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Placental metal concentrations and birth outcomes: The Environment and Childhood (INMA) project

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Objective: To examine the association of placental levels of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), manganese (Mn), and chromium (Cr) with birth outcomes (birth weight, length, and head circumference, low birth weight [LBW], gestational age, preterm delivery, and small for gestational age [SGA]) in mother-child pairs from the Environment and Childhood (INMA) Project in Spain.

Methods: Metal concentrations were measured in placenta tissue samples randomly selected from five INMA cohorts. Data on birth outcomes were obtained from medical records. Associations were assessed in a sub-sample of 327 mother-infant pairs by regression models adjusted for confounding factors and for all metals simultaneously. Effect modification by sex was also evaluated.

Results: Elevated placental Cd levels (> 5.79 *vs.* < 3.30 ng/g) were associated with reduced birth weight (−111.8 g, 95%CI = −215.6; −8.06, p-trend = 0.01) and length (−0.62 cm, 95%CI = −1.20; −0.04, p-trend = 0.02), while a 10% increase in Cd was associated with 1.21-fold increased odds (95%CI = 1.01; 1.43) of LBW in the global sample but with 14% lower odds (95%CI = 0.78; 0.96) of preterm delivery in males ($P_{interaction}$ = 0.10). Detected (*vs.* undetected) Hg was associated with reduced head circumference (−0.49 cm, 95%CI = −1.00; 0.03) in females ($P_{interaction}$ = 0.03). A 10% increase in placental Mn was associated with slight increases in gestational age (0.04 weeks, 95%CI = 0.01; 0.07) in the global sample and in head circumference (0.05 cm, 95%CI = −0.01; 0.10) in females ($P_{interaction}$ = 0.03). Elevated Cr levels (> 99.6 *vs.* < 56.1 ng/g) were associated with reduced birth length (−0.68 cm, 95%CI = −1.33; −0.04, p-trend = 0.02) and slightly increased gestational age (0.35 weeks, 95%CI = −0.07; 0.77, p-trend = 0.08) in the global sample. As and Pb were detected in few placentas (27% and 13%, respectively) and were not associated with any studied birth outcome.

Conclusions: Data suggest that *in utero* exposure to Cd, Hg, and Cr could adversely affect fetal growth, whereas Mn and Cr appear to have a positive effect on gestational age. Given the relatively small number of subjects, sex-specific associations should be interpreted with caution.

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1. Introduction

Adverse birth outcomes such as low birth weight (LBW) and preterm birth are multifactorial conditions with environmental, socio-cultural, physiological, and nutritional components. LBW and preterm birth are major determinants of neonatal morbidity and mortality and are closely associated with the risk of developmental problems and chronic diseases (Barker, 2004; Crump et al., 2011). LBW can result from preterm delivery and/or intrauterine growth restriction, commonly assessed as small for gestational age (SGA). Arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) are well-known environmental heavy metal(loid)s that top the list of priority hazardous substances in the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 1999, 2007a; 2007b, 2012). They are recognized as toxic during critical windows of fetal and infant development and may cause intrauterine growth restriction and fetal loss.

A number of studies of populations in areas with very high environmental levels of As and Cd, such as Bangladesh, India, Japan, China, and Chile, have shown adverse associations between prenatal exposure to As and/or Cd and birth outcomes, including reductions in birth weight, length, and head circumference, placental weight, and gestational age (Al-Saleh et al., 2015; Esteban-Vasallo et al., 2012; Kippler et al., 2012a, 2012b; Laine et al., 2015; Lin et al., 2011; Menai et al., 2012; Milton et al., 2017; Quansah et al., 2015; Sun et al., 2014; Zhang et al., 2004), and a higher risk of preterm delivery (Milton et al., 2017; Rahman et al., 2018; Yang et al., 2016) and SGA (Al Saleh et al., 2015; Menai et al., 2012). However, few data have been published on the developmental effects of low-to-moderate As and Cd exposure (Quansah et al., 2015).

Although epidemiological studies increasingly support the association of *in utero* exposure to Pb and Hg with adverse pregnancy outcomes, the evidence is less consistent than for As and Cd, especially in the case of Hg (Vrijheid et al., 2016). Results of studies in populations with different environmental Pb exposure levels have not been conclusive in relation to birth size (Al-Saleh et al., 2014; Esteban-Vasallo et al., 2012; Johnston et al., 2014; Lamichhane et al., 2018; Sun et al., 2014; Taylor et al., 2015; Wang et al., 2017a; Zhu et al., 2010), preterm delivery (Taylor et al., 2015; Vigeh et al., 2011; Zhu et al., 2010), or SGA (Rodosthenous et al., 2017; Wang et al., 2017a; Zhu et al., 2010). Conflicting findings have also been observed for Hg, and an association of prenatal exposure to moderate Hg levels with preterm delivery and reduced size at birth has been found by some authors (Al-Saleh et al., 2014; Gundacker et al., 2010; Karagas et al., 2012; Vigeh et al., 2018; Xue et al., 2007) but not by others (Bashore et al., 2014; Drouillet-Pinard et al., 2010; Guo et al., 2013; Karagas et al., 2012).

Some essential metals such as manganese (Mn) and chromium (Cr) can also be toxic to humans if present at high levels. Excessive prenatal exposure to Mn has mainly been associated with neurodevelopmental delay in children (Rodríguez-Barranco et al., 2013). However, there has been little research into the effects of prenatal Mn exposure on birth outcomes, and conflicting findings have been published on the association with birth size and gestational age (Röllin et al., 2014; Tsai et al., 2015; Vigeh et al., 2008; Zota et al., 2009). Likewise, there has been scant investigation of the effects on fetal growth of excessive Cr levels during pregnancy; however, two recent studies associated higher maternal Cr exposure with increased risk of LBW (Xia et al., 2016) and preterm delivery (Pan et al., 2017).

As, Pb, and Hg can readily pass through the placental barrier from the maternal bloodstream into the fetal circulation (Iyengar and Rapp, 2001), as is also the case for Mn (Krachler et al., 1999) and Cr (Ziaee et al., 2007), while the placenta is only a partial barrier to the transfer of Cd (Esteban-Vasallo et al., 2012). Once they have reached the placenta, these metallic/metalloid elements may interfere with placental transport systems and enzymes, affecting the normal placental function (Esteban-Vasallo et al., 2012). Thus, given the role of the placenta as a mediator in the transfer of nutrients and contaminants to the fetus, placental biomarkers may provide valuable information on intrauterine exposure to these potentially toxic elements. Moreover, the placenta is readily available and provides information on the exposure of both mother and fetus (Esteban-Vasallo et al., 2012). However, few studies have used the placenta to assess prenatal exposure to metals such as As, Hg, or Mn in relation to birth outcomes (Al-Saleh et al., 2014, 2015; Esteban-Vasallo et al., 2012).

Previous data from the INMA – INfancia y Medio Ambiente [Environment and Childhood] Project, a birth cohort study in Spain, showed that total Hg level in cord blood from > 1800 newborns was inversely associated with placental weight and head circumference but not with the length of gestation (Murcia et al., 2016). Cord blood total Hg was also associated with a reduced biparietal diameter at 12 weeks of pregnancy (Ballester et al., 2018). With the above background, this study aimed to examine the association of placental As, Cd, Pb, Hg, Mn, and Cr concentrations with birth weight and length, head circumference, LBW, gestational age at birth, preterm delivery, and SGA in a sub-sample of mother-child pairs from the INMA Project. There is increasing evidence that the sex of the newborn may modify the association of prenatal exposure to toxic metals with birth outcomes (Kippler et al., 2012b, 2012b; Lamichhane et al., 2018; Wang et al., 2017b; Xia et al., 2016) and subsequent neurodevelopmental effects (Llop et al., 2016). However, studies on the sex-specific effects of prenatal metal exposure on birth outcomes remain scarce, and their results are controversial. Therefore, the potential role of sex as modifier of the studied associations was also evaluated.

2. Methods

2.1. Study population

The INMA Project is a multicenter population-based cohort study designed to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development (http://www.proyectoinma.org). The study is composed of seven mother-child cohorts established in different geographic areas of Spain (Guxens et al., 2012). A total of 490 placentas (approximately one out of six births) were randomly collected at delivery from five of the INMA cohorts, *i.e.*: Asturias, Gipuzkoa (Basque Country), Granada (Andalusia), Sabadell (Catalonia), and Valencia, from 2000 through 2008. Participants were recruited during the first prenatal visit (10–13 weeks of gestation) (2003–2008) in Asturias, Gipuzkoa, Sabadell, and Valencia but at birth in Granada (2000–2002), with a total of 3294 babies born to women in these cohorts. All cohorts included male and female newborns except for Granada, which recruited only male newborns. The details of the INMA Project, including methods of recruitment and enrollment, have been previously reported (Guxens et al., 2012). Out of the 490 placentas collected, 366 (75%) were randomly selected for metal content analysis. The present study included 327 mother-infant pairs with available data on placental metal levels, anthropometrical measures, gestational age at birth, and relevant covariates. Information on head circumference was only available for 293 newborns. All procedures associated with the INMA study were approved by the institutional review boards of the participating centers.

2.2. Laboratory analysis

The processing of placentas and the analytical and quality control procedures used to determine metal concentrations are reported elsewhere (Amaya et al., 2013; Gil et al., 2006). Briefly, half of the placenta (including maternal and fetal sides as well as central and peripheral parts) was homogenized to ensure homogeneity of the placental tissue. Cd, Pb, Mn, and Cr concentrations in placenta homogenates were determined by graphite furnace atomic absorption spectrometry, using an AAnalyst 800 Atomic Absorption Spectrometer (Perkin–Elmer, Norwalk, CT) equipped with Zeeman background correction system and

AS-800 auto-sampler. Total As content was determined in a hydride generation atomic absorption spectrometer (Perkin–Elmer FIAS-100) and total Hg content in a Perkin–Elmer 560 Atomic Absorption Spectrometer equipped with Power Supply Lamp System and MHS-10 Mercury Hydride System. Placental metal levels were expressed as ng per g of placenta tissue (wet weight). Limits of detection (LD) were 0.004 ng/g for As, 0.235 ng/g for Cd, 0.016 ng/g for Hg, 6.500 ng/g for Pb, 0.940 ng/g for Mn, and 1.488 ng/g for Cr. Certified Reference Material for whole blood (two levels, ref. 201505 and 201605) were supplied freeze-dried by Seronorm (Billingstad, Norway) and reconstituted by adding 5 mL of water (Table S1).

2.3. Birth outcomes

Data on neonatal anthropometric measurements (birth weight in g and birth length and head circumference in cm) were obtained by the attending midwife or nurse. Gestational age was calculated as the number of weeks from the self-reported last menstrual period to the end of pregnancy. In Asturias, Gipuzkoa, Sabadell, and Valencia cohorts, early fetal crown–rump length measurement by ultrasound was used to correct gestational age when the two estimations differed by 7 days or more (12%) (Murcia et al., 2016). LBW was defined by a birth weight of less than 2500 g at term, and preterm birth by live birth before 37 weeks of pregnancy. Newborns were defined as SGA when below the 10th percentile of the expected weight according to the Spanish birth weight curve adjusted for gestational age and sex (Carrascosa et al., 2008).

2.4. Confounding variables

Socio-demographics and lifestyle data were collected by interviewer-administered questionnaires during the 1st and 3rd trimesters of pregnancy (Asturias, Gipuzkoa, Sabadell, and Valencia cohorts) or at the birth (Granada cohort). Information on the type of delivery (cesarean or vaginal) was obtained from clinical records. Variables considered as potential confounders in this study were: newborn sex; cohort; maternal age; maternal and father's education level at delivery (primary, secondary, or university schooling); maternal smoking during pregnancy (yes or no); maternal working during pregnancy (yes or no); pre-pregnancy body mass index (BMI); parity (1, 2, or ≥3); and maternal place of residence during pregnancy (rural or urban/sub-urban). Pre-pregnancy BMI was obtained by dividing self-reported pre-pregnancy weight in kg by the square of the current height and categorized as underweight/normal weight ($< 25.0\,kg/m^2$) or overweight/obese ($\geq 25.0\,kg/m^2$). Confounding assessment also included parental social class, considering the higher-classified maternal or father's occupation during the pregnancy. Social class was categorized as classes I + II (higher classes), III (other non-manual classes), or IV + V (manual classes). Additionally, the average of mean daily intakes of fish and seafood reported at 12 and 32 weeks of gestation, adjusted for total caloric intake, was considered as the daily intake (in g/day) during the pregnancy. Information on food intake frequency during pregnancy was gathered from the women in the Asturias, Gipuzkoa, Sabadell, and Valencia cohorts using a validated semiquantitative questionnaire of 101 food items (Vioque et al., 2006). Fish intake during pregnancy is known to be a potential source of fetal exposure to toxic metals (Herreros et al., 2008). However, fish is also a rich source of proteins, vitamins, iron, and long-chain unsaturated fatty acids, and its intake during pregnancy might protect against metal toxicity and adverse birth outcomes (Murcia et al., 2016; Olsen and Secher, 2002).

2.5. Statistical analysis

Concentrations of As, Hg, and Pb were below the LD in a high proportion of samples. Therefore, we did not impute values < LD of the assays for placental levels of these metals, and they were categorized as detected or undetected. Cd concentrations < LD (1.8% of placentas) were assigned a value one-half of the LD. Descriptive data on placental metal content are shown as detection frequencies, median values, and 25th and 75th percentiles of concentrations. Spearman's correlation test was used to assess relationships between Cd, Mn, and Cr levels. For birth outcomes, absolute and relative frequency, mean, median, and 25th and 75th percentiles were used for descriptive purposes. Associations of parental characteristics and newborn sex with birth weight and length, gestational age, and SGA were examined by univariate linear and logistic regression, and those showing an association at $p < 0.20$ entered a multivariate regression model to identify predictors of each outcome. Variables were retained in the multivariate model if associated at $p < 0.10$ with the outcome. The covariates associated with birth weight in the multivariate model were newborn sex, maternal smoking or working during pregnancy, and pre-pregnancy BMI; those associated with birth length were newborn sex and cohort; and those associated with head circumference were newborn sex and maternal smoking during pregnancy. Maternal education alone was a significant predictor of gestational age, while the father's education, maternal working during pregnancy, and the variable cohort remained associated with SGA.

Adjusted linear regression models were created to assess the association of metal levels with anthropometric parameters and gestational age at birth. Associations with binary outcomes (LBW, preterm delivery, and SGA) were explored by logistic regression. Models were adjusted for outcome predictors and cohort and simultaneously adjusted for all metals. Because a reduction in gestation length is a major determinant of birth weight, models with birth weight, LBW, birth length, and head circumference were additionally adjusted for gestational age. The 10% change-in-estimate criterion was used to select confounders for each outcome, retaining variables in the final model if they modified the effect of metal level on outcome (b regression coefficient) by > 10%. LBW and preterm delivery models were adjusted for the same covariates as those included in birth weight and gestational age models, respectively. Cd, Mn, and Cr concentrations showed skewed distributions and were therefore transformed into natural logarithms before regression analyses. Random effect models were used to report final estimates from the main and sex-stratified analyses to account for the heterogeneity among cohorts (Table 2 and Table S3). Estimates of models with Cd, Mn, and Cr and continuous outcomes are reported as change in outcome for each 10% increase in metal level, while results for logistic regression models are reported as the odds of each outcome for every 10% increase in placental levels of Cd, Mn, or Cr. All linear regression models were tested for multicollinearity by variance inflation factor (VIF), using a cutoff of 3.5, and for outliers by standardized residuals and for potential influential observations (Cook's distance).

In order to explore nonlinear associations of metal levels with birth weight and length, head circumference, and gestation age, further regression analyses were conducted with Cd, Mn, and Cr categorized in tertiles, and with Hg grouped as < LD, between LD and median detected value, and above this median. The likelihood ratio test was performed to test the significance of linear trends across tertiles/categories of metal levels. The effect modification by sex was evaluated by interaction terms and stratified regression models. Generalized additive models (GAMs) were also used to evaluate the linearity of the relationships of Cd, Mn, and Cr with continuous outcomes, comparing linear and nonlinear models by using Akaike Information Criteria (AIC). GAMs were fitted with the mgcv library of R, using penalized thin plate regression splines as smoothing functions and the default basis dimension ($k = 10$). In a final sensitivity analysis, associations with birth size, LBW, and SGA were examined after excluding preterm births, and the potential confounding of maternal fish intake was assessed in a subsample of women with data available on dietary intake during pregnancy ($N = 217$). R statistical software version 3.4.3 (fmsb, mtest, and mgcv libraries) was used for the data analyses.

Table 1
Characteristics of study participants, INMA Project, Spain (N = 327).

Variables	Descriptive statistic
Cohort, N (%)	
Asturias	47 (14.4)
Gipuzkoa	57 (17.4)
Granada	106 (32.4)
Sabadell	67 (20.5)
Valencia	50 (15.3)
Maternal age (years), mean (SD)	32 (4)
Maternal education, N (%)	
Up to primary	95 (29.1)
Secondary	136 (41.6)
University	96 (29.4)
Father's education, N (%)	
Up to primary	125 (38.2)
Secondary	123 (37.6)
University	79 (24.2)
Parental social class, N (%)	
I + II	104 (31.8)
III	86 (26.3)
IV + V	137 (41.9)
Maternal smoking during pregnancy, N (%)	85 (26.0)
Maternal working during pregnancy, N (%)	250 (76.4)
Pre-pregnancy BMI ≥ 25 kg/m^2 (overweight/obese), N (%)	92 (28.1)
Residing in a rural area during pregnancy, N (%)	55 (16.8)
Cesarean delivery, N (%)	47 (14.4)
Parity, N (%)	
1	128 (39.1)
2	165 (50.5)
3 or more	34 (10.3)
Male newborn, N (%)	220 (67.3)
Birth weight (g), mean (SD)	3318 (428)
Birth length (cm), mean (SD)	50.0 (2.2)
Head circumference (cm), mean (SD)[a]	34.5 (1.4)
Gestational age at birth (weeks), mean (SD)	39.61 (1.38)
LBW (< 2500 g), N (%)	11 (3.4)
Preterm birth (< 37 weeks of gestation), N (%)	8 (2.4)
SGA, N (%)	20 (6.1)

LBW: Low birth weight; SGA: Small for gestational age; SD: Standard deviation.
[a] Available for 293 subjects.

3. Results

No significant differences in characteristics of study participants were found between those included in the current study (N = 327) and those with data on birth outcomes but not on placental metal levels or relevant covariates (N = 2811), with the exception of a higher percentage of children from Granada (32 *vs* 19%) and a lower percentage of women residing in rural areas (17 *vs* 25%) and of women working during pregnancy (76 *vs* 84%) in the included *versus* excluded children (Supplementary material, Table S2). Table 1 describes the characteristics of parents and newborns in the study sample. Up to 29% of fathers and 38% of mothers had primary schooling, while 42% of parents belonged to lower social classes. Among the mothers, 26% reported having smoked during pregnancy, 76% worked throughout the pregnancy, 28% were overweight or obese before pregnancy, 14% gave birth by cesarean section, 39% were primiparous, and only 4% were over 40 years old at delivery. Nearly one-third of the newborns under study were female. The mean birth weight, length, and head circumference were 3318 g, 50 cm, and 34.5 cm, respectively. Mean gestational age was 39.6 weeks. LBW was recorded in 3%, premature birth in 2%, and SGA in 6% of the deliveries. None of the newborns had a very LBW (< 1500 g) or was extremely preterm (< 32 weeks).

The non-essential metal found in the highest percentage of placentas was Cd (98%), followed by Hg (62%), As (27%), and Pb (13%). However, metal content varied among cohorts, with the women from Valencia having higher As, Hg, and Cd levels; women from Sabadell having higher Mn and Cr levels; and those from Granada having lower As, Hg, and Cd levels but higher Pb levels (Table 2). Mn was positively

Table 2
Placental concentrations of arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), manganese (Mn), and chromium (Cr) (ng/g wet weight).

	N	As			Hg			Pb			Cd			Mn			Cr		
		% > LD	Median	P25 – P75	% > LD	Median	P25 – P75	% > LD	Median	P25 – P75	% > LD	Median	P25 – P75	% > LD	Median	P25 – P75	% > LD	Median	P25 – P75
All cohorts	327	27.5	< 0.004	< 0.004–0.856	62.4	4.427	< 0.016–17.11	13.3	< 6.50	< 6.50	98.2	4.452	2.786–6.487	100	65.40	52.20–82.24	99.4	80.50	46.50–111.9
Asturias	47	34.0	< 0.004	< 0.004–1.731	59.6	6.156	< 0.016–10.04	2.1	< 6.50	< 6.50	97.9	5.758	3.472–8.284	100	61.17	50.86–77.69	100	40.87	34.61–54.47
Gipuzkoa	57	56.1	1.157	< 0.004–5.070	71.9	8.397	< 0.016–15.15	5.3	< 6.50	< 6.50	91.2	3.895	2.227–5.857	100	65.15	54.82–86.26	100	68.25	52.70–105.6
Granada	106	0.0	< 0.004	< 0.004	29.2	< 0.016	< 0.016–0.017	34.0	< 6.50	< 6.50–24.32	100	3.901	2.350–5.625	100	64.55	48.40–83.77	98.1	57.60	34.02–118.7
Sabadell	67	17.9	< 0.004	< 0.004	88.1	17.51	8.810–25.36	3.0	< 6.50	< 6.50	100	4.161	2.948–5.874	100	69.69	55.90–87.58	100	102.2	90.43–121.3
Valencia	50	60.0	1.442	< 0.004–5.409	90.0	20.25	11.76–27.20	2.0	< 6.50	< 6.50	100	6.144	4.275–8.224	100	64.99	56.27–75.77	100	96.97	81.92–116.8

P25, P75: 25th and 75th percentiles; LD (limit of detection) = As: 0.0038 ng/g; Hg: 0.015663 ng/g; Pb: 6.5000 ng/g; Cd: 0.2349 ng/g; Mn: 0.93976 ng/g; Cr: 1.48795 ng/g.

Table 3
Adjusted associations between placental metal levels and birth outcomes.

Metals	Birth weight (g)		LBW		Birth length (cm)		Head circumference (cm)		Gestational age (weeks)		Preterm birth		SGA	
	Beta	95% CI	OR	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI	OR	95% CI	OR	95% CI
Detected[a] As	−38.83	−136.3; 58.59	0.52	0.11; 3.25	−0.10	−0.67; 0.47	−0.15	−0.50; 0.17	0.17	−0.20; 0.54	0.69	0.08; 6.84	1.26	0.38; 2.21
Detected[a] Hg	37.60	−52.01; 127.2	1.01	0.34; 8.54	−0.47	−0.99; 0.05*	−0.02	−0.33; 0.29	0.04	−0.31; 0.38	1.36	0.66; 6.42	0.79	0.21; 2.40
Detected[a] Pb	54.57	−70.84; 180.0	2.94	0.38; 28.34	−0.26	−0.97; 0.44	−0.10	−0.57; 0.36	−0.11	−0.57; 0.36	0.40	0.04; 4.70	1.69	0.53; 8.82
Cd	−3.70[b]	−8.55; 1.12	1.21[c]	1.02; 1.43**	−0.02[b]	−0.05; 0.00*	−0.01	−0.03; 0.00	0.01[b]	0.00; 0.03*	0.92[c]	0.84; 0.99**	1.03[c]	0.96; 1.10
Mn	1.73[b]	−7.76; 11.22	0.89[c]	0.62; 1.07	0.01[b]	−0.04; 0.06	0.01	−0.02; 0.04	0.04[b]	0.01; 0.07**	1.08[c]	0.91; 1.26	0.96[c]	0.86; 1.09
Cr	0.65[b]	−4.72; 6.03	1.02[c]	0.87; 1.19	−0.01[b]	−0.04; 0.02	−0.01	−0.03; 0.01	0.02[b]	0.00; 0.04*	0.96[c]	0.90; 1.03	1.01[c]	0.93; 1.06

Models included 327 mother-infant pairs except for head circumference models, which included 293 mother-infant pairs.
All models are adjusted for cohort (random effect), newborn sex, and for all metals simultaneously.
Birth weight and LBW models are additionally adjusted for gestational age, maternal smoking during pregnancy, maternal working during pregnancy, and pre-pregnancy BMI.
Birth length models are additionally adjusted for gestational age and maternal smoking during pregnancy.
Head circumference models are additionally adjusted for gestational age, maternal smoking during pregnancy, pre-pregnancy BMI, and cesarean delivery.
Gestational age and preterm birth models are additionally adjusted for maternal educational level.
SGA models are additionally adjusted for father's education and maternal working during pregnancy.
$^{**}p < 0.05$; $^{*}p < 0.10$.
[a] Level above the limit of detection; [b] Change in outcome for each 10% increase in metal levels; [c] Odds of outcome for each 10% increase in metal levels.

correlated with both Cd (Spearman coefficient, $r = 0.22$; $p < 0.001$) and Cr ($r = 0.34$; $p < 0.001$), whereas no significant correlation was found between Cd and Cr.

Multivariate regression analysis (Table 3) revealed that a 10% increase in Cd levels were associated with 1.21-fold increased odds (95%CI = 1.01; 1.43) of LBW but with a 9% lower risk (95%CI = 0.84; 0.99) of preterm delivery. In addition, a weak and marginal association was found between increased Cd level and reduced birth length (−0.02 cm, 95%CI = −0.05; 0.00). Detected (*vs.* undetected) Hg levels were associated with a non-significant reduction in birth length (−0.47 cm, 95%CI = −0.99; 0.05). In contrast, a 10% increase in placental Mn and Cr, respectively, was associated with a slightly longer gestational period (0.04 weeks, 95%CI = 0.01; 0.07 for Mn and 0.02 weeks, 95%CI = 0.00; 0.04 for Cr) (see also GAM plots in Fig. 2). Regression analysis with categorized metal levels and continuous outcomes (Table 4) revealed a decrease of −111.8 g (95%CI = −215.2; −8.06) in birth weight, a decrease of −0.62 cm (95%CI = −1.20; −0.04) in birth length, and an increase of 0.38 weeks (95%CI = 0.00; 0.76) in gestational age for women with placental Cd levels in the highest (*vs.* lowest) tertile, with the three outcomes showing significant linear trends across Cd tertiles (see also Figs. 1 and 2). In comparison to women with undetected placental Hg, those with low levels showed a decrease of −0.63 cm (95%CI = −1.18; −0.08) in birth length, although there was no evidence of a linear trend (Table 4). Placental Cr was inversely associated with birth length, with a decrease of −0.68 cm (95%CI = −1.33; −0.04) for women with Cr levels in the highest (*vs.* lowest) tertile (p-trend = 0.02), whereas Cr levels in the middle and highest tertile were associated, respectively, with an increase in gestational age of 0.56 (95%CI = 0.16; 0.97) and 0.35 (95%CI = −0.07; 0.77) weeks (p-trend = 0.08) (Table 4, Figs. 1 and 2). GAM analysis suggested possible nonlinear relationships between Mn level and anthropometric parameters, finding a slight inverted U-shaped relationship with birth weight and length (Fig. 1). However, linear models showed lower AIC values, indicating no significant improvement in nonlinear models. As and Pb were not associated with any of the birth outcomes. No multicollinearity problems, outliers, or influential observations were detected in any model.

The sex-stratified analysis revealed that head circumference was associated inversely with detected Hg (−0.49 cm, 95%CI = −1.00; 0.03, $P_{interaction} = 0.03$) and positively with Mn (0.05 cm, 95%CI = −0.01; 0.10 for 10% increased Mn, $P_{interaction} = 0.03$) in female newborns alone. There was also a significant association between Cd and lower risk of preterm birth in male newborns alone (OR = 0.86, 95%CI = 0.78; 0.96 for 10% increased Cd, $P_{interaction} = 0.10$). In addition, Mn and Cr were marginally and positively associated with gestational age in males (0.05 weeks, 95%CI = 0.00; 0.09 for 10% increased Mn and 0.02 weeks, 95%CI = 0.00; 0.05 for 10% increased Cr), but no statistically significant interactions were found.

Sensitivity analysis performed by excluding preterm births did not show substantially different results (Tables S4 and S5) except for a weakening of the associations of Cd and Cr with gestational age and an apparent increasing trend in gestational age across Mn tertiles (Table S5). In models adjusted for maternal fish intake (N = 217), inverse associations of Cd with birth weight and length and of Cr with birth length were strengthened (Tables S6 and S7), while there were significant associations of Mn with increased birth weight and head circumference and of Cd with reduced head circumference (Table S7). Stratified analysis after excluding preterm births strengthened the positive association between Mn and gestational age in males (0.60 weeks, 95%CI = 0.21; 0.98 for 10% increased Mn, $P_{interaction} = 0.15$), while associations of gestational age with Cr and Cd in males lost statistical significance. Models adjusted for maternal fish intake showed no substantial differences in the sex-stratified analysis. GAM analysis showed no evidence of nonlinear relationships in sex-stratified models (data not shown).

Table 4
Adjusted associations of categorical metal levels with birth weight, height, and head circumference, and gestational age.

Metals	Birth weight (g)		Birth length (cm)		Head circumference (cm)		Gestational age (weeks)	
	Beta	95% CI	Beta	95% CI	Beta	95% CI	Beta	95% CI
Hg								
< 12.66 ng/g *vs* undetected	19.50	−80.82; 119.8	−0.63	−1.18; −0.08**	0.01	−0.26; 0.36	0.14	−0.23; 0.51
≥12.66 ng/g *vs* undetected	63.72	−45.17; 172.6	−0.11	−0.78; 0.55	−0.05	−0.49; 0.37	−0.17	−0.59; 0.25
p-trend	0.42		0.59		0.83		0.82	
Cd								
3.30–5.79 *vs* < 3.30 ng/g	−23.23	−122.8; 76.33	−0.14	−0.69; 0.40	−0.25	−0.60; 0.10	0.04	−0.33; 0.40
> 5.79 *vs* < 3.30 ng/g	−111.8	−215.6; −8.06**	−0.62	−1.20; −0.04**	−0.33	−0.69; 0.03	0.38	0.00; 0.76**
p-trend	0.01		0.02		0.19		0.04	
Mn								
57.1–75.6 *vs* < 57.1 ng/g	3.94	−72.10; 108.0	−0.27	−0.83; 0.29	0.04	−0.33; 0.39	0.12	−0.26; 0.50
> 75.6 *vs* < 57.1 ng/g	55.25	−45.60; 154.5	0.19	−0.39; 0.77	0.31	−0.04; 0.65*	0.21	−0.18; 0.61
p-trend	0.24		0.43		0.13		0.23	
Cr								
56.1–99.6 *vs* < 56.1 ng/g	45.52	−58.40; 149.4	−0.20	−0.83; 0.42	0.01	−0.42; 0.44	0.56	0.16; 0.97**
> 99.6 *vs* < 56.1 ng/g	−27.59	−132.4; 77.27	−0.68	−1.33; −0.04**	−0.31	−0.75; 0.09*	0.35	−0.07; 0.77*
p-trend	0.47		0.02		0.11		0.08	

Models included 327 mother-infant pairs except for head circumference models, which included 293 mother-infant pairs.
Cd, Mn, and Cr were categorized in tertiles; Hg was grouped as undetected, between LD and median detected value, and above this median.
All models are adjusted for cohort (random effect), newborn sex, and for all metals simultaneously.
Birth weight models are additionally adjusted for gestational age, maternal smoking, maternal working during pregnancy, and pre-pregnancy BMI.
Birth length models are additionally adjusted for gestational age and maternal smoking during pregnancy.
Head circumference models are additionally adjusted for gestational age, maternal smoking during pregnancy, pre-pregnancy BMI, and cesarean delivery.
Gestational age models are additionally adjusted for maternal educational level.
$^{**}p < 0.05$; $^{*}p < 0.10$.

4. Discussion

In this multicenter cohort study, we found some adverse associations between placental metal concentrations and birth size. Specifically, 1) Cd level was associated with reduced birth weight and length in a linear manner, and with increasing odds of LBW in the global sample; 2) placental Hg level was related to reduced head circumference in female newborns; and 3) Cr level was associated with reduced birth length in the global sample. These associations were independent of gestational age or preterm birth, suggesting that exposure to these metals influences fetal growth. Interestingly, Cd levels were associated with a slight increase in gestational age and lower odds of preterm delivery. There was also evidence of positive associations of Mn with gestational age in males and with head circumference in females and of Cr with gestational age in the global sample.

The placental metal levels found in the INMA cohorts fall within the low-intermediate range of previous reports, as already described (Amaya et al., 2013; Freire et al., 2018). Studies on placental biomarkers of exposure to toxic metals in relation to birth outcomes have published conflicting results (Al-Saleh et al., 2014, 2015; Esteban-Vasallo et al., 2012; Falcón et al., 2003; Gundacker et al., 2010; Guo et al., 2013; Kippler et al., 2010; Llanos and Ronco, 2009; Ronco et al., 2005; Stasenko et al., 2010; Zhang et al., 2004). Thus, a large study (N > 1500) in Saudi Arabia found that placental Hg levels were negatively associated with head circumference but not with birth weight or length, and that placental Cd or Pb was not related to birth anthropometry (Al-Saleh et al., 2014, 2015). The mean placenta Hg, Cd, and Pb levels in their population were several times higher than in our study (Hg: 64 *vs.* 4.43 ng/g; Cd: 45 *vs.* 4.45 ng/g; Pb: 579 *vs.* < 6.5 ng/g, respectively). Studies in China (Guo et al., 2013) and in a small sample (N = 53) in Austria (Gundacker et al., 2010) observed no association between placental Hg and birth weight, length, or head circumference, finding similar Hg levels as those in our population (3.58 and 1.9 ng/g, respectively, *vs.* 4.43 ng/g). In the Austrian study, however, placental Pb content, which was higher than in our study (25.8 *vs.* < 6.5 ng/g), was inversely related to birth weight and length but not with gestational age (Gundacker et al., 2010). A Chilean study of 20 women reported higher placental As, Cd, and Pb levels in those delivering LBW *vs.* normal-weight newborns (Llanos and Ronco, 2009), while other studies indicated a relationship between placental Cd and reduced birth weight (Kippler et al., 2010; Ronco et al., 2005; Stasenko et al., 2010), length (Stasenko et al., 2010), and chest circumference (Kippler et al., 2010). In contrast, two small studies in China (Zhang et al., 2004) and Spain (Falcón et al., 2003) found no association of placental Cd and Pb levels, which were much higher than in the current study, with birth weight, length, or head or abdominal circumference. Other studies found no association between placental levels of Hg, Pb, or Cd and birth outcomes (Esteban-Vasallo et al., 2012).

4.1. Cadmium

Because Cd accumulates in the placenta, higher Cd levels would be expected in placentas from longer pregnancies. This may explain the observed positive association with gestational age; however, this is not supported by previous findings of no or inverse associations between placental Cd and gestational age (Esteban-Vasallo et al., 2012; Falcón et al., 2003; Kippler et al., 2010; Yang et al., 2016), and the possibility of residual confounding cannot be entirely ruled out. Despite its partial retention in the placenta, Cd appeared to exert the most negative effect on birth size in our study population, and placental Cd levels may be an indicator of maternal smoking during pregnancy (Ronco et al., 2005). Accordingly, placental Cd content was significantly higher in the present mothers who smoked during pregnancy than in those who did not (median: 5.57 *vs.* 4.10 ng/g). Although studies using the placenta as exposure matrix have reported varied results regarding Cd and birth size (Al-Saleh et al., 2014; Falcón et al., 2003; Kippler et al., 2010; Llanos and Ronco, 2009; Ronco et al., 2005; Stasenko et al., 2010; Zhang et al., 2004), our results are in agreement with the finding by various large studies linking Cd levels in maternal blood, urine, or cord blood to reduced birth weight (Johnston et al., 2014; Kippler et al., 2012a; Menai et al., 2012; Sun et al., 2014) and length (Al-Saleh et al., 2015; Zhang et al., 2004). However, in contrast to our finding, a recent study of > 5000 Chinese women found that maternal urinary Cd levels were not associated with birth size (Yang et al., 2016). Cd is known to cause oxidative stress and placental Cd may interfere with the transfer of Zn to the fetus (Kippler et al., 2010), which would likely have an

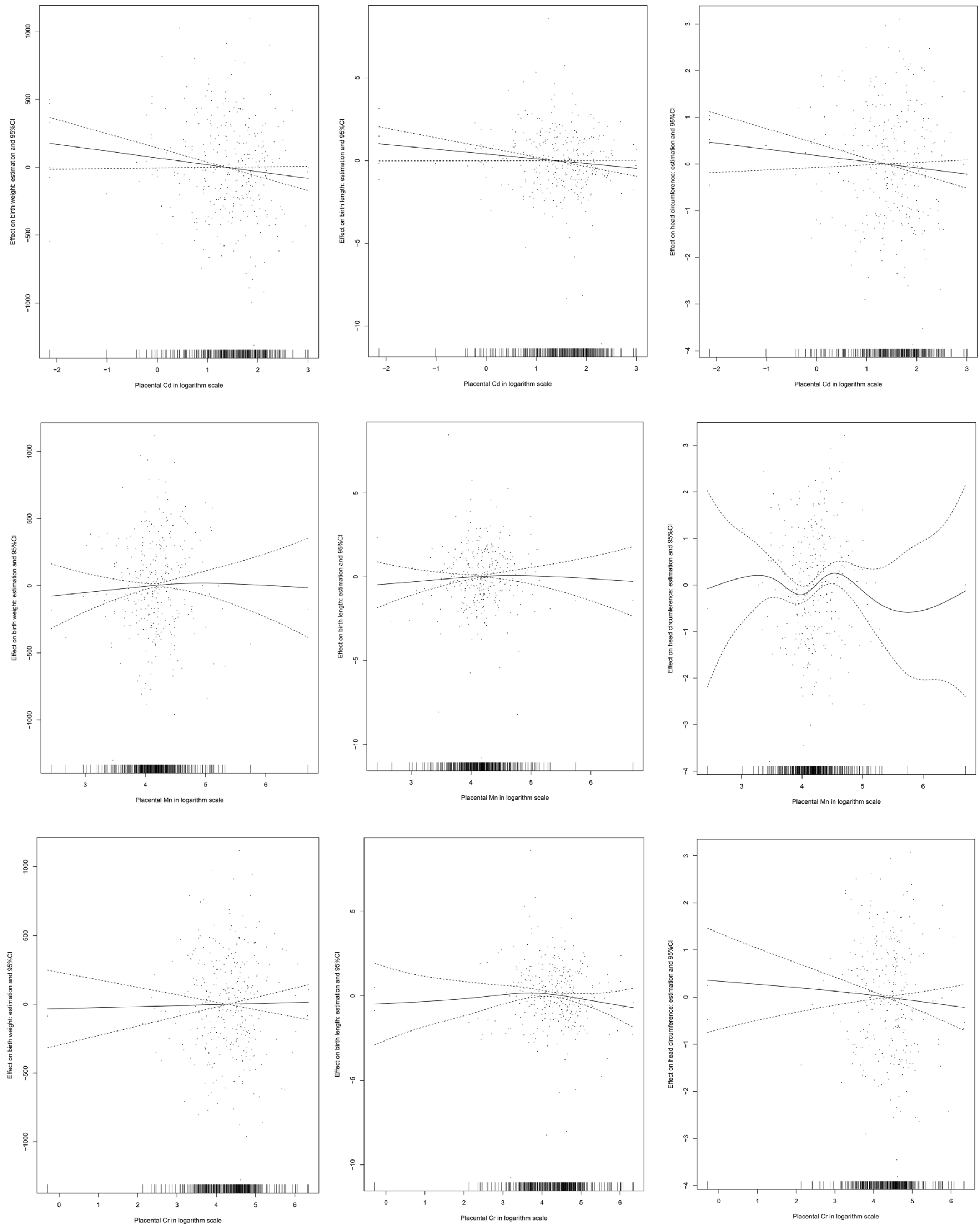

Fig. 1. Smoothing associations of placental Cd, Mn, and Cr levels with birth weight, length, and head circumference in multivariate analysis.

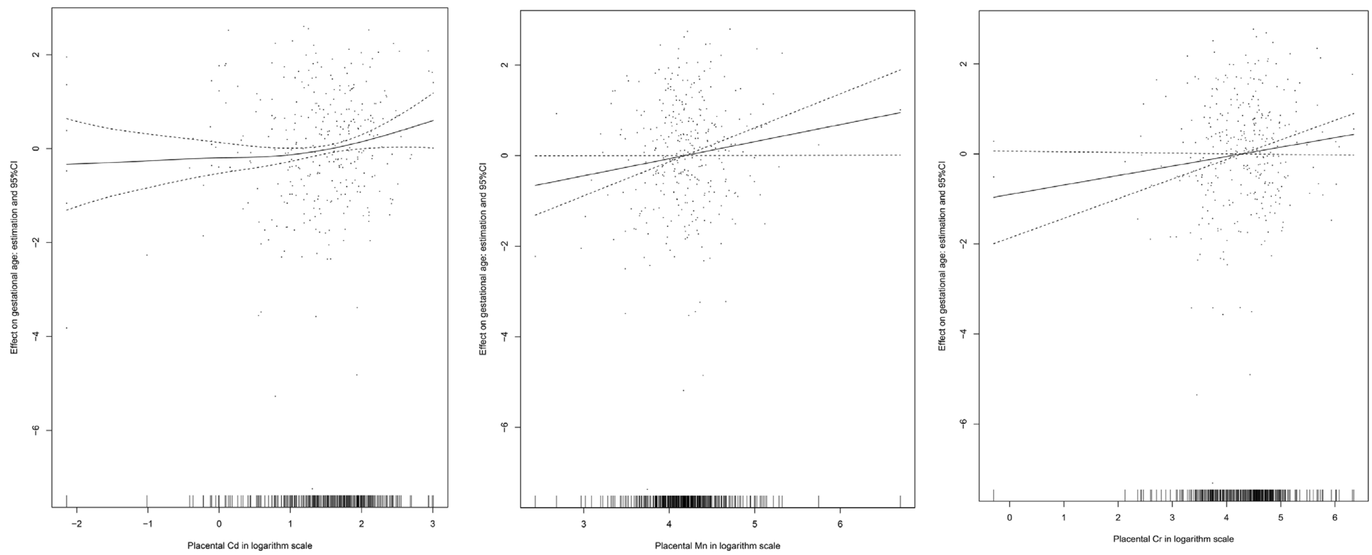

Fig. 2. Smoothing associations of placental Cd, Mn, and Cr levels with gestational age at birth in multivariate analysis.

adverse effect on fetal growth. Cd may also reduce placental leptin and progesterone synthesis and inhibit the migration of human trophoblast cells (Jacobo-Estrada et al., 2017; Stasenko et al., 2010). Cd has also been identified as an endocrine disruptor, interfering with the estrogen receptor (Kortenkamp, 2011). *In vitro* studies have shown that Cd reduces the expression of the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (Yang et al., 2006). This enzyme protects the fetus from high maternal glucocorticoid levels, which may lead to fetal growth restriction, and female fetuses have been shown to be more sensitive than male fetuses to increased glucocorticoid levels (Murphy et al., 2006). In this line, Kippler et al. (2012a, 2012b) reported that prenatal Cd exposure in Bangladesh was negatively related to birth weight and head circumference in females alone, and that the association with fetal size was stronger for the females. Our results do not support previously published findings of sex-specific effects of Cd on birth size, and this issue requires further research.

4.2. Arsenic

Exposure to high naturally occurring levels of As in drinking water has been associated with adverse birth outcomes, especially a significant reduction in birth weight (Laine et al., 2015; Milton et al., 2017; Quansah et al., 2015; Rahman et al., 2018). Several recent studies in the US have corroborated the association of prenatal As exposure as measured by drinking water concentration (inorganic As) and maternal levels (total As), with reduced birth weight at lower levels of exposure (Almberg et al., 2017; Claus Henn et al., 2016; Gilbert-Diamond et al., 2016), while some evidence, although still inconsistent, has suggested that relatively lower levels of maternal total As are also related to reduced head circumference (Claus Henn et al., 2016; Gilbert-Diamond et al., 2016). In the present study, placental total As level was not related to birth size or gestational age, probably due to low levels of exposure, especially in the Granada cohort, and the fact that As exposure in our population was mostly from fish in which As is largely found in its less toxic organic form (Fort et al., 2014).

4.3. Mercury

Conflicting results have been published on the relationship between birth outcomes and total Hg levels measured in cord blood or in maternal hair or blood. Some authors have reported a relationship with reduced birth size (Al-Saleh et al., 2014; Gundacker et al., 2010; Murcia et al., 2016; Vigeh et al., 2018; Wells et al., 2016) and premature delivery (Xue et al., 2007), whereas others found no association with birth anthropometry (Bashore et al., 2014; Guo et al., 2013; Drouillet-Pinard et al., 2010; Karagas et al., 2012) or gestation length (Bashore et al., 2014; Drouillet-Pinard et al., 2010; Murcia et al., 2016; Wells et al., 2016). Studies of placental Hg levels have also yielded contradictory results (Al-Saleh et al., 2014, 2015; Esteban-Vasallo et al., 2012). The present study provides evidence of a negative effect of placental Hg on head circumference in females and suggests that the apparent negative effect on birth length is confounded by maternal fish intake, as previously observed in relation to cord blood Hg in the INMA study (Murcia et al., 2016). Some previous studies have reported a negative association of prenatal Hg exposure with head circumference (Al-Saleh et al., 2014; Murcia et al., 2016). Cord blood total Hg levels in four of the present INMA cohorts were also associated with reduced head circumference, independently of maternal fish intake, but no difference was observed between the sexes (Murcia et al., 2016). It is noteworthy that a high proportion of the INMA cord blood samples had elevated levels of total Hg, which were influenced by maternal intake of large oily fish (Llop et al., 2012), and were positively correlated with placental Hg levels (data not shown). Discrepancies between findings from INMA and other studies may be attributable to differences in sample sizes, exposure biomarkers, degree of Hg exposure, or to the lack of adjustments for maternal fish intake and other relevant confounders, among other factors.

The negative association observed between Hg and head circumference in females is consistent with the finding by our previous investigation of a negative relationship between cord blood Hg and psychomotor development at 1 year of age in females alone (Llop et al., 2012), given that birth head circumference may be linked to subsequent cognitive development. Methyl-Hg has been found to induce oxidative stress (Caballero et al., 2017; Jauniaux et al., 2006), which may contribute to the effects of Hg on impaired fetal growth. However, further research is required to elucidate the biological mechanism underlying the sex-specific association observed between Hg exposure and birth size.

4.4. Lead

Prenatal Pb exposure is known to affect embryonic development and is associated with several adverse outcomes from infancy through adolescence. It has been postulated that even low-level Pb exposure may impair infant growth and development (Bellinger, 2008). However, a review in 2008 described the epidemiological evidence for an association of *in utero* Pb exposure with fetal growth and preterm birth as limited (Wigle et al., 2008). Since then, several studies in populations with different levels of exposure (mostly measured in maternal blood) have reported adverse associations of Pb levels with birth weight, length and head circumference, gestational age, LBW, SGA, and preterm delivery (Gundacker et al., 2010; Lamichhane et al., 2018; Rodosthenous et al., 2017; Taylor et al., 2015; Vigeh et al., 2011; Wang et al., 2017a; Zhu et al., 2010). Placental Pb content was low in the present population, especially in younger cohorts, confirming previous INMA findings of cord blood levels several times lower than the threshold established by the World Health Organization (Llop et al., 2011). In fact, among women with data available on placental and cord blood Pb ($N = 219$), 95% of those with placental Pb levels below the LD had undetected cord blood Pb. As in the case of As, the low placental Pb levels in our study population may explain the apparent lack of an adverse association between Pb and birth outcomes.

4.5. Manganese and chromium

Studies addressing prenatal exposure to Mn have shown varied results in relation to fetal growth and gestation length (Röllin et al., 2014; Tsai et al., 2015; Vigeh et al., 2008; Zota et al., 2009), with some experimental studies finding reduced fetal weight in pregnant mice exposed to Mn (Colomina et al., 1996; Sanchez et al., 1993). In the present study, there was a positive effect of Mn on gestational age and an apparent sex-specific effect on increased head circumference, which remains unclear and warrants further investigation. Mn is involved in the functioning of numerous organ systems and is needed for immune function, digestion, and bone growth, but excessive Mn exposure is considered harmful to the fetus (Aschner and Aschner, 2005). However, present results do not appear to support the hypothesis that fetal growth is favored by lower Mn levels but impaired by elevated levels, in disagreement with the finding by Zota et al. (2009) of an inverse U-shaped relationship between maternal blood Mn and birth weight.

Higher urinary Cr levels in Chinese pregnant women from the general population were associated with an increased risk of LBW in a case-control study (Xia et al., 2016) and with shorter gestation length and greater risk of preterm delivery in a large ($N > 7000$) cohort study (Pan et al., 2017), and these associations seemed to be modified by the sex of the newborn (Pan et al., 2017; Xia et al., 2016). In contrast, cord blood Cr levels were not associated with newborns' birth size in a recent study conducted in a non-industrialized island in the Canary archipelago, Spain (Cabrera-Rodríguez et al., 2018). Although the toxicity of Cr is mainly attributable to the hexavalent form (Cr + 6), excessive trivalent Cr (Cr + 3), which is the biologically active form of Cr, may also be hazardous for health. As in the Chinese studies (Pan et al., 2017; Xia et al., 2016), total Cr levels were recorded in the present investigation but our results do not support their conclusions, because we found that elevated Cr levels were associated with reduced birth length but longer gestation. The lack of adverse association found in our study between Cr and other anthropometrical parameters or gestational age may possibly be due to the low levels of exposure to Cr (Cr + 3 and Cr + 6) in our study population, as also observed in the Canary islands (Cabrera-Rodríguez et al., 2018).

4.6. Strengths and limitations

Major strengths of the present study include the relatively large number of participants in comparison to previous studies on placental metal levels, the adjustment for several confounders, the performance of sensitivity analysis (sex-stratified analysis, adjustment for fish intake, and exclusion of preterm births), and the use of the placenta as exposure matrix. In addition, this is one of the few studies to examine Mn and Cr in relation to birth outcomes. Given the accumulation of metals such as Cd in the placenta during pregnancy, it offers a singular opportunity to assess chronic maternal exposure to toxic chemicals, avoiding the need for repeated maternal blood or urinary sampling (Esteban and Castaño, 2009). Nonetheless, the relationship between this measurement and exposure at critical times during pregnancy remains unknown. Our study is also limited by the fact that genders were not equally distributed (the Granada cohort recruited only boys); therefore, sex-specific associations should be interpreted with caution. It should also be taken into account that some significant associations observed could result from chance because multiple analyses were performed. However, some of the statistically significant associations found were in line with the literature and seem unlikely to be the result of chance or residual confounding, particularly the dose-response relationships observed for Cd and Mn in relation to birth size. Furthermore, the low numbers of LBW, premature, and SGA newborns with available data on placental metal levels likely reduced the precision of our estimates. Finally, the measures of birth outcomes were recorded according to each hospital protocol, and our findings may have been subject to non-differential measurement error.

5. Conclusions

This study of Spanish mother-child pairs from the general population found that *in utero* exposure to Cd and Cr, as measured by placental levels, was associated with reduced birth size with no apparent sex-specific effects, and that placental Hg was associated with reduced head circumference in females. Although the magnitude of the estimated effects is not necessarily clinically relevant for newborns, the ubiquity of exposure to these metals, especially to Hg through fish intake, represents an important public health issue. Furthermore, results obtained suggest that Mn exposure may have a beneficial effect on gestation length and female head circumference and that Cr may also have a positive impact on gestational length, but further investigation is required to verify this possible effect. Larger prospective studies are also needed to confirm the adverse associations observed and to identify developmental windows of particular susceptibility to potentially toxic metals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2018.12.014.

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