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MATERNAL AND FETAL HEALTH IN RELATION TO LITHIUM IN DRINKING WATER

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Maternal and Fetal Health in Relation to Lithium in Drinking Water

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family

“When a thing is new, people say:

‘It is not true.’

Later, when its truth becomes obvious, they say:

‘It is not important.’

Finally, when its importance cannot be denied, they say:

‘Anyway, it is not new.”

William James

ABSTRACT

Lithium is an alkali metal commonly used for treating mood disorders. A more common source of lithium exposure worldwide is drinking water, including bottled water, although few measurements have been performed. Based on clinical and experimental studies, lithium at therapeutic doses may impair fetal growth and development. Also, lithium may impair the thyroid and calcium homeostases in lithium-treated patients, but data on people exposed to lithium through drinking water are very limited.

The overall aim of this PhD thesis was to elucidate the potential impact of the exposure to lithium via drinking water during pregnancy on maternal and fetal health. Specifically, we aimed at elucidating the transfer of lithium through the placenta and mammary gland and the potential impact of lithium exposure during pregnancy on fetal size and maternal thyroid and calcium homeostases.

By analyzing lithium in banked samples from a small mother-child cohort (n=11) recruited in 1996 in San Antonio de los Cobres, an area with elevated lithium in the drinking water in northern Argentina, we evidenced a marked transfer of lithium through the placenta. The lithium concentration in cord blood was at least as high as in maternal blood and both were highly correlated (r_s=0.82). In line with this, the lithium concentration in the newborns' first urine in life was highly elevated. The urinary lithium concentration of the infants decreased during exclusive breastfeeding, consistent with the observed lower transfer of lithium through the mammary gland into breast milk.

To clarify the potential impact of lithium exposure on fetal and birth size and underlying mechanisms, we recruited a larger mother-child cohort from October 2012 to December 2013 (n=194, participation rate 88%) covering most of the Andean part of the Province of Salta in northern Argentina. The lithium concentrations in the drinking water were about 700 μg/L in the main village of San Antonio de los Cobres and from 5.0 to 242 μg/L in the surrounding nine villages. The selected biomarker of lithium exposure was blood lithium (overall median 25 μg/L) which showed a wide range of distribution (1.9-145). Lithium concentration in blood correlated very well with that in plasma (r_s=0.99) and urine (r_s=0.84), and, to a lesser extent, with that in water (r_s=0.40).

In multivariable-adjusted linear regression models, we observed that maternal blood lithium concentrations were inversely associated with fetal size. A 25 μg/L increment in the blood lithium concentrations was associated with a statistically significant decrease of 0.5 cm in birth length. Newborns to mothers in the highest tertile of lithium exposure (median blood lithium 47 μg/L) were on average 0.8 cm shorter than those in the lowest tertile of exposure (median blood lithium 11 μg/L).

Based on multivariable-adjusted quantile regression across pregnancy, blood lithium concentrations were positively associated with thyrotropin (TSH) and inversely associated with free (fT3) and total triiodothyronine (T3) and with transthyretine (TTR).

Using multivariable-adjusted linear mixed-effects models across pregnancy, we observed blood lithium to be inversely associated with plasma vitamin D_3 concentrations and with urinary calcium and magnesium, and positively associated with serum magnesium. A 25 μg/L

increment in the blood lithium concentrations was associated with an odds ratio of 3.5 for having vitamin D_3 concentrations <50 nmol/L, and an odds ratio of 4.6 for having vitamin D_3 concentrations <30 nmol/L, an association independent of season of sampling.

Taken together, the results of this thesis provide evidence of a marked transfer of lithium through the placenta and a consequent lithium exposure to the fetus. This elevated fetal exposure seemed to impair the fetal size. Findings of a potential lithium-related impaired homeostasis of the thyroid and calcium systems in the mother during pregnancy might be underlying mechanisms of action of lithium. Further studies are indeed warranted.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-IV:

I. **Harari F**, Ronco AM, Concha G, Llanos M, Grandér M, Castro F, Palm B, Nermell B, Vahter M. Early-life exposure to lithium and boron from drinking water. *Reproductive Toxicology*. 2012 Dec;34(4):552-60.

II. **Harari F**, Langeén M, Casimiro E, Bottai M, Palm B, Nordqvist H, Vahter M. Environmental exposure to lithium during pregnancy and fetal size: a longitudinal study in the Argentinean Andes. *Environment International*. 2015 Apr;77:48-54.

III. **Harari F**, Bottai M, Casimiro E, Palm B, Vahter M. Exposure to lithium and cesium through drinking water and thyroid function during pregnancy: a prospective cohort study. *Thyroid*. 2015. In press.

IV. **Harari F**, Åkesson A, Casimiro E, Lu Y, Vahter M. Exposure to lithium through drinking water and calcium homeostasis during pregnancy: a longitudinal study. *Submitted.*

LIST OF OTHER SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

- **Harari F**, Engström K, Concha G, Colque G, Vahter M, Broberg K. N-6-adenine-specific DNA methyltransferase 1 (*N6AMT1*) polymorphisms and arsenic methylation in Andean women. *Environmental Health Perspectives*. 2013 Jul;121(7):797-803.

- Castro F, **Harari F**, Llanos M, Vahter M, Ronco AM. Maternal-child transfer of essential and toxic elements through breast milk in a mine-waste polluted area. *American Journal of Perinatology*. 2014 Nov;31(11):993-1002.

- Lu Y, Ahmed S, **Harari F**, Vahter M. Impact of Ficoll density gradient centrifugation on major and trace element concentrations in erythrocytes and blood plasma. *Journal of Trace Elements in Medicine and Biology*. 2015 Jan;29:249-54.

- Lu Y, Kippler M, **Harari F**, Grandér M, Palm B, Nordqvist H, Vahter M. Alkali dilution of blood samples for high throughput ICP-MS analysis-comparison with acid digestion. *Clinical Biochemistry*. 2015 Feb;48(3):140-7.

- Ameer SS, Engström K, **Harari F**, Concha G, Vahter M, Broberg K. The effects of arsenic exposure on blood pressure and early risk markers of cardiovascular disease: Evidence for population differences. *Environmental Research*. 2015 Jul;140:32-6.

- Wojdacz TK, **Harari F**, Vahter M, Broberg K. Discordant pattern of *BRCA1* gene epimutation in blood between mothers and daughters. *Journal of Clinical Pathology*. 2015 Jul;68(7):575-7.

CONTENTS

LIST OF ABBREVIATIONS

1-α-OHase	1-alpha-hydroxylase
$1,25(OH)_2$-D	1,25-dihydroxyvitamin D
$24,25(OH)_2$D	24,25 dihydroxyvitamin D
$25(OH)$-D_3	25-hydroxyvitamin D3
5'DI	5'-deiodinase I
5'DII	5'-deiodinase II
As	Arsenic
AC	Abdominal circumference
B	Boron
BMI	Body mass index
BPD	Biparietal diameter
Ca	Calcium
CI	Confidence intervals
Cs	Cesium
CYP24A1	24-hydroxylase
DBP	Vitamin D binding protein
FGF23	Fibroblast growth factor 23
FL	Femur length
fT3	Free triiodothyronine
fT4	Free thyroxine
FW	Fetal weight
GD	Gestational day
GW	Gestational week
HC	Head circumference
hCG	Human chorionic gonadotropin
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
INTA	Institute of Nutrition and Food Technology
iPTH	Intact parathyroid hormone
LBM	Lean body mass

Li	Lithium
LMP	Last menstrual period
LOD	Limit of detection
Mg	Magnesium
MMA	Methylarsonic acid
NH_4OH	Ammonium hydroxide
NIST	National Institute of Standards and Technology
OFD	Occipitofrontal diameter
P	Phosphorus
PTH	Parathyroid hormone
RBC	Red blood cells
Se	Selenium
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-binding protein
Tg	Thyroglobulin
THr	Thyroid hormone receptor
TPO	Thyroperoxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyrotropin or thyroid-stimulating hormone
TTR	Prealbumin or transthyretin
UVB	Ultraviolet B

1 INTRODUCTION

The focus of this thesis is the potential impact of maternal and fetal exposure to lithium from drinking water on fetal growth and maternal thyroid and calcium homeostases during pregnancy. Although lithium's pharmaceutical use as therapy for bipolar disease has been extensively studied (Grandjean and Aubry 2009b), very little is yet known concerning environmental exposure to lithium from drinking water and food, and in particular, the potential adverse health effects of such exposure on maternal and fetal health. It is particularly important to assess the exposure and potential health effects in early life since this is considered the most critical window of exposure for many contaminants (Barouki et al. 2012) and effects at this stage in life may persist or aggravate in adulthood (Barker et al. 2013; Barker et al. 2002). Besides reviewing the methods used and the results found, the present summary also focuses on certain methodological problems encountered as well as in more depth description of the potential involved mechanisms of lithium toxicity.

2 BACKGROUND

2.1 LITHIUM

2.1.1 Chemistry and use of lithium

The alkali metal lithium (chemical symbol Li, atomic number 3, atomic weight 6.9) is the 27th most abundant element on earth and is naturally found at varying concentrations in rocks, soil and water (Oruch et al. 2014). Naturally-occurring lithium is composed of two stable isotopes, ^{6}Li and ^{7}Li, the latter being the most abundant (92.5%) (Oruch et al. 2014). Today, lithium is primarily mined for use in the manufacturing of ceramics and glass (29%), batteries (27%) and, to a lesser extent, medicines (2%) (**Figure 1**). The largest lithium-producing countries are Australia and Chile, followed by China and Argentina (Jaskula 2015).

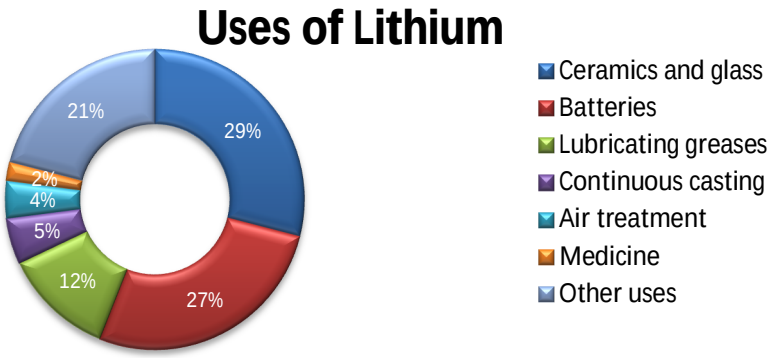

Figure 1. Uses of lithium. Data obtained from the U.S. Geological Survey (Jaskula 2012).

2.1.2 Lithium therapy

Lithium has been used in the therapy for mood disorders, particularly bipolar disease, for more than 50 years. Lithium efficacy is largely dose dependent, although there is a clear inter-individual variation in the response (Grandjean and Aubry 2009a). The daily dose of lithium, prescribed for mood disorders, ranges between 375 and 1,300 mg. Serum or plasma concentrations of lithium need to be regularly monitored in the patients. The therapeutic window of lithium concentration in plasma is usually between 0.8 and 1.2 mmol/L (i.e. 5,550–8,330 µg/L) (Grandjean and Aubry 2009a; Malhi et al. 2011).

Several side effects are reported in patients undergoing lithium therapy, particularly, increased risk of kidney failure, hypothyroidism, hyperparathyroidism and weight gain (McKnight et al. 2012). During pregnancy, lithium therapy has been associated with increased risk for miscarriages and prematurity, as well as malformations, hypothyroidism and goiter in the offspring (Cohen et al. 1994; Diav-Citrin et al. 2014; Gentile 2012; Grandjean and Aubry 2009c; Oyebode et al. 2012).

2.1.3 Lithium in the environment

2.1.3.1 Lithium in drinking water

A source of general environmental exposure to lithium is drinking water, although there are very few studies on both the exposure and the potential health consequences. Lithium concentrations in drinking water have been reported to vary from <1 to 219 µg/L in Texas (Schrauzer and Shrestha 1990), Japan (Ohgami et al. 2009), Italy (Pompili et al. 2015), Greece (Giotakos et al. 2015) and England (Kabacs et al. 2011); while in a few regions in Austria (Kapusta et al. 2011), northern Chile (Zaldivar 1980), southern Bolivia (Ormachea Munoz et al. 2013) and northern Argentina (Concha et al. 2010), the concentrations exceed 1,000 µg/L (**Figure 2**). However, the lithium concentration in drinking water sources is still unknown in most countries.

Several ecological studies have investigated the relationship between lithium concentrations in public drinking water and suicide rates (Bluml et al. 2013; Giotakos et al. 2013; Giotakos et al. 2015; Helbich et al. 2012, 2015; Ishii et al. 2015; Kabacs et al. 2011; Kapusta et al. 2011; Ohgami et al. 2009; Pompili et al. 2015; Schrauzer and Shrestha 1990; Sugawara et al. 2013). However, the results are inconsistent and due to the nature of these studies, many potential confounding factors are not considered at the individual level. It should be noted that the lithium concentrations in these studies are low (<1-219 µg/L, most <20 µg/L), and lower than those in the study area of the present thesis.

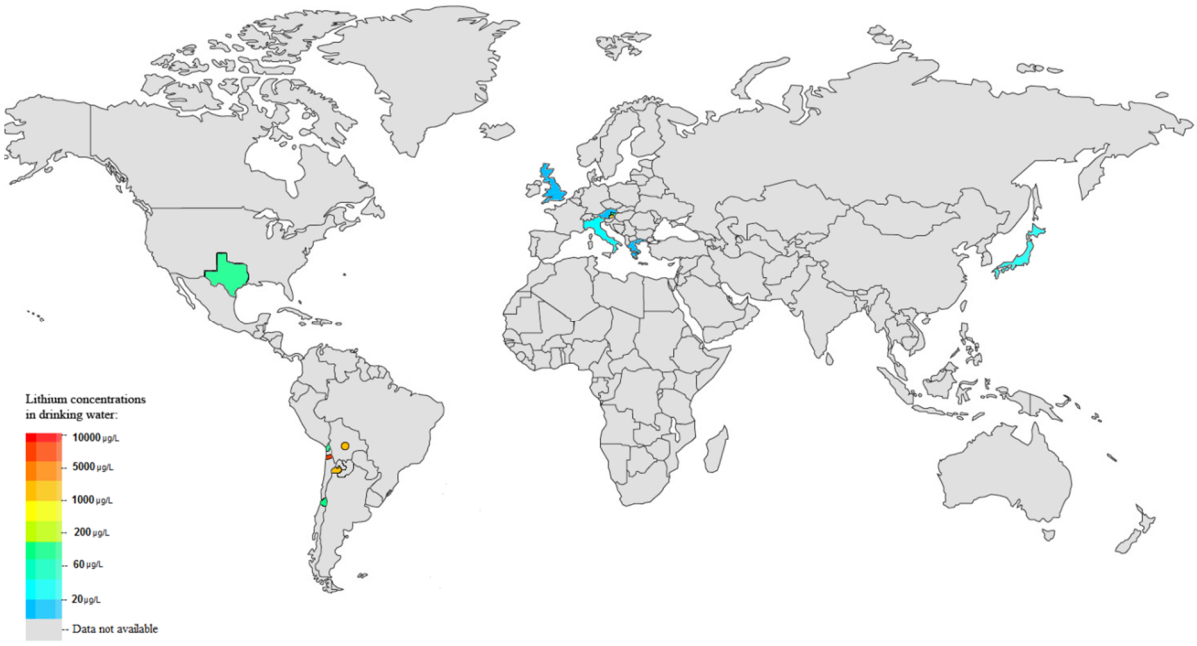

Figure 2. Available data on lithium concentrations in drinking water worldwide. World map modified from: www.worldatlas.com. References of the lithium concentrations in the different countries are provided in the text.

Certain brands of bottled water also contain high concentrations of lithium, e.g. up to 5,000 µg/L in products from Germany and Yugoslavia, about 5,500 µg/L in a product from France and almost 10,000 µg/L in one from Slovakia (Allen et al. 1989; Krachler and Shotyk 2009; Reimann 2010). At the same time, lithium is usually not analyzed in the regular control of drinking water quality and only Russia and Ukraine have set limits for lithium in drinking water (<30 µg/L) (Reimann 2010).

2.1.3.2 Lithium in food

Environmental exposure to lithium could also occur via food, although the concentrations are usually very low. Grains and vegetables, in particular spinach, appear to contain the highest concentrations of lithium (0.5-4.6 µg/g), while dairy products contain about 0.50 µg/g and meat about 0.012 µg/g (Ammari et al. 2011; Schrauzer 2002). One brand of low-sodium salt, extracted from lithium-rich salt flats, was found to contain 44 µg/g (our unpublished data). Thus, consumption of 10 g of such salt a day would result in an intake of 440 µg of lithium. The lithium intake in the U.S. has been estimated to 9-44 µg/kg/day (Schrauzer 2002). The same author has proposed that lithium is essential for humans but the evidence for this is scarce and no mechanisms have been established.

Other routes of exposure such as inhalation or dermal absorption seem to contribute very little to the lithium concentrations in serum (Moore 1995).

2.1.4 Toxicokinetics

After oral ingestion, lithium is rapidly and completely absorbed from the gastrointestinal tract by passive diffusion through pores in the small intestinal membrane and, to a much lesser extent, actively transported in exchange for sodium (Bauer et al. 2006). Once in the body, lithium is widely distributed in the interstitial fluid. Lithium is not subject to metabolic transformation and 95% is excreted through the kidneys as a free ion, while 1% can be found in the feces and 4% in sweat (Grandjean and Aubry 2009a; Morgan et al. 2003). The elimination half-life of lithium in plasma varies between 16 and 30 hours in patients with adequate kidney function. However, it increases in lithium-treated patients with time of medication to up to 60 hours in patients treated for more than 1 year (Goodnick et al. 1981). The kinetics of low-dose lithium from environmental sources is largely unknown.

Lithium crosses the blood-brain barrier and its concentration in the human brain is 50-80% of that found in serum in lithium-treated patients (Soares et al. 2000). Lithium accumulates mainly in bone (half-life several months), but also in the kidney, thyroid, brain (~10 days) and bile (>24 hours) (Birch and Hullin 1972; Grandjean and Aubry 2009a; Spirtes 1976).

2.1.4.1 Toxicokinetics during pregnancy and lactation

In patients on lithium therapy, lithium crosses the placenta, as indicated by the high concentration of lithium in the amniotic fluid [about 10,000 µg/L; (MacKay et al. 1976)] as well as by the similar lithium concentrations in plasma of women and their newborns (Newport et al. 2005; Schou and Amdisen 1975; Sykes et al. 1976). There are no similar studies concerning women environmentally exposed to lithium.

It has been suggested that lithium passes freely into breast milk (Moore 1995). However, there seems to be a wide inter-individual variation in the transfer of lithium through the mammary gland in lithium-treated patients; and the lithium concentration in breast milk has been reported to constitute anything from 10 to 80% of that found in maternal serum (Grandjean and Aubry 2009c; Schou and Amdisen 1973; Viguera et al. 2007).

2.2 HEALTH EFFECTS OF LITHIUM

2.2.1 Fetal size and lithium

The embryonic and fetal development are periods that require careful regulation of processes involving cell proliferation, formation and functionality of cell lineages and interaction between cell types (Harding and Bocking 2001). The whole embryonic and fetal development takes about 280 days (40 weeks). The first days involve fertilization and blastocyst formation, followed by implantation and gastrulation, i.e. formation of the three germ layers ectoderm, mesoderm, and endoderm (**Figure 3**). Then, the process of organogenesis starts (week 3-8), which is the period with the highest risk for malformations (Langman and Sadler 2000). The final fetal stage is characterized by cell proliferation and growth (week 9-40).

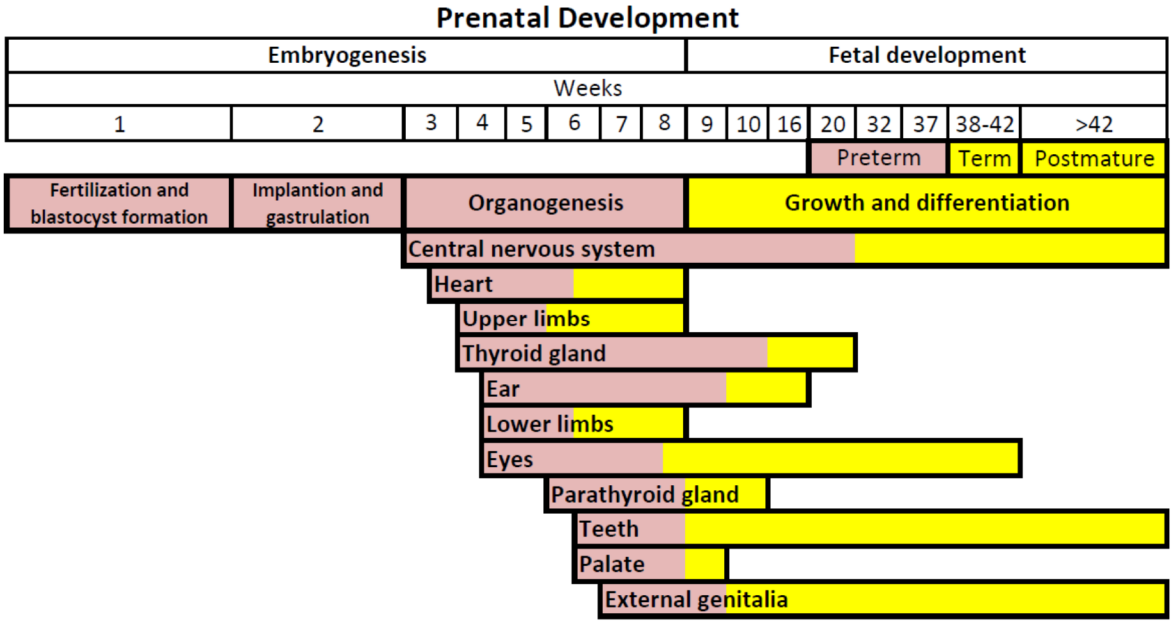

Figure 3. Stages of embryonic and fetal development (the pink color indicates stages with the highest risk for malformations and mortality and yellow color indicates lower risk). Modified from (Moore and Persaud 2003).

Lithium is classified as teratogenic (category D) by the U.S Food and Drug Administration and it is recommended not to use lithium in early pregnancy (Grandjean and Aubry 2009c). A few human studies and several experimental studies (Morgan et al. 2003), particularly with mice and rats, have investigated the potential teratogenicity of lithium and its impact on fetal growth and development, but their results are inconsistent (**Table 1**).

Table 1. Summary of studies on lithium exposure during pregnancy and fetal or post-natal size.

Study		Sample size	Li-exposure levels	Results of interest in the Li-exposed group
Human studies				
Diav-Citrin et al. 2014	Prospective observational	Bipolar Li therapy n=183 Bipolar no Li therapy n=72 Control n=748	N/A	Lower birth weight (40g., borderline significant), higher proportion of preterm births and miscarriages.
Jacobson et al. 1992	Prospective observational	Patients Li therapy n=138 Control n=148	N/A	Higher birth weight (94 g, p=0.025).
Newport et al. 2005	Prospective observational	Bipolar Li therapy n=12 Control n=12	300-1800 mg/day	Lower Apgar score, longer hospital stay, higher proportion of low birth weight, preterm births and newborns with complications.
Experimental studies in mice				
Chernoff and Kavlock. 1982	Mice	Li-exposed n=25 Control n=30	400 mg/kg/day orally	No effect on pup weight. Reduced litter size.
Giles and Bannigan. 1993	Mice	Li-exposed n=8-20 Control n=8-20	200, 300, 350, 400 mg/kg injection	No effect on embryo or fetal weight.
Laborde and Pauken. 1995	Mice	Li-exposed n= N/A Control n= N/A	0.5, 1, 1.5, 2, 2.5 mg/mL water orally	Reduced fetal weight and crown-rump length. No dose-response was reported.
Matsumoto et a. 1974	Mice	Li-exposed n= N/A Control n= N/A	400 mg/kg orally	Reduced fetal weight and ossification centers in digits of hindlimbs and tail vertebrae.
Messiha 1986	Mice	Li-exposed n=5 Control n=5	Drinking water with ~7mg/L *ad libitum*	Lower liver and kidney weight in females and lower spleen weight in females and males.
Messiha 1989	Mice	Li- and Cs exposed n=3 Control n=3	Drinking water with ~7mg/L *ad libitum*	Effects were only seen in the Li-treated group. Lower brain weight in females and males, lower kidney weight in females, increased liver and spleen weight during weaning.
Messiha 1993	Mice	Li-exposed n=5 Control n=5	Drinking water with ~7mg/L *ad libitum*	Lower post-natal total weight as well as brain (males and females), kidney (females) and testis (males) weight.
Mroczka et al. 1983	Mice	Li-exposed n=252 Control n=283	70, 140, 210, 350, 700 and 1400 mg/day orally	Lower post-natal brain, liver, kidney and heart weight, higher post-natal mortality, more frequently in the females. No dose-response was reported.
Seidenberg et a. 1986	Mice	Li-exposed n=28 Control n=28	400 mg/kg/day orally	No effects on post-natal pup weight.

Li: lithium; Cs: cesium; GD: gestational day; G: group; N/A: not available.

Table 1 (cont). Summary of studies on lithium exposure during pregnancy and fetal or post-natal size.

Study		Sample size	Li-exposure levels	Results of interest in the Li-exposed group
Experimental studies in rats				
Christensen et al. 1982	Rats	Li-exposed n=20 Control n=20	280 and 420 mg/kg orally	No effects on pup birth weight.
Fritz. 1988	Rats	Li-exposed n=16, 19, 14 Control n=20	100 mg/kg/day at GD: 6-10, 11-15, 16-20, orally in food	Reduced body weight in all Li-treated groups.
Glockner et al. 1989	Rats	Li-exposed n=30 Control n=20	140 mg/L in water orally	No effects on pup body weight.
Gralla and McIlhenny. 1972	Rats	Li-exposed n=20 Control n=20	4.7, 14.2, 28.4 mg/kg/day orally	Reduced post-natal pup body weight at 28.4 mg/kg/day.
Hsu and Rider. 1978	Rats	Li-exposed n=13 Control n=10	7mg/kg orally	Reduced body weight at weaning.
Ibrahim and Canolty. 1990	Rats	Li-exposed n=13 Control n=11	188 mg/kg food , orally	Reduced birth weight in Li-treated group. Reduced growth at weaning in the Li-treated post-natally.
Johansen and Ulrich. 1969	Rats	Li-exposed n=22 Control n=13	G1: 6.9 mg/kg/day G3: 20.8 mg/kg/day orally	Retarded post-natal growth in G3 group.
Marathe and Thomas. 1986	Rats	Li-exposed n=23 Control n=20	50 and 100 mg/kg/day orally	Reduced fetal weight at 100 mg/kg/day.
Rider and Hsu. 1976	Rats	Li-exposed n=13 Control n=10	140 mg/kg/day orally	Reduced liver weight and pup weight at weaning.
Rider et al. 1978	Rats	Li-exposed n=12 Control n=11	105 mg/L in water orally	Reduced birth weight. Reduced spleen weight in females.
Sechzer et al. 1986	Rats	Natural Li salts n=15 Li-6 n=15 Li-7 n=13 Control n=16	13,9 mg/kg/day orally	Lower birth weight in all Li-treated groups.
Sharma and Rawat. 1986	Rats	Li-exposed n=77 Control n=100	7 mg/kg intragastrically for 10 days	Reduced fetal body weight and size
Texeira et al. 1995	Rats	Li-exposed n=44 Control n=59	70 mg/kg orally	Reduced pup weight at weaning.
Trautner et al. 1958	Rats	Li-exposed n=25 Control n=30	140 mg/kg/day orally	Slower post-natal growth.
Experimental studies in monkeys				
Gralla and McIlhenny. 1972	Monkeys	Li-exposed n=6 Control n=5	4.7 mg/kg/day orally	Reduced fetal and post-natal body weight.
In vitro				
Klug et al. 1992	Rat embryos		0, 50, 100, 150, 200 mg/L	Crown-rump length reduced with exposures $\geq$150 mg/L

Li: lithium; Cs: cesium; GD: gestational day; G: group; N/A: not available.

Two human studies have found a higher proportion of low birth weight babies born to mothers undergoing lithium treatment, compared to a control group (Diav-Citrin et al. 2014; Newport et al. 2005), while a third study found increased birth weight in the newborns to mothers in the treated group (Jacobson et al. 1992). In the latter study, however, no association was found between birth weight and the lithium dose (range 50-2400 mg/day).

At different lithium doses (7-400 mg/kg/day, **Table 1**), several experimental studies have reported a reduced birth weight in mice (Laborde and Pauken 1995; Matsumoto et al. 1974) and rats (Ibrahim and Canolty 1990; Marathe and Thomas 1986; Rider et al. 1978; Sechzer et al. 1986; Sharma and Rawat 1986), as well as reduced post-natal organ and body weight in mice (Messiha 1986, 1993, 1989; Mroczka et al. 1983) and rats (Fritz 1988; Gralla and McIlhenny 1972; Hsu and Rider 1978; Ibrahim and Canolty 1990; Johansen and Ulrich 1969; Rider and Hsu 1976; Rider et al. 1978; Texeira et al. 1995; Trautner et al. 1958). All these studies used doses representing those of lithium therapy. An *in vitro* study on cells from rat embryos found a decreased crown-rump length with lithium doses ≥150 mg/L (Klug et al. 1992).

On the contrary, a few studies on mice and rats, using doses of 140 mg/L in the drinking water or ≥200 mg/kg/day (orally or by injection), have found no effects in birth or post-natal weight (Chernoff and Kavlock 1982; Christensen et al. 1982; Giles and Bannigan 1993; Glockner et al. 1989; Seidenberg et al. 1986).

2.2.2 Thyroid function and lithium

A brief overview of the normal thyroid function is provided in **Figure 4**. The thyroid gland releases thyroxine (T4) and triiodothyronine (T3) which are in charge of controlling several functions in the body, e.g. heart rate, body weight and body temperature (Guyton and Hall 2006). During pregnancy, thyroid hormones are essential for normal fetal growth and development (Polak 2014). Thyrotropin (TSH) production increases in early pregnancy due to the influence of the human chorionic gonadotropin hormone (hCG) released by the hypothalamus, which has a TSH-like activity. There is also an increase in the thyroxine-binding protein (TBG) production, in the transplacental transfer of thyroid hormones and in the renal iodine excretion (Kennedy et al. 2010). Due to the increase in the TBG production, T4 and T3 decrease, particularly in early pregnancy (Lockitch 1993).

Disruption of the thyroid function is a well-known side effect of lithium therapy (Grandjean and Aubry 2009c). Disturbances of the thyroid function during pregnancy may lead to gestational hypertension, placental abruption, pre-term delivery and fetal loss (Allan et al. 2000; Casey 2005) as well as lower birth weight, congenital hypothyroidism and impaired neurological function (Chen et al. 2014; Haddow et al. 1999; Zimmermann 2012).

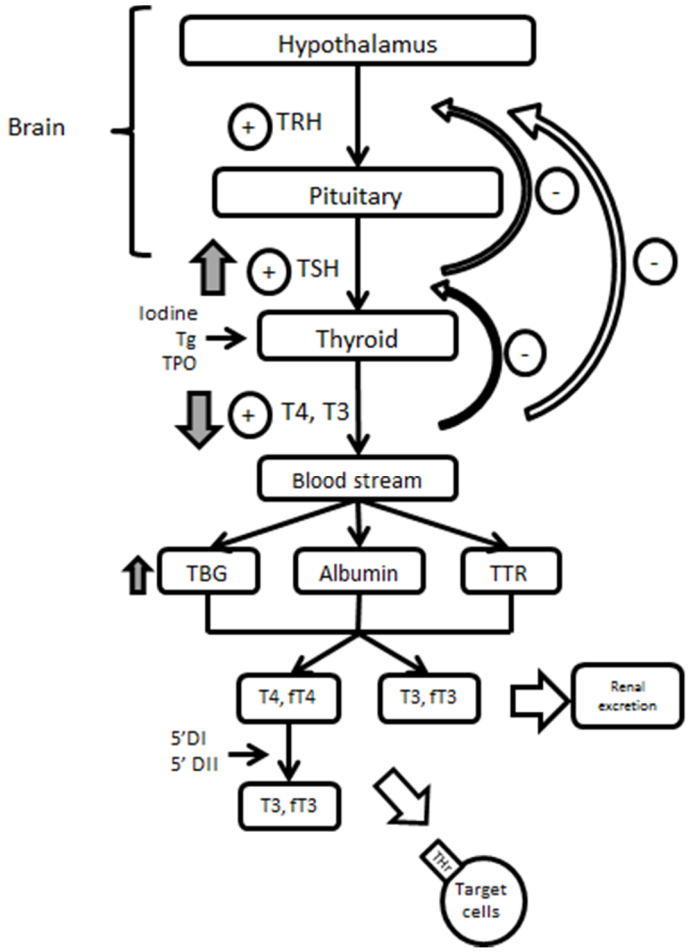

Figure 4. Normal thyroid function. TRH: thyrotropin-releasing hormone, TSH: thyrotropin or thyroid-stimulating hormone, T4: thyroxine, T3: triiodothyronine, Tg: thyroglobulin, TPO: thyroperoxidase, TBG: thyroxine-binding protein, TTR: prealbumin or transthyretin, 5'D: deiodinases I and II, THr: thyroid hormone receptor. Gray arrows represent changes in the thyroid function during pregnancy.

Stimulation of the hypothalamus induces the production of TRH which in turn stimulates the production of TSH. The latter has several functions. Particularly, TSH increases the Tg proteolysis, the iodine uptake by the thyroid gland, the iodination of T4, and the size and production of the thyroid follicles. After T4 and T3 are released to the blood stream, they bind partially to TBG, albumin and TTR. Increment in TSH inhibits the TRH production, and increment in T4 and T3 inhibits TSH and TRH (Guyton and Hall 2006).

Most studies investigating thyroid function in relation to lithium exposure are based on patients undergoing lithium therapy. A meta-analysis including case-control studies showed a 6-fold increased risk (95% CI 2.72; 13.4) of developing clinical hypothyroidism in patients on lithium therapy compared with controls (McKnight et al. 2012). A randomized double-blind placebo-controlled clinical trial showed a higher mean TSH concentration in the lithium-treated group (Frye et al. 2009) and a prospective double-blind clinical trial showed a reduction in thyroid morbidity when decreasing the lithium dose to a serum lithium concentration <0.79 mmol/L (5.5 mg/L) (Coppen et al. 1983).

A recent study including women exposed to lithium through drinking water, the only one with environmental exposure, found a positive association of urinary lithium concentrations with TSH, as well as an inverse association with free T4 (fT4) (Broberg et al. 2011).

2.2.3 Calcium homeostasis and lithium

The calcium homeostasis is closely related to that of parathyroid hormone (PTH), vitamin D, magnesium and phosphorus. A summarized scheme of the calcium-phosphorus-magnesium-vitamin D homeostatic systems is shown in **Figure 5**. Although much is known about these homeostatic systems and their interrelation, everything is not yet fully understood.

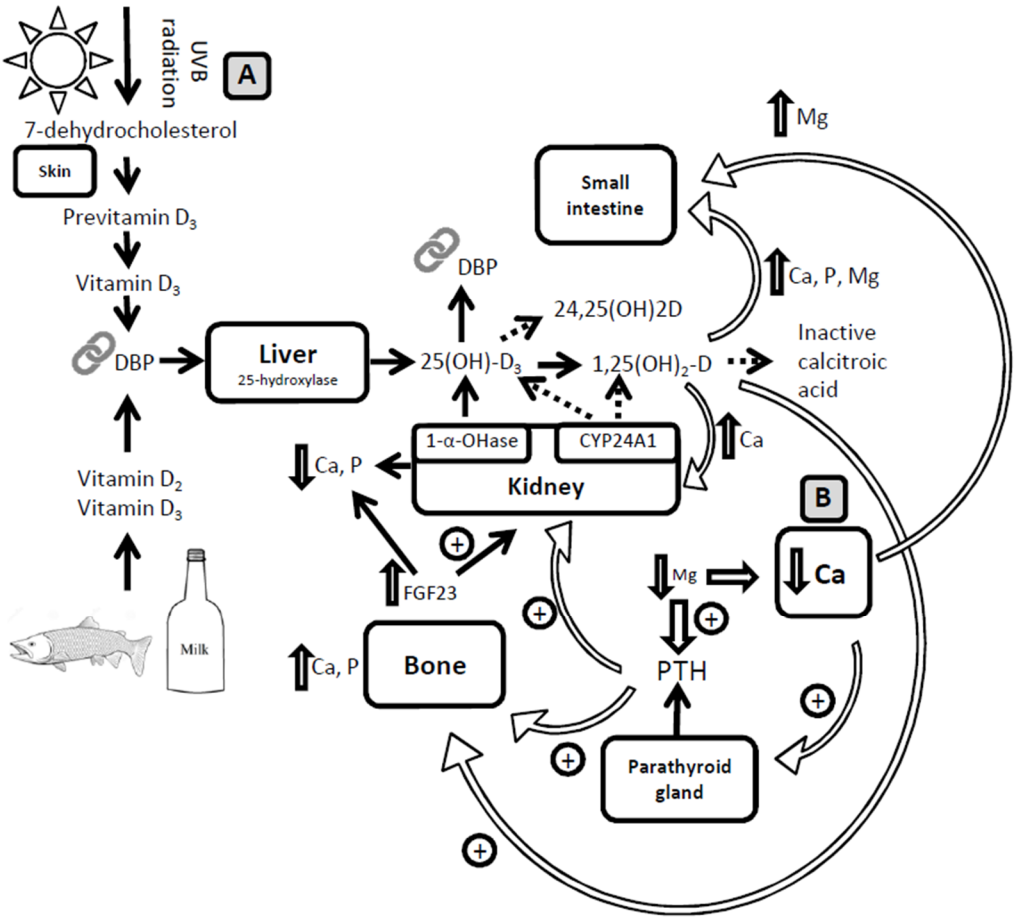

Figure 5. Schematic overview of the calcium-phosphorus-magnesium-vitamin D homeostatic systems.
DBP: vitamin D binding protein; Ca: calcium, P: phosphorus, Mg: magnesium, PTH: parathyroid hormone, 25(OH)-D_3: 25-hydroxyvitamin D_3, 1,25$(OH)_2$-D: 1,25-dihydroxyvitamin D, 1-α-OHase: 1-alpha-hydroxylase, CYP24A1: 24-hydroxylase, 24,25$(OH)_2$D: 24,25 dihydroxyvitamin D, FGF23: fibroblast growth factor 23, UVB: ultraviolet B. Dashed lines represent vitamin D catabolic pathways.

A: Vitamin D is mainly produced from the conversion of 7-dehydrocholesterol to previtamin D_3 in the skin by UVB radiation. Once in the body, vitamin D_3 is converted to 25(OH)-D_3 and then to 1,25$(OH)_2$-D (the active form). CYP24A1 catabolizes both vitamin D forms to be excreted as 24,25$(OH)_2$D or as inactive calcitroic acid through the kidneys.

B: A decrease in the circulating Ca concentrations induces the production of PTH in the parathyroid gland, which releases Ca and P from the bone to the blood and decreases the renal Ca and P excretion. Decreased Ca concentrations will also increase the intestinal absorption of Mg, P and Ca directly in the small intestine or by stimulating the production of 1,25$(OH)_2$-D.

Approximately 99% of the total amount of calcium in the body is stored in the bones and the remaining 1% is located in soft tissue and the extracellular fluid, including blood (Guyton and Hall 2006). Due to calcium's critical role in many physiological processes, the calcium concentration in serum is strictly regulated. Similar to calcium, most phosphorus (85%) is stored in the bones, while 14-15% is located intracellularly and 1% in the extracellular compartment. Both calcium and phosphorus levels are controlled by the parathyroid hormone, which is produced in the parathyroid gland. Magnesium is the second most abundant essential element within the cells and only 1% of it is found extracellularly, while 60%, 20% and 19% is found in bone, skeletal muscle and other tissues, respectively (Burtis and Ashwood 1999).

Vitamin D is also an important component of this homeostatic system. Sources of vitamin D include UVB radiation, which converts 7-dehydrocholesterol to previtamin D_3 in the skin, as well as food (Brannon and Picciano 2011). Once in the body, vitamin D goes through a series of enzymatic reactions to finally be converted to 1,25-dihydroxyvitamin D, the active form (**Figure 5**).

During pregnancy, the homeostasis of these systems is critical for ensuring normal fetal growth and development. Particularly, disturbances in the homeostasis of vitamin D and in the parathyroid system are associated with preeclampsia, infectious diseases as well as impaired fetal development (Brannon and Picciano 2011; Karras et al. 2014).

Impairment of the calcium homeostasis, diagnosed often as hyperparathyroidism, is another known side effect of lithium therapy (Grandjean and Aubry 2009c). A systematic review of case-control studies with patients undergoing lithium therapy found a significant increase in serum calcium and in the concentrations of parathyroid hormone (McKnight et al. 2012). Three studies, based on patients on lithium therapy, have investigated the potential impact of lithium on vitamin D and reported a decrease in the 25-hydroxyvitamin D_3 concentrations in the lithium-treated group, compared to controls (Haden et al. 1997; Oliveira et al. 2014; van Melick et al. 2014). None of these studies included pregnant women.

3 AIMS

The overriding aim of our ongoing research concerning environmental lithium exposure was to clarify the potential impact of exposure to lithium via drinking water during pregnancy on maternal and fetal health.

Specifically, this PhD thesis aimed at elucidating:

- The transfer of lithium from drinking water through the placenta and the mammary gland.
- The potential impact of exposure to lithium during pregnancy on fetal size.
- The association between lithium exposure and maternal thyroid function during pregnancy.
- The association between lithium exposure and maternal calcium homeostasis during pregnancy.

4 MATERIALS AND METHODS WITH DISCUSSION

This section summarizes the materials and methods used in this thesis. For further details, the reader is referred to the individual papers (**Papers I-IV**). A broader description of the study area, recruitment and sample collection, as well as a discussion of the exposure assessment, is also provided in this section.

4.1 STUDY AREA

The present thesis was performed in the Puna region, on the eastern side of the Andes, in the province of Salta in northern Argentina. The study site included the whole Los Andes Department and part of La Poma and Rosario de Lerma Departments (**Figure 6**). The study area is located at 3,180-4,070 meters above the sea level, latitude from -23.31 to -24.35, and longitude from -65.51 to -67.23. The total area is about 32,000 km^2 with a population density of only 0.2 inhabitants/km^2. By December 31st 2013, the total population of this area was 8,135 inhabitants, out of whom 5,893 lived in the main village of San Antonio de los Cobres (**Figure 7**), and the rest in the nine surrounding villages: Santa Rosa de los Pastos Grandes, Tolar Grande, Salar de Pocitos, Olacapato, Cobres, Las Cuevas, El Toro, El Palomar and Esquina de Guardia (**Table 2**).

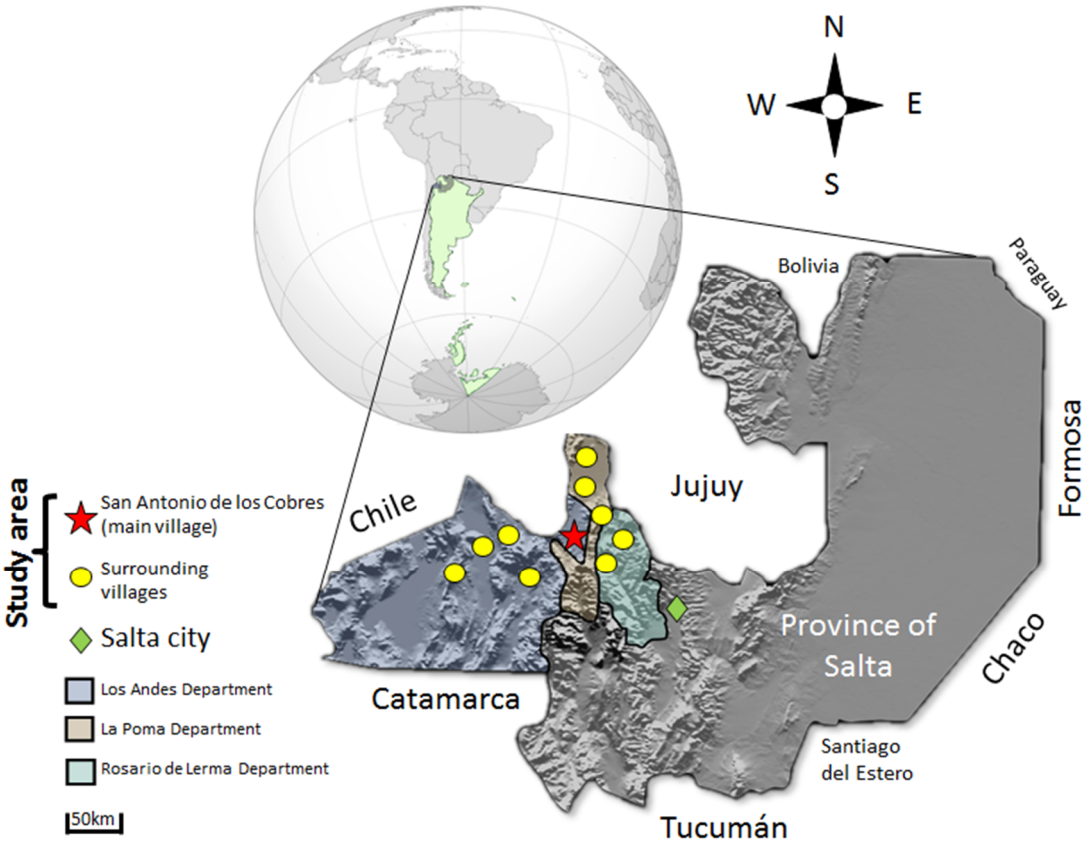

Figure 6. Map of the study area. Topographic map of Salta obtained from: NASA/JPL/NIMA; World map modified from Dexxter via Wikimedia Commons [CC BY 3.0 (http://creativecommons.org/licenses/by/3.0)].

Figure 7. San Antonio de los Cobres, the main village. Photographer: Florencia Harari.

The main village San Antonio de los Cobres is located 160 km from the city of Salta, the capital of the province. The nine other villages are located at 40-216 km from the main village (**Table 2**). During the rain period (from January to March), the dirt roads are usually destroyed, limiting the accessibility from San Antonio de los Cobres to the surrounding villages, as well as to the city of Salta.

In order to facilitate the organization of the health care services in the area, the Ministry of Health in Salta divided San Antonio de los Cobres geographically into 11 areas and grouped all the 20 “administrative areas” (11 in San Antonio de los Cobres and the nine surrounding villages) into a so-called “Operative Area XXIX”. The hospital Dr. Nicolás Cayetano Pagano is located in the main village and basic health service to the entire area is provided by three medical doctors, several nurses and nurse assistants. The Primary Health Care office is located next to the hospital and is integrated by 11 primary health care workers and 3 supervisors. Each surrounding village has a primary health care clinic, where assistance is provided by a nurse and a primary health care worker, with the exception of Cobres and Tolar Grande where also a medical doctor is available. In total, there are 23 primary health care workers who visit the families in all villages on a regular basis, from 1 to 4 times every 3 months (depending on the specific needs of the families) to update demographic information, including migration, health status and pregnancies. The annual birth rate is about 200 in the study area and the overall infant mortality rate is about 41 per 1000 born infants (Alduncin et al. 2005).

The Puna region is an arid mountain highland characterized by dry summers (December-February) with daytime temperatures around 20° C and cold and windy winters (June-August, -20° C and wind speed up to 80km/h). Inhabitants are mostly of indigenous origin, almost exclusively belonging to the Kolla community. Only a few inhabitants belong to the Atacama and Tastil communities. About 2/3 of the families own their houses, often built of adobe, with mud or cement floor and roofs made of tin/stones or straw/wood (**Figure 8**). The local economy is based on trading (e.g. handcrafts) and breeding of llamas, goats and sheep, as well as working in mines. The diet is largely of animal origin (meat and some dairy products but essentially no fish) with vegetables, potatoes and corn.

Figure 8. House built of adobe with roof made of straw located in Casa Colorada (40 km from San Antonio de los Cobres). Tanks contain drinking water. Photographer: Florencia Harari.

The source of drinking water in San Antonio de los Cobres is a natural spring ("Agua de Castilla") located approximately 10 km away. From this spring, water is first pumped to a series of sand filters, and since a few years back, to an arsenic treatment plant. Thereafter, the water is transferred to a chlorination station before being distributed throughout the village. The presence of elevated arsenic concentrations in the drinking water in San Antonio de los Cobres is known since long (Vahter et al. 1995). More recently, the presence of elevated lithium, boron and cesium concentrations were discovered (Concha et al. 2010). The arsenic treatment plant was installed a few months before the start of the present study, decreasing the arsenic concentrations in the drinking water of San Antonio de los Cobres from ~200 µg/L (Concha et al. 2010) down to about 30 µg/L during the study period (**Figure 15)**.

4.2 STUDY DESIGN AND PARTICIPANTS

The study in **Paper I** was designed to evaluate the kinetics of lithium and boron at delivery and in the post-partum period based on samples available from two studies performed previously. The studied women were from San Antonio de los Cobres (n=11), i.e. our main study site in northern Argentina (with elevated lithium and boron in the drinking water), and from two other study areas: one was Arica (n=24) in northern Chile with low lithium (60 μg/L) but high boron (8,000 μg/L) concentrations in the drinking water, and the other was Santiago (n=11), the capital of Chile, with low concentrations of both lithium (~20 μg/L) and boron (~190 μg/L) in the drinking water (**Figure 9**). The women from San Antonio de los Cobres (included in **Paper I**) were recruited in 1996 as part of another study investigating the early-life exposure to arsenic (Concha et al. 1998a). The women from Arica and Santiago were recruited in 2010, as part of a study investigating early-life exposure to lead and arsenic through breast-feeding.

For the purpose of the studies in **Papers II-IV**, we invited all pregnant women living in the Operative Area XXIX with estimated delivery date between October 2012 and December 2013 to participate in a longitudinal mother-child cohort designed to evaluate potential health effects of early-life exposure to lithium and other water pollutants. Pregnant women were recruited with the assistance of the primary health care personnel, who knew the women and their addresses. The study was designed to see the pregnant women at least once during pregnancy; but preferably 2-3 times in order to obtain repeated measures of exposures and outcomes. The women were followed-up at delivery and 0-3 and 3-6 months later, when also the infants were included in the study. In **Papers II-IV**, only data during pregnancy and infant size measures at birth were included.

Prior to each visit, a formal invitation was sent out to the pregnant women in each village with a suggested date and time. In case a woman was not able to attend at the scheduled date, she was offered to come any day before or after in order to achieve the highest possible rate of participation in the study. Since some women could not be located, we announced and welcomed all women to get involved in the study, using the local radio. Altogether, we visited the study area on seven occasions for collection of data and samples (October 2012, January, April, June-July, September-October and December 2013, and March 2014). In between on-site visits, close contact with the hospital and the primary health care workers was maintained regularly via e-mail and telephone.

In total, 221 women were pregnant during the study period out of whom 194 became enrolled (participation rate: 88%). Reasons for not participating included delivery before the recruitment (n=11), twin pregnancy (n=1), fetal loss before the recruitment (n=5), refusal or not located (n=6), and migration (n=4) (**Figure 10 and Table 2**).

4.3 SAMPLING AND DATA COLLECTION

For the study in **Paper I** (**Figure 9**), data regarding maternal age, parity, years of residency, gestational age at birth, birth weight and length were available. In San Antonio de los Cobres, samples of cord blood, maternal blood and urine, and infant urine had been collected at delivery as well as maternal blood, urine and breast milk, and infant urine at 2-4 weeks, 2-4 months and 4-6 months after delivery (Concha et al. 1998a). All samples were kept frozen since collection. In Arica and Santiago, samples of maternal blood (RBC and plasma), urine and breast milk, and infant urine had been collected 2-4 months after delivery in 2010. Water samples had also been collected from each area.

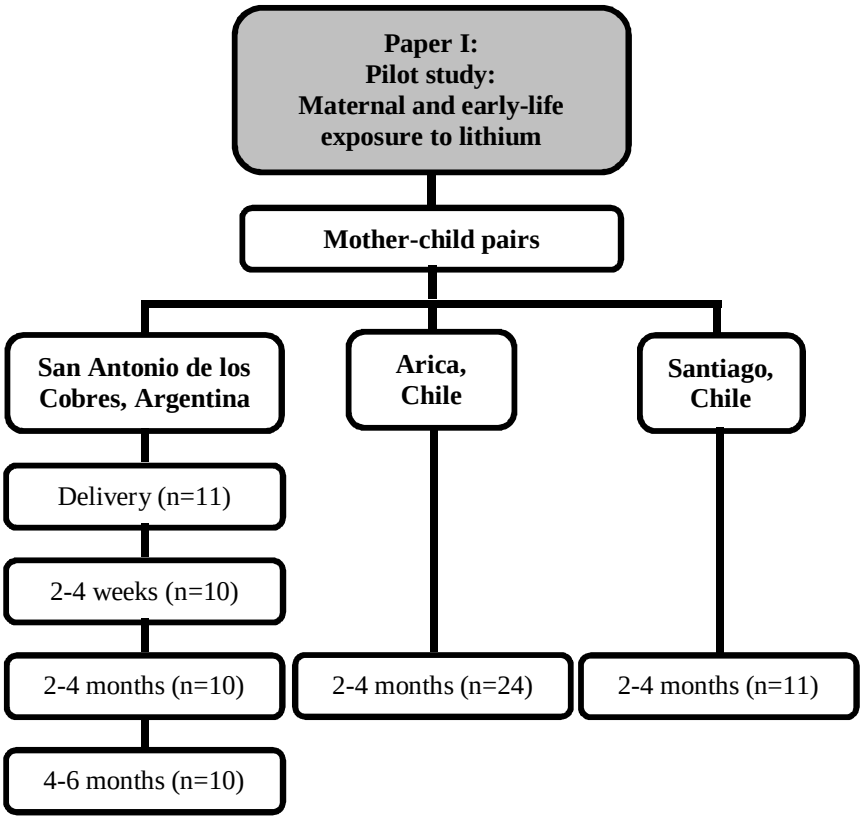

Figure 9. Overview of study in Paper I.

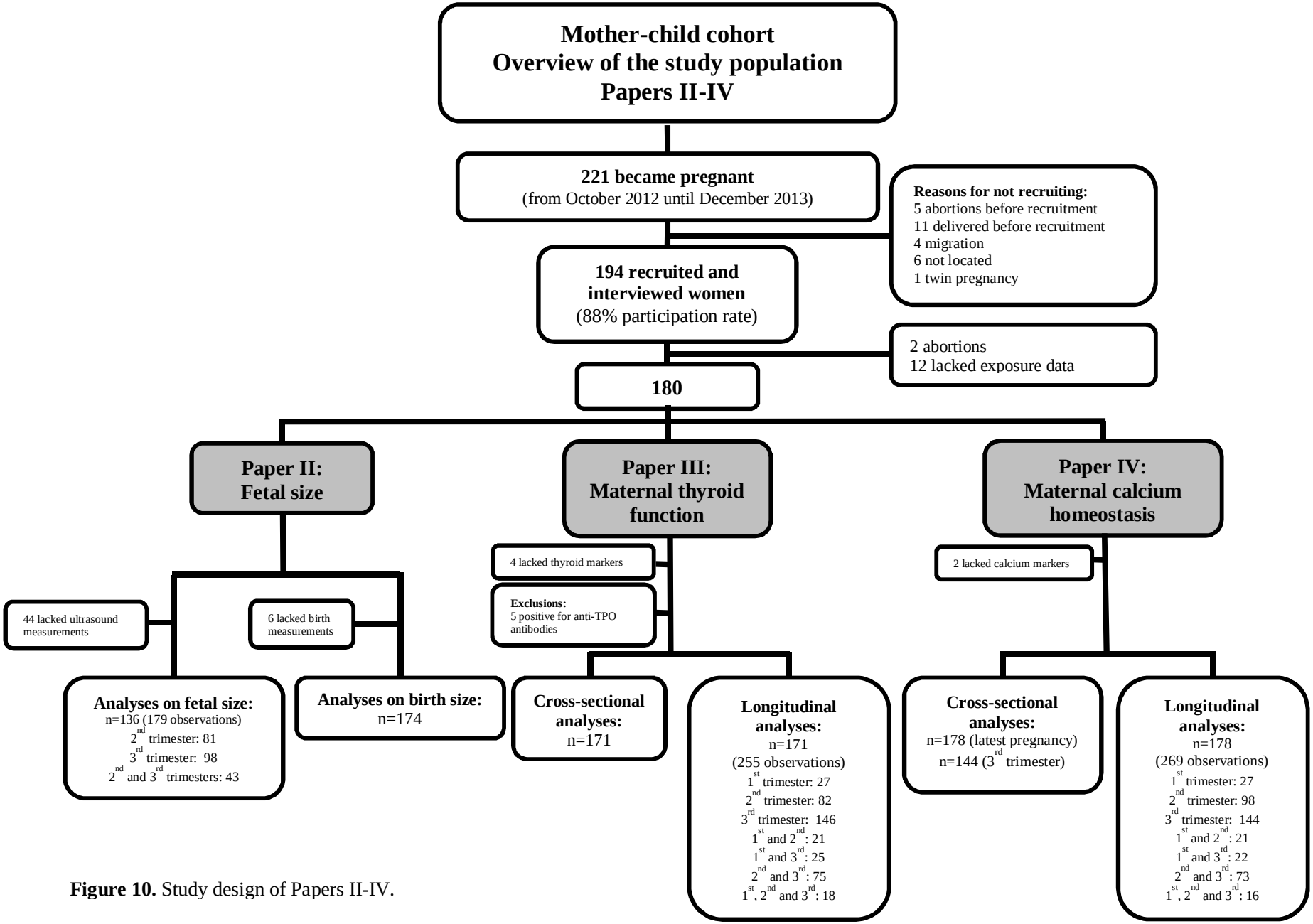

Figure 10. Study design of Papers II-IV.

Table 2. Overview of the study sites and of the recruited pregnant women and collected samples in each village for Papers II-IV between October 2012 and December 2013.

Village	Distance from San Antonio de los Cobres	Inhabitants by Dec. 31st, 2013	N Pregnant women from Oct. 2012 to Dec. 2013	Total recruited and sampled		Trimesters		
						1st	2nd	3rd
				N women	N samples	N women	N women	N women
San Antonio de los Cobres	--	5,893	165	138	220	26	77	117
Cobres	60km	350	12	11	18	3	5	10
El Palomar	78km	198	4	4	5	0	2	3
El Toro	80km	198	1	1	1	0	0	1
Esquina de Guardia	>45km	182	6	2	3	1	2	0
Las Cuevas	40km	183	7	4	5	0	1	4
Olacapato	60km	462	10	9	17	2	6	9
Pocitos	110km	165	2	1	1	0	1	0
Santa Rosa de los P. G.	60km	307	8	7	9	0	4	5
Tolar Grande	216km	197	6	3	3	0	1	2
Total		8,135	221	180	282	32	99	151

For the studies in **Papers II-IV**, we interviewed the women during the first visit about age, place of birth, time and place of residency, ancestry, education years, occupation, family income, living conditions, personal and familial history of diseases, parity and other gyneco-obstetrical history, dietary habits, tap water sources and type of water consumed (tap/bottled water), smoking habits, passive smoking, alcohol consumption, coca chewing, last menstrual period (LMP) and pre-pregnancy body weight, and we measured participant's height. Gestational age was calculated based on the reported date of LMP (counting from the first day of the LMP), which was compared with fetal ultrasound-based estimation (see below). Height and pre-pregnancy body weight were used to calculated the pre-pregnancy body mass index (BMI; calculated as the pre-pregnancy body weight in kilograms divided by height in meters squared) and lean body mass (LBM) using the equation proposed by Watson and collaborators [LBM=(-2.097 + (0.1069*Height) + (0.2466*weight))/0.73; (Watson et al. 1980)]. At each visit during pregnancy, we asked the women about potentially encountered health problems, collected blood and urine samples and measured body weight (HCG-210QM, GA.MA ® professional, Italy; accurate to 100 g), LBM (Body Fat Monitor, HBF-302, OMRON®, Tokyo, Japan) and blood pressure (aneroid sphygmomanometer, AB Henry Eriksson, Stockholm, Sweden).

All women were asked to donate blood and spot-urine samples at baseline and at each follow-up visit. We also repeatedly collected water samples (20 mL polyethylene bottles) during the whole study period. All samples were collected at the hospital, at the local primary health care clinics or, in a few cases, at home. Whole blood samples were collected in Trace Elements Sodium Heparin tubes (Vacuette®; Greiner bio-one, Kremsmünster, Austria) and

Trace Elements Serum Clot Activator tubes (Vacuette ®; Greiner bio-one, Kremsmünster, Austria) using butterfly needles (BD Safety-Lok™, Vacutainer®, Bencton, Dickinson and Company, Franklin Lakes, USA). We extracted plasma and serum by centrifugation for 10 minutes at 3000 rpm, 15 minutes after blood withdrawal. Hemoglobin levels were measured in whole blood using HemoCue® 201+ (HemoCue AB, Ängelholm, Sweden).

Spot-urine samples were collected using disposable plastic cups and transferred to 24 mL trace-element free polyethylene bottles. All participating women received instructions on wet wipe cleaning and appropriate mid-stream urine sample collection in order to avoid contamination. During the fieldwork, we measured the urinary specific gravity using a hand refractometer (Atago, Japan) and we checked the content of glucose, proteins, blood, pH, ketones and nitrites using Combur®-7 test stripes (Roche Diagnostics, Mannheim, Germany). We also measured urinary albumin in all samples using HemoCue® Albumin 201 System (HemoCue AB, Ängelholm, Sweden).

All samples were kept frozen at -20°C until transported to Karolinska Institutet, Sweden, where they were stored at -80°C until analysis. Samples were analyzed within 2 months after collection.

4.4 EXPOSURE ASSESSMENT

To assess the exposure to lithium in **Paper I** we measured the lithium concentrations in maternal whole blood and urine samples collected after delivery, as well as in cord blood, breast milk, infant urine and water. For **Papers II-IV**, the concentrations of lithium were measured in maternal whole blood and urine during pregnancy and in drinking water. Other elements found at elevated concentrations in the drinking water, such as boron, arsenic and cesium, were also analyzed in the different media.

Lithium and all other trace elements were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). All sample preparation and trace element analyses were performed at the Unit of Metals and Health, Institute of Environmental Medicine, Karolinska Institutet in Stockholm, Sweden.

4.4.1 Water lithium

Water samples were collected at each visit during the whole study period. Each village has its own water supply, used for drinking water purposes by most pregnant women. In a very few cases, when women lived outside the villages and obtained water from different sources/springs, water samples were taken from those sources as well.

Because of the occurrence of highly varying concentrations of lithium but also of arsenic, boron and cesium in the drinking water in the study area, all these elements were measured by ICP-MS [Agilent 7500ce (for **Paper I**) and Agilent 7700x (for **Papers II-IV**), Agilent Technologies, Tokyo, Japan], with the collision/reaction cell in no gas mode (lithium, boron and cesium) or helium mode (arsenic). Before analysis, water samples were diluted 1:10 with 1% nitric acid (65% w/w, ppb-trace analysis grade, Scharlau, Scharlab S.L., Sentmenat, Spain).

4.4.2 Biomarkers

4.4.2.1 Blood lithium

In order to test for potential trace element contamination of the sampling equipment used in the studies in **Papers II-IV**, that would invalidate the measured specimen concentrations, we performed trace element tests for each type of blood sampling tube using overnight extraction by weak nitric acid (0,03 M HNO_3, ppb-trace analysis grade, Scharlau, Scharlab S.L., Sentmenat, Spain) at room temperature. The obtained concentrations of the trace elements of interest are presented in **Table 3**. The Trace Elements Serum Clot Activator tubes showed a severe contamination of lithium (37 µg/L extracted from the tubes, as compared to an overall median lithium concentration of 24 µg/L in blood collected in non-contaminated tubes in **Papers II-IV**), invalidating the use of lithium serum measurements in samples collected in such tubes.

Table 3. Trace elements concentrations (µg/L) from leaking tests for blood sampling tubes used for extraction of serum and plasma**.**

Element	LOD*	Trace Elements Serum Clot Activator tubes	Trace Elements Sodium Heparin tubes	Trace Elements Sodium Heparin tubes + Butterfly needle
Arsenic	0.0021	<LOD	0.0036	0.0035
Boron	0.00047	0.26	0.73	0.41
Calcium	0.29	20	12	11
Cesium	0.00042	0.007	0.003	0.0038
Lithium	0.0046	**37**	<LOD	0.068
Magnesium	0.033	226	153	158
Phosphorus	5.4	<LOD	<LOD	<LOD
Selenium	0.0077	<LOD	<LOD	<LOD

*Limit of detection (µg/L).

The therapeutic window of lithium in patients undergoing lithium therapy is commonly assessed by the lithium concentrations in the patients' plasma or serum (Grandjean and Aubry 2009a). Due to the severe lithium contamination in the blood sampling tubes (Trace Elements Serum Clot Activator tubes, Vacuette ®; Greiner bio-one, Kremsmünster, Austria), we could not make use of serum lithium concentrations as exposure marker in **Papers II-IV**. To evaluate the validity of the lithium concentrations in whole blood as a biomarker of exposure, we compared the concentrations with those measured in a subgroup of plasma samples (n=20) collected in non-contaminated tubes. We found an excellent correlation between lithium concentrations in whole blood and in plasma (**Figure 11**), indicating that the lithium concentration in whole blood is indeed a reliable marker of internal dose. Also, for the purpose of the present study, blood lithium is a more relevant measure as it has the possibility to cross the placenta and reach the fetus and thus, it better reflects the fetal exposure than does the lithium concentration in water or urine.

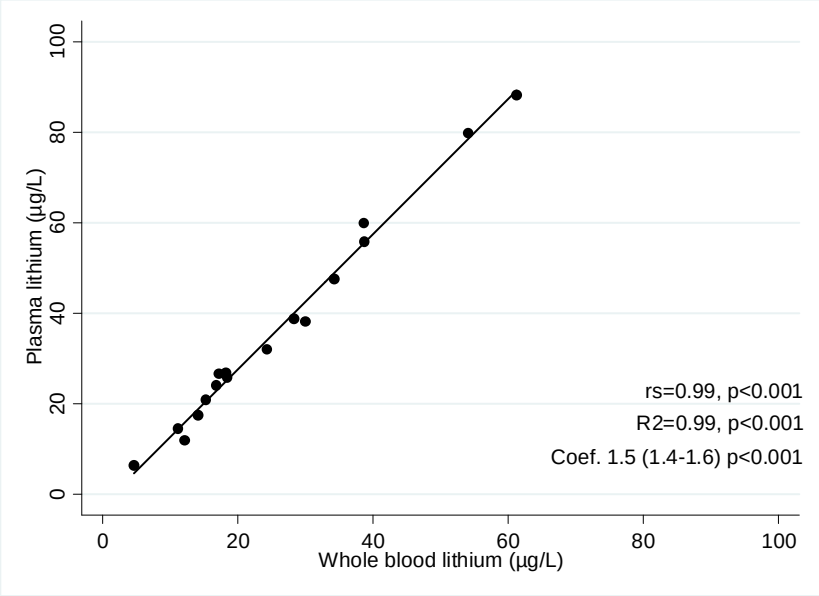

Figure 11. Scatter plot of lithium concentrations in plasma and blood (n=20).

For the study in **Paper I**, prior to the analyses of the available whole blood samples by ICP-MS, approximately 0.5 g of each blood sample was first mixed with 3 mL of deionized water and 2 mL of nitric acid (65% suprapur, Merck, Darmstadt, Germany) and digested at 250° C for 30 minutes using a Milestone ultraCLAVE II microwave digestion system (EMLS, Leutkirch, Germany). This method has been used for other elements as described previously (Kippler et al. 2009). After digestion, samples were diluted to a final nitric acid concentration of 20%.

For the studies in **Papers II-IV**, an alkali dilution method for lithium and multiple other trace elements in blood was developed (Lu et al. 2015). We used aliquots of only 0.2 g of blood samples and diluted them 1:25 with an alkali solution and internal standards, as described in **Papers II-IV** and elsewhere (Lu et al. 2015). The mixture was sonicated for 5 minutes and centrifuged at 2000 rpm for 5 minutes before analyzing the samples by ICP-MS. This method was found to provide a better limit of detection (LOD) and analytical stability for blood lithium measurements compared with the conventional acid digestion (Lu et al. 2015).

4.4.2.2 Urinary lithium

Urine samples were analyzed to compare the trace element concentrations with those in blood and water. In **Papers I-IV**, urine samples were analyzed for lithium, boron and cesium, using the same method as for the water samples. Assessment of arsenic exposure was based on the sum concentrations of inorganic arsenic and its methylated metabolites [methylarsonic acid (MMA) and dimethylarsinic acid] in urine, determined using high-performance liquid chromatography coupled with hydride generation and ICP-MS (Fangstrom et al. 2008; Harari et al. 2013). The correlation between concentrations of sum of arsenic metabolites and total arsenic (measured using direct ICP-MS with the method described for water arsenic) was 0.97 ($p<0.001$), supporting reliable analytical data.

For comparison and quality control of the lithium measurements, all the urine samples from **Papers II-IV** were also analyzed using an alkali method in which the samples were diluted 1:10 using 0.1% ammonium hydroxide (NH_4OH 25% w/w, Suprapur®, Merck, Darmstadt, Germany). The acid and alkali methods gave essentially the same results (r_s=0.98; n=288; **Figure 12**). Thus, the lithium measurements, analyzed using the acid method, were used for exposure assessment.

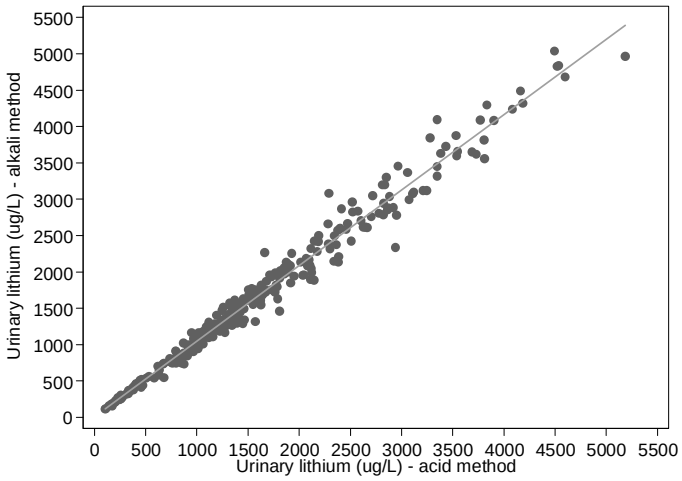

Figure 12. Scatter plot of lithium urinary concentrations analyzed with the acid vs. alkali method (n=288).

In order to investigate the inter-day and intra-day variations in the lithium concentrations in urine, we measured lithium in urine samples from a previous study on arsenic in the same study area (Concha et al. 2002). Inter-day variation was assessed by collection of daily spot urine samples (at the same time of the day) from 15 women during 5 consecutive days. The intra-day variation was assessed by collection of all urine (each void in a separate container) produced during 72 hours in 7 women.

The mean intra-day variation in the specific gravity-adjusted lithium concentrations in urine was +/-17% (**Figure 13A**), while the mean inter-day variation was +/-9% (**Figure 13B**). The average lithium concentrations in urine of day 1, 2 and 3 (from the intra-day samples) were highly correlated [r_s=0.50 (day 1 *vs.* 2), r_s=0.71 (day 1 *vs.* 3) and r_s=0.86 (day 2 *vs.* 3)] and similar correlations were found in samples from the inter-day comparison (r_s ranging from 0.43 to 0.74). A diurnal variation in the lithium concentrations in urine might be due to reported circadian variations in the renal excretion of lithium (Kyroudis et al. 1987; Shetty et al. 2012). In our study, the lithium concentrations in urine collected during morning/day time (average of day 1, 2 and 3: 1,845 µg/L, range 1,204-2,486) did not differ statistically from that in urine collected during evening/night time (1,997 µg/L, range 1452-2,543). Thus, lithium measurements in spot-urine samples seem to estimate the actual exposure to lithium. Although 24-hour urine samples (or first morning urine) would definitely provide a better exposure estimate than spot-urine samples, the collection of 24-hour urine samples has a high probability of incomplete sampling (Johansson et al. 1999) and was not feasible in the present study. Similarly, first morning urine sampling was not feasible.

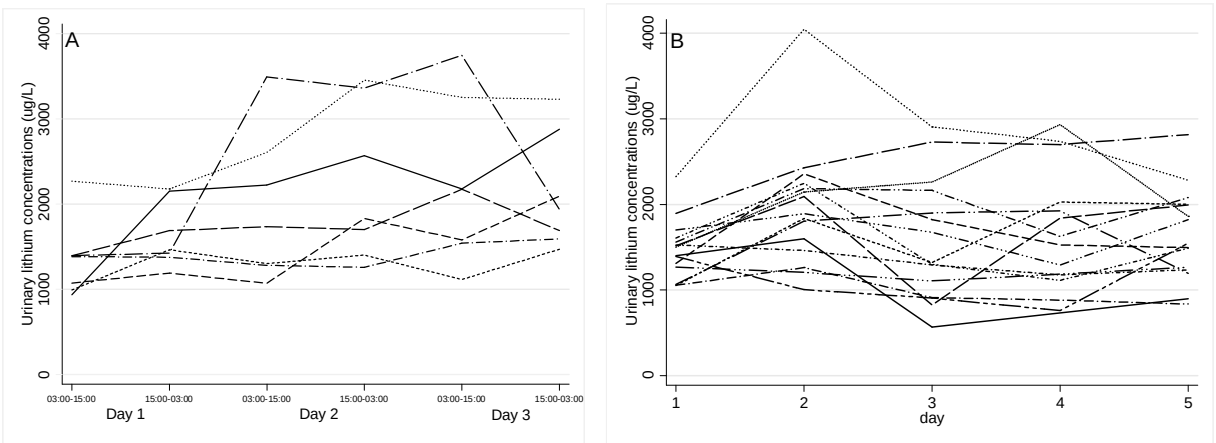

Figure 13. Individual intra-days (n=7; A) and inter-days (n=15; B) variation of the lithium concentrations in urine (adjusted for specific gravity).

To compensate for variations in the dilution of urine, we adjusted the measured element concentrations to the mean specific gravity (1.019 g/mL), measured by a hand refractometer (Atago, Japan) during the fieldwork, as well as to the mean urinary osmolality (694 mOsm/kg; range 141–1174), measured later at Karolinska Institutet by a digital cryoscopic osmometer (OSMOMAT® 030, Gonotec Gesellschaft für Meß- und Regeltechnik mbH, Berlin, Germany). The lithium concentrations in urine adjusted for specific gravity were highly correlated with those adjusted for osmolality (r_s=0.98, p<0.001, n=288, **Figure 14**). Urinary lithium concentrations adjusted for osmolality were chosen as an exposure biomarker in urine in **Papers II-IV** since the specific gravity is influenced by urinary protein and glucose (Parikh et al. 2002). Creatinine adjustment, other commonly-used alternative, was not chosen as it is known to be markedly affected by muscle mass, age and meat intake (Nermell et al. 2008; Suwazono et al. 2005).

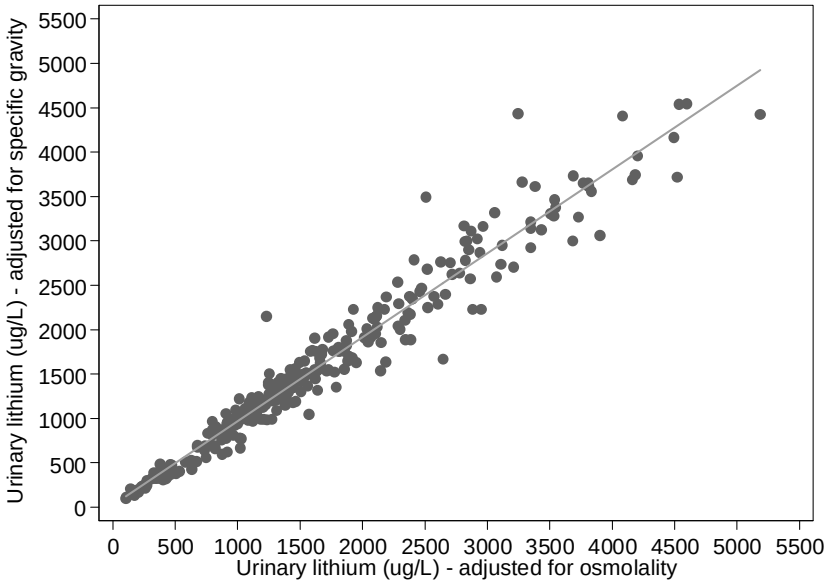

Figure 14. Scatter plot of the lithium concentrations in urine adjusted for osmolality and specific gravity (n=288).

4.4.2.3 Breast milk

In **Paper I**, breast milk samples were analyzed for lithium using the same method as for the blood samples in the same study, i.e. acid digestion of samples prior the ICP-MS analysis.

4.4.3 Reference materials and limits of detection

In order to secure the analytical accuracy, we analyzed all the samples together with reference materials. To the best of our knowledge, there are no certified reference materials for lithium in blood, serum, RBC, urine or breast milk. Instead, we used commercially available reference materials with recommended values. For lithium in drinking water, we used the certified reference material SRM 1643e Trace Elements in Water [National Institute of Standards and Technology (NIST), Gaithersburg, USA].

In general, the values that we obtained were in satisfactory agreement with the reference/recommended values. Results of reference materials used for other elements are summarized in each individual paper.

Limits of detection were calculated as 3 times the standard deviation of the blanks. Results for the used reference materials and LOD for lithium are presented in **Table 4**.

Table 4. Limits of detection and obtained and reference values of the reference materials used for analytical quality control for lithium measurements.

Paper	Quality control for	Method	LOD (µg/L)	n	Reference material	Reference value	Obtained value
I	Blood and RBC	Acid	0.18 and 0.028	6	Seronorm™ 0503109 L-2[a]	1.9 (1.8-2.0)[a]	1.5 (1.2-2.0)[a]
	Breast milk and plasma	Acid	0.028 and 0.14	10	Seronorm™ NO0371y L-2[b]	10.6 (9.8-11)[b]	9.1 (7.1-13)[b]
	Urine	Acid	0.032	10	Seronorm™ NO2525[a]	7.9 (7.4-8.5)[a]	9.0 (4.9-14)[a]
				9	Seronorm™ OK4636[a]	15.8 (15-17)[a]	13 (7.7-19)[a]
	Water	Acid	0.032	11	NIST 1643e[a]	17.4 (16-19)[a]	16 (12-18)[a]
II-IV	Whole blood	Alkali	0.0073	21	Seronorm™ 1103128[a]	0.46	0.63 (0.48-0.89)
				21	Seronorm™ 1103129[a]	0.44 (0.41-0.47)	0.67 (0.21-0.96)
	Urine	Acid	0.20	36	Seronorm™ 1011644 L-1[a]	7.0	7.9 (6.3-11)
				37	Seronorm™ 1011645 L-2[a]	7.0	7.6 (5.8-11)
				14	NIST2670a High level[a]	N/A	14 (12-16)
	Water	Acid	0.008	14	NIST1643e[a]	17.4 (16-19)	16 (15-18)

[a] µg/L; [b] mg/L; N/A: not available; RBC: red blood cells; LOD: limit of detection; NIST: National Institute of Standards and Technology.

4.5 OUTCOMES

4.5.1 Fetal and birth size measurements

At 1-3 time-points during pregnancy, fetal growth was assessed by ultrasound measurement of the biparietal diameter (BPD), occipitofrontal diameter (OFD), head circumference (HC), abdominal circumference (AC), femur length (FL) and estimation of fetal weight (FW). The detailed description of the performance of the fetal measurements can be found in **Paper II**. Out of the 180 women included in the study, ultrasound measurements were obtained in 136, measured in October 2012, January 2013 and April 2013. The examinations were performed by an experienced senior obstetrician, using a portable ultrasound machine FFSonic, UF-4100, (Fukuda Denshi, Tokyo, Japan) available at the hospital in San Antonio de los Cobres. Each measurement was performed three times, each time using a new picture, after which the average was computed.

In addition, weight, length and head circumference were measured at birth by the nurses at the hospital in San Antonio de los Cobres in most of the cases. Prior to the study start, we informed all health care personnel about the project and trained the medical personnel to perform the anthropometric measurements of the newborns at birth and to collect the samples of cord blood and placenta. Also, we established collaboration with the main gyneco-obstetric hospital in Salta and provided instructions for data and sample collection as well as sample collection kits. In this way, independently if women delivered in San Antonio de los Cobres or Salta, all data and sample collection at birth was performed in a standardized way.

Thirty seven percent of the women delivered at the main hospital of the city of Salta, 3.5% in the ambulance on the way to Salta, 3.5% in the surrounding villages, 4% at home and the rest (52%) at the hospital in San Antonio de los Cobres.

4.5.2 Markers of thyroid function

To assess the maternal thyroid function, we selected the following markers in serum: TSH, T4, fT4, T3, fT3, Tg and TTR. All analyses were performed at the Department of Clinical Chemistry at the University Hospitals in Lund and Malmö, Sweden. The detailed description of each particular method can be found in **Paper III**.

4.5.3 Markers of calcium homeostasis

We assessed the calcium homeostasis by the concentrations of intact parathyroid hormone (iPTH) in serum, 25-hydroxyvitamin D_3 in plasma, and the serum and urinary concentrations of total calcium, phosphorus and magnesium. We also analyzed serum albumin to adjust the serum calcium concentrations. Analysis of iPTH, 25-hydroxyvitamin D_3 and albumin were performed at the Department of Clinical Chemistry at the University Hospitals in Lund and Malmö, Sweden. Calcium, phosphorus and magnesium were analyzed by ICP-MS using the method described above for lithium measurements in blood and urine. The detailed description of each particular method can be found in **Paper IV**.

4.6 ETHICAL CONSIDERATIONS

Studies in **Papers I-IV** were approved by the regional ethical committee at Karolinska Institutet, Stockholm, Sweden, and by the Ministry of Health, Salta, Argentina. The study in **Paper I** was also approved by the Ethical Committee of the Institute of Nutrition and Food Technology (INTA), University of Chile.

Prior to recruitment of the pregnant women, we obtained written informed consent from all women after oral and written explanation of study details. For women below 18 years of age, informed consent was also obtained from the closest care giver. At the end of every meeting with each woman and her baby, the woman received a report of the results of the measurements performed during the visit (i.e. blood pressure, urine tests, hemoglobin, body weight, BMI, blood glucose, as well as weight, length and head circumference of her baby). Without prior announcement, in the last follow-up visit, every woman received a baby garment and a tissue bag as acknowledgement for their participation.

In case any of the measurements showed abnormal results, these were communicated to the local medical doctors. Abnormalities found during the ultrasound examinations [i.e. heart malformation (n=1), renal cysts (n=1), polyhydramnios (n=1) oligoamnios (n=1)] were immediately reported to the local medical doctors who referred the patients to the main obstetric clinic in Salta. After completion of the analyses of thyroid function, abnormal values of TSH were reported to the hospital in San Antonio de los Cobres for additional follow-ups of the patients at the thyroid hospital “Dr. Arturo Oñativia” in Salta.

On regular basis, we reported to and discussed the measured concentrations of the different trace elements in drinking water with the hospital in San Antonio de los Cobres, the primary health care clinics in the surrounding villages and the Ministry of Health in Salta. Also, results of the published articles have been reported to the Ministry of Health in Spanish. During the last visit to San Antonio de los Cobres, we prepared a meeting with the medical and health care personnel from all the villages to present preliminary results of the study on lithium and other elements during pregnancy.

We also participated in meetings with the “Arsenic Group” in the city of Salta. This consists of representatives of different local governmental agencies and universities aiming at discussing and following up results concerning arsenic in drinking water in the province of Salta. We have assisted in the search of an alternative water source for San Antonio de los Cobres by collecting water samples and measuring the concentrations of multiple elements.

4.7 STATISTICAL ANALYSES

For a detailed description of the different statistical methods used in this thesis, the reader is referred to the individual papers (**Papers I-IV**).

Depending on the nature of the data, different statistical approaches were applied. All statistical analyses were performed using either IBM ® SPSS ® Statistics 18.0 (SPSS, Chicago, IL, USA), Stata (StataCorp LP. 2012. Stata Statistical Software: Release 12.1. College Station, TX, USA) or R [version 3.2.1, (R Core Team 2015)].

Relationships between exposure measures, outcome variables and covariates were assessed using Spearman's rank correlation coefficients or linear mixed-effects regression for longitudinal data. We used scatter plots to visually evaluate the different relationships. We tested differences between groups (e.g. tertiles of blood lithium) using Kruskal-Wallis *H* rank test or chi square test.

Associations between lithium exposure and the different outcomes were assessed using linear regression (cross-sectional data) or linear mixed-effects regression models (longitudinal data). For dichotomous outcome variables (e.g. vitamin D_3 concentrations < and ≥50 nmol/L), we used mixed-effects logistic regression with random intercept (longitudinal data) and binomial logistic regression (cross-sectional data).

We also performed quantile regression analyses (for both cross-sectional and longitudinal data) to assess the association of blood lithium on the whole distribution of the outcomes. In all cases, we based the estimates, confidence intervals (CI), and *p*-values on 500 bootstrap samples and used Wald test to evaluate differences between the estimated regression coefficients across the quantiles.

The regression models were adjusted for covariates known to affect the outcome measures or that influenced the estimates more than 10% (Sullivan 2008). Whenever necessary, i.e. to get the best fit of the data with normally distributed residuals, the exposure variables were log2-transformed.

Different sensitivity analyses were performed to test e.g. the influence of extreme values as well as the impact of diseases (infections and preeclampsia) in the different associations.

5 RESULTS AND DISCUSSION

In this section, the main findings of the thesis are presented and discussed, with reference to the actual paper. Some unpublished data are also presented. For further details, the reader is referred to the individual papers (**Papers I-IV**).

5.1 MATERNAL AND EARLY-LIFE EXPOSURE TO LITHIUM

5.1.1 Lithium and other elements in drinking water

Elevated concentrations of lithium, boron and cesium in drinking water in our study area were discovered about five years ago (Concha et al. 2010), while elevated arsenic concentrations have been known for several decades (Vahter et al. 1995). The highest lithium concentrations in the drinking water were found in San Antonio de los Cobres (mean 718 µg/L, range 528-837, n=58; **Table 5**) and in a small natural spring 20 km east of Cobres providing water for just one family (range 958-1,660 µg/L, n=3). The concentrations of lithium, arsenic and cesium in the other villages were at least 1/5 of those in San Antonio de los Cobres, with the lowest found in Tolar Grande. The highest boron concentrations were found in San Antonio de los Cobres, Esquina de Guardia and Cobres and the lowest in Tolar Grande and Palomar (**Table 5**).

Table 5. Average concentrations (range) of lithium, boron, arsenic and cesium (all expressed in µg/L) in the drinking water in the 10 study villages from October 2012 until April 2014.

Village	n	Lithium		Boron		Arsenic		Cesium	
San Antonio de los Cobres	58	718	(528-837)	5,610	(3,421-6,925)	114	(41-244)	323	(101-410)
Cobres	13	77	(29-162)	3,433	(1,356-6,655)	14	(1.7-89)	0.25	(0.01-1.7)
El Palomar	9	13	(5.0-71)	541	(314-1,866)	4.0	(1.0-17)	0.047	(0.01-0.22)
El Toro	17	66	(7.6-203)	1,806	(449-5,995)	13	(2.3-71)	0.084	(0.02-0.20)
Esquina de Guardia	8	117	(52-123)	5,781	(3,311-6,239)	13	(6.2-13)	4.4	(3.0-6.2)
Las Cuevas	15	98	(17-159)	1,521	(388-2,256)	27	(0.83-48)	0.37	(0.02-2.6)
Olacapato	8	23	(17-29)	668	(448-919)	9.3	(4.1-13)	5.5	(3.2-7.0)
Pocitos	6	99	(22-132)	869	(438-1,081)	44	(17-58)	14	(5.0-22)
Santa Rosa de los Pastos Grandes	6	112	(18-242)	2,403	(426-4,620)	46	(12-141)	34	(10-65)
Tolar Grande	1	8.2		377		3.2		0.060	

Water samples have been repeatedly collected in San Antonio de los Cobres since 1994, when the first studies on arsenic in this area were performed (Vahter et al. 1995). We analyzed all the samples available since then (n=104), for arsenic, lithium, boron and cesium and investigated changes in the concentrations over time (**Figure 15**). The lithium and boron

concentrations had similar patterns of variation, with the highest concentrations found in December 2008 (summer) and the lowest in November 2012 and September 2013 (spring), but there were no clear seasonal variations or influence of the rain period (January-March). Lithium and boron concentrations in the drinking water were highly correlated (r_s=0.91). Lithium was also highly correlated with water arsenic (r_s=0.88) and cesium (r_s=0.79).

The cesium concentrations in drinking water appeared to be more stable over time than the other elements and slightly lower concentrations were found in July, September and December 2013. The arsenic concentrations were quite stable until November 2011, as previously reported (Concha et al. 2006). However, following the installation of the arsenic filter close to the main water source, the arsenic concentrations dropped to about 30 µg/L. Conversely, arsenic concentrations seemed to increase again after the completion of this study, in April 2014, suggesting that the filter might not be working or in use.

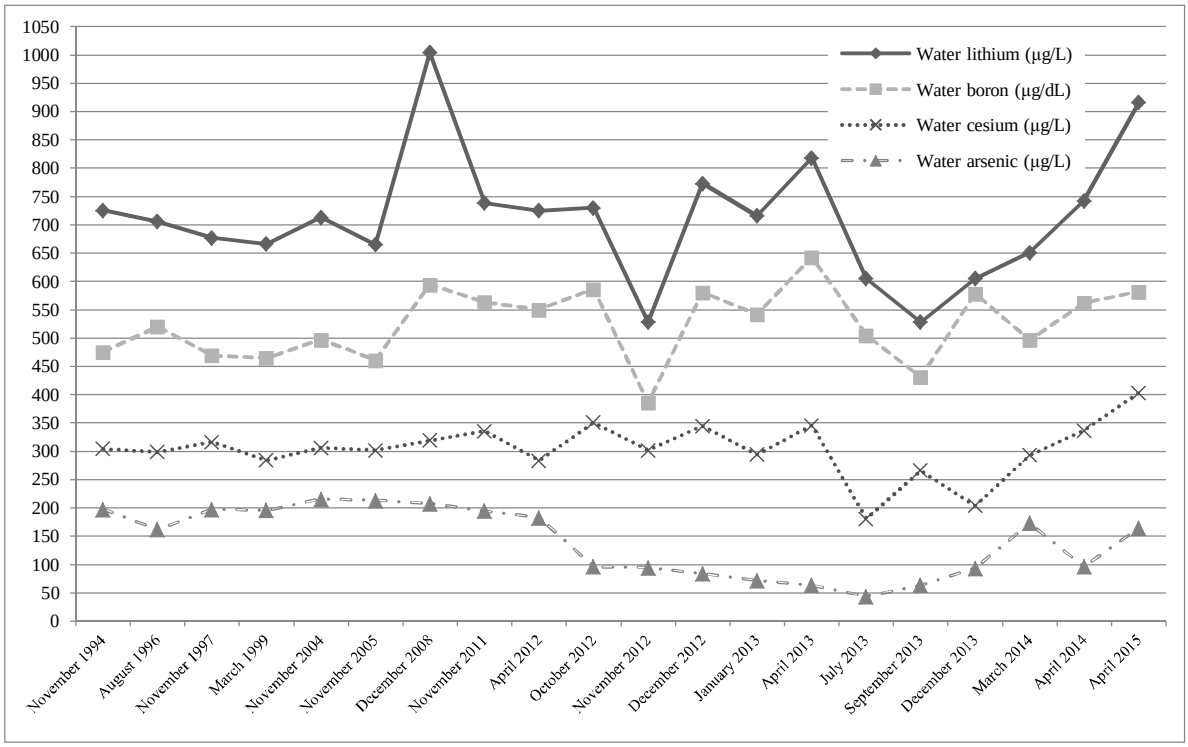

Figure 15. Average lithium, boron, cesium and arsenic concentrations in drinking water in San Antonio de los Cobres from November 1994 till April 2015 (n=104). Lithium, arsenic and cesium concentrations are expressed in µg/L, and, to fit the scale, boron concentrations are expressed in µg/dL.

5.1.2 Lithium in maternal blood and urine

5.1.2.1 Lithium concentrations in blood and urine during pregnancy

As mentioned above, the main biomarker selected to assess lithium exposure was blood lithium. The overall median lithium concentration in maternal blood and urine during pregnancy was 25 µg/L and 1,404 µg/L, respectively, both with wide ranges of distribution (1.9-145 and 105-5,185, respectively; **Papers II-IV** and **Figure 16A** and **16B**). Thus, the concentrations in urine were 56 times higher, on average, than those in blood.

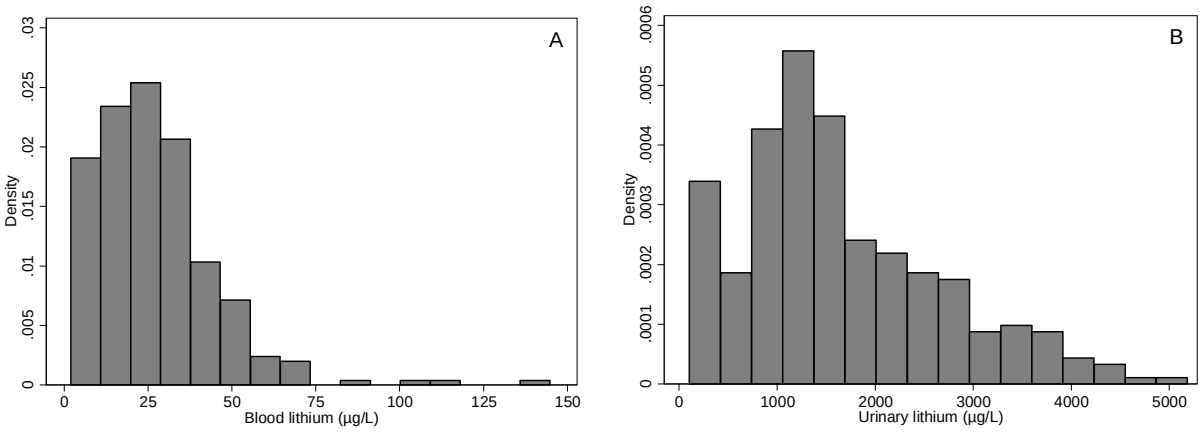

Figure 16. Distribution of the lithium concentrations (average for women with 2-3 measurements) in maternal blood (A) and urine (B) during pregnancy (n=180) (**Papers II-IV**).

The lithium concentrations in blood and urine (average for women with 2-3 measurements) were highly correlated (r_s=0.84, **Paper II, Figure 17B**). To a lesser extent, lithium concentrations in blood were correlated with those in water (r_s=0.40) (**Figure 17A**). Similar correlations were found between urinary lithium and drinking water (r_s=0.44). These weaker correlations between blood/urinary lithium and water lithium are likely explained by differences in the intake of drinking water, potential variations in the lithium concentrations in the water, and the intake of lithium-free bottled water. Indeed, the correlation between blood and urinary lithium with that in drinking water was higher in women reporting exclusive intake of tap water (r_s=0.48 for blood and r_s=0.50 for urine), compared with those reporting additional intake of bottled water (r_s=0.31 for blood and r_s=0.40 for urine). In addition, exposure through other sources such as food might have contributed to the weaker correlation with lithium in the tap water.

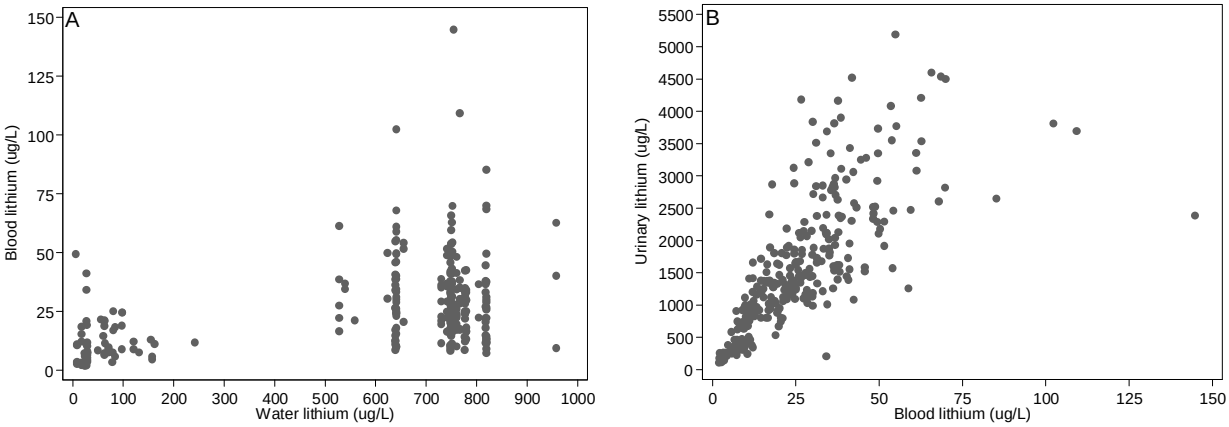

Figure 17. Scatter plot showing the lithium concentrations in blood and drinking water (A), and those in blood and urine (B) (n=180) (**Papers II-IV**).

A marked inter- and intra-individual variation in the lithium concentrations in urine of lithium-treated patients has been reported (Kyroudis et al. 1987) as well as a much weaker correlation between plasma and urinary lithium [r_s=0.23, p=0.10; (Shetty et al. 2012)] compared with that in our study, suggesting that the correlation between plasma/blood and urinary lithium might be dependent on the dose, the dosage regimen or genetic differences in the excretion of lithium. In the case of continuous lithium exposure, such as from water and food, the concentration in blood is likely to reach a steady state and to estimate a more long-term exposure.

Lithium concentrations in plasma or serum are commonly used for monitoring the therapeutic range in patients on lithium therapy (Grandjean and Aubry 2009a). In our study, the lithium concentrations in plasma appeared to be about 1.5 times that measured in whole blood in the same individuals (**Figure 11**), with very low inter-individual variation (95% CI 1.4-1.6, n=20). Transforming our highest observed blood lithium concentration (i.e. 161 µg/L) into corresponding plasma concentration (242 µg/L), it would still be only 7% of the lower range of serum lithium levels during lithium therapy (~5,500-8,300 µg/L, i.e. 0.8-1.2 mmol/L) (Grandjean and Aubry 2009a; Malhi et al. 2011). However, it was at least 30 times higher than what was described in the Chilean study (range 4.1-8.0 µg/L; **Paper I**). In unexposed people, the lithium concentration in plasma is generally about 1 µg/L (Alimonti et al. 2005).

5.1.2.2 Lithium concentrations in blood and urine during pregnancy and post-partum

Based on linear-mixed effects models with random intercept (longitudinal analyses), the maternal blood lithium concentrations in our study seemed to increase progressively during

pregnancy and to 3 months post-partum (0.83 µg/L per week, 95% CI 0.67-0.99; **Figure 18**), along with an increase in the urinary lithium concentrations (19 µg/L per week, 95% CI 6.9-30). After this period, at 3-6 months after delivery, the lithium concentrations in urine seemed to decrease again (**Figure 19**). Please note that the results from samples collected during pregnancy are described in **Papers II-IV**, while those from the post-partum period are presented only in the **Figures 18** and **19**.

Our findings are in disagreement with the reported decrease in the lithium concentrations in plasma in lithium-treated pregnant women during late pregnancy (Grandjean and Aubry 2009a), which is suggested to happen due to the physiological increased glomerular filtration rate and plasma expansion occurring during gestation. This is the reason why lithium doses are recommended to be increased in late gestation (Grandjean and Aubry 2009a).

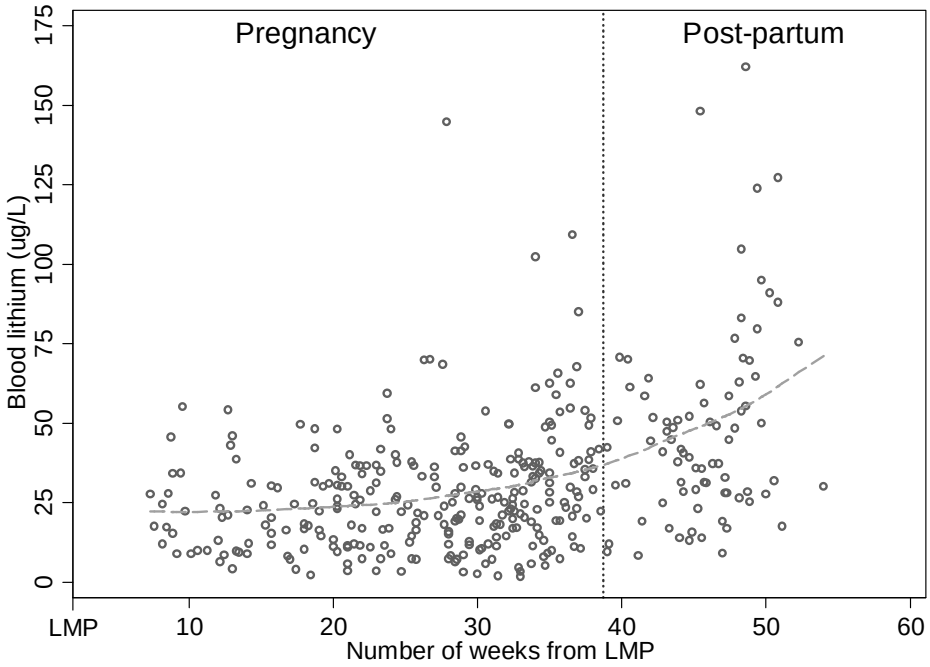

Figure 18. Scatter plot of maternal blood lithium concentrations (µg/L) during pregnancy and post-partum (n=360 measurements, 1-4 measurements per woman, n=180 women). LMP: last menstrual period. Blood samples were collected only up to 3 months after delivery. Dashed line represents a lowess line between blood lithium concentrations and the number of days from the LMP. Dotted line represents the average gestational age at delivery.

The increased glomerular filtration rate during pregnancy would only support the finding of increased lithium concentrations in urine and not in blood in the present study. It can be speculated that the increased lithium concentrations in blood during pregnancy and post-partum might instead be due to an increased activity of the sodium-lithium counter transporter in the erythrocytes (Worley et al. 1982). Increased consumption of drinking water during pregnancy could be an alternative explanation. Moreover, because a large part of the lithium that remains the longest in the body is accumulated in the bone (Birch and Hullin

1972), it is likely that an increased bone resorption during late pregnancy and after delivery (Åkesson et al. 2004) releases the accumulated lithium from the bones, explaining the increase in both blood and urinary concentration of lithium observed up to 3 months after delivery. The decrease in urinary lithium concentrations at 3-6 months post-partum is in agreement with the resolution of the physiological kidney changes that occur during pregnancy (Cheung and Lafayette 2013).

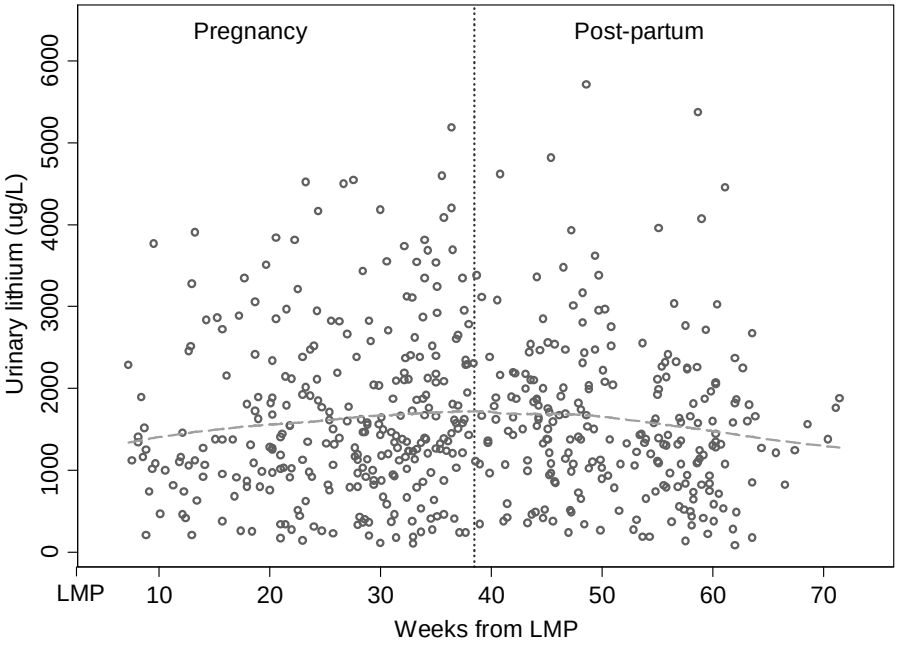

Figure 19. Scatter plot of maternal urinary lithium concentrations (µg/L) during pregnancy and puerperium (n=360 measurements, 1-4 measurements per woman, n=180 women). LMP: last menstrual period. Urine samples were collected up to 6 months after delivery. Dashed line represents a lowess line between urinary lithium concentrations and the number of days from the LMP. Dotted line represents the average gestational age at delivery.

5.1.3 Lithium transfer through the placenta

The geometric mean lithium concentration in the cord blood samples from 1996 collected in San Antonio de los Cobres (**Paper I,** n=10) was 70 µg/L (range 30-105), compared to 47 µg/L (range 20-77) in the maternal blood at delivery, indicating passage of lithium through the placenta. Furthermore, the lithium concentrations in cord blood and maternal blood at delivery were highly correlated (r_s=0.82, n=10, **Figure 20**). Similarly, in our recent mother-child cohort recruited in 2012-2014, the median cord blood lithium concentration was 48 µg/L (range 9.5-156; n=76, in San Antonio de los Cobres), compared to 26 µg/L (range1.9-109) in maternal blood in the third trimester (GW 28-42). The correlation between cord blood

and maternal lithium was lower (r_s=0.48, n=76), likely due to the marked variation in maternal blood lithium with increasing gestational week also within the third trimester.

In support of a pronounced transfer of lithium to the fetus (**Paper I**), we found that the geometric mean lithium concentration in the first infant urine in life was 600 µg/L (range 140-1,680, n=7), almost half of that in maternal urine at delivery (geometric mean 1,400 µg/L, range 810-2,300, n=11).

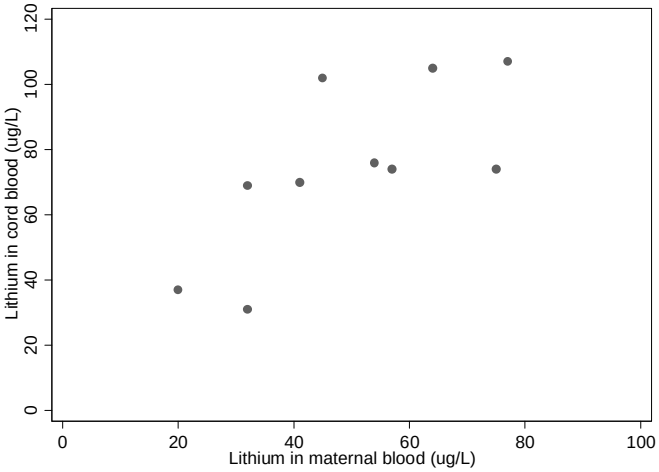

Figure 20. Scatter plot of lithium concentrations in maternal and cord blood (**Paper I**).

A case-study of a patient on lithium therapy showed similar lithium concentrations (about 2,000 µg/L) in maternal and newborn serum at birth (Sykes et al. 1976), supporting our findings of a marked transfer of lithium through the placenta at both low and high exposure levels. Indeed, a more recent clinical study showed good agreement between concentrations in cord and maternal blood within a range of lithium doses of 300-1800 mg/day (Newport et al. 2005).

The transfer of lithium through the placenta occurs most probably during the whole gestation, including the sensitive embryonic period when the organogenesis takes place. This is supported by the fact that lithium therapy has been associated with the occurrence of a series of developmental abnormalities, particularly in experimental studies [**Table 1** and (Morgan et al. 2003)] and therefore, lithium is classified as teratogenic (category D) by the U.S. Food and Drug Administration. Suggested mechanisms of lithium transfer through the placenta are: bidirectional passive diffusion, as well as fetal-maternal unidirectional active transfer involving sodium/potassium ATPase (Palavinskas et al. 1982).

5.1.4 Lithium transfer through the mammary gland

The lithium concentrations in the infant urine decreased from 600 µg/L in the very first urine produced, to 120 µg/L (84-600) already at 2-4 weeks after birth (**Paper I**). Thereafter, the lithium concentrations in infant urine increased again to 380 µg/L at 4-6 months of age. The lithium concentrations in breast milk were on average 23 µg/L (range 8.8-53) at 2-4 weeks after delivery, 31 µg/L (17-53) at 2-4 months and 36 µg/L (24-54) at 4-6 months.

The observed rapid decreased in the newborns' urinary lithium concentrations, to 120 µg/L 2-4 weeks after birth, reflects a fast neonatal renal excretion of lithium and a much lower exposure through breast-milk than prenatally. Thus, breast-feeding appeared to provide certain protection against infant lithium exposure. Previous studies indicated a similar protection level against arsenic in the drinking water (Concha et al. 1998b). Evidently, formula prepared from the drinking water containing elevated lithium concentrations had provided much higher exposure levels.

The lithium concentrations in breast milk in our study were about half of that in maternal blood and both increased slightly but constantly after delivery (**Paper I**) and were highly correlated (r_s=0.80, **Figure 21**). In line with the high maternal blood lithium concentrations in women on lithium therapy, also the concentrations in breast milk are much higher in treated mothers [700-3,500 µg/L; (Viguera et al. 2007)].

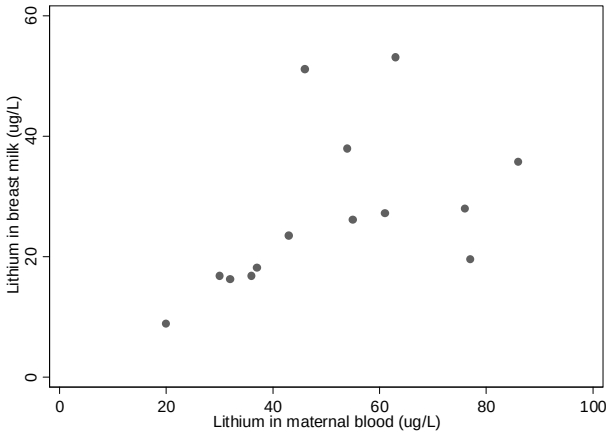

Figure 21. Scatter plot of lithium concentrations in maternal blood and breast milk (**Paper I**).

5.2 LITHIUM EXPOSURE AND FETAL SIZE

The study in **Paper II** is the first ever to investigate the potential impact of maternal exposure to lithium from drinking water on human fetal development. The study was based on fetal ultrasound measurements performed during pregnancy as well as measurements at birth.

5.2.1 Lithium exposure and fetal measurements

Out of the 180 women with available exposure data, 134 had at least one ultrasound measurement of the fetal size [head (BPD, OFD, HC), abdomen (AC), length (FL) and estimated weight (FW)] in the 2nd and/or 3rd trimester. Measurements in the 1st trimester were not included, as mainly crown-rump length was measured in the first weeks of pregnancy. Blood lithium was used as exposure biomarker, supported by the lithium concentrations in urine.

The correlation between the different fetal measurements was strong within the 2nd (r_s>0.98) and the 3rd (r_s>0.83) trimesters, but those were not correlated with blood or urinary lithium, urinary arsenic, serum boron, blood cesium, maternal age, parity, educational level, height, residence time or parental monthly income. In individuals with two fetal measurements (n=43), the different measurements were also strongly correlated between the 2nd and the 3rd trimesters (r_s=0.72-0.83).

In the multivariable-adjusted linear regression models (n=136 women, n=179 measurements), both blood and urinary lithium were inversely associated with all measures of fetal size in the 2nd trimester, although these associations did not generally reach significance (**Figure 22**). The effect sizes were in the order of 1/8 SD for all fetal measures. In the 3rd trimester, there was no association between blood lithium and fetal size measurements, although the associations with urinary lithium were generally inverse but not statistically significant. Neither urinary arsenic, nor serum boron or blood cesium were significant in the statistical models.

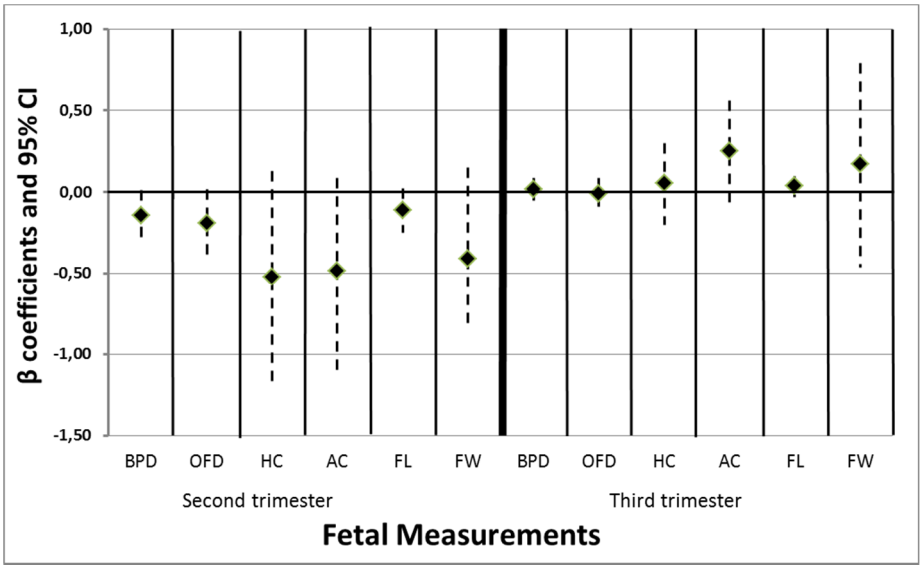

Figure 22. Multivariable-adjusted estimates (Beta coefficients and 95% CI) for the associations between blood lithium and fetal measurements. BPD: biparietal diameter (cm), OFD: occipitofrontal diameter (cm), HC: head circumference (cm), AC: abdominal circumference (cm), FL: femur length (cm), FW: fetal weight (in grams divided by 100 to fit the scale). Estimates expressed as changes per every 25 μg/L increment in blood lithium.

Because early embryonic and fetal stages are the most sensitive periods in life (**Figure 3**), one could speculate that, despite the fact that none of the associations were clearly statistically significant, lithium impairs the fetal development already in early gestation. This early embryonic and fetal stages are also the most sensitive periods for the exposure to other chemicals (Barouki et al. 2012). Maybe the lack of associations in the 3rd trimester could also be due to the known increased variation in the ultrasound measurements with increasing gestational age (Geirsson 1991), along with the above mentioned variation in the blood and urinary concentrations of lithium during pregnancy and the limited sample size for these analyses to overcome such variations.

5.2.2 Lithium exposure and measurements at birth

The average birth weight in the study population was similar in both boys and girls and was on average 3,022 g (range 1,250-4,500), which is lower than the average birth weight in European countries [~3,500 g, (Pedersen et al. 2013)] and that reported in Buenos Aires, the capital of Argentina [~3400 g, (Grandi 2003)]. Eight percent of the newborns had low birth weight (<2,500 g) and 18% were born preterm [average gestational age at birth 38.5 weeks (range 29-42)]. The average birth length and head circumference were 48 and 34 cm, respectively.

After adjusting for gestational age at birth, parity, parental monthly income, maternal height, infant sex, arsenic in urine, boron in serum and cesium in whole blood, the linear regression models showed an inverse association between maternal blood lithium and birth length (about 0.5 cm per every 25 μg/L increment in the average blood lithium concentrations across pregnancy) (**Figure 23**). Similarly, newborns to mothers in the highest tertile of lithium exposure (median blood lithium 42 μg/L) were on average 0.8 cm shorter than those in the lowest tertile of exposure (median blood lithium 11 μg/L).

Neither urinary arsenic, nor serum boron or blood cesium were statistically significantly associated with fetal size measurements in the multivariable-adjusted models. However, the estimates for the association between blood lithium and birth length were attenuated after adjusting the model for serum boron. Experimental studies have suggested that boron may impair the fetal size (WHO 2011). Thus, we cannot fully rule out that the associations observed in the present study are actually due to a combination of lithium and boron exposure. On the other hand, due to the high correlation between these two elements in drinking water (r_s=0.91) and blood (blood lithium *vs.* serum boron, r_s=0.77), the observed attenuation could be due to collinearity. In contrast, urinary arsenic and blood cesium did not

influence the estimates. The arsenic concentrations in the drinking water were lower during the study period due to the installation of an arsenic filter in the main study village, decreasing the exposure to arsenic. Also, women in the study area are known to metabolize arsenic more efficiently than other populations such as that in Bangladesh (Engstrom et al. 2011). This, together with a lower arsenic exposure, might, at least partially, explain why arsenic was not statistically significant in the multivariable-adjusted models; i.e. women with a more efficient arsenic metabolism will excrete the most toxic arsenic metabolite (i.e. MMA) more rapidly, exposing the fetus to lower levels of such metabolite.

The findings of associations between maternal lithium exposure and birth size are supported by those found with fetal measurements performed with ultrasound. That lithium may affect fetal growth has some support in the literature. Particularly, two studies in humans with lithium-treated patients reported lower birth weight in babies born to women undergoing the therapy, compared to controls (**Table 1**). Also, a series of experimental studies in mice, rats and monkeys, using doses from 7 to 400 mg/kg/day, as well as an *in vitro* study, have reported a decreased birth size in the exposed group (**Table 1**).

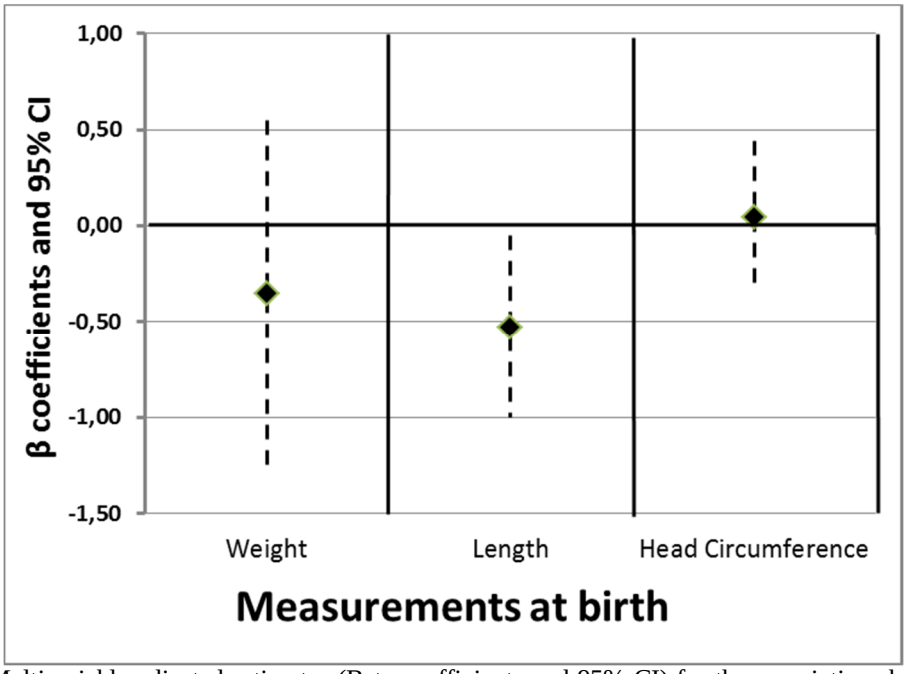

Figure 23. Multivariable-adjusted estimates (Beta coefficients and 95% CI) for the associations between blood lithium and measurements at birth [weight (grams), length (cm) and head circumference (cm)]. Estimates for birth weight were divided by 100 to fit the scale. Estimates are expressed as changes per every 25 µg/L increment in blood lithium.

5.3 POTENTIAL MECHANISMS

5.3.1 Disruption of the maternal thyroid function

Impairment of the thyroid function is a common side effect of lithium medication (McKnight et al. 2012). Therefore, we wanted to elucidate whether lithium exposure through drinking water could influence the maternal thyroid function during pregnancy (**Paper III**). If so, this could be a potential underlying mechanism for the associations between maternal lithium exposure and fetal size. We measured a number of different thyroid markers in maternal serum and investigated their association with lithium exposure during pregnancy. The selected thyroid markers included: TSH, fT4, T4, fT3, T3, Tg and TTR. Nineteen, twelve and thirteen percent of the women showed TSH concentrations above the reference value in the 1st (0.1-2.5 mIU/L), 2nd (0.2-3.0) and 3rd (0.3-3.5) trimesters, respectively. Eighty-three percent of those with abnormal values belonged to the highest tertiles of blood lithium (median blood lithium 42 μg/L, range 34-145). Also, 6% of the women had low fT3 values (reference value 3.7-7.7 pmol/L) in the 3rd trimester, and 78% of those belonged to the highest tertiles of lithium exposure. Only half of the women appeared to be iodine sufficient (i.e. urinary iodine ≥150 μg/L).

The multivariable-adjusted quantile regression models showed that the blood lithium concentrations were positively associated with TSH, particularly in the lowest tail of TSH concentrations. Also, blood lithium was inversely associated with fT3, total T3 and TTR, particularly in the highest percentiles of the outcomes. No associations were found between blood lithium and fT4, T4 or Tg.

Unexpectedly, blood cesium was also inversely associated with fT3 and T3 in the highest percentiles of the outcomes. Neither urinary arsenic, nor serum boron were significant in the models and they did not influence the associations with lithium and cesium.

Impairment of the thyroid function during pregnancy might be detrimental for the fetal growth and development (Forhead and Fowden 2014). To the best of our knowledge, there is only one previous study investigating the thyroid function in relation to lithium exposure from drinking water (Broberg et al. 2011). This study was performed in the same study area, i.e. with the same level of exposure, in adult non-pregnant women (median age 34, range 12-80), where urinary lithium was positively associated with TSH and negatively associated with fT4. No other thyroid markers were measured in that study. The lack of associations between

blood lithium and fT4 or T4 in this thesis might be explained by an elevated variation of the fT4 and T4 concentrations during pregnancy (Lockitch 1993).

In medication and experimental studies, using much higher doses than those in the present thesis, lithium is suggested to impair the thyroid function in different ways. For example, by inhibiting the iodine uptake, the iodotyrosine coupling, the thyroxine secretion and the conversion of T4 to T3, or by altering the Tg structure (Berens et al. 1970; Burrow et al. 1971; Terao et al. 1995). Lithium effects on the TSH levels could occur by inhibiting its stimulation by TRH, as lithium is known to cross the blood–brain barrier and accumulate in the hypothalamus (Mukherjee et al. 1976). This would in turn, inhibit TSH secretion and alter the whole chain of thyroid hormone production, which will conversely stimulate the TSH secretion (**Figure 4**) to abnormal levels.

Another probable hypothesis is an inhibition of the production and release of thyroid hormones in the thyroid gland, as lithium is also known to accumulate there (Berens et al. 1970). The latter would also explain both the increase in TSH and decrease in fT3 and T3 levels. Also, the inhibition of the 5'deiodinase I, in charge of forming T3 from T4 (Bauer et al. 2006), would explain the decrease in the T3 levels. Inhibition of iodine uptake might occur by the competition of lithium with iodine in, for example, the sodium-iodine symporter, which is the way iodine is taken up in the thyroid gland (Eskandari et al. 1997).

The associations with TTR have, however, not been reported previously. Therefore, if confirmed in other studies, the indicated decrease in the TTR levels due to lithium might be a novel mechanism of action. Importantly, TTR is one of the proteins responsible for transporting T4 in the body and the most abundant T4 transporter in the cerebrospinal fluid (Schreiber et al. 1995). Also, TTR is produced in placental trophoblastic cells and is in charge of transporting thyroid hormones to the fetus (McKinnon et al. 2005). The decrease in TTR concentrations can, thus, result in lower fetal thyroid hormone levels (Darnerud et al. 1996).

5.3.2 Impairment of the maternal calcium homeostasis

Another common side-effect of lithium medication is the impairment of the calcium homeostasis (McKnight et al. 2012). Therefore, we tested whether also environmental exposure to lithium might have similar effects in pregnant women (**Paper IV**). It could, like the disruption of thyroid function, be a potential underlying mechanism of the inverse association of lithium with fetal size. To elucidate a potential impact of lithium exposure through drinking water on the calcium homeostasis, we measured different markers in

maternal serum and investigated their association with blood lithium concentrations during pregnancy. The selected markers of calcium homeostasis included: serum calcium (total and albumin-adjusted), urinary calcium, serum PTH, plasma 25-hydroxivitamin D_3 (vitamin D_3), as well as total phosphorus and magnesium in serum and urine. The correlation between the different markers in late pregnancy varied, with the highest between urinary magnesium and calcium (r_s=0.51), serum magnesium with total serum calcium (r_s=0.37), serum phosphorus and albumin-adjusted serum calcium (r_s=0.22) and PTH and vitamin D_3 (r_s=0.22). Six women had elevated albumin-adjusted serum calcium concentrations, while 58% of the women had vitamin D_3 concentrations <50 nmol/L and 19%, <30 nmol/L.

Vitamin D_3 concentrations were strongly influenced by the season of sampling, showing the lowest concentrations in the winter (June-August) and the highest in the summer (December-February). One of the main sources of vitamin D_3 is UVB radiation which converts 7-dehydrocholesterol to previtamin D_3 in the skin. The study area is located at a latitude between -23.31 and -24.35, and although sunlight is present at least 10 hours/day during the winter season, the characteristic weather of the Puna region, with cold and very windy winters, impose people to use more clothes and to stay indoors longer time, reducing the exposure time to sunlight.

In multivariable-adjusted linear mixed-effects models (longitudinal analyses across pregnancy), blood lithium was inversely associated with plasma vitamin D_3 concentrations, as well as with urinary calcium and magnesium, and positively associated with serum magnesium. A 25 μg/L increment in the blood lithium concentrations was associated with an odds ratio of 3.5 for having vitamin D_3 concentrations <50 nmol/L, and with an odds ratio of 4.6 for having vitamin D_3 concentrations <30 nmol/L. The influence of season of sampling on the Vitamin D_3 concentrations was independent of that of lithium.

Low vitamin D concentrations during pregnancy could be unfavorable for the maternal and fetal health, as they are associated with an increased risk for preeclampsia and infectious diseases during pregnancy in the mother, as well as with impaired fetal programming, low birth size, increased risk for inflammatory and immune disorders during infancy and a poorer infant bone health (i.e. lower bone mineral density) later in life (Brannon and Picciano 2011; Karras et al. 2014).

There are different mechanisms that could be involved in the inverse associations observed between blood lithium and vitamin D_3. Due to the lack of associations between lithium and PTH, it seems likely that the present associations might be explained by mechanisms

occurring outside the parathyroid gland. One possible mechanism is the stimulation of fibroblast growth factor 23 (FGF23) by lithium, leading to an enhanced activity of 24-hydroxylase (CYP24A1) and a consequent increased catabolism of both 25-hydroxyvitamin D_3 and 1,25-hydroxyvitamin D_3 (Fakhri et al. 2014). This would in turn lead to a decrease in the urinary excretion of calcium and phosphorus (Brannon and Picciano 2011; Fakhri et al. 2014). Since we found no clear associations with the levels of phosphorus in our study, this hypothesis is not completely compatible with our findings. Nevertheless, findings concerning phosphorus have to be interpreted with caution as we measured total phosphorus in serum and not only inorganic phosphate.

Another hypothesis is that the lithium-related decrease of the vitamin D_3, urinary calcium and urinary magnesium concentrations occurs directly at the kidney level. Impairment of the kidney function is a known adverse effect of lithium therapy (McKnight et al. 2012). In fact, we found positive associations between blood lithium and urinary albumin. An impaired kidney function is associated with lower serum 25-hydroxyvitamin D_3 concentrations (Damasiewicz et al. 2013; de Boer et al. 2011), although a severe kidney impairment might be needed to cause a decrease in the vitamin D_3 concentrations. The inverse associations between blood lithium and urinary magnesium and calcium could also be explained by such a mechanism, considering that their homeostasis is mainly regulated in the kidneys (Blaine et al. 2015).

5.3.3 Other potential mechanisms

It is possible that additional mechanisms are involved in the association of lithium exposure with fetal size, but these were not investigated in this thesis. For example, it could be speculated that the accumulation of lithium in other endocrine organs could play a role. A lithium-related increase in the maternal circulating levels of cortisol, as shown in lithium-treated patients (Bschor et al. 2002; Platman and Fieve 1968) and in rats (Sugawara et al. 1988), could lead to a decreased weight, length and head circumference at birth (Duthie and Reynolds 2013).

It is also likely that both mechanisms of lithium toxicity explored in this thesis and other suggested mechanisms are complementary, rather than mutually exclusive. More efforts are, however, necessary to fully understand the biological processes underlying the potential impairment of fetal development due to lithium exposure through drinking water in the general population.

5.4 METHODOLOGICAL CONSIDERATIONS

The study in **Paper I** was based on a few mother-child pairs from San Antonio de los Cobres (n=11), in Argentina, and Arica (n=24) and Santiago (n=11), in Chile. We took advantage of two previous studies and could clarify that environmental lithium exposure during pregnancy results in high fetal exposure, as indicated by the high concentrations in both cord blood and the first infant urine in life. In this study, blood, urine and breast milk samples in San Antonio de los Cobres were sampled repeatedly, allowing us to see changes over time during the first 6 months after delivery/birth. A drawback of this study, in addition to the small sample size, was the lack of data concerning factors that could affect the transfer of lithium through the placenta and mammary gland (e.g. nutrition).

For **Papers II-IV**, we recruited a larger mother-child cohort (n=194) covering most of the Andean region of the Province of Salta, and sampled different exposure and outcome markers repeatedly during and after pregnancy. Indeed, the population-based longitudinal design is a strength in addition to the fact that the women in the cohort had wide ranges of exposures. Also, all women were nonsmokers and reported very low alcohol consumption, reducing such sources of confounding in this cohort. Moreover, in each study, we considered several measures of the outcomes, along with the exposure measurements and evaluated potential mechanisms and susceptibility factors. In addition, we evaluated other exposures, such as cesium, boron and arsenic, also present in the drinking water, and adjusted models accordingly. The lower arsenic exposure during the study period (due to the installation of an arsenic filter) decreased the possibilities of a combined effect of arsenic with other metals. On the contrary, in **Paper II**, we could not elucidate if the associations found between lithium and birth length were a result of lithium exposure exclusively or a consequence of the combined exposure to both lithium and boron. Moreover, in **Paper III**, cesium was also inversely associated with fT3 and T3, but not with TSH nor even with TTR, and the adjustment of the latter models for cesium did not affect the estimates.

Studies in **Papers II-IV** present some limitations. As in all observational studies, our findings are just associations and do not infer causality, and we cannot rule out the possibility of residual or unmeasured confounding. Therefore, we put much emphasis on the mechanistic studies. A drawback of the studies is the lack of measurements of both exposure and outcome markers in the first trimester for all women. The main reason for this was the limited accessibility to reach the far away villages, especially during the rain period (January-March), when the sandy roads often were destroyed, which made it impossible to reach some of the study areas. Also, women in the study area might not report, suspect or confirm their

pregnancies until the 2nd trimester starts, and thus, the chances of recruiting women in the very early gestation were low. Moreover, the wide-spread study area and the study logistics did not allow us to collect samples at the same time of the day, and thus variation in the biomarkers could not be avoided.

Due to the discovered contamination of the blood sampling tubes with lithium, serum lithium measurements could not be used. This could have made the lithium concentrations comparable with those in clinical studies. However, we found a remarkably high correlation between the lithium concentrations in plasma (from non-contaminates tubes) and those in whole blood, validating the use of the lithium concentrations in whole blood as exposure marker.

Although much effort was put into considering potential confounding factors in the design and analyses of each study, remaining or unmeasured confounding could still exist. For example, besides the use of psychiatric medication during pregnancy, the maternal mental health itself is known to influence the fetal growth (Boden et al. 2012; Grigoriadis et al. 2013; Huybrechts et al. 2014). Although none of the women reported any psychiatric diagnoses or psychiatric medication in response to our questions about mental health, we cannot completely rule out the possibility of underreporting or recall bias.

The study area is located at high altitude with low atmospheric oxygen concentrations. This causes stress on pregnancy and decreases the fertility rate, and it may have certain effect on the thyroid function (Kametas et al. 2004; Sarne 2000; Soria et al. 2013; Vitzthum 2013). Although these effects seem to be less common among indigenous people living at this high altitude for generations than among those with Caucasian influence (Kametas et al. 2004; Soria et al. 2013; Vitzthum 2013) and most women in our study reported to belong to indigenous communities, mainly Kolla, we did not have specific markers to control for ethnicity. Therefore, we cannot rule out the involvement of ethnicity in the findings of lithium with maternal and fetal health.

We did not measure TBG, which increases in early pregnancy and thereby, alters the T3 levels (Lockitch 1993). Nonetheless, this should have further increased the variation in the T3 measurements, decreasing the possibilities of finding associations with lithium or cesium. Also, DBP and various genetic polymorphisms were not measured, that could have affected the vitamin D_3 and PTH concentrations, and thereby masked potential associations.

Although the sample size in Papers II-IV is much larger than that in Paper I, the cohort is still relatively small and we lacked statistical power to perform certain analyses such as stratifications by urinary iodine and infant sex, which might have revealed additional potential mechanisms, or by categories of exposure (high boron *vs.* low lithium, etc). Thus, a larger sample size in this study could have facilitated the interpretation of the results. However, only about 200 women get pregnant every year in the study area and we did recruit 88% of all pregnant women during October 2012 and December 2013. Also, there is limited access and transportation to the surrounding villages, a problem that is still worse in the rain period and that challenged the field work of the study. Indeed, the findings of this thesis need to be followed-up and hopefully this breakthrough pilot study will stimulate the performance of similar studies in larger cohorts and in other populations exposed to lithium through drinking water.

6 CONCLUSIONS

Taken together, the results of this thesis provide evidence that elevated exposure to lithium through drinking water during pregnancy may adversely affect prenatal development, possibly through endocrine disruption. In particular, we found that:

- Lithium was easily transferred through the placenta, giving rise to considerable exposure to lithium during fetal life, a potential susceptible window of toxic exposure. The transport through the mammary gland was much lower and breast-feeding seemed to provide some protection of the infants against lithium exposure.

- Lithium exposure through drinking water during pregnancy was associated with shorter birth length.

- Lithium exposure was associated with impaired thyroid function. Maternal blood lithium concentrations during pregnancy were positively associated with thyrotropin and inversely associated with the active thyroid hormones (fT3 and T3) as well as with transthyretin, a transporter of thyroid hormones over e.g. the placenta.

- Lithium exposure was also associated with altered calcium homeostasis. Blood lithium concentrations were inversely associated with plasma 25(OH)vitamin D_3 and with almost 5 times higher odds of having vitamin D_3 concentrations <30 nmol/L during pregnancy.

If confirmed in other and larger studies, the findings have implications for the public health and reinforce the need for thorough screening of lithium concentrations in drinking and bottled water and further evaluation of the health effects.

7 FUTURE RESEARCH

Future efforts should focus on the following:

- Determination of lithium concentrations in drinking water, including bottled water, worldwide in order to identify highly exposed individuals and populations.
- Follow-up of the children of the presently studied women, to elucidate potential effects of the prenatal lithium exposure on infant growth and other potential adverse effects, including e.g. impairment of the thyroid and calcium homeostases, as well as epigenetic alterations.
- Investigation of health effects in the infants due to the continued elevated exposure to lithium after weaning.
- Investigation of fetal growth and development in relation to lithium exposure in several and larger mother-child cohorts, to confirm the findings of the present thesis and to facilitate the elaboration of risk assessments for lithium and the decision upon health-based guideline values for drinking and bottled water.
- Elucidation of other mechanisms underlying the early-life toxicity of lithium.
- Identification of susceptibility factors (e.g. polymorphisms in genes related to lithium or to vitamin D kinetics, nutritional status, epigenetic mechanisms) that could enhance the risk for lithium toxicity.
- Consideration of additional outcome markers such as vitamin D-binding protein, 1-apha-hydroxilase and CYP24A1 activity, and thyroid-binding protein.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Litium är en metall som förekommer naturligt i miljön, inklusive dricksvatten i varierande halter. Trots att det finns rapporterat att mineralvatten på flaska kan innehålla höga halter litium, så analyseras det ytterst sällan i dricksvatten. De få studier som finns visar att dricksvatten i visa områden i Österrike, Chile och Argentina kan innehålla upp till ett par tusen mikrogram/liter, medan i andra länder, så som Italien, Grekland, Japan och England verkar koncentrationerna vara betydligt lägre. Bara Ryssland och Ukraina har nationellt gränsvärde för litium i dricksvatten.

Litium används bland annat för att tillverka batterier och glas men också i medicinering mot bipolär (manodepressiv) sjukdom. Litiumbehandlingen har setts kunna ge bieffekter i form av påverkad funktion av sköldkörteln och njurarna, samt störa kalciumomsättningen i kroppen. Under graviditet är litiumbehandling problematisk och riktlinjen är att så långt som möjligt undvika medicinering i början av graviditeten. Man vet från kliniska och experimentella studier att litiumbehandling kan påverka fostertillväxten samt orsaka missbildningar. Vi vet dock ytterst lite om litiumexponeringen från dricksvatten och om den kan påverka hälsan; det finns bara en enda studie som indikerar påverkan på sköldkörtelfunktionen.

Huvudsyftet med denna avhandling var att undersöka potentiella hälsoeffekter av litiumexponering under graviditeten hos kvinnor och deras barn. Vi studerade framför allt överföringen av litium till fostret genom moderkakan (placentan) och till det ammade barnet genom bröstmjölken, samt påverkan på fostertillväxt. För att utreda eventuella orsaker till en antydd fosterpåverkan, så undersökte vi även om litiumexponering under graviditet kan störa sköldkörteln och kalciumomsättningen hos mödrarna.

För fem år sedan rapporterade vår forskargrupp att dricksvattnet i San Antonio de los Cobres, ett område i Anderna i norra Argentina, innehöll höga halter litium, förutom höga halter arsenik, vilket var känt sedan tidigare. Av denna anledning analyserade vi litium i prover som insamlats i detta område under 1996 från en liten mor-barn kohort (11 kvinnor och deras barn). Litiumhalterna i navelsträngsblod befanns vara minst lika höga som de i mammas blod, vilket indikerar att litium passerar över moderkakan till fostret. Litiumhalterna i bröstmjölken var däremot betydligt lägre, vilket tyder på att amningen delvis skyddar barnen mot de höga litiumnivåerna i dricksvattnet.

För att försöka klargöra eventuella effekter av denna exponering på kvinnorna och deras barn, rekryterade vi en större mor-barn kohort i samma by samt i ett antal närliggande byar. Vi bjöd

in samtliga kvinnor som blev gravida från oktober 2012 till december 2013 att delta. Totalt rekryterades 180 kvinnor (88% svarsfrekvens). Vi intervjuade kvinnorna under 1 till 3 tillfällen under graviditeten och tog blod-, urin- och dricksvattenprover för analys av litium. I blod mättes också olika markörer för sköldkörtelfunktion och kalciumomsättning. Fosterstorlek mättes med hjälp av ultraljud under graviditeten, samt vikt, längd och huvudomfång vid födseln.

De statistiska analyserna visade att det fanns ett samband mellan en högre litiumexponering via dricksvatten och lägre fostertillväxt. På motsvarande sätt fann vi samband mellan litiumexponering och förändrade halter av olika sköldkörtelhormoner hos mamman, vilket antyder att litium kan störa sköldkörtelfunktionen under graviditet. Vidare fann vi samband mellan litiumexponeringen och vitamin D-nivåerna hos mamman som kan betyda att litium sänker vitamin D-nivån.

Sammanfattningsvis så bidrar denna avhandling med ny kunskap om hälsoeffekter av litiumexponeringen via dricksvatten. Resultaten visar att en exponering motsvararande mindre än 1 procent av den vid litiumbehandling kan påverka fostrets tillväxt negativt. Då både sköldkörtelhormoner och hormonet vitamin D är nödvändiga för normal fostertillväxt och utveckling, kan det indikerade sambandet mellan litium och dessa hormoner vara en möjlig förklaring till påverkan på fostertillväxten. Fynden bör beläggas i större studier i andra populationer, men de kan visa sig vara viktiga ur folkhälsosynpunkten och belyser behovet av analyser av litium i både dricksvatten och mineralvatten på flaska.

9 RESUMEN CIENTÍFICO POPULAR

Litio es un metal que está presente naturalmente en el ambiente, incluso en el agua potable en concentraciones variables. A pesar de que hay reportes que indican que agua embotellada puede contener altas concentraciones de litio, este metal es rara vez analizado en el agua potable. Los pocos estudios disponibles muestran que el agua potable en ciertas regiones en Austria, Chile y Argentina contiene hasta 2 miligramos/litro, mientras que el agua potable en países como Italia, Grecia, Japón e Inglaterra parece tener concentraciones mucho más bajas. Sólo Rusia y Ucrania tienen valores límite para litio en el agua potable a nivel nacional.

Litio se utiliza, entre otras cosas, para fabricar baterías y vidrio pero también para tratar la enfermedad bipolar. Se ha visto que el tratamiento con litio puede ocasionar efectos adversos a la salud tales como afección de la función tiroidea y renal, así como trastornos en el metabolismo del calcio. Durante el embarazo, el tratamiento con litio es problemático y guías clínicas recomiendan que se evite, tanto como sea posible, la medicación con litio en las primeras semanas de embarazo. Se sabe, por estudios clínicos y experimentales, que la exposición a litio puede afectar el crecimiento fetal y causar malformaciones congénitas. Sin embargo, se sabe muy poco sobre la exposición a litio en el agua potable y sus efectos a la salud; existe sólo un estudio y éste indica efectos adversos en la función tiroidea.

El objetivo principal de esta tesis fue investigar potenciales efectos sobre la salud en mujeres y sus hijos debido a la exposición a litio durante el embarazo. Particularmente, estudiamos el traspaso de litio al feto a través de la placenta y a los niños a través de la leche materna, así como sus efectos en el crecimiento fetal. Para explorar causas potenciales de la afección del crecimiento fetal, investigamos también si la exposición a litio puede ocasionar alteraciones de la función tiroidea y del metabolismo del calcio en las mujeres embarazadas.

Hace 5 años, nuestro grupo de investigación reportó que el agua de San Antonio de los Cobres, un área en los Andes en el norte Argentino, contiene altas concentraciones de litio, además del arsénico que ha sido estudiado previamente. Por esta razón, analizamos litio en muestras recolectadas en esta área en 1996, de una cohorte pequeña de madres y niños (11 mujeres y sus hijos). Las concentraciones de litio en sangre del cordón umbilical fueron iguales o más altas que aquellas en sangre materna, lo cual indica que el litio traspasa la placenta hacia el feto. Por el contrario, las concentraciones de litio en la leche materna fueron mucho más bajas, lo cual sugiere que el amamantamiento exclusivo protege, al menos parcialmente, a los niños de la exposición a concentraciones altas de litio en el agua potable.

Para tratar de elucidar potenciales efectos sobre la salud debido a esta exposición en las mujeres embarazadas y sus hijos, reclutamos una cohorte de madres y niños más grande en la misma área y en áreas aledañas. Nosotros invitamos a participar en el estudio a todas las mujeres que estuvieron embarazadas desde octubre del 2012 hasta diciembre del 2013. En total reclutamos 180 mujeres (tasa de participación del 88%). Entrevistamos a las mujeres de 1 a 3 veces durante el embarazo y recolectamos muestras de sangre, orina y agua potable en las cuales se analizó litio. En sangre se analizó también marcadores de función tiroidea y del metabolismo del calcio. El tamaño fetal se midió a través de ultrasonido (ecografía) durante el embarazo, y también se midió peso, talla y perímetro cefálico al nacer.

Los análisis estadísticos mostraron asociaciones entre la exposición a litio a través del agua potable y un tamaño fetal más pequeño. De la misma manera, encontramos asociaciones entre la exposición a litio y alteración de las concentraciones de las hormonas tiroideas maternas durante el embarazo, lo cual indica que el litio puede afectar la función tiroidea durante el embarazo. Además, encontramos asociaciones entre la exposición a litio y las concentraciones de la vitamina D en las mujeres, lo cual sugiere que el litio parece disminuir las concentraciones de la vitamina D.

En resumen, esta tesis contribuye con conocimiento sobre efectos a la salud de la exposición a litio a través del agua potable. Los resultados muestran que la exposición, correspondiente a menos del 1% de aquella usada para medicar, podría afectar negativamente el crecimiento fetal. La función tiroidea así como la vitamina D son esenciales para el desarrollo y crecimiento fetal normal, por lo cual, las asociaciones de litio con estas hormonas podrían ser una explicación potencial de la afección del crecimiento fetal. Los hallazgos necesitan ser repetidos en cohortes más grandes y en diferentes poblaciones, pero de cualquier manera, los resultados son importantes desde el punto de vista de la salud pública y recalcan la necesidad de realizar mediciones de litio en agua potable y embotellada.

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