0.08–0.12)
fT3:tT3 ratio	548	1.6 (1.3–1.9)
fT3:fT4 ratio	548	0.33 (0.26–0.44)
Erythrocyte Cd (μg/kg)	544	0.29 (0.14–0.77)
Urinary Cd (μg/L)[a]	542	0.10 (0.04–0.26)
Erythrocyte Pb (μg/kg)	544	11 (6.0–27)
Erythrocyte Hg (μg/kg)	544	1.5 (0.29–4.2)
Urinary iodine (μg/L)[a]	542	113 (53–306)
Plasma selenium (μg/L)[b]	548	67 (46–93)

Abbreviations: BMI, body mass index; Cd, cadmium; fT3, free triiodothyronine; fT4, free thyroxine; Hg, mercury; Pb, lead; TSH, thyroid stimulating hormone; tT3, total triiodothyronine; tT4, total thyroxine.

[a] Concentrations adjusted to the mean specific gravity of 1.017.

[b] Concentrations converted from μg/kg to μg/L by assuming a plasma density of 1.026 kg/L.

Table 2
Multivariable-adjusted regression analyses of metal concentrations in erythrocytes (μg/kg; log_2-transformed) and urine (μg/L; log_2-transformed) with concurrent hormone levels in pregnancy.

	Multivariable-adjusted models[a]				
	n	B	95% CI	p	Sharpened q-value[b]
fT4 (pmol/L)					
Ery-Cd	544	0.012	(−0.16, 0.19)	0.90	0.79
U-Cd	542	0.015	(−0.14, 0.17)	0.85	0.79
Ery-Pb	544	0.014	(−0.21,0.18)	0.89	0.79
Ery-Hg	544	0.018	(−0.089, 0.13)	0.74	0.71
tT4 (nmol/L)					
Ery-Cd	544	0.37	(−1.8, 2.5)	0.73	0.71
U-Cd	542	**2.7**	**(0.78, 4.6)**	**0.006**	**0.028**
Ery-Pb	544	0.90	(−1.5, 3.3)	0.46	0.59
Ery-Hg	544	−0.89	(−2.2, 0.42)	0.18	0.30
fT3 (pmol/L)					
Ery-Cd	544	−0.016	(−0.064, 0.031)	0.50	0.59
U-Cd	542	**0.060**	**(0.017, 0.10)**	**0.007**	**0.028**
Ery-Pb	544	0.036	(−0.018, 0.090)	0.19	0.30
Ery-Hg < 1 μg/kg	148	0.010	(−0.037, 0.057)	0.67	0.69
Ery-Hg > 1 μg/kg	396	**−0.11**	**(−0.16, −0.052)**	**<0.001**	**0.003**
tT3 (nmol/L)					
Ery-Cd	544	−0.010	(−0.057, 0.037)	0.67	0.69
U-Cd	542	**0.092**	**(0.050, 0.13)**	**<0.001**	**0.003**
Ery-Pb	544	0.038	(−0.015, 0.091)	0.16	0.29
Ery-Hg < 1 μg/kg	148	0.002	(−0.044, 0.048)	0.93	0.79
Ery-Hg > 1 μg/kg	396	**−0.11**	**(−0.16, −0.055)**	**<0.001**	**0.003**
TSH (mIU/L)					
Ery-Cd	544	0.17	(−0.013, 0.35)	0.069	0.16
Ery-Cd^2	544	0.045	(−0.009, 0.098)	0.10	0.20
U-Cd < 0.09 μg/L	198	−0.19	(−0.40, 0.014)	0.067	0.16
U-Cd > 0.09 μg/L	344	0.16	(0.023, 0.30)	0.022	0.062
Ery-Pb	544	−0.023	(−0.13, 0.087)	0.68	0.69
Ery-Hg	544	0.051	(−0.009, 0.11)	0.094	0.20
fT4:tT4 ratio					
Ery-Cd	544	−0.0003	(−0.002, 0.001)	0.58	0.67
U-Cd	542	**−0.002**	**(−0.003, −0.001)**	**<0.001**	**0.003**
Ery-Pb	544	−0.001	(−0.002, 0.001)	0.25	0.33
Ery-Hg	544	0.001	(0.0001, 0.002)	0.022	0.062
fT3:tT3 ratio					
Ery-Cd	544	−0.001	(−0.021, 0.019)	0.93	
U-Cd	542	**−0.034**	**(−0.052, −0.016)**	**<0.001**	**0.003**
Ery-Pb	544	−0.009	(−0.031, 0.014)	0.45	0.59
Ery-Hg	544	0.014	(0.002, 0.027)	0.021	0.062
fT3:fT4 ratio					
Ery-Cd	544	−0.001	(−0.007, 0.005)	0.65	0.69
U-Cd	542	0.005	(−0.001, 0.010)	0.096	0.20
Ery-Pb	544	0.004	(−0.003, 0.011)	0.25	0.33
Ery-Hg < 1 μg/kg	148	0.003	(−0.003, 0.009)	0.33	0.46
Ery-Hg > 1 μg/kg	396	**−0.013**	**(−0.020,-0.006)**	**<0.001**	**0.003**

Abbreviations: Ery-Cd, erythrocyte cadmium; Ery-Hg, erythrocyte mercury; Ery-Pb, erythrocyte lead; fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid stimulating hormone; tT3, total triiodothyronine; tT4, total thyroxine.
[a]Adjusted for parity (two groups: '0' and '>0'), maternal education (two groups: 'lower than university', and 'university'), maternal pre-pregnancy smoking (two groups: 'never', and 'sometimes or daily').
[b]Sharpened false discovery rate q-values are a form of adjusted p-values (representing the probability of type I error after adjustment for multiple testing).

(median: 1.6 and 1.3 μg/kg, respectively; $p < 0.001$), but lower in pre-pregnancy smokers than never-smokers (median: 0.80 and 1.5 μg/kg, respectively; $p < 0.001$).

3.3. Concentrations of thyroid biomarkers

The mean concentration of fT4 and tT4 was 12 pmol/L (SD: 1.6) and 123 nmol/L (SD: 19), respectively, and for fT3 and tT3 the mean concentrations were 4.1 pmol/L (SD: 0.44) and 2.6 nmol/L (SD: 0.44), respectively. The mean concentration of plasma TSH was 1.7 mIU/L (SD: 0.88). The mean ratio of fT4:tT4 and fT3:tT3 was 0.10 (SD: 0.01) and 1.6 (SD: 0.18), respectively. The mean ratio of fT3:fT4 was 0.34 (SD: 0.06). Among the measured hormones, fT3 and tT3 displayed the strongest correlation (r_s: 0.74; Supplementary Table S2), followed by fT4 and tT4 (r_s: 0.68), and tT4 and tT3 (r_s: 0.44). TSH was weakly and inversely correlated with both fT4 (r_s: −0.14) and tT4 (r_s: −0.10). For the hormone ratios, the strongest correlation was observed between the fT4:tT4 and fT3:tT3 ratios (r_s: 0.80).

3.4. Associations between metal exposure and thyroid biomarkers

In the multivariable-adjusted regression analyses (Table 2), urinary Cd (long-term exposure marker) was linearly and positively associated with concentrations of tT4, fT3, and tT3; a doubling of urinary Cd was associated with a mean increase in tT4 of 2.7 nmol/L [95% CI: 0.78, 4.6 (corresponding to a change of 0.14 SD)], fT3 of 0.06 pmol/L [95% CI: 0.02, 0.10 (0.14 SD)], and tT3 of 0.09 nmol/L [95% CI: 0.05, 0.13 (0.21 SD)]. Further, urinary Cd was non-linearly associated with TSH. Above the urinary Cd concentration of 0.09 μg/L, a doubling in Cd resulted in a mean increase in TSH of 0.16 mIU/L (95% CI: 0.02, 0.30), a change corresponding to 0.18 SD, but the association did not persist after FDR adjustment (sharpened q-value > 0.05). Urinary Cd was also inversely associated with the ratio of both fT4:tT4 and fT3:tT3, with a mean decrease in total:free hormone ratio of −0.002 (95% CI: −0.003,

−0.001) for T4 and −0.03 (95% CI: −0.05, −0.01) for T3, both associations corresponding to a 0.18 SD decrease in the respective ratio.

Maternal erythrocyte Cd, a more short-term exposure marker (3–4 months), was not significantly associated with any of the hormones, neither was erythrocyte Pb. Erythrocyte Hg, however, was non-linearly associated with fT3, tT3, and the fT3:fT4 ratio. For Hg levels above 1 μg/kg, a doubling in Hg concentration was associated with a mean decrease in both fT3 of −0.11 pmol/L (95% CI: −0.16, −0.05), and in tT3 of −0.11 nmol/L (95% CI: −0.16, −0.06), corresponding to a change of 0.24 and 0.25 SD, respectively, and with a mean decrease of −0.01 (95% CI: −0.02, −0.01) in fT3:fT4 (0.23 SD). Also, Hg appeared to be positively associated with the free:total ratio of both T4 and T3, but the associations were no longer significant after FDR adjustment (sharpened q-value > 0.05).

In sensitivity analyses, further adjusting for gestational week at sampling and season at sampling had essentially no impact on the observed associations (Supplementary Table S3). Similarly, restricting the analyses to only never-smokers had very little impact on the observed metal-hormone relationships (Supplementary Table S3).

In the BKMR models including urinary Cd, erythrocyte Pb, and erythrocyte Hg as the exposure, urinary Cd had the highest posterior inclusion probability (PIP; representing the relative importance of each exposure in relation to the outcomes; Supplementary Table S4), in relation to fT4, tT4, and the fT4:tT4 ratio, while Hg had the highest PIP in relation to fT3, tT3, TSH, and the fT3:tT3 and fT3:fT4 ratios. The BKMR did not indicate any joint effects of mixed metal exposure on the hormone concentrations (Supplementary Fig. S4A-H), nor any clear interactions between the three metals in relation to the hormones (Supplementary Fig. S5A-H).

3.5. Influence of iodine and selenium on the metal-hormone associations

In the multivariable-adjusted regression models, the observed statistically significant associations between the toxic metals and the hormones were essentially unaffected when also adjusted for maternal urinary iodine and plasma selenium concentrations, except for the inverse association between Hg and tT4 which became more pronounced after the adjustment (Supplementary Table S5). In the BKMR analyses including urinary iodine, plasma selenium, and the toxic metals as exposure, urinary iodine had the highest PIP in relation to fT4 and TSH, while urinary Cd still had the highest PIP for tT4, and Hg for fT3, tT3, and the fT3:tT3 and fT3:fT4 ratios (Supplementary Table S4). Plasma selenium had the highest PIP in relation to the fT4:tT4 ratio. The BKMR did not indicate any overt interactions between the metals and the essential elements in relation to the hormone levels (Supplementary Fig. S5A-H), although a slightly more pronounced positive association between Hg and the fT4:tT4 ratio was observed when selenium was fixed at its 10th percentile compared to when selenium was fixed at the 90th percentile. This was therefore further explored by including both Hg and selenium, as well as a multiplicative interaction term between the two, in a regression model (otherwise adjusted as our main model), but this did not indicate a statistically significant interaction (p-interaction > 0.10; data not shown).

4. Discussion

The present findings suggest that exposure to Cd and MeHg, but not Pb, at levels that are globally prevalent through the diet, may affect thyroid function in pregnancy. We found that the women's long-term exposure to Cd, measured by concentrations in urine, was positively associated with circulating tT4, fT3, and tT3, in a linear manner. Erythrocyte Hg concentrations, mainly reflecting MeHg exposure from fish consumption, was inversely associated with fT3, tT3, and the fT3:fT4 ratio, at concentrations above 1 μg/kg, which was found in 73% of the women. We found no associations between the women's Pb exposure and their hormone levels, but the Pb exposure was low [median in erythrocytes: 11 μg/kg, corresponding to approximately 4.3 μg/L in whole blood (Gustin et al. 2020)]. The BKMR analysis did not indicate any mixed exposure effects of the toxic metals, or any clear interactions with iodine and selenium, in relation to the hormone concentrations, despite a fairly wide variation in both urinary iodine and plasma selenium concentrations, including insufficiency (Stråvik et al, submitted). Thus, the potential effects of Cd and Hg on maternal thyroid function did not appear to be substantially aggravated by poor iodine and/or selenium status.

Beside the positive associations with tT4, fT3, and tT3, urinary Cd, above the concentration of 0.09 μg/L (which 63% of the women had), was also positively associated with TSH, although not clearly significant after FDR adjustment. These findings are in accordance with the results from a large US study, including women and men from the National Health and Nutrition Examination Survey (NHANES; n = 4409) (Chen et al. 2013). In a Chinese study by Wang et al. (2020), maternal urinary Cd concentrations in late first trimester (median: 0.57 μg/L; n = 389) were positively associated with TSH levels in the second trimester (mean gestational week: 24.6), but not with T4 or T3. In another Chinese study (Sun et al. 2019), urinary Cd in early pregnancy (before gestational week 18) was not associated with concurrent levels of T4, T3, or TSH, despite a similar Cd exposure (median: 0.62 μg/L; n = 675). However, it should be noted that comparing Cd-related associations with T4, T3, and TSH between different stages of pregnancy is not appropriate because of the drastic changes in thyroid physiology that occurs in pregnancy, especially during the first and second trimester (Glinoer 1997; Korevaar et al. 2017).

The mechanisms of action by which toxic metals may affect levels of thyroid hormones are largely unknown. It has been suggested that metals like Cd may accumulate in the thyroid, due to its high expression of metallothionein (Suzuki et al. 1996). However, we would then have anticipated a negative association between Cd and T4, as T4 is produced in the thyroid, but instead we observed the opposite with both T4 and T3 across the whole range of Cd exposure, more indicative of indirect effects of Cd. Further, we observed inverse associations between urinary Cd and the fT4:tT4 and fT3:tT3 ratios, suggesting a relatively higher increase in total hormone levels compared to free levels, with increasing Cd. The major thyroid hormone transport protein is thyroxine-binding globulin (TBG). In pregnancy, the levels of TBG increase drastically due to rising estrogen levels (Glinoer 1997). Cadmium is known to have estrogen-mimicking properties (Johnson et al. 2003), which may explain the observed associations. Also, Cd exposure has been shown to inhibit the expression of thyroid hormone receptors and type III deiodinase in experimental studies (Wu et al. 2017).

In contrast to the Cd exposure, the women's exposure to MeHg [>1 μg Hg/kg in erythrocytes, roughly corresponding to 0.40–4.2 μg/L in whole blood (Gustin et al. 2020)] was inversely associated with both fT3 and tT3, and with the fT3:fT4 ratio. Non-linear associations, with no observable association up to a certain exposure level, are commonly observed for non-carcinogenic toxicants and may be indicative of threshold effects. The few existing studies assessing thyroid function in relation to MeHg exposure in pregnancy are either very small or suffer from certain methodological issues. In a study of 75 pregnant women in Slovakia, maternal blood MeHg (median: 0.22 μg/L) at delivery was inversely associated with tT3 in sub-group analyses (Ursinyova et al. 2012). In a much larger Spanish study of 1407 mothers (Llop et al. 2015), total Hg concentrations in umbilical cord blood (geometric mean: 7.7 μg/L) was nearly significantly inversely associated with tT3 in early pregnancy (B: −0.05; 95% CI: −0.10, 0.01). However, the fact that the exposure (cord blood Hg) was measured several months after the outcome (early pregnancy) raises concern.

In experimental studies, MeHg has been shown to inhibit type II deiodinase activity (Mori et al. 2007), which is responsible for converting T4 into T3, and inhibition of its activity could potentially lead to reduced T3 levels. The fact that we also observed an inverse association between erythrocyte Hg and the fT3:fT4 ratio is further indicative of

reduced type II deiodinase activity with increasing MeHg exposure. Also, increased placental type III deiodinase activity, responsible for inactivation of T3 (Huang and Bianco 2008), has been observed in pregnant mice exposed to MeHg (Watanabe et al. 1999). However, there were no discernable effect of MeHg exposure on maternal plasma T3 levels in the studied mice.

The strengths of this study include the individual exposure assessments of three toxic metals and two thyroid-related essential elements by measuring concentrations of suitable biomarkers with a highly sensitive ICP-MS method. We observed generally low metal concentrations, yet only two samples had a concentration below the LOD for Hg, while Pb and Cd were detected in all samples. We were also able to adjust for several important covariates, as well as maternal iodine and selenium concentrations. A major limitation is that only about 10% of all the women (about 5000) giving birth at Sunderby hospital during the recruitment period participated in the study (Englund-Ögge et al., submitted). Consequently, the participating women may not be representative of pregnant women in the area in general. However, the exposure to Cd and Hg appears similar to those previously reported for pregnant women in southern and central Sweden (Akesson et al. 2002; Vahter et al. 2000), and to the Cd exposure reported for women in reproductive age in northern Sweden (Wennberg et al. 2017). Another limitation is that erythrocyte Hg was not speciated, why we cannot exclude that a small fraction of erythrocyte Hg was inorganic Hg, although part of that would then be due to demethylation of MeHg (Berglund et al. 2005). Since dental amalgams, a main source of inorganic Hg exposure (Bjornberg et al. 2005; Clarkson and Magos 2006), was gradually phased out in the 1990s in Sweden, it seems unlikely that the women were exposed to inorganic Hg to any significant extent. Further, we used single spot-urine samples to assess maternal iodine status. Urinary iodine is a short-term biomarker that can vary substantially within and between days and it is therefore suboptimal for assessing individual iodine status. In the present study, we measured free thyroid hormone concentrations in plasma by immunoassays which may be sensitive to the pregnancy-related increase in TBG, however, it was not possible to calculate the fT4 or fT3 index (Villanger et al. 2017). Finally, this is a cross-sectional observational study, why the causality is not known, and, as with all observational studies, we cannot exclude that our findings were influenced by unmeasured or residual confounding.

5. Conclusion

The present findings indicate that exposure to Cd and MeHg, at levels globally prevalent through the diet, may interfere with maternal thyroid function during pregnancy. This is of public health concern as even fairly small changes in the hormones during pregnancy may be disadvantageous for both maternal and fetal health. The associations appeared independent of the iodine and selenium levels, which both were low among many of the women. Studies on the potential impact on fetal thyroid hormones are warranted, especially as MeHg is actively transported across the placenta (EFSA 2012) and Cd accumulates in the placenta, a crucial site of type III deiodinase activity (Huang et al. 2003). Importantly, since exposure to Cd and MeHg mainly comes from the consumption of food with high nutritional value, such as whole-grain cereals and seafood (Gustin et al. 2020), our findings highlight the importance of reducing food contamination.

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CRediT authorship contribution statement

Klara Gustin: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Malin Barman:** Data curation, Writing – review & editing, Project administration. **Helena Skröder:** Writing – review & editing. **Bo Jacobsson:** Writing – review & editing. **Anna Sandin:** Resources, Project administration, Funding acquisition, Writing – review & editing. **Ann-Sofie Sandberg:** Project administration, Funding acquisition, Writing – review & editing. **Agnes E. Wold:** Project administration, Funding acquisition, Writing – review & editing. **Marie Vahter:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing. **Maria Kippler:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

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