Exposure to lithium through drinking water and calcium homeostasis during pregnancy: A longitudinal study

Florencia Harari [a], Agneta Åkesson [b], Esperanza Casimiro [c], Ying Lu [a], Marie Vahter [a,*]

[a] Unit of Metals and Health, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[b] Unit of Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[c] Atención Primaria de la Salud, Área Operativa XXIX, Hospital Dr. Nicolás Cayetano Pagano, San Antonio de los Cobres, Salta, Argentina

ARTICLE INFO

ABSTRACT

There is increasing evidence of adverse health effects due to elevated lithium exposure through drinking water but the impact on calcium homeostasis is unknown. This study aimed at elucidating if lithium exposure through drinking water during pregnancy may impair the maternal calcium homeostasis. In a population-based mother-child cohort in the Argentinean Andes (n=178), with elevated lithium concentrations in the drinking water (5–1660 µg/L), blood lithium concentrations (correlating significantly with lithium in water, urine and plasma) were measured repeatedly during pregnancy by inductively coupled plasma mass spectrometry and used as exposure biomarker. Markers of calcium homeostasis included: plasma 25-hydroxyvitamin D_3, serum parathyroid hormone (PTH), and calcium, phosphorus and magnesium concentrations in serum and urine. The median maternal blood lithium concentration was 25 µg/L (range 1.9–145). In multivariable-adjusted mixed-effects linear regression models, blood lithium was inversely associated with 25-hydroxyvitamin D_3 (-6.1 nmol/L [95%CI -9.5; -2.6] for a 25 µg/L increment in blood lithium). The estimate increased markedly with increasing percentiles of 25-hydroxyvitamin D_3. In multivariable-adjusted mixed-effects logistic regression models, the odds ratio of having 25-hydroxyvitamin D3 < 30 nmol/L (19% of the women) was 4.6 (95%CI 1.1; 19.3) for a 25 µg/L increment in blood lithium. Blood lithium was also positively associated with serum magnesium, but not with serum calcium and PTH, and inversely associated with urinary calcium and magnesium. In conclusion, our study suggests that lithium exposure through drinking water during pregnancy may impair the calcium homeostasis, particularly vitamin D. The results reinforce the need for better control of lithium in drinking water, including bottled water.

1. Introduction

A recently discovered environmental health problem of potential public health relevance is the presence of elevated concentrations of lithium in drinking water (Concha et al., 2010; Ormachea Munoz et al., 2013; Reimann and Birke, 2010). Lithium has been suggested to be essential for humans (Pickett and O'Dell 1992; Schrauzer, 2002), however, the evidence is weak. On the other hand, lithium exposure has been associated with impaired thyroid function in women (Broberg et al., 2011), including pregnant women (Harari et al. 2015a), in northern Argentina, where the lithium concentrations in drinking water range 5–1660 µg/L. Lithium readily crosses the placenta (Harari et al., 2012) and an inverse association between maternal blood lithium

concentrations during pregnancy and birth length has also been observed (Harari et al., 2015b).

Lithium has long been used in the treatment of bipolar disease (Grandjean and Aubry, 2009). The side effects of lithium therapy include impaired calcium homeostasis, often as hyperparathyroidism (McKnight et al., 2012; Shine et al., 2015). Whether lithium in drinking water can have similar effects in the general population is not known. The calcium homeostasis is strictly regulated because of calcium's important role in bone formation, intracellular signaling and muscle contraction. Parathyroid function and circulating concentrations of magnesium, phosphorus and vitamin D are vital parts of this regulation (Brannon and Picciano, 2011).

During pregnancy, deficient vitamin D and elevated parathyroid hormone concentrations have been associated with impaired maternal and fetal health, specially preeclampsia, infectious diseases, fetal skeletal development and developmental programming (Brannon and Picciano, 2011; Karras et al., 2014). Therefore, we aim at elucidating the potential impact of lithium

exposure through drinking water on maternal calcium home-ostasis during pregnancy in a mother-child cohort in the Argentinean Andes.

2. Methods

2.1. Study population

The study was performed within our mother-child cohort in San Antonio de los Cobres and surrounding nine small villages in the Andean part (3180–4070 m above sea level; latitude -24; about 8000 inhabitants, mostly indigenous) of the Salta province, northern Argentina (Harari et al., 2015b). The drinking water in this area, mostly public water supplies, contains varying concentrations of lithium (5–1660 µg/L), frequently also arsenic, cesium and boron (Concha et al., 2010; Harari et al., 2015b). All pregnant women in the study area with an estimated delivery date between October 2012 and December 2013 were invited to participate in the study. Out of a total of 221 pregnant women, 194 became enrolled. We excluded two women who had spontaneous abortions and 14 who lacked exposure or calcium biomarkers giving a final number of 178 women. More details about the study area and recruitment of this cohort have been described elsewhere (Harari et al., 2015b).

2.2. Data and sample collection

This cohort was designed to obtain repeated exposure and outcomes measures during pregnancy. At the first meeting, we interviewed the pregnant women about age, last menstrual period (LMP), pre-pregnancy weight, parity, family income, education level, smoking, alcohol consumption, coca chewing, and personal and familial history of diseases. We measured height at the first visit. At each visit, we asked about potentially encountered health problems during pregnancy, collected blood and urine samples, and measured weight, lean body mass (LBM) and blood pressure (Harari et al., 2015b). We measured urinary albumin using HemoCue® Albumin 201 System (HemoCue AB, Ängelholm, Sweden) and calculated the gestational age, pre-pregnancy body mass index and LBM (Harari et al., 2015b).

Venous blood samples were collected in Trace Elements Sodium Heparin tubes and in Trace Elements Serum Clot Activator tubes (Vacuette®; Greiner bio-one, Kremsmünster, Austria). We fractionated serum and plasma by centrifugation (3000 rpm, 10 min) exactly 15 min after blood withdrawal. Serum samples were used for analyses of Parathyroid hormone (PTH), calcium, phosphate, magnesium, boron and albumin. Plasma intended for analyses of 25-hydroxyvitamin D_3 concentrations was carefully protected from UV-light. Urine was collected in disposable trace element-free plastic cups and immediately transferred to 20-mL polyethylene bottles (Zinsser Analytic GMBH, Frankfurt, Germany). Water samples were collected in 20-mL polyethylene bottles during the study period. All samples were kept at $-20\,°C$ until transported to Karolinska Institutet, Sweden, where they were stored at $-80\,°C$ until analysis.

2.3. Exposure assessment

The assessment of lithium exposure was based on the concentrations in whole blood and we compared those with the concentrations in urine and drinking water. We were not able to use serum lithium concentrations as the Trace Elements Serum Clot Activator tubes were severely contaminated by lithium (Lu et al., 2015), but in a sub-set (N=20) of the women we found an excellent correlation (r_s=0.99) between lithium concentrations in whole blood and plasma (from non-contaminated tubes). The plasma lithium concentrations were on average 1.5 times that in whole blood (range of ratios 1.4–1.6) (Harari, 2015). We also measured arsenic (urine and water), cesium and boron (blood/serum, urine and water), as they were also present in the drinking water in the study area (Concha et al., 2010; Harari et al., 2015b). All elements were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies, Tokyo, Japan), with the collision/reaction cell in no gas mode (lithium, boron and cesium) or helium mode (arsenic). Before analysis, the urine and water samples were diluted 1:10 with 1% nitric acid (HNO_3 65% w/w, ppb-trace analysis grade, Scharlab S.L., Sentmenat, Spain). Arsenic metabolites in urine were separated by high-performance liquid chromatography coupled with hydride generation and ICP-MS (Harari et al., 2015b). To compensate for variations in the dilution of urine, we adjusted the element concentrations to the mean urinary osmolality (694 mOsm/kg; range 141–1174) (Harari et al., 2015b).

For determination of lithium and cesium in blood and boron in serum, aliquots (0.2 mL) of the samples were diluted 1:25 with an alkali solution (1-butanol 2% (w/v), EDTA 0.05% (w/v), triton X-100 0.05% (w/v), NH_4OH 1% (w/v)) (Lu et al., 2015). This method provided a better limit of detection (LOD) and analytical stability for blood lithium than the conventional acid digestion (Lu et al., 2015).

Three serum samples had boron concentrations below the LOD (calculated as three times the standard deviation of the blanks) and those were replaced by LOD/$\sqrt{2}$. Commercially available reference materials were analyzed with the collected samples to validate analytical accuracy (Supplemental Table 1).

2.4. Markers of calcium homeostasis

Assessment of calcium metabolism was based on the concentrations of serum intact parathyroid hormone (PTH), plasma 25-hydroxyvitamin D_3, as well as total concentrations of calcium, phosphorus and magnesium in serum and urine. Total calcium, phosphorus and magnesium were analyzed by ICP-MS as described above (Harari et al., 2015b; Lu et al., 2015). Analyses of PTH, 25-hydroxyvitamin D_3 (also refer to as "vitamin D_3") and albumin, were performed at the Department of Clinical Chemistry at the University Hospitals in Lund and Malmö, Sweden. PTH was analyzed using a one-step sandwich method with electro-chemi-luminescence immunoassay (ECLI; Roche Diagnostics, Mannheim, Germany) and measured subsequently using a Cobas® 6000/8000 analyzer system (Roche Diagnostics International Ltd. Switzerland). Vitamin D_3 concentrations in plasma were measured using liquid chromatography connected to a triple quadrupole mass spectrometer (LC-MS/MS, API™ 4000, AB-Sciex, Concord, Ontario, Canada). Albumin was analyzed using an immunoturbidimetric method and measured using a Cobas® 6000/8000 analyzer system (Roche Diagnostics International Ltd., Switzerland). As calcium is mostly bound to albumin in the blood, serum calcium concentrations were adjusted for serum albumin (Payne et al., 1973). Total and albumin-adjusted serum calcium concentrations were used as outcomes.

2.5. Statistical analyses

Univariate associations were initially assessed using Spearman's rank correlation (r_s) or linear mixed-effects regression (for longitudinal data). We visually evaluated scatter plots of the outcomes versus exposure measures and covariates. Differences in the exposure and outcome markers, and covariates across tertiles of blood lithium were evaluated using Kruskal–Wallis H rank test or chi square test. We evaluated the associations between lithium exposure and biomarkers of calcium homeostasis (apparently

linear in the scatter plots) longitudinally during pregnancy (1st, 2nd and 3rd trimesters) using linear mixed-effects regression with random intercept and maximum likelihood estimation, based on 1–3 measurements per woman, and using linear regression for data in the 3rd trimester only. For dichotomous outcome variables, we used mixed-effects logistic regression with random intercept (for longitudinal analyses) and binomial logistic regression for data in the 3rd trimester only. We performed quantile regression analyses in the 3rd trimester based on five quantiles (5th, 25th, 50th, 75th and 95th) to assess the association of blood lithium on the whole distribution of the outcomes. Estimates, confidence intervals (CIs), and p-values were based on 500 bootstrap samples. Wald test was used to test for differences between the estimated regression coefficients across the quantiles.

Models were adjusted for covariates known to affect the calcium homeostasis or that influenced the estimates more than 10%. Initially, (Model 1) we adjusted the models for gestational age (weeks) and season of sampling (summer/fall/spring/winter), because these covariates were associated with most of the exposure and outcome measures. In model 2, we additionally adjusted for maternal age and urinary arsenic metabolites. The final multivariable-adjusted model 3 included also serum boron (μg/L), as this markedly influenced some of the associations. Maternal education level, maternal height, parity, parental monthly income, coca chewing, hemoglobin concentrations, lean body mass, cesium exposure and infections during pregnancy as well as ethnicity (kolla vs. others) did not influence the associations between blood lithium and the outcomes. Data on pre-pregnancy weight was missing for 17.3% of the women and, because this variable did not alter the coefficients of the various associations (tested by comparing coefficients before and after adjustment for weight in the models with complete data), it was not included in the final models.

Statistical analyses were performed using Stata (StataCorp LP. 2012. Stata Statistical Software: Release 12.1. College Station, TX, USA) and R (version 3.2.1, (R Core Team, 2015)). All tests were two sided and p-values < 0.05 were considered statistically significant.

3. Results

The highest water lithium concentration was observed in the largest village San Antonio de los Cobres (mean 718 μg/L, range 528–837; n=58 from one and the same source), as compared with 5.0–324 (n=83) in the other 9 villages. In spite of the moderate variation in the lithium concentrations in the public water in the main village, we observed wide variations in the concentrations of blood lithium (median 25 μg/L, range 1.9–145 μg/L; n=178) and urine lithium (1491 μg/L, 105–4598, n=178). Still, water lithium correlated well with blood lithium (r_s=0.40) and urine lithium (r_s=0.44).

In total, we obtained 269 observations of combined exposure and outcome measures from the 178 participating women; 31 in the 1st trimester, 94 in the 2nd and 144 in the 3rd. Only one woman reported smoking (seldom) and five used alcohol (very moderately). Blood lithium correlated strongly with urinary lithium (r_s=0.84, p < 0.001). Both blood lithium and urinary lithium increased with increasing gestational age, but the concentrations were not affected by the season of sampling. On the contrary, we observed a clear variation in the vitamin D_3 concentration by season of sampling (Fig. 1), but no influence by gestational age (p=0.85).

As shown in Table 1, there were no major differences in the maternal basic characteristics across tertiles of blood lithium concentrations (mean concentrations were used for women with several measurements). All the other exposure biomarkers (in late
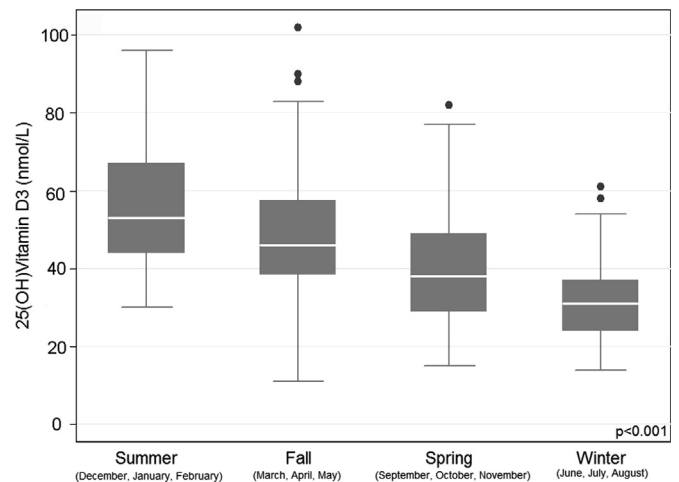

Fig. 1. Plasma concentrations of 25-hydroxyvitamin D_3 (all observations, n=269) by season of sampling. Box layers describe the 75th, 50th and 25th percentiles.

pregnancy for the purpose of comparison) increased with increasing blood lithium. Serum phosphorus and magnesium increased significantly while urinary calcium decreased across the tertiles of blood lithium (Table 1). The vitamin D_3 concentration did not differ across the tertiles of blood lithium or between the 10 villages (p=0.49). The overall median vitamin D_3 concentration was 41 nmol/L (mean ± SD 44 ± 17 nmol/L); and 58% of the women had vitamin D_3 concentrations < 50 nmol/L (< 20 ng/ml) and 19% had < 30 nmol/L (< 10 ng/ml). No woman had abnormal PTH (> 7.15 pmol/L) or serum magnesium concentrations (> 25 mg/L) and only 6 women (4%) had elevated albumin-adjusted serum calcium concentrations (> 10.4 mg/L). The correlation matrix of all biomarkers of exposure and outcomes and basic characteristics (Supplemental Fig. 1) showed a clear cluster of high correlations among the exposure markers. Also, calcium and magnesium in serum and urine were correlated, but not phosphorous in serum and urine.

Table 2 shows the linear mixed-effects regression analysis of the different outcome markers in relation to the maternal blood lithium concentrations. In the multivariable-adjusted model 2, we observed inverse associations of blood lithium with vitamin D_3, urinary calcium and magnesium and positive associations with serum phosphorus. After additional adjustment for serum boron (fully-adjusted model 3), the associations became stronger for vitamin D_3 (Fig. 2) and serum and urinary magnesium, while the association with serum phosphorus became non-significant (Table 2). Neither urinary arsenic, nor serum boron, was associated with any of the calcium markers. The cross-sectional linear regression analyses restricted to data in the 3rd trimester (n=144) basically confirmed the longitudinal results, but showed stronger estimates for the associations between blood lithium and vitamin D_3 and serum and urinary phosphorus (Supplemental Table 2). Season of sampling remained statistically significant in all the models with vitamin D_3. Stratification of model 3 by season of sampling (summer/fall vs. spring/winter) did not reveal any major differences in the association between lithium exposure and vitamin D_3 by season (data not shown). Exclusion of outliers (> 75 μg/L, n=4) did not change the estimates in any of the models. Restricting the analyses to the women in San Antonio de los Cobres, who had the highest lithium exposure, gave slightly stronger estimate for blood lithium and vitamin D_3 (−9.5 nmol/L, 95%CI −16; −3.0; model 3).

In the multivariable-adjusted mixed-effects logistic regression (all women), every 25 μg/L increase in blood lithium concentrations was associated with 3.5 higher odds (95%CI 1.0; 12) of having

Table 1
Maternal characteristics at baseline and outcome and exposure measures in late pregnancy by tertiles of mean blood lithium during pregnancy[a] (n=178).

	Tertile 1 (n=60)		Tertile 2 (n=59)		Tertile 3 (n=59)		p-Value[b]
Blood lithium (µg/L)	11	(1.9–18)	25	(19–31)	47	(31–145)	
Maternal characteristics at baseline							
Maternal age (years)	24	(15–41)	22	(14–37)	24	(13–40)	0.74
Parity (n)	1.0	(0.0–11)	1.0	(0.0–12)	1.0	(0.0–8.0)	0.84
Maternal education level (years)	9.0	(0.0–16)	9.0	(0.0–17)	10	(0.0–15)	0.59
Parental monthly income (ARS)	2150	(0.0–8000)	1550	(0.0–6000)	1000	(0.0–8000)	0.69
Height (cm)	153	(144–169)	153	(144–168)	152	(134–161)	0.11
Pre-pregnancy weight (kg)	53	(40–86)	54	(42–74)	50	(38–77)	0.12
Systolic blood pressure (mmHg)	105	(80–148)	105	(80–130)	100	(80–195)	0.99
Diastolic blood pressure (mmHg)	65	(40–95)	65	(40–90)	70	(45–105)	0.71
Coca chewing (n (%))	26	(33%)	24	(30)	29	(37%)	0.64
Outcomes in late pregnancy							
Serum iPTH (pmol/L)	2.1	(0.65–6.2)	1.8	(0.73–5.4)	1.9	(0.53–5.3)	0.83
Plasma 25(OH)Vitamin D_3 (nmol/L)	44	(21–90)	39	(11–102)	37	(13–88)	0.11
Total serum calcium (mg/L)	89	(78–109)	88	(69–101)	88	(78–98)	0.14
Adjusted serum calcium (mg/L)[c]	97	(83–121)	98	(82–109)	98	(86–114)	0.84
Urinary calcium (mg/L)[d]	102	(2.1–319)	108	(7.8–375)	68	(1.9–510)	0.015
Serum phosphorus (mg/L)	142	(103–187)	152	(99–195)	154	(98–203)	0.0020
Urinary phosphorus (mg/L)[d]	376	(65–1106)	377	(160–892)	335	(127–1049)	0.68
Serum magnesium (mg/L)	18	(14–22)	18	(13–21)	19	(15–22)	0.0074
Urinary magnesium (mg/L)[d]	56	(10–128)	62	(19–131)	54	(9.4–185)	0.24
Urinary albumin (mg/L)	6	(0–710)	6	(0–240)	7	(0–5900)	0.49
Exposure biomarkers in late pregnancy							
Urinary lithium (µg/L)[d]	714	(105–1855)	1460	(671–5185)	2397	(1031–4598)	< 0.001
Blood cesium (µg/L)	63	(2.5–576)	115	(6.2–711)	142	(11–362)	< 0.001
Urinary cesium (µg/L)[d]	278	(14–1748)	474	(26–1696)	603	(17–2248)	< 0.001
Urinary arsenic (µg/L)[d]	60	(13–1826)	136	(25–556)	158	(43–2450)	< 0.001
Serum boron (µg/L)	77	(0.13–262)	133	(0.13–447)	201	(25–658)	< 0.001

Abbreviations: ARS: Argentinian pesos, iPTH: intact parathyroid hormone.

[a] Median and range (minimum-maximum) or n(%) are presented by the tertiles of lithium (based on the average concentrations) during pregnancy.
[b] Comparison across tertiles of lithium based on Kruskal-Wallis rank test or chi square test.
[c] Serum calcium concentrations were adjusted by serum albumin levels.
[d] Urinary concentrations were adjusted for the mean urinary osmolality from all urine samples (694 mOsm/Kg).

Table 2
Linear mixed effect models of associations between maternal exposure to lithium through drinking water and concentrations of different markers of calcium homeostasis during pregnancy (n=178, observations=269).

Outcomes	Model 1[a]		Model 2[b]		Model 3[c]	
	B (95% CI)[d]	p	B (95% CI)[d]	p	B (95% CI)[d]	p
Intact serum PTH (pmol/L)	−0.015 (−0.21; 0.18)	0.88	0.029 (−0.18; 0.24)	0.78	0.019 (−0.25; 0.29)	0.89
Plasma 25(OH)Vitamin D_3 (nmol/L)	−3.4 (−5.8; −1.0)	0.005	−3.7 (−6.2; −1.2)	0.004	−6.1 (−9.5; −2.6)	0.001
Total serum calcium (mg/L)	−0.84 (−1.6; −0.13)	0.020	−0.57 (−1.3; 0.15)	0.12	−0.15 (−1.1; 0.84)	0.76
Albumin-adjusted serum calcium (mg/L)[e]	−0.021 (−0.093; 0.051)	0.58	−0.029 (−0.10; 0.046)	0.45	0.016 (−0.087; 0.12)	0.76
Urinary calcium (mg/L)[f]	−22 (−37; −7.0)	0.004	−29 (−44; −13)	< 0.001	−29 (−51; −7.2)	0.009
Serum phosphorus (mg/L)	4.1 (1.2; 6.9)	0.005	4.4 (1.5; 7.4)	0.004	2.2 (−1.9; 6.4)	0.29
Urinary phosphorus (mg/L)[f]	−14 (−47; 19)	0.41	−18 (−53; 17)	0.30	−3.7 (−52; 45)	0.88
Serum magnesium (mg/L)	0.22 (−0.044; 0.48)	0.10	0.19 (−0.089; 0.45)	0.19	0.40 (0.008; 0.79)	0.045
Urine magnesium (mg/L)[f]	−3.9 (−8.6; 0.81)	0.11	−5.7 (−11; −0.79)	0.023	−8.9 (−16; −2.1)	0.010

[a] Adjusted for: Gestational age (weeks) and season of sampling (summer/fall/spring/winter).
[b] Additionally adjusted for: Maternal age (years) and urinary arsenic (µg/L). Model for total serum calcium was also adjusted for serum albumin (g/L).
[c] Additionally adjusted for: Serum boron (µg/L).
[d] Coefficients are expressed as changes in the outcomes for every 25 µg/L increase in blood lithium concentration.
[e] Serum calcium concentrations were adjusted by serum albumin levels.
[f] Urinary concentrations are adjusted for the mean urinary osmolality from all urine samples (694 mOsm/Kg).

vitamin D_3 concentrations < 50 nmol/L and 4.6 higher odds (95% CI 1.1; 19) of having vitamin D_3 concentrations < 30 nmol/L (Table 3).

The quantile regression analyses, performed in the 3rd trimester to explore potential variation in the association across the whole distribution of vitamin D_3 showed higher estimates in the highest quantiles of vitamin D_3. After adjustment for gestational age, season of sampling, maternal age, urinary arsenic and serum boron, the changes per 25 µg/L increment in blood lithium were as follows: 5th percentile: −2.7 nmol/L (95%CI−10; 4.9), 25th: −5.7 (−10; −1.4), 50th: −5.0 (−12; 1.5), 75th: −8.3 (−15; −1.6) and 95th: −17 (−23; −10; corresponding to 1 SD; p for trend < 0.001).

To investigate the potential involvement of impaired kidney function in the association between blood lithium and vitamin D_3,
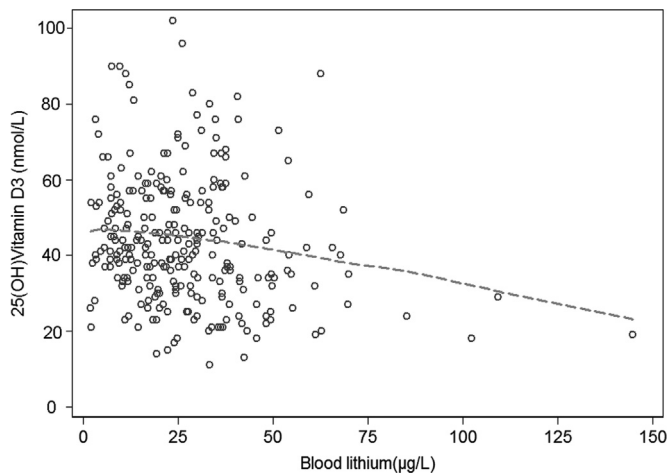
Fig. 2. Associations of maternal blood lithium with 25(OH)vitamin D_3 concentrations during pregnancy. Dashed lines represent the fitted model of blood lithium and 25(OH)vitamin D_3 concentrations adjusted for gestational age, season of sampling (summer/fall/spring/winter), maternal age, urinary arsenic and serum boron.

Table 3
Maternal risk (95% confidence intervals) of having vitamin D_3 concentrations < 50 nmol/L or < 30 nmol/L in relation to exposure to lithium through drinking water across pregnancy and in the 3rd trimester.

	Cases (n (%))	Odds ratio (95% CI)[a]	*p*-value
25(OH)vitamin $D_3 < 50$ nmol/L			
Across pregnancy[b]	181 (67)	3.46 (1.040; 11.5)	0.043
Only 3rd trimester[c]	100 (70)	3.43 (1.23; 9.55)	0.018
25(OH)vitamin $D_3 < 30$ nmol/L			
Across pregnancy[b]	57 (21)	4.64 (1.11; 19.3)	0.035
Only 3rd trimester[c]	34 (24)	2.98 (1.21; 7.32)	0.017

[a] Adjusted for: Gestational age (weeks), season of sampling (summer/fall/spring/winter), maternal age (years), urinary arsenic (µg/L) and serum boron (µg/L). Odds ratios expressed for every 25 µg/L increase in blood lithium.

[b] n=178, observations=269.

[c] n=144.

we additionally adjusted the linear mixed-effects model 3 for urinary albumin, which resulted in a 23% attenuation of the estimate (-4.7 nmol/L; 95%CI -8.4; -1.0, $p=0.012$). Additional adjustment for urinary albumin similarly decreased the association between lithium and serum magnesium (from 0.40 mg/L (95%CI 0.008, 0.79) to 0.22 mg/L (-0.20; 0.64) per 25 µg/L increment of serum lithium). In addition, blood lithium was associated with 6.2 higher odds (95%CI 1.3–30; per every 25 µg/L increment) of having urinary albumin concentrations > 30 mg/L (adjusted for the same co-variates as model 3), and urinary albumin was inversely associated with vitamin D_3 ($p=0.003$) and serum magnesium concentrations ($p=0.007$).

4. Discussion

This longitudinal study suggests, for the first time, that elevated lithium exposure through drinking water may impair the calcium homeostasis during pregnancy. In particular, a 25 µg/L increment in blood lithium concentrations (range 1.9–145 µg/L) was associated with almost 5 times higher risk of having vitamin D_3 concentrations < 30 nmol/L (19% of the women). Furthermore, blood lithium was associated with higher serum magnesium and

lower urinary excretion of magnesium and calcium. The associations were robust and became generally stronger after adjustment for multiple potential confounders and effect modifiers. In contrast, we observed no association between blood lithium and PTH or serum calcium. Interestingly, we found by far the strongest association of lithium in the women with the highest serum vitamin D_3 concentrations (six times stronger association, compared with those with lowest vitamin D), which might indicate a genetic variation in susceptibility, e.g. in the vitamin D-binding protein gene (Powe et al., 2013).

Only few previous studies have measured 25-hydroxyvitamin D_3 concentrations in relation to lithium and all those involved patients on lithium treatment, i.e. with much higher lithium concentrations (serum lithium generally > 5000 µg/L, corresponding to about 3500 µg/L in whole blood). Three studies (n=7, n=111 and n=35) found lower 25-hydroxyvitamin D_3 concentrations in lithium-treated patients compared to controls (Haden et al., 1997; Oliveira et al., 2014; van Melick et al., 2014). Also in line with the present findings, clinical studies have shown an increase in serum magnesium (Transbol et al., 1978) and a decrease in urinary calcium concentrations in lithium-treated patients (Haden et al., 1997; Mak et al., 1998).

The observed inverse association between blood lithium and vitamin D_3 may be explained by different mechanisms. Because blood lithium was not associated with PTH in the present study, possibly explained by the known ability of lithium to reset the calcium "set-point" (Brown, 1981), i.e. the level of serum calcium at which PTH production is inhibited, non-parathyroid mechanisms could be involved. One possibility is that lithium stimulates the fibroblast growth factor 23 (FGF23), which in turn stimulates the production of 24-hydroxylase (CYP24A1), essential for the catabolism of 25-hydroxyvitamin D_3 and 1,25-hydroxyvitamin D_3 (Fakhri et al., 2014). A stimulation of FGF23 would increase the urinary excretion of bone minerals (Brannon and Picciano 2011; Fakhri et al., 2014), supporting our findings of inverse associations of blood lithium with urinary calcium, magnesium and phosphorus. Another possibility is that the apparent lithium-related decrease in vitamin D is a consequence of impaired kidney function, which is a known side effect of lithium therapy (McKnight et al., 2012). Indeed, we found that a 25 µg/L increment in blood lithium was associated with 6 times higher adjusted odds of having urinary albumin above 30 mg/L – a marker of impaired kidney function (Levey et al., 2015). Impaired kidney function has previously been associated with lower serum 25-hydroxyvitamin D_3 concentrations (Damasiewicz et al., 2013; de Boer et al., 2011), in line with the finding in the present study. The associations of lithium with magnesium and calcium might be explained by such a mechanism as well, because the homeostasis of these elements is mainly regulated by the kidneys (Blaine et al., 2015). In support to this, we found that also the association between lithium and serum magnesium was attenuated by urinary albumin. We found no association between blood lithium and blood pressure in the women; however, few women (n=3) had elevated blood pressure. Further investigations of a potential impact of drinking water lithium on kidney function are warranted.

Our findings have clinical relevance, since low vitamin D_3 concentrations during pregnancy may be detrimental for both maternal and fetal health (Brannon and Picciano, 2011). Particularly, low vitamin D_3 concentrations have been associated with preeclampsia, reduced intrauterine growth, shorter post-natal size and impaired neurodevelopment (Brannon and Picciano, 2011; Eckhardt et al., 2015; Moon et al., 2015). In the present study, 58% of the pregnant women had vitamin D_3 concentrations < 50 nmol/L and 19% had concentrations < 30 nmol/L, indicating apparent deficiency. Whether a lithium-related impairment of the vitamin D_3 metabolism, possibly in combination with the

previously indicated impairment of thyroid function in these women (Harari et al., 2015b), may explain the observed shorter birth length in relation to the lithium exposure (Harari et al., 2015b) is presently unknown.

The findings of low vitamin D concentrations in this study population raise the need for a more thorough follow-up of pregnant women and children in particular. High prevalence (97%) of vitamin D_3 levels < 50 nmol/L among children in the same study area was recently reported (Hirschler et al., 2013). Despite residing in a high altitude area with plenty of sunlight to enable endogenous vitamin D production (Wacker and Holick, 2013), the cold and windy climate of the Puna necessitates much clothing, leaving small areas of the fairly dark skin to be UVB-exposed. Indeed, we observed a marked decrease in vitamin D concentrations from summer to winter, an association independent of lithium. Similarly low serum vitamin D concentrations have been observed in elderly women in the highlands of Guatemala (mean 48.4 ± 11.6 nmol/L) (Sud et al., 2010) and in Swedish pregnant women in the 3rd trimester (mean 47.4 ± 18 nmol/L) (Brembeck et al., 2013). Among pregnant women in the U.S., the prevalence of low serum vitamin D_3 levels was higher among Hispanic Americans and African Americans, compared to Caucasians (Johnson et al., 2011), demonstrating the influence of skin pigmentation. In addition, dietary intake of vitamin D in our study area is likely low as they lack access to vitamin D-rich food such as oily fish and fortified foods.

The drinking water concentration of lithium varied considerably in the present study area, from 5 to 1660 µg/L (Concha et al., 2010; Harari et al., 2015b). Similar lithium concentrations (1000–2000 µg/L) have been reported for certain areas in Austria and northern Chile (Kapusta et al., 2011; Zaldivar, 1980), as well as for several brands of bottled water (Reimann and Birke, 2010). Lithium concentrations up to about 200 µg/L have been reported for drinking water in the U.S. (Texas), Greece, Japan, England and Italy (Pompili et al., 2015; Vita et al., 2015). To note, lithium is seldom included in the routine drinking water analysis.

The strengths of our study include the population-based longitudinal design, covering most of the Andean part of the Salta province, with wide ranges of lithium concentrations in drinking water. We used multiple biomarkers of calcium homeostasis collected longitudinally across pregnancy along with the measurement of the exposure biomarkers, and evaluated potential mechanisms and susceptibility factors. Also, we took special care to protect the collected blood samples from UV-light, to avoid biased vitamin D results.

As in all observational studies, our findings are associations and do not infer causality. Thus, we cannot rule out the possibility of residual or unmeasured confounding related to variations in the vitamin D status. Although the 25-hydroxyvitamin D_3 concentrations reflect the total exposure from dietary intakes and the endogenous production and are used to assess adequacy (Brannon and Picciano, 2011), the fact that they are influenced by factors such as thyroid hormone concentrations, kidney function and vitamin D receptor polymorphisms (Lips, 2007) makes this marker less useful to assess calcium homeostasis. Also, there is a lack of agreement in reference values. However, we carefully evaluated other potential explanations for the associations observed in relation to lithium exposure and found no indication of such influence. For example, restricting the analyses to women in the largest village, who have a more homogeneous lifestyle and ethnicity, did not change the results. The lack of associations of lithium exposure with PTH, calcium and phosphorus might have been caused by the known circadian variations in the plasma concentrations of these markers (Redmond et al., 2014). Due to the study logistics and the long distances between villages it was impossible to collect the samples at a specific time of the day. Another limitation is the lack

of determination of vitamin D-binding protein and various polymorphisms, which may influence the vitamin D levels. Investigation of these factors is warranted in future follow ups. Also, the interpretation of the association with serum phosphorus in the present study is uncertain as our measurements of total phosphorus do not only reflect the inorganic phosphate. Finally, the cohort was fairly small and we did not manage to obtain samples for all women at all time-points. Thus, the findings need to be followed-up in larger studies and in other populations.

5. Conclusions

Our study suggests that elevated lithium exposure through drinking water during pregnancy may impair the calcium homeostasis, particularly vitamin D. If confirmed, our findings are of public health relevance and reinforce the need for better control of drinking water sources, including bottled water.

Competing financial interest declaration

The authors declare that no competing financial interests exist.

Ethics

The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study was approved by the Ministry of Health, Salta, Argentina and by the regional ethical committee at Karolinska Institutet, Stockholm, Sweden. All women gave written informed consent after oral and written explanation of the study. For subjects below 18 years of age, we also obtained informed consent from a caregiver.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.01.031.

References

Blaine, J., Chonchol, M., Levi, M., 2015. Renal control of calcium, phosphate, and magnesium homeostasis. Clin. J. Am. Soc. Nephrol. 10 (7), 1257–1272.
Brannon, P.M., Picciano, M.F., 2011. Vitamin D in pregnancy and lactation in humans. Annu. Rev. Nutr. 31, 89–115.
Brembeck, P., Winkvist, A., Olausson, H., 2013. Determinants of vitamin D status in pregnant fair-skinned women in Sweden. Br J. Nutr. 110 (5), 856–864.

Broberg, K., Concha, G., Engstrom, K., Lindvall, M., Grander, M., Vahter, M., 2011. Lithium in drinking water and thyroid function. Environ. Health Perspect. 119 (6), 827–830.

Brown, E.M., 1981. Lithium induces abnormal calcium-regulated PTH release in dispersed bovine parathyroid cells. J. Clin. Endocrinol. Metab. 52 (5), 1046–1048.

Concha, G., Broberg, K., Grander, M., Cardozo, A., Palm, B., Vahter, M., 2010. High-level exposure to lithium, boron, cesium, and arsenic via drinking water in the andes of northern Argentina. Environ. Sci. Technol. 44 (17), 6875–6880.

Damasiewicz, M.J., Magliano, D.J., Daly, R.M., Gagnon, C., Lu, Z.X., Sikaris, K.A., et al., 2013. Serum 25-hydroxyvitamin D deficiency and the 5-year incidence of CKD. Am. J. Kidney Dis. 62 (1), 58–66.

de Boer, I.H., Katz, R., Chonchol, M., Ix, J.H., Sarnak, M.J., Shlipak, M.G., et al., 2011. Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. Clin. J. Am. Soc. Nephrol. 6 (9), 2141–2149.

Eckhardt, C.L., Gernand, A.D., Roth, D.E., Bodnar, L.M., 2015. Maternal vitamin D status and infant anthropometry in a US multi-centre cohort study. Ann. Hum. Biol. 42 (3), 215–222.

Fakhri, H., Pathare, G., Fajol, A., Zhang, B., Bock, T., Kandolf, R., et al., 2014. Regulation of mineral metabolism by lithium. Pflug. Arch. 466 (3), 467–475.

Grandjean, E.M., Aubry, J.M., 2009. Lithium: updated human knowledge using an evidence-based approach: part I: plinical efficacy in bipolar disorder. CNS Drugs 23 (3), 225–240.

Haden, S.T., Stoll, A.L., McCormick, S., Scott, J., Fuleihan, Ge-H., 1997. Alterations in parathyroid dynamics in lithium-treated subjects. J. Clin. Endocrinol. Metab. 82 (9), 2844–2848.

Harari, F., 2015. Maternal and Fetal Health in Relation to Lithium in Drinking Water (Thesis for doctoral degree (Ph.D.)). Karolinska Institutet, Stockholm. ISBN: 978-91-7676-033-8. ⟨https://openarchive.ki.se/xmlui/handle/10616/44792⟩.

Harari, F., Bottai, M., Casimiro, E., Palm, B., Vahter, M., 2015a. Exposure to lithium and cesium through drinking water and thyroid function during pregnancy: a prospective cohort study. Thyroid 25 (11), 1199–1208.

Harari, F., Langeen, M., Casimiro, E., Bottai, M., Palm, B., Nordqvist, H., et al., 2015b. Environmental exposure to lithium during pregnancy and fetal size: a longitudinal study in the Argentinean andes. Environ. Int. 77, 48–54.

Harari, F., Ronco, A.M., Concha, G., Llanos, M., Grander, M., Castro, F., et al., 2012. Early-life exposure to lithium and boron from drinking water. Reprod. Toxicol. 34 (4), 552–560.

Hirschler, V., Maccallini, G., Molinari, C., Aranda, C., 2013. Low vitamin D concentrations among indigenous Argentinean children living at high altitudes. Pediatr. Diabetes 14 (3), 203–210.

Johnson, D.D., Wagner, C.L., Hulsey, T.C., McNeil, R.B., Ebeling, M., Hollis, B.W., 2011. Vitamin D deficiency and insufficiency is common during pregnancy. Am. J. Perinatol. 28 (1), 7–12.

Kapusta, N.D., Mossaheb, N., Etzersdorfer, E., Hlavin, G., Thau, K., Willeit, M., et al., 2011. Lithium in drinking water and suicide mortality. Br. J. Psychiatry 198 (5), 346–350.

Karras, S.N., Anagnostis, P., Bili, E., Naughton, D., Petroczi, A., Papadopoulou, F., et al., 2014. Maternal vitamin D status in pregnancy and offspring bone development: the unmet needs of vitamin D era. Osteoporos. Int. 25 (3), 795–805.

Levey, A.S., Becker, C., Inker, L.A., 2015. Glomerular filtration rate and albuminuria for detection and staging of acute and chronic kidney disease in adults: a systematic review. J. Am. Med. Assoc. 313 (8), 837–846.

Lips, P., 2007. Relative value of 25(OH)D and 1,25(OH)2D measurements. J. Bone Miner. Res. 22 (11), 1668–1671.

Lu, Y., Kippler, M., Harari, F., Grander, M., Palm, B., Nordqvist, H., et al., 2015. Alkali dilution of blood samples for high throughput ICP-MS analysis-comparison with acid digestion. Clin. Biochem. 48 (3), 140–147.

Mak, T.W., Shek, C.C., Chow, C.C., Wing, Y.K., Lee, S., 1998. Effects of lithium therapy on bone mineral metabolism: a two-year prospective longitudinal study. J. Clin. Endocrinol. Metab. 83 (11), 3857–3859.

McKnight, R.F., Adida, M., Budge, K., Stockton, S., Goodwin, G.M., Geddes, J.R., 2012. Lithium toxicity profile: a systematic review and meta-analysis. Lancet 379 (9817), 721–728.

Moon, R.J., Harvey, N.C., Cooper, C., 2015. Endocrinology in pregnancy: influence of maternal vitamin D status on obstetric outcomes and the fetal skeleton. Eur. J. Endocrinol. 173 (2), R69–R83.

Oliveira, T.C., Campos Neto, I.A., Aguiar-Oliveira, M.H., Pereira Fde, A., 2014. Evaluation of parathyroid function and mineral metabolism in psychiatric patients using lithium salts. Arq. Bras. Endocrinol. Metab. 58 (6), 619–624.

Ormachea Munoz, M., Wern, H., Johnsson, F., Bhattacharya, P., Sracek, O., Thunvik, R., et al., 2013. Geogenic arsenic and other trace elements in the shallow hydrogeologic system of Southern Poopo Basin, Bolivian Altiplano. J. Hazard. Mater. 262, 924–940.

Payne, R.B., Little, A.J., Williams, R.B., Milner, J.R., 1973. Interpretation of serum calcium in patients with abnormal serum proteins. Br. Med. J. 4 (5893), 643–646.

Pickett, E.E., O'Dell, B.L., 1992. Evidence for dietary essentiality of lithium in the rat. Biol. Trace Elem. Res. 34 (3), 299–319.

Pompili, M., Vichi, M., Dinelli, E., Pycha, R., Valera, P., Albanese, S., et al., 2015. Relationships of local lithium concentrations in drinking water to regional suicide rates in Italy. World J. Biol. Psychiatry, 1–8.

Powe, C.E., Evans, M.K., Wenger, J., Zonderman, A.B., Berg, A.H., Nalls, M., et al., 2013. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N. Engl. J. Med. 369 (21), 1991–2000.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. Available: ⟨http://www.R-project.org/⟩.

Redmond, J., Jarjou, L.M., Zhou, B., Prentice, A., Schoenmakers, I., 2014. Ethnic differences in calcium, phosphate and bone metabolism. Proc. Nutr. Soc. 73 (2), 340–351.

Reimann, C., Birke, M., 2010. Geochemistry of European Bottled Water. Gebr. Borntraeger Verlagsbuchhandlung, Stuttgart, Germany.

Schrauzer, G.N., 2002. Lithium: occurrence, dietary intakes, nutritional essentiality. J. Am. Coll. Nutr. 21 (1), 14–21.

Shine, B., McKnight, R.F., Leaver, L., Geddes, J.R., 2015. Long-term effects of lithium on renal, thyroid, and parathyroid function: a retrospective analysis of laboratory data. Lancet.

Sud, S.R., Montenegro-Bethancourt, G., Bermudez, O.I., Heaney, R.P., Armas, L., Solomons, N.W., 2010. Older Mayan residents of the western highlands of Guatemala lack sufficient levels of vitamin D. Nutr. Res. 30 (11), 739–746.

Transbol, I., Christiansen, C., Baastrup, P.C., Nielsen, M.D., Giese, J., 1978. Endocrine effects of lithium. III. Hypermagnesaemia and activation of the renin-aldosterone system. Acta Endocrinol. 88 (3), 619–624.

Wacker, M., Holick, M.F., 2013. Sunlight and Vitamin D: a global perspective for health. Dermatoendocrinology 5 (1), 51–108.

van Melick, E.J., Wilting, I., Ziere, G., Kok, R.M., Egberts, T.C., 2014. The influence of lithium on calcium homeostasis in older patients in daily clinical practice. Int. J. Geriatr. Psychiatry 29 (6), 594–601.

Vita, A., De Peri, L., Sacchetti, E., 2015. Lithium in drinking water and suicide prevention: a review of the evidence. Int. Clin. Psychopharmacol. 30 (1), 1–5.

Zaldivar, R., 1980. Geographical gradients of lithium concentration in surface and drinking waters and in plasma of exposed subjects in Northern Chile (author's transl). Rev. Med. Chile 108 (9), 822–824.