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Elevated arsenic exposure and efficient arsenic metabolism in indigenous women around Lake Poopó, Bolivia

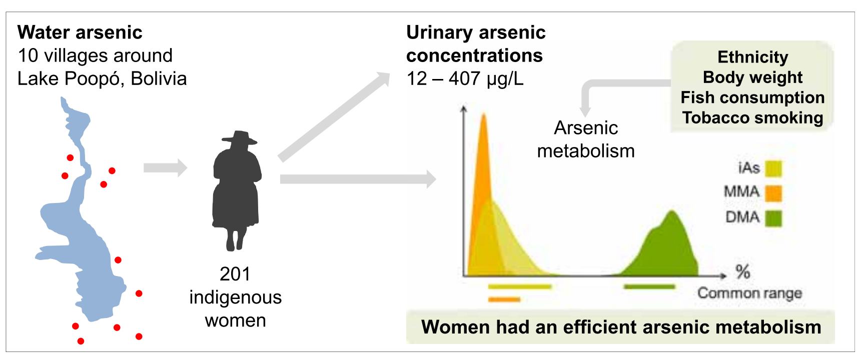
Jessica De Loma [a], Noemi Tirado [b], Franz Ascui [c], Michael Levi [a], Marie Vahter [a], Karin Broberg [a,*], Jacques Gardon [d]

[a] Institute of Environmental Medicine, Unit of Metals and Health, Karolinska Institutet, Stockholm, Sweden
[b] Genetics Institute, Genotoxicology Unit, Universidad Mayor de San Andrés, La Paz, Bolivia
[c] Programa de Salud Familiar Comunitaria e Intercultural (SAFCI), Ministerio de Salud Bolivia, Bolivia
[d] Hydrosciences Montpellier, Institut de Recherche pour le Développement, CNRS, University of Montpellier, France

HIGHLIGHTS

- Studies on human exposure to arsenic (As) in Bolivia are limited.
- Women living around Lake Poopó had a wide range of urinary As (12–407 μg/L).
- Women presented an efficient As metabolism with low %MMA and high %DMA in urine.
- Ethnicity was a major determinant for As metabolism efficiency.

GRAPHICAL ABSTRACT



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ABSTRACT

Elevated concentrations of inorganic arsenic, one of the most potent environmental toxicants and carcinogens, have been detected in well water around Lake Poopó, Bolivia. This study aimed to assess human exposure to arsenic in villages around Lake Poopó, and also to elucidate whether the metabolism and detoxification of arsenic in this population is as efficient as previously indicated in other Andean areas. We recruited 201 women from 10 villages around Lake Poopó. Arsenic exposure was determined as the sum concentration of arsenic metabolites (inorganic arsenic; monomethylarsonic acid, MMA; and dimethylarsinic acid, DMA) in urine (U-As), measured by HPLC-HG-ICP-MS. Efficiency of arsenic metabolism was assessed by the relative fractions of the urinary metabolites. The women had a wide variation in U-As (range 12–407 μg/L, median 65 μg/L) and a markedly efficient metabolism of arsenic with low %MMA (median 7.7%, range: 2.2–18%) and high %DMA (80%, range: 54–91%) in urine. In multivariable-adjusted linear regression models, ethnicity (Aymara-Quechua vs. Uru), body weight, fish consumption and tobacco smoking were associated with urinary arsenic metabolite fractions. On average, the Uru women had 2.5 lower % (percentage unit) iAs, 2.2 lower %MMA and 4.7 higher %DMA compared with the Aymara-Quechua women. Our study identified several factors that may predict these women's arsenic methylation capacity, particularly ethnicity. Further studies should focus on mechanisms underlying these differences in arsenic metabolism efficiency, and its importance for the risk of arsenic-related health effects.

Abbreviations: BMI, body mass index; DMA, dimethylarsinic acid; HPLC-HG-ICP-MS, high-performance liquid chromatography online with hydride generation and inductively coupled plasma-mass spectrometry; iAs, inorganic arsenic [sum of As(III) and As(V)]; MMA, monomethylarsonic acid; U-As, urinary arsenic (sum of iAs, MMA and DMA concentrations).

* Corresponding author at: Institute of Environmental Medicine, Unit of Metals and Health, Karolinska Institutet, Box 210, 17177 Stockholm, Sweden.
E-mail address: karin.broberg@ki.se (K. Broberg).

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1. Introduction

Arsenic concentrations in drinking water above the WHO guideline of 10 μg/L affect an estimated number of 140 million people worldwide (Ravenscroft et al., 2009). Arsenic is a potent group 1 carcinogen (IARC, 2012), which has also been associated with nephrotoxicity, diabetes, and cardiovascular, pulmonary and skin diseases (Minatel et al., 2018).

In Latin America, elevated water arsenic concentrations, usually from natural-occurring volcanic sources, have been described in 14 out of 20 Latin American countries, and >10 million people are estimated to be exposed to arsenic (Bundschuh et al., 2012). In Argentina and Chile, human exposure to inorganic arsenic in arsenic-rich areas are rather well-defined (Concha et al., 1998; Francisca and Carro Pérez, 2009; Nicolli et al., 2010; Smith et al., 2018; Vahter et al., 1995), whereas the situation in most other Latin American countries, is still unknown.

In the area around Lake Poopó, situated in the Andean highlands of Bolivia, arsenic concentrations between 5.2 and 250 μg/L in ground and surface water have been reported (Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012). Still, the actual human exposure to arsenic of people living in this area has not been studied. In fact, very limited studies have described human arsenic exposure in Bolivia (Archer et al., 2005; Smolders et al., 2006).

The capacity to metabolize arsenic differs between individuals and populations, and this variation matters for arsenic toxicity (Ameer et al., 2016; Engström et al., 2015; Lindberg et al., 2008b; Vahter, 2002; Vahter and Concha, 2001). Arsenic is methylated, via the addition of methyl groups in the one-carbon cycle, into monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are excreted in urine (Vahter, 2002). Generally, high fractions of MMA and remaining, non-methylated inorganic arsenic in urine are associated with higher risk for arsenic-related adverse health effects (Dulout et al., 1996; Huang et al., 2009; Kuo et al., 2015; Lindberg et al., 2008b; Steinmaus et al., 2006; Tseng et al., 2005), indicating that a more efficient metabolism (high fractions of DMA) is beneficial for the excretion and protection of the body against arsenic (Vahter, 2007). Importantly, in previous studies of northern Argentinean Andean communities, indigenous people had a particularly efficient metabolism of inorganic arsenic (Vahter et al., 1995). Recently it was showed that, as a consequence of multiple generations living in arsenic-rich areas, women from that region had been selected towards a more efficient arsenic-detoxifying phenotype (Schlebusch et al., 2015, 2013). This finding highlights the need to extend these studies towards other Andean areas to investigate if this phenomenon occurred in parallel in other indigenous populations. Therefore, the aim of this study was to characterize human exposure to arsenic and its metabolism in areas previously reported to have elevated water arsenic, focusing on indigenous communities surrounding Lake Poopó.

2. Materials and methods

2.1. Study area and participants

Lake Poopó is situated approximately 300 km southeast of La Paz, in the southern part of the Andean Plateau in Bolivia (17S–20S; 66W–68W) with a mean elevation of 3686 m above sea level. The lake is a shallow saline lake; once the second largest water body in Bolivia, but declared in emergency state in 2015 due to global warming and increased use of water for irrigation (Satgé et al., 2017).

Villages in the study were initially selected based on previous literature describing elevated arsenic concentrations in water sources of the area (Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012) and based on an initial screening of arsenic concentrations in drinking water in the villages, carried out with an on-site quick test kit (MQuant, Merck, Darmstadt. Germany). In addition, water samples from the major sources of drinking water were collected in each village, mainly from taps, wells or tanks (Fig. 1).

Fig. 1. Photograph of a drinking water well in San Felipe de Chaytavi. (Photo taken in March 2017 by Jessica De Loma.)

The 10 villages included (all belonging to the Oruro Department) were: Poopó, Pampa Aullagas, Villañeque, Llapallapani, Bengal Vinto, Santuario de Quillacas, Sevaruyo, Puñaca, Crucero Belén and San Felipe de Chaytavi (Fig. 2). In order to inform about the water arsenic problem and the current project, we organized meetings before and during recruitment with the assistance of the health authorities, local authorities and medical professionals. The recruited women were a convenience sample from the study area. Women were informed about the project and invited to participate through direct information by the local medical personnel at the health centers or by personal visits to the women's houses, or via local television announcement. Men were not included since they were often away from the village for work and likely had a different exposure to arsenic. Between September 2015 and November 2017 five field trips were organized and a total of 201 apparently healthy women (one excluded due to having a brain tumor and tuberculosis) were recruited on a voluntary basis from multiple small communities surrounding Lake Poopó.

The study group included individuals from two different indigenous communities: Uru and Aymara-Quechua. The Uru communities around Lake Poopó (Uru-Murato) have historically been isolated and excluded from the other surrounding villages (de la Barra Saavedra et al., 2011), making it feasible to assess ethnicity based on location of residence. Individuals living in the southern villages of Llapallapani, Villañeque and Puñaca (and an Uru minority originally from Puñaca living in Poopó) were of Uru-Murato descent, while the remaining participants were of Aymara-Quechua descent. The Aymara-Quechua population is much larger than the Uru population, which explains the difference in sample size between ethnic groups despite the fact that we included all Uru villages around Lake Poopó. Both groups mainly depend on agricultural activities and trading of handcrafts. Traditionally, the Uru people were dependent on fishing activities, but this was not the case at the time of recruitment, due to the exceptional dry period mentioned above.

The study was approved by the Comité Nacional de Bioética in Bolivia, and by the Regional Ethic Committee in Stockholm, Karolinska Institutet. Prior to recruitment and sampling, oral and written description of the project was given to the participants, and written informed consent was obtained.

2.2. Anthropometric and questionnaire data

The women's body weight and height were measured at the local health centers during the field trips. Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters. The study participants were interviewed in Spanish about age, parity, time

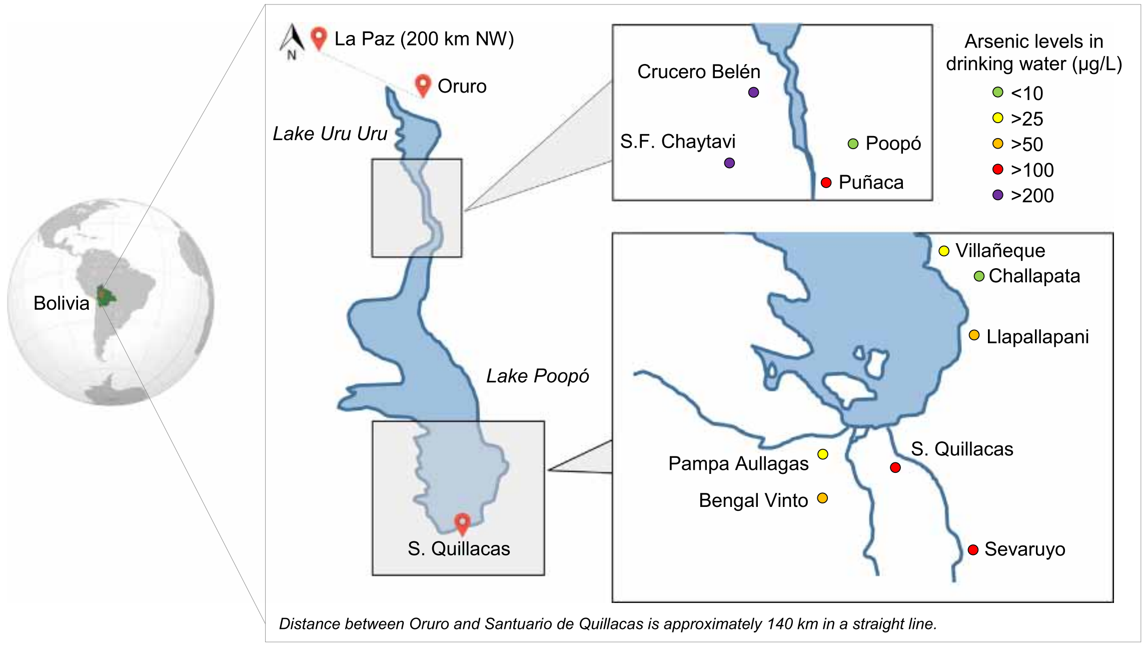

Fig. 2. Map of villages around Lake Poopó included in the study and mean arsenic levels (μg/L) in drinking water from the sources sampled.

and place of residence, family origin, water sources and type of water consumed, food consumption and dietary habits, tobacco smoking, alcohol consumption, coca chewing, and personal and family history of diseases. We assessed ethnicity based on reported birthplace, complemented with information of the residency of the participant's parents and grandparents.

2.3. Water and urine sampling

Water, from multiple sources in each village, and spot urine samples were collected in 20 mL polyethylene bottles, previously checked for trace element leakage. Whenever water was collected from a tap, the water was flushed for about a minute before sampling. The women were given instructions on wet wipe cleaning and mid-stream urine collection to minimize sample contamination. Urine sticks (Combur-7 Test strips, Roche, Basel, Switzerland) were used to measure urinary pH, glucose, ketones, leucocytes, nitrites and protein immediately after sampling. Hemoglobin measurements were performed in venous blood using HemoCue201+ (HemoCue, Ängelholm, Sweden) at the study sites. Results regarding hemoglobin concentration, presence of glucose in urine and indication of urinary tract infection were reported on the spot to the participants.

Urine and water samples were stored at −18 °C during each field trip (generally 3 days long) in a portable freezer (ARB, Alice Springs, Australia). Samples were then transported to the Genetics Institute at Universidad Mayor de San Andrés (La Paz, Bolivia) and stored at −20 °C until further shipment. Samples were transported on dry ice to Karolinska Institutet (Stockholm, Sweden), where all further analyses took place. Upon arrival, the water samples were aliquoted and HNO_3 1:200 (67% w/w, NORMATOM Ultrapure for trace metal analysis, VWR Chemicals, Philadelphia, US) was added to avoid precipitation of metals. Water and urine samples were analyzed within 3 months from collection and the results were reported back to the health authorities and municipalities in Bolivia.

2.4. Arsenic (and additional elements) determination in water and urine

We measured total arsenic in drinking water and urine, as well as the concentrations of lithium, boron, strontium and cesium, as those elements have previously been reported to be elevated in water in the Andes Mountains, including in Bolivia (Concha et al., 2010; Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012). All trace element concentrations were measured by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7700×, Agilent Technologies, Waldbronn, Germany). Samples were thawed at 7 °C overnight and left at room temperature before diluting 10 times with 1% HNO_3 (67% w/w, NORMATOM Ultrapure for trace metal analysis). All tips and tubes were previously acid-washed to avoid trace element contamination of the samples. The ICP-MS was operated under the conditions described in Supplementary material, Table S1A. To ensure analytical accuracy of the measurements, all runs included suitable commercial reference materials, as described in Supplementary material, Table S2. This table also includes the limits of detection (LOD) and coefficients of variation (CV), based on the average of different runs for arsenic, lithium, boron, strontium and cesium. In general, there was a good agreement between the obtained concentrations and reference values.

Assessment of inorganic arsenic exposure was based on the sum of the concentrations of arsenic metabolites (iAs + MMA + DMA) in urine (referred to as U-As). Prior to the analyses, approximately 1 mL of each urine sample was filtered through a 0.20-μm syringe filter (Sarstedt, Nümbrecht, Germany). The arsenic metabolite concentrations in urine were determined using high-performance liquid chromatography online with hydride generation and ICP-MS (HPLC-HG-ICP-MS) (HPLC: Agilent 1110 series, Agilent Technologies, Waldbronn, Germany; Hamilton Column PRP-X100, Reno, US; ICP-MS: Agilent 7500ce, Agilent Technologies, Waldbronn, Germany), as described in Lindberg et al. (2006) and in Supplementary material, Table S1B. This method is able to separate and detect trivalent [As(III)] and pentavalent [As(V)] forms of inorganic arsenic, as well as MMA and DMA. Because of the rapid oxidation of As(III) to As(V), we used only iAs [sum of As(III) and As(V)] in the further evaluations. Arsenic methylation efficiency was evaluated based on the relative concentrations (%) of the different arsenic metabolites in urine (Vahter, 2002).

The LODs, CVs and suitable reference materials for arsenic speciation are presented in Supplementary material, Table S3. The obtained concentrations of methylated metabolites in the reference materials were in agreement with the reference values. Regarding the concentrations of As(III) and As(V) in the reference materials, the obtained values were not in agreement with the reference concentrations, due to oxidation, but the values of the sum of inorganic forms were in accordance.

Also, there was an excellent correlation between U-As (as the sum of inorganic metabolite concentrations by HPLC-HG-ICP-MS) and the total arsenic concentration in urine measured directly by ICP-MS ($r_S = 0.985$, $p < 0.001$; Supplementary material, Fig. S1), supporting accurate analyses.

To compensate for variations in the dilution of urine, all urinary concentrations were adjusted to the mean urinary osmolality of the total study group (727 mOsm/kg; range 129–1226). Urinary osmolality was measured by a digital cryoscopic osmometer (OSMOMAT 030, Gonotec, Berlin, Germany). We also measured specific gravity (mean 1.02; range 1.004–1.052) using a digital refractometer (RD712 clinical refractometer, EUROMEX, Arnhem, the Netherlands), as it was previously shown to be more useful for urinary arsenic than creatinine excretion (Nermell et al., 2008). In this study group specific gravity and osmolality were highly correlated ($r_S = 0.987$, p-value < 0.001, $n = 201$; Supplementary material, Fig. S2). However, adjustment to osmolality was chosen throughout this study since it is less affected by urinary protein and glucose (Parikh et al., 2002), which some of these women had.

2.5. Statistics

All statistics were done in RStudio Version 1.1.383 (R version 3.4.1). Wilcoxon test and z-test of proportions were used initially to assess differences in characteristics between ethnic groups. Spearman correlation tests were performed for: factors possibly influencing arsenic exposure and metabolism, U-As (the sum of metabolites in urine) and the total arsenic (total arsenic in urine, including all organic forms like arsenobetaine), different urinary dilution adjustments, and U-As against arsenic concentrations in water.

Multivariable-adjusted linear regression analyses were used to assess factors predicting the urinary arsenic metabolite fractions, and these predictors were initially selected from the literature. In the final models (the same for all arsenic metabolites to allow for comparisons) we kept the variables that modified the estimate for any of the arsenic metabolites >10% in a backward elimination approach, starting with the less significant variable. The included predictors in the final models were U-As, ethnicity (coded as 0 for Aymara-Quechua and 1 for Uru), age, body weight, fish consumption (yes/no in the questionnaire), meat consumption (number of meals containing meat per month) and tobacco smoking (yes/no in the questionnaire). Despite not modifying any of the effect estimates >10%, U-As was included in the final models to facilitate comparison with the literature. In addition, the Akaike information criterion estimator was calculated for models including or not including U-As. Keeping U-As did not decrease the quality of the model. We did not include hemoglobin level, height, alcohol or coca chewing in the final models as they had no influence on the estimates for arsenic metabolites (data not shown). The distribution of %iAs, %MMA, %DMA and U-As were slightly skewed. However, the linear regression models presented residuals that were not violating the assumptions of the regression analysis, based on the residuals vs. fitted and normal Q-Q scale-location diagnostic plots for regression analysis.

In addition, to assess to which extent different factors could predict U-As, a linear regression model with the same selected predictors as above was carried out with U-As as a dependent variable.

3. Results

3.1. Study participant characteristics

The study group consisted of 201 women living in 10 villages close to Lake Poopó (Fig. 2). Approximately 84% of the women were of Aymara-Quechua ethnicity, the rest being of Uru ethnicity. The Aymara-Quechua women were significantly older and weighed less (Table 1).

The majority of the study participants (95%) reported eating meat at least once a week; the median number of meals including meat per month was 28 (range 0–40). Fish consumption was not as common; 56% of the women reported not eating fish at all (Aymara-Quechuas 58% vs. Urus 48%, $p = 0.43$). It should be noted that during the sampling period, Lake Poopó was undergoing a severe dry period and no fishing activity was taking place, as known from personal communication with the residents. Therefore, we assume most fish was imported. The study participants rarely consumed alcohol; only two women out of the 201 reported consuming alcoholic beverages every other week. Also, only 3% of the women reported smoking tobacco (yes/no), whereas 73% declared chewing coca leaves.

3.2. Arsenic exposure in residents around Lake Poopó

Overall, U-As ranged 12–407 μg/L, adjusted to the mean urinary osmolality of 727 mOsm/kg, with median iAs making up 12%, MMA 7.7% and DMA 80% of U-As. The Aymara-Quechua women had significantly higher U-As, and also higher %MMA and lower %DMA in urine, compared with Uru women (Table 1). There was a wide range of median total urinary arsenic concentrations (including all organic forms) in the different areas around Lake Poopó: from 32 μg/L in Poopó ($n = 4$) to 191 μg/L in Sevaruyo ($n = 30$) (Supplementary Table S4). The villages with the highest total urinary arsenic concentrations were Puñaca (median 101 μg/L, $n = 2$), Villañeque (120 μg/L, $n = 4$), Bengal Vinto (123 μg/L, $n = 11$) and Sevaruyo (191 μg/L, $n = 30$). Also, there was a wide range of total urinary arsenic within each village, for example in Sevaruyo (range: 28–630 μg/L, $n = 30$) and in Santuario de Quillacas (range: 23–298, $n = 58$). Generally, there was a good agreement between U-As (as the sum of inorganic metabolites) and the total arsenic concentration ($r_S = 0.985$, $p < 0.001$; Supplementary material, Fig. S1), indicating that the exposure was mainly to inorganic arsenic.

The water arsenic concentrations varied between the villages and ranged from 3.3 to 571 μg/L (Supplementary material, Table S4). Only As(III) and As(V) were detected in the water samples. Also, villages where Uru women were living had lower arsenic concentrations in water (median 46 μg/L, range 3.3–222 μg/L) compared to the Aymara-Quechua villages (median 130 μg/L, range 25–571 μg/L). Importantly, there was a wide variation within some villages for which we had more than one tested water source; for example, in Santuario de Quillacas the water arsenic concentrations varied between 25 and 176 μg/L. To assess if drinking water was the main source of arsenic exposure for the women, we plotted U-As against the values of water arsenic concentrations sampled in each village (mean value when multiple sources were sampled in one village; Fig. 3). We did not find a strong

Table 1
Characteristics of the studied women around Lake Poopó (total study group and stratified by ethnicity).

Characteristic	Total population	Aymara-Quechua	Uru	p-Value[a]
Number of participants	201	168	33	NA
Age (years)	35 (14–85)	36 (15–85)	30 (14–65)	0.007
Height (cm)	149 (120–162)	149 (120–162)	149 (140–162)	0.386
Body weight (kg)	60 (36–97)	60 (36–89)	67 (47–97)	0.038
BMI (kg/m^2)	27 (16–44)	26 (16–39)	30 (21–44)	0.011
Parity	2 (0–13)	2 (0–12)	2 (0–13)	0.884
Tobacco smoking (yes/no)	3%	3%	3%	>0.999
Hemoglobin (g/dL)	15 (8.2–20)	15 (8.2–19)	15 (11–20)	0.329
U-As (μg/L)	65 (12–407)	66 (12–407)	55 (16–143)	0.016
%iAs	12 (3.2–34)	12 (3.2–34)	10 (4.9–23)	0.257
%MMA	7.7 (2.2–18)	8.1 (2.9–18)	5.9 (2.2–10)	<0.001
%DMA	80 (54–91)	79 (54–91)	84 (68–91)	0.0018

Abbreviations: BMI, body mass index; U-As, sum of arsenic metabolite concentrations in urine adjusted to average osmolality; iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid.
Note: Data are presented as median (minimum–maximum) or percentage (%).
[a] p-Value for Wilcoxon rank-sum (Mann-Whitney) test when comparing medians and for z-test when comparing proportions.

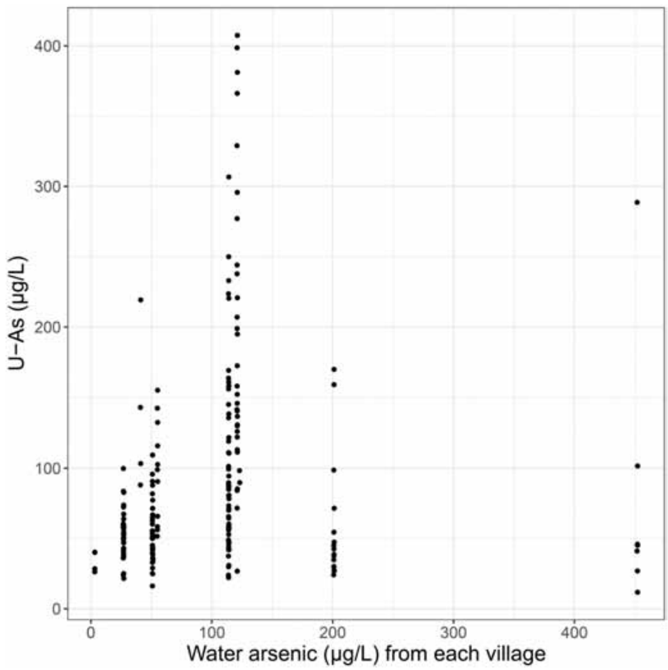

Fig. 3. Scatter plot of urinary arsenic concentrations (U-As, as the sum of arsenic metabolites adjusted to average osmolality, Y-axis) against the arsenic concentration in water in each respective village (X-axis). Whenever more than one drinking water source was sampled per village, the mean concentration is indicated (X-axis), as people often used several sources. The villages are, from left to right: Poopó (3.3 μg/L of arsenic), Pampa Aullagas (27), Villañeque (41), Llapallapani (51), Bengal Vinto (55), S. Quillacas (114), Sevaruyo (121), Puñaca (123), Crucero Belén (201), S.F. de Chaytavi (452).

correlation between U-As and water arsenic concentrations ($r_S = 0.30$, $p < 0.001$). Additionally, we measured arsenic in rice, since it is a source of arsenic in multiple areas around the world (Davis et al., 2017). We collected rice samples from the main markets around Lake Poopó located in Challapata and Crucero Belén, as a proxy of what type of rice our study group ate. The mean total arsenic concentration of 7 different rice samples was 51 ± 21 ng/g of dry rice (range 28–89 ng/g).

In order to characterize the drinking water in relation to results from other Andean areas, including previous work from Lake Poopó (Concha et al., 2010; Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012), we also measured the concentrations of lithium, boron, strontium and cesium in the water and urine samples. The results are presented by village in Supplementary Table S4. Water arsenic correlated moderately with water lithium ($r_S = 0.47$, $p = 0.015$) and water boron ($r_S = 0.57$, $p < 0.001$), and U-As correlated in a similar way with urinary lithium ($r_S = 0.47$, $p < 0.001$) and urinary boron ($r_S = 0.62$, $p < 0.001$). Comparison of element concentrations in water samples collected repeatedly from the same source indicated fairly small temporal variations (Supplementary Table, S5).

3.3. Arsenic metabolism and influencing factors

The U-As was neither correlated with %iAs, %MMA nor %DMA (correlations are presented as a Spearman correlation heat map in Fig. 4). Body weight was inversely correlated with %MMA ($r_S = -0.40$, $p < 0.001$) and positively with %DMA ($r_S = 0.15$, $p = 0.04$). Height was not correlated with any arsenic metabolite fraction.

To further identify factors influencing the capacity to metabolize arsenic, multivariable-adjusted linear regression analyses were performed with each arsenic metabolite fraction as a dependent variable. In the final model (Table 2) ethnicity was associated with %iAs, %MMA and %DMA, and it was the factor with the strongest association with %DMA: on average the Uru women had 2.5 lower % (percentage unit) iAs, 2.2 lower %MMA and 4.7 higher %DMA compared with Aymara-Quechua women. Also, increasing age was weakly associated with lower %iAs, and higher body weight was associated with lower %MMA. Consuming fish (yes/no) was associated with lower %iAs and higher %DMA, whereas tobacco smoking was associated with higher %iAs and %MMA, and correspondingly lower %DMA. The U-As was not associated with any arsenic metabolite and neither was meat consumption. The models explained between 11 and 26% of the variability in the percentages of urinary arsenic metabolites (see R^2 in Table 2).

We also assessed the association of different factors with U-As (Table 2). Because we did not have individual data on water arsenic, this was not included in the model. On average, the Uru ethnicity was associated with 35 μg/L lower U-As compared to Aymara-Quechua ethnicity. Being a smoker was associated with 70 μg/L higher U-As, however, few women smoked and the model explained only 6% of the variation in U-As.

4. Discussion

This study shows an elevated, varying exposure to inorganic arsenic among indigenous women living in villages around Lake Poopó in Bolivia. The found arsenic concentrations in water are consistent with those previously reported around the area (Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012). The studied women presented an efficient and unusual arsenic metabolism, characterized by low %MMA and high %DMA in urine, similar to other indigenous women in the Andes (Engström et al., 2011; Vahter et al., 1995). Also, arsenic exposure (U-As) did not seem to impair the methylation efficiency of arsenic. The main predictors for arsenic methylation efficiency appeared to be ethnicity, body weight, fish intake and tobacco smoking.

4.1. Factors influencing arsenic metabolism

An important finding of the present study was that several factors, including ethnicity, were associated with the urinary arsenic metabolite fractions, a measure of the efficiency of metabolism and, presumably, detoxification of arsenic (Vahter and Concha, 2001). On average, the

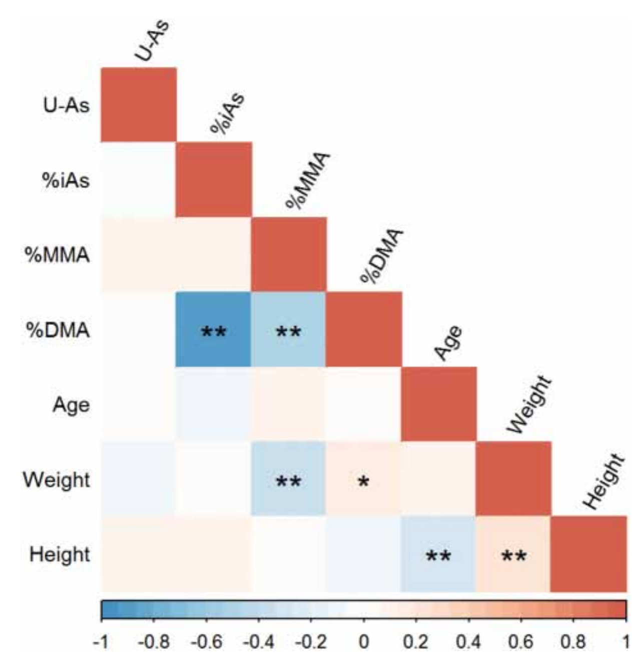

Fig. 4. Heat map of Spearman correlations between arsenic fractions and influencing factors. Two or one asterisks indicate p-values < 0.01 or < 0.05, respectively. Abbreviations and units: U-As, sum of arsenic metabolite concentrations in urine adjusted to average osmolality (μg/L); iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; Age (years); Body weight (kg); Height (cm).

Table 2
Linear regression analyses of factors associated with arsenic metabolite fractions in urine and U-As from women living around Lake Poopó, Bolivia (n = 197[a]).

	%iAs		%MMA		%DMA		U-As	
	B (95% CI)	β	B (95% CI)	β	B (95% CI)	β	B (95% CI)	β
U-As	−0.01 (−0.02, 0.004)	−0.08	0.001 (−0.004, 0.01)	0.02	0.01 (−0.01, 0.02)	0.06	NA	NA
Ethnicity[b]	−2.5* (−4.9, −0.03)	−0.15	−2.2** (−3.3, −1.2)	−0.29	4.7** (2.0, 7.4)	0.26	−35* (−66, −3.4)	−0.17
Age	−0.06* (−0.11, −0.002)	−0.15	0.01 (−0.01, 0.04)	0.07	0.04 (−0.02, 0.10)	0.10	−0.05 (−0.74, 0.65)	−0.01
Body weight	0.05 (−0.03, 0.12)	0.09	−0.08** (−0.11, −0.05)	−0.35	0.04 (−0.04, 0.12)	0.10	−0.08 (−1.0, 0.84)	−0.01
Fish[c]	−3.0** (−4.6, −1.3)	−0.25	0.03 (−0.68, 0.74)	0.01	2.9** (1.1, 4.8)	0.22	−9.3 (−31, 12)	−0.06
Meat (per month)	−0.06 (−0.16, 0.04)	−0.09	−0.04 (−0.08, 0.01)	−0.12	0.10 (−0.01, 0.20)	0.13	0.12 (−1.1, 1.4)	−0.01
Tobacco smoking[c]	4.6 (−0.05, 9.3)	0.14	2.4* (0.39, 4.5)	0.15	−7.1** (−12, −1.9)	−0.18	70* (10, 131)	0.16
R^2	0.109		0.260		0.155		0.062	

Abbreviations and units: iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; U-As, sum of arsenic metabolite concentration in urine adjusted to average osmolality (μg/L); NA, not available; Age (years); Body weight (kg).
Note: Results are presented as B coefficients (95% confidence interval) and β standardized coefficients from multivariable-adjusted linear regression models (*p < 0.05, **p < 0.01).
[a] Four individuals did not report food habits.
[b] Ethnicity coded as 0 for Aymara-Quechua and 1 for Uru.
[c] Information was reported as yes/no in the questionnaire.

Uru women had 2.5 lower % (percentage unit) iAs, 2.2 lower %MMA, and 4.7 higher %DMA, compared to the Aymara-Quechua women, indicating that the Uru population has a more efficient arsenic metabolism. However, also the Aymara-Quechua women appeared to be more efficient in methylating arsenic (median 8.1% MMA and 79% DMA) compared to most other studied populations, which usually have 10–30% iAs, 10–20% MMA and 60–80% DMA in urine (Vahter and Concha, 2001). Previously, indigenous women in the Argentinean Andes were found to have lower %MMA than other studied populations (median 2.2%, range 0.0–11%), indicating a unique efficient arsenic metabolism (Vahter et al., 1995), which later was explained by selection for metabolizing arsenic efficiently during generations living in an arsenic-containing environment (Schlebusch et al., 2015, 2013). However, ethnicity (Hispanic or non-Hispanic) was not associated with arsenic metabolites fractions in studies from southwest US and northern Mexico (Gomez-Rubio et al., 2011). The indicated difference in arsenic exposure between ethnicities in the present study was accounted for by including U-As as a covariate in the regression models, and no other assessed characteristic could explain the different arsenic metabolism efficiency between ethnic groups. Therefore, follow-up analyses are warranted to properly understand if there is a genetic basis for the observed variation in arsenic metabolism between ethnic groups around Lake Poopó.

Other factors appeared to also influence arsenic methylation efficiency. Higher body weight was associated with lower %MMA in urine in the studied women (0.8 lower %MMA in urine for every 10 kg increase in body weight). An association between BMI and efficient arsenic metabolism has previously been observed in Uruguayan children (Kordas et al., 2016), and in several US study groups (Gribble et al., 2013; Hudgens et al., 2016). A hypothesis is that higher body weight reflects higher intake of proteins and methyl groups. In fact, it is known that poor nutritional status and low intake of proteins results in a lower capacity to methylate arsenic (Steinmaus et al., 2005; Vahter and Marafante, 1987). The women around Lake Poopó in Bolivia had a median BMI of 27 kg/m^2 (range: 16–44), but were generally short (half of them were shorter than 149 cm), resulting in higher BMI values. Nevertheless, this is in agreement with other Andean populations in Chile, Argentina and Ecuador (Espinoza-Navarro et al., 2011; Harari et al., 2015; Macdonald et al., 2004).

Also fish consumption was associated with a more efficient arsenic methylation, possibly due to higher intake of proteins from fish. Those who ate fish had significantly higher %DMA (82%) compared to those who did not (79%; p = 0.001). In a US adult study group, individuals with lower protein intake had higher %MMA and lower %DMA (Steinmaus et al., 2005). In addition, seafood intake (mainly bivalves and algae) has been connected with higher exposure to arsenic in the form of DMA, which can translate into higher DMA concentrations in urine (deCastro et al., 2014; Navas-Acien et al., 2011). However, in our study, urinary DMA concentrations were not significantly different between those who reported consuming fish (other seafood were not specified in the questionnaire) and those who did not (51 vs. 50 μg/L DMA, respectively; p = 0.996 Wilcoxon test).

Only 6 out of the 201 studied women reported smoking tobacco, but smoking was associated with markedly higher %iAs and %MMA, and lower %DMA. Similarly, smokers in rural Bangladesh had slightly higher median U-As, higher %MMA and lower %DMA (Lindberg et al., 2010). Smoking may inhibit enzymes participating in arsenic methylation or impair the one-carbon metabolism, since smokers tend to have lower levels of folate and vitamins B6 and B12, and a higher level of homocysteine (O'Callaghan et al., 2002).

Elevated arsenic exposure may inhibit the metabolism from inorganic arsenic to DMA. In vitro studies showed that enzymes involved in arsenic metabolism were inhibited by excess substrate (Buchet and Lauwerys, 1988; Styblo et al., 1996), and epidemiological studies have found that increasing U-As is associated with lower %DMA (Hopenhayn-Rich et al., 1996; Olmos et al., 2015; Skröder Löveborn et al., 2016; Sun et al., 2007). However, this phenomenon was not observed in the present Bolivian study group, neither in the Andean population in Argentina (Vahter et al., 1995), possibly related to the efficient methylation.

4.2. Arsenic exposure and its possible sources

Arsenic concentrations in water in Bolivia have been studied and reviewed in Bundschuh et al. (2012). In the present study we analyzed 21 drinking water sources in 10 villages, selected based on reported data on elevated arsenic concentrations, and found similar concentrations as reported (Ormachea Muñoz et al., 2013; Ramos Ramos et al., 2012). In our study 14 out of 21 wells had arsenic concentrations above the national Bolivian limit of 50 μg/L of arsenic in agricultural water (Bolivian Agricultural Water Standards, 1995), and 20 out of 21 wells had arsenic concentrations above 10 μg/L, the limit for drinking water proposed by WHO. It should be noted though, that our study focused on villages with elevated water arsenic and therefore the results are not representative for the entire area and all individuals living around Lake Poopó.

Despite arsenic in water being a well-described problem in Bolivia, very few studies have focused on human arsenic exposure in this country. Archer et al. (2005) evaluated human arsenic exposure in villages in the upstream areas of Pilcomayo River in the western Andean region of Bolivia. There, drinking water arsenic concentrations ranged 0.2–112 μg/L (all but the highest were below 10 μg/L), and total arsenic in urine (not as the sum of metabolites) ranged 11–891 μg/L. In the present study, the measured water arsenic (average per village when available) was weakly correlated with U-As, indicating that the women used other drinking water sources than those sampled and/or had additional exposure sources. For example, in villages with elevated water arsenic, especially those situated in the northern part of Lake Poopó, people were aware of the presence of arsenic in the drinking water and drank water brought from Oruro, the nearest city, as they informed us during our visits. Further, they also reported in the questionnaires that they consumed water from nearby rivers and rain water.

Another exposure source to arsenic is the diet. As reviewed in Saifullah et al. (2018), rice is one of the most important exposure sources of inorganic arsenic, after drinking water. Based on the measured arsenic concentrations in the present study, a portion of 100 g (dry weight) of rice would contribute about 5 μg of arsenic, indicating a probable low exposure through rice. However, the potential human arsenic exposure from rice and other foodstuff remains to be assessed in Bolivia. In addition, the area near Lake Poopó is very arid and a further possible exposure route is through dust, either deposited in the superficial drinking water sources and on crops, or directly inhaled. Recently, Goix et al. (2016) concluded that 7-year-old children in the mining city of Oruro might be highly exposed to airborne arsenic, based on measured concentrations in dust and aerosols.

4.3. Other elements

Our values for lithium and boron are higher than those reported in Ormachea Muñoz et al. (2013) and Ramos Ramos et al. (2012) around Lake Poopó, but in line with those found in San Antonio de los Cobres, a village in the Argentinean Andes (Concha et al., 2010). The correlations found between arsenic, lithium and boron were similar to those in Concha et al. (2010), probably reflecting a shared geochemistry of the bedrock in the altiplano. In San Antonio de los Cobres, health outcomes such as birth size and thyroid function were associated with lithium and boron exposure, stressing the need for further research on these elements around Lake Poopó (Harari et al., 2015; Igra et al., 2016).

4.4. Additional considerations

A limitation of our study is that the questions about food habits were not part of a validated food-frequency questionnaire or a nutritional evaluation. The diet questions were included with the intention of describing the population's general routines and to analyze if these showed large differences between villages or ethnic groups, which they did not. A further restriction was not having individual water exposure measurements, which was not possible considering the multiple ways drinking water is obtained in the area. Additionally, taking accurate anthropometric measurements in remote villages of the Andes Mountains has inherent challenges. Weighing the women without clothes was not feasible, due to the limited investigation facilities and prevalent low temperatures. Therefore, the women only removed bags, shoes and hats, and we proceeded to use these estimated measurements recognizing the underlying limitations, but assuming a similar weight of the clothes across all women. Lastly, only women were included in the study and therefore, there is a need for further evaluation of potential gender differences, as found by Lindberg et al. (2008a).

The strengths of this study included the assessment of arsenic concentrations, along with speciation of methylated arsenic metabolites, using well-established methods that had low limits of detection (Lindberg et al., 2007). Also, having two indigenous groups that did not show major differences in living conditions and diet, allowed addressing the influence of ethnicity on arsenic metabolism. To access remote, isolated villages and contact study groups with restricted foreign social interactions enabled us to identify potential public health problems related to arsenic exposure.

5. Conclusions

This is the most comprehensive study so far regarding arsenic exposure in humans around Lake Poopó, showing a wide variation in exposure to inorganic arsenic. Further, it is the first documentation of an efficient and unusual arsenic metabolism in indigenous people in Bolivia, and we identified several factors that may predict their arsenic methylation, particularly ethnicity (Aymara-Quechua vs. Uru), but also body weight, fish consumption and tobacco smoking. Generally, Uru women showed a more efficient metabolism compared with Aymara-Quechua women. To what extent this will affect susceptibility to arsenic toxicity remains to be elucidated. This study warrants further analyses to clarify the underlying mechanisms behind the indicated ethnic differences in arsenic metabolism, and it emphasizes the need for solutions for water security and proper arsenic exposure assessment in this area.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.11.473.

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