**Prenatal exposure to heavy metal mixtures and anthropometric birth outcomes**

**BACKGROUND:** Numerous studies have suggested significant associations between prenatal exposure to heavy metals and newborn anthropometric measures. However, little is known about the effect of various metal mixtures at relatively low concentrations. Hence, this study aimed to investigate the associations between prenatal exposures to a wide scope of individual metals and metal mixtures to newborns' anthropometric measures.

**METHODS:** We recruited 975 mother-term infant pairs, from two major hospitals in Israel. Associations between eight metals (arsenic, cadmium, chromium, mercury, nickel, lead, selenium, and thallium) detected in maternal urine samples on the day of delivery to weight, length, and head circumference at birth were estimated using linear and Bayesian Kernel Machine Regression (BKMR) models.

**RESULTS:** Most metals examined in our study were observed in lower concentrations than in other studies, except for selenium. in the linear models, birthweight was negatively associated with levels of chromium and thallium. In BKMR analyses, a positive association was found between selenium and birthweight, and an inverse association was detected between selenium and birth length; positive associations were found between nickel and both weight and length. Cubic-shaped associations found between exposure to chromium and selenium and weight and length suggested interactions that were further analyzed. Associations between most metals and head circumference were U-shaped, suggesting interactions among them.

**CONCLUSION:** Maternal urinary concentrations of chromium and thallium were individually associated with decreased birth weight. Although other significant associations between metals and the anthropometric measures were not detected, our findings suggest various relations between metals and anthropometric measures, resulting from complex biochemical processes, that should be further investigated.

**KEYWORDS**: Anthropometric Measures; Prenatal Exposure; Pregnancy; Metals; BKMR;

**1. INTRODUCTION**

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least five times greater than water. Some of these heavy metals are essential nutrients in the body and a deficiency of one of them might result in diseases 1. Over-consumption and exposure to high levels, on the other hand, were previously associated with adverse health outcomes 2–4. Over-exposure of both mother and fetus during pregnancy5 was previously associated with preterm birth and reduced birth size 6–8.

While the mechanisms accounting for heavy metals’ over-exposure effects on newborns development are still subjects of ongoing studies9,10, some heavy metals, including cadmium (Cd), mercury (Hg), lead (Pb), and selenium (Se), were found to cross the placental barrier11, and accumulate in the fetal blood circulation. The associations between prenatal exposure to these metals and adverse birth outcomes were widely studied and raised possible associations with shorter birth length12, low birthweight 13, and small head circumference14. Prenatal exposures to other metals including arsenic (As), thallium (Tl), nickel (Ni), and chromium (Cr) were studied less extensively but were also found to be significantly associated with various adverse birth outcomes15–17. Prenatal assessment of heavy-metal exposures during pregnancy is challenging and is usually conducted using analysis of maternal blood and urine samples, assuming exposure traces found in these specimens are correlated with cord blood and newborn's circulation levels18,19.

In recent years, many epidemiologic studies examined the associations between metals measured in maternal urine and various newborns’ adverse health outcomes; including low birth weight20, birth size6, and various congenital abnormalities 21. While these findings alone are associated with morbidity in early childhood22 and adulthood23, they might have resulted from a complicated sequence of intrauterine events24 that could be associated with many other future complications, including behavioral changes in early childhood25, obesity during late childhood26 and various endocrine disruptions27. Hence, it is crucial to investigate the associations between various prenatal metals exposures and measurable and sensitive birth outcomes. Until now, most studies conducted in this field focused on populations exposed to relatively high levels of metals28,29, rather than levels similar to the background population averages, where no exceptional exposures occur.

In the current study, we examine the association between prenatal exposure to a mixture of metals as measured in maternal urine and newborn anthropometric measures. We investigated concentrations of eight metals (As, Cd, Cr, Hg, Ni, Pb, Se, Tl) in maternal urine samples, and examined their association to anthropometric measures, individually and by using a modeling approach that accounts for possible non-linear associations, as well as interactions between the metals30.

**2. METHODS**

**2.1. STUDY SAMPLE**

Beginning in 2016, pregnant women and their newborns were recruited to delivery rooms of two hospitals in Israel: (1) "Rambam" Medical Center – the biggest hospital in the Northern District of Israel, which accounts for ~5500 births annually, and (2) "Shamir" Medical Center – located in the Central region of Israel, and accounts for ~8000 deliveries annually. Women were considered eligible if they were Hebrew speaking, 18 years or older, and pregnant with a singleton. Exclusion criteria included: (1) preterm birth (<37 weeks of gestational age); (2) pregnancies considered by the medical staff with a high risk of complications (e.g. Autoimmune diseases, hypertension, diabetes)31; (3) congenital minor and major malformations as defined by the Center for Disease Control and Prevention (CDC) and by the European network of population-based registries for the epidemiological surveillance of congenital anomalies (EUROCAT)32,33. A specialized study coordinator in each hospital had obtained written informed consent from each woman before participation and filled out a questionnaire covering variables including sociodemographic characteristics, tobacco exposure, health status, pregnancy, and obstetric history. A total of 975 mother-newborn pairs were recruited from both hospitals: 509 from Rambam Medical Center and 466 from Shamir Medical Center. Maternal urine samples were collected from all participants on the day of delivery, and newborns' anthropometric measures were examined by specialized neontologists.

**2.2. URINARY METALS AND CREATININE**

Each participant was asked to collect a single urine sample. The samples were frozen at −80°C immediately after receiving, and then transported at −20°C for further analysis at the Central Public Health laboratory of the Israeli Ministry of Health (Abu-Kabir). we measured levels of As, Cd, Cr, Hg, Ni, Pb, Se, and Tl by inductively coupled plasma mass spectrometry (ICP-MS), using Agilent 7800x ICP-MS Instrument, equipped with Integrated Sample Introducing System (ISIS) and High Matrix Introducing mode (HMI). The procedure involved acid dilution of urine and direct injection to ICP-MS, followed by "helium dilution" in instrument HMI. The method followed standard quality assurance and quality control procedures. Urinary metal concentrations were quantified using internal standard calibration procedures and certified analytical standards. Quality control was performed by analyzing aliquots of control material in each series (every ten samples) and accuracy was validated by the annual successful participation in the international proficiency test (G-EQUAS) for all parameters. Urine creatinine was measured using a well-established colorimetric method at the Central Teratology lab at the Shamir Medical Center and was used to standardize the metal concentration detected in the urine samples.

**2.3. NEWBORNS HEALTH and ANTHROPOMETRIC MEASURES**

As part of a routine physical examination performed upon all infants after birth by trained neonatologists, birth weight, length, and head circumference were measured. The data was documented under an anonymous number each mother-child pair received. A total of 975 measures of weight and head circumference were conducted, as well as 887 length measures. For reliability, each measurement was repeated three times, and mean values were computed. All results were documented in the medical records.

**2.4. COVARIATES**

Using the comprehensive data collected from each mother via the questionnaires and data collected from maternal medical registries, we were able to adjust our final models to possible confounders including maternal age (continuous, in years), newborn's gender, previous parities (Null puros vs. multiparous) tobacco exposure during pregnancy (yes vs. no), sociodemographic status (SES) (standardized score) and geographic area. The maternal standardized SES index was calculated individually using matching of maternally reported home address zip codes and geographical distribution of SES as reported yearly by the Central Bureau of Statistics34 via geographical information systems (GIS).

Since gestational age could function as a mediator affecting the pathway between exposure and outcome 35, and therefore leading to over-or under-estimation of the true effects36 this variable was not included in the analysis.

Information on cigarette, cigar, or pipe smoking and the degree to which women were exposed to environmental tobacco smoke during pregnancy was self-reported by participants. Women were smoke-exposed if they reported either being an active smoker or were exposed to environmental tobacco smoke for 1 hour or more per week during at least one half of the pregnancy.

**2.5. STATISTICAL ANALYSIS**

Distributional plots and descriptive statistics were examined for all variables by the recruitment center (Rambam and Shamir). Mean values and standard deviations (SDs) were used to describe continuous variables, and independent t-tests were used to compare the differences between groups. Median values, interquartile range (IQRs), and Mann-Whitney U tests were used to describe and compare maternal urinary metal concentrations between the groups. We used frequencies and Chi-square tests to present and compare categorical variables between the groups. All metal concentrations were modeled as natural log-transformed and standardized for IQR, to achieve a common scale and account for the positive skewness detected. The mean values of repeated anthropometric measurements were calculated and then standardized to the mean and SD of the study population.

For further analysis, statistical significance was 2-sided and set at p < .05. All statistical processes were performed using R (version 4.1.1; R Foundation for Statistical Computing) including the R packages: *'data.table'*, '*ggplot2*', '*dplyr*', '*lubridate*' and '*BKMR*'.

**2.5.1. MULTIVARIABLE LINEAR REGRESSION**

First, we evaluated the associations of exposure to individual metals during pregnancy and standardized anthropometric measures using multivariate linear regression models adjusted for maternal age, previous parities, newborn's gender, tobacco exposure, SES, and geographic area. Newborn's standardized birth weight was also included as an independent variable in models that examined the association of the exposure with birth length and head circumference. First, models were adjusted for covariates without any consideration of interactions among the metals. Then, two-way and three-way interactions of metal concentrations were included in the models. Results were presented as mean differences in SD of anthropometric measures (with 95% confidence intervals) per IQR change in the log-transformed urine metal concentrations.

**2.5.2. BAYESIAN KERNEL MACHINE REGRESSION (BKMR)**

Alongside the single pollution models, possible effects of joint exposures were examined. To examine possible interactions between metals on and their associations to the standardized birth weight, length, and head circumference, Bayesian Kernel machine regression (BKMR) models were conducted. This novel non-parametric method enables a Bayesian variable selection framework for mixtures analysis conduction without any prior assumption of linearity of the associations30 and has been widely used in prenatal exposure studies37–39. Each model (*Equation 1*) accounted for an anthropometric outcome; Yi, an independent exposure-response function; h(), as well as covariates (Zi) and their corresponding coefficients (β).

*Equation 1*: $Y\_{i}=h\left(AS\_{i},Cd\_{i},Cr\_{i},Hg\_{i},Ni\_{i},Pb\_{i},Se\_{i},Tl\_{i}\right)+ βZ\_{i}+ϵ\_{i}$

In our study, BKMR models were fit using the Markov Chain Monte Carlo algorithm, with 25,000 iterations using the Gaussian Kernel40. All metals have entered the model as one group, and the posterior inclusion probabilities (group PIP) representing the contribution of each metal to the overall association were computed and reported.

For group PIP, a minimal threshold of .50 was previously suggested41 to determine whether a single exposure is important. Dose-response relations were assessed for each metal individually, by fixing other exposure agents at their median values. Further exposure-response relationships between the metals were explored as mean changes in the anthropometric measurements were calculated for IQR change in each metal's log concentration, while the other metals' concentrations were fixed at their 25th, 50th, and 75th percentiles. For further examination of the possible bivariate metals-response associations, we visualized the anthropometric measures as functions of two exposures while concentrations of one metal change and the second is fixed at its 10th, 50th, and 90th percentiles.

**2.5.3. SENSITIVITY ANALYSIS**

Several sensitivity analysis processes were conducted; (1) Linear regressions were repeated with all metals coded in quartiles – and comparisons were made between the 4th, 3rd, and 2nd quartiles to the 1st of each metal concentration. (2) Linear, as well as BKMR models, were conducted again including gestational age (in days, calculated as the difference between the date of delivery and last menstrual period date and excluding newborns who were small and large for gestational age (SGA and LGA respectively).

**3. RESULTS**

Among 975 mother-newborn pairs recruited for the study, the mean maternal age (SD) was 32.34 (4.58) years, and the mean (SD) gestational age at delivery was 39.47 (1.33) weeks; 509 newborns (52.2%) were male, and 466 (47.8%) were female. The mean birth weight (SD) was 3299.73 (442.0) gr, the mean length at birth was 49.59 (2.16) cm while head circumference was 34.61 (1.27) cm.

The overall metal concentrations corrected for creatinine (μg/g creatinine) detected in maternal urine samples are shown in Table 2. Correlations between metals were tested, and Spearman's coefficients appear inFigure 1.

**3.1. MULTIVARIATE LINEAR REGRESSION ANALYSIS**

Linear regressions’ results are presented in Figure 2. While adjusting for covariates a 1-IQR increase in log Cr concentration [μg/g creatinine] was associated with an average decrease of .118 SD (95% CI: -.183 to -.054; P = .003) in birth weight. A 1-IQR increase in log Tl concentration [μg/g creatinine] was also associated with an average decrease in birthweight of .077 SD (95% CI: -.146, to -.009; P = .039) in birthweight. No statistically significant two-way interactions were detected, yet few three-ways interactions were found to be significant: Cr-Tl-As, Cr-Tl-Hg, Cr-Tl-Pb (β = .078 SD; P = .018; β = .112 SD; P < .001; β = .092 SD; P = .012; respectively). Newborn's birth length was not associated with any of the metals, and no significant linear interactions were found among the metals. Head circumference at birth, was also not associated with any of the exposures, yet significant two- and three-ways interaction were detected among the metals: Ni-As, and Cd-Pb (-.048 SD; 95% CI: -.002, .010, P=.039; and .076 SD; 95% CI: .016, .136, P=.012, respectively), as well as Pb-Ni-Hg, Pb-Ni-Tl and Pb-As-Se (.046 SD; 95% CI: .003, .090, P=.035; .055 SD; 95% CI: .011, .100, P=.013; .086 SD; 95% CI: .014, .158, P=.018; respectively).

**3.2. BKMR ANALYSIS**

BKMR was implemented to obtain estimates of the joint exposure-response function of all metals examined in our study. We first examined the dose-response relationship of each creatinine corrected metal (IQR centered log concentrations) in the mixture with weight, length, and head circumference at birth when all other metals are fixed at their median. Models are adjusted for the above-mentioned covariates and presented in Figure 3**.** PIPs (Posterior Inclusion Probabilities) of the birthweight model are reported inTable 3., and were above 0.5 for Cr, Tl, and Pb (.880; .621; .519 respectively), the other metals had PIPs between .4 to .5. Similar to the findings obtained from the linear model of birth weight, an inverse dose-response association was found between Cr and Tl concentrations to birthweight. Positive linear associations were detected between Hg as well as Ni, Pb, and Se and birthweight As and Cd were negatively associated with birth weight.

To further investigate possible effect modifications by metals, based on the non-linear association detected, we estimated the associations of a 1-IQR increase in each metal while the other seven metals were fixed at their 25th, 50th, and 75th percentiles (Figure 4). A possible interaction was suggested if the estimates obtained for each metal varied while concentrations of other metals were changed. When examining the estimates of birth weight at birth, a slight reduction was detected for IQR increases in log Cr concentrations: .007 SD (95% CI: -.183, .034), .008 SD (95% CI: -.182, .028), 008 SD (95% CI: -.183, .033), while other metals were set at their 25th, 50th and 75th percentiles respectively. A 1-IQR increase in log Pb concentrations was also associated to changes in the estimates: .017 SD (95% CI: -.051, .085), .015 SD (95% CI: -.047, .078), .013 SD (95% CI: -.049, .074). An Increase in a 1-IQR of log Tl concentrations had a consistent estimate for all metals concentrations: -.034 (95% CI: -.129, .061). As shown in Figure S1, a visual examination of the two-metals interactions plot supported the results obtained from the linear models, as interactions seemed to occur between Cr-As and Cr-Pb.

PIPs calculated for the metals in the length model were all higher than .50 (Table 3) with .701 for Se, .688 for Cr, and .665 for Tl and Hg with .656. Length at birth appeared as a decreasing cubic function of Se and Cr, suggesting possible interactions between these metals and other metals. Except for Cr and Se concentrations, all other metals had non-linearly positive associations to length at birth yet with either low magnitude or wide confidence interval. The further analysis supported possible interactions of Cr with other metals as a 1-IQR increase in log Cr concentration [μg/g creatinine] was found associated with a nonsignificant decrease in birth length: -.014 SD (95% CI: -.075, .047), -.018 SD (95% CI: -.078, .043), and -.020 SD (95% CI: -.083, .043), while other metals were set at their 25th, 50th and 75th percentiles respectively. A 1-IQR increase in log concentration of Se [μg/g creatinine] was associated with estimates of: -.022 SD (95% CI: -.088, .044), -.026 SD (95% CI: -.095, .043) and -.027 SD (95% CI: -.098, .045). While increase in Tl had a consistent estimate for all metals concentrations (.024 SD, 95% CI: -.045, .092), Hg was associated to decreasing yet positive estimates: .021 SD (95% CI: -.041, .082), .019 SD (95% CI: -.039, .076) and .014 SD (95% CI: -.041, .070). A comparison of the slopes obtained from the two-metals interaction plots (Figure S2) suggested possible interactions between Cr-Ni, Cr-Hg, and Cr-Pb. In addition, it seems that when metals interacted with Cr and Se, calculated estimates were lower while both were set to their 90th percentile compared to their 10th.

Calculated PIPs for the head circumference model (Table 3) were lower than .5 for all metals. The highest and closest to .50 was Se with .493, and As was the second highest with a PIP of .177. Findings suggested a non-linear, U-shaped association between Se and head circumference. A non-linear positive association with a relatively prominent magnitude was also observed between As and head circumference. While head circumference was not significantly associated with none of the metal's concentrations in the linear models, it was found insignificantly and negatively associated with an IQR increase in Se levels: -.007 SD (95% CI: -.055, .024), -.006 SD (95% CI: -.051, .023), and -.005 SD (95% CI: -.050, .023), while other metals were set at their 25th, 50th and 75th percentiles respectively. A 1-IQR increase in As was found positively associated to head circumference, as estimates increased with metals concentrations: .004 SD (95% CI: -.030, .039), 005 SD (95% CI: -.030, .041), 006 SD (95% CI: -.034, .045) respectively. Using the bivariate metal-response charts (Figure S3) obtained from the BKMR analysis it was suggested that Se interacted with all other metals, especially when its concentrations were high (90th percentile), suggesting it is associated with a reduction in head circumference even in presence of other exposure. The two-way interaction suggested in the linear models, was also observed using BKMR.

**3.3. SENSITIVITY ANALYSIS**

Associations of the z-scored birthweight and metals concentrations divided into quantiles (Figure 5) were consistent with the results obtained from the linear models of the log-transformed metals – as birth weight among newborns exposed to the 4th quartile of log Cr concentration [μg/g creatinine] was significantly lower than of those exposed to the 1st quartile (SD = -.177; 95% CI: -.291 to -.063; P = .002). The estimates of birth weight seemed to decrease as log Tl concentration [μg/g creatinine] increased from 1st to 4th quartiles (SD = -.010; 95% CI -.125,.104; P = .859; SD = -.072; 95% CI -.187,.423; P = .216; SD = -.144; 95% CI -.260,.028; P = .014), yet were found significant only for the highest quartile. Associations between z-scored length at birth, head circumference, and metals were consistent with the linear models, as none of the metal concentrations were significantly associated with either of them. Inclusion of gestational age in the linear models, as well as the exclusion of SGA and LGA newborns (Figure S4), did not affect the significance levels of the estimates: a 1-IQR increase in log Cr concentration [μg/g creatinine] was associated with an average decrease of .127 SD (95% CI: -.185 to -.069; P = .029) in birth weight. A 1-IQR increase in log Tl concentration [μg/g creatinine] was also associated with an average decrease in birthweight of .078 SD (95% CI: -.148 to -.008; P = .028). Neither did significant changes observed in the models divided into percentiles (Figure S5).

Estimates of all anthropometric measures examined in this study were compared to those obtained from the BKMR models conducted with and without gestational age (Figure S6). PIPs are reported in Table S1 and decreased for all metals. As for estimates of weight, PIPs of Cr and Tl remained highest: .698 and .521 respectively. All other PIPs were lower than .10. Weight now seemed to behave as a non-linear function of Cr, yet no changes were detected regarding the function of Tl. The magnitude of the estimate of weight for a 1-IQR change in log Cr and Tl concentrations [μg/g creatinine] (Figure S7) decreased and became consistent as percentiles of other metals changed. Magnitudes of all metals decreased compared to the models without gestational age that included the whole study population.

PIPs for length models decreased for all metals and were highest for Cr (PIP = .519) – PIPs of Tl and Hg were the next highest with .197 and .109 respectively while all others were now below .10. Similar to the association of Cr and weight – was still negative but turned into a linear association. Compared to the previous model, estimates of length still presented a variation with an increase of a 1-IQR in log concentrations of Cr.

For head circumference, all PIPs calculated for each metal were lower than for other anthropometric measures, with the highest PIP for Se (.272) followed by Tl with PIP = .116, while for all other metals PIPs were below .10. In the dose-response charts association between head circumference and Se was still U-shaped yet with a lower magnitude. The non-linear associations between As and head circumference had now smaller magnitude – in fact, magnitude of all other metals seemed to decrease. Head circumference estimates calculated for a 1-IQR increase of log Se concentrations [μg/g creatinine] were no different from those obtained from the model including SGA and LGA newborns without gestational age.

**4. DISCUSSION**

As shown in Table S2 compared to other studies conducted in the field, medians of most metals/creatinine concentrations (μg/g) detected in our study (Table 2) were lower20,42–48, except for Se which showed higher levels compared to other studies (geometric mean = 38.68 μg/g; median = 38.35μg/g; IQR: 30.64-48.42 μg/g). Yet was similar to the amounts detected among pregnant women in Boston47, the US by Kim et al (2019); geometric mean = 35.4 μg/g (IQR: 18.0 – 57.4 μg/g). The relatively low concentrations of metals detected in urine samples of our study population enabled us to examine the possible associations between anthropometric measures at birth, and prenatal exposure to metals at levels like the background population averages.

Using linear regression models and BKMR analysis of eight metals, we found a significant decrease in newborn birth weight associated with increasing levels of Cr concentrations detected in maternal urine at delivery. The reduction in birth weight associated with an increase in Cr levels was supported both by linear and BKMR models, as well as the sensitivity analysis conducted. The association between Cr levels and length at birth in the linear models was insignificant, yet the exposure-response analysis conducted using the BKMR models suggested a possible interaction between Cr and other metals in the mixture. Interactions involving Cr and other metals were also detected in all models conducted for birthweight estimates. These interactions can support the inconsistency with other studies conducted in this field; Several studies have previously reported a possible decrease in newborn birth size and weight associated with increasing levels of Cr in maternal urine samples at birth49 and during pregnancy50. However, other studies did not support these findings 51,52 yet none of them accounted for possible associations between the outcomes and metals mixtures. Growing evidence suggests that Cr in maternal blood is associated with placental insufficiency 53 increasing the placental oxidative stress, and associated with possible lower birth weight, and pregnancy complications54. Besides the indirect mechanism, Saxena et.al55 suggest that Cr can cross the placenta, accumulate in the fetal tissues, and could directly induce DNA-damage56 and affect the intrauterine growth50.

Like Cr, increasing levels of Tl were significantly associated with lower birth weight as shown in the linear models. The BKMR models, as well as the sensitivity analysis, conducted supported these findings. These results are consistent with findings of several studies44,57,58 where Tl was found associated with decreased birth weight. It was previously suggested that Tl, like Cr, can increase the placental, as well as the fetal oxidative stress59, thus associated with intrauterine growth restrictions60. Prenatal exposure to Tl was found associated with a decrease in maternal and fetal thyroid activity61, which could be directly and indirectly related to developmental impairments27. However, while Tl levels were negatively and significantly associated with newborn's weight, they were not found associated with length at birth in none of the linear models conducted yet seemed associated with an increase in length in the BKMR models, but barely contributed to the model when gestational age included (Table S1). The inconsistency of its association to length at birth among models raises questions regarding its mechanism of action in relatively low concentrations. As far as we know, this result is not supported by other studies and although insignificant should be further investigated in larger samples studies. This insignificant trend could be explained by the relatively low levels of Tl detected in maternal urine samples, resulting in high variance and lack of consistency. Since our study included only term newborns, no other sensitive adverse health outcomes including early delivery58 that were previously found associated with Tl exposure could be examined and should be further investigated in future studies.

Se was insignificantly associated with increased birth weight and reduced birth length in the BKMR models conducted but seemed to contribute to the models of the latter only when gestational age was not included as an independent variable. The decrease in birth length associated with an increase in Se concentration in maternal urine was previously reported by Lozano et al. (2019)62. Although Se did not contribute to the birthweight models according to the calculated PIP, its insignificant association with an increase in birth weight is consistent with a study conducted previously by Solé-Navais et al (2020)63. In their study, increase prenatal Se levels detected in the blood of Norwegian pregnant women were found significantly and positively associated with birth weight. Monangi et al. (2021)64 suggested increasing levels of Se in maternal blood were associated with longer gestation, hence could contribute to the increase in birth weight. The mechanism behind the involvement of Se in gestational duration is not fully understood but could be explained by its activity in the suppression of mediators involved in the activation of labor in human fetal membranes and the myometrium65. Another study66 suggested Se was able to form chemical bonds, reduce the effect of teratogenic metals, and promote fetal growth. In our study, Se in high concentrations did seem (Figure S1) to minimize the reduction of birth weight associated with other metals, yet the mechanisms behind this possible interaction, and its association to anthropometric measures are beyond the scope of this study and should be further investigated.

Previous studies that examined Ni’s association with fetal growth have been inconclusive, however, several studies67,68 did report positive associations between Ni and fetal growth. The positive association between Ni concentration observed in the current study and fetal growth is consistent with results obtained from our BKMR models, and could be contributed to some of the nutritional benefits of Ni; having a biological function in metabolic pathways in which vitamin B12 is important69, Ni could potentially affect the stages in fetal growth when consumption of B12 is enhanced70. The cubic function describing the association between Ni, weight, and length was also observed by Howe et al (2022)67 and increases the validity of our findings.

The association of maternal urine Hg concentrations to anthropometric measures of newborns was large investigated43,66. While some studies did suggest an inverse association between prenatal exposure to Hg and anthropometric measures at birth71, most studies did not suggest any significant association43,66 and were conducted among women exposed to median Hg levels five to six-fold higher than those observed in our study (Table 2). In general, Hg levels detected in our study were lower than those detected among the US population 72, and significantly lower than the upper limit suggested73 for pregnant women by the world health organization (WHO) (5-7 μg/g creatinine). Increasing levels of Hg were found positively but insignificantly associated with weight and length, and negatively associated to head circumference. Only the latter is consistent with other study74, yet Hg levels detected among participants in our study were low and had a narrow range (IQR = .08-.38 μg/g creatinine) compared to other studies, thus the associations to anthropometric measures should be considered carefully, and should be further studied among populations with higher variance.

Since Pb and Cd levels exceeded the LOQ in less than 70% of participants in our study, and had a prominently lower range and mean compared to other studies75–77 it is difficult to relate the dose-response relations observed for these metals with changes in the anthropometric measures. In the sensitivity analysis conducted, concentrations of both metals were recoded into quartiles, a method that could account for the low concentrations and variances. Estimates calculated for each quartile compared to the 1st were not statistically significant and did not show any possible dose-response association. Yet it is possible that future studies analyzing data of participants with higher metals concentrations would have the statistical power needed to detect small differences.

Previous literature on the association between As exposure and anthropometric measures of the newborn is relatively limited and reports had mixed findings: while some failed to reject the null hypothesis78,79, others reported an inverse association between increasing concentrations of As and birthweight80, as well as birth size81. Although insignificant, the association between As concentration in maternal urine and newborn weight did seem inverse (Figure 3) and consistent with previous studies27,80. Interestingly, As concentrations were found positively associated to head circumference, similar to previous findings suggested previously by Shih et al. (2020)17. However, many other studies have reported inverse28,82,83 or null84,85 associations between As levels in maternal blood or urine and the head circumference of the newborn. Given the inconsistency with previous studies, and the lack of adequate biological mechanism, the positive association between As concentrations and head circumference might be spurious.

None of the metals was found significantly associated to head circumference in none of the models conducted. Using the BKMR models for head circumference, PIPs detected were lower than .50 for all metals, and most single exposure associations appeared as U-shaped functions, suggesting various interactions. In general, more interactions among metals existed in the models assessing head circumference compared to the weight and length models. These varied associations between metals and head circumference were previously shown by Rahman et al. (2021)14 who examined the associations between metals detected in maternal erythrocytes and newborn anthropometric measures. An infant's head circumference was previously found associated with many prenatal and environmental factors including; newborns gender, gestational age86, and maternal nutrition87; however it is dominantly determined by inheritance87 and pathways involving many genes and transcription factors88, therefore alternations in head circumference characterize many genetic disorders89,90, and were extensively studied. Since many metals included in this study were previously found to act as genetic modifiers 91–93; suppressing or enhancing fetal expression of genes, it is not unlikely to assume that interactions between these metals themselves65, or other proteins94, including transcription factors could lead to various alternations in newborns phenotypes. Recent studies suggest that metals could also interact with epigenetic processes that could be crucial to intrauterine development95, especially in the context of metal mixtures. Investigating the biochemical mechanisms which contribute to the genomic-metals interactions should be a key area for future research, that might require the collection of samples, such as placental tissue and cord blood.

The current study has several strengths; the large sample size; the examination of multiple metals; the use of classic as well as advanced mixture modeling analysis and the heterogeneous population recruited from two different geographical areas and hospitals. However, there were also several limitations. Since our study included only term newborns, any association between prenatal maternal exposure to metals and preterm deliveries could not be examined64,75,82. Levels of metals observed in our study were relatively low; enabling us to examine the possible effect of daily exposures on one hand but on the other limiting the scope of outcomes associated with high concentrations and wide variance. Although metals could be measured in urine and were corrected to maternal hydration condition, they had various half-lives with some concentrations reflecting exposure that occurred in the past few days (eg. As, Ni, Pb, Se, Tl), and others reflecting exposures over past weeks and months (eg. Cd, Cr, Hg)96–99. Thus, our findings cannot reflect the association of duration and prenatal timing of exposure to any of the anthropometric measures. It is worth mentioning that metals measured in urine did not reflect the existence of many possible potent forms in the human body: eg. methylHg100, selenomethionine101, and lead-protein complexes102.

**5. CONCLUSION**

Using a large sample size and multi-metal mixture data, we delineated a potential association between prenatal maternal exposure and newborn's weight, length, and head circumference. Our findings suggested inverse associations between Tl and Cr, and positive for Se to birthweight. An inverse association of Se and birth length was detected, and a positive association between Ni and both weight and length. Although some findings were not consistent with other studies, levels of metals observed in our study were relatively low with low variance, hence some associations detected might be spurious, and should be further investigated in future epidemiological as well as in-vitro and in-vivo biochemical studies.