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Toxic metals and essential trace elements in placenta and their relation to placental function

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ABSTRACT

Introduction: Placental function is essential for fetal development, but it may be susceptible to malnutrition and environmental stressors.
Objective: To assess the impact of toxic and essential trace elements in placenta on placental function.
Methods: Toxic metals (cadmium, lead, mercury, cobalt) and essential elements (copper, manganese, zinc, selenium) were measured in placenta of 406 pregnant women in northern Sweden using ICP-MS. Placental weight and birth weight were obtained from hospital records and fetoplacental weight ratio was used to estimate placental efficiency. Placental relative telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) were determined by quantitative PCR (n = 285). Single exposure-outcome associations were evaluated using linear or spline regression, and joint associations and interactions with Bayesian kernel machine regression (BKMR), all adjusted for sex, maternal smoking, and age or BMI.
Results: Median cadmium, mercury, lead, cobalt, copper, manganese, zinc, and selenium concentrations in placenta were 3.2, 1.8, 4.3, 2.3, 1058, 66, 10626, and 166 μg/kg, respectively. In the adjusted regression, selenium (>147 μg/kg) was inversely associated with placental weight (B: −158; 95 % CI: −246, −71, per doubling), as was lead at low selenium (B: −23.6; 95 % CI: −43.2, −4.0, per doubling). Manganese was positively associated with placental weight (B: 41; 95 % CI: 5.9, 77, per doubling) and inversely associated with placental efficiency (B: −0.01; 95 % CI: −0.019, −0.004, per doubling). Cobalt was inversely associated with mtDNAcn (B: −11; 95 % CI: −20, −0.018, per doubling), whereas all essential elements were positively associated with mtDNAcn, individually and joint.
Conclusion: Among the toxic metals, lead appeared to negatively impact placental weight and cobalt decreased placental mtDNAcn. Joint essential element concentrations increased placental mtDNAcn. Manganese also appeared to increase placental weight, but not birth weight. The inverse association of selenium with placental weight may reflect increased transport of selenium to the fetus in late gestation.

1Introduction

During pregnancy, the fetal development is largely influenced by the placenta, a highly vascularized and complex maternal-fetal organ with a life span of about 280 days. It ensures the transport of energy and nutrients to the fetus and waste products back to the mother. Both low and

Abbreviations: AIC, Akaike information criterion; BKMR, Bayesian kernel machine regression; BMI, body mass index; DAG, directed acyclic graph; GAM, generalized additive model; ICP-MS, inductively coupled plasma-mass spectrometry; LOD, level of detection; mtDNAcn, mitochondrial DNA copy number; NICE, Nutritional impact on Immunological maturation during Childhood in relation to the Environment; PIP, posterior inclusion probability; qPCR, quantitative polymerase chain reaction; SD, standard deviation; TL, telomere length.

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high placental weight and placental dysfunction have been associated with adverse perinatal outcomes (McNamara et al., 2014). Birth weight is closely linked to placental weight (Pathak et al., 2010; Sanin et al., 2001), and the relationship between them, i.e., the fetoplacental weight ratio, is often used as a crude proxy for placental efficiency, particularly the placenta's ability to transfer nutrients to the fetus (Hayward et al., 2016).

Some toxic metals are known to accumulate in the placenta and/or affect fetal growth (Caserta et al., 2013; Fagerstedt et al., 2015; Herlin et al., 2019; Kippler et al., 2010; Osman et al., 2000; Gustin et al., 2020). However, there is limited and inconclusive data on the impact of toxic metals on placental size and efficiency. In a couple of studies, placenta cadmium concentrations have been reported to be inversely associated with placental weight and positively associated with placental efficiency (Mikelson et al., 2019; Punshon et al., 2019), especially at low zinc and selenium concentrations (Punshon et al., 2019). No association has been observed with placenta lead (Mikelson et al., 2019; Punshon et al., 2019) or mercury concentration (Punshon et al., 2019), and the impact of placental cobolt has not been assessed.

The length of the telomeres, located at the end of the chromosomes, serves as a cellular clock (Kohlrausch and Keefe, 2020). The large number of cell divisions required for placental growth leads to high telomere attrition rates (Sabharwal et al., 2018; Phillippe, 2022), and when a critical telomere length (TL) is reached, it results in replicative senescence and eventually cell death (Shay and Wright, 2019; Phillippe, 2022; Biron-Shental et al., 2010; Kohlrausch and Keefe, 2020). Shorter placental TL have been observed in obstetrical complications such as preeclampsia and intrauterine growth restriction (Kohlrausch and Keefe, 2020; Manna et al., 2019). Placental growth and maintenance require much energy and, consequently, mitochondria are enriched in the placenta (Joo et al., 2021; Sultana et al., 2017). Mitochondria have their own small circular DNA [mitochondrial DNA (mtDNA)] and aberrations of mtDNA, such as point mutations, deletions, and lower copy number may lead to decreased mitochondrial function (Kopinski et al., 2021) and decerased placental function.

Metal-related oxidative stress, as well as an unbalanced intake of essential trace elements, may cause shorter TL and lower mtDNA copy number (mtDNAcn) (Herrera-Moreno et al., 2023; Ryva et al., 2023; Freitas-Simoes et al., 2016; Priliani et al., 2019; Vahter et al., 2020; Herlin et al., 2019). However, studies of toxic metals and essential elements in placenta with placental TL and mtDNA are either very limited or lacking. Placental cadmium has been inversely correlated with placental TL (Lin et al., 2013), and placental concentrations of manganese, zinc, and selenium have been positively associated with placental mtDNAcn (Aparicio et al., 2023).

In the present study we tested the hypotheses that toxic metals (cadmium, lead, mercury, and cobalt) and essential trace elements (copper, manganese, zinc, and selenium) in placenta may affect placental weight, efficiency, TL, and/or mtDNA, either separately or joint, and that associations of the toxic metals with these outcomes may depend on the concentrations of the essential elements. The placental ability to adapt or respond to environmental stress may differ between the sexes (Rosenfeld, 2015) and, therefore, we also explored differences by fetal sex.

2. Materials and methods

2.1. Study area and participants

This study was performed using samples and data from the Swedish birth cohort "Nutritional impact on Immunological maturation during Childhood in relation to the Environment" (NICE; ClinicalTrials.gov Identifier: NCT05809479), established in the catchment area of Sunderby Hospital in Norrbotten county between 2015 and 2018 (Barman et al., 2018). The inclusion criteria were participant consent, communication skills in written and spoken Swedish, residential address in the catchment area of Sunderby Hospital, and planning to give birth at Sunderby Hospital. In total, the NICE cohort consists of 655 pregnancies of which 629 were eligible pregnancies for the present study (Supplementary Fig. S1), after excluding second pregnancies (n = 18), twin pregnancies (n = 3), miscarriages and stillbirths (n = 4), and one study withdrawal (n = 1). Of these pregnancies, a placenta sample was collected at 433 deliveries. In our main analyses, we included 406 women with complete data on placental trace element concentrations, placental weight, birth weight, and relevant covariates (Supplementary Fig. S1). For 391 of the women, we compared the concentrations in placentas with those in maternal blood (erythrocyte fractions) to estimate the placental accumulation. Of the 433 placenta samples collected, 285 were analyzed for TL and mtDNAcn in placenta. The reasons for the reduced sample size were that a different DNA extraction kit was used for some samples (n = 111), which affected the TL and mtDNA analysis, and that too little or insufficient DNA quality was obtained (n = 35), and also missing data on maternal pre-pregnancy smoking (n = 2).

The study has been approved by the Regional Ethical Review Board in Umeå, Sweden, (2013-18-31 M, 2018-256-32 M) and was conducted in accordance with the Declaration of Helsinki. Informed consent from the women was obtained beforehand, and they were informed that they could drop out at any time without any further explanation and withdraw all their data from the study.

2.2. Sample collection

Placentas were collected by the study nurses at delivery and placed in plastic bags and stored at 4 °C until processed at the research laboratory of Sunderby Hospital the same day (if weekend delivery, on the following workday). A triangular cross-section (formed as a pie slice), taken from the cord insert to the outer edge of the placenta, was sampled using a metal-free ceramic knife, placed into a plastic bag, and stored at −80 °C (Barman et al., 2018), until transported frozen to Karolinska Institutet, Stockholm. The samples were thawed, the chorion and decidua were removed and a biopsy of about 5 cm^3 villous parenchyma tissue (fetally derived tissue) was sampled in the middle of the larger placental slice, using an acid-washed cutting board and a ceramic knife. About one third of the biopsy was used for DNA extraction and the other part for trace element analysis. The samples were then stored at −80 °C until analysis.

Venous blood samples were collected from the women during a visit to the local maternity health clinic around gestational week 29 (mean: 29; range: 24–36), using a 6 mL trace element-free sodium heparin tube (Greiner bio-one, Kremsmünster, Austria) (Barman et al., 2018; Gustin et al., 2020). Blood samples were stored at the local maternity clinic at 4 °C awaiting transport to the hospital laboratory the same or following workday. Once at the hospital laboratory, the blood samples were centrifuged for 5 min at 2400 rpm (Hettich Rotina 420, Hettich Lab Technology, Tuttlingen, Germany) and separated into erythrocyte and plasma fractions. The blood fractions were frozen and stored at −80 °C.

2.3. Toxic metal and essential trace element analyses

Placental wet weight and erythrocyte concentrations of cadmium, mercury, lead, and cobalt and the essential elements copper, manganese, zinc, and selenium were measured. We did not explore potential effects of arsenic in the placenta, because we have previously shown that the women in the NICE cohort are exposed mainly to dietary arsenobetaine (Stravik et al., 2023), a non-toxic organic form of arsenic. Although cobalt is an essential part of cobalamins (vitamin B12), it was herein grouped among the toxic metals as humans cannot synthesize B12 from inorganic cobalt (Osman et al., 2021), which is the most common form of dietary cobalt (Lison, 2022), and, importantly, cobalt accumulates in placenta (Fagerstedt et al., 2015). We measured total mercury concentrations in placenta, since we have previously shown that the women in NICE are mainly exposed to methylmercury from seafood intake (Gustin

et al., 2020; Stravik et al., 2023).

All concentrations of metals and essential trace elements were measured using inductively coupled plasma mass spectrometry (ICPMS; Agilent 7900, Agilent Technologies, Tokyo, Japan) at the Institute of Environmental Medicine, Karolinska Institutet, Stockholm. Cadmium (isotope 111), cobalt (isotope 59), copper (isotope 63), manganese (isotope 55), selenium (isotope 78), and zinc (isotope 66) were measured in helium mode, whereas lead (isotope 208) and mercury (isotope 202) were measured in no gas mode. Prior to the analysis, 0.5 g of each biopsy was placed into teflon tubes together with 3 mL deionized water and 2 mL nitric acid (65 % Scharlau, Scharlab S.L Sentmenat Spain, ppb trace analysis grade) and digested using a Milestone Ultra-CLAVE microwave digestion system (EMLS, Leutkirch, Germany) (Fagerstedt et al., 2015). Subsequently, the solutions were transferred to low density polyethylene tubes and diluted with deionized water to a nitric acid concentration of 20 %.

The maternal erythrocyte samples were diluted 1:25 in an alkali solution [2 % (w/v)1-butanol, 0.05 % (w/v) EDTA, 0.05 % (w/v) Triton X-100, 1 % (w/v) NH_4OH and 20 μg/L internal standard (germanium, rhodium, lutetium, and iridium)] as described in detail elsewhere (Lu et al., 2015; Gustin et al., 2020).

The limit of detection (LOD; the standard deviation of the blank concentrations multiplied by three) for each respective element in placental tissue and maternal erythrocytes are presented in Supplementary Table S1. None of the elements had a concentration below their respective LOD, except for mercury for which two of the placenta samples, and one of the erythrocyte samples, had a concentration below LOD and therefore these concentrations were replaced by LOD/$\sqrt{2}$. As quality control, two different commercial whole blood reference materials were analyzed in each analytical run of placenta [Seronorm whole blood L-1 (1702821) and L-2 (1702825)] and maternal erythrocytes [Seronorm whole blood L-1 (1406263) and L-2 (1406264)], and in general there was a good agreement between the obtained values and the reference values (Supplementary Table S1).

2.4. Placental size and efficiency

Placental weight (g) and birth weight (g) were measured to the nearest gram by midwives after birth and obtained from hospital records. Placentas were weighed with membranes and umbilical cord attached, in accordance with clinical practice (Sunde et al., 2017). Placental efficiency was estimated as the grams of newborn per grams of placenta (Hayward et al., 2016), calculated as $\log_2$ (birth weight)/$\log_2$ (placental weight).

2.5. Measurements of TL and mtDNAcn

Qiagen DNeasy Blood & Tissue Kit (Qiagen) was used for extracting total DNA from 25 to 30 mg of villous parenchyma tissue. Each placenta sample was incubated with 180 μL of ATL buffer and 20 μL of Proteinase K, and then mixed with a rotor-stator homogenizer (TissueRuptor II, Qiagen). Lysates were then incubated for 1 h at 56 °C with thorough vortex every 15 min. Subsequently, DNA was extracted and purified following the manufacturer instruction and finally eluted two times with 100 μL of AE Buffer (Qiagen). Thermo Scientific NanoDrop One Microvolume UV–Vis Spectrophotometer (Thermo Scientific) was used to evaluate the quantity and purity of DNA. The 260 nm/280 nm absorbance ratio was ≥1.8 for all samples (mean = 1.89, range 1.82–2.01), indicating pure DNA. The DNA samples were diluted in deionized water to 20 ng/μL and stored at −80 °C until further use.

Placental relative TL and relative mtDNAcn were measured by quantitative Polymerase Chain Reaction (qPCR) based on the method developed by Cawthon (2002) with minor adjustments as described previously (Alhamdow et al., 2020; Xu et al., 2019). In short, TL and mtDNAcn (primers for the *MT-ND1* gene encoding mitochondrially encoded NADH dehydrogenase 1), and the nuclear encoded reference gene HBB (encoding hemoglobin beta) were measured from independent qPCR runs using DNA standard curves from the same pooled DNA samples, included in each run. The standard curve was prepared from a pooled DNA sample (total of eight individual placental DNA samples) at 20 ng/μL and further two-fold serial diluted with a concentration range from 20 to 0.625 ng/μL. The total PCR reaction volume per well was 10 μL (7.5 μL master mix and 2.5 μL DNA at 5 ng/μL). The master mix for the telomere assay contained 1.25 U HOT FIREPol DNA Polymerase (Solis BioDyne), 1X HOT FIREPol buffer B1 (Solis BioDyne), 2 mM $MgCl_2$ (Solis BioDyne), telomere-specific primers (900 nM of each primer, Invitrogen), 0.2 mM dNTP mix (Invitrogen), and 1.5 μM SYTO9 fluorescent dye (Invitrogen). For the *HBB* and mtDNA assays, 1x qPCRBIO SyGreen Blue Mix (PCR Biosystems) was used with *HBB*- and mtDNA-specific primers (300 nM of each primer, Invitrogen). All PCR runs were carried out in 384-well plates compatible with a LightCycler 480 II real-time PCR platform (Roche Diagnostics). All samples, including the standard curves and negative controls, were prepared in triplicates. Standard deviation (SD) for triplicate samples was accepted for cycle threshold (Ct) values at SD ≤ 0.2. The coefficient of determination (R^2) for all PCR runs was >0.95. Two internal DNA control samples were included in all PCR runs and the intra- and inter-assay coefficients of variation were 5.5 % and 7.5 % for TL and 6.3 % and 15.0 % for mtDNAcn. PCR conditions and primer sequences are summarized in Supplementary Table S2.

2.6. Covariates

Data on maternal age (years), body mass index [BMI (kg/m^2); based on body weight (kg) and height (cm) measured at registration at the maternity clinic in the first trimester], parity (number of previous parturitions), education (elementary school, high school, or university), pre-pregnancy tobacco smoking (never, sometimes, or daily), and alcohol consumption during pregnancy (never, sometimes, or daily), as well as newborn sex and gestational age at delivery (days) were extracted from hospital records. Data on preeclampsia (yes/no) was also collected from hospital records. As none of the mothers reported consumption of alcohol during pregnancy, it was not considered in the covariate selection. Placental TL has previously been shown to vary by ambient temperature (Martens et al., 2019), and therefore season of birth was classified into four seasons (spring: March–May; summer: June–August; fall: September–November; winter: December–February). The date of birth and the date of placenta sample preparation were used to calculate the time intervals (days) for which the placentas were stored in the fridge before samples for TL and mtDNA analyses were collected and frozen. We also collected information on maternal diet during pregnancy, using a semi-quantitative food frequency questionnaire administered around gestational week 34, as previously described (Stravik et al., 2019). For the present study, we included only information on maternal total intake of seafood (including marine and freshwater fish and shellfish), estimated in grams per day, from reported intake frequency and picture-based portion size.

2.7. Statistical analysis

Statistical analyses were conducted using software Stata/IC 15.0 (StataCorp, TX, USA) and R 4.1.2. P-values were considered to show statistical significance below 0.05. To investigate bivariate correlations, the non-parametric Spearman rank correlation and Kruskal-Wallis tests were used. As an attempt to assess metal and essential element incorporation in placenta, the placental trace element concentrations were divided by the maternal erythrocyte concentrations in the third trimester and explored descriptively, where a higher ratio was considered to reflect a higher placental trace element accumulation. The concentrations of metals and essential elements in placental tissue were right skewed and were therefore $\log_2$-transformed.

Linearity of associations between exposures (metals and essential

elements) and outcomes (placental weight, efficiency, TL, and mtDNAcn) was evaluated using generalized additive models (GAMs) with two degrees of freedom and based on the p-gain, defined as the difference in normalized deviance between the GAM and the linear model (Royston and Ambler, 1998). Deviation from linearity was observed for selenium in relation to placental weight (p-gain<0.05), and therefore this association was explored adding a spline knot at the turning point concentration. Three different spline knot positions (visually determined from the GAM plot) were explored and the one that gave the lowest Akaike information criterion (AIC) was kept in subsequent regression analyses (Supplementary Fig. S2). The linear and splined linear regression models were used to assess associations of single metals and essential elements in placenta (all log_2-transformed) with the four different placental outcomes.

A directed acyclic graph (DAG) was used to determine minimal sufficient covariate adjustment (i.e., the fewest number of included covariates that would block all causal pathways that may induce bias) for associations of metals and essential elements with placental weight and efficiency (Supplementary Fig. S3) and placental telomere length and mtDNA (Supplementary Fig. S4). In the DAG, arrows between variables were drawn if there was a plausible causal relationship between them and a statistically significant association was observed in the data. We explored unadjusted associations in Model 1, while Model 2 was adjusted for the minimal sufficient adjustment suggested by the DAG. For the associations with placental weight or efficiency as the outcomes, the minimal sufficient adjustment according to the DAG was for early-pregnancy BMI and infant sex, but we also included adjustment for pre-pregnancy tobacco smoking (yes/no), since it is an important source of metals, especially cadmium, and may influence placental function (Beltran-Castillo et al., 2023), and has been identified as important covariates in previous studies (Punshon et al., 2019; Zhao et al., 2020). For the associations with TL or mtDNAcn as the outcomes, the minimal sufficient adjustment was for maternal age (years) and infant sex. Tobacco smoking was again included in Model 2 based on previous studies linking smoking to these outcomes (Janssen et al., 2017; Osorio-Yanez et al., 2020). Gestational age at birth was not adjusted for in any of the models as it may be in the line of pathway for placental weight and efficiency (i.e., a mediator; Supplementary Fig. S3) or a collider in the causal pathways to TL or mtDNA (Supplementary Fig. S4).

To explore potential sex differences, we also included multiplicative interaction terms between the toxic metals or essential elements with infant sex in Model 2. In a secondary step, we stratified Model 2 by infant sex, after checking that none of the covariates included in the models (i.e., maternal BMI, age, and pre-pregnancy smoking) differed between the sex strata (p = 0.17–0.85). Due to limited power, we did not use splined selenium concentrations in these analyses.

In sensitivity analyses, models of placental mercury with the placental outcomes were further adjusted for maternal total seafood intake during pregnancy (g/day) as it has previously been shown that associations with especially contaminates originating from fish may be masked by beneficial nutrients in fish (Strain et al., 2015). Preeclampsia among the included women was not statistically associated with any of the placental outcomes or any of the placental metals or essential elements. However, because preeclampsia is strongly linked to impaired placental function, although the cause-and-effect relationship is not established (Myatt, 2002), we performed sub-analyses excluding preeclamptic women (n = 15) in all the linear regression analyses (otherwise adjusted as Model 2). Gestational age at birth was identified as a mediator (for placental weight and efficiency as outcomes) or a collider (for TL and mtDNA as outcomes) in the DAGs, but in additional models we explored the influence of additionally controlling for gestational age (also adjusted for the identified confounders).

Potential interactions between metals and essential elements in relation to the four outcomes were explored with Bayesian kernel machine regression (BKMR) (Bobb et al., 2015) using the R package *bkmr* (Bobb, 2017). Prior to the BKMR analysis, the log_2-transformed toxic metal and essential element concentrations were centered (mean-subtracted) and scaled (divided by the SD). Because BKMR is sensitive to extreme values, concentrations ±3 SD from the mean were excluded (Supplementary Fig. S1). As a sensitivity analysis, we also run all the BKMR models including extreme values. This showed that extreme values had little influence on the estimated exposure-response relationships, but diagnostic plots suggested poorer model convergence (data not shown). Thus, the following placental concentrations were omitted for placental weight or efficiency as outcomes: cadmium>15 μg/kg (n = 2), mercury<0.20 μg/kg (n = 4), lead>19 μg/kg (n = 3), copper>2071 (n = 4), and zinc<5001 (n = 1), resulting in a final sample size of 392. For TL and mtDNAcn as outcomes, the following concentrations were omitted: cadmium>15 μg/kg (n = 1), mercury<0.19 μg/kg (n = 3), lead>23 μg/kg (n = 2), copper>2222 (n = 2), and zinc<5001 (n = 1), as well as mtDNAcn<0.01 (n = 1), resulting in a final sample size of 275. Joint metal and essential trace element associations were assessed by plots. Since all the essential elements were significantly associated with mtDNA, we performed an additional BKMR including only the essential element with mtDNA as the outcome to assess the joint effect of only the essential elements (n = 281; Supplementary Fig. S1). All BKMR models were adjusted as Model 2, described above, and we applied the option of variable selection (to obtain posterior inclusion probabilities, discussed below) and 100,000 iterations (for algorithm convergence) by the Markov chain Monte Carlo algorithm. Metal and essential element interactions were assessed using plots, as was the joint micronutrient association with mtDNAcn. The relative metal and essential element importance in relation to each outcome was assessed in the BKMR by posterior inclusion probabilities (PIPs), which is a ranking measure where the highest PIP indicates the most important trace element for the given outcome (Bobb et al., 2015). In case the BKMR plots suggested interactions between any of the metals and essential elements, this was further explored in stratified linear regression. This was only observed for placental lead and selenium in relation to placental weight. Therefore, the association of lead with placental weight was stratified by median selenium concentrations in linear regression models adjusted as Model 2.

3. Results

3.1. Participant characteristics

The main general characteristics of the included and non-included mother-child dyads from the NICE cohort are presented in Table 1 The median age of the 406 mothers was 30 years (range: 20–45 years), and their median early-pregnancy BMI was 25 kg/m^2 (range: 17–50 kg/m^2). Seventy-one percent of the women had a university education and 52 % were primiparous. Only 5 % of the women reported that they smoked prior to pregnancy, and 3.7 % had preeclampsia. The median placental weight was 600 g (range: 250–1315 g). Out of the 406 newborns, 46 % were boys. The median birth weight and gestational age was 3557 g (range: 1200–5165 g) and 281 days (range: 200–298 days), respectively. Five percent of the newborns were born preterm (<37 weeks of gestation). As shown in Table S1, the women included in the analyses with placental weight or efficiency as outcomes (n = 406) were more likely to be primiparous (52 %) compared to the women who were not included (n = 223; of which 43 % were primiparous). The included women were also more likely to have given birth during the winter and spring seasons (26 and 24 % versus 17 and 21 %, respectively) and less likely to have given birth during fall (21 % versus 32 %) than excluded women. Similar differences were observed between the women included in the analyses with TL or mtDNAcn as outcomes (n = 285) and those excluded (n = 344), with the addition that the included women were more highly educated than the excluded women (74 % versus 64 % had a university degree).

Table 1
Main characteristics of the mothers and children in the two study cohort groups (for evaluation of placental weight/estimated placenta efficiency and TL/mtDNAcn, respectively), and comparisons with mothers and children that were not included in the present analyses.

Characteristics	Mother-child dyads with placental weight and efficiency		Mother-child dyads not included			Mother-child dyads with TL and mtDNAcn		Mother-child dyads not included		
Mother	n	mean ± SD or percentage	n[a]	mean ± SD or percentage	p-value	n	mean ± SD or percentage	n[c]	mean ± SD or percentage	p-value[b]
Age (years)	406	30 ± 4.8	222	31 ± 4.7	0.47	285	30 ± 4.6	343	31 ± 4.8	0.24
Early-pregnancy BMI (kg/m^2)	406	25 ± 5.0	208	25 ± 4.7	0.77	279	26 ± 4.9	335	25 ± 4.9	0.27
Education (%)[d]	404	1/28/71	216	4/30/66	0.087	284	2/24/74	346	3/32/65	***0.040***
Primiparous (% yes)	406	52	215	43	***0.025***	285	56	336	43	***0.002***
Preeclampsia (% yes)	406	3.7	215	2.3	0.36	285	5.3	336	1.5	***0.008***
Never smokers (%)[e]	406	95	212	91	0.093	285	95	333	92	0.21
Placental weight (g)	406	614 ± 133	190	615 ± 131	0.67	278	617 ± 140	318	613 ± 124	0.97
Fetoplacental weight ratio[f]	406	1.28 ± 0.03	187	1.28 ± 0.03	0.61	277	1.28 ± 0.03	316	1.28 ± 0.03	0.46
Total seafood intake (g/day)[g]	386	29 ± 20	188	31 ± 23	0.72	268	30 ± 23	306	29 ± 20	0.95
Newborn										
Sex (% boys/girls)	406	46/54	218	48/52	0.69	285	46/54	339	53/47	0.76
Season of birth (%)[h]	406	24/29/21/26	219	21/31/32/17	***0.006***	285	24/24/22/29	340	21/34/26/18	***0.001***
Gestational age (days)	406	279 ± 13	215	279 ± 11	0.28	285	279 ± 13	336	279 ± 11	0.24
Birth weight (g)	406	3583 ± 553	213	3599 ± 565	0.67	284	3559 ± 555	335	3613 ± 558	0.25

Abbreviations: BMI, Body Mass Index; mtDNAcn, mitochondrial DNA copy number; TL, telomere length.
[a] The total number of eligible participants that were excluded was 223, and lower numbers indicate missing data.
[b] Mann-Whitney *U* test (continuous characteristics variables) or Chi2 test (categorical characteristics variables) for comparison between the included and the eligible excluded mother-child dyads.
[c] The total number of eligible participants that were excluded was 344, and lower numbers indicate missing data.
[d] Elementary school/High school/University or higher education.
[e] Before pregnancy.
[f] Calculated as $\log_2$ (birth weight)/$\log_2$ (placental weight).
[g] Estmated from semi-quantitative food frequency questionnaires administered in gestational week 34.
[h] Spring (March–May), summer (June–August), fall (September–November), winter (December–February).

3.2. Toxic metals and essential elements in placenta, their intercorrelations, and associations with maternal and infant characteristics

The median concentration of cadmium, mercury, lead, and cobalt in placenta was 3.2 μg/kg, 1.8 μg/kg, 4.3 μg/kg, and 2.3 μg/kg, respectively (Table 2). The concentrations of the toxic metals in placenta were not correlated with each other besides positive correlations for cobalt with cadmium (r_s: 0.20, $p < 0.001$; Supplementary Table S3) and lead (r_s: 0.18, $p < 0.001$). The cadmium concentrations in placenta were positively correlated with all the essential elements in placenta (r_s: 0.19–0.25; $p < 0.001$), and placental mercury concentrations were weakly positively correlated with placental selenium (r_s: 0.11; $p = 0.024$). The placental lead concentrations were positively correlated with copper and manganese in placenta (r_s: 0.24 and 0.19, respectively; $p < 0.001$) and inversely correlated with selenium (r_s: −0.25; $p < 0.001$). All toxic metal concentrations in placenta were positively correlated with those in maternal erythrocytes in early third trimester (r_s: 0.31–0.81, $p < 0.001$; Table 2), lead showing the weakest correlation. Cobalt showed the highest placenta:erythrocyte concentration ratio (median: 22.5; Table 2), followed by cadmium (median: 11.3).

The median concentrations of copper, manganese, zinc, and selenium in placenta were 1058 μg/kg, 66 μg/kg, 10626 μg/kg, and 166 μg/kg, respectively (Table 2) and they were all positively correlated with each other (r_s: 0.22–0.44; $p < 0.001$; Supplementary Table S3). The concentrations of manganese, zinc, and selenium showed only weak positive correlations with those in maternal erythrocytes in early third trimester (r_s: 0.21, 0.13, and 0.20, $p < 0.001$, 0.011, and <0.001, respectively; Table 2), while no correlation was observed between

Table 2
Metal concentrations (μg/kg) in placentas at birth (n = 406) and their relation to maternal erythrocyte concentrations in the third trimester (n = 391).

Trace element	Placental concentration (μg/kg)[a]		Maternal erythrocyte concentrations (μg/kg)	Placental and maternal erythrocyte correlation[b]	Placenta/maternal erythrocyte concentration ratio
	Median (5–95th perc.)	Range	Median (5–95th perc.)	r_s (p-value)	Median
Toxic metals					
Cadmium	3.2 (1.5–7.7)	1.1–33	0.29 (0.14–0.68)	0.65 (<0.001)	11.3
Mercury	1.8 (0.51–4.4)	<0.05–7.9	1.6 (0.26–4.2)	0.81 (<0.001)	1.16
Lead	4.3 (2.0–10)	1.4–24	11 (6.2–26)	0.31 (<0.001)	0.38
Cobalt	2.3 (1.4–4.9)	1.0–6.7	0.11 (0.05–0.23)	0.40 (<0.001)	22.5
Micronutrients					
Copper	1058 (781–1539)	617–3325	779 (622–972)	0.001 (0.88)	1.37
Manganese	66 (45–100)	34–146	22 (12–37)	0.21 (<0.001)	3.05
Zinc	10626 (7577–15201)	5000–19660	9724 (7587–12091)	0.13 (0.011)	1.11
Selenium	166 (112–203)	97–269	106 (76–145)	0.20 (<0.001)	1.54

[a] Concentrations are reported per wet weight of placental tissue.
[b] Asssesed with Spearman rank correlation.

placental and erythrocyte copper. The essential element concentrations in placenta were generally similar to those in maternal blood, with manganese showing the highest placenta:erythrocyte concentration ratio (median: 3.05; Table 2).

Associations of metals and essential elements with maternal and infant characteristics are shown in Supplementary Table S4. Placental cadmium increased slightly with increasing maternal age (r_s: 0.10; p = 0.048) and decreased with maternal early-pregnancy BMI (r_s: −0.18; p < 0.001) and parity (median: 3.4 and 3.0 μg/kg; for primi- and multiparous women, respectively; p = 0.005). Placental mercury concentrations increased with maternal age (r_s: 0.11; p = 0.022), education (median: 2.0 and 1.6 μg/kg; for women with and without a university degree, respectively; p = 0.001), and increasing seafood consumption (r_s: 0.45; p < 0.001). Furthermore, placental mercury decreased with maternal BMI (r_s: −0.19; p < 0.001) and was lower among pre-pregnancy smokers (median: 1.0 μg/kg; n = 22) compared to never-smokers (median: 1.9 μg/kg; n = 384). Placental lead concentrations increased slightly with gestational age and varied by season, being highest in winter (median: 5.2 μg/kg; n = 107) and lowest in fall (median: 4.0 μg/kg; n = 84). Placentas from multiparous women contained higher cobalt concentrations (median: 2.6 μg/kg) compared to placentas from primiparous women (median: 2.2 μg/kg; p < 0.01) and placental cobalt concentrations were weakly correlated with birth weight (r_s: 0.11; 0.030). Copper was slightly higher in male placentas (median: 1.1 mg/kg; n = 187) than in female placentas (median: 1.0 mg/kg; n = 219; p = 0.032). Placental manganese increased marginally with gestational age at birth (r_s: 0.11; p = 0.027) and decreased with increasing early-pregnancy BMI (r_s: −0.17; p < 0.001). Placental selenium decreased slightly with increasing maternal BMI (r_s: −0.11; p = 0.024) and was also inversely correlated with gestational age at birth (r_s: −0.11; p = 0.029) and birth weight (r_s: −0.17; p < 0.001).

3.3. Placental outcomes

Placental weight (mean: 614 g; range: 250–1315 g) and placental efficiency (ratio of birth weight to placental weight; mean: 1.28; range: 1.15–1.36) were inversely correlated (r_s: −0.80; p < 0.001). Placental weight was not correlated with placental TL (r_s: 0.07; p = 0.28) or mtDNAcn (r_s: 0.04; p = 0.51), and placental TL (mean: 0.94; range: 0.58–1.64) and mtDNAcn (mean: 1.0; range: <0.01–3.37) were not correlated (r_s: 0.002; p = 0.97). There was a weak negative correlation between placental efficiency and TL (r_s: −0.12; p = 0.045), but not with mtDNAcn (p = 0.53). Associations between the placental outcomes and maternal or child characteristics are presented in Supplementary Table S4.

3.4. Associations of single toxic metals and essential elements with placental weight and efficiency

In the main linear regression analyses, none of the toxic metals were associated with placental weight, neither in the unadjusted nor in the adjusted models (Table 3). Manganese concentrations in placenta were positively associated with placental weight (B: 41 g per doubling; 95 % CI: 5.9, 77 g; p = 0.022) and inversely associated with placental efficiency (B: −0.012 per doubling; 95 % CI: −0.019, −0.004; p = 0.003). Selenium concentrations in placenta were non-linearly associated with placental weight, with an inverse association at concentrations above 147 μg/kg (B: −158 g per doubling; 95 % CI: −246, −71; p < 0.001), but no association below this selenium concentration. We found no significant interaction between any of the toxic metals or essential elements with infant sex in relation to either placental weight or efficiency (Table 3). Additionally adjusting the models with placental mercury for maternal fish intake reduced the already weak positive model estimate in relation to placental weight by about 90 %, while the estimate for placental efficiency was unchanged, and, thus, both associations remained statistically non-significant. Excluding preeclamptic women had essentially no impact on the associations and did not alter any of the findings (Supplementary Table S5). Additionally adjusting the models for gestational age had overall little influence on the observed association (Supplementary Table S6). However, the positive association

Table 3
Linear regression analyses (splined for selenium) of metal or essential element concentrations in placenta at birth (μg/kg; log_2-transformed) with placental weight (g) and efficiency [log_2 (g birth weight)/log_2 (g placental weight)].

	Model 1		Model 2		
	B (95 % CI)	p	B (95 % CI)	p	$p_{sex\ interaction}$
Placental weight	n = 406		n = 406		
Metals					
Cadmium	−9.9 (−28.3; 8.5)	0.29	−3.4 (−21.8; 15.1)	0.72	0.94
Lead	0.21 (−15.4; 15.9)	0.98	0.15 (−15.6; 15.9)	0.99	0.18
Mercury	−1.5 (−14.9; 12.0)	0.83	3.1 (−11.0; 17.3)	0.66	0.66
Cobalt	7.9 (−18.3; 34.1)	0.55	12.5 (−13.1; 38.0)	0.34	0.31
Essential elements					
Copper	−26.7 (−71.9; 18.5)	0.25	−30.1 (−75.9; 15.8)	0.20	0.80
Manganese	27.2 (−10.3; 64.6)	0.15	**41.2 (5.9; 76.5)**	**0.022**	0.54
Zinc	−15.9 (−62.3; 30.5)	0.50	−7.8 (−52.4; 36.8)	0.73	0.47
Selenium≤147 μg/kg[a]	71.7 (−27.6; 171)	0.20	63.1 (−32.6; 159)	0.20	0.12
Selenium>147 μg/kg[b]	**−177 (-264; 88.9)**	**<0.001**	**−158 (-246; 70.5)**	**<0.001**	0.12
Placental efficiency	n = 406		n = 406		
Metals					
Cadmium	0.003 (−0.001; 0.008)	0.10	0.002 (−0.002; 0.006)	0.39	0.88
Lead	0.002 (−0.002; 0.005)	0.36	0.001 (−0.002; 0.005)	0.45	0.56
Mercury	0.0002 (−0.003; 0.003)	0.92	−0.001 (−0.004; 0.002)	0.53	0.89
Cobalt	0.003 (−0.002; 0.008)	0.24	0.002 (−0.003; 0.007)	0.43	0.76
Essential elements					
Copper	0.003 (−0.008; 0.013)	0.63	0.003 (−0.008; 0.013)	0.64	0.87
Manganese	−0.008 (−0.017; 0.0001)	0.052	**−0.012 (-0.019; -0.004)**	**0.003**	0.82
Zinc	0.007 (−0.003; 0.017)	0.15	0.005 (−0.005; 0.014)	0.33	0.32
Selenium	0.011 (−0.00005; 0.021)	0.051	0.009 (−0.001; 0.020)	0.081	0.78

Model 1: unadjusted.
Model 2: adjusted for maternal body mass index (kg/m^2), maternal smoking before pregnancy (yes/no), and newborn sex.
[a] n = 109.
[b] n = 297.

between manganese and placental weight decreased by 22 % and was no longer statistically significant (B: 32; 95 % CI: −3.4, 67; p = 0.077), while the indicated positive association between selenium and placental efficiency increased by 22 % (B: 0.011; −0.001, 0.021; p = 0.037).

3.5. Associations of single metals and essential elements with TL and mtDNAcn in placenta

In multivariable-adjusted linear regression models, none of the toxic metals or essential elements were associated with TL (Table 4). Similarly, the toxic metals were not associated with mtDNAcn in placenta (Table 4), except for cobalt which showed an inverse association with mtDNAcn (B: −0.11 per doubling; 95 % CI -0.20; −0.02; p = 0.022). In contrast, all essential element concentrations in placenta were positively associated with mtDNAcn. The size of the effect estimates decreased in the order copper > zinc > manganese > selenium (B:0.18–0.49 per doubling). The associations of copper, manganese, and selenium in relation to mtDNAcn had p-for-sex interaction terms below 0.10 (Table 4), and these positive associations were only statistically significant among boys (Supplementary Table S7). Additionally adjusting the models with mercury for maternal fish intake had essentially no impact on the association with TL or mtDNAcn (data not shown).

Table 4
Linear regression analyses of metal and essential element concentrations in placenta at birth (log_2-transformed) with relative telomere length (TL) and mitochondrial DNA copy number (mtDNAcn) in placenta.

	Model 1		Model 2		
	B (95 % CI)	p	B (95 % CI)	p	$p_{sex\ interaction}$
TL	n = 285		n = 285		
Metals					
Cadmium	−0.015 (−0.047; 0.018)	0.37	−0.023 (−0.055; 0.010)	0.17	0.31
Lead	−0.014 (−0.048; 0.019)	0.40	−0.012 (−0.044; 0.020)	0.45	0.63
Mercury	0.016 (−0.009; 0.041)	0.21	0.011 (−0.013; 0.035)	0.37	0.22
Cobalt	0.032 (−0.014; 0.078)	0.18	0.029 (−0.016; 0.074)	0.20	0.93
Essential elements					
Copper	−0.006 (−0.11; 0.094)	0.91	0.003 (−0.094; 0.10)	0.95	0.22
Manganese	0.038 (−0.026; 0.10)	0.24	0.036 (−0.028; 0.10)	0.27	0.58
Zinc	−0.020 (−0.097; 0.057)	0.61	−0.008 (−0.081; 0.066)	0.84	0.69
Selenium	0.050 (−0.029; 0.13)	0.22	0.040 (−0.039; 0.12)	0.32	0.74
mtDNAcn	n = 285		n = 285		
Metals					
Cadmium	−0.016 (−0.083; 0.050)	0.63	−0.013 (−0.086; 0.059)	0.72	0.42
Lead	0.010 (−0.047; 0.068)	0.72	−0.004 (−0.062; 0.054)	0.89	0.94
Mercury	−0.014 (−0.069; 0.040)	0.60	−0.017 (−0.073; 0.039)	0.55	0.96
Cobalt	**−0.11 (-0.20; -0.016)**	**0.022**	**−0.11 (-0.20; -0.018)**	**0.019**	0.68
Essential elements					
Copper	**0.52 (0.31; 0.72)**	**<0.001**	**0.49 (0.29; 0.68)**	**<0.001**	**<0.001**
Manganese	**0.18 (0.043; 0.33)**	**0.011**	**0.18 (0.037; 0.32)**	**0.014**	0.062
Zinc	**0.27 (0.11; 0.44)**	**0.001**	**0.24 (0.081; 0.41)**	**0.003**	0.19
Selenium	**0.18 (0.022; 0.34)**	**0.026**	**0.20 (0.034; 0.36)**	**0.018**	0.052

Model 1: unadjusted.
Model 2: adjusted for maternal age (years), maternal smoking before pregnancy (yes/no), and newborn sex.

3.6. Metal and essential element interactions and joint associations

In the BKMR analysis including all metals and essential elements, placental selenium, followed by placental lead, showed the highest PIPs (i.e., variable importance) in relation to placental weight (Supplementary Table S8). The association between placental lead and placental weight was inverse at lower placental selenium concentrations (Supplementary Fig. S5A), suggesting an interaction between the two elements. Therefore, we proceeded with linear regression of lead and placental weight, stratified by the median placental selenium concentrations (166 μg/kg). At placental selenium concentrations ≤166 μg/kg (n = 204), placental lead concentrations were weakly inversely associated with placental weight (B: −23.6 g per doubling; 95 % CI: −43.2, −4.0; p = 0.019), while no significant association was observed between placental lead and placental weight at higher selenium concentrations. There was no indication of interactions between any of the other elements in relation to any of the outcomes (Supplementary Figs. S5A–D).

Since all the essential elements (copper, manganese, zinc, and selenium) were associated with mtDNAcn in the linear regression analyses, we explored joint essential element associations with mtDNAcn using BKMR. The results showed a significant positive association of joint essential elements with mtDNAcn (Fig. 1). In the joint exposure plots including both the toxic metals and the essential elements (Supplementary Figs. S6A–D), the association with mtDNA was still significantly positive (Supplementary Fig. S6D), mainly driven by copper

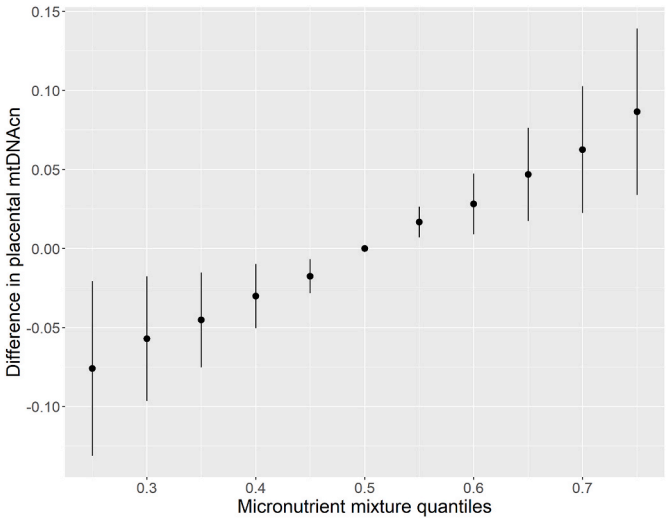

Fig. 1. Changes in placental mitochondrial DNA copy number (mtDNAcn) with increasing joint essential element concentrations in placenta, assessed by Bayesian kernel machine regression. The graph shows the estimated difference in mtDNA, with 95 % credible intervals, when all five essential elements (copper, manganese, zinc, and selenium) are held at different concentration percentiles compared to when all five essential elements are held at the 50th percentile (the median). Essential element concentrations (μg/kg; log_2-transformed) were centered and scaled, and the model was adjusted for maternal age, newborn's sex, and maternal pre-pregnancy smoking.

(Supplementary Table S8). An inverse joint association of all elements was observed with placental weight (Supplementary Fig. S6A), mainly driven by selenium (Supplementary Table S8).

4. Discussion

We evaluated the impact of commonly occurring toxic metals and certain essential trace elements on four placental outcomes (placental weight, efficiency, TL, and mtDNAcn), all of which are related to placental function (Workalemahu et al., 2016; Salafia et al., 2006; Niu et al., 2019; Lattuada et al., 2008). Placental concentrations of toxic metals did not appear to influence placental size or efficiency, measured as the ratio between birth weight and placental weight, possibly because of low exposure levels with rather narrow ranges. The only exception was a weak inverse association of placental lead with placental weight among women with lower placental selenium concentrations (≤median). At higher concentrations, selenium was found to be inversely associated with placental weight. Manganese, on the other hand, was positively associated with placental weight, and inversely associated with placental efficiency. Among the toxic metals, cobalt was inversely associated with placental mtDNAcn, while all essential elements were positively associated with mtDNAcn. Neither the toxic metals nor the essential elements appeared to influence placental TL, suggesting that the observed concentrations of metals and essential elements do not impact placental ageing.

Placental weight is known to be associated with birth weight and perinatal health (McNamara et al., 2014; Pathak et al., 2010). We have previously found maternal exposure to cadmium during pregnancy to be inversely associated with birth weight in the present cohort (Gustin et al., 2020), and placental cadmium accumulation, which was apparent herein, has been reported to impair placenta function, especially nutrient transfer (Kippler et al., 2010; Tekin et al., 2012). Nevertheless, we did not observe any associations of placental cadmium concentrations with placental weight or efficiency in the present study. This contrasts with two previous studies that found placental cadmium concentrations to be inversely associated with placental weight and positively correlated with placental efficiency, expressed as newborn weight per unit of placental weight (Mikelson et al., 2019; Punshon et al., 2019). The discrepancies may be due to higher placental cadmium concentrations in the studies by Punshon et al. (0.07–22 μg/kg) and Mikelson et al. (0.2–14 μg/kg; estimated from dry weight concentrations; Esteban-Vasallo et al., 2012), which is likely due to a higher prevalence of tobacco smoking than in our study. Indeed, smoking is an important source of cadmium (Elinder et al., 1983; Barregard et al., 2010) and it is also known to negatively affect placental weight and function (Jaitner et al., 2023). Importantly, we found that placental cadmium correlated positively with all essential elements measured herein, likely because the sources of cadmium exposure included root vegetables, vegetables, whole grain, rice, and nuts and seeds (Gustin et al., 2020). Thus, it is possible that a healthy diet might have ameliorated any oxidative stress-related adverse effect of the placental cadmium accumulation on placental function.

In line with the findings of Punshon et al. (2019) and Mikelson et al. (2019), we did not observe any association of placental lead or mercury with placental weight or efficiency in our main analyses. However, when we stratified the analyses of placental lead and placental weight by the median placental selenium concentration, an inverse association was observed in the lower selenium group. This is an interesting finding as antagonistic behavior between lead and selenium has been observed in several *in vivo* and *in vitro* studies (Rahman et al., 2019; Miao et al., 2022). On the other hand, the placental lead concentrations were not associated with placenta efficiency even at low selenium concentrations. Also, we did not observe any association of maternal erythrocyte lead concentrations with birth weight in the present cohort (Gustin et al., 2020).

We found that placental selenium was inversely associated with placental weight above ~150 μg/kg. To our knowledge, only the study by Mikelson et al. (2019) has previoulsy reported an inverse correlation of placenta selenium with placental weight. An inverse association of selenium with placental weight seem counterintuitive, as selenium is essential for multiple biological functions, including antioxidative defense, thyroid hormone regulation, and anti-inflammatory effects (Tinggi, 2008), and placental selenium has been associated with improved Doppler markers of placental function (Gomez-Roig et al., 2021). Also, adequate selenium intake has been associated with higher birth weight (Solé-Navais et al., 2021). We have previously shown that the intake of selenium among the studied women (about 40 μg/d) was below recommended intakes, leading to insufficient status with a median plasma selenium concentration of 65 μg/L in the third trimester (Stråvik et al., 2021). Thus, we propose reverse causality, as the transport of selenium to fetus is prioritized, especially in late pregnancy, for the formation of prenatal reserves (Lonnerdal et al., 2017; Stravik et al., 2021; Adams et al., 2021). Indeed, we presently found a decrease in placental selenium with increasing gestational age, and with increasing birth weight.

Manganese is an essential micronutrient required for numerous metabolic functions (Haase, 2018; Lee et al., 2006; Rondanelli et al., 2021; Santamaria, 2008) and necessary for activation of various enzymes involved in, for example, gluconeogenesis, cholesterol synthesis, and protection against oxidative stress (Studer et al., 2022), all of which are important for placental growth and function. Accordingly, we found that placenta manganese concentrations were positively associated with placental weight, partly explained by gestational age at birth. Mikelson et al. (2019) reported that placental manganese concentrations [slightly lower (53 μg/kg wet weight) than in this study (66 μg/kg)] were inversely correlated with placental weight, but the correlation was unadjusted.

Small amounts of cobalt are essential in the form of vitamin B12, but elevated exposure to inorganic cobalt may induce oxidative stress and cause toxicity (Chen and Lee, 2023). We found that cobalt, like cadmium, accumulated in the placenta, in line with a previous Swedish study on toxic metals in placenta (Fagerstedt et al., 2015). In addition, increased cobalt concentrations in placenta were associated with lower placental mtDNAcn, which may suggest a toxic effect of the placental cobalt accumulation on mitochondrial function. Experimental studies have found that elevated cobalt exposure interferes with the mitochondrial respiratory chain enzymes, reduces the generation of ATP production by aerobic cellular respiration (Hantson, 2019), and decreases the fidelity of DNA synthesis (Beyersmann and Hartwig, 1992). In adults, reduced blood mtDNAcn has been associated with hypertension and cardiovascular disease (Hagg et al., 2021; Wang et al., 2019; Vostatek et al., 2023), neuroticism, and neurodegenerative disease (Oppong et al., 2022). In pregnancy, low placenta mtDNAcn has been associated with macrosomia (Lin et al., 2022). In our study, we did not find any correlation of placental mtDNAcn with placental weight, efficiency, birth weight or preeclampsia, and further studies on mtDNAcn and health outcomes over the life course are needed.

To the best of our knowledge, this is the only study so far which has reported associations of several essential elements in placenta with placental mtDNAcn. The BKMR showed that the joint positive association of essential elements with placental mtDNAcn appeared to be driven mainly by copper, followed by manganese. In a multi-pollutant study in Belgium, cord blood concentrations of copper and manganese were not identified as important predictors of mtDNAcn in placenta (Vriens et al., 2017). Nevertheless, our blood copper concentrations did not reflect placental copper concentrations, and in our previous study on toxic metals in placenta (Fagerstedt et al., 2015), placental concentrations of both copper and manganese were much higher than those of maternal and newborn blood. In support of a beneficial influence on mtDNAcn, copper is highly important for several cuproenzymes in the mitochondria (i.e., respiratory complex IV, cytochrome *c* oxidase, superoxide dismutase 1), making it crucial for mitochondrial function and

signaling involving bioenergetics, dynamics and mitophagy (Ruiz et al., 2021).

The main strengths of the present study include the measurements of several toxic metals and essential trace elements in placenta using ICP-MS, which is a sensitive and reliable method (Nageswara Rao and Talluri, 2007). We were able to assess several potential confounders and potential joint effects and trace element-interactions. An important limitation of the present study is the restricted sample size, especially for the evaluation of associations with TL and mtDNA. The women included in the present analyses had higher education and were more likely to be primiparous compared to excluded women, which may affect the generalizability of the present findings, although these differences in maternal characteristics do not seem to influence associations of maternal characteristics and birth outcomes in the study population (Englund-Ogge et al., 2022). Another limitation is that we did not obtain maternal plasma concentrations for all essential elements and, thus, used maternal erythrocyte concentrations to assess essential element accumulation in placenta. Also, the fetoplacental weight ratio is a crude and somewhat flawed estimate of placental efficiency (Christians et al., 2018), and another estimate of placental efficiency has been suggested (using regression residuals; Christians et al., 2018) but has not yet been applied within the present field. We have assessed several exposures against several outcomes, which may raise concerns regarding multiple testing. However, we have cautiously collected our data and carefully assessed potential confounding and rather than dismissing any associations found by using a stricter limit for statistical significance, the validity of these findings is better confirmed or refuted by further studies (Goldberg and Silbergeld, 2011). Finally, as with all observational studies, we cannot rule out unmeasured or residual confounding in the present analyses.

5. Conclusions

Despite a marked placental accumulation of cadmium, we found no evidence of an impact on the studied placenta markers. Cobalt, which also accumulated in placenta, appeared to decrease mtDNAcn. Lead, although not accumulating in placenta, appeared to negatively impact placental weight when selenium concentrations were low. Manganese was positively associated with placental weight, but this did not seem to influence birth weight. The joint placental essential element concentrations increased placental mtDNAcn, outweighing the negative influence of cobalt. The observed inverse association of placental selenium with placental weight is likely not a causal relationship but related to the increased transport of selenium to the fetus in late gestation. Further studies on toxic metals and essential elements in relation to placental function are warranted.

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Ethical review

The study has been approved by the Regional Ethical Review Board in Umeå, Sweden, (2013-18-31 M, 2018-256-32 M).

CRediT authorship contribution statement

Marijke Grundeken: Investigation, Writing – original draft. **Klara Gustin:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Marie Vahter:** Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft. **Mathilde Delaval:** Data curation, Investigation, Validation. **Malin Barman:** Validation, Writing – review & editing. **Anna Sandin:** Resources, Writing – review & editing. **Ann-Sofie Sandberg:** Funding acquisition, Writing – review & editing. **Agnes E. Wold:** Funding acquisition, Writing – review & editing. **Karin Broberg:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Maria Kippler:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118355.

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