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Boron exposure through drinking water during pregnancy and birth size

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ABSTRACT

Background: Boron is a metalloid found at highly varying concentrations in soil and water. Experimental data indicate that boron is a developmental toxicant, but the few human toxicity data available concern mostly male reproduction.
Objectives: To evaluate potential effects of boron exposure through drinking water on pregnancy outcomes.
Methods: In a mother-child cohort in northern Argentina ($n = 194$), 1–3 samples of serum, whole blood and urine were collected per woman during pregnancy and analyzed for boron and other elements to which exposure occurred, using inductively coupled plasma mass spectrometry. Infant weight, length and head circumference were measured at birth.
Results: Drinking water boron ranged 377–10,929 μg/L. The serum boron concentrations during pregnancy ranged 0.73–605 μg/L (median 133 μg/L) and correlated strongly with whole-blood and urinary boron, and, to a lesser extent, with water boron. In multivariable-adjusted linear spline regression analysis (non-linear association), we found that serum boron concentrations above 80 μg/L were inversely associated with birth length (B − 0.69 cm, 95% CI − 1.4; − 0.024, $p = 0.043$, per 100 μg/L increase in serum boron). The impact of boron appeared stronger when we restricted the exposure to the third trimester, when the serum boron concentrations were the highest (0.73–447 μg/L). An increase in serum boron of 100 μg/L in the third trimester corresponded to 0.9 cm shorter and 120 g lighter newborns ($p = 0.001$ and 0.021, respectively).
Conclusions: Considering that elevated boron concentrations in drinking water are common in many areas of the world, although more screening is warranted, our novel findings warrant additional research on early-life exposure in other populations.

1. Introduction

Boron (B) is a metalloid found in the bedrock bound to oxygen as boric acid and borax, and it is mined for industrial applications such as borosilicate glass manufacturing (WHO, 2009). Boron in the ground may dissolve in the surrounding water, and may therefore appear in drinking water sources. Boron concentrations in drinking water up to 1 mg/L have been observed in northern France (Yazbeck et al., 2005), 1.4 mg/L in Public Water Systems in the U.S. (EPA, 2008), 3 mg/L in southern Sweden (SSI, 2008), 6 mg/L in the Argentinean Andes (Concha et al., 2010), 11 mg/L in Muenster, Germany (Queste et al., 2001) and Arica, Chile (Cortes et al., 2011), and 29 mg/L in Balikesir province, Turkey (Sayli et al., 1998). Also, bottled water may contain elevated boron concentrations; one brand from Slovakia as much as 120 mg/L (Reimann and Birke, 2010). The World Health Organization (WHO) health-based guideline value for drinking water boron is 2.4 mg/L (WHO, 2011). Food items rich in boron include nuts, legumes, fruit and vegetables (Rainey et al., 1999), but data on the typical daily intake of boron through food and drinking water are scarce. One U.S. study estimated the mean intake to about 1 mg/day (Rainey et al., 1999).

Boron is easily absorbed in the gastrointestinal tract and fairly rapidly excreted, mainly in urine (Moore, 1997). The toxicology of boron is largely unknown. Experimental studies on rats indicate that boron causes male reproductive toxicity, especially low sperm count (Weir and Fisher, 1972), and developmental toxicity (fetal skeletal malformations and impaired fetal growth) (Price et al., 1996). Only a few epidemiological studies are available, mainly concerning male reproduction, and these showed no convincing evidence of impaired semen quality (Başaran et al., 2012; Robbins et al., 2010). Boron passes through the placenta (Harari et al., 2012), and because the fetus may be particularly sensitive to boron toxicity (Huel et al., 2004), this study aimed at evaluating potential effects of boron on birth outcomes in a mother-child cohort in northern Argentina exposed to varying boron concentrations in the drinking water.

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2. Materials and methods

2.1. Study population

This study is based on a mother-child cohort study in the arid, high-altitude Andean part of the Province of Salta in northern Argentina. Details of the study area and recruitment have been described elsewhere (Harari et al., 2015). Briefly, we recruited pregnant women in the main village San Antonio de los Cobres and nine surrounding villages (in total about 8000 inhabitants), where we previously had discovered varying concentrations of boron (range 335–5956 μg/L), arsenic (3.5–322 μg/L), cesium (0.03–322 μg/L), and lithium (8.0–1005 μg/L) in the drinking water (Concha et al., 2010). Concentrations of other elements in the drinking water in this region are mostly low (Supplementary Table S1). Also, this remote rural area has no major industries besides lithium and boron mining, and the traffic is very low. The diet is primarily based on meat and vegetables, with essentially no fish, dairy products or rice. Taken together, this would indicate limited exposure to other toxic elements such as methylmercury, lead and cadmium.

All women being pregnant between October 2012 and December 2013 were invited to participate in the project with the assistance of the health care personnel at the hospital in San Antonio de los Cobres and the small health care centers in the surrounding villages. In total, 194 women were recruited (response rate = 88%; see Fig. S1). Reasons for not participating included twin pregnancy (one case identified by ultrasound), deliveries before recruitment (11), fetal loss (5), refusal or not located (6), and migration (4). Out of the 194 women enrolled in the project, 182 were interviewed and provided samples before delivery and out of these, two had a miscarriage. At enrollment, the women were interviewed regarding family characteristics, including known diseases, preferred diet, last menstrual period (LMP), and pre-pregnancy weight. The study was designed to see the pregnant women at least once during pregnancy; preferably 2–3 times (once per trimester) in order to obtain repeated measures of exposure. Blood and spot-urine samples were collected at each visit, at which time the women were also interviewed about encountered health problems.

The study was approved by the regional ethical committee at Karolinska Institutet, Stockholm, Sweden, and by the Ministry of Health, Salta, Argentina. After detailed explanation of the study, we obtained oral and written informed consent from all women before recruitment. Informed consent was also obtained from the closest caregiver if the women were younger than 18 years of age. We regularly report the findings to the medical personnel at the study area and to the Health Ministry in Salta.

2.2. Exposure assessment

Because it is the serum fraction of nutrients and potentially other substances that may reach the fetus through the placenta, we assessed the exposure to boron by the concentrations in serum. However, as a biomarker of boron exposure is not well established, we also measured boron concentrations in whole blood, urine and drinking water for validation. The boron concentration in serum was highly correlated with that in whole blood ($r_s = 0.87$, $p < 0.001$) and urine ($r_s = 0.71$, $p < 0.001$) and to a much lesser extent with the boron concentration in water ($r_s = 0.28$, $p < 0.001$). The correlation between urinary and water boron was $r_s = 0.37$ ($p < 0.001$). Urinary boron has been used as a biomarker for ongoing boron exposure, especially among occupationally exposed people (Sutherland et al., 1999). However, it represents the excreted boron (with short half-life) (Sutherland et al., 1999), and not the fetal exposure to the same extent as serum boron.

Serum samples were fractionated from whole blood samples collected in Trace Elements Serum Clot Activator tubes (Vacuette®, Greiner bio-one, Kremsmünster, Austria) by centrifugation at 3000 rpm for 10 min, exactly 15 min after blood drawing. Another sample of whole blood was collected in sodium heparin tubes (Vacuette®, NH Trace Elements; Greiner bio-one, Kremsmünster, Austria) for measurements of multiple exposures. Spot urine samples were collected in disposable trace element-free plastic cups and transferred to 20-mL polyethylene bottles (Zinsser Analytic GMBH, Frankfurt, Germany). Water samples were repeatedly collected during the study period using 20-mL polyethylene bottles (Concha et al., 2010).

Boron concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700×, Agilent Technologies, Tokyo, Japan), as described elsewhere (Harari et al., 2015; Lu et al., 2015). Serum and whole blood samples were diluted 1:25 in acid-washed polypropylene tubes with an alkali solution containing 2% 1-butanol, 0.05% EDTA, 0.05% triton X-100, and 1% NH_4OH (Lu et al., 2015). Urine and water samples were diluted 1:10 with 1% nitric acid (Suprapur; Merck, Darmstadt, Germany) before analysis. For comparison, we measured all urine samples, as well as other urine samples from the study site, after alkali dilution also, and acid and alkali dilution gave very similar results for urinary boron ($r_s = 0.98$, p-value < 0.001; $n = 667$). The limit of detection (LOD) for boron was 1.0 μg/L in serum, 0.23 μg/L in whole blood, 6.2 μg/L in urine and 6.4 μg/L in water. Only three serum samples had boron concentrations below the LOD value and those were replaced by LOD/√2.

Reference materials were prepared in an identical manner as the samples and were analyzed after about every 20 samples. As there are no certified standard reference materials (SRM) available for the determination of boron concentrations in serum, whole blood and urine, we used Seronorm™ reference samples (SERO AS, Billingstad, Norway). For water samples, we used certified SRM 1643e Trace Elements in Water (National Institute of Standards and Technology (NIST), Gaithersburg, USA). The results of boron in the reference materials used are shown in Table S2.

Because arsenic, cesium and lithium were also present at elevated concentrations in the drinking water (Supplemental Table S1 and Concha et al., 2010), and these elements may also impair fetal growth (Harari et al., 2015; Rahman et al., 2009), the exposure to these was considered. We additionally measures lead, cadmium and selenium to test for confounding. Cesium and lithium were measured in whole blood and urine, lead and cadmium in whole blood and selenium in serum, all by ICP-MS. Exposure to arsenic was assessed by the sum concentrations of inorganic arsenic (iAs) and its mono- and dimethylated metabolites (MMA and DMA) in urine, measured using HPLC-HG-ICPMS. The analytical details have been described elsewhere (Concha et al., 2010; Fängström et al., 2009; Harari et al., 2015; Lu et al., 2015).

To compensate for the variability in urine dilution, the metal concentrations were adjusted to the average urinary osmolality according to the equation: urinary concentration ∗ [(average osmolality of all women (694 mOsm/kg)) / (individual's osmolality)]. The boron concentrations in urine adjusted for specific gravity were highly correlated with those adjusted for osmolality ($r_s = 0.96$, $p < 0.0001$).

2.3. Outcomes and covariates

The pregnancy outcomes considered in this study were birth weight (g), length (cm) and head circumference (cm), measured by the health care personnel immediately after birth (for most women) or after a few hours for seven women (3.9%) who delivered at home. Birth weight was measured with a baby scale (Seca 725 Mechanical Beam Baby Scale, Brooklyn, NY, U.S.A.), birth length was measured to the nearest 5 mm using a portable wood infantometer with the child in supine position, and head circumference was measured with a soft, non-stretchable plastic tape line with mm indications.

Gestational age at birth was calculated by subtracting the last menstrual period (LMP) date from the date of birth. In a few cases where the date for LMP was not available, we used the ultrasound estimation. The season of birth was noted as winter during the months April–September, and summer October–March. We collected information about maternal age, parity (number of born children), parental monthly income, years of maternal education, smoking, alcohol consumption, chewing of coca leaves, and prenatal vitamin supplementation through personal interviews at recruitment and at the follow-up visits. In a standardized way, we measured the women's weight (HCG-210QM, GA.MA® professional, Italy; accurate to 100 g) at each visit as well as height. Body mass index (BMI) was calculated as the maternal body weight in kilograms divided by height in meters squared (kg/m^2).

To control for the co-exposure to arsenic, cesium and lithium in the statistical models, we chose the biomarker that correlated the least with serum boron in order to reduce problems with collinearity. Thus, cesium in whole blood and lithium and arsenic (metabolites) in urine, all relevant exposure biomarkers, were included as co-variables in the serum boron models. Only one woman reported smoking (rarely) during pregnancy, so adjustment for smoking was not considered. Consumption of alcohol during pregnancy was also very rare (2.8%, infrequently); but 45% of the recruited women reported chewing coca leaves.

2.4. Statistical analyses

Descriptive statistics (mean, standard deviation (SD), median, range, 5th–95th percentiles) for the mother-child pairs were calculated by tertiles of serum boron concentrations during pregnancy (for women with 2 or more measurements we first calculated their average serum boron concentrations during pregnancy). We used Spearman's rank correlation to evaluate the correlation between exposure biomarkers. Kruskal-Wallis *H* test was used to compare differences across tertiles of serum boron for continuous variables and chi squared for categorical variables. Initially, we visually evaluated the associations between the exposure markers and the outcomes (birth weight, birth length and birth head circumference) by scatter plots with Lowess, running-line least squares, and found them to be non-linear, indicative of an inverted U-shape (Supplementary Figs. S3–S6). We therefore used linear spline regression models, with a knot at 80 μg/L for serum boron, to further evaluate the associations between boron exposure (average concentrations during pregnancy for those with 2 or more measurements) and birth outcomes. We first calculated B-coefficients and 95% confidence intervals (CI) for regression models adjusted for gestational age at birth and gestational age at birth (quadratic term) only (basic adjustment). We then additionally adjusted for all variables considered to be of importance for the outcomes (parity, maternal height, parental monthly income, infant sex, cesium in whole blood and lithium in urine). All measures, except for parental monthly income, were used as continuous variables. Parental monthly income was reputed more relevant than the income of the mother alone, and it was expressed as a categorical variable (above or below the median 1700 Argentinean pesos). Maternal height was chosen instead of BMI as people in this population are fairly short and differences in body frame largely disappeared by the BMI measurement. Maternal age was not included in the models due to high correlation with parity ($r_s = 0.79$, $p \leq 0.0001$), and because parity influenced the estimates more than maternal age. We also controlled for the co-exposure to lithium and cesium in all models. Arsenic in urine, lead and cadmium in blood and selenium in serum were also tested in the models but were finally not included as they only marginally changed the estimates for boron. Passive smoking, coca chewing, years of maternal education and prenatal vitamin supplementation were not found to affect the models and were therefore not included in the final models. Two individuals with extreme boron concentrations in serum (above 500 μg/L) were excluded from the final models as they disproportionally influenced the results.

Depending on the time point of recruitment, the women had 1–3 measures of serum concentrations of boron during pregnancy, and we first evaluated if boron concentrations varied during pregnancy using mixed-effects linear models. Because this showed that the boron concentrations increased during pregnancy, and the susceptibility of the fetus may differ at the different stages of development, we repeated the analyses of birth outcomes in relation to boron exposure during the third trimester. At this time point we had serum boron data for most of the women ($n = 152$).

In sensitivity analysis, we performed the multivariable-adjusted linear regression models considering only the children born at term (gestational week at birth ≥37). In addition, we adjusted the regression models for lithium concentrations in whole blood instead of urine, although whole blood lithium and serum boron were highly correlated ($r_s = 0.77$, $p \leq 0.0001$). We also adjusted the models for pre-pregnancy BMI or pre-pregnancy weight in addition to maternal height. Furthermore, the models were stratified by infant sex and by pre-pregnancy BMI (above or below the median), the latter as a general measure of nutritional status. We also stratified the models by season of birth (birth date between April–September or between October–March) or by season during early pregnancy (expressed as LMP during the winter or the summer months).

Statistical analyses were performed with Stata (StataCorp. 2012. Stata Statistical Software: Release 12.1. College Station, TX: StataCorp LP, USA) and a *p*-value < 0.05 was considered to be statistically significant.

3. Results

The main characteristics of the study participants and their newborns are summarized by tertiles of serum boron concentrations in Table 1. More detailed descriptive statistics of the investigated birth outcomes are presented in Supplementary Table S3. In total 180 of the recruited women were interviewed, provided blood and urine samples during pregnancy, and gave birth to live babies (Supplementary Fig. S1). The pregnant women were on average 25 years old (total range 13–41 years) and 38% were primiparous. Their average pre-pregnancy weight was 55 kg (range 38–86 kg) and height 153 cm (range 134–169 cm), and, partly due to their low stature which highly affects the BMI, 24% had a BMI above 25. The mean birth weight of the infants was 3022 ± 459 g (range 1250–4500 g), with 9.4% weighing <2500 g. The average birth length was 48 ± 2.3 cm (range 39–53 cm) and the head circumference 34 ± 1.7 cm (range 26–40 cm). The average gestational age at birth was 39 weeks (range 29–42 weeks), and 18% of the infants were born pre-term (before 37 gestational weeks). Almost all women (96%) had lived in the study area for several years (mean time 18 years, range 0.1–40 years). In the main village (San Antonio de los Cobres), drinking water samples collected repeatedly during the study period showed similarly high concentrations of boron, lithium and cesium (median values 6072, 750 and 351 μg/L, respectively) as measured a few years ago (Concha et al., 2010). The concentrations of arsenic in San Antonio de los Cobres had, however, decreased (median 99 μg/L, range 48–157 μg/L) due to installation of a filter. In all villages together, all water element concentrations showed wide ranges, e.g. for boron 377–10,929 μg/L.

The boron concentrations in serum (among all women having 1–3 samples each) ranged 0.73–605 μg/L (median 133 μg/L) and were similar to those in whole blood (median 134 μg/L, range 12–542 μg/L) (Table 1). Much higher concentrations were found in urine (10,494 μg/L, 1590–35,551 μg/L), reflecting the short half-life, but the correlation with serum boron was still high ($r_s = 0.71$; $p < 0.0001$). Also, as expected, the concentrations of lithium in blood and urine, cesium in blood and arsenic in urine increased significantly with increasing tertiles of serum boron (Table 1). Serum boron concentrations correlated with lithium concentrations in urine and whole blood ($r_s = 0.68$ and $r_s = 0.77$, respectively), but to a lesser extent with cesium in urine and whole blood ($r_s = 0.41$ and $r_s = 0.48$, respectively) as well as arsenic in urine ($r_s = 0.37$). Measurement of lead and cadmium in whole blood and selenium in serum showed a median concentration during pregnancy of 21 μg/L (range 6.9–99), 0.16 μg/L (0.042–0.47) and 86 μg/L (50–136), respectively.

Biomarker concentrations of boron, lithium, cesium and arsenic exposure per pregnancy trimester are presented in Table 2. We regressed

Table 1
Maternal and infant characteristics by tertiles of serum boron concentrations during pregnancy.

	Tertile 1 ($n = 60$)		Tertile 2 (n = 60)		Tertile 3 (n = 60)		
Variable	Median	5th–95th	Median	5th–95th	Median	5th–95th	*p*-Value[a]
Mothers							
Age (years)	23	16–37	24	16–37	25	15–37	0.84
Parity (n)	1	0–7.5	1	0–6	1	0–8	0.73
Pre-pregnancy weight (kg)	54	45–80	53	42–73	50	42–70	0.095
Height (cm)	153	145–166	153	146–161	152	144–158	0.079
Pre-pregnancy Body Mass Index (kg/m^2)	22	19–31	23	18–31	22	18–33	0.45
Education years (years)	9.0	3.0–14	9.0	2.0–14	9.0	0.5–14	0.96
Monthly income family (Argentinean pesos)	2050	0–6000	1700	0–5800	1000	0–4250	0.52
Boron in drinking water (μg/L)	5246	377–6525	5965	402–6525	6072	846–10,929	0.0049
Biomarker concentrations during pregnancy[b]							
Serum boron (μg/L)	75	11–101	133	111–161	205	168–381	0.0001
Whole blood boron (μg/L)	80	24–127	135	95–179	189	139–437	0.0001
Urinary boron (μg/L)	6592	1808–12,466	10,742	6132–15,832	13,684	7187–29,314	0.0001
Whole blood lithium (μg/L)	13	3.0–26	27	10–53	37	12–85	0.0001
Urinary lithium (μg/L)	916	169–1653	1631	451–3390	2106	1070–3846	0.0001
Whole blood cesium (μg/L)	75	4.3–198	108	9.8–223	166	11–362	0.0001
Urinary arsenic (μg/L)	74	24–273	145	32–274	129	43–469	0.0001
Infants							
Sex (boys, %)	51%		61%		51%		0.48
Gestational age at birth (weeks)	39	34–42	39	35–42	38	32–42	0.037
Birth weight (g)	3100	2320–3950	3040	2380–3650	3000	2110–3680	0.39
Birth length (cm)	49	42–51	48	42–51	47	43–50	0.037
Birth head circumference (cm)	34	31–37	34	31–36	34	32–36	0.99

[a] Kruskall Wallis *H*-test (for continuous variables) and chi-squared test (for categorical variables) across tertiles.
[b] Average during pregnancy for those with 2 or more measurements.

the serum boron concentrations across pregnancy against gestational week and adjusted for parity, weight of the mothers at the time of the visit (which was considered relevant as it increased during pregnancy) and monthly income of the family (high, low). The adjusted mixed-effect linear models showed that the serum boron concentration increased by 3.1 μg/L per gestational week on average (95% CI 1.9; 4.4, p-value < 0.001). The increase is visualized in Supplementary Fig. S2.

We found significant differences across the tertiles of serum boron for gestational age at birth and birth length (Table 1). The indicated differences in gestational age at birth in relation to serum boron were, however, influenced by two extreme values. The linear regression models adjusted for gestational age (weeks) and gestational age (quadratic term) only showed generally positive associations between serum boron exposure (average in pregnancy) and birth size below the spline at 80 μg/L (indicated in the scatter plots), and inverse associations above the splines. This was most apparent for birth length, for which the associations were statistically significant ($n = 130$; Table 3). In the multivariable-adjusted linear regression models, the estimates changed very little. For each 10 μg/L increase in serum boron concentration, above the spline knot at 80 μg/L, the birth length decreased by 0.07 cm. Including the 2 women with the highest serum boron concentrations (571 and 605 μg/L) resulted in an increment of the effect coefficient by 20% (B −0.083, 95% CI −0.13; −0.033, p-value = 0.001). Below the spline knot, each 10 μg/L increase in serum boron concentration was associated with a 0.26 cm increase in birth length.

The regression models based on exposure measures during the third trimester only ($n = 152$ women; median serum boron 135, range 0.73–447 μg/L) showed strong inverse associations above the spline with both birth length and weight (Table 4). The effect estimate for the inverse association between serum boron concentrations (above 80 μg/L) and birth length increased by 28% when considering only the third trimester instead of the whole pregnancy (B −0.088 for each 10 μg/L increase in serum boron concentration, 95% CI −0.14; −0.036, p-value = 0.001). Also, the inverse association between serum boron concentrations (above 80 μg/L) and birth weight was statistically significant, and the fully adjusted effect estimate increased >2.5 times (from −4.5 to −12 g per 10 μg/L increase in serum boron) when considering only exposure in the third trimester. We found no statistically significant association between serum boron concentrations above 80 μg/L and birth head circumference in any model.

In sensitivity analyses, when we restricted the analyses to children born at term, the coefficients of the inverse associations between serum boron concentrations above 80 μg/L (both during whole pregnancy and only during the third trimester) and birth length increased and gained even stronger significance (Tables 3 and 4), while the positive associations between serum boron concentrations below 80 μg/L and birth length became weaker and no longer significant (Tables 3 and 4). Also, we adjusted the whole pregnancy and the third trimester models for pre-pregnancy BMI or pre-pregnancy weight in addition to maternal height, but the effect coefficients changed very little or not at

Table 2
Biomarker concentrations of boron, lithium, cesium and arsenic in the pregnant women by trimenter.

	1st trimester ($n = 31$)		2nd trimester ($n = 99$)		3rd trimester ($n = 152$)	
	Median	5th–95th	Median	5th–95th	Median	5th–95th
Gestational week	12	7.6–14	22	16–27	33	28–38
Serum boron (μg/L)	118	32–232	131	20–273	135	26–315
Whole blood boron (μg/L)	131	55–245	119	38–210	139	47–280
Urinary boron (μg/L)	10,076	3107–19,681	9881	2803–23,058	10,307	2972–21,144
Whole blood lithium (μg/L)	21	6.6–54	23	4.1–52	26	5.7–63
Urinary lithium (μg/L)	1117	209–3768	1398	262–3509	1465	273–3732
Whole blood cesium (μg/L)	132	12–288	107	8.3–220	111	8.9–253
Urinary arsenic (μg/L)	98	31–458	104	26–282	129	33–414

Table 3
Linear spline regression analyses of birth outcomes in relation to maternal boron concentrations in serum (women's average concentrations during pregnancy).

	Basic adjustment[a,b]		Further adjustment[b,c]	
Outcomes	B (95% CI)	p-Value	B (95% CI)	p-Value
Birth length (n = 161)				
≤80 μg/L (n = 31)	0.27 (0.060, 0.47)	0.012	0.26 (0.061, 0.47)	0.011
>80 μg/L (n = 130)	−0.061(−0.12, −0.0022)	0.042	−0.069 (−0.14, −0.0024)	0.043
Birth weight (n = 167)				
≤80 μg/L (n = 31)	38 (−2.6, 78)	0.067	36 (−3.0, 76)	0.070
>80 μg/L (n = 136)	−7.1 (−18, 4.3)	0.22	−4.5 (−17, 8.1)	0.48
Birth head circumference (n = 150)				
≤80 μg/L (n = 28)	0.077 (−0.11, 0.26)	0.41	0.13 (−0.055, 0.31)	0.17
>80 μg/L (n = 122)	−0.0050 (−0.043, 0.054)	0.84	0.021 (−0.033, 0.075)	0.45
Children born at term (≥37 gestational weeks)				
Birth length (n = 134)				
≤80 μg/L (n = 25)	0.24 (0.035, 0.45)	0.022	0.19 (−0.010, 0.39	0.063
>80 μg/L (n = 109)	−0.081(−0.14, −0.024)	0.006	−0.11 (−0.17, −0.042)	0.001
Birth weight (n = 139)				
≤80 μg/L (n = 25)	34 (−7.3, 75)	0.11	28 (−12, 68)	0.17
>80 μg/L (n = 114)	−9.7 (−21, 1.6)	0.093	−8.7 (−21, 3.9)	0.18
Birth head circumference (n = 123)				
≤80 μg/L (n = 22)	0.093 (−0.11, 0.30)	0.38	0.12 (−0.093, 0.33)	0.27
>80 μg/L (n = 101)	−0.00027 (−0.052, 0.052)	0.99	0.019 (−0.041, 0.079)	0.53

[a] Basic models adjusted for gestational age at birth (weeks) and gestational age at birth squared (weeks^2).
[b] Coefficient per 10 μg/L change of boron concentration in serum.
[c] Further adjusted models include gestational age at birth (weeks), gestational age at birth squared (weeks^2), parity (number of children), height of the mother (cm), monthly family income (above or below 1700 Argentinean pesos), infant sex, cesium in whole blood (μg/L) and lithium in urine (μg/L).

all. The inverse associations between third trimester serum boron above 80 μg/L and birth length and birth weight (Table 4) were still significant when the model was additionally adjusted for other exposure biomarkers, i.e. lithium in whole blood and cesium in urine.

Stratifying the models for infant sex did not indicate any sex differences in the effect of boron (data not shown). When stratifying all the models for nutritional status, assessed as pre-pregnancy BMI above or below the median of 22.5 kg/m^2, we found a stronger positive association between serum boron below the spline knot at 80 μg/L and birth length in the lower BMI stratum (B 0.40 cm per each 10 μg/L increase in serum boron, 95% CI 0.12; 0.69, p-value = 0.007) as compared with the women in the higher BMI stratum (B 0.18 cm per each 10 μg/L increase in serum boron, 95% CI −0.14; 0.49, $p = 0.27$), but the inverse association between serum boron above 80 μg/L and birth length was similar in both groups. This was also the case for the associations between serum boron (below the spline knot at 80 μg/L) and birth weight (B 63 g per each 10 μg/L increase in serum boron, 95% CI 53; 122, $p = 0.033$, for women in the lower BMI stratum vs. B 18 g per each 10 μg/L increase in serum boron, 95% CI −38; 73, $p = 0.53$, for women in the higher BMI stratum). We found no differences when we compared women with LMP in the winter or the summer, or among births during the different seasons.

4. Discussion

This cohort study, involving pregnant women exposed to a wide range of boron concentrations in drinking water, provides the first epidemiological indication of a potential adverse effect of elevated boron

Table 4
Linear spline regression analyses of birth outcomes in relation to maternal boron concentrations in serum during the third trimester.

	Basic adjustment[a,b]		Further adjustment[b,c]	
Outcome	B (95% CI)	p-Value	B (95% CI)	p-Value
Birth length (n = 138)				
≤80 μg/L (n = 31)	0.23 (0.053, 0.41)	0.012	0.22 (0.049, 0.39)	0.012
>80 μg/L (n = 107)	−0.072 (−0.12, −0.026)	0.003	−0.088 (−0.14, −0.036)	0.001
Birth weight (n = 144)				
≤80 μg/L (n = 31)	47 (11, 82)	0.010	38 (4.9, 71)	0.025
>80 μg/L (n = 114)	−14 (−23, −4.9)	0.003	−12 (−22, −1.8)	0.021
Birth head circumference (n = 130)				
≤80 μg/L (n = 29)	0.16 (−0.011, 0.32)	0.067	0.19 (0.020, 0.35)	0.029
>80 μg/L (n = 101)	−0.026 (−0.066, 0.014)	0.21	−0.0065 (−0.053, 0.040)	0.78
Children born at term (≥37 gestational weeks)				
Birth length (n = 117)				
≤80 μg/L (n = 24)	0.16 (−0.0035, 0.33)	0.055	0.13 (−0.024, 0.29)	0.095
>80 μg/L (n = 93)	−0.071 (−0.11, −0.029)	0.001	−0.095 (−0.14, −0.048)	<0.001
Birth weight (n = 122)				
≤80 μg/L (n = 24)	31 (−3.5, 65)	0.078	24 (−8.5, 56)	0.15
>80 μg/L (n = 98)	−13 (−21, −3.9)	0.005	−11 (−21, −1.5)	0.025
Birth head circumference (n = 109)				
≤80 μg/L (n = 22)	0.13 (−0.057, 0.31)	0.17	0.15 (−0.039, 0.34)	0.12
>80 μg/L (n = 87)	−0.023 (−0.065, 0.020)	0.30	−0.0044 (−0.056, 0.047)	0.87

[a] Basic models adjusted for gestational age at birth (weeks) and gestational age at birth squared (weeks^2).
[b] Coefficient per 10 μg/L change of boron concentration in serum.
[c] Further adjusted models include gestational age at birth (weeks), gestational age at birth squared (weeks^2), parity (number of children), height of the mother (cm), monthly family income (above or below 1700 Argentinean pesos), infant sex, cesium in whole blood (μg/L) and lithium in urine (μg/L).

exposure on size at birth. Serum boron concentrations increased during pregnancy and the exposure during the third trimester (median 135 μg/L, range 0.73–447 μg/L) appeared to be more critical than that earlier in pregnancy. At serum boron concentrations higher than 80 μg/L, the turning point in the apparent inverted U-shaped dose-effect curve, an increase of 100 μg/L in late pregnancy was associated with a reduction in birth length of 0.9 cm, corresponding to about 0.4 SD, and a decrease in birth weight of 120 g (0.3 SD). The associations were robust, and the estimates changed only marginally when adjusting for multiple factors known to affect fetal growth, including co-exposure to cesium and lithium through drinking water. The boron concentrations in serum were strongly correlated with those in whole blood and urine and appeared to be the most suitable biomarker of the prenatal exposure.

Although this is the first human study on impaired fetal development by boron, there is some support for such an effect in experimental studies. As reviewed in the background document for development of WHO Guidelines for Drinking-water Quality (WHO, 2009), experimental studies in rats, mice and rabbits have found reduced fetal growth and skeletal malformations among the critical effects of in utero boron exposure. The mode of action of boron toxicity is unknown, but in postmenopausal women, boron has been shown to interact with calcium and phosphorus (Hunt et al., 1997). Another study indicated an inverse association of boron in placental tissue ($N = 197$) from French mothers with the activity of δ-aminolevulinic acid dehydratase in umbilical cord blood (Huel et al., 2004). A study in young female pigs given a diet supplemented with 5 mg B/kg/day showed alterations in the serum thyroid hormone concentrations compared to those given the basal diet containing 1–2 mg/kg boron (Armstrong et al., 2001).

In a parallel study, we found that lithium exposure was inversely associated with birth length, but not birth weight (Harari et al., 2015). Although lithium, which was also present in the drinking water, was adjusted for in the models, we cannot rule out that the observed diminished birth length is the result of a combined exposure to both boron and lithium. Further studies looking at other populations exposed to elevated boron concentrations would be enlightening on this matter. The potential consequences of smaller size at birth for health and disease later in life, including adulthood, have become evident in recent years (Barker et al., 2013; Walker et al., 2007). In particular, associations between impaired prenatal growth and enhanced risk of early childhood morbidity and mortality, as well as impaired brain function and greater reproductive risks and morbidity later in life, have been well established (Barker et al., 2013; Berngard et al., 2013).

The associations between serum boron concentration and birth length and weight were non-linear, with an indicated positive slope below 80 μg/L serum boron. The positive slopes appeared, however, less obvious for children born at term and the sample size was not large enough for evaluation of preterm births separately. While boron is an essential element to plants (Wimmer and Eichert, 2013), and possibly zebra fish (Rowe and Eckhert, 1999), its essentiality for humans and other mammals is still controversial. Boron is naturally found in healthy food items such as vegetables, fruits and nuts (e.g. peaches contain up to 5.3 μg boron/g; peas 4.6 μg/g; carrots 3.0 μg/g), while meat, eggs and dairy products usually contain <0.4 μg/g (Hunt and Meacham, 2001; Rainey et al., 1999; Ysart et al., 1999). As the diet in the study area is largely based on vegetables, potatoes, maize and rice, a possible explanation for the observed increase in size at birth with increasing serum boron concentrations up to 80 μg/L may be that it merely reflects the intake of nutrients via food. Such an explanation is supported by the observed stronger positive associations between serum boron and birth length and weight in mothers with low BMI, which was used as a marker of nutritional status. On the contrary, higher concentrations of boron were evidently the result of consumption of drinking water with high content of boron. In fact, most women (93%) with serum boron above 80 μg/L had >3 mg/L in their drinking water.

We observed that boron concentrations in serum increased during pregnancy. This increase in serum boron might have been due to release of boron that had accumulated in the bones (Chapin et al., 1997) during the pregnancy-related increase in bone turnover (Zeni et al., 2003). Because of this increase in serum boron concentrations during pregnancy, the time of blood sampling is a factor to consider when comparing concentrations across studies. Available literature data on serum boron concentrations in pregnant women mainly concern the second trimester, and were reported to be on average 23 ± 13 μg/L in an American study (Pahl et al., 2001), 15.5 ± 4.2 μg/L in Turkey (Caglar et al., 2012), and 122 ± 39 μg/L in the city of Beer Sheva in Israel (Silberstein et al., 2014). The latter concentrations are surprisingly high, and close to those in the second trimester in the present study (140 ± 87 μg/L). Unfortunately, none of the studies referred to above did evaluate any adverse effects of the boron exposure. Obviously, more intense assessment of boron in drinking water and related health effects is warranted.

The main strengths of this study are the prospective design and the high participation rate (88%), ensuring that most pregnant women living in San Antonio de los Cobres and surrounding villages were included. We were able to adjust for multiple potential confounders, including other potentially toxic elements in the drinking water. However, the potential impact of glomerular filtration rate, which may change during pregnancy (Vesterinen et al., 2015), could not be adjusted for and thus, we cannot rule out the presence of residual confounding. We also validated serum boron as a biomarker of exposure by comparison with concentrations in whole blood and urine. The main limitations of this study were the fairly small sample size and the lack of samples in both early and late pregnancy for all women. The latter was due to the fact that some women were recruited during a later stage of pregnancy, and that a few were unavailable for follow-up in late pregnancy. Moreover, there are no certified reference materials available for the measurements of boron in serum, whole blood or urine but we could use the certified reference material NIST SRM 1643e for the analysis of trace elements in water samples.

5. Conclusions

This is the first report showing inverse associations between elevated environmental boron exposure and birth size. Many other areas in the world have boron concentrations in drinking water, including bottled water, exceeding the WHO guideline level of 2.4 mg/L. Thus, the findings may have major public health consequences, as the potential consequences of smaller size at birth for health and disease later in life, including adulthood, have become increasingly evident in recent years (Barker et al., 2013; Berngard et al., 2013; Walker et al., 2007). However, studies on other populations and, in particular, studies to reveal the mechanisms of action, are needed.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.07.017.

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