Maternal Heavy Metal Exposure, Thyroid Hormones, and Birth Outcomes: A Prospective Cohort Study

Xiaojie Sun,[1] Wenyu Liu,[1] Bin Zhang,[2] Xiantao Shen,[1] Chen Hu,[1] Xiaomei Chen,[1] Shuna Jin,[1] Yangqian Jiang,[1] Hongxiu Liu,[1] Zhongqiang Cao,[2] Wei Xia,[1] Shunqing Xu,[1] and Yuanyuan Li[1]

[1]State Key Laboratory of Environment Health (Incubation), Key Laboratory of Environment and Health, Ministry of Education, Key Laboratory of Environment and Health (Wuhan), Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China; and [2]Women and Children Medical and Healthcare Center of Wuhan, Wuhan, Hubei 430030, China

ORCiD numbers: 0000-0002-2513-633X (Y. Li).

Context: Maternal thyroid hormones during pregnancy play a critical role in fetal development. However, whether maternal heavy metal exposure affects their thyroid hormones and the effects on fetal growth are still unclear.

Objective: To explore the effect of heavy metal exposure on maternal thyroid hormones and the potential mediation role of thyroid hormones on birth outcomes.

Methods: Concentrations of heavy metals in urine samples and thyroid hormones in blood samples of 675 pregnant women were measured during early pregnancy in a cohort study conducted in China. Multivariable linear regressions were applied to explore the associations of maternal urinary heavy metal levels with both maternal thyroid hormones and birth outcomes. Mediation analyses were performed to assess the mediation role of thyroid hormones in these associations.

Results: Maternal urinary vanadium (V) exhibited an inverse association with free T3 (FT3) and FT3/free T4 (FT4) ratio levels. Urinary arsenic (As) and lead (Pb) had inverse relationships with FT3. We also observed the positive associations of maternal FT3 and FT3/FT4 ratio with birthweight. The mediation analyses suggested that 5.33% to 30.57% of the associations among V, As, and Pb levels and birth size might be mediated by maternal FT3 or FT3/FT4 ratio.

Conclusions: We have shown that maternal exposures to V, As, and Pb at early pregnancy were associated with decreased maternal FT3 or FT3/FT4 ratio, which might contribute to reduced birthweight. Mediation analyses indicated that maternal thyroid hormone was a possible mediator of the association between urinary heavy metals and birth size. **(*J Clin Endocrinol Metab* 104: 5043–5052, 2019)**

Thyroid hormones have multiple fundamental physiological functions that are critical for maintaining a normal pregnancy and fetal growth (1). In the first half of a pregnancy, the fetus relies entirely on the supply of maternal thyroid hormone for development (2). During the remaining part of a pregnancy, the maternal thyroid homeostasis also contributes substantially to the fetus growth (2). There was a well-documented link between maternal hypo- or hyperthyroidism and adverse birth outcomes, including reduced fetal growth and impaired

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Abbreviations: As, arsenic; BMI, body mass index; Cd, cadmium; Ce, cesium; Cr, chromium; FT3, free T3; FT4, free T4; IE, indirect effect; Li, lithium; ln, natural log; LOD, limit of detection; Pb, lead; SG, specific gravity; Tl, thallium; V, vanadium.

neuropsychological development (3–6). In addition, more recent evidence suggests that even minor variations in maternal thyroid hormone levels may also have important effects on these outcomes (7–12). For example, researchers have observed that higher maternal free T4 (FT4) levels were negatively associated with birthweight of newborns whose mothers had FT4 and TSH levels within the normal ranges (8–12). Therefore, the imbalance in maternal thyroid hormone of embryonic and fetal development has been associated with delayed growth of fetus.

Thyroid hormone homeostasis is highly vulnerable to environmental chemical exposure. In recent years, epidemiological studies have focused on the relationships between exposure to heavy metals such as arsenic (As) and lead (Pb) and thyroid functions among the occupational (13) and general populations (14–16), whereas less attention was paid to pregnant women, who are more sensitive and susceptible to adverse effects of environmental contaminant exposures (17). Only a few studies have addressed the potential disrupting effects of heavy metals on thyroid homeostasis among pregnant women. The associations between reduced maternal thyroid hormones and heavy metal concentrations in maternal blood [Pb (18, 19)], As, manganese, antimony(20), mercury (21, 22), lithium (Li), cesium (Ce) (23), and cord blood [mercury (24)] have been found in some studies. However, other studies reported exposures to As and Pb (20, 23) had no effect on thyroid hormones. Differences among these studies might be attributed to the different biological matrices used for exposure assessment or that some studies were conducted in areas with relatively high heavy metal levels (18, 19, 23).

Heavy metals are ubiquitous in the environment as a result of natural processes across the Earth's crust or anthropogenic activities, including oil combustion, vehicle emission, and mining (25, 26). Humans are exposed daily to metals via inhalation, digestion, and skin contact. Extensive research has shown that maternal exposure to heavy metals during pregnancy might have adverse effects on fetal growth. Previous epidemiological studies have revealed that prenatal exposures to As (27), cadmium (Cd) (28), chromium (Cr) (29), Pb (30), thallium (Tl) (31), and vanadium (V) (32, 33) were associated with adverse birth outcomes, such as intrauterine growth retardation, low birthweight, and preterm birth. These adverse effects on birth outcomes are major determinants of early childhood morbidity and disability (34) and relate to a series of health problems in adulthood, such as respiratory disorders and cardiovascular disease (34, 35).

Although there was a well-documented link between metal exposure and adverse birth outcomes, the role of maternal thyroid function in these relationships is still unclear. Here, we posed the hypothesis that metal exposure might affect maternal thyroid hormone levels during pregnancy and then exhibited adverse effects on birth outcomes. We tested this hypothesis in a prospective cohort by exploring: (i) whether heavy metal exposure was associated with altered thyroid hormones in pregnant women and (ii) whether changes of thyroid hormones mediated the relationships between maternal heavy metal exposure and birth outcomes.

Method

Study population

Pregnant women in present study were selected from a birth cohort conducted in Women and Children Medical and Health Care Center of Wuhan, China (28). We included pregnant mothers in this study with the following criteria: (i) living in Wuhan; (ii) conceiving a singleton baby at gestational age <16; and (iii) willing to have prenatal visit and give birth at the study hospital. Mothers were invited to provide urine samples at enrollment. From October 2014 through March 2016, there were 694 women who had provided urine samples and had thyroid function test before 18 weeks of gestation. We excluded 16 mothers who had thyroid diseases such as gestational hyper- or hypothyroidism and 3 infants with birth defects, leaving 675 mother-infant pairs for analysis. All pregnant women provided written informed consent at enrollment and the study had ethical approval which was obtained from the ethics committees of the Women and Children Medical and Health Care Center of Wuhan and Tongji Medical College, Huazhong University of Science and Technology.

Birth outcomes

Birth outcomes in this study included birthweight, birth length, and the gestational age at delivery, which were retrieved from medical records. After birth, the nurses measured the weight and length of the newborn immediately and recorded the data through standardized procedures. We calculated gestational age at delivery by the weeks between delivery date and the date of the last menstrual period, which was reported by participants and was also estimated according to ultrasound measurements in the first trimester. If the difference between two methods was more than 7 days, the estimated date was used.

Covariates

The following data of the participants were extracted from medical records: maternal age, thyroid hormone test date, parity, the diagnosis of gestational hyper- or hypothyroidism and gestational diabetes and hypertension, and infant sex. The self-reported prepregnancy weight extracted from records and the height measured with a stadiometer at first prenatal visit were used to calculate the maternal prepregnancy body mass index (BMI). Information on mothers' educational level, smoking, passive smoking, and alcohol consumption during pregnancy was achieved from standardized face-to-face interviews, which were conducted by nurses at the study hospital. Exposure of mothers to cigarette smoke during pregnancy (the father or other persons smoking in the household or workplace) was defined as passive smoking (36).

Urine collection and exposure measurement

The maternal urine samples were collected at enrollment and frozen at −20°C until further analysis. The methods of measurements of urine metals have been described elsewhere (28). In brief, the obtained urine samples were diluted with 3% nitric acid overnight nitrification, and were further digested by ultrasound for 1 hour. Urinary metal concentrations were measured using inductively coupled plasma mass spectrometry (Agilent Technologies, Santa Clara, CA). In each batch, the certified reference material human urine SRM2670a (National Institute of Standards and Technology, Gaithersburg, MD) was used as an external quality control. Values of urinary metal concentrations less than the limit of detection (LOD) were replaced with one-half of the LOD. The LODs (μg/L) of metals were 0.020 for As, 0.001 for Cd, 0.010 for Cr, 0.008 for Pb, 0.020 for Tl, and 0.002 for V. We measured urinary specific gravity (SG) to control the variation effect of urine dilutions by a pocket refractometer (Atago PAL-10S). The SG-corrected urinary metal concentrations were calculated according to the formula: $Pc = P \times [(SG_m - 1)/(SG_i - 1)]$, where Pc = SG-corrected metal concentration, P = observed metal concentration, SGi = SG of the individual urine sample, and SG_m = median SG for all measurements (1.013).

Thyroid hormone measurements

Maternal thyroid function was routinely screened at early pregnancy when participants underwent prenatal care at the hospital. Fasting blood was collected in the procoagulation tube and centrifuged at 3500 rpm for 5 minutes to obtain serum. Serum TSH (uIU/mL), FT4 (pmol/L), and T3 (FT3; pmol/L) concentrations were measured using Unicel Dxi800 (Beckman Coulter, Fullerton, CA) at the clinical laboratory of the study hospital.

Statistical analyses

Distributions of concentrations of thyroid hormones and urinary metals, tested by the Kolmogorov-Smirnov normality test, were right-skewed. Thus, the geometric mean and percentiles of the urinary metal concentrations and maternal thyroid hormone levels were calculated. Maternal urinary metal concentrations and serum thyroid hormone concentrations were both natural log-transformed to improve the normality of the distributions. Multivariable linear regressions were performed to evaluate the associations between urinary metal concentrations and serum thyroid hormone concentrations or birth outcomes, and between thyroid hormones levels and birth outcomes. In addition, maternal urinary metal concentrations were divided into quartiles to determine possible nonlinear relationships. The linear trends were tested by modeling the median value of each quartile of urinary metals as a continuous variable. Maternal thyroid hormones were fitted as natural log-transformed continuous variables in the models with urinary metals, and were analyzed as both continuous and categorical variables to estimate the effects on birth outcomes. If a covariate was associated with outcomes and exposures in prior researches, or correlated with the outcomes or exposures in bivariate analysis in this study ($P < 0.1$), we adjusted it in multivariable linear regression models. All birth outcome models were adjusted for maternal age, education, prepregnancy BMI, parity, passive smoking, and infant sex. Birthweight and length models were also adjusted gestational age at delivery. All thyroid hormone models were adjusted for maternal age, education, prepregnancy BMI, gestational age at thyroid function test, and passive smoking. Only one mother in our study reported smoking and no mother reported alcohol consumption during pregnancy, so we did not adjust maternal smoking and drinking. We conducted mediation analyses to investigate the role of thyroid hormones in associations of urinary metal levels with birth outcomes. The total effect, direct effect, indirect effect, and the proportion of mediation were calculated using R software (version 3.5.1, mediation package version 4.4.6). The direct effect represented the effect of metal exposure on birth outcomes after controlling for thyroid hormones; the indirect effect was the estimated effect of metal exposure during pregnancy operating through thyroid hormones. The ratio of indirect pathways among the total effect was calculated to indicate the proportion of mediation by maternal thyroid hormones. In mediation analysis, the same confounders were adjusted for as multivariable linear regression models.

To assess the robustness of our results, we replicated all analyses restricted to women without gestational hypertensive disorders or gestational diabetes in the sensitivity analyses. All statistical analyses except mediation analysis were made using SAS (version 9.4; SAS Institute Inc., Cary, NC). Two-tailed P value < 0.05 was identified as statistically significant.

Result

Participant characteristics

A description of the basic characteristics of 675 mother-infant pairs is shown in Table 1. The mean age of mothers was 29.0 years (range, 18 to 43). More than one-half of mothers had prepregnancy BMI between 18.5 and 23.9 kg/m^2 (65.0%). Only one mother reported smoking during pregnancy. The mean values (± SD) of birthweight, birth length, and gestational age of infants at delivery were 3361.4 (± 422.1) g, 50.5 (± 1.7) cm and 39.3 (± 1.1) weeks, respectively.

Maternal urinary heavy metal and serum thyroid hormone concentrations

Table 2 shows distributions of maternal urinary metals and serum thyroid hormone concentrations. The measured metals were highly detectable in maternal urine samples, with detecting rates ranging from 95.9% to 100%. Average gestational age at thyroid function test was 13.5 weeks (range, 10 to 18 weeks). The geometric means (± SD) of maternal serum TSH (mIU/L), FT4 (pmol/L), FT3 (pmol/L), and FT3/FT4 ratio were 1.09 (± 2.26), 9.87 (± 1.17), 4.44 (± 1.14), and 0.45 (± 1.20) respectively.

Maternal urinary metal concentrations and birth outcomes

The relationships between maternal urinary metal concentrations and birth outcomes are presented in Table 3. We observed significant inverse relationships of birthweight and length with urinary V. Each natural log (ln)-unit increase in V was associated with 90.16 g (95% CI, −141.74 to −38.57) decrease in birthweight and

Table 1. Basic Characteristics of Study Population (N = 675)

Characteristics	n (%)
Age, y	
18–24	46 (6.8)
25–29	388 (57.5)
30–34	187 (27.7)
≥35	54 (8.0)
Body mass index	
Underweight (<18.5)	138 (20.4)
Normal (18.5–23.9)	439 (65.1)
Overweight (≥24)	98 (14.5)
Education	
Less than high school	40 (5.9)
High school education	95 (14.1)
More than high school	540 (80.0)
Occupation	
Employed	590 (87.4)
Unemployed	68 (10.1)
Missing	17 (2.5)
Parity	
1	552 (81.8)
≥2	123 (18.2)
Smoking during pregnancy	
No	674 (99.9)
Yes	1 (0.2)
Passive smoking	
No	489 (72.4)
Yes	185 (27.4)
Missing	1 (0.2)
Gestational hypertensive disorders	
No	655 (97.0)
Yes	20 (3.0)
Gestational diabetes	
No	624 (92.4)
Yes	51 (7.6)
Infant sex	
Boy	354 (52.4)
Girl	321 (47.6)
Birthweight, g[a]	3361.4 (422.1)
Birth length, cm[a]	50.5 (1.7)
Gestational age at delivery, wk[a]	39.3 (1.1)

[a]Mean (SD).

0.35 cm (95% CI, −0.56 to −0.15) decrease in birth length. The associations between quartiles of urinary V concentrations and birthweight [β (95% CI): first quartile = reference; fourth quartile = −133.75 (−212.25 to −55.25); P for trend <0.01] and birth length [β (95% CI): first quartile = reference; fourth quartile = −0.65 (−0.96, −0.34); P for trend < 0.01] have confirmed the dose-response relationships in linear models (37). We did not find any associations between any urinary metals and gestational age at delivery.

Maternal urinary metal concentrations and maternal thyroid hormones

The relationships between maternal urinary metal concentrations and maternal thyroid hormones were plotted in Fig. 1. Urinary V, As, and Pb concentrations exhibited inverse associations with FT3 or FT3/FT4 ratio. Specifically, each unit increase in ln-transformed urinary V was associated with 0.019 to 0.028 reductions in ln-FT3 and ln-FT3/FT4 ratio. Compared with pregnant women in the lowest quartile of urinary V concentrations, those in the highest quartiles had 0.034 and 0.046 unit decreases in serum ln-FT3 (95% CI, −0.061 to −0.008, P for trend = 0.01) and ln-FT3/FT4 ratio (95% CI, −0.085 to −0.007; P for trend = 0.01) (37). For each unit increase in ln-transformed urinary As and Pb levels, there was a 0.015 and 0.011 lowering in serum ln-FT3. We did not observe relationships between any urinary metal and serum TSH and FT4 concentrations.

Maternal thyroid hormone concentrations and birth outcomes

After adjustment for potential confounders, each unit increase in ln-FT4 was associated with 215.50 g (95% CI, −403.77 to −27.24) decrease in birthweight (Table 4). Maternal FT3 and FT3/FT4 ratio showed positive associations with birthweight and length. One unit increase in ln-FT3 was associated with 385.03g (95% CI, 159.61 to 610.45) increase in birthweight. For each unit increase in ln-FT3/FT4 ratio, there was a 324.11g (95% CI, 171.04 to 477.18) increase in birthweight and 0.72 cm (95% CI, 0.10 to 1.34) increase in birth length, respectively. Additionally, compared with women in the lowest quartiles of FT3 or FT3/FT4 levels, those in highest quartiles had offspring with higher birthweight (37).

Mediation effects of maternal thyroid hormones

A mediator should be related to both exposure and outcome (38). Because maternal FT3 and FT3/FT4 ratio were associated with urinary metals and birth outcomes, mediation analyses were performed to assess whether maternal FT3 and FT3/FT4 ratio could be mediators of the associations of metal levels with birth outcomes (Table 5). The results suggested that maternal FT3 levels explained 21.63% [indirect effect (IE), −5.90 g; 95% CI, −13.23 to −0.49], 30.57% (IE, −4.21 g; 95% CI, −9.06 to −0.74), and 8.08% (IE, −7.16 g; 95% CI, −15.40 to −1.03) for the associations of As, Pb, and V levels with birthweight. In addition, the relationship of urinary V with birthweight [10.11% (IE,−8.97 g; 95% CI, −20.30 to −0.66)] was mediated by maternal serum FT3/FT4 ratio concentrations. Because maternal TSH and FT4 did not meet the assumptions for mediation, we did not perform mediation analyses for TSH and FT4.

Sensitivity analyses

In sensitivity analyses, the results from models conducted among women who did not have hypertensive

Table 2. Distributions of Specific Gravity Adjusted Urinary Metal and Blood Thyroid Hormone Levels of the Pregnant Women (N = 675)

Analytes	>LOD%	GM	Percentiles				
			5th	25th	50th	75th	95th
Metals, μg/L							
As	100.00	20.03	8.24	13.12	18.54	28.78	67.80
Cd	99.70	0.63	0.24	0.43	0.62	0.96	1.91
Cr	98.81	1.12	0.41	0.75	1.07	1.68	4.26
Pb	99.11	2.54	1.01	1.66	2.42	3.75	9.01
Tl	99.41	0.38	0.18	0.29	0.38	0.50	0.88
V	100.00	0.96	0.44	0.73	0.95	1.29	2.36
Thyroid hormones							
TSH, uIU/mL	100.00	1.09	0.23	0.71	1.27	1.89	3.28
FT4, pmol/L	100.00	9.87	7.72	8.88	9.91	10.94	12.61
FT3, pmol/L	100.00	4.44	3.63	4.10	4.42	4.81	5.47
FT3/FT4 ratio	100.00	0.45	0.34	0.39	0.45	0.50	0.64

Abbreviations: GM, geometric mean; LOD, limit of detection.

disorders or gestational diabetes during the whole pregnancy were similar to the main analyses (37). The proportions of mediation of maternal thyroid hormones in the associations of maternal heavy metal exposure with birth outcomes were numerically higher, but the estimation sizes and the statistical significance were consistent with those in the main analyses (37).

Discussion

In this prospective birth cohort study, we observed that maternal urinary V, As, and Pb concentrations were inversely associated with maternal FT3 or FT3/FT4 ratio levels. In addition, maternal FT3 and FT3/FT4 ratio might mediate the adverse effects of maternal V, As and Pb exposure on offspring birthweight. We estimated a potential mediating role of maternal thyroid hormones in the association of maternal heavy metal exposure with adverse birth outcomes.

To date, only four early studies have assessed effects of maternal exposure to V, As, and Pb on maternal thyroid hormones. Three were conducted in areas with relatively high heavy metal levels. Kahn *et al.* observed that women from Mitrovica, a town with a Pb-processing plant, had lower FT4 in midpregnancy than women from Pristina, a town with low environmental Pb, and maternal blood Pb was inversely associated with maternal FT4 in two towns (19). However, Lamb *et al.* also reported the inverse association between maternal Pb and FT4 in Mitrovica, but not in Pristina (18). In another village with relatively high-level natural Li, Ce, and As, the authors observed the association of thyroid parameters with blood Li levels in pregnancy, but not with As and Ce (24). Associations between heavy metals and thyroid hormones were also found in pregnant women from areas with normal environmental heavy metal levels. A recent cross-sectional birth cohort study conducted in Hangzhou, a city with lower environmental heavy metal levels than other cities in China, reported that maternal blood As, manganese, and

Table 3. Adjusted Regression Coefficients [β (95% CI)] for Birth Outcomes Associated With ln-Transformed Maternal Urinary Metal Concentrations

Metals	Birthweight		Birth Length		Gestational Age at Delivery	
	β (95% CI)[a]	P	β (95% CI)[a]	P	β (95% CI)[b]	P
As	−28.29 (−66.83 to 10.25)	0.15	−0.13 (−0.28 to 0.03)	0.11	0.00 (−0.11 to 0.12)	0.94
Cd	−27.22 (−64.95 to 10.51)	0.16	0.01 (−0.14 to 0.16)	0.94	0.07 (−0.04 to 0.18)	0.22
Cr	−19.88 (−50.48 to 10.72)	0.20	−0.01 (−0.13 to 0.11)	0.86	−0.01 (−0.10 to 0.08)	0.84
Pb	−14.10 (−45.29 to 17.09)	0.38	−0.08 (−0.20 to 0.05)	0.23	−0.01 (−0.10 to 0.08)	0.82
Tl	−14.78 (−66.32 to 36.75)	0.57	−0.10 (−0.31 to 0.11)	0.34	0.05 (−0.10 to 0.20)	0.55
V	−90.16 (−141.74 to −38.57)	<0.01	−0.35 (−0.56 to −0.15)	<0.01	0.06 (−0.09 to 0.21)	0.45

[a] Adjusted for maternal age, maternal education, prepregnancy body mass index, parity, passive smoking during pregnancy, gestational age at delivery, and infant sex.

[b] Adjusted for all the covariates in the first footnote except gestational age at delivery.

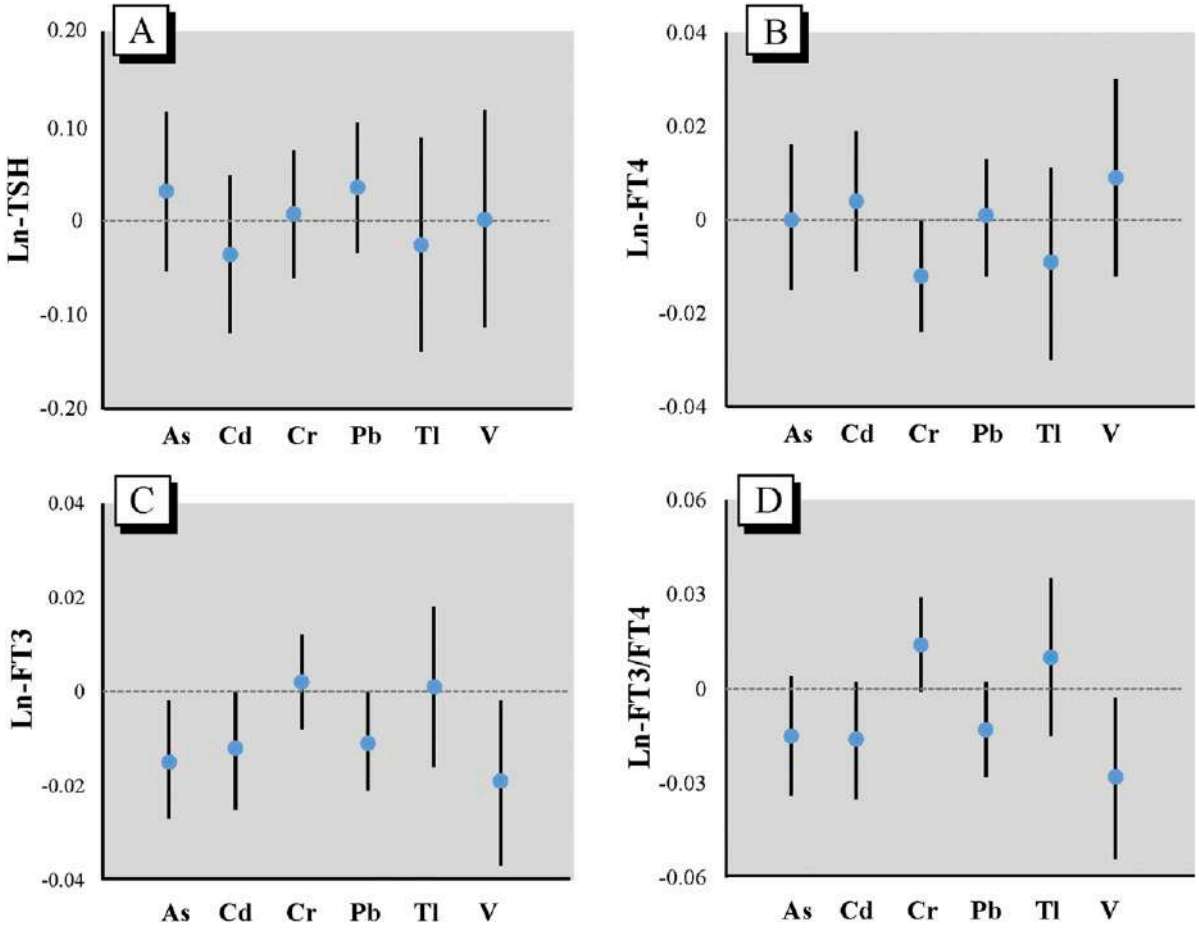

Figure 1. Adjusted associations between maternal urinary metal levels and serum thyroid hormone concentrations. (A) TSH, (B) FT4, (C) FT3, and (D) FT3/FT4 ratio. Regression coefficients and 95% CIs for associations of maternal urinary metal concentrations with thyroid hormone were estimated using multivariate linear regression models. Models were adjusted for maternal age, maternal education, prepregnancy body mass index, parity, gestational age at thyroid function test, and passive smoking during pregnancy. Both urinary metal concentrations and thyroid hormone levels were ln-transformed.

antimony were negatively associated with maternal FT3 or FT4 during the second trimester (20). In the current study, we not only observed the similar association between maternal urine As concentration and FT3, but also the inverse relationship of V and Pb levels with maternal FT3. The differences in the samples for exposure assessment, exposure levels, and timing of thyroid hormone measurement might contribute to the heterogeneity of the results between these studies and the current study. Further research is warranted to clarify the potential effects of heavy metal exposure on thyroid function in pregnant women.

Mothers provide all of the thyroid hormones required for fetal development during the first half of pregnancy.

Table 4. Adjusted Regression Coefficients [β (95% CI)] for Birth Outcomes Associated With ln-Transformed Maternal Thyroid Hormones

	Birth Weight		Birth Length		Gestational Age at Delivery	
Thyroid Hormones	**β^a (95% CI)**	***P***	**β^a (95% CI)**	***P***	**β^b (95% CI)**	***P***
TSH	−10.73 (−45.23 to 23.77)	0.54	0.00 (−0.13 to 0.14)	0.95	−0.01 (−0.11 to 0.09)	0.91
FT4	−215.50 (−403.77 to −27.24)	0.02	−0.57 (−1.32 to 0.18)	0.14	0.40 (−0.15 to 0.94)	0.15
FT3	385.03 (159.61 to 610.45)	<0.01	0.73 (−0.18 to 1.63)	0.11	0.14 (−0.51 to 0.80)	0.67
FT3/FT4 ratio	324.11 (171.04 to 477.18)	<0.01	0.72 (0.10 to 1.34)	0.02	−0.20 (−0.65 to 0.25)	0.38

[a] Adjusted for maternal age, maternal education, prepregnancy body mass index, gestational age at thyroid function test, parity, passive smoking during pregnancy, gestational age at delivery, and infant sex.

[b] Adjusted for all the covariates in the first footnote except gestational age at delivery.

Table 5. Mediation Effect Investigating Whether Maternal Thyroid Hormones Mediate the Association Between Maternal Heavy Metal Exposure and Birth Outcomes

Exposure and Outcome	Mediator	Total Effect	Direct Effect	Indirect Effect	Proportion of Mediation, %
As and birthweight	FT3	−27.28 (−61.98 to 9.22)	−21.38 (−55.52 to 14.84)	−5.90 (−13.23 to −0.49)[a]	21.63
Pb and birthweight	FT3	−13.77 (−45.33 to 16.70)	−9.56 (−40.47 to 21.81)	−4.21 (−9.06 to −0.74)[a]	30.57
V and birthweight	FT3	−88.60 (−132.91 to −45.48)[a]	−81.44 (−125.26 to −39.25)[a]	−7.16 (−15.40 to −1.03)[a]	8.08
V and birthweight	FT3/FT4	−88.70 (−133.00 to −45.38)[a]	−79.70 (−122.00 to −37.41)[a]	−8.97 (−20.30 to −0.66)[a]	10.11
V and birth length	FT3/FT4	−0.35 (−0.57 to −0.16)[a]	−0.33 (−0.54 to −0.15)[a]	−0.02 (−0.05 to 0.00)	5.33

Urinary metal concentrations and serum thyroid hormones were both ln-transformed.

[a] $P < 0.05$.

Even mild variation in maternal thyroid hormones in this period may affect the fetal growth. Considerable research has explored the association of maternal thyroid hormones with birth outcomes (7–12). The consistent findings from these studies have shown that elevations in FT4 among generally euthyroid pregnant women might contribute to decreased birthweight, which agrees with our findings regarding maternal FT4 and birthweight. Medici *et al.* reported that maternal FT4 at early pregnancy was inversely associated with birthweight in a dose-dependent manner (10), and similar results were also found in pregnant women living in United States (9) and Spain (11). In addition, Kahr *et al.* reported that birthweight was negatively associated with maternal FT4 levels, but positively associated with maternal FT3 or FT3/FT4 ratio (8). Consistent with these results, our study found decreased birth size with increased maternal FT4 and decreased FT3 or FT3/FT4 ratio. FT3/FT4 ratio is an indicator of how effectively deiodinase is able to convert FT4 into FT3 (39). It has been reported that both higher FT4 and lower FT3/FT4 ratio in euthyroid subjects was correlated with lower maternal glucose and triglycerides levels, which might result in decreased glucose transferred to the fetus and consequent fetal weight gain (40). We cannot rule out the possibility that low FT3 is not causative factor of low birthweight because low FT3 is expected to increase TSH. Also, we cannot fully explore other factors such as oxidative stress, which would also result in altered fetal growth. We also attempted to explore the relationships between maternal thyroid hormones and gestational age at delivery. However, we did not observe the relationships between thyroid hormones and gestational age.

Epidemiological studies have shown that heavy metal exposure during pregnancy might be associated with fetal growth restriction (27, 29, 31, 32, 41). We observed an overall negative relationship between metals and birth size in our study, although only the relationship between V and birth size was statistically significant. Previous studies have proposed the potential mechanisms underlying the associations of maternal heavy metal exposure with birth size, which mainly focused on the causing oxidative stress, inflammation, and disruption of endocrine system such as thyroid gland, which would result in altered fetal growth (19, 42–45). However, much less is known about the mediation role of maternal thyroid hormones in these associations.

We found maternal FT3 and FT3/FT4 ratio might be the intermediaries in the pathway between V, As, and Pb exposure and birthweight. Experiments on rats documented the role of V in affecting iodine metabolism and thyroid function by stimulating the thyroid peroxidase activity (46). Recently an *in vivo* study has found that V was able to induce the secretion of T-helper 1 chemokines into the thyroid and then promoted the induction and perpetuation of inflammatory reaction in the thyroid (47). Moreover, the induced inflammatory reaction in the thyroid might also inhibit the deiodinase activity (48). Animal studies have shown that exposure to As repressed thyroid hormone receptor and Pb inhibited the activity of an enzyme, type I deiodinase, which converts FT4 to FT3 (49.50). The mediation role of FT3 in the associations between As and Pb exposure and birthweight should be interpreted with caution because of the nonsignificant dose-response relationships between As and Pb exposure and FT3 in our study. Additional research is needed to confirm our findings in other populations. The prospective cohort design enabled us to evaluate the mediating role of maternal thyroid hormones in the associations of maternal heavy metal exposure with birth outcomes. Also, the mediation analysis supported our hypothesis that the variance of thyroid hormones might be an intermediate in mechanisms relating metal exposure to birth size. Our study also has some limitations.

First, we did not have data on maternal iodine status, which was relevant to maternal thyroid hormone synthesis. However, iodine is fortified in salt in China (49), and those pregnancies reflecting thyroid disease were excluded in this study. Therefore, our results may be less biased by the maternal iodine status. Second, the blood levels of some metals (*e.g.,* Pb) are more suitable for exposure assessment, whereas in our study we measured metal levels in urine samples. Both blood and urine have been frequently used for assessing exposure to a variety of metals, and moderate to high correlation (correlation coefficients: 0.58 for Cd, 0.72 for Pb, and 0.85 for As) between urine and blood heavy metal levels has been reported (14, 50). We measure metal levels in urine samples because urine is a noninvasive sample and is much easier to collect. Third, although results of *in vivo* and *in vitro* studies have shown that heavy metal exposure could affect thyroid hormone levels (47, 51, 52), the sampling time of single-spot urine for metal measurements is too close to the time for thyroid hormone test, which means we cannot totally rule out the possibility that changes in metals might be caused by thyroid hormone. More research with earlier sampling time like prepregnancy before thyroid hormone test are needed. Fourth, a recent study showed that the free thyroid hormone might be less accurate by immunoassays than LC-tandem mass spectrometry (53). Additionally, we used the FT3/FT4 ratio rather than a measurement of deiodinase. Further studies with the LC-tandem mass spectrometry and measurement of deiodinase activity are then needed to confirm the results.

Conclusion

In summary, exposure to heavy metal was associated with maternal thyroid hormones and birth outcomes. Exposures to V, As, and Pb in early pregnancy were associated with deceased maternal FT3 and FT3/FT4 ratio, which might contribute to the reduced birth weight. Mediation analysis indicated that maternal thyroid hormone was a possible mediator of the association between urinary heavy metals and birth size.

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Correspondence and Reprint Requests: Yuanyuan Li, PhD, State Key Laboratory of Environment Health (Incubation), Key Laboratory of Environment and Health, Ministry of Education, Key Laboratory of Environment and Health (Wuhan), Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei 430030, China. E-mail: liyuanyuan@hust.edu.cn.

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