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Urinary Arsenic Metabolism and Birth Outcomes in Tacna, Peru, 2019: A Prospective Cohort Study

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Abstract

Arsenic exposure during pregnancy might affect foetal development. Arsenic metabolism may modulate the potential damage to the foetus. Tacna has the highest arsenic exposure levels in Peru. However, this region has the highest birth weight in Peru. It is not known if arsenic exposure is affecting maternal-perinatal health in Tacna. The study aimed to evaluate the association between urinary arsenic metabolism and birth outcomes, specifically birth weight and gestational age at birth in Tacna, Peru. A prospective cohort study was conducted, involving 158 pregnant women in Tacna, Peru, during January-November 2019. Participants were enrolled in their second trimester and followed-up until birth. Urine samples were collected in the second and third trimester. Urine samples were analyzed for total arsenic concentration and its species. Generalized estimating equations (GEE) analysis was used to evaluate the association of interest. Inter-differences in arsenic toxicokinetics, calculated with principal component

analysis (PCA) was included as an interaction term. Analysis was stratified by pregnancy trimester. The median total urinary arsenic (tAs) concentration was 33.34 µg/L. Inorganic arsenic (iAs) and Dimethylarsinic acid (DMA) were higher in the second trimester. Dimethylarsinic acid (DMA) was the predominant component (84.78% of total urinary arsenic). No significant association was found between urinary arsenic exposure and birth weight or gestational age at birth. The association was not affected by arsenic metabolism. Stratified analyses by pregnancy trimester also showed no significant associations. Urinary arsenic was not associated with birth weight, and this null relationship remained unaffected by arsenic toxicokinetic differences reflected in urine.

Keywords: Birth weight, Foetal development, Gestational age, Toxicity, Pregnant women, Latin America

Introduction

Arsenic is a naturally occurring element found in the earth's crust, soil, water, and air. It is a toxic substance and a known carcinogen, causing skin, lung, bladder, and kidney cancers.[1] Arsenic is also known to have adverse effects on foetal and infant health.[2] Pregnant women who are exposed to high levels of arsenic are at an increased risk of adverse birth outcomes, including stillbirth, preterm birth (<37 weeks of gestational age), low birth weight (<2500 g at term), and congenital abnormalities.[3] In recent years, there has been growing concern about the impact of arsenic exposure on maternal and child health.

The ingestion of water containing high concentration of arsenic is one of the most common routes of exposure. It is estimated that 107 countries around the world are affected by high levels of arsenic in water [4], with groundwater being the most common source, although high levels are also found in surface water.[5] A study from our group has determined that around two-thirds of the Tacna (a province in southern Peru) population is exposed to levels higher than 10 µg/L in tap water.[6] However, this province has the highest birth weight in Peru.[7]

Urinary arsenic and its metabolites are commonly used as biomarker of arsenic exposure in epidemiological studies.[8] Arsenic and its metabolites are excreted primarily in urine, and urinary arsenic levels have been shown to correlate with the internal dose of arsenic exposure.[8] Several studies have reported a significant association between maternal urinary arsenic levels and adverse birth outcomes, although the findings have been inconsistent across studies.[3, 9] It is important to note that individuals have varying proficiencies in metabolizing arsenic, and this could modulate the potential damage to the fetus.[10]

Given the potential health risks associated with arsenic exposure during pregnancy, there is a need for further research to better understand the impact of arsenic on maternal and child health. This study aims to evaluate the association between urinary arsenic metabolism and birth outcomes, specifically birth weight and gestational age at birth.

Materials and methods

Study design and study area

We conducted a longitudinal cohort study during January-November 2019, in which a total of 158 pregnant women that lived in the province of Tacna, in their second trimester of pregnancy who attend to their antenatal care-controls were enrolled and followed-up until birth. The province of Tacna is in southern Peru, with a total area of 8,170 km^2, and it is characterized for its desertic geography.

Enrolment of participants and follow-up

The recruitment of the pregnant women is described elsewhere [6]. In brief, a total of 16 health establishments within the 5 most populated districts in the province of Tacna were selected for the enrolment to take place. We were granted authorization to consult the prenatal health care record that included information about the date of last antenatal care consultation, gestational age by the time of consultation, age, address, and telephone number.

To be considered as a potential participant for the study, the women were 18-40 years-old, lived in Tacna for at least 5 years, and were pregnant for <24 weeks by the time of the recruitment. Eligible women were recruited via telephone call. Those invited to participate in the study were then visited in their homes or in the health establishment a total of 2 times for urine sampling. A final visit was scheduled after birth, in which data from their baby was collected, such as birth weight and gestational age at birth.

Urine sampling and arsenic quantification

One urine sample was taken in the second and third trimester of pregnancy. During the recruitment the women were given two sterile plastic flasks for urine specimen collection. They were asked to avoid consuming fish or seafood for the last three days prior the sampling. They were instructed in how to do the self-collection of the sample, indicating that they should eliminate the first few milliliters of the morning void. Once the sample was collected, participants were asked to store it in the freezer until the research personnel were able to collect them. The samples were transported at 4°C to the laboratory for storage. Samples were homogenized and then aliquoted in cryovials of 2 mL, and stored at -20°C. For arsenic quantification and speciation, the samples were delivered on dry ice to the LEADER laboratory at Emory University in Atlanta, GA, USA. Procedure is described elsewhere.[11]

Statistical Analysis

Descriptive statistics were used to display median with interquartile range for non-normal distributed data. Categorical variables are presented as absolute and relative frequencies. Arsenic species concentrations and their relative percent (%) are presented.

Relative percent of the species were obtained as follows:

$$\%iAs = \frac{[As^{III}] + [As^{V}]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

$$\%MMA = \frac{[MMA]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

$$\%DMA = \frac{[DMA]}{[As^{III}] + [As^{V}] + [MMA] + [DMA]}$$

To compare total urinary arsenic and arsenic species concentration between the second and third trimester of pregnancy, we used Wilcoxon's sign-rank test. To compare %iAs, %MMA and %DMA we used Student's t-test for paired data. We performed a principal component analysis (PCA) to identify the main sources of variability in the urinary arsenic data and its species (arsenic toxicokinetics differences between pregnant women). The PCA was conducted on the concentration of urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The principal components correlations and eigenvectors can be found in **Supplementary Material 1**.

Arsenic exposure was considered as the residuals of the following model:

$$tAs = \beta_1 * Asb + \beta_2 * Asb^2 + constant$$

Where. -

tAs : Total urinary arsenic (µg/L)

Asb: Arsenobetaine (µg/L)

Generalized estimating equations (GEE) with Gaussian family analysis was employed to evaluate the association between arsenic and birth weight, and if this association was affected by arsenic toxicokinetic differences between pregnant women This same approach was applied to examine the association with gestational age at birth but scaling the variable dividing it by 1000 for better interpretation, since coefficients were small. An analysis stratified by pregnancy trimester was performed using linear regression. All statistical analyses were conducted using STATA 17.0 software with a significance level of $p < 0.05$.

Ethical aspects

The study protocol was approved by Universidad Peruana Cayetano Heredia IRB (R-29420-20). Informed consent was obtained from each participant.

Results

The study sample of pregnant women had a mean age of 28.15 years at the time of recruitment, and mean body mass index of 26.73 kg/m^2 before pregnancy.

Only five women (3.13%) declared to be smokers during pregnancy and 13 consumed alcohol (8.16%). Thirty-six of the women (22.50) were single mothers, and 61 of them (38.13%) had any higher education (at least bachelor's degree). In **Table 1** we present the distribution of urinary arsenic species concentrations as median and interquartile range (IQR). Median total urinary arsenic (tAs) was 33.34 µg/L and ranged between 2.50 – 167.48 µg/L. We observed variation in tAs across visits, being lower in visit 2. DMA was the most present arsenic component (84.78%).

Table 1. Urinary arsenic species concentration and relative content across pregnancy.

Arsenic specie	**Total**		**Second trimester**		**Third trimester**		**p-value**
(µg/L)	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
tAs	33.34	30.58	41.57	33.95	28.32	20.67	<0.001
AsIII	1.57	1.57	2.08	1.9	1.24	1.03	<0.001
AsV	1.36	1.3	1.36	1.36	1.36	1.21	0.553
iAs	2.99	2.8	3.54	2.99	2.68	2.03	0.001
MMA	2.1	1.79	2.17	2.07	2.07	1.35	0.165
DMA	28.36	26.86	35.55	29.06	23.36	16.75	<0.001
Asb	2.37	2.55	2.64	3.05	2.09	2.24	0.002
%iAs*	8.85	2.72	8.3	2.59	9.49	2.73	<0.001
%MMA*	6.37	2.21	5.41	1.87	7.47	2.06	<0.001
%DMA*	84.78	4.05	86.28	3.56	83.03	3.89	<0.001

tAs: Total urinary arsenic.
Asb: Arsenobetaine.
MMA: Monomethylarsonic acid.
DMA: Dimethylarsinic acid.
Wilcoxon's sign-rank for total arsenic and arsenic species concentration; and paired Student's t-test for arsenic species relative content (%).
*Values are shown as mean and standard deviation.

Mean birth weight was 3618 ± 477.38 grams. As seen in **Table** 2, there was no significant association between urinary arsenic and birth weight (adjusted =0.16, 95%CI -1.07 ; 1.39, p=0.800). The interaction between urinary arsenic and arsenic toxicokinetics difference between women (PCA Score 1) showed a reduction in birth weight, nonetheless, this was non-significant (adjusted =-0.05, 95%CI -0.76 ; 0.65, p=0.882).

Table 2. Association between urinary arsenic and interaction with arsenic metabolism with birth weight.

Variable	- coeff	95% CI	a - coeff	a95% CI
Urinary Arsenic	0.04	-1.27 ; 1.36	0.16	-1.07 ; 1.39
Score 1	0.62	-16.09 ; 17.33	1.27	-14.11 ; 16.65
Urinary arsenic*Score 1	-0.10	-0.89 ; 0.69	-0.05	-0.76 ; 0.65
Mother's age	4.40	-7.81 ; 16.60	3.63	-7.97 ; 15.23
Pregestational BMI	**23.76**	**9.59 ; 37.92**	**20.65**	**6.94 ; 34.35**
Education				
Elementary	Ref.		Ref.	
Secondary	305.65	-1.68 ; 612.97	**371.28**	**72.67 ; 669.93**
Tertiary	212.99	-101.37 ; 527.36	**312.73**	**8.70 ; 616.77**

Residuals were calculated from the model
tAs~ $_1$(Arsenobetaine)+ $_2$(Arsenobetaine)^2
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.
a -coeff: Adjusted -coefficient.
a95% CI: Adjusted 95% Confidence Interval.
Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.
BMI: Body mass index
Bold letters indicate a $p<0.05$.

Regarding gestational age at birth, as seen in **Table 3**, we found a non-significant increase of 0.02 weeks (95%CI -2.37 ; 2.40, p=0.989), while the interaction term presented a decrease, although not significant, in gestational age at birth (=-0.17, 95%CI -1.53 ; 1.19, p=0.802).

Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Variable	-coeff	95% CI	a -coeff	a95% CI
Urinary Arsenic	-0.08	-2.48 ; 2.32	0.02	-2.37 ; 2.40
Score 1	0.01	-0.02 ; 0.04	0.01	-0.02 ; 0.04

Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Urinary Arsenic*Score 1	-0.19	-1.63 ; 1.24	-0.17	-1.53 ; 1.19
Mother's age	**-0.03**	**-0.06 ; -0.004**	**-0.03**	**-0.06 ; -0.001**
Pregestational BMI	**-0.04**	**-0.07 ; -0.01**	-0.03	-0.07 ; 0.003
Education				
Elementary	Ref.		Ref.	
Secondary	0.62	-0.12 ; 1.36	0.48	-0.27 ; 1.23
Tertiary	0.55	-0.21 ; 1.31	0.32	-0.45 ; 1.08

Residuals were calculated from the model
tAs~ $_1$(Arsenobetaine)+ $_2$(Arsenobetaine)^2
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.
a -coeff: Adjusted
-coefficient.
a95% IC: Adjusted 95% IC.
BMI: Body mass index.
Bold letters indicate a
$p<0.05$.
Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.

We then evaluated if arsenic or the interaction term with arsenic toxicokinetic differences were associated with both outcomes, stratifying it by pregnancy trimester. As seen in **Table 4**, there was no association between urinary arsenic exposure and the interaction term with birth weight and gestational age at birth.

Table 4. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by visit.

Trimester	**Regression term**	**Birth weight**		**Gestational age at birth**	
		a (95%CI)	**p-value**	**a (95%CI)**	**p-value**
Second	Urinary Arsenic	1.61 (-1.44 ; 4.67)	0.298	-5.11 (-14.43 ; 4.20)	0.28
	Urinary Arsenic * Score 1	-1.36 (-3.32 ; 0.59)	0.170	-5.13 (-12.00 ; 1.75)	0.142
Third	Urinary Arsenic	-1.91 (-6.09 ; 2.27)	0.368	7.88 (-5.81 ; 21.57)	0.257

Table 4. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by visit.

	Urinary Arsenic	1.60 (-0.84 ;	0.197	-0.81 (-8.19 ;	0.828
	* Score 1	4.05)		6.57)	

Regressions were adjusted for mother's age, pregestational body mass index and education.
Coefficients for gestational age at birth are scaled.
PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabolic capability.
a : adjusted -coefficient.

Discussion

The present study aimed to evaluate the association between urinary arsenic and metabolism with birth weight and gestational age at birth. It was found no association with these outcomes, and this null relationship is unaffected by arsenic toxicokinetic differences reflected in urine.

No association may have been found because exposure levels might not be high enough to exert an effect. Previous studies have found a decrease in birth weight with increasing levels of urinary arsenic, at exposure levels 100 µg/L.[3] In this study, the median level for the cohort across pregnancy was 33.34 µg/L with a range of 2.50 – 167.48 µg/L. A total of 25 and 36 women showed tAs levels 100 µg/L in the second and third trimester of pregnancy, respectively, but no difference in birth weight was found (**Supplementary material 2**). In some previous studies, low levels of arsenic in urine (1.8 – 27.7 µg/L) have not been found to be associated with a decrease in birth weight.[12] However, other studies with similar exposure levels in urine have found a significant association with birth weight or estimated fetal weight.[13, 14] A Wuhan cohort study that showed median urinary arsenic levels of 31.22 µg/L for the first, 25.23 µg/L for the second, and 24.98 µg/L for the third trimester found a significant decrease of 24.27 g in birth weight only for the third trimester.[9] This suggests that even low exposure levels might be harmful for fetal development. That there might be other characteristics of our population affecting this association such as genetics or nutrition, which were not measured.

In a cohort study from Bangladesh, it was found that arsenic association with birth weight was mediated by gestational age.[15, 16] In the present study, pregnancy duration, seen as gestational age at birth, was not associated with arsenic exposure. It has been found that low arsenic levels in biological samples such as umbilical cord (3.82 ± 3.81 µg/L) and whole blood (4.13 ± 3.21 µg/L) were associated with a decrease in gestational age by 0.342 weeks.[17] On the contrary, in a study that included a total of 212 mother-infant pairs, no association was found between total urinary arsenic (median 7.77 µg/L) and urinary DMA (3.44 µg/L) with gestational age.[18] Some studies did not find any significant

association with adverse birth outcomes, even with exposure levels 10 µg/L.[19] Similarly, with birth weight, the lack of association could be due to an exposure below harmful levels, or to unmeasured nutritional, genetical and other factors.

Arsenic can be metabolized, and a higher arsenic methylation capability of the body can reduce this metalloid toxicity.[20] Higher concentration of urinary MMA and urinary iAs are shown to have the biggest impact in decreasing birth weight and birth length, respectively [10]; evidence is less clear for DMA; Nonetheless, a higher proportion of DMA, which means a better arsenic metabolism, is associated with better health outcomes compared to those with lower DMA, such as general health status of children [21] and neurodevelopment in low birth weight preterm children.[22] We have observed in pregnant women from Tacna, Peru that DMA at 84.78% (total urinary arsenic minus arsenobetaine) represents the main arsenic component present in urine. This may explain the low negative impact of arsenic on birthweight and gestational age at birth; and suggests that the difference in arsenic toxicokinetics might modify the association.

The effect modification of arsenic toxicokinetics was also assessed in the study by including the interaction term of arsenic with the PCA Score 1. For both birth weight and gestational age at birth, differences in arsenic metabolism seemed to modify the association by reducing these outcomes, although it was non-significant. Despite not finding an association, there might be an interaction between arsenic exposure and metabolism, as suggested in a Romanian longitudinal pilot study, in which women that had low birth weight children higher had a percentage of iAs and MMA [23], suggesting a slower or reduced metabolism.

Consideration of arsenic species and speciation is essential for a better understanding of exposure, not only in research studies but also in nationwide screenings such as the one done in the NHANES survey.[24, 25] Currently, the Peruvian NHANES (ENDES, in Spanish) is not assessing arsenic exposure.

It is possible that birth weight was not affected due to the variation in arsenic exposure between pregnancy trimesters. Other studies showed that there are seasonal variations in arsenic concentration [26-28], although depending on the area, the change can be very small (3.3 µg/L in well water between the dry and rainy season).[29] The first study visit was conducted in summer and autumn, while the second visit occurred during winter and spring. At the second visit, median tAs was 28.32 µg/L, compared with 41.57 µg/L found in the first study visit. In the stratified analysis, no association was found with arsenic exposure, nor with toxicokinetic differences.

The fetus experiences the fastest weight gain during the third trimester [30], and different arsenic exposures in this developmental window have been found to reduce birth weight [31], although some authors have found that early pregnancy arsenic exposure might be the critical window for birth weight and other pregnancy outcomes.[32] Nonetheless, trimester-based analysis might not reflect an adequate association.[33] Daily exposure assessment is difficult for exposures that

need biological samples such as urinary arsenic. Arsenic has been found to be associated with a decrease in birth weight and gestational age at birth, possibly through lowering thyroid hormones ratio during early pregnancy.[13] Seasonal variation in exposure, along with the analysis of pregnancy-relevant hormones should be considered for a better evaluation and interpretation.

It is still unclear why pregnant women from Tacna, despite living the highest arsenic-exposed region in Peru, also have one of the highest mean birth weights.[34] One hypothesis is that in Tacna there is a considerable proportion of people from Aymara ethnicity.[6, 11] This is an indigenous group that is predominantly located in high altitude settings and is characterized by a higher birth weight compared to other high-altitude populations.[35] Considering that Tacna is located at sea level, this effect on birth weight might be increased. In our sample, neonates of pregnant women that self-reported as Aymara have higher mean birth weight compared to the other ethnic groups (**Supplementary material 3)**.

When considering arsenic metabolism, polymorphisms in the *AS3MT* gene related increased arsenic metabolic capability [36-39], were found in Aymara populations of Argentina.[40] However, while 55.41% of our sample self-identified as Aymara, %DMA was not different between ethnic groups in our study (**Supplementary material 4**). These hypotheses should be explored in further studies.

The study has some limitations. There were unmeasured confounders such as the consumption of folates, which are part of the one-carbon metabolism and methyl donors for arsenic metabolism, which could modify the association between arsenic metabolism and birth weight.[41] Based on the Peruvian national program on pregnancy, it is mandatory to supplement women with folic acid; therefore, the folate deficiency in our population is reduced, however it should be considered in further studies. Covariates such as gestational weight gain should also be evaluated since it is strongly associated with birth weight, especially during the first half of gestation.[42] The exposure assessment at the beginning of pregnancy (first trimester) is encouraged, since it would also allow testing arsenic effects on placenta formation, as has been suggested in both human [43] and animal studies.[44] This would also allow for a better evaluation of seasonal variation in arsenic exposure. Pregnancy itself could impact the As metabolism, although we used specific gravity to adjust arsenic concentration, which is a preferred method compared to creatinine adjustment.[45]

Conclusions

Arsenic was not associated with birth weight or gestational age at birth in this study, and this null relationship was unaffected by arsenic toxicokinetic differences reflected in the analysis. This should not be interpreted as if the Tacna population is protected against arsenic toxicity. Further studies should include other variables to better understand this phenomenon and the mechanism(s) behind it, including the evaluation of other clinical outcomes. Additionally,

the inclusion of arsenic exposure assessment and its speciation in national programs should be encouraged for better monitoring, along with the elimination of arsenic contamination in drinking water.

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CRediT authorship contribution statement

DFS: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing-Original Draft **MOG:** Conceptualization, Methodology, Formal Analysis, Writing – Review & Editing, Visualization, Supervision **CVV:** Investigation, Writing – Review & Editing **CRA:** Conceptualization, Resources, Writing – Review & Editing **JA:** Conceptualization, Resources, Writing – Review & Editing **JKW:** Conceptualization, Writing – Review & Editing, Supervision **MYL:** Conceptualization, Writing – Review & Editing, Supervision **DBB:** Validation, Investigation, Resources, Writing – Review & Editing **GFG:** Conceptualization, Resources, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interests

The authors declare that they have no competing interests.

Ethics statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Universidad Peruana Cayetano Heredia (Project identification code R-121-12-23).

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Table 1. Urinary arsenic species concentration and relative content across pregnancy.

Arsenic specie (µg/L)	**Total**		**Second trimester**		**Third trimester**		**p-value**
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
tAs	33.34	30.58	41.57	33.95	28.32	20.67	<0.001
AsIII	1.57	1.57	2.08	1.9	1.24	1.03	<0.001
AsV	1.36	1.3	1.36	1.36	1.36	1.21	0.553
iAs	2.99	2.8	3.54	2.99	2.68	2.03	0.001
MMA	2.1	1.79	2.17	2.07	2.07	1.35	0.165
DMA	28.36	26.86	35.55	29.06	23.36	16.75	<0.001
Asb	2.37	2.55	2.64	3.05	2.09	2.24	0.002
%iAs*	8.85	2.72	8.3	2.59	9.49	2.73	<0.001
%MMA*	6.37	2.21	5.41	1.87	7.47	2.06	<0.001
%DMA*	84.78	4.05	86.28	3.56	83.03	3.89	<0.001

tAs: Total urinary arsenic.
Asb: Arsenobetaine.
MMA: Monomethylarsonic acid.
DMA: Dimethylarsinic acid.

Wilcoxon's sign-rank for total arsenic and arsenic species concentration; and paired Student's t-test for arsenic species relative content (%).
*Values are shown as mean and standard deviation.

Table 2. Association between urinary arsenic and interaction with arsenic metabolism with birth weight.

Variable	- coeff	95% CI	a - coeff	a95% CI
Urinary Arsenic	0.04	-1.27 ; 1.36	0.16	-1.07 ; 1.39
Score 1	0.62	-16.09 ; 17.33	1.27	-14.11 ; 16.65
Urinary arsenic*Score 1	-0.10	-0.89 ; 0.69	-0.05	-0.76 ; 0.65
Mother's age	4.40	-7.81 ; 16.60	3.63	-7.97 ; 15.23
Pregestational BMI	**23.76**	**9.59 ; 37.92**	**20.65**	**6.94 ; 34.35**
Education				
Elementary	Ref.		Ref.	
Secondary	305.65	-1.68 ; 612.97	**371.28**	**72.67 ; 669.93**
Tertiary	212.99	-101.37 ; 527.36	**312.73**	**8.70 ; 616.77**

Residuals were calculated from the model
tAs~ $_1$(Arsenobetaine)+ $_2$(Arsenobetaine)^2
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.
a -coeff: Adjusted -coefficient.
a95% CI: Adjusted 95% Confidence Interval.
Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.
BMI: Body mass index
Bold letters indicate a $p<0.05$.

Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Variable	-coeff	95% CI	a -coeff	a95% CI
Urinary Arsenic	-0.08	-2.48 ; 2.32	0.02	-2.37 ; 2.40
Score 1	0.01	-0.02 ; 0.04	0.01	-0.02 ; 0.04
Urinary Arsenic*Score 1	-0.19	-1.63 ; 1.24	-0.17	-1.53 ; 1.19
Mother's age	**-0.03**	**-0.06 ; -0.004**	**-0.03**	**-0.06 ; -0.001**

Table 3. Association between urinary arsenic and interaction with arsenic metabolism with gestational age at birth

Pregestational BMI	**-0.04**	**-0.07 ; -0.01**	-0.03	-0.07 ; 0.003
Education				
Elementary	Ref.		Ref.	
Secondary	0.62	-0.12 ; 1.36	0.48	-0.27 ; 1.23
Tertiary	0.55	-0.21 ; 1.31	0.32	-0.45 ; 1.08

Residuals were calculated from the model
tAs~ $_1$(Arsenobetaine)+ $_2$(Arsenobetaine)^2
Models were adjusted for mother's age, mother's education level, pre-gestational body mass index.
a -coeff: Adjusted -coefficient.
a95% IC: Adjusted 95% IC.
BMI: Body mass index.
Bold letters indicate a p<0.05.
Score 1 (arsenic toxicokinetics difference between women), obtained from principal components analysis, is higher when %DMA is lower, meaning a reduced metabolic capability.

Table 4. Association between urinary arsenic and interaction with arsenic metabolism with birth weight and gestational age at birth stratified by visit.

Trimester	**Regression term**	**Birth weight**		**Gestational age at birth**	
		a (95%CI)	**p-value**	**a (95%CI)**	**p-value**
Second	Urinary Arsenic	1.61 (-1.44 ; 4.67)	0.298	-5.11 (-14.43 ; 4.20)	0.28
	Urinary Arsenic * Score 1	-1.36 (-3.32 ; 0.59)	0.170	-5.13 (-12.00 ; 1.75)	0.142
Third	Urinary Arsenic	-1.91 (-6.09 ; 2.27)	0.368	7.88 (-5.81 ; 21.57)	0.257
	Urinary Arsenic * Score 1	1.60 (-0.84 ; 4.05)	0.197	-0.81 (-8.19 ; 6.57)	0.828

Regressions were adjusted for mother's age, pregestational body mass index and education.
Coefficients for gestational age at birth are scaled.
PCA Score 1 (arsenic toxicokinetics difference between women) is higher when %DMA is lower, meaning a reduced metabolic capability.
a : adjusted -coefficient.