Placental Concentrations of Essential, Toxic, and Understudied Metals and Relationships with Birth Outcomes in Chattanooga, TN

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Abstract

Background: Comprehensive examinations of placental metal concentrations and correlations with infant parameters are under-investigated. Chattanooga, Tennessee's consistently high incidence of low birth weight and potential for metal exposure provides an ideal opportunity to investigate potential correlations.

Objectives: To investigate the associations between a wide variety of metals in placental tissue and multiple infant parameters.

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Methods: A total of 31 elements were screened via ICP-MS in 374 individual placental samples. Of those, 14 were quantifiable in >86% of the samples. We examined correlations between metal concentrations and infant parameters (birth weight, gestational age, birth weight centile, placental weight, birth length and head circumference). We fit multivariable regression models to estimate the covariate-adjusted associations of birth weight with ln-transformed concentrations of each of the 14 metals and used generalized additive models to examine nonlinear relationships.

Results: Some of the strongest relationships with infant parameters came from several lesser-studied metals. Placental rhodium concentrations were negatively correlated with almost all infant parameters. In the fully adjusted regression model, birth weight was significantly associated with several metals. On an IQR (25th to the 75th percentile) basis, estimated changes in birthweight were: for cobalt (82.5 g, IQR=6.05 μg/kg, p = 0.006), iron (−51.5 g, IQR=171800 μg/kg, p = 0.030), manganese (−27.2 g, IQR=152.1 μg/kg, p = 0.017), lead (−72.7 g, IQR=16.55 μg/kg, p = 0.004) and rhodium (−1365.5 g, IQR=0.33 μg/kg, p < 0.001). Finally, a generalized additive model showed significant nonlinear relationships between birth weight and concentrations of Co and Rh.

Conclusions: Our comprehensive examination of placental metals illustrate many strong associations between lesser-studied metals and infant parameters. These data, in combination with our correlations of well-studied metals, illustrate a need to consider *in utero* exposure to a broad array of metals when considering fetal development.

Keywords

birth weight; placenta; metals; rhodium; lead; manganese

Introduction

Singleton, full-term infants are classified as low birth weight (LBW) if weighing less than 2500 g and very low birth weight (VLBW) if weighing less than 1500 g. Birth weight is well known to correlate with infant mortality and myriad adult health indicators. Infants born on the lower end of the normal birth weight range also have an increased risk of adult chronic diseases, such as heart disease and type 2 diabetes (Barker et al. 1989; Yajnik et al. 1995). Hamilton County, TN, has a persistently high LBW rate. According to the Hamilton County Health Department (Fagerstedt et al. 2015), 27 of the 28 zip code areas in Hamilton County have an incidence of LBW infants that is consistently 50% higher than the national average. Race alone does not explain these observations. For example, white Hamilton County (HC) infants are consistently born LBW at a greater incidence than white United States (US) infants; similarly, black HC infants are consistently born LBW at a greater incidence than white or black US infants (Hamilton County Health Department 2015). While many factors are known to contribute to LBW (e.g., diet, smoking, access to health care, poverty) (Krans and Davis 2014; Lagiou et al. 2004; Magee et al. 2004; Miller and Korenman 1994), environmental pollution is another potential causative factor. Indeed, the potential for metal and metalloid intrauterine exposure must be understood for proper mitigation of LBW/VLBW.

Prior to environmental regulations, metal foundry sand was commonly used as fill dirt for Chattanooga area home construction which ultimately resulted in a large portion of

Chattanooga residential soils being named a Superfund Site (EPA 2018). Foundry sand is often contaminated with multiple metals. For example, the EPA conducted a risk assessment of foundry sand from multiple types of metal smelting (Aluminum, Iron, Steel (EPA 2014)). That risk assessment focused on an array of metals and metalloids: Antimony (Sb), Arsenic (As), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn) and Nickel (Ni), as these were the most commonly associated with all types of metal smelting. Because Hamilton County has a high incidence of LBW and because the majority resides in Chattanooga among widespread legacy foundry sand, exposure to co-occurring metals needs to be considered as potential contributors to LBW in Hamilton County, TN.

The placenta is a temporary organ that provides for the exchange of nutrients and wastes between fetus and mother. Along with this vital exchange, there is the potential for the passage of deleterious compounds from mother to fetus for intrauterine exposure. As such, placentae can serve as a non-invasive, long-term (gestational length) sample that can be studied for transplacental toxicant transfer (Iyengar and Rapp 2001). Several studies have addressed these questions of placental transfer by evaluating the ratio of metal concentration between fetal and maternal blood supply. The higher the ratio, the greater the passage of the metal through the placenta. Sakamoto (2010) found Cd had the lowest correlation coefficient of maternal and fetal blood supplies. Other metals measured had increased passage through the placenta (Se < Pb < As), with the greatest correlation between maternal and fetal blood samples being mercury. Similarly, Rudge et al. (2009) saw strong correlations between lead, cobalt, arsenic and selenium between maternal and fetal blood samples, while cadmium, manganese, copper and zinc have a decreased pathway through the placenta, suggesting tighter regulation.

Correlations of placental metal concentrations with birth weight are well documented. Osada (2002) quantified essential elements that are required for proper growth (magnesium (Mg), manganese, iron (Fe), copper (Cu), zinc (Zn), and selenium (Se)) and elements that are non-essential (rubidium (Rb), strontium (Sr), cadmium (Cd), cesium (Cs)) in placenta tissue of 30 appropriate for gestational age (AGA) infants and 21 intrauterine growth restricted (IUGR) infants in Japan. Se and Mg were significantly greater in the AGA infants compared to the IUGR infants. Zadrozna (2009) reported significantly greater placental concentrations of Zn and Cu in normal pregnancies compared to pregnancies complicated by IUGR. Both of these metals also had a significant, positive correlation with birth weight. In the same study however, Se was greater in placenta from IUGR cases compared to a control group. Llanos and Ronco (2009) reported lower birth weight as Cd, Pb, and As concentrations increased in the placenta. Ronco et al. (2009) also found that Cd exposure directly caused reduced birth weight in rats and depressed the transfer of Zn (a beneficial metal) across the placenta. Greater Pb concentrations were reported in placentae from associated with IUGR infants (Llanos and Ronco 2009). In addition, lead (Pb) has been found to readily pass through the placenta and previous work has shown that cord blood Pb concentrations were negatively correlated with birth weight, length and head circumference (Osman et al. 2000).

Several metals, including rhodium (Rh), barium (Ba), and aluminum (Al) are under reported in placenta tissue. Rh has been detected globally in urban environments, surface water, river

sediment, freshwater isopods, well-water and many other environmental matrices (Gómez et al. 2001; Ravindra et al. 2004). To date, there are no published data on Rh in placental tissue. Sharma and Mishra (2006) studied the effects of Al on pregnant rats and showed that oral exposure to Al resulted in uptake to fetal and maternal brain tissues, as well as placental tissues. Subsequently, birth weight, placental weight and fetal growth were all significantly reduced compared to the control group. Al and Ba were detected in amniotic fluid (16–19 weeks gestation), at an average of 93.4 and 22.4 ug/L, respectively (Hall et al. 1983). Ba has also been detected in stillborn babies, which indicates passage through the placental barrier (IPCS 1990), as well as reduced birth weight in rats given an oral dose of Ba (ATSDR 2007).

To that end, the present study quantified and compared concentrations of 14 elements against birth weight (male and female births separately) as well as gestational age, birth weight centile (BWC), placental weight, birth length, and head circumference. In the absence of complete information about the effects of these 14 metals on birth outcomes, we consider this a hypothesis generating study.

Methods:

Placental collection was conducted at Erlanger Hospital (Baroness campus), Chattanooga, TN from singleton births of HIV and hepatitis negative mothers over 18 years of age. Infants born at or greater than 34 weeks, with no major morphological or chromosomal abnormalities, were included in the sample population. Over the study period, 690 women were asked to participate in the study. From those requests, 665 placentae were successfully collected across a large spectrum of pregnancies. When processing samples for elemental analysis, samples from normal weight births were a random subset of a larger population (n = 292). Because low birth weight samples were the minority of the births, all LBW placentae above the gestational date cutoff were processed (n= 82). Maternal and infant data were collected from questionnaire interviews and medical records. All specimen and data collection methods were approved by the UT College of Medicine Institutional Review Board (#05–031, FWA# 2301). As samples became available, consent was sought out and obtained within the day, or in the case of a night delivery, first thing the following morning. In the rare case the consent was not obtained or refused to be given, the samples were discarded. Post-delivery, placentae were refrigerated in a plastic container. Placentae were collected after no more than 12 hrs after delivery. After umbilical cord and amniotic sac were removed with ceramic scissors, the placenta was weighed, cut in half and placed in a sterile plastic bag and frozen at −20□ C until processing (as illustrated in Figure 1). The placental half was thawed and both fetal and maternal membranes (decidua basalis and chorionic plate, respectively) were removed, which allowed for sampling of the syncytiotrophoblast layers to be maximized, the area of fetal/maternal exchange (Sun et al. 1997). Because the spatial distribution of metals through the placenta is not uniform (Lagerkvist et al. 1996) the entire sample was homogenized and subsampled for metal analysis (Miller et al. 1988). Following tissue digestion, 14 elements were consistently quantified: aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), rhodium (Rh), selenium (Se), titanium (Ti), and zinc (Zn). Placental metal concentrations were compared between normal

and low birth weight infants as well as between BWC groupings (small, average and large for gestation).

Birth Weight Centile Calculation

An assessment based on infant weight alone ignores other important variables that influence birth weight, including gestational age at delivery, maternal height and weight, parity, ethnicity and infant sex (Gardosi et al. 1992). With the incorporation of these factors, infants can be grouped according to an individualized birth weight centile that can provide a more nuanced assessment of whether or not fetal growth met the expectations of the pregnancy. The BWC is grouped as small for gestational age (< 10th centile), average (11th-89th centile), or large for gestational age (>90th centile, SGA, AGA, LGA). GROW software was obtained for the United States coefficients (Gardosi and Francis 2009). Data for the gestational age adjustment (parity, maternal height/weight, ethnicity, infant sex, and weight) were obtained from the associated medical charts. Gestational age was based on best obstetric estimate that incorporated the use of first trimester ultrasound that was within +/− 3 days of last missed period (LMP) or a second trimester (< 20 weeks gestation) that was +/− 7 days of LMP. Although calculating a customizable birth weight centile has some limitations and adds complexity (Hutcheon 2014), we feel that the multi-ethnic population of Chattanooga merits these added measures of fetal growth and ultimately birth weight (Gardosi et al. 2018; Melamed et al. 2014).

Elemental Analysis

Placental concentration of each element was quantified at the Laboratorio Chimico Merceologico (Naples, Italy) by means of quadrupole inductively coupled plasma-mass spectrometry, ICP-MS (model 820-MS, Bruker Daltonics, Billerica, MA), equipped with a collision reaction interface (CRI) for suppression of polyatomic, isobaric interferences. The CRI utilized high purity He (99.9999%, SALDOGAS Srl, Italy) during analysis of Cr and high purity H_2 (99.9999%, using DBS H_2 generator PGH2–300) for all other elements. The plasma was generated using an RF power of 1400 W and the nebulizer gas flow rate was 0.98 L/min. All reagents were Optima grade (Fisher Scientific) and 18.2 MΩ·cm water was produced from a Direct Q3 Milli-Q system (Millipore, Bedford, MA). All 374 placentae samples were analyzed for Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, Tl, U, V, Zn in quantitative mode using external calibration. Multi-elemental standard stock solutions of 20 mg/L (Ultra Scientific, North Kingstown, USA) were used to prepare 10 calibration solutions ranging from 0.1 – 1000 μg/L for all elements except Hg (3-point calibration; range 0.1 – 10 μg/L). An internal standard (10 μg/L ^{89}Y) was used for signal normalization and added via peristaltic pump to each standard and sample during analysis. Calibration curves used for quantitation were constructed by plotting internal standard-normalized signal intensities versus standard concentration and all had R^2 values   0.999. After every 10 samples, a reagent blank and at least 2 standard samples were analyzed to verify instrument calibration. Analyses were stopped and the instrument re-calibrated if the blanks or standard samples differed by ±15% of the correct value. Additionally, Re, Ta, Rh, Ru, Ti, Ge, Pd, In, I, W, Os, Pt were measured in semi-quantitative mode using an average response factor instead of a calibration curve. Combined, a total of 31 elements were analyzed in placenta tissue.

Prior to and after each analytical session, bovine liver standard reference material (NIST SRM 1577c) was analyzed in order to assess the accuracy of analysis. The results were deemed to be reliable if the measured value was within the quoted uncertainty of the certified value for the SRM. Throughout the analytical campaign, a total of 23 SRM samples were digested, processed, and analyzed exactly as the samples. Concentrations determined with this method were in good agreement for all elements certified by NIST (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Se, V, Zn). Samples were prepared as follows. Before use, all glassware and plastic containers were cleaned by washing with 5% nitric acid for at least 24 h, and then copiously rinsed with ultra-pure water. The chorionic plate and decidua basalis were removed with acid-washed ceramic scissors, leaving the villous core tissue to homogenize. Samples were oven-dried at 60°C to constant weight and then powderized. As a result, all concentration data are reported on a dry-weight basis. Aliquots of dried placenta with nominal mass 0.25 g, determined to nearest 0.1 mg, were placed in acid-rinsed, 50 mL polypropylene digestion vessels. Hot plate digestion was carried out with a total of 5 mL of concentrated nitric acid and 3 mL of 30% hydrogen peroxide. Following digestion to a clear, yellow solution, samples were dried down and reconstituted to 10 mL with 2% nitric acid. Finally, samples were 0.2 um filtered, using a Teflon filter, prior to ICP-MS analysis.

Statistical Analysis

Statistical analyses were performed using R version 3.3.2. One infant had a birth weight of only 1275 g despite a gestational age of 275 days (39.3 weeks), which was very unlike all other subjects. This subject, the only infant of VLBW, was excluded from all statistical summaries and models, and consequently our model results only apply to normal or LBW infants. Correlations between birth weight, BWC, gestational age, head circumference, birth length and placental weight, and the log-transformed detectable metals were analyzed using Pearson's correlation. Negative correlations indicate that the magnitude of a given outcome (e.g., birth weight) decreases as the element concentration increases, whereas positive correlations indicate that the outcome tends to increase as element concentration increases.

The distribution of the raw metal concentrations is summarized in Table 1, and median metal values within birth weight and birth weight centiles is summarized in Table 6. In all other analyses, 1 was added to the metal concentrations and then converted to the natural logarithm (Wicklin 2011). These values are referred to as the ln-transformed concentrations, for simplicity. The addition of 1 prior to the log-transform was necessary to reduce the influence of extremely small metal concentrations. To examine the relationship between birth weight and metal concentrations, separate multiple regression models were used for each metal, controlling for the following covariates: maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no). Model assumptions were checked using standard methods (Weisberg 2005). Linearity was assessed by partial regression plots and by plots of studentized residuals against the predicted values, and in some cases nonlinearity was noted. There was no evidence of heteroscedastisticy, as assessed by plots of studentized residuals versus unstandardized predicted values. There was no evidence of multicollinearity, as assessed by small variance inflation factors (all were < 1.1). Q-Q plots suggested that the assumption of normality was reasonable. Unusual data points were

evaluated for via Cook's distance, and studentized residuals. After exclusion of the VLBW subject (noted above), there were no very unusual values (all studentized residuals were < 3.5 in absolute value, and all Cook's distances were < 0.1). Due to the violation of linearity in some cases, generalized additive models (Hastie and Tibshirani 1990) were fit to evaluate possible nonlinearity in the relationships between birth weight and the ln-transformed metal concentrations, while controlling for the same covariates of the aforementioned multivariable regressions.

Results

Concentrations of metals/metalloids

A total of 31 elements were screened in 374 individual placental samples. Of those, 17 elements (Be, V, Ge, Pd, In, Sb, W, Os, Pt, Hg, Ru, Cr, U, Tl, Re, Ta, and I) were considered non-quantifiable (e.g., less than 40% of the samples contained quantifiable concentrations). Of the 14 quantifiable elements, 7 (Ti, Rh, Ba, Fe, Cu, Zn, and Se) were quantifiable in 100% of the samples while the other 9 were quantifiable in >86% of the samples (Al, As, Cd, Co, Mn, Ni, Pb,) (Table 1). For all placental samples containing one of the 14 metals at non-quantifiable concentrations, half of the detection limit was inserted as the concentration value (EPA 2000). This was done to provide a conservative estimate for those elements present at concentrations near the detection limit (EPA 2000). Descriptive statistics of the quantifiable metals present in placental samples are summarized in Table 1. Maternal descriptive statistics are summarized in Table 2; infant descriptive statistics are summarized in Table 3.

Correlations Between Metal/Metalloid Concentrations and Infant Outcomes

A Pearson's correlation matrix (Table 4) of transformed placental concentrations shows that most of the metal pairs are positively correlated. Only Rh and Zn were significantly negatively correlated. Metals quantified in placental tissue have been reported before with an accompanying correlation matrix, like those in Table 4 (Punshon et al. 2016). Pearson's correlation were calculated between transformed placental metal concentrations and all infant outcomes (Table 5). Rh, Mn and Pb were all significantly negatively correlated with birth weight. Rh was significantly negatively correlated with BWC. Rh, Mn, and Cd were all significantly negatively correlated with birth length. Rh and Mn were all significantly negatively correlated with head circumference. Cu, Fe, Se, Rh, Mn, Pb, Al and Cd were all significantly negatively correlated with placental weight. The fetal/placental ratio, generally considered a measure of placental efficiency (larger placenta size is theorized to compensate for exchange inefficiencies/defects), was significantly positively correlated with Al, Cd, Co, Cu, Fe, Se, Zn. No metal concentrations significantly correlated with gestational age. The distribution of each metal/metalloid concentration was further grouped on the basis of birth weight and birth weight centile (Table 6). The median Co placental concentration was significantly smaller in LBW infants. Cd, Co, Rh, and Zn were significantly different across BWC groupings.

Regression analysis

We fitted multivariable regression models that adjusted for confounding variables that affect birth weight (maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no). Of the 14 metals, 5 metals were significantly associated with birth weight after adjusting for covariates (Table 7). Increasing levels of two metals were associated with a greater birth weight. Each unit increase in ln-Co was associated with an 83.7 g (95% CI: 23.9 to 143.5) birth weight increase. Increasing levels of four metals were associated with significant decreases in birth weight. Birth weight was predicted to decrease by 138.2 g (95% CI: −263.4 to −13) per ln-increase in Fe (Figure 2a), by 57.2 g (95% CI: −104.3 to −10.1) per ln-increase in Mn (Figure 2b), by 58.3 g (95% CI: −97.9 to −18.8) per ln-increase in Pb (Figure 2c), and by −1490.3 g (95% CI: −1767.1 to −1213.5) per ln-increase in Rh (Figure 2d). These same results can also be expressed as a percent change; a 1% increase in Rhodium would result in a 14.9 gram decrease in birth weight (p < 0.001). Lead, manganese and iron were strong predictors of birth weight, decreasing birth weight by 0.58, 0.57 and 1.38 g respectively with a 1% increase in placental concentration (p < 0.030). Cobalt was significantly positively associated with birth weight, increasing birth weight 0.84 g with a 1% increase in Co placental concentration (p = 0.006). Additionally, we calculated the change in birth weight between the 25 and 75 percentile of the ln-metal distribution (Table 7). Between these two percentiles, Co increased birth weight by 82.5 g. Additionally, birth weight decreased when comparing the 25th and 75th percentiles in Fe, Mn, Pb and Rh distribution (51.1, 27.2, 72.7, and 1365.5 g respectively).

The same multivariable regression models were refit with the addition of a sex by ln-metal interaction, to understand whether the slope for metal on birthweight differed for female v. male infants. The interaction term was not significant for any of these models, suggesting that the effect of the ln-metals on birth weight was not different for female and male, and that the models that assume the same metal slope for both sexes are reasonable.

Results from generalized additive models showed that there were significant nonlinear relationships between two ln-metals and birth weight, after adjusting for covariates. For ln-Co, birth weight is predicted to increase at low levels until ln-Co is about 2.5 ug/kg (on the ln-transformed value, corresponding to an untransformed value of about 11 ug/kg), with a leveling off thereafter (df=3 for smoothing parameter; *p=0.0008)* for the non-linear effect) (Figure 3). For ln-Rh, birth weight was predicted to be relatively unaffected at low Rh levels, followed by a steep decrease in birth weight for ln-Rh above about 0.18 ug/kg (corresponding to an untransformed value of about 0.2 ug/kg, df=2 for smoothing parameter; *p = 0.005*) (Figure 4). In all models, gestational age showed a significant nonlinear relationship with birth weight. Birth weight is predicted to increase somewhat slowly for gestational age < 250 days, increase more quickly until gestational age of about 280 days, and then level off (Figure 5).

Discussion:

The present study quantified 14 metals/metalloids that were consistently detected in 374 placentae collected in Chattanooga, TN. Using Pearson's correlation, we compared metal

concentrations with a variety of fetal growth parameters. In addition, we used linear regression models to examine the relationships between birth weight and 14 placental metal concentrations in covariate-adjusted models. Because of the hypothesis-generating nature of this study and not wanting to rely solely on p-values for inference, we did not make any adjustment for multiple comparisons. Our aim with the hypothesis generating nature of our study was to underscore the complex nature of these elements, the placenta, and birth outcomes from the literature. To streamline this effort, metals that are discussed below are only those with significant associations (either essential or non-essential in the placenta or very under-reported in the placenta). Mn, Fe, Co and Zn serve essential functions, though at some concentrations, fetal growth can be impacted. Non-essential Cd and Pb are well documented to have deleterious effects on fetal growth. Several metals (Rh, Ti, Ba and Al) have limited to no published data on placental concentrations. As, Se, Cu and Ni have been previously studied in placental tissue and were not significantly associated with birth outcomes, and so are not extensively discussed below.

Essential metals significantly associated with fetal parameters

Manganese serves a vital role in the cell and is an essential nutrient (Leach and Harris 1997). In the present study, placental manganese concentrations were negatively correlated with birth weight, length and placental weight. A past study found that Mn concentrations in placental tissue was not significantly different between IUGR or low birth weight infants and normal weight infants (Osada et al. 2002). A few studies have investigated maternal blood Mn and infant parameters. When measuring maternal Mn in blood, Zota et al. (2009) reported an inverted U-shaped dose-response curve for birth weight; birth weights increased with increasing maternal whole blood Mn concentrations up to 3.1 μg/L, with a reduction of birth weight occurring past that threshold. However, Osada et al. (2002) did not find a significant difference in maternal blood Mn concentrations of normal and IUGR infants at concentrations that were at or below the 3.1 μg/L for both AGA and IUGR samples. Vigeh et al. (2008) reported responses at Mn concentrations well above 3.1 μg/L; maternal blood Mn concentrations for AGA infants were significantly higher than the IUGR infants (16.7 ± 4.8 μg/L for IUGR and 19.1 ± 5.9 μg/l for AGA infants). However, this trend was reversed in the infant cord blood samples (Mn = 44.7 ± 19.1 μg/L for IUGR and 38.2 ± 13.1 μg/l for AGA newborns). We found no evidence of non-linearity between ln-Mn and birth weight; however, we did find a significant decrease in birth weight with increasing ln-Mn levels. The results of the present study and other studies suggest that the relationship between Mn concentrations (in maternal tissues, *in utero* tissues, and transplacental transfer) and infant outcomes warrants further investigation.

Cobalt concentrations were greater in normal weight birth placentae and lower in SGA births compared to LGA births, and were a significant predictor of birth weight after adjusting for covariates. A generalized additive model allowing for nonlinearity in the effects of the continuous covariates and Co demonstrated a significant nonlinear relationship between Co and birth weight. Cobalt is an essential cofactor in Vitamin B-12, which plays a role in energy production (Banerjee and Ragsdale 2003). Data on Co and human pregnancy outcomes are limited. However, there have been reports linking Co deficiencies in sheep with high prenatal mortality. In one such study by Duncan et al. (1981), ewes were placed on

a Co deficient diet for a period prior to conception. That Co deficiency lead to high perinatal mortality of the associated lambs. Cobalt appears to undergo transplacental transfer. Indeed, deSouza et al. (2012), examined women who had undergone hip replacement surgery (the metal of the replacement hip contains Co) and found that concentrations in the umbilical cord blood were approximately half that of maternal blood levels, demonstrating transplacental transport.

Zinc is an essential element in more than 1000 proteins required for biological functions ranging from antioxidants to cell signaling and gene expression. As such, it is extremely important for fetal growth (Maret 2009; Prasad 2008; Wilson et al. 2017). Zadro na et al. (2009) found that Zn levels were significantly lesser in IUGR placentae than the control group, which mirrors the reduction of zinc in our population SGA infants (Table 6). Through the use of a Zn deficient mouse model, Wilson et al. (2017) found an 8% reduction in placental and birth weight, and a 32% reduction in weaning weight compared to mothers with a Zn supplementation.

Non-essential metals significantly associated with fetal parameters

Both lead and cadmium are well-documented toxic metals, even when considering the unique matrix such as the placenta (both of these metals specifically quantified in the placenta, have merited a review (Esteban-Vasallo et al. 2012)). In the present study, there was a significant negative correlation between placental cadmium concentration and birth length and placental weight. Guo et al. (2010) examined two cities in China, one of which is a major processor of e-waste (electronic waste). Placental Cd concentrations were not significantly different than the control city (where e-waste was not being processed), nor was there any significant correlations with placental Cd and birth weight. In the present study, no significant difference in Cd concentrations was found between LBW and normal weight infants, which is similar to past studies (Osada et al. 2002), although Cd has been found to be was significantly greater in IUGR/LBW placentae than normal placentae in other studies (Klapec et al. 2008; Llanos and Ronco 2009). Cadmium (Cd) is an endocrine disrupter (Henson and Chedrese 2004) that has been shown to inhibit 11β-HSD2 activity in the human placenta (Yang et al. 2006). Impaired fetal growth and intrauterine growth restriction (IUGR) are linked to reduced levels of 11β-HSD2 activity/expression in the placenta (Dy et al. 2008; McTernan et al. 2001; Mikelson et al. 2015). Further studies on the potential for cadmium to directly (transplacental transfer) or indirectly (myriad pathways) affect infant parameters is warranted.

Lead was significantly negatively correlated with birth weight and placental weight. Lead has been found to readily pass through the placenta (Rudge et al. 2009; Sakamoto et al. 2010). In a study of Swedish women, lead cord blood levels were negatively correlated to birth weight, length and head circumference (Osman et al. 2000), and higher Pb concentrations were reported in placenta from IUGR infants (Llanos and Ronco 2009), but this association has not always been found in other studies examining birth weight or IUGR infants (Al-Saleh et al. 2011; Falcón et al. 2003; Klapec et al. 2008). Guo et al. (2010) also did not find a significant correlation between placental lead and gestational age (similar to

the present study), nor a significant correlation of lead with birth weight (dissimilar to the present study).

Underrepresented metals significantly associated with fetal parameters

The present study significantly contributed to a relatively extensive database of some placental metals (e.g., Pb and Zn) and the relationship each has with birth weight and other infant parameters. However, some of the strongest relationships that are presented herein came from several metals underrepresented in placental and birth outcome literature. For example, concentrations of Rh have not been reported in placental tissue, yet Rh was the strongest predictor of birth weight of all metals examined here. Indeed, placental Rh was consistently negatively correlated with many infant parameters. As noted earlier, the generalized additive model for ln-Rh demonstrated a significant nonlinear relationship between Rh and birth weight, after adjusting for covariates. In contrast to the other metals in the present study, Rh was not correlated with multiple metals; Rh correlated only with Zn, suggesting the source of exposure to Rh was different from exposure to most other metals. Rhodium is a relatively non-toxic metal (CDC 1994; Schertzinger et al. 2017); therefore, mechanisms for reduced birth weight are not established. Minor DNA damage has been observed at high concentrations (micromolar) in cell culture which may indicate a danger for those in industry (Iavicoli et al. 2012), but overall, Rh toxicity is low. When compared to more well-studied metals, cell culture LC_{50} values (tested on BEAS-2B, a human bronchial cell line) were significantly higher for Rh (1.2 mmol/L) as compared to Ni (0.8 mmol/L), and Cd salts (0.005 mmol/L) (Schmid et al. 2007). Through the use of catalytic converters, Rh release has been on the rise (Barbante et al. 2001), and with that, a concern for exposure from heavy, urban traffic environments (Kamala et al. 2015). Blood samples in police officers occupationally exposed to heavy traffic showed increased Rh blood levels with increasing experience and time on the force (Kamala et al. 2015). Thus, airborne concentration of Rh would be the most likely source of maternal exposure. Because Rh is not a Criteria Air Pollutant or considered to pose a threat to human health and the environment, the Clean Air Act does not require that it be regularly monitored or reported in the Toxic Release Inventory (E.P.A. 2006, 2017). Considering the increased abundance of automobiles in the US and the developing world, environmental monitoring of Rh and other platinum group elements has expanded, but is still very under-reported. (Ajmone-Marsan and Biasioli 2010; Barbante et al. 2001; Ravindra et al. 2004). Given our data and increased automobile emissions, Rh is a relevant metal to further investigate regarding fetal growth outcomes.

Aluminum negatively correlated with placental weight, but positively correlated with the fetal/placental ratio. Significant neurological development delays and decreased postnatal body weight gain were observed when dams were administered 300 and 600 mg/kg/day $AlCl_3$ (Abu-Taweel et al. 2012; Bondy 2010). Sharma and Mishra (2006) studied the effects of Al on pregnant rats. Female rats were given 345 mg/kg day oral dose of aluminum chloride for 16 days pre- and post-partum. Al was quantified in fetal and maternal brain tissues, as well as placental tissues. Birth weight, placental weight, and fetal growth were all reduced compared to the control group. Neither Al transplacental passage nor related fetal outcomes have been extensively studied in humans. Al concentrations have been reported,

but the focus was on distribution within the placenta rather than health outcomes (Kruger et al. 2010).

While the present study did not find any significant correlations of barium and titanium with birth outcomes, our data are important because reports of barium and titanium concentrations in placental tissues are very limited. A few studies have examined Ba concentrations in maternal tissues and associated birth outcomes, but none have examined correlations to birth weight (Han et al. 2011; Jain 2013; Odland et al. 2001). Titanium dioxide nanoparticles (TiO_2 NP) are prominent sources which have increased in daily use and manufacturing (Rollerova et al. 2015). When administered to mice, a 1 mg/kg/day oral TiO_2 NP treatment significantly reduced the ratio of placenta/body weight on gestational day 13; however, placental weight nor litter size was affected (Zhang et al. 2018).

Potential Mechanisms for Effects on Fetal Growth

The comprehensive group of metals and metalloids investigated herein likely involves many biochemical pathways associated with birth weight and fetal growth. Oxidative stress is one general mechanism that has been shown to affect birth weight and is related to maternal metal concentrations. Additionally, studies have examined how certain essential and non-essential metals affect placental morphology/function.

Oxidative Stress within the Placenta Due to Essential and Non-essential Metals

Oxidative stress in any cell or tissue is of great concern. Concentrations of free radical species in excess of antioxidant defenses causes damage to cells, mainly the cell membrane through lipid peroxidation (Betteridge 2000). A myriad of conditions including cardiovascular diseases, asthma, and various cancers, among many others, are thought to be related to oxidative stress (Phaniendra et al. 2015). Though some signaling pathways rely on reactive oxygen species in the placenta, if excess free radicals are not sequestered, oxidative stress can occur. Spontaneous abortion, preeclampsia, and IUGR are all associated with oxidative stress (Sultana et al. 2017; Wu et al. 2016).

The toxicity of iron-catalyzed reactive oxygen species (ROS) is well established. Shastri et al. (2016) measured hemoglobin (Hb) in the first trimester of pregnancy and grouped women with high Hb levels (120 g/l) and compared them to women with normal to low Hb levels (< 110 g/l). Both groups continued to take an iron supplement for the duration of the pregnancy. Measured during the first trimester, the high Hb mothers had a significant increase in plasma lipid peroxides and lower paraoxonase-1 (PON-1) activity (a common enzyme to mitigate lipid peroxidation) compared with women with low/normal Hb. Further, routine supplementation of Fe to these high Hb women for the duration of gestation resulted in persistent lower PON-1 activity in cord blood and lighter birth weights. Although these results were not measured in placental tissue, our correlations of Fe with birth weight, after covariate adjustments, is very similar to the relationship with birth weight reported by Shastri et al. (2016), which may be related to over exposure to iron and subsequent increase in oxidative stress. Cadmium and lead reduce total antioxidant potential by depleting glutathione and protein-bound sulfhydryl groups that serve to neutralize free radicles (Stohs

and Bagchi 1995). Schmid et al. (2007) tested rhodium for ROS generation, but instead found that ROS decreased with Rh concentrations above 0.09 mol/L.

Several of the metals measured in the present study are important trace metals that serve a role as enzyme cofactors that help control oxidative stress in tissue. For example, superoxide dismutase, contains zinc and copper (Cu-Zn SOD) (Mistry and Williams 2011). Reactive oxygen species naturally generated in the mitochondria are captured with the antioxidant enzyme, manganese superoxide dismutase (MnSOD). Several other studies that examined metal concentrations in the placenta collected data on biomarkers of metals; such as metallothioneins (MT), delta-aminolevulinic acid dehydratase (ALAD), and lipid peroxidation (LPO) (Serafim et al. 2012), cytochrome c oxidase (CCO), glucose-6-phosphate dehydrogenase (G6PDH), Cu–Zn superoxide dismutase (Cu–Zn SOD), glutathione peroxidase (GSH-Px), and glutathione (GSH) (Zadro na et al. 2 9). These biomarkers are related either to metal sequestration (MT), a measure of or abetment of oxidative stress (LPO, Cu–Zn SOD, GSH/GSH-Px). Or, are involved in a biochemical pathway or ATP production (e.g., CCO, G6PDH, ALAD), that have been shown to correlate with fetal health outcomes (Zadro na et al. 2 9). Measuring such biomarkers and biochemical pathways was well beyond the scope of the present study. However, our data, particularly those on underrepresented metals, warrants future mechanistic investigations.

Effects of metals on placental morphology and function

Beyond their effects on biochemical pathways, metals may also affect placental morphology, and / or function. Zinc deficiencies are associated with significant decreases (16%) in trophoblast volume and trophoblast barrier thickness and increases in the volume density and surface area density of fetal capillaries in mice (Wilson et al. 2017). A 10 mg/kg/day dose of TiO_2 NPs disrupted placental labyrinth vascularization and significantly inhibited placental cell proliferation and increased placental apoptosis (Zhang et al. 2018), suggesting a morphological change to the placenta. Cd is commonly detected in the placenta, especially in smokers (as seen in our data set). In addition to directly increasing placental oxidative stress, Cd in the placenta also alters the detoxifying mechanism of the placenta by downregulating a transport protein, ABCG2, in rats (Liu et al. 2016). ABCG2 is an ATP-binding cassette (ABC) transporter, that is well known to move heavy metals (e.g., Cd, Pb, Hg) from syncytiotrophoblast to maternal circulation, decreasing fetal exposure (Kummu et al. 2012). Further, Wier et al. (1990) reported decreased Zn transport into fetal circulation, altered ultrastructural changes and fetal vasculature with CdCl human placental perfusion.

Considerations for Infant Sex-specific effects of metals and Fetal/Placental Ratio

Several studies have reported sex-specific differences in metal toxicity. Karagas et al. (2012) cited two studies (Gao et al. 2007; Philibert et al. 2008) in a review of methyl mercury toxicity that examined sex-specific effects and called for an increased analysis of this important variable. Cd showed a sex-specific mechanism in a study conducted by Kippler et al. (2012), who measured maternal urine Cd concentrations and found a significant negative correlation with birth weight and head circumference in female infants only. A review by Clifton (2010) highlights several broad areas of fetal health that have shown a sex-biased difference (different growth rates in response to environmental stress, cytokine expression,

insulin-like growth factor pathways, placental response to cortisol, preterm delivery placental function). Additionally, Clifton states, "ignor[ing] the sex of the placenta is no longer sound scientific practice." We did not find any evidence that the effects of ln-metals on birth weight differed by sex in this study. However, we concur that sex-specific effects on infant parameters should be considered in future studies.

Another approach to assessing fetal growth is calculating the ratio of fetal weight to placental weight, or how much fetal growth can be supported per gram of placenta (Matsuda et al. 2015). The fetal/placental ratio is one based on the theory that a relatively large placenta represents an inefficiency in nutrient transfer across the placenta (i.e., more tissue is needed to exchange molecules); thus, placental growth is stimulated to compensate for exchange inefficiencies (Salafia et al. 2006). As normal fetal growth proceeds and birth weight increases, the fetal/placental ratio value increases (which is to imply that proper fetal growth outpaces the growth of the placenta) (Lurie et al. 1999; Matsuda et al. 2015). When correlated with ln transformed metals, Al, Cd, Co, Cu, Fe, Se and Zn all had positive correlations (e.g., as Al increased, so did the fetal/placenta ratio). This was the only birth outcome with positive correlations for those metals, meaning that as metal concentrations increased so did the fetal/placental ratios, indicating improved placental function. This is interesting considering the well-known deleterious and non-essential functions of Al and Cd. Little research in this area has been conducted, but more investigation is warranted.

Study Limitations:

Limitations in the present study must be taken into consideration. The maternal records lacked complete information on maternal income and dietary habits. Dietary habits and vitamin supplementation are well documented as being significant influences on the metal status of the mother and fetus (Hovdenak and Haram 2012; Mistry and Williams 2011; Scholl 2005). After an analysis of six metals and several biomarkers, Serafim et al. (2012) found that diet was a significant influence on the metal status of the placenta (as well as tobacco use). Additionally, examination of individual metals does not address potentially additive or synergetic effects, but an analysis of the effects of metal mixtures is beyond the scope of the current manuscript. Like all observational studies, we can only assess association, but not causation. The results of this study cannot be construed as showing that exposure to high levels of these metals causes changes in birth outcomes. Additionally, we can only review and consider potential mechanisms or biomarkers that would directly link fetal growth to these placental concentrations. Finally, though our sample size is relatively large, and we made great efforts to eliminate any selection bias from our sample population, it is not guaranteed that our selection process would affect all metals in the same way. Although the relationship between a factor that affects participation and birth weight will remain constant across variables, the relationship between these factors and the metals may vary.

The main objective of the present study was to quantify the placental concentrations of a wide spectrum of metals and describe correlations with fetal parameters – regardless of the source or associated mechanisms of action. To that end, the present study expands the

database on underrepresented placental metals and provides one of the most comprehensive examinations of placental metals and correlations with fetal parameters to date.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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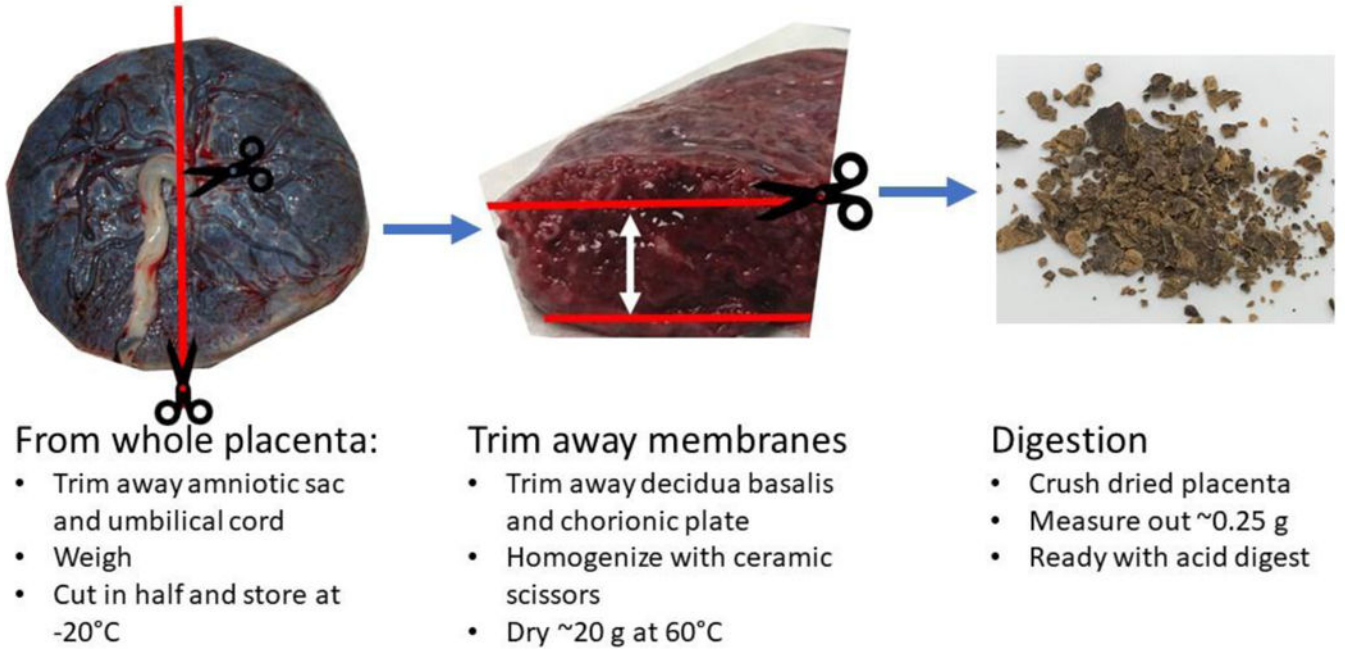
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Highlights:

- Placental Rh concentrations are negatively correlated with many infant parameters.

- Each unit increase in ln-Rh was associated with a 1490 gram decrease in birth weight

- Pb and Mn were significantly negatively correlated with many of the infant parameters

- Cobalt was significantly positively associated with birth weight.

- Significant nonlinear relationships between birth weight and placental Co and Rh.

From whole placenta:

- Trim away amniotic sac and umbilical cord
- Weigh
- Cut in half and store at -20°C

Trim away membranes

- Trim away decidua basalis and chorionic plate
- Homogenize with ceramic scissors
- Dry ~20 g at 60°C

Digestion

- Crush dried placenta
- Measure out ~0.25 g
- Ready with acid digest

Figure 1.
Placenta processing procedure.

Relationship between ln-iron and birthweight

slope = -138.22
p = 0.03

— Estimated slope
- - 95% Confidence band

Birth Weight (g)

ln-iron (ug/kg)
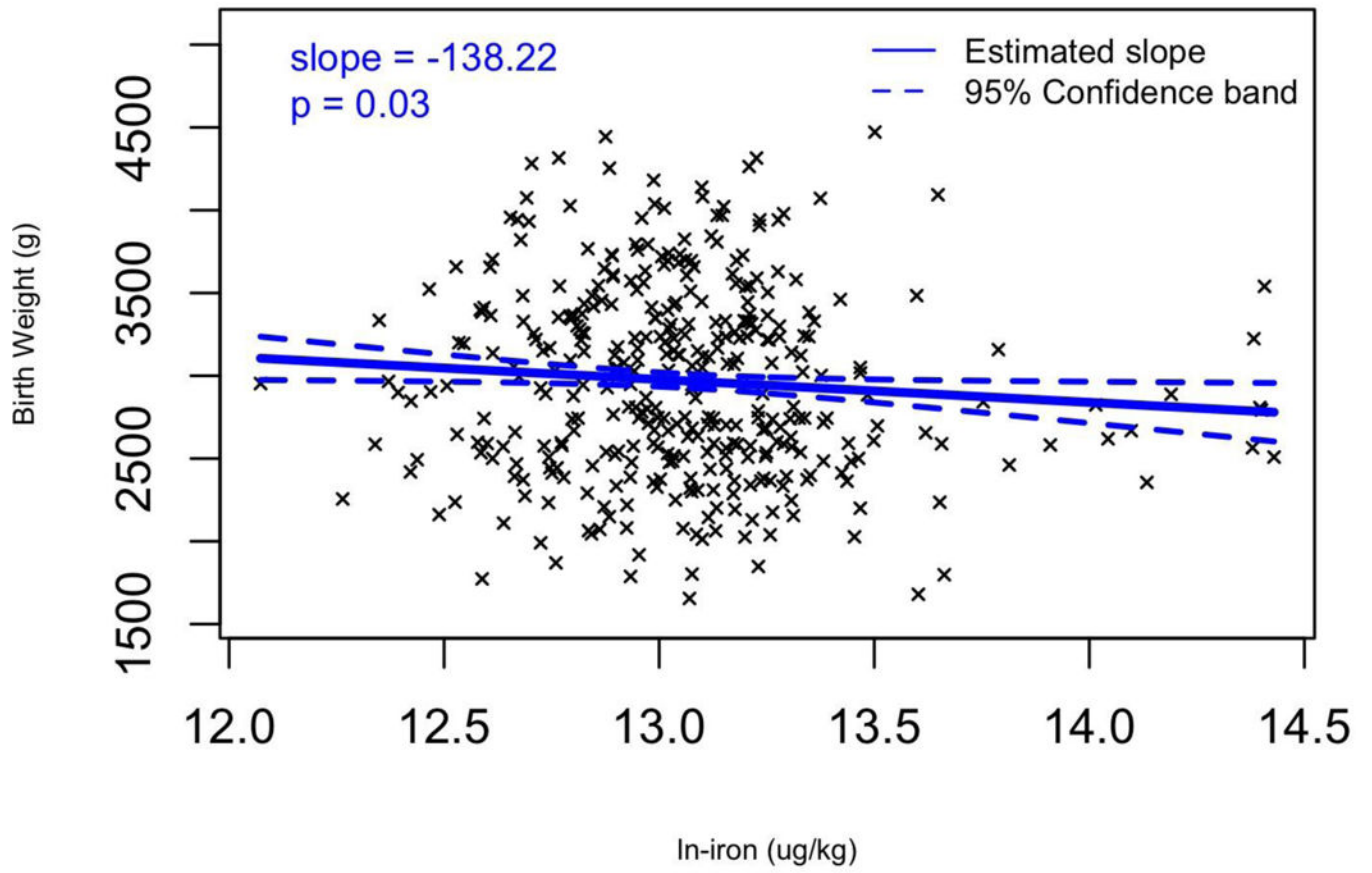
Relationship between ln-manganese and birthweight

slope = -57.2
p = 0.02

—— Estimated slope
– – 95% Confidence band

Birth Weight (g)

ln-manganese (ug/kg)
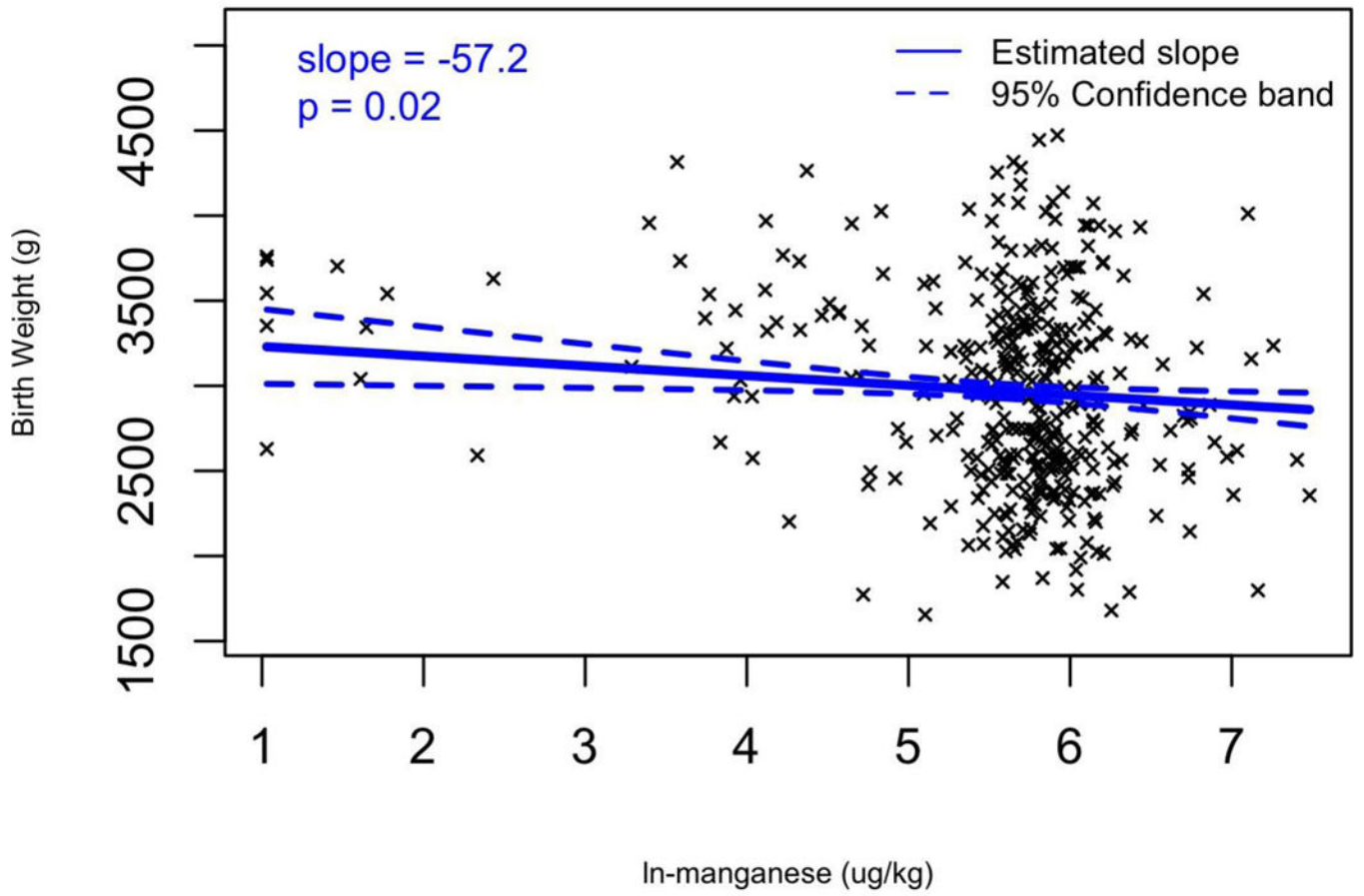
Relationship between ln-lead and birthweight
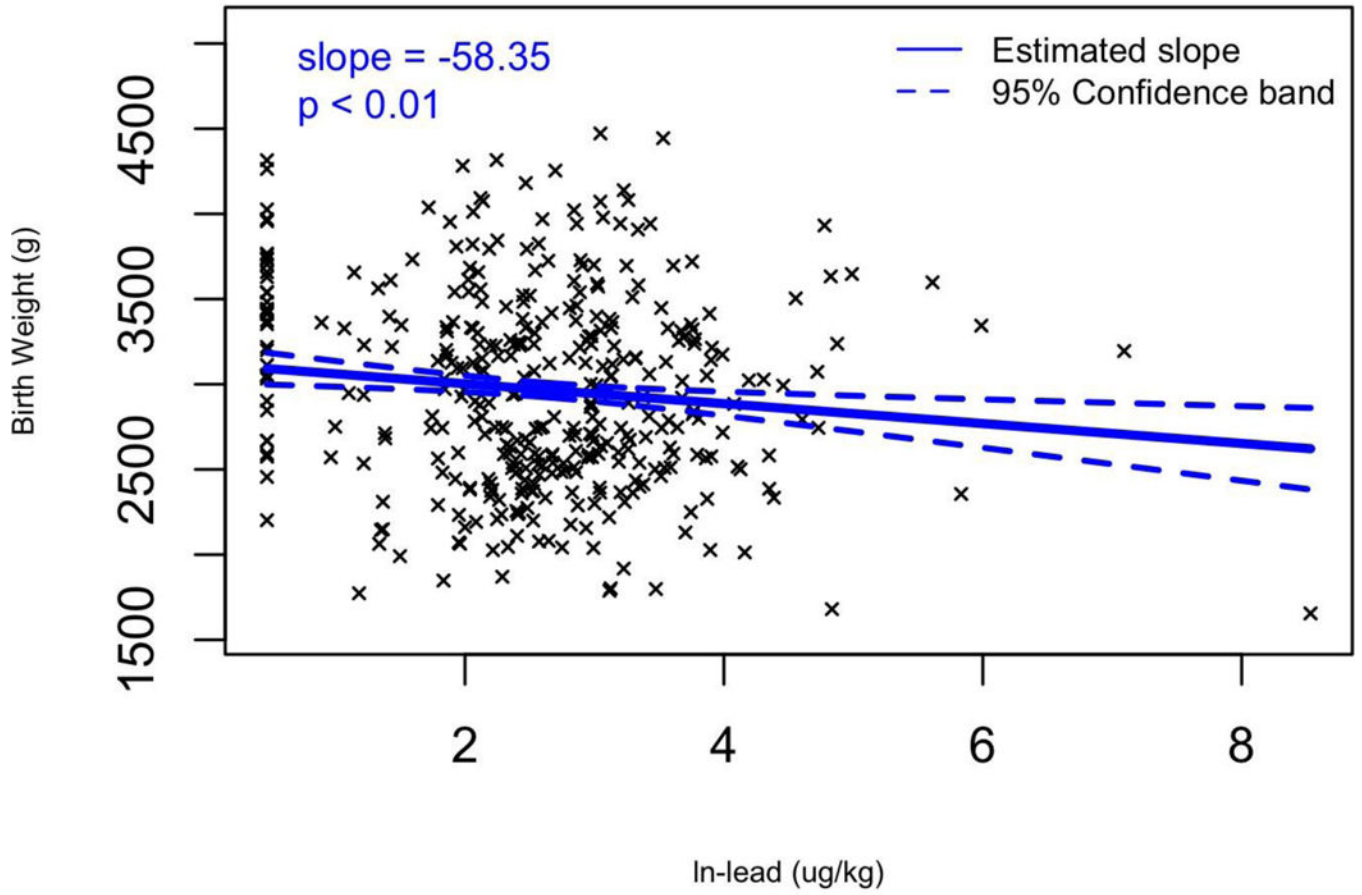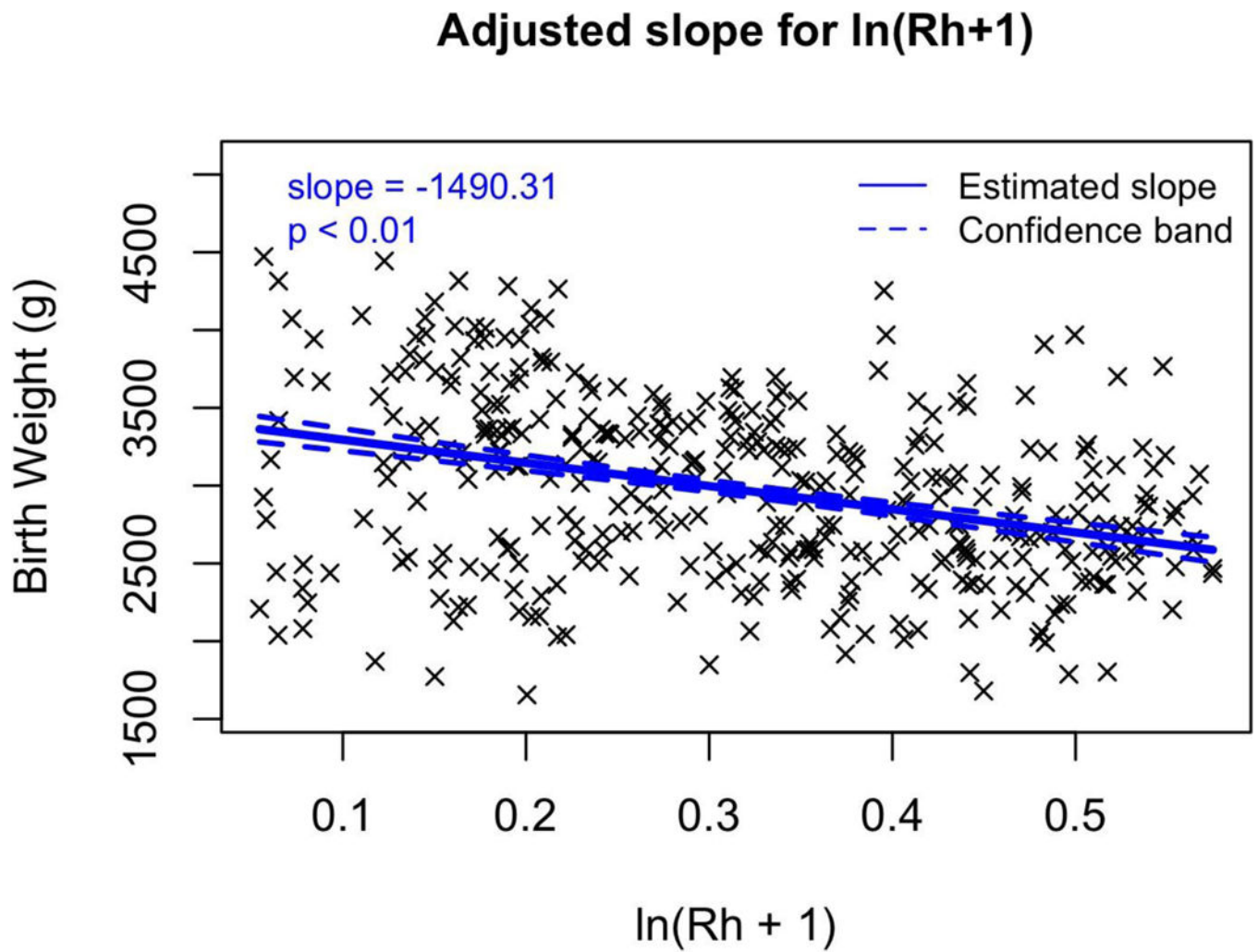
Figure 2.
Multivariable regression models for birth weight (g) versus transformed (ln+1) metal concentrations for a) Fe, b) Mn, c) Pb, and d) Rh which all have significant slopes (β). Regression coefficients controlled for the following covariates: maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no).

Relationship between ln-cobalt and birthweight
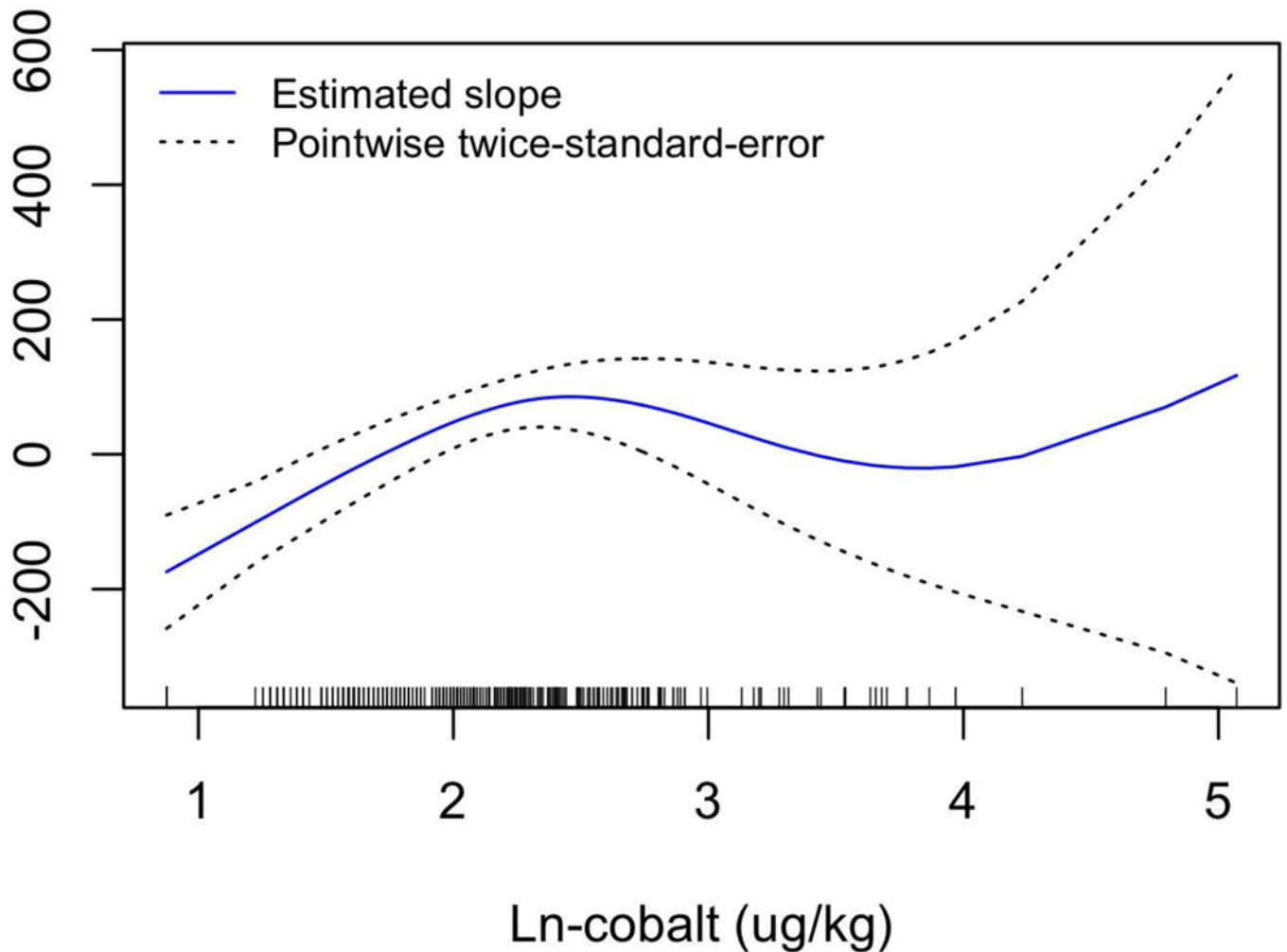
Figure 3.
The estimated covariate-adjusted effect of log(cobalt + 1) on birthweight from a generalized additive model with 3 degrees of freedom for this relationship. Hash marks at the bottom of the plot show observed values for ln-cobalt. Regression coefficients controlled for the following covariates: maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no).

Relationship between ln-rhodium and birthweight
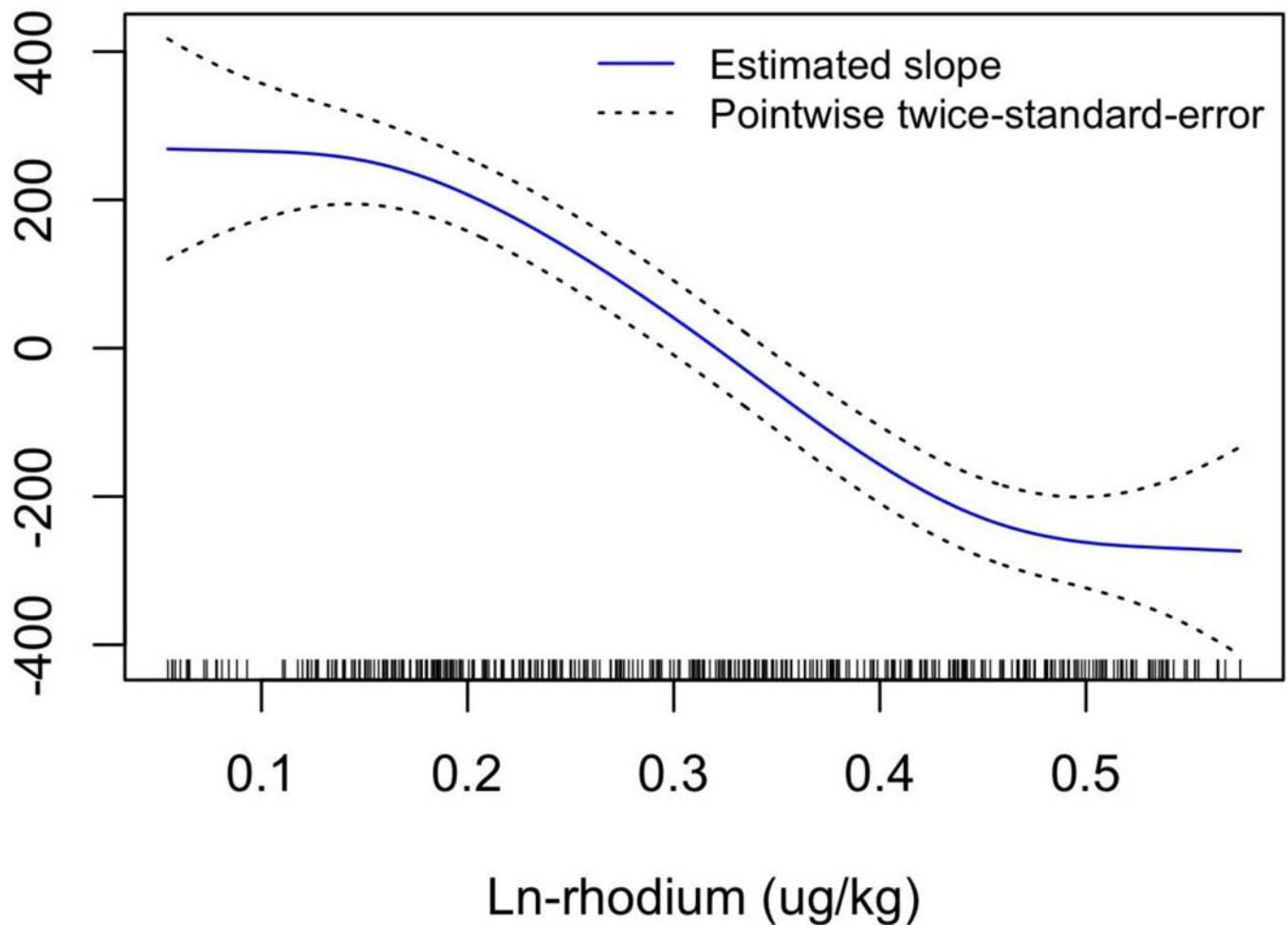
Figure 4.
The estimated covariate-adjusted effect of log(rhodium + 1) on birthweight from a generalized additive model with 3 degrees of freedom for this relationship. Hash marks at the bottom of the plot show observed values for ln-rhodium. Regression coefficients controlled for the following covariates: maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no).

Relationship between gestational age and birthweight
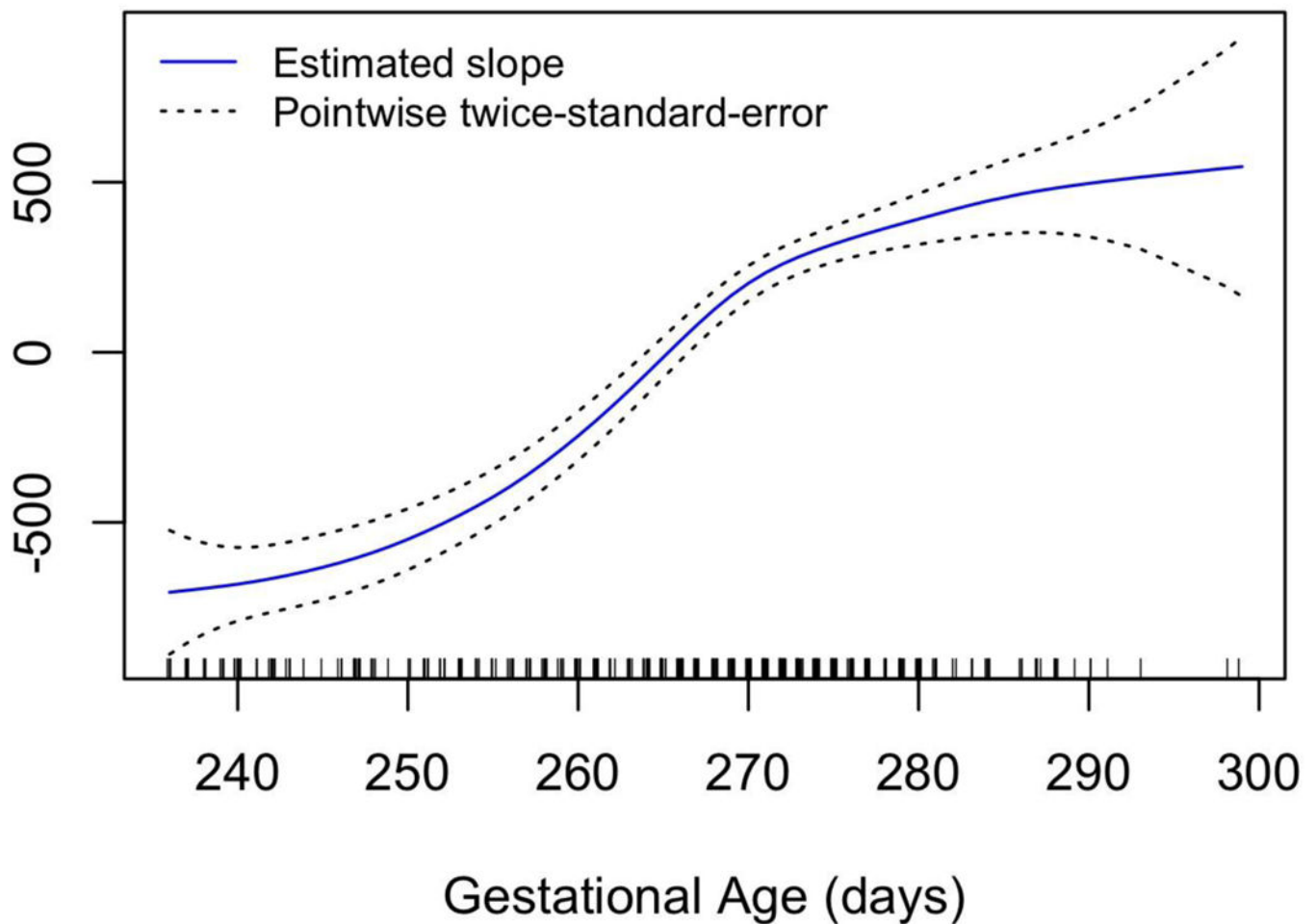
Figure 5.
The estimated covariate-adjusted effect of gestational age on birthweight from a generalized additive model with 5 degrees of freedom for this relationship. The plot shown is for the model that adjusts for rhodium exposure; the relationship between gestational age and birthweight is extremely similar for all exposures. Hash marks at the bottom of the plot show observed gestational ages. Regression coefficients controlled for the following covariates: maternal pre-pregnancy BMI, maternal age, and gestational age as continuous variables, as well as three categorical covariates: race (white or minority), infant sex, and smoking (yes or no).

Table 1:

Dry-weight basis elemental concentrations (µg/kg) in placenta tissue collected from Hamilton County, TN (n=374).

Element	Mean	Standard Deviation	Median	25%	75%	I.Q.R.	Min.	Max.	Detection Limit [a]	Detection Limit (ug/L)
Al	1080	1990	385.3	120.9	1061	940.1	1.800	14740	3.6	0.09
As	43.01	15.03	42.14	33.66	52.79	19.13	1.800	105	3.6	0.09
Ba	91.62	146.0	47.41	26.67	93.08	66.41	1.200	1657	2.4	0.06
Cd	18.54	11.99	15.81	10.40	23.80	13.4	1.200	84.22	2.4	0.06
Co	8.890	12.72	6.600	3.600	9.650	6.05	1.400	158.3	2.8	0.07
Cu	3889	2480	3413	3001	3923	922	1356	20560	2	0.05
Fe	503200	232800	463500	380900	552700	171800	175100	184800	4.4	0.11
Mn	346.2	228.0	318.2	249.6	401.7	152.1	1.800	1774	3.6	0.09
Ni	111.0	219.5	60.50	23.79	117.5	93.71	1.600	2886	3.2	0.08
Pb	37.97	270.5	12.03	6.680	23.23	16.55	0.6000	5073	1.2	0.03
Rh	0.390	0.190	0.380	0.220	0.550	0.33	0.0600	0.780	0.0008	0.00002
Se	1504	383.0	1442	1329	1584	255	566.3	3773	2.4	0.06
Ti	381.7	77.21	380.7	315.4	448.4	133	238.0	532.7	0.0032	0.00008
Zn	55120	24120	50180	45870	55480	9610	18910	332500	5.6	0.14

[a] Assuming 0.25 g of sample

Table 2.

Descriptive statistics of maternal parameters associated with placentae from Hamilton County, TN (n=374).

Maternal characteristic	N	%
Race		
Caucasian	225	60
African Am.	86	23
Latina	61	16
Other	2	1
Maternal age (years)		
18–24	172	46
25–29	110	30
30–34	59	16
35+	33	9
Cigarette/day		
Non-smoking	287	77
Smoking	87	23
Delivery		
C-section	131	35
SVD	243	65
Marital Status		
Married	194	52
Single	180	48
Pre-pregnancy BMI		
Underweight (18.5)	16	4
Average (18.5–24.9)	168	45
Overweight (25–29.9)	101	27
Obese (3)	89	24
Education		
< high school	105	28
HS/GED	104	28
any college	123	32
Unknown	42	11
Parity		
0	115	30
1	138	37
2	67	18
3+	54	15
Gestational Age		
40+ weeks	43	11
40–37 weeks	230	62
34–36 weeks	101	27

Table 3.

Descriptive statistics of infant parameters associated with placentae from Hamilton County, TN.

	Mean	SD	Median	Minimum	Maximum	Range	n
Birth Weight (g)	2969	577.9	2942	1655	4472	2817	374
Birth Weight Centile (%)	38.4	30.8	30.8	0.1	100	99.9	374
Placental Weight (g)	576.6	135.7	562	306	1005	699	238[a]
Head Circumference (cm)	34.6	17.4	33	13.5	337	324	317[a]
Birth Length (cm)	49.3	3.8	50	20	59.3	39.3	324[a]
Gestational Age (days)	266	13	268	236	299	63	374
Maternal Age (years)	25.9	5.5	25	18	47	29	374

[a] Data missing from placental weight, head circumference and birth length compared to the original sample size due to incomplete clinical records.

Table 4:

Pearson's correlation matrix (r-values) between 14 ln-metals (defined as the natural logarithm of 1 + the metal concentration in placental tissues) (n = 374).

	Al	As	Ba	Cd	Co	Cu	Fe	Mn	Ni	Pb	Rh	Se	Ti	Zn
Al	1													
As	0.00	1												
Ba	0.10	−0.03	1											
Cd	**0.15**[*]	0.03	0.06	1										
Co	**0.2**[*]	−0.01	**0.14**[*]	**0.31**[*]	1									
Cu	**0.45**[*]	−0.04	**0.14**[*]	**0.32**[*]	**0.43**[*]	1								
Fe	**0.27**[*]	0.00	0.08	**0.18**[*]	**0.22**[*]	**0.62**[*]	1							
Mn	**0.35**[*]	−0.06	0.09	**0.27**[*]	**0.16**[*]	**0.60**[*]	**0.29**[*]	1						
Ni	0.06	−0.09	0.06	**0.14**[*]	**0.20**[*]	**0.37**[*]	**0.26**[*]	**0.27**[*]	1					
Pb	**0.26**[*]	−0.08	**0.19**[*]	**0.21**[*]	**0.12**[*]	**0.41**[*]	**0.29**[*]	**0.43**[*]	0.07	1				
Rh	−0.01	−0.05	−0.08	0.01	−0.06	−0.02	0.02	0.04	−0.02	0.00	1			
Se	**0.24**[*]	0.01	**0.12**[*]	**0.27**[*]	**0.40**[*]	**0.70**[*]	**0.43**[*]	**0.23**[*]	**0.31**[*]	0.08	−0.02	1		
Ti	0.00	−0.04	−0.01	−0.02	0.00	−0.02	−0.05	−0.02	−0.09	0.05	0.09	0.00	1	
Zn	**0.34**[*]	−0.05	**0.31**[*]	**0.26**[*]	**0.53**[*]	**0.69**[*]	**0.42**[*]	**0.35**[*]	**0.41**[*]	**0.20**[*]	**−0.11**[*]	**0.65**[*]	−0.01	1

Significant correlations:

[*] p<0.05

Table 5:

Pearson's correlation coefficients (r-value) between ln-metal concentrations and birth weight, BWC, gestational age, length, head circumference and placental weight.

	Birth Weight (g)	Birth Weight Centile (%)	Gestational Age (days)	Birth Length (cm)	Head Circumference (cm)	Placental Weight (g)	Fetal/Placental Ratio
n	374	374	374	324[a]	317[a]	238[a]	238[a]
Al	−0.005	0.006	−0.006	−0.006	0.033	**−0.195**[**]	**.183**[**]
As	−0.019	−0.006	−0.009	−0.024	0.061	−0.081	0.055
Ba	0.058	0.002	0.091	0.09	0.023	0.011	0.095
Cd	−0.069	−0.096	0.048	**−0.111**[*]	−0.102	**−0.195**[**]	**.134**[*]
Co	0.052	0.043	0.057	0.027	0.037	−0.023	**.189**[**]
Cu	−0.071	−0.038	0.001	−0.086	−0.048	**−0.252**[***]	**.237**[**]
Fe	−0.081	−0.052	0.014	−0.077	−0.047	**−0.228**[**]	**.196**[**]
Mn	**−0.172**[**]	−0.096	−0.096	**−0.173**[**]	−0.093	**−0.281**[***]	−0.015
Ni	0.013	0.033	−0.02	−0.004	0.018	0.049	−0.030
Pb	**−0.111**[*]	−0.077	−0.053	0.019	−0.014	**−0.135**[*]	0.043
Rh	**− 0.329**[***]	**− 0.463**[***]	0.032	**− 0.144**[**]	**−0.141**[*]	**−0.156**[*]	−0.086
Se	0.002	−0.001	0.036	−0.019	−0.008	**−0.179**[**]	**.220**[**]
Ti	−0.044	−0.083	0	−0.055	−0.065	0.029	−0.084
Zn	0.053	0.062	0.005	0.008	0.028	−0.108	**.265**[**]

Significant correlations:

[*] $p < 0.05$

[**] $p < 0.01$

[***] $p < 0.0001$

[a] Data missing from placental weight, head circumference and birth length compared to the original sample size due to incomplete clinical records.

Table 6:

Comparison of dry-weight basis placental element concentration to birth weight and birth weight centile groupings (LGA, AGA, SGA). Median values and interquartile range (in parentheses) for untransformed metal values are reported in μg/kg (n=374).

	Birth Weight		Birth Weight Centile		
	Low	Normal	SGA[a]	AGA	LGA
n	82	292	89	253	32
Al	351.3 (970.2)	393.4 (924.3)	309.5 (726.4)	409.2 (1029)	540.8 (1085)
As	43.5 (20)	41.8 (19.3)	41.9 (16.4)	42.7 (20.5)	37.7 (17.5)
Ba	39.8 (47.1)	49.6 (72.9)	47.4 (66.6)	47 (63.3)	49.8 (104.1)
Cd	16.3 (12.5)	15.4 (14.1)	**18 (14.5)**	**14.6 (13.4)**	**13.8 (9.8)**[*]
Co	**5 (5.5)**	**6.8 (6.1)**[*]	**4.9 (7.1)**	**6.9 (5.9)**	**8.3 (4.2)**[*]
Cu	3281 (625.3)	3489 (955.8)	3282 (821.5)	3447 (923.8)	3696 (1110)
Fe	475800 (185000)	460500 (160200)	482400 (179600)	453000 (165000)	459800 (168200)
Mn	332.9 (149.2)	314.5 (145.7)	335.7 (153)	313.8 (143)	329.1 (133.6)
Ni	58.9 (108.9)	60.7 (88.6)	53.4 (90.3)	62.2 (91.1)	43.3 (55.3)
Pb	11.5 (13.6)	12.2 (17.2)	15.1 (14.8)	11.4 (16)	12.7 (17.5)
Rh	0.5 (0.4)	0.4 (0.3)	**0.5 (0.3)**	**0.4 (0.3)**	**0.2 (0.1)**[***]
Se	1396 (256.4)	1445 (254.2)	1391 (291.3)	1449 (242.4)	1447 (186.5)
Ti	369.5 (121.4)	382.5 (136.9)	401.6 (116.8)	372.3 (142.4)	367.7 (92.5)
Zn	49070 (10180)	50690 (9008)	**48000 (10250)**	**50550 (8617)**	**51850 (11640)**[*]

Significance:

[*] $p < 0.05$

[***] $p < 0.0001$

[a] LGA (large for gestational age, >90% BWC), AGA (average for gestational age, 11–89% BWC), SGA (small for gestational age, <10% BWC).

Table 7:

Summary of ln-transformed metal coefficients in models fit for birth weight (grams), controlling for the following covariates: maternal pre-pregnancy BMI, maternal age and gestational age and three additional categorical variables: race (white or minority), infant sex and smoking while pregnant (yes or no) (n=374).

Metal	Estimated Coefficient	95% CI	p-value	Estimated change in BW from 25th-75th percentile	95% CI
Al	2.7	(−16.5, 21.9)	0.783	5.9	(−35.8, 47.5)
As	−26	(−110, 58.1)	0.543	−11.7	(−49.5, 26.1)
Ba	16.3	(−27.8, 60.3)	0.468	20.4	(−34.7, 75.4)
Cd	−38.1	(−114.7, 38.5)	0.328	−31.5	(−95, 31.9)
Co	**83.7**	**(23.9, 143.5)**	**0.006**	**82.5**	**(23.6, 141.5)**
Cu	−17	(−134.5, 100.6)	0.776	−4.6	(−36, 26.9)
Fe	**−138.2**	**(−263.4,−13)**	**0.030**	**−51.5**	**(−98.1,−4.9)**
Mn	**−57.2**	**(−104.3,−10.1)**	**0.017**	**−27.2**	**(−49.6,−4.8)**
Ni	12.2	(−18.8, 43.3)	0.438	19.5	(−30, 69.1)
Pb	**−58.3**	**(−97.9,−18.8)**	**0.004**	**−72.7**	**(−122, −23.4)**
Rh	**−1490.3**	**(−1767,−1214)**	**0.000**	**−1365.5**	**(−1629, −1119)**
Se	117.6	(−88.2, 323.4)	0.262	20.7	(−15.5, 56.8)
Ti	−104.6	(−312.5, 103.3)	0.323	−36.8	(−109.9, 36.4)
Zn	154.1	(−4.5, 312.8)	0.057	29.3	(−0.9, 59.5)

*
p<0.05

**
p<0.01

***
p<0.001