**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 heavy metal mixtures at relatively low concentrations. Hence, this study aimed to investigate associations between prenatal exposures to a wide range of individual heavy metals and heavy metal mixtures with anthropometric measures of newborns.

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

**RESULTS:** Most heavy metals examined in our study were observed in lower concentrations than in other studies, except for selenium. In the linear models, birth weight was negatively associated with levels of chromium and thallium. In the BKMR analyses, a positive association was found between selenium and birth weight, 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 between exposure to chromium and selenium and weight and length suggested interactions that were further analyzed. Associations between most heavy 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 heavy metals and anthropometric measures were not detected, our findings suggest various relationships between heavy 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 that of water. Some of these heavy metals are essential nutrients in the body, and a deficiency in one of them can result in diseases 1. Over-consumption and exposure to high levels of heavy metals, on the other hand, have been associated with adverse health outcomes 2–4. Over-exposure of both mother and fetus to heavy metals during pregnancy5 has been associated with preterm birth and reduced birth size 6–8.

While the mechanisms underlying the effect of over-exposure to heavy metals on the development of newborns remain the subject of ongoing studies9,10, some heavy metals, including cadmium (Cd), mercury (Hg), lead (Pb), and selenium (Se), have been 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 have been widely studied and raised possible associations with shorter birth length12, low birth weight 13, and small head circumference14. Prenatal exposure to other metals, such as arsenic (As), thallium (Tl), nickel (Ni), and chromium (Cr), has been less extensively studied but was 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 by analyzing maternal blood and urine samples, assuming exposure traces found in these specimens are correlated with cord blood and a newborn’s circulation levels18,19.

In recent years, many epidemiologic studies have examined the associations between heavy metals measured in maternal urine and various adverse health outcomes among newborns, including low birth weight20, birth size6, and various congenital abnormalities 21. While these findings alone may be associated with morbidity in early childhood22 and adulthood23, they may 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 any associations between prenatal exposure to various heavy metals and measurable and sensitive birth outcomes. Until now, most studies conducted in this field have focused on populations exposed to relatively high levels of heavy metals28,29, rather than levels similar to the average background population averages, where no exceptional exposures occur.

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

**2. METHODS**

**2.1. STUDY SAMPLE**

Beginning in 2016, pregnant women and their newborns were recruited in delivery rooms of two hospitals in Israel: (1) Rambam Medical Center – the largest hospital in the Northern District of Israel, which accounts for around 5500 births annually, and (2) Shamir Medical Center – located in the Central region of Israel and which accounts for around 8000 deliveries annually. Women were considered eligible if they were Hebrew speaking, aged 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 to have a high risk of complications (e.g., autoimmune diseases, hypertension, diabetes)31; (3) minor or major congenital malformations as defined by the Center for Disease Control and Prevention (CDC) and the European network of population-based registries for the epidemiological surveillance of congenital anomalies (EUROCAT)32,33. A specialized study coordinator in each hospital obtained written informed consent from each woman prior to her participation and completed 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 the 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 taken by specialized neontologists.

**2.2. URINARY METALS AND CREATININE**

Each participant was asked to provide a single urine sample. The samples were frozen at −80°C immediately after receiving them 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 using inductively coupled plasma mass spectrometry (ICP-MS), on an Agilent 7800x ICP-MS instrument equipped with an Integrated Sample Introducing System (ISIS) and High Matrix Introducing mode (HMI). The procedure involved acid dilution of urine and direct injection to the ICP-MS instrument, followed by helium dilution in the HMI instrument. 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 Laboratory 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 by trained neonatologists performed upon all infants following birth, birth weight, length, and head circumference were measured. The data were documented under an anonymized number for each mother–child pair. A total of 975 measurements 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 newborns' 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 account for possible confounders including maternal age (continuous, in years), newborn’s gender, previous parities (nulliparous vs. multiparous), tobacco exposure during pregnancy (yes vs. no), socioeconomic status (SES) (standardized score), and geographic area. The maternal standardized SES index was individually calculated by matching maternally reported home address zip codes and the geographical distribution of SES as reported yearly by the Central Bureau of Statistics34, using a geographical information system (GIS).

As gestational age could function as a mediator affecting the pathway between exposure and outcome35, and therefore potentially lead 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 considered to be 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 their 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 differences between groups. Median values, interquartile ranges (IQRs), and Mann–Whitney U tests helped describe and compare maternal urinary metal concentrations between groups. We used frequencies and chi-square tests to present and compare categorical variables between 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 two-sided and set at p < 0.05. All statistical processes were performed using R (version 4.1.1; R Foundation for Statistical Computing) and the data.table, ggplot2, dplyr, lubridate, and BKMR packages.

**2.5.1. MULTIVARIATE LINEAR REGRESSION**

First, we evaluated the associations between 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. The standardized birth weight of newborns was also included as an independent variable in models that examined the association between exposure with birth length and head circumference. First, models were adjusted for covariates without any consideration of interactions among the metals. Then, two- and three-way interactions of metal concentrations were included in the models. The results are presented as mean differences in SD of anthropometric measures (with 95% confidence intervals, CI) 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 with the standardized birth weight, length, and head circumference, Bayesian kernel machine regression (BKMR) models were run. This novel non-parametric method enables a Bayesian variable selection framework to conduct analyses of mixtures 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*:

In our study, BKMR models were fit using the Markov chain Monte Carlo algorithm, with 25,000 iterations using the Gaussian kernel40. All metals were entered into 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 0.50 was previously suggested41 to determine whether a single exposure is important. Dose–response relationships were individually assessed for each metal, 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 changes in the log concentration of each metal, while the concentrations of the other metals were fixed at their 25th, 50th, and 75th percentiles. For further examination of the possible bivariate metal–response associations, we visualized the anthropometric measures as functions of two exposures while concentrations of one metal change and the second was fixed at their 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 with the 1st quartile of each metal concentration. (2) Linear, as well as BKMR models, were conducted again and included gestational age (in days, calculated as the difference between the date of delivery and the date of the last menstrual period); newborns who were small or large for gestational age (SGA and LGA, respectively) were excluded.

**3. RESULTS**

Among 975 mother–newborn pairs recruited for the study, the mean maternal age (SD) was 32.347 (4.580) years, and the mean (SD) gestational age at delivery was 39.472 (1.338) weeks; 509 newborns (52.2%) were male, and 466 (47.8%) were female. The mean birth weight (SD) was 3287.693 (441.475) g, the mean length at birth was 49.557 (2.203) cm, and the mean head circumference was 34.611 (1.272) 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 are shown inFigure 1.

**3.1. MULTIVARIATE LINEAR REGRESSION ANALYSIS**

The linear regression results are shown in Figure 2. When adjusting for covariates, a 1-IQR increase in log Cr concentration [μg/g creatinine] was associated with an average decrease of 0.118 SD (95% CI: -0.183 to -0.054; P = 0.003) in birth weight. A 1-IQR increase in log Tl concentration [μg/g creatinine] was also associated with an average decrease in birth weight, of 0.077 SD (95% CI: -0.146 to -0.009; P = 0.039). No statistically significant two-way interactions were detected, although some three-way interactions were found to be significant: Cr-Tl-As, Cr-Tl-Hg, and Cr-Tl-Pb (β = 0.078 SD, P = 0.018; β = 0.112 SD, P < 0.001; and β = 0.092 SD; P = 0.012, respectively). The birth length of newborns 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, although significant two- and three-way interactions were detected among the metals: Ni-As and Cd-Pb (-0.048 SD, 95% CI: -0.002, 0.010, P=0.039 and 0.076 SD, 95% CI: 0.016, 0.136, P=0.012, respectively), as well as Pb-Ni-Hg, Pb-Ni-Tl, and Pb-As-Se (0.046 SD, 95% CI: 0.003, 0.090, P=0.035; 0.055 SD, 95% CI: 0.011, 0.100, P=0.013; and 0.086 SD, 95% CI: 0.014, 0.158, P=0.018, 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 were fixed at their median. Models were adjusted for the above-mentioned covariates and are shown in Figure 3.The PIPs of the birth weight model are shown inTable 3 and were above 0.5 for Cr, Tl, and Pb (0.880, 0.621, and 0.519, respectively); the other metals had PIPs between 0.4 and 0.5. Similar to the findings obtained from the linear model of birth weight, an inverse dose–response association was found between Cr and Tl concentrations with birth weight. Positive linear associations were detected between Hg as well as Ni, Pb, and Se and birth weight, while As and Cd were negatively associated with birthweight.

To further investigate possible effect modifications by metals, based on the non-linear associations 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 the concentrations of other metals remained unchanged. When examining the estimates of birth weight, a slight reduction was detected for IQR increases in log Cr concentrations: 0.007 SD (95% CI: -0.183, 0.034), 0.008 SD (95% CI: -0.182, 0.028), and 0.008 SD (95% CI: -0.183, 0.033), while other metals were set at their 25th, 50th, and 75th percentiles, respectively. A 1-IQR increase in log Pb concentrations was also associated with changes in the estimates: 0.017 SD (95% CI: -0.051, 0.085), 0.015 SD (95% CI: -0.047, 0.078), and 0.013 SD (95% CI: -0.049, 0.074). An increase of 1-IQR in log Tl concentrations gave a consistent estimate for all metal concentrations: -.034 (95% CI: -0.129, 0.061). As shown in Figure S1, a visual examination of the two-metal interaction plots 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 0.50 (Table 3): 0.701 for Se, 0.688 for Cr, 0.665 for Tl, and 0.656 for Hg. Length at birth appeared to be 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-linear, positive associations with length at birth, albeit with either a 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 to be associated with a non-significant decrease in birth length: -0.014 SD (95% CI: -0.075, 0.047), -0.018 SD (95% CI: -0.078, 0.043), and -0.020 SD (95% CI: -0.083, 0.043), 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: -0.022 SD (95% CI: -0.088, 0.044), -0.026 SD (95% CI: -0.095, 0.043), and -0.027 SD (95% CI: -0.098, 0.045) respectively. While an increase in Tl gave a consistent estimate for all metal concentrations (0.024 SD, 95% CI: -0.045, 0.092), Hg was associated with decreasing yet positive estimates: 0.021 SD (95% CI: -0.041, 0.082), 0.019 SD (95% CI: -0.039, 0.076), and 0.014 SD (95% CI: -0.041, 0.070). A comparison of the slopes obtained from the two-metal 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, the calculated estimates were lower when both were set to their 90th percentile compared with their 10th.

Calculated PIPs for the head circumference model (Table 3) were lower than 0.5 for all metals. The highest and closest to 0.50 was Se, at 0.493, with As the second highest with a PIP of 0.177. The findings suggested a non-linear, U-shaped association between Se and head circumference. A non-linear, positive association of relatively large magnitude was also observed between As and head circumference. While head circumference was not significantly associated with any of this metal’s concentrations in the linear models, it was found to be non-significantly and negatively associated with an increase in IQR in Se levels: -0.007 SD (95% CI: -0.055, 0.024), -0.006 SD (95% CI: -0.051, 0.023), and -0.005 SD (95% CI: -0.050, 0.023), while other metals were set at their 25th, 50th, and 75th percentiles, respectively. A 1-IQR increase in As was found to be positively associated with head circumference, as estimates increased with the metal's concentrations: 0.004 SD (95% CI: -0.030, 0.039), 0.005 SD (95% CI: -0.030, 0.041), and 0.006 SD (95% CI: -0.034, 0.045), respectively. Based on the bivariate metal–response charts (Figure S3) obtained from the BKMR analysis, it appeared that Se interacted with all other metals, especially when its concentrations were high (90th percentile), suggesting Se is associated with a reduction in head circumference even in the presence of other exposures. The two-way interaction suggested in the linear models was also observed when using BKMR.

**3.3. SENSITIVITY ANALYSIS**

Associations of the z-scored birth weight and metal 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 those exposed to the 1st quartile (SD = -0.177; 95% CI: -0.291 to -0.063; P = 0.002). The estimates of birth weight appeared to decrease as log Tl concentration [μg/g creatinine] increased from the 1st to the 4th quartiles (SD = -0.010; 95% CI -0.125, 0.104; P = 0.859; SD = -0.072; 95% CI -0.187, 0.423; P = 0.216; and SD = -0.144; 95% CI -0.260, 0.028; P = 0.014), yet were found to be significant for the highest quartile only. 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 these measures. 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 0.127 SD (95% CI: -0.185 to -0.069; P = 0.029) in birth weight. A 1-IQR increase in log Tl concentration [μg/g creatinine] was also associated with an average decrease in birth weight of 0.078 SD (95% CI: -0.148 to -0.008; P = 0.028). Neither did significant changes observed in the models divided into percentiles (Figure S5).

Estimates of all anthropometric measures examined in this study were compared with those obtained from the BKMR models conducted with and without gestational age (Figure S6). PIPs are shown in Table S1 and decreased for all metals. As for estimates of weight, the PIPs of Cr and Tl remained highest, at 0.698 and 0.521, respectively. All other PIPs were less than 0.10. Birth weight now seemed to behave as a non-linear function of Cr, although no changes were detected regarding the function of Tl. The magnitude of the estimate of birth weight for a 1-IQR change in log Cr and Tl concentrations [μg/g creatinine] (Figure S7) decreased and became consistent as the percentiles of other metals changed. The magnitudes of all metals decreased compared with the models without gestational age that included the entire study population.

PIPs for birth length models decreased for all metals and were highest for Cr (PIP = 0.519); the PIPs of Tl and Hg were the next highest, at 0.197 and 0.109, respectively, while all of the others were now less than 0.10. Similar to the association between Cr and birth weight, the association was still negative but became a linear association. Compared with the previous model, birth length estimates still showed 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 the other anthropometric measures, with the highest PIP for Se (0.272), followed by Tl with a PIP of 0.116, while for all other metals the PIPs were less than 0.10. In the dose–response charts the association between head circumference and Se was still U-shaped but with a lower magnitude. The non-linear associations between As and head circumference showed a smaller magnitude – in fact, the magnitude of all the other metals appeared to decrease. Head circumference estimates calculated for a 1-IQR increase in log Se concentrations [μg/g creatinine] were no different from those obtained from the model that included SGA and LGA newborns without gestational age.

**4. DISCUSSION**

As shown in Table S2, compared with other studies conducted in this field, the medians of most of the metal/creatinine concentrations (μg/g) detected in our study (Table 2) were lower20,42–48, except for Se, which showed higher levels compared with other studies (geometric mean = 38.68 μg/g; median = 38.35 μg/g; IQR: 30.64–48.42 μg/g). However, this was similar to the amounts detected among pregnant women in the US47 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 from our study population enabled us to examine possible associations between anthropometric measures at birth and prenatal exposure to metals at levels similar to the background 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 by both the 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 not significant, although 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 birth weight estimates. These interactions may explain the inconsistencies compared with other studies conducted in this field. Several studies have 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 findings51,52, although none of them accounted for possible associations between the outcomes and mixtures of metals. There is increasing evidence to suggest that Cr in maternal blood is associated with placental insufficiency53, increasing placental oxidative stress, and possible lower birth weight and pregnancy complications54. Besides this 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 intrauterine growth50.

Similar to 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, supported these findings. These results are consistent with the findings of several studies44,57,58 where Tl was found to be associated with decreased birth weight. It was previously suggested that Tl, as with Cr, can increase the placental as well as the fetal oxidative stress59 and is thus associated with intrauterine growth restriction60. Prenatal exposure to Tl has been found to be associated with a decrease in maternal and fetal thyroid activity61, which could be directly and indirectly related to developmental impairments27. However, while we found Tl levels were negatively and significantly associated with a newborn’s weight, they were not found to be associated with length at birth in any of the linear models conducted. They did appear to be associated with an increase in length in the BKMR models, but barely contributed to the model when gestational age was included (Table S1). The inconsistency of the association between Tl and length at birth among the 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 non-significant should be further investigated in studies with larger sample sizes. This non-significant trend could be explained by the relatively low levels of Tl detected in maternal urine samples, resulting in high variance and a lack of consistency. As our study included only term newborns, no other sensitive adverse health outcomes including early delivery58 that were previously found to be associated with Tl exposure could be examined and should be further investigated in future studies.

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

Previous studies that examined the association of Ni with fetal growth have been inconclusive; however, several studies67,68 have reported 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 attributed to some of the nutritional benefits of Ni. As it has 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 thus increases the validity of our findings.

The association of maternal urine Hg concentrations with anthropometric measures of newborns has been 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 population72 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 to be positively but non-significantly associated with weight and length and negatively associated with head circumference. Only the last of these is consistent with the findings of another study74, although the Hg levels detected among participants in our study were low and had a narrow range (IQR = 0.08 to 0.38 μg/g creatinine) compared with other studies. Therefore, the associations with anthropometric measures should be considered carefully and studied further among populations with greater variances.

As Pb and Cd levels exceeded the limit of detection (LOD) in less than 70% of participants in our study, and had a prominently lower range and mean compared with other studies75–77, it is difficult to relate the dose–response relationships 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 with the 1st were not statistically significant and did not show any possible dose–response associations. However, it is possible that future studies analyzing data from participants with higher concentrations of metals 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 have had mixed findings: while some failed to reject the null hypothesis78,79, others reported an inverse association between increasing concentrations of As and birth weight80, as well as birth size81. In our study, although non-significant, the association between As concentration in maternal urine and newborn weight did seem to be inverse (Figure 3) and consistent with previous studies27,80. Interestingly, As concentrations were found to be positively associated with head circumference, similar to previous findings reported 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 an adequate explanatory biological mechanism, the positive association between As concentrations and head circumference might be spurious.

None of the metals was found to be significantly associated with head circumference in any of the models run. Using the BKMR models for head circumference, the PIPs detected were less than 0.50 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 with the weight and length models. These varied associations between metals and head circumference were previously shown by Rahman et al. (2021)14, who examined associations between metals detected in maternal erythrocytes and newborn anthropometric measures. An infant’s head circumference was previously found to be associated with many prenatal and environmental factors including the newborn’s gender and gestational age86 and maternal nutrition87. However, it is predominantly determined by inheritance87 and pathways involving many genes and transcription factors88, therefore alternations in head circumference characterize many genetic disorders89,90 and have been extensively studied. As many of the 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 unreasonable to assume that interactions between these metals themselves65, or with proteins94, including transcription factors, could lead to various alterations in newborns’ phenotypes. Recent studies have suggested that metals could also interact with epigenetic processes that may be crucial to intrauterine development95, especially in the context of metal mixtures. Investigating the biochemical mechanisms that contribute to genomic–metal interactions should be a key area for future research and might require the collection of samples such as placental tissue and cord blood.

The current study had 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. As our study included only term newborns, any association between prenatal maternal exposure to metals and preterm deliveries could not be examined64,75,82. The levels of metals observed in our study were relatively low; this enabled us to examine the possible effect of daily exposures on one hand but on the other hand, it limited the scope of outcomes associated with high concentrations and wide variances. Although metals could be measured in urine and were corrected to maternal hydration condition, they had a variety of half-lives, with some concentrations reflecting exposure that had occurred in the past few days (e.g., As, Ni, Pb, Se, and Tl), and others reflecting exposures over past weeks and months (e.g., Cd, Cr, and Hg)96–99. Thus, our findings cannot reflect any association of duration and prenatal timing of exposure with 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, e.g., methyl-Hg100, 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 to heavy metals and newborns’ weight, length, and head circumference. Our findings suggested inverse associations between Tl and Cr with birth weight and a positive association between Se with birth weight. An inverse association between Se and birth length was detected, while a positive association between Ni and both birth weight and length was detected. Although some findings were not consistent with those of other studies, the levels of heavy metals observed in our study were relatively low, with low variances, hence some associations detected might be spurious and should be further investigated in future epidemiologic studies as well as in vitro and in vivo biochemical studies.