Environment International 147 (2021) 106310

Contents lists available at ScienceDirect

Environment International

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

Association of biomarkers of exposure to metals and metalloids with maternal hormones in pregnant women from Puerto Rico

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ARTICLE INFO

Handling Editor: Shoji F. Nakayama

Keywords:
Metals
Metalloids
Essential metals
Maternal hormones
Pregnancy

ABSTRACT

Background: Metal(loid)s have been associated to adverse birth outcomes in experimental and epidemiological studies, but the underlying mechanism(s) are not well understood. Endocrine disruption may be a mechanism by which the metal(loid)s impact birth outcomes.

Methods: Pregnant women were recruited through the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT). Urine, blood, demographic and pregnancy-related data were collected at recruitment and subsequent visits. Sixteen metal(loid)s were analyzed in urine and blood samples, while nine maternal hormones (corticotropin-releasing hormone (CRH), sex-hormone binding globulin (SHBG), estriol (E3), progesterone, testosterone, thyroid-stimulating hormone (TSH), total triiodothyronine (T3), total thyroxine (T4), and free thyroxine (fT4)) were measured in serum samples from 815 singleton pregnancies. Linear mixed models with random intercepts were used to examine associations between metal(loid)s in blood and urine with hormone concentrations.

Results: Arsenic blood concentrations were significantly associated with increased levels in CRH (%Δ: 23.0, 95% CI: 8.4–39.6) and decreased levels in testosterone (%Δ: ⁻16.3, 95%CI: ⁻26.2-⁻5.1). Cobalt, manganese, and lead blood concentrations were associated with small increases in SHBG (%Δ range: 3.3–4.2), E3 (%Δ range: 3.9–8.7) and progesterone (%Δ range: 4.1–6.3) levels, respectively. Nickel blood concentration was inversely associated with testosterone levels (%Δ ⁻13.3, 95%CI: ⁻18.7-⁻7.6). Significant interactions were detected for the association between nickel and study visit in relation to CRH ($p < 0.02$) and testosterone levels ($p < 0.01$).

Conclusion: Our analysis suggests that metal(loid)s may act as endocrine disruptors by altering prenatal hormone levels. This disruption may depend on specific windows of exposure during pregnancy. Additionally, some essential metal(loid)s such as managense and cobalt may be contributors to adverse maternal and fetal outcomes. The study of metal(loid)s as endocrine disruptors is in the early stages of epidemiological research and future studies are needed to further investigate these associations.

1. Introduction

A delicate hormonal balance orchestrates pregnancy from conception to delivery and perturbations of this balance may negatively impact both mother and fetus. In fact, epidemiological studies have shown that variation in maternal and placental hormone levels during pregnancy is related to pregnancy complications as well as adverse birth outcomes including growth restriction, preterm birth and low birth weight (Gilles et al., 2018; Jelliffe-Pawlowski et al., 2010; Kumar et al., 2018; Mucci et al., 2003; Noyola-Martinez et al., 2019; Wadhwa et al., 2004). Emerging work from the developmental origins of health and disease (DOHaD) perspective, furthermore, suggests that the prenatal hormonal

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https://doi.org/10.1016/j.envint.2020.106310
Received 30 June 2020; Received in revised form 23 October 2020; Accepted 26 November 2020

milieu may contribute to children's later health and disease risk including neurodevelopmental impairment, polycystic ovary syndrome (PCOS), endometriosis, prostate and breast cancer (Day et al., 2020; Filippou and Homburg, 2017; Gore et al., 2015; Parazzini et al., 2017). Interestingly, how disruptions in prenatal hormones impact long-term health is understudied.

As science moves forward to better understanding the early origins of disease, investigating how maternal exposures impact the prenatal hormonal milieu is critical. Metal(loid)s are ubiquitous in the environment and as such, there is widespread exposure among the general population, including pregnant women and their children (Hendryx and Luo, 2018; Papadopoulou et al., 2019; Woodruff et al., 2011). The adverse effects of toxic metal(loid)s such as lead (Pb) and mercury (Hg) on human health is well known. Both Hg and Pb are neurotoxicants associated with miscarriage, birth defects and preterm birth, while arsenic (As), and cadmium (Cd) have been associated with growth restriction (Edwards, 2014; Liao et al., 2018; Omeljaniuk et al., 2018). Additionally, a growing body of literature has reported associations between essential trace metals and adverse birth outcomes. For example, elevated copper (Cu) and nickel (Ni) levels have been associated with increased risk of preterm birth (Chen et al., 2018; Kim et al., 2018). Other reports suggest inverted U-shaped dose–response curves for the associations between cobalt (Co) and manganese (Mn) and birth weight (Mikelson et al., 2019; Zota et al., 2009). We previously reported that elevated Mn and zinc (Zn) exposure were associated with higher odds of preterm birth and shorter gestational age (Ashrap et al., 2020a). Given the strong evidence of association between metal(loid)s and adverse birth outcomes, investigating possible mechanisms underlying such associations is an important next step.

One potential mechanism by which metal(loid)s impact pregnancy and fetal health is through endocrine disruption. Experimental studies have demonstrated that metal(oid)s alter maternal and placental hormones including estradiol, progesterone, testosterone, and thyroid-stimulating hormone (TSH) among others (Iavicoli et al., 2009; Rana, 2014). For example, Cd and Hg exposure stimulate progesterone production while Hg and Pb reduce plasma testosterone levels in experimental animals (Davis et al., 2001; Ronis et al., 1998; Vachhrajani and Chowdhury, 1990). Cd can also alter the secretory patterns of pituitary hormones including TSH (Lafuente et al., 2003). Furthermore, Cd may impact steroidogenesis by downregulation of critical enzymes such as steroidogenic acute regulatory proteins (StAR) and 3ß-hydroxyl steroid deydrogenase (3ß-HSD) in the placenta (Xiong et al., 2020). Metal(loid)s can also act as estrogens by binding to the estrogen receptors and initiating transcription of estrogen-activated genes (Tilghman et al., 2010). Despite evidence from experimental studies that metal(loid)s can alter hormones production and function, only two epidemiological studies have examined the association between prenatal hormones and metal(loid)s, which focused only on thyroid hormones (Guo et al., 2018; Sun et al., 2019).

The present study addresses that gap by examining metal(loid)s in relation to maternal hormones in a large study of pregnant women in Puerto Rico (PR), United States. PR has higher rates of adverse birth outcomes including PTB and endocrine-related diseases compared to other U.S. states and territories (CDC, 2012; MoD, 2016; Perez et al., 2008; Rivera-Soto et al., 2010). Interestingly, metal(loid) exposure is particularly relevant in this population because PR has one of the highest rates of Superfund sites of any of the U.S. jurisdictions with 19 active sites at the time of this study (EPA, 2020). Our previous work indicates that among pregnant women, metal(loid)s exposure is higher in PR than in women from the continental U.S. (Ashrap et al., 2020b). The primary aim of this study was to examine associations between metal(loid)s and maternal hormones including corticotropin-releasing hormone (CRH), sex-steroid, and thyroid hormones during pregnancy. Secondarily, we considered potential effect modification of these relationships by timing of exposure and infant sex.

2. Methods

2.1. Study participants

Participants in this study were enrolled in the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. Initiated in 2010, PROTECT is an ongoing prospective birth cohort designed to study environmental exposures in pregnant women and their children residing around the northern karst zone of Puerto Rico (Cantonwine et al., 2014; Meeker et al., 2013). Inclusion criteria for recruitment included women with (1) singleton pregnancies, (2) between 18 and 40 years, (3) resident of the northern karst zone, (4) report no major obstetrical or medical complication (5) no use of oral contraceptives in the three months before pregnancy, (6) no use of *in vitro* fertilization to achieve the current pregnancy. Women were recruited at approximately 14 ± 2 weeks and completed up to three prenatal visits (18 ± 2 weeks [Visit 1], 22 ± 2 weeks [Visit 2] and 26 ± 2 weeks [Visit 3]). A series of detailed questionnaires were administered to gather participants' demographics, occupation, in addition to physical examination parameters at different time points in pregnancy (Meeker et al., 2013). Blood samples were collected at 1st (18 ± 2 weeks) and 3rd (26 ± 2 weeks) visits and urine samples were collected at all three visits corresponding to windows of rapid fetal growth and development. A total of 815 women recruited between 2011 and 2017 with metal(loid)s and hormones measurements from at least one of the visits were included in this study. This research protocol was approved by the Ethics and Research Committees of the University of Puerto Rico and participants clinics, the University of Michigan, School of Public Health, and Northeastern University. Each participant received a full description of the study and informed consent was obtained before enrollment.

2.2. Measurements of metals

Blood samples were collected in metal free whole tubes and urine samples were collected in sterile polypropylene cups and aliquoted within an hour after collection. All samples were frozen at −80 °C and shipped on dry ice to NSF International (Ann Arbor, MI) for metal(loid) analysis. Metal(loid)s were selected based on experimental evidence for endocrine disruption and/or impact in hormone secretion and metabolism (Iavicoli et al., 2009; Rana 2014). Concentrations of 16 metal (loid)s were measured in both spot urine and blood samples: As, barium (Ba), beryllium (Be), Cd, Co, chromium (Cr), cesium (Cs), Cu, Hg, Mn, Ni, Pb, titanium (Ti), uranium (U), vanadium (V), and zinc (Zn); an additional 6 metal(loid)s were measured in urine only: Mo, platinum (Pt), antimony (Sb), selenium (Se), tin (Sn), and tungsten (W) (Ashrap et al., 2020b). Analysis was conducted using a Thermo Fisher (Waltham, MA, USA) ICAPRQ inductively coupled plasma mass spectrometry (ICPMS) and CETAC ASX-520 autosampler following established protocols (see Supplemental Material for quality control and assurance details) (Kim et al., 2018). Urine measurements of 15 metals (up to 3 measurements per women) and blood measurements of 8 metals (up to 2 measurements per women) that had a high detection rate (70%> limit of detection) were included in the current analysis as continuous variables, while blood As (49%>LOD) and Cd (61%>LOD) were included as binary variables (above vs below LOD). Overall, 502 women provided urine samples, from those 372 and 130 had one and two urine samples (Visit 1 = 391 and Visit 3 = 241), respectively. Seven hundred and three women provided urine samples, from those 450 and 245 had one and two samples (Visit 1 = 529 and Visit 3 = 419).

2.3. Measurements of hormones

Serum samples were shipped to and analyzed at the Central Ligand Assay Satellite Services laboratory in the Department of Epidemiology at the University of Michigan, School of Public Health. Reproductive and thyroid hormones were selected based on animal and human studies to

be associated with environmental exposures and/or adverse pregnancy outcomes (McGrath et al., 2002; Patel et al., 2011; Wadhwa et al., 2004). Progesterone (Siemens, catalog no. 1586287) (ADVIA), sex hormone binding globulin [SHBG] (Siemens, catalog no. 6520781) (ADVIA), testosterone (Siemens, catalog no. 5476206) (ADVIA), total triiodothyronine [T3] (Siemens, catalog no. 8427516) (ADVIA) total thyroxine [T4] (Siemens, catalog no. 9236439) (ADVIA), free T4 (fT4; Siemens, catalog no. 6490106) (ADVIA), and TSH (Siemens, catalog no. 8700387) (ADVIA) were measured using a chemiluminescence immunoassay. Estriol [E3] (DiaMetra, catalog no. DKO019) (DiaMetra) and CRH (LifeSpan, catalog no. LS-F5352) (LifeSpan) were measured using an enzyme immunoassay. The ratios of progesterone/E3 and T3/T4 were calculated and assessed as these ratios may be better indicators of adverse pregnancy outcome than individual hormone measurement (Dietrich et al., 2012; Romero et al., 1988). Overall, 815 women had hormone measurements, from those 513 and 302 had one and two measurements (Visit 1 = 636, Visit 3 = 481), respectively.

2.4. Statistical analysis

Metal(loid)s and hormone concentrations below the LOD were replaced by the LOD divided by the square root of two (Lubin et al., 2004; Schisterman et al., 2006). Distributions of all metal(loid)s in urine and blood were right skewed and thus were natural log transformed for all analyses. Because As and Cd had 50 and 40% of samples below LOD respectively, we dichotomized their concentration (below LOD and above LOD) in all analyses. Distributions of CRH, E3, progesterone, TSH, testosterone, and progesterone/E3 ratio were also right skewed and natural log transformed for all analyses. Distributions of SHBG, fT4, T3, T4 and T3/T4 ratio were approximately normal and thus were not transformed.

We used linear mixed models (LMM) to regress hormone concentrations on blood or urine metal(loid)s, with separate models for each exposure biomarker and random intercepts to account for correlation of repeated measurements. All results were presented as percent changes (%Δ) and 95% confidence intervals (CIs) in hormone concentrations associated with an interquartile range (IQR) increase in blood or urine metal(loid) concentration. The crude models only included the metal (loid) concentration as the exposure; for models regressing on urinary metals, log-transformed concentrations were further corrected for specific gravity (Ashrap et al., 2020b). Potential confounders were selected *a priori* from existing literature, available PROTECT data and directed acyclic graph evaluation. The covariates considered were maternal age, gravidity, gestational age at prenatal visit, infant sex, pre-pregnancy body mass index (BMI), marital status, insurance type, maternal education, employment status, smoking, exposure to second-hand smoking, alcohol consumption, time of sample collection, and prenatal study visit. The final set of covariates were selected in a stepwise procedure if they altered the beta coefficient of metal exposure by 10% or more. Final models were adjusted for maternal age (continuous), education (categorical), pre-pregnancy BMI (categorical) and prenatal visit. Testosterone models were further adjusted for SHBG concentrations to account for SHBG bound-testosterone (Dunn et al., 1981). In order to simplify our results, we present main analysis using metal(loid)s in blood, and urinary analysis are presented as supplemental.

We conducted additional analyses to examine windows of susceptibility, effect modification by fetal sex and nonlinear metal(loid)-hormone relationships. First, to evaluate different windows of susceptibility, we additionally included interaction terms between metal concentration and each visit indicator into the LMMs to generate separate models for each visit. Using this method, the effect estimates of the covariates were still assessed using the LMM structure. The visit-metal interaction terms were tested for significance and the visit-specific metal effect estimates were also abstracted from the LMMs. Second, to assess if the change in hormone levels differed by fetal sex, all single LMMs were refitted with the addition of an interaction term between metal concentrations and fetal sex indicator. Third, to assess nonlinear dose–response relationships between metal(loid)s and hormones concentrations, we used generalized additive mixed models (GAMM) using smooth function for metal(loid)s and adjusted for the same covariates. To further evaluate nonlinear dose–response relationships on metal (loid)s showing evidence of non-linearity, metal(loid) concentration was categorized (tertiles) and included in additional GAMMs. An α level of 0.05 was used to indicate statistical significance. All statistical analyses were conducted using R version 3.6.2.

3. Results

3.1. Demographics

A total of 948 blood samples and 632 urine samples from 815 women were analyzed in this study (Table 1). Participants were 27 ± 5 years old on average and most (78%) were married or in a domestic partnership. Overall, they had above a high school education (77%), were employed (61%), and had private insurance (60%). >80% of women never smoked while <2% smoked during pregnancy and 6% reported second-hand smoking exposure (>1h per day). No alcohol consumption within the last few months was reported by almost all (92%) participants.

3.2. Distribution of metal(loid)s and maternal hormones

Descriptive statistics and Spearman correlations between different metal(loid)s have been previously reported (Ashrap et al., 2020a, b). Overall, metal(loid)s were detected in most blood and urine samples with the exception of As (50%>LOD) and Cd (60%>LOD), and Be, Cr, Ti, U, V which were detected in very few samples and not included in our analyses (Supplemental Table 1). Similarly, descriptive statistics for all maternal hormones have been previously described (Supplemental Table 2) (Aker et al., 2018; Cathey et al., 2019). Overall, all hormones except testosterone (n = 5 below LOD) were detected in 100% of blood samples and concentrations of E3, SHBG, fT4, progesterone, and testosterone were all significantly higher at Visit 3 than at Visit 1 (p-values: <0.001, <0.001, <0.001, 0.002, and 0.001, respectively).

3.3. CRHa

Main unadjusted and adjusted LMM results between blood metal (loid)s and maternal serum hormones are shown in Fig. 1. In main adjusted models, women with As concentrations above the LOD had 23% higher CRH levels (95%CI: 8.4–39.6) compared to women with As below the LOD. This association was higher in Visit 1 (%Δ: 28.6, 95%CI: 9.1–51.5) compared to Visit 3 (%Δ: 16.8, 95%CI: ⁻2.3–39.6) but the interaction term was not significant. No other significant associations were observed between any other metal(loid) exposure biomarker and CRH levels in main models. However, in visit-specific analyses, an IQR increase in Ni concentrations was associated with an increase in CRH levels only at Visit 3 (%Δ: 12.6, 95%CI: 2.1–24.1) (Supplemental Table 3). Furthermore, we reported a significant interaction between prenatal visit and blood Ni concentration in the association with CRH levels (p = 0.02) (Fig. 2). Finally, we also observed a significant nonlinear relationship (inverted u-shape) between Ni concentrations and CRH levels (estimated degree of freedom (edf) = 2.99 for smooth parameter; p = 0.0177) (Fig. 3a, Supplemental Table 4).

3.4. Sex-steroid hormones

In main adjusted models, IQR increases in blood Co, Mn, and Pb concentrations were associated with small increases in SHBG levels (%Δ: 4.2, 95%CI: 1.6–6.8, %Δ: 3.6, 95%CI: 0.6–6.7, %Δ: 3.3, 95%CI: 0.3–6.3, respectively).

IQR increases in Co, Mn, Ni and Pb concentrations were also associated with changes in E3 levels (%Δ: 8.7, 95%CI: 4.0–13.5, %Δ: 6.1,

Table 1
Demographic characteristics of 815 women enrolled in PROTECT (2011–2017).

Variable	n(%)
Maternal age at enrollment (years)	26.7(5.5)[a]
Mean gestational age at delivery (weeks)	38.9(2.1)[a]
Maternal age (years)	
<25	311(38.2)
25–30	299(36.7)
>30	204(25.0)
missing	1(0.1)
Gravidity (# pregnancies)	
0	338(41.5)
1	294(36.1)
>1	170(20.9)
missing	13(1.6)
Marital status	
single	165(20.2)
married or living together	635(77.9)
missing	15(1.8)
Maternal Education	
≤High school/GED	173(21.2)
Some College or technical school	280(34.4)
College degree	250(30.7)
Masters degree or higher	94(11.5)
missing	18(2.2)
Employment Status	
employed	496(60.9)
unemployed	300(36.8)
missing	19(2.3)
Household Income (U.S.$)	
<$10,000	220(27.1)
≥$10,000 to <$30,000	235(28.8)
≥$30,000 to <$50,000	160(19.6)
≥$50,000	85(10.4)
missing	115(14.1)
Insurance type	
Private	487(59.8)
Public (Mi Salud)	278(34.1)
missing	50(6.1)
Pre-pregnancy BMI (kg m^{-2})	
≤25	418(51.3)
>25 to ≤ 30	213(26.1)
>30	138(16.9)
missing	46(5.6)
Smoking	
Never	682(83.7)
Ever	109(13.4)
Current	10(1.2)
missing	14(1.7)
Exposure to second hand smoking	
None	672(82.5)
Up to 1 h	34(4.2)
>1 h	52(6.4)
missing	57(7)
Alcohol consumption	
None	397(48.7)
Before pregnancy	351(43.1)
Yes within the last few months	50(6.1)
missing	17(2.1)
Fetal sex	
Female	367(45.0)
Male	407(49.9)
missing	41(5.0)

BMI: body mass index.
[a] Mean(standard deviation).

95%CI: 1.2–11.3, %Δ: 3.9, 95%CI: 0.2–7.7, and %Δ: 5.0, 95%CI: 0.4–9.9, respectively). In visit-specific analyses, associations remained significant only for Visit 1 ([Co]: %Δ: 10.0, 95%CI: 4.7–15.5), [Mn]: %Δ: 7.7, 95%CI: 1.3–14.5), [Ni]: %Δ: 5.5, 95%CI: 0.9–10.4), [Pb]: %Δ: 7.5, 95%CI: 1.6–13.7) (Supplemental Table 3).

In main adjusted models, an IQR increase in Co concentration was associated with increases only in progesterone levels (%Δ: 6.3, 95%CI: 2.1–10.7). Similar to E3, this association was significant only at Visit 1 (%Δ: 6.7, 95%CI: 2.0–11.7). An IQR increase in Ni concentrations was associated with a decrease in testosterone levels (%Δ: ⁻13.3, 95%CI: ⁻18.7- ⁻7.6). As concentrations above the LOD were also associated with decreases in testosterone levels (%Δ: ⁻16.3, 95%CI: ⁻26.2- ⁻5.1). Results in the testosterone models were unchanged with further adjustment for SHBG levels (data not shown). In visit-specific analyses, these relationships were mostly driven by Visit 3 ([As]: %Δ: ⁻19.2, 95%CI: 32.0- ⁻4.0; [Ni] %Δ: ⁻20.4, 95%CI: ⁻6.9- ⁻2.5). We also observed a significant interaction between prenatal visit and Ni concentration in the association with testosterone levels (p = 0.01) (Fig. 2).

3.5. Thyroid hormones

In main adjusted analysis, an IQR increase in Co concentrations was associated with an increase in TSH concentrations (%Δ: 6.1, 95%CI: 0.6–12.0). Cd concentrations above the LOD were also associated with increases in TSH concentrations (%Δ: 9.7, 95%CI: 0.6–19.8). In visit-specific analyses, an IQR increase in Co concentrations was found to be associated with TSH levels only in Visit 1 (%Δ: 6.3, 95%CI: 0.2–12.9). An IQR increase in Ni concentrations was also associated with increases in TSH levels but only in Visit 3 (%Δ: 7.5, 95%CI: 0.8–14.5). An IQR increase in Ni concentration was associated with an increase in T3 levels (%Δ: 4.8, 95%CI: 2.6–7.0). This association remained significant in visit-specific analyses but interaction terms were not statistically significant. IQR increases in Cu and Hg concentrations were associated with small increases in T4 concentrations (%Δ: 1.3, 95%CI: 0.4–2.3, %Δ: −4.8, 95%CI: 2.6–7.0, respectively). These small increases in T4 levels in relation to Cu concentration remained significant in visit-specific analyses. No significant association were observed between fT4 levels and any blood metal(loid)s.

3.6. Effect modification by fetal sex

Interaction terms between fetal sex and metal(loid) concentration were not statistically significant for any models except for the association between Hg and SHBG levels (p = 0.03) (Supplemental Fig. 1). Visit-specific analyses showed that the effect of Hg concentrations in SHBG levels was only significant among mothers delivering female fetuses (p = 0.02), for whom an IQR increase in Hg concentration was associated with 5% decrease in serum SHBG concentration (%Δ: ⁻4.8, 95%CI: ⁻9.0- ⁻0.6). A marginally significant interaction (p = 0.06) was observed between Pb concentrations and progesterone levels in mothers delivering male fetuses (%Δ: 7.8, 95%CI: 2.1–13.8) compared to mothers delivering female fetuses (%Δ: ⁻0.8, 95%CI: ⁻7.6–6.53).

Although no other interaction term was statistically significant, other differences were observed in visit-specific analyses by fetal sex. The percent difference in CRH levels in relation to an IQR increase in As concentration was larger in women delivering female fetuses (%Δ: 29.0, 95%CI: 7.6–54.7) compared to women delivering male fetuses (%Δ: 14.6, 95%CI: −4.3–37.4). A similar trend was observed for changes in progesterone levels and As concentrations, where women delivering female fetuses (%Δ: 10.8, 95%CI: 1.1–21.4) had larger percent differences in progesterone levels than women delivering male fetuses (%Δ: 3.4, 95%CI: −5.4–13.1). Finally, the percent increase in TSH levels associated with Ni concentrations was larger in mothers delivering female fetuses (%Δ: 6.6, 95%CI: 0.2–13.5) compared to mothers delivering male fetuses (%Δ: 2.0, 95%CI: −4.6–8.5).

3.7. Metals in urine

Overall, results from models using metal(loid)s measured in urine were similar to those presented in our main analysis using the blood biomarker with the exception of Mn. Much stronger associations were observed for urinary Mn compared to blood Mn (Supplemental Fig. 2). An IQR increase in Mn concentration was associated with an increase in CRH levels (%Δ: 34.0, 95%CI: 26.5–42.0). This %Δ in CRH levels was 10 times larger than the %Δ for CRH in the blood biomarker (%Δ: 3.5, 95%

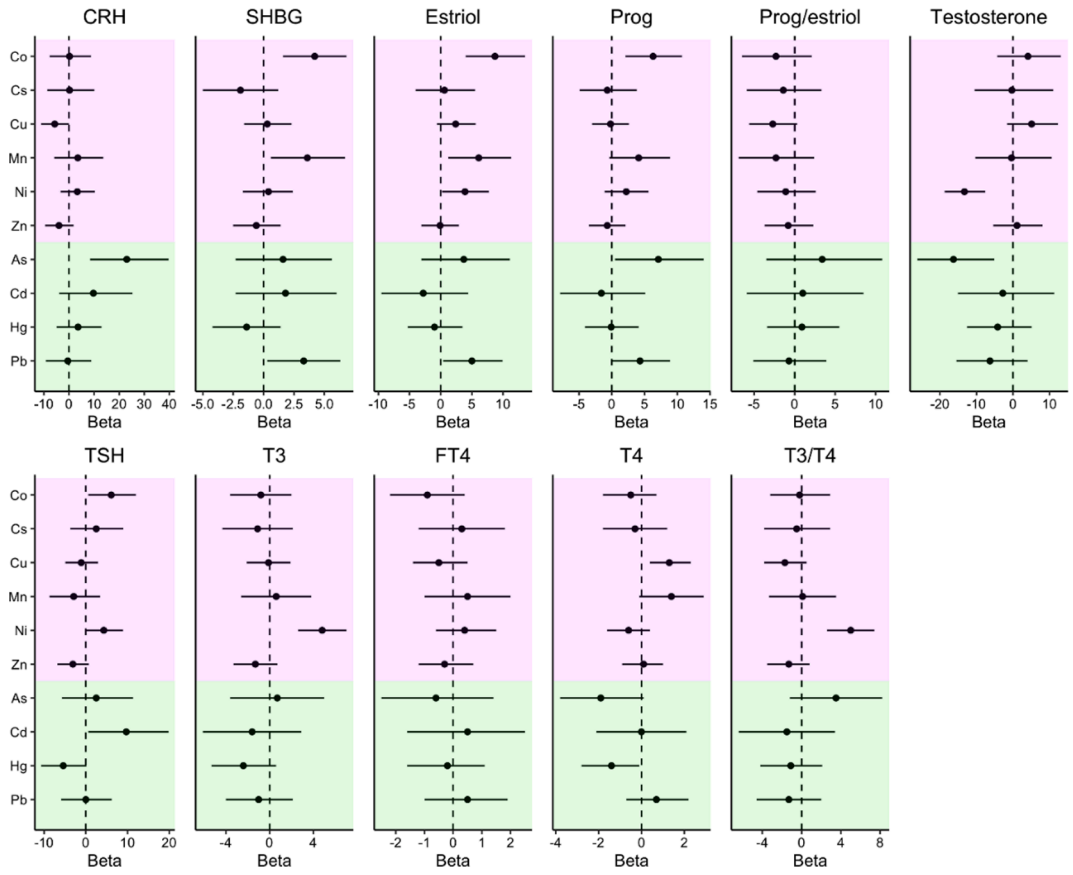

Fig. 1. The percent difference in serum hormone concentration (95% confidence interval) in non-transformed units per interquartile range (IQR) increase in natural log-transformed metal(loid) concentration in blood. CRH: corticotropin-releasing hormone, SHBG: sex hormone binding globulin, E3: estriol, progesterone, testosterone, TSH: thyroid-stimulating hormone, T3: total triiodothyronine, T4: total thyroxine, and fT4: free thyroxine. Green: non-essential metal(loid)s, Purple: essential metal(loid)s. Models adjusted for maternal age (continuous), education (categorical), pre-pregnancy body mass index (categorical) and study visit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

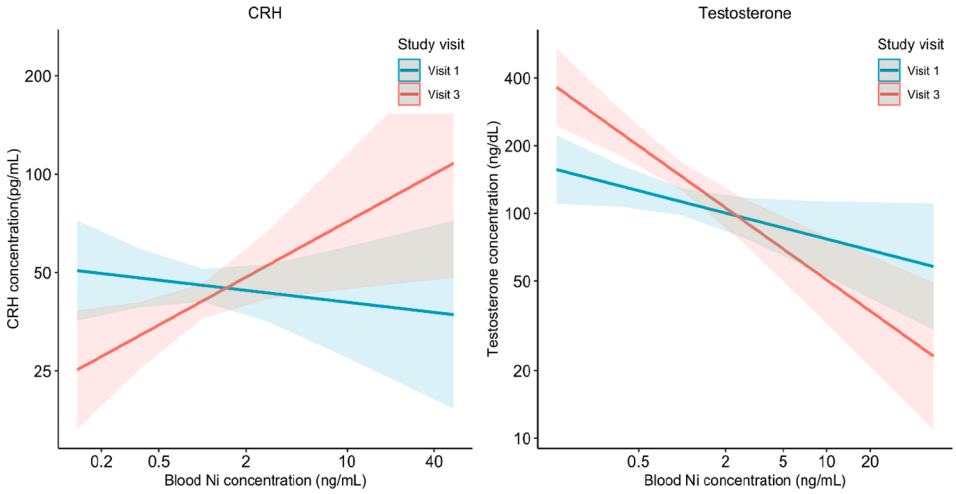

Fig. 2. Interaction effect of study visit on the association between the IQR increase in Ni concentration in blood and % difference in serum CRH and testosterone levels. CRH: corticotropin-releasing hormone, IQR: interquartile range. Models adjusted for maternal age (continuous), education (categorical) and pre-pregnancy body mass index (categorical).

CI: ⁻5.8–13.6). In visit-specific analyses, no difference was found between Visit 1 and Visit 3. However, in analysis stratified by fetal sex, we observed that mothers delivering female infants had a larger increase in CRH levels in relation to an IQR increase in Mn concentration (%Δ: 45.9, 95%CI: 31.6–61.8) compared to women delivering male fetuses (%Δ: 27.7, 95%CI: 18.7–37.3). Interaction terms for both prenatal visit and infant sex were not statistically significant. An IQR increase in Mn concentrations was associated with lower testosterone levels (%Δ: ⁻28.0, 95%CI: ⁻32.3-⁻23.5) but no differences were observed by prenatal visit. In analyses stratified by fetal sex, women delivering female fetuses

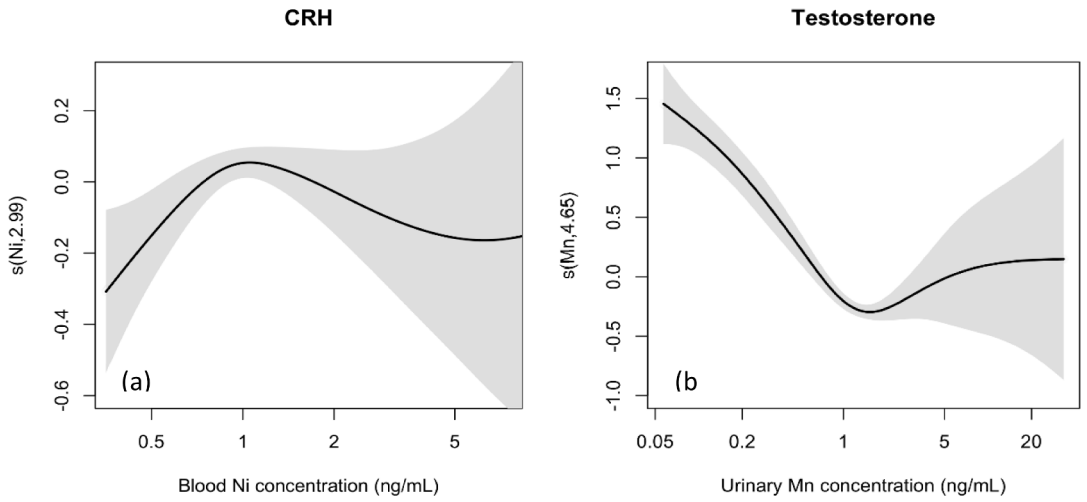

Fig. 3. Nonlinear relationship between (a) blood Ni concentration and CRH levels, and (b) urinary Mn concentration and testosterone levels. Generalized additive mixed model of log-Ni concentration in log-CRH level and log-Mn concentration and log-T. Hash marks at the bottom of the plot show observed values for log-Ni concentration or log-Mn concentration. Model adjusted for maternal age (continuous), education (categorical) and pre-pregnancy body mass index (categorical). CRH: corticotropin-releasing hormone.

had larger decreases in testosterone levels (%Δ: ⁻35.4, 95%CI: ⁻42.1-⁻27.9) with an IQR increase in Mn concentration compared to those delivering male fetuses (%Δ: ⁻23.7, 95%CI: ⁻29.2- ⁻17.7) but the interaction term was not significant. Additionally, a significant nonlinear relationship (u-shape) was observed between Mn concentrations and testosterone levels (estimated degree of freedom (edf) = 4.65 for smooth parameter; $p < 0.001$) (Fig. 3b, Supplemental Table 4). Six metal(loid)s were measured only in urine: Ba, Mo, Pt, Sb, Se, and Sn. Results and a brief discussion for these are presented in the Supplemental Material.

4. Discussion

In this study we examined the association between biomarkers of prenatal metal(loid) exposure and maternal serum hormones measured at two times points during pregnancy. Gestational age at prenatal visit impacted some of the metal(loid)-hormone relationships, potentially indicating the importance of timing of exposure during pregnancy. Contrary to other endocrine disruptors (Barrett et al., 2017; Wenzel et al., 2018), minimal sex-dependent associations were observed between metal(loid) exposure and hormone concentrations. Finally, essential metals Ni, Co and Mn were associated with significant differences in maternal hormone concentrations, adding to recent literature on the possible contributions of elevated levels of essential metals to adverse prenatal outcomes. This analysis was largely exploratory since the study of metal(loid)s as endocrine disruptors is in the early stages of epidemiological research. To our knowledge, this is the first study that examines toxic and essential metals exposure in relation to prenatal sex-steroid concentrations.

4.1. CRHa

CRH is a major stress hormone produced by the hypothalamus as well as the placenta and fetal membranes. As parturition approaches, CRH produced by the placenta and fetal membranes signals the up-regulation of key hormones including cortisol. Cortisol perpetuates this positive feedback loop by promoting further placental CRH (pCRH) expression. This creates exponential production of glucocorticoids which synchronizes fetal organ maturation and labor (McGrath et al., 2002). As a result, pCRH has been proposed as a "placental clock" that critically regulates the timing of labor. Surprisingly, minimal work has been directed into the potential impact of endocrine disruptors on this critical hormone. Experimental data suggest that endocrine disruptors such as bisphenols, phthalates, and zeranol may enhance pCRH expression (Huang et al., 2012; Wang et al., 2016; Wang et al., 2013). Our study found a positive association between As exposure and circulating CRH levels. It is known that As is transported through the placenta, possibly by GLUT1, one of the main transplacental glucose transporters (Jones et al., 2007). Experimental data suggest that As interferes with the glucocorticoid receptor signaling system (GR) in both the placenta and the fetal brain (Caldwell et al., 2015; Goggin et al., 2012; Martinez-Finley et al., 2009). It is possible that As interferences in the GR may influence CRH production leading to alterations in the cycle and affecting fetal organ maturation and labor. Further research investigating As impact in the GR, should include examination of CRH production and function.

4.2. Sex-steroid hormones

Although there are no epidemiological studies assessing the impact of metal(loid)s on prenatal hormones levels, several studies have examined prenatal sex-steroid hormones in relation to other endocrine disruptors including phthalates, parabens, BPA and some pesticides (Aker et al., 2019; Araki et al., 2018; Johns et al., 2015; Kolatorova et al., 2018; Mulder et al., 2019; Sathyanarayana et al., 2014; Sathyanarayana et al., 2017). The Infant Development and the Environment Study (TIDES), a large pregnancy cohort including women from four U.S. cities ($n = 591$), reported that first trimester phthalate exposure was positively associated with estrone and estradiol measured during the first half of pregnancy (<20 week) (Sathyanarayana et al., 2017). We also showed positive associations between E3 and various metals (Co, Mn, Ni, Pb) mainly driven by associations in Visit 1 (week 18 ± 2 of gestation). In a smaller U.S. cohort, certain phthalate metabolites were inversely associated with free and total testosterone when measured during late pregnancy (≥20 weeks) (Sathyanarayana et al., 2014). Similar work in PROTECT observed that the relationship between some phthalate metabolites and testosterone levels was modified by timing of prenatal visit (Cathey et al., 2019). Here we showed that timing of prenatal visit is important for some of the metal(loid)-hormone associations, including an interaction between Ni concentration and prenatal visit in relation to testosterone levels. Metal(loid)s concentrations may vary across pregnancy due to various factors including their unique physiochemical properties and toxicokinetics. Additionally, metal(loid)s concentrations may be influenced by changes in fetal and maternal nutrient supply

(King, 2000). Pregnancy also represent metabolic changes including variation in glomerular filtration rate and plasma volume expansion which may impact metal(loid)s concentration in a particular point in time (Cheung and Lafayette, 2013; Hytten, 1985). These results also suggest that gestational age may play a critical role in the association between endocrine disruptors, including metal(loid)s, and sex-steroid hormones. This is consistent with the DOHaD concept that the impact of a given exposure depends on its timing in relation to critical and sensitive windows of development. Longitudinal assessments of prenatal hormones have shown that the major increases in estrogens during pregnancy occur during the first 20 weeks of gestation while testosterone levels gradually increase during pregnancy, peaking in late pregnancy (O'Leary et al., 1991; Schock et al., 2016). These windows of rapid estrogen and testosterone production are particularly important points at which endocrine disruptors may interact with normal production and function of sex-steroid hormones and alter fetal development. Alterations in estrogen and testosterone during pregnancy can lead to a wide range of adverse birth outcomes including abnormalities of the reproductive system, miscarriage, intrauterine growth restriction, and preterm labor (Noyola-Martinez et al., 2019; Rey and Picard 1998).

4.3. Thyroid hormones

Two studies have examined metal(loid)s exposure in relation to prenatal thyroid hormones (Guo et al., 2018; Sun et al., 2019). The Hangzhou Birth Cohort Study in China (n = 915) reported inverse associations between blood As, Mn, Ni and Sb concentrations and thyroid hormones levels measured around 25 weeks of gestation (Guo et al., 2018). In contrast, As, Mn, and Sb concentrations were not associated with thyroid hormones in the current study, but we observed a positive association between Ni concentrations and TSH and T3 levels. Similar to sex-steroid hormone studies, other endocrine disruptors have been studied in relation to prenatal thyroid hormone levels. The Health Outcomes and Measured Study (HOME) reported an inverse association between multiple phthalate exposures and T4 levels measured at 16 weeks of gestation (Romano et al., 2018). The Generation X birth cohort in the Netherlands reported no association between organophosphate pesticides and thyroid hormones measured at three points during pregnancy (<18, 18–25, >25 weeks) (Mulder et al., 2019). Assessing disruption of thyroid hormones is particularly important for a number of birth and neurodevelopmental outcomes. During pregnancy, the fetus relies mostly on the transplacental transfer of maternal thyroid hormones until 18 to 22 weeks of gestation with a critical role in fetal development (Patel et al., 2011). Even after the fetal thyroid has been established, maternal thyroid hormone production continues to contribute to the overall hormonal balance. Pregnancy related thyroid disorders have been associated to adverse birth outcomes such as spontaneous abortion and pregnancy complications (Teng et al., 2013) as well as neurodevelopmental impairments later in childhood (Chung et al., 2013).

4.4. Essential metal(loids)

Overall, most epidemiological studies examining adverse effects of prenatal exposure to metals focused on toxic metals specifically, Pb, Cd, As, and Hg. However, substantially less is known about adverse effects of essential metal(loid)s and even less about their endocrine disrupting potential. We report associations for some essential metal(loid)s and maternal hormone concentrations, most notably for Ni. In humans, the essentiality of Ni has been debated but the general consensus is that Ni is necessary at very low concentrations for enzyme synthesis and carbohydrate metabolism (Denkhaus and Salnikow, 2002). On the other hand, Ni is very resistant to corrosion at low and high temperatures, and has magnetic and electronic properties. These properties have led to its widespread use in industrial and commercial products and as a result, resulting in increased environmental pollution and human exposure (Das et al., 2018). It is known that Ni can trigger oxidative stress and inflammation leading to adverse effects in the immune system and increased risk of cancer but little is known about its endocrine disrupting properties (Kakela et al., 1999; Viemann et al., 2007). In rats, Ni can transfer across the placenta and can be found in the fetal blood and amniotic fluid (Hou et al., 2011). In fact, prenatal Ni exposure has been associated with adverse birth outcomes including small for gestational age and preterm birth (Ashrap et al., 2019; Chen et al., 2018; McDermott et al., 2015). We observed that Ni concentration is associated with significant changes in the levels of CRH, E3, testosterone, TSH and T3 and, as discussed above, study visit modified the association between Ni with CRH and testosterone levels. Experimental data suggests that exposure to Ni nanoparticles may increase FSH, luteinizing hormone (LH) and lower estradiol and testosterone levels (Kong et al., 2014) but more research is needed to determine the potential of Ni as an endocrine disruptor in humans.

We also observed small but consistent increases in maternal hormone levels in relation to Co exposure. Co is an essential trace metal which plays a key role in a number of biochemical processes, including nucleic acid and amino acid synthesis, erythrocyte formation, and specifically, as a cofactor in Vitamin B-12 production. Epidemiological studies of pregnant women have found associations between Co deficiency and anemia, pregnancy induced hypertension, and increased risk of preterm birth (Fort et al., 2015; Li et al., 2019; Liang et al., 2018). In our previous work, we reported a positive association between Co levels at Visit 3 and birthweight z-score (0.14, 96%CI: 0.03–0.25) (Ashrap et al., 2020a). Interestingly, Mikelson et al., (2019) reported an inverted U-shaped dose–response curve for the association between Co and birth weight (Mikelson et al., 2019) but this finding was not observed in relation to hormone levels in our data. Finally, we detected small increases in prenatal hormones in relation to blood Mn concentrations. In models examining urinary Mn, this increase was 10 times larger than the difference reported in models using the blood biomarker. Previously, we calculated the urine/blood ratio for Mn to be<1 possibly indicating generally higher concentrations in blood vs. urine (Ashrap et al., 2020b). This finding corresponds with toxicokinetics studies indicating that only small amounts of Mn are found in urine compared to blood (ATSDR 2012). However, differences between blood and urine biomarkers may be directly related to frequency, duration, magnitude and variability of exposure as well as Mn speciation which is an important factor affecting uptake and disposition. Mn is an essential micronutrient with a vital role in activation and function of many enzymes (e.g., pyruvate carboxylase, kinases, decarboxylases) (Tsai et al., 2015). However, Mn is also a well-documented neurotoxin and experimental data shows its potential for endocrine disruption by stimulating LH, FSH, testosterone and gonadotropin secretion from the hypothalamus (Lee et al., 2006; Lee et al., 2007; Levy and Nassetta 2003; Pine et al., 2005; Wasserman et al., 2006). Recently, Yao et al., (2019) showed associations between Mn exposure and higher levels of testosterone in female adolescents using NHANES 2011–2012 (Yao et al., 2019). Although less is known about the impact of Mn on sex-steroid hormones during human pregnancy, epidemiological studies have reported associations with birth weight, including a u-shape dose–response, and preterm birth (Ashrap et al., 2020a; Mikelson et al., 2019; Zota et al., 2009). Importantly, although there is an established normal range of Mn for adults (ATSDR 2012), it is unknown how this may vary during pregnancy or in a fetus. Future research should be directed to study the essential nutrients and their contributions to altered endocrine function and adverse birth outcomes.

From the DOHaD perspective, the impact of prenatal metal(loid) exposure in prenatal hormones levels can have enormous consequences beyond health at birth. For example, alterations in sex-steroid hormones during pregnancy have been associated to inadequate fetal growth which leads to low birthweight (Mucci et al., 2003). Size at birth is strongly associated with infant and children growth, and influences pubertal trajectories and risk of chronic diseases including obesity and breast cancer (Adair, 2001; Bukowski et al., 2012; Richards et al., 2002).

Similarly, alterations in CRH levels have been associated to preterm birth which leads to numerous adverse outcomes including neurodevelopmental impairment, asthma and metabolic disease among others (Markopoulou et al., 2019; Sonnenschein-van der Voort et al., 2014; Sucksdorff et al., 2015). The prenatal hormonal milieu is a critical component for fetal growth and development which contribute to children's later health and disease risk, and ultimately public health.

In summary, our study is the first to assess associations between prenatal sex-steroid hormones and metal(loid)s exposure. The PROTECT study's longitudinal design allowed us to examine different windows of exposure as well as two different biomarker matrices, blood and urine. We were also able to examine essential and underrepresented metal (loid)s as prenatal environmental exposures that may merit future research. Metals such as Mn and Co play important roles in human physiology but their contribution to adverse maternal and fetal health at low or high levels of exposure is less understood. Finally, the study of CRH in relation to environmental exposures is understudied, and given the major role of this hormone in pregnancy and parturition, further studies should evaluate it as a potential mechanism by which environmental chemicals may impact birth outcomes. In spite of this innovative design and extensive panel of metal(loid)s and maternal hormones, our study had some limitations. A few metal(loid)s including As and Cd were detected in a smaller proportion of blood samples (<50%) precluding examination of continuous measurements. However, since these two metal(loid)s are less variable in urine, we presented results using the urine biomarkers as part of the supplemental material. The serum biomarker of circulating hormone levels does not provide information on which of the sources of prenatal hormones (i.e., maternal, fetal, placental) is being impacted by metal(loid) exposure. Additionally, metal(loid)s concentrations in blood indicate circulating levels but these levels may not reflect uterine and fetal compartments especially for metal(loid)s that will cross the placental barrier. However, blood concentrations may reflect part of the activity at the maternal-fetal interface in addition to being much more feasible and less expensive to collect and process. Finally, although we measured exposure at two windows of rapid fetal growth, two time points may not be enough to characterize exposure across pregnancy.

5. Conclusion

Overall, our analysis suggests that metal(loid)s may act as endocrine disruptors by altering prenatal hormone concentrations during pregnancy. This disruption may depend on specific windows of exposure during pregnancy. Our results also suggest that some essential metal (loid)s may be contributors to adverse maternal and fetal outcomes through endocrine-related pathways. Future research steps should include investigation on how changes in markers of endocrine function mediate adverse birth and other health outcomes, toxicological and epidemiological research on essential metal(loid)s in relation to maternal and fetal health, and studying metal(loid)s as mixtures in relation to markers of endocrine function.

Funding

This study was supported by the Superfund Research Program of the National Institute of Environmental Health Sciences (NIEHS; R01ES032203), National Institutes of Health (NIH; grant number P42ES017198). Additional support was provided from NIEHS grant number P30ES017885 and the Environmental influences on Child Health Outcomes (ECHO) program grant number UH3OD023251. ECHO is a nationwide research program supported by the NIH, Office of the Director to enhance child health. First author was supported by NIEHS 3R01ES029275-02S1.

CRediT authorship contribution statement

Zorimar Rivera-Núñez: Methodology, Writing - original draft. **Pahriya Ashrap:** Formal analysis, Validation, Visualization. **Emily S. Barrett:** Writing - review & editing. **Deborah J. Watkins:** Writing - review & editing. **Amber L. Cathey:** Writing - review & editing. **Carmen M. Vélez-Vega:** Project administration. **Zaira Rosario:** Data curation, Project administration. **José F. Cordero:** Funding acquisition, Investigation. **Akram Alshawabkeh:** Funding acquisition, Investigation. **John D. Meeker:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing - review & editing.

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.

Acknowledgments

We are extremely grateful to all PROTECT study participants and their families. The authors also thank the nurses and research staff who participated in cohort recruitment and follow up, as well as the Federally Qualified Health Centers (FQHC) in Puerto Rico that facilitated participant recruitment, including Morovis Community Health Center, Prymed in Ciales, Camuy Health Services, Inc. and the Delta OBGyn Group in Manati, as well as the Manati Medical Center and the Metro Pavia Hospital in Arecibo.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106310.

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