Assessment of placental metal levels in a South African cohort

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Abstract The placenta plays an important role in mediating the effect of maternal metal exposure on fetal development, acting as both barrier and transporter. Term-placenta metal levels serve as an informative snapshot of maternal/fetal exposure during pregnancy and could be used to predict offspring short- and long-term health outcomes. Here, we measured term-placenta metal levels of 11 metals in 42 placentas from the Soweto First 1000 days cohort (S1000, Soweto-Johannesburg, SA). We compared these placental metal concentrations with previously reported global cohort measurements to determine whether this cohort is at increased risk of exposure. Placental metals were tested for correlations to understand potential interactions between metals. Since these samples are from a birth cohort study, we also performed exploratory analyses to determine whether metal levels were associated with placenta and birth outcomes. Most S1000 placental metal levels were similar to other cohorts; however, cadmium (Cd) levels up to 50-fold lower, and essential elements nickel (Ni) and chromium (Cr) level up to 6- and 16-fold lower, respectively. Cd, Se, and Ni were associated with placenta and birth outcomes. Studies are ongoing to examine underlying mechanisms and how these developmental differences affect long-term health.

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Introduction

Metals naturally occur in the environment but can act as toxicants with adverse developmental effects, particularly neurodevelopmental and birthweight outcomes (Lawn et al. 2014; Wright and Baccarelli 2007; Parajuli et al. 2013; Luo et al. 2017; Thomas et al. 2015). Vanadium, cadmium, and lead were shown to be negatively associated with birthweight, while mercury and arsenic were associated with an increased risk for small for gestational age (SGA) (Bloom et al. 2014; Shirai et al. 2010; Hu et al. 2017; Sun et al. 2014; Xie et al. 2013). For some of these metals and others such as manganese, zinc, chromium, copper, and nickel, global industrialization in the last century has created an added burden on the environment (Tchounwou et al. 2012; Singh et al. 2011; He et al. 2005). However, even low exposure to metals is linked to adverse effects. For example, arsenic, lead, cadmium, and mercury can be hazardous at low exposure levels particularly during sensitive developmental windows or due to bioaccumulation (Tchounwou et al. 2012; Jaishankar et al. 2014; Wirth and Mijal 2010). Therefore, the role of metals as pollutants and their impact on public health is a growing concern.

Metal exposure is of particular concern during pregnancy, since maternal exposure to harmful levels is linked to adverse fetal/birth outcomes (Luo et al. 2017; Shirai et al. 2010; Arbuckle et al. 2016). On the other hand, maternal deficiency of essential metals (e.g., zinc) has also been linked to adverse pregnancy outcomes and prenatal development resulting in SGA babies, intrauterine growth retardation, or reduced birth weight (Shah and Sachdev 2006; Keen et al. 1998; Mariath et al. 2011). However, a systematic review of the literature reported conflicting results between studies and further analyses are required to elucidate the full impact on human development (Chaffee and King 2012). The placenta likely plays an important role in mediating the effects of metals on fetal development since it serves as the interface between mother and child, supplying essential elements and acting as a barrier to harmful elements. Differences in harmful or essential metal levels could alter the capacity of the placenta to be an effective transporter and barrier through changes in placental formation, function, and pathology (Donnelly and Campling 2016; Saenz et al. 2013; Vaughan et al. 2011; Cooke 2014; Mattison 2010). Furthermore, differences in transplacental transport of metals to expose the fetus may directly impact fetal development. However, this remains poorly characterized for most metals (Myllynen et al. 2005).

This is the first study to measure placental metal levels in a South African population. Our samples are from a cohort located in a region of South Africa with a history of mining for gold, uranium, manganese, platinum, and copper (Statistics South Africa 2017). To characterize the placental metal levels in these samples, we investigated the risk of metal exposure and interactions between metals, by comparing placental metal levels with previous placental measurements and testing for correlations between the metals. We assessed eleven metals having differing levels of transport through the placenta—cadmium (Cd), which can accumulate in the placenta (Esteban-Vasallo et al. 2012), while arsenic (As), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) are transferred to the fetus (Punshon et al. 2015; Ziaee et al. 2007; Hardman et al. 2007; Takser et al. 2004; Gundacker and Hengstschläger 2012; Odland et al. 1997; Shennan 1988; Ford 2004). We also assessed palladium (Pd) and platinum (Pt) levels for which placental transport has not been determined. Previous work in this population measured metal levels in maternal and fetal blood. Lead, arsenic, and selenium levels were found to be correlated between maternal and fetal blood samples, suggesting that maternal exposure is reflective of fetal exposure for certain metals (Rudge et al. 2009). Due to the potential for metal exposure to affect fetal development, we also assessed relationships between the metal levels and placenta and birth outcomes.

Although not the direct topic of this study, it is important to note that S1000 samples assayed here are enriched for pregnancies with gestational diabetes mellitus (GDM), a metabolic disorder diagnosed during pregnancy. GDM is linked to changes in placental physiology such as altered maturity and branching of placental villi, which could explain the altered nutrient transport seen in GDM placentas (Taricco et al. 2009; Daskalakis et al. 2008; Cvitic et al. 2014; Schäfer-Graf et al. 1998; Gauster et al. 2012; Castillo-Castrejon and Powell 2017). GDM is also linked to increased placenta size likely caused by longitudinal vascular growth and enhanced branching angiogenesis (Taricco et al. 2009; Gauster et al. 2012; Edu et al. 2016; Daskalakis et al. 2008), possibly in response to the increased oxygen needs of the fetus in a hyperglycemic environment (Babawale et al. 2000; Jirkovská et al. 2002; Leach and Mayhew 2005).

Methods

Sample population

Forty-two de-identified placenta samples and matched maternal, placental, and infant phenotype data were obtained from the Soweto First 1000 Days Pregnancy Cohort (S1000, MRC Developmental Pathways for Health Research Unit at the University of Witwatersrand). S1000 is a pregnancy cohort consisting of women of African descent located in Soweto, South Africa. Soweto is a highly transitioned poor urban area southwest of Johannesburg and is enclosed by dormant gold mine dumps. S1000 participants with gestational diabetes mellitus (GDM) had access to treatment at a tertiary hospital. The 42 samples were selected to be HIV-negative, half GDM ($n = 21$) and half nonGDM ($n = 21$), and equal proportions of non-obese and obese in each disease group. For our sample group, 2 participants reported smoking before or during pregnancy and 2 reported alcohol consumption during pregnancy.

Placenta sampling

Placentas were weighed at delivery and samples were collected within 1 h of delivery. Tissue punches selected from this study were taken from the fetal side of the placenta disc, avoiding the umbilical cord and at least 3 cm from the edge of the placenta. Blood was not removed from the tissue, but care was taken to avoid any visible lesions as well as areas that look distinctly abnormal. Tissue punches were stored at − 80 °C until use (The INTERBIO-21st Consortium 2012).

Trace metal measurement

Placental trace metal analysis was performed by the University of North Carolina Biomarker Mass Spectrometry Core Facility. In brief, placental samples were digested with 70% nitric acid at room temperature for 5 h before incubation at 85 °C overnight. Samples were cooled to room temperature and 30% hydrogen peroxide was added followed by an additional incubation at 85 °C for 24 h. Samples were then diluted to 4 mL with deionized water and total concentrations of As, Cd, Cr, Cu, Pb, Mn, Ni, Pd, Pt, Se, and Zn were measured using Agilent Technologies 7500cx inductively coupled plasma mass spectrometer (ICP-MS), (Santa Clara, CA USA). External calibration and quality control standards were prepared from National Institute Standards Technology (NIST) traceable solutions (High Purity Standards, Charleston, SC, USA) (Laine et al. 2015).

For placental metal levels comparisons with previous cohorts, concentrations from previous studies that were reported relative to dry weight of placenta were converted to approximate wet weights by dividing dry weight by a conversion factor of 6.285 representing the average ratio of dry/wet weights, as previously reported (Iyengar and Rapp 2001).

Data transformations and variable score calculations

To normalize distributions, Cd, Cr, Cu, Pb, Mn, Ni, Pd, and Pt were log10 transformed before analyses. As, Se, and Zn were normally distributed and therefore not log transformed. Placenta weight *Z*-scores were calculated using means and standard deviations of placenta weight from pregnancies matched for gestational age (GA) and offspring sex, as previously described (Almog et al. 2011). Birth weight and length *Z*-scores were calculated using the Intergrowth 21st Neonatal Size Calculator for newborn infants between 24 and 42 weeks gestation (Intergrowth 21st 2017). The ratio of birthweight to placenta weight [birthweight (g)/placenta weight (g)] was calculated as an indicator of placenta efficiency (Hayward et al. 2016). Ponderal index [weight (g) × 100/[height (cm)] was calculated as an indicator of newborn adiposity (Armangil et al. 2011). Socioeconomic status (SES) scores were created using a principal component analysis on household access to electricity, ownership of a television, refrigerator, personal computer, bicycle, vehicle, and the number of household rooms following the International Wealth Index (Smits and Steendijk 2015). From the principal component analysis, the first component explained the most variance in the sample population and was chosen as the SES indicator (mean ± STD, $-1.17 \times 10^{-8} \pm 1.7$; median, 0.168; range, − 7.35–1.86).

Statistical analyses

A two-tailed *t* test or chi-square tests were used to test for differences between GDM status for each outcome and covariate. Multivariate regression was used to test for associations between GDM status and metal levels. Spearman's correlation was used to assess correlations between untransformed placental metal levels.

Linear regression models were run using STATA 15 (StataCorp, TX), to assess the association between placental metal levels and pregnancy outcomes: placenta weight *Z*-score, placenta efficiency, birthweight *Z*-score, ponderal index, and birth length *Z*-score. All models were adjusted for GDM, maternal age, maternal BMI, gestational age, parity, offspring sex, and socioeconomic status (SES). A significance threshold of $p <$ 0.05 was used for all models

Sensitivity analyses were conducted to identify major outliers in outcome variables, defined as individual data points that alone substantially influenced the significance of the results. As a result, we removed one outlier from the ponderal index dataset reducing sample size for ponderal index to 41. Sensitivity analyses were also conducted for maternal smoking status and showed no differential effect on the associations.

Results

Maternal and placenta clinical characteristics and birth outcomes

Placentas from forty-two pregnancies were selected from the S1000 cohort. Table 1 describes the maternal, placental, and birth outcome characteristics from these pregnancies. S1000 was enriched for gestational diabetes mellitus (GDM) pregnancies; therefore, we selected an equal number of GDM and nonGDM samples. GDM samples were on average from pregnancies with slightly higher maternal age (p = 0.002), maternal parity (p = 0.003), and proportion of male offspring (p = 0.0001) but did not significantly differ for other maternal, placental, or birth outcomes.

Table 1 Characteristics of the 42 mother-infant dyads from the S1000 Days cohort

	Mean ± SD/(%) of total	Range
Maternal characteristics (n=42)		
Maternal BMI (first trimester, kg/m^2)	29.4 ± 6.8	18.2–48
Maternal age (years)	30.2 ± 5.6	19–43
Gestational age (weeks)	38.4 ± 2.0	33–41
Parity (no. of full-term pregnancies)	1.2 ± 0.9	0–4
Mode of Delivery (% cesarean)	59%	*n.a.*
Placenta outcomes (n=33)		
Placenta weight (g)	478.7 ± 89.7	290–628
Placenta efficiency (grams fetus/gram placenta)	6.5 ± 1.0	4.6–8.9
Birth outcomes (n=42)		
Birth weight (kg)	3.1 ± 0.5	1.9–4.1
Birth length (cm)	48.4 ± 3.3	40.7–55.2
Ponderal index (g × 100/cm^3)	2.7 ± 0.3	1.8–4.1
Sex (% female)	38%	*n.a.*

n.a., not applicable

Placental levels of Cd, Cr, and Ni are lower in S1000 compared with other cohorts

To infer potential risk of exposure to metals during pregnancy in the S1000 pregnancy cohort, we compared S1000 placental levels of eleven metals (As, Cd, Cr, Cu, Pb, Mn, Ni, Pd, Pt, Se, and Zn) with previously described cohorts from other geographical locations (Table 2). This is necessary since reference doses and/or recommended exposure limits for placental metal levels have not yet been determined since the direct health risk associated with different levels of placental metals is unclear. Most of the metals exhibited similar or lower levels in S1000 compared with other populations. Cd, Cr, and Ni levels in S1000 were substantially lower compared with other reports (Table 2). For similarly measured wet weight concentrations, S1000 Cd levels were ~ 50-fold lower than a Chinese cohort (Guo et al. 2010); Cr levels were ~ 8- and 16-fold lower than a Turkish and Chinese cohorts, respectively (Guo et al. 2010; Arica et al. 2013); and Ni levels were ~ 6- and 2-fold lower than a Turkish and Chinese cohorts, respectively (Guo et al. 2010; Arica et al. 2013) (Table 2). S1000 As, Cu, Pb, Mn, Se, and Zn levels were mostly similar (less than 2-fold different) to other populations, while no reported data could be found for Pd and Pt (Table 2). The directionality of these comparative results remained the same even when GDM samples were excluded from the S1000 dataset.

S1000 placental metal levels show several strong positive correlations between metals

To better understand the potential interactions between metals, we assessed correlations between metal levels. We detected strong positive correlations ($r > 0.70$) between Pd and Pt and Se and Zn (r = 0.836 and 0.908, respectively) (Fig. 1). Moderate correlations ($0.50 < r < 0.70$) were detected between Cd and Zn, Cr and Ni, Cu and Pb, Cu and Mn, Pb and Pd, Mn and Pd, and Mn and Se (r = 0.579, 0.652, 0.630, 0.672, 0.551, 0.575, and 0.509, respectively) (Fig. 1). All other significant correlations found were considered weakly correlated ($r <$ 0.5). No significant negative correlations were found.

Table 2 Placental metal levels for 42 placental samples from S1000 Days South African cohort compared with previous studies

Trace metal	*N*	Arithmetic mean ± SD	Median	Range	Reports from other cohorts (approximate values)				
					Location	Mean	Median	Range	Reference
Arsenic (ppt)	42	1685.7 ± 661.9	1662	537–3351	USA	NR	*760.0*	*10.0*–**18,350.0**	Punshon et al. (2015)[1]
					Mexico	**2600.0**	NR	*500.0*–**6000.0**	Diaz-Barriga et al. (1995)[1]
					Multiple	**6000.0**	NR	**3000.0–12,000.0**	Iyengar and Rapp (2001)[2]
Cadmium (ppb)	38	2.5 ± 1.5	2.1	0.5–6.9	Sweden	NR	**5.2**	**1.1–19.1**	Osman et al. (2000)[1]
					China	NR	**104.2**	**2.3–393.5**	Guo et al. (2010)[1]
					Italy	NR	**5.1**	**2.1–28.6**	Roverso et al. (2015)[2]
Chromium (ppb)	41	26 ± 36.2	14.6	3.9–159.7	Turkey	**220.7**	NR	NR	Arica et al. (2013)[1]
					China	NR	**228.4**	**83.5–6,638.9**	Guo et al. (2010)[1]
					Italy	NR	**20.7**	*2.4*–**300.7**	Roverso et al. (2015)[2]
Copper (ppb)	42	1318.1 ± 809.1	1107	363–5129	USA	**1598.0**	NR	NR	Karp and Robertson (1977)[1]
					Sweden	NR	*953.0*	**635.5**–*1270.9*	Osman et al. (2000)[1]
					Italy	NR	*795.5*	**588.7**–*4932.4*	Roverso et al. (2015)[2]
Lead (ppb)	42	21.5 ± 17.9	15.9	4.4–92.4	Sweden	NR	*5.4*	*0*–**130.5**	Osman et al. (2000)[1]
					China	NR	**165.8**	**4.5–3,176.1**	Guo et al. (2010)[1]
					Multiple	**34.0**	NR	**5**–*60*	Iyengar and Rapp (2001)[2]
Manganese (ppb)	42	110.6 ± 68.1	91.3	48–439.8	USA	**115.0**	NR	NR	Karp and Robertson (1977)[1]
					Sweden	NR	*65.9*	*35.7–280.2*	Osman et al. (2000)[1]
					Italy	NR	*52.5*	*11.8*–**795.5**	Roverso et al. (2015)[2]
Nickel (ppb)	40	20.3 ± 50.9	6.7	1.9–303.6	Turkey	**124.2**	NR	NR	Arica et al. (2013)[1]
					China	NR	**14.3**	*1.76*–**593.7**	Guo et al. (2010)[1]
					Multiple	**36.0**	NR	**9**–*62*	Iyengar and Rapp (2001)[2]
Palladium (ppb)	42	21.5 ± 11.9	17.9	7.0–55.4	NR	NR	NR	NR	NR
Platinum (ppt)	42	754.5 ± 743.2	521	174–4784	NR	NR	NR	NR	NR
Selenium (ppb)	42	147.7 ± 37.3	150.9	56–220.5	Sweden	NR	**189.0**	**157.9–260.6**	Osman et al. (2000)[1]
					Croatia	NR	150.0	**100.0–240.0**	Klapec et al. (2008)[1]
					Italy	NR	*100.2*	*55.7–151.2*	Roverso et al. (2015)[2]
Zinc (ppm)	42	7.8 ± 2.0	7.9	3.1–11.4	USA	**10.2**	NR	NR	Karp and Robertson (1977)[1]
					Sweden	NR	**10.5**	**7.9–18.3**	Osman et al. (2000)[1]
					Italy	NR	**8.1**	*1.4*–**15.9**	Roverso et al. (2015)[2]

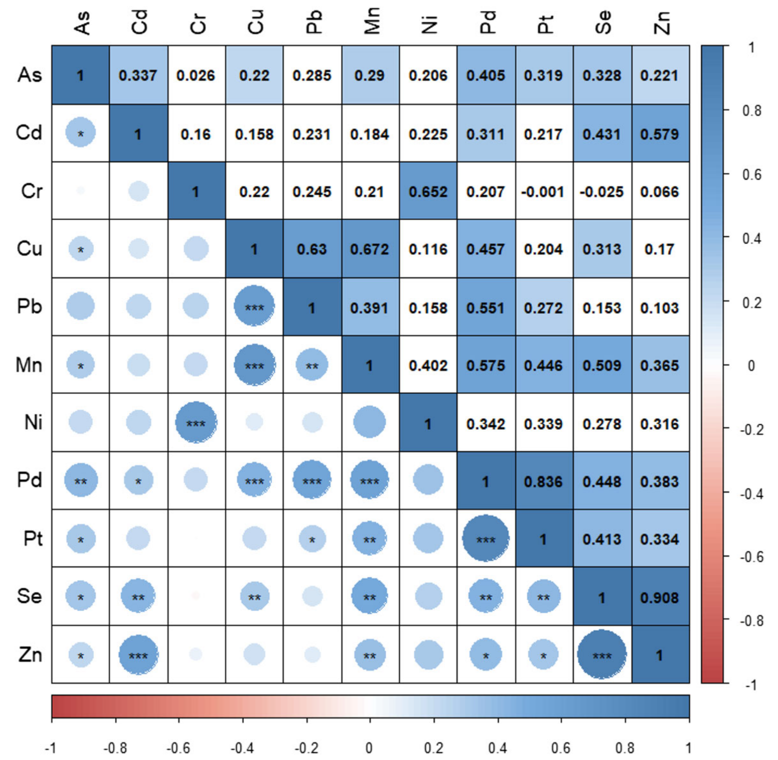
Italics indicates lower levels and boldface indicates higher levels reported for other cohorts compared with S1000. Concentration relative to wet weight[1] or dry weight converted to wet weight[2] using conversion factor of 6.285 as previously described (Iyengar and Rapp 2001). *NR*, not reported

S1000 placental levels of Se, Cd, and Ni are associated with placental and/or birth outcomes

To determine whether placental levels of the eleven metals assessed in S1000 are associated with offspring developmental outcomes, we used measurements of placenta weight *Z*-score and placenta efficiency (defined as the ratio of fetal to placental weight) as placental outcomes; and birth weight *Z*-score, birth length *Z*-score, and ponderal index as birth outcomes. For placental outcomes, Se was significantly negatively associated with placenta weight *Z*-score (Table 3), such that every 1-unit decrease of Se was associated with a 7.74 × 10^3-unit increase in placenta weight *Z*-score. Cd was significantly positively associated with placenta efficiency (Table 3), such that every log transformed-unit increase of Cd was associated with a 1.06 increase in placenta efficiency (Table 3).

For birth outcomes, Ni placental levels were negatively associated with ponderal index (Table 4), such that every log transformed-unit decrease in Ni was associated with a 0.0995-unit (g × 100/cm^3) increase in ponderal index (Table 4). None of the metal levels were associated with birth weight or birth length *Z*-score after adjustments for covariates (Table 4).

All regression models were adjusted for GDM, which would remove any effect of GDM on birth outcomes. To test separately whether there could be a causal effect of



	As	Cd	Cr	Cu	Pb	Mn	Ni	Pd	Pt	Se	Zn
As	1	0.337	0.026	0.22	0.285	0.29	0.206	0.405	0.319	0.328	0.221
Cd	*	1	0.16	0.158	0.231	0.184	0.225	0.311	0.217	0.431	0.579
Cr			1	0.22	0.245	0.21	0.652	0.207	-0.001	-0.025	0.066
Cu	*			1	0.63	0.672	0.116	0.457	0.204	0.313	0.17
Pb				***	1	0.391	0.158	0.551	0.272	0.153	0.103
Mn	*			***	**	1	0.402	0.575	0.446	0.509	0.365
Ni			***				1	0.342	0.339	0.278	0.316
Pd	**	*		***	***	***		1	0.836	0.448	0.383
Pt	*				*	**		***	1	0.413	0.334
Se	*	**		**		**		**	**	1	0.908
Zn	*	***				**		*	*	***	1

Fig. 1 Correlations between placental metal levels. Spearman correlation was used to assess the correlation between untransformed placental metal levels. *R* values are shown in the top-right portion of the graph. N = 38–42. Only the statistically significant correlations are denoted in the lower-left portion of the graph (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). All r values with light blue colors had weak correlations ($-0.2 < r < 0.2$). The color scheme denotes the strength of the r values, with positive correlations in blue and negative correlations in red

metal exposure on GDM, we used the placental metal levels as a proxy for maternal exposure and tested the association between metal levels as a predictor and GDM status as an outcome. No significant associations were found.

Discussion

We have assessed the levels of eleven metals in a subset of the S1000 pregnancy cohort of Soweto-Johannesburg, South Africa, compared the levels with previous populations, and determined relationships with birth and placental outcomes. To the best of our knowledge, none of the previous populations used for comparison were specifically reported to have known/suspected increased risk of exposure or deficiency. Although we propose that the placental metal levels reflect differences in environmental exposure levels, unrelated differences in intrinsic features of the population such as placental metabolism or transport of metals, or technical differences in how the metals were measured, may also play a role. Nonetheless, this comparison remains a valuable assessment to infer high vs. low risk of metal exposure vs. deficiency where limited data are available.

Cd is the only metal we measured known to accumulate in the placenta (Esteban-Vasallo et al. 2012). Our samples had substantially lower levels of Cd in comparison with previous populations and were moderately correlated with Zn levels (r = 0.579, Fig. 1). This relationship between Cd and Zn in the placenta was previously reported in a Ukrainian population, although it was a weak correlation (r = 0.26) (Zadorozhnaja et al. 2000). Cd is known to interact with essential metals, like zinc, by competitively binding to metal-binding proteins named metallothioneins (Brzóska and Moniuszko-Jakoniuk 2001). Cd has been shown to accumulate in the liver and kidneys leading to increased retention of Zn in those same organs (Brzóska and Moniuszko-Jakoniuk 2001). The positive correlation between Cd and Zn in the placenta may be due to this interaction previously seen in other organs and is particular cause for concern during pregnancy. Zn supplementation in mice was shown to cause a > 30% reduction of kidney and liver Cd levels (Pabis et al. 2018), perhaps Cd exposure during pregnancy could be mitigated by Zn supplementation. Interestingly, S1000 samples exhibited a positive association with placenta efficiency. This may be the result of more efficient placenta having higher barrier function, such that more efficient

Table 3 Association between placental metal levels and placenta outcomes ($n = 33$)

Metal measured in placenta	Placenta weight *Z*-score		Placenta efficiency	
	Unadjusted β (*p* value)	Adjusted β (*p* value)	Unadjusted β (*p* value)	Adjusted β (*p* value)
Arsenic	− 0.000219 (0.199)	− 0.000142 (0.475)	0.000168 (0.528)	0.000062 (0.846)
Cadmium[1]	− 0.328 (0.143)	− 0.464 (0.105)	**0.842** **(0.011*)**	**1.06** **(0.011*)**
Chromium[1]	0.0886 (0.602)	− 0.0757 (0.707)	0.125 (0.634)	0.234 (0.461)
Copper[1]	− 0.0773 (0.753)	− 0.182 (0.519)	− 0.0437 (0.908)	− 0.0755 (0.867)
Lead[1]	0.0702 (0.703)	0.109 (0.634)	− 0.315 (0.264)	− 0.569 (0.109)
Manganese[1]	− 0.152 (0.612)	− 0.0138 (0.968)	− 0.0774 (0.868)	− 0.38 (0.484)
Nickel[1]	− 0.0205 (0.898)	− 0.125 (0.474)	0.206 (0.416)	0.289 (0.334)
Palladium[1]	− 0.264 (0.194)	− 0.254 (0.259)	0.446 (0.154)	0.349 (0.331)
Platinum[1]	**− 0.353** **(0.040*)**	− 0.306 (0.108)	0.489 (0.067)	0.376 (0.219)
Selenium	**− 0.00712** **(0.023*)**	**− 0.00774** **(0.044*)**	0.00749 (0.129)	0.00838 (0.181)
Zinc	− 0.000116 (0.051)	− 0.000108 (0.125)	0.000115 (0.220)	0.000104 (0.364)

[1] log-transformed

Asterisks & Boldface indicate statistically significant associations (p<0.05)

Adjusted for GDM, maternal age, gestational age, maternal BMI, parity, offspring sex, and SES

placentas accumulate higher levels of Cd with increased environmental exposure.

We found lower S1000 placental levels of essential elements Cr, Ni, and Zn compared with other reports, which may indicate that this population is at risk for deficiency. Maternal deficiency of Se and Zn was previously shown to be associated with increased risk for preterm birth and SGA (Iyengar et al. 1978; Ward et al. 1987). We could not test for these outcomes specifically here but did show that Se was negatively associated with placenta weight *Z*-score. Se and Cd were both associated with placenta outcomes and significantly correlated (Fig. 1), which may be reflective of the retention effects of Cd on essential nutrients. Despite a strong correlation between Se and Zn levels (Fig. 1), Zn was not associated with any outcomes. Ni levels were positively correlated with Cr levels ($r = 0.652$, Fig. 1). No current studies have shown this interaction between Ni and Cr in the placenta; however, Cr was recently shown to be positively correlated to the essential element Mn (Freire et al. 2019) but this correlation was not significant in our study. In the case that our findings reflect maternal deficiency in both Cr and Ni, this population should be studied further as Ni deficiency in animal models has also been shown to affect development including reduced birth weight, decreased weight gain, and increased risk of preweaning mortality (Anke et al. 1978). In S1000, Ni was negatively associated with ponderal index. The relationships found here may reflect adverse effects of inadequate levels of these metals on development; however, further studies are required to fully elucidate these effects.

Conclusions

This study provides an important examination of placental metal levels in a previously unassessed South African cohort and gives some preliminary evidence suggesting a link with

Table 4 Association between placental metal levels and birth outcomes (*n* = 42)

Metal measured in placenta	Birth weight *Z*-score		Birth length *Z*-score		Ponderal index (n = 41)	
	Unadjusted β (*p* value)	Adjusted β (*p* value)	Unadjusted β (*p* value)	Adjusted β (*p* value)	Unadjusted β (*p* value)	Adjusted β (*p* value)
Arsenic	− 0.000289 (0.182)	− 0.000116 (0.569)	− 0.000018 (0.964)	0.00028 (0.521)	− 0.0000487 (0.471)	− 0.0000759 (0.326)
Cadmium[1]	− 0.397 (0.110)	− 0.189 (0.459)	− 0.734 (0.126)	− 0.588 (0.286)	0.0493 (0.554)	0.035 (0.720)
Chromium[1]	0.2151 (0.176)	0.166 (0.228)	0.458 (0.113)	0.397 (0.184)	**− 0.106 (0.025*)**	− 0.098 (0.056)
Copper[1]	− 0.229 (0.452)	− 0.484 (0.081)	− 0.561 (0.311)	− 0.873 (0.146)	0.091 (0.314)	0.0905 (0.383)
Lead[1]	− 0.193 (0.385)	− 0.234 (0.234)	− 0.161 (0.692)	− 0.265 (0.535)	− 0.0371 (0.577)	− 0.0278 (0.703)
Manganese[1]	− 0.155 (0.639)	− 0.196 (0.516)	− 0.230 (0.702)	− 0.411 (0.527)	0.0286 (0.771)	0.0409 (0.713)
Nickel[1]	0.203 (0.131)	0.196 (0.071)	0.456 (0.059)	0.423 (0.082)	**− 0.114 (0.004*)**	**− 0.0995 (0.016*)**
Palladium[1]	− 0.0196 (0.943)	− 0.0914 (0.703)	− 0.182 (0.717)	− 0.300 (0.561)	− 0.0835 (0.332)	− 0.0865 (0.351)
Platinum[1]	− 0.159 (0.481)	− 0.148 (0.456)	− 0.349 (0.397)	− 0.388 (0.363)	− 0.0381 (0.589)	− 0.0506 (0.517)
Selenium	− 0.00728 (0.055)	− 0.00549 (0.156)	**− 0.0152 (0.026*)**	− 0.0145 (0.080)	0.00165 (0.152)	0.0012 (0.412)
Zinc	**− 0.000147 (0.039*)**	− 0.0000754 (0.290)	**− 0.000309 (0.016*)**	− 0.000272 (0.072)	0.0000323 (0.140)	0.0000276 (0.313)

[1] log-transformed

Asterisks & Boldface indicate statistically significant associations (p<0.05)

Adjusted for GDM, maternal age, gestational age, maternal BMI, parity, offspring sex, and SES

birth outcomes. Comparisons with findings in other cohorts suggest that S1000 may be potentially deficient for Cr, Ni, and Zn. Further studies should investigate the roles of these important metals in maternal and child health.

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Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions We declare that this study was conceptualized and designed by FI with input from LA and LM. Cohort recruitment and management and sample collection and selection was supervised by SN. Sample preparation for metal measurements was supervised by FI. Data QC and statistical analyses were performed by LM under FI supervision with input from LA. Manuscript was drafted and revised by LM and FI with input from LA and SN. All authors have seen and approved the final version of the manuscript.

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Compliance with ethical standards

Ethics approval The work reported here was conducted in accordance with The Code of Ethics of the World Medical Association, approved by the Human Research Ethics Committee

(Medical) at the University of the Witwatersrand, and reviewed and cleared by the Office of Human Research Ethics at the University of North Carolina at Chapel Hill.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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