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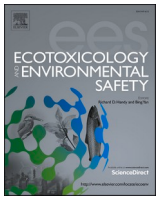

# Arsenic exposure and biomarkers for oxidative stress and telomere length in indigenous populations in Bolivia

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ABSTRACT

*Background:* Women living in the Bolivian Andes are environmentally exposed to arsenic, yet there is scarce information about arsenic-related effects in this region. Several biomarkers for telomere length and oxidative stress (mitochondrial DNA copy number, mtDNAcn; 8-Oxo-2′-deoxyguanosine, 8-oxo-dG; and 4-hydroxy nonenal mercapturic acid, 4-HNE-MA) have been previously linked to arsenic, and some of which are prospective biomarkers for cancer risk.

*Objective and hypothesis:* To evaluate associations between arsenic exposure and telomere length, mtDNAcn, 8-oxo-dG, and 4-HNE-MA in Bolivians. Arsenic exposure was hypothesized to be positively associated with all four toxicity biomarkers, particularly in individuals with a less efficient arsenic metabolism.

*Methods:* The study encompassed 193 indigenous women. Arsenic exposure was assessed in urine as the sum of inorganic arsenic metabolite concentrations (U-As) measured by HPLC-HG-ICP-MS, and in whole blood as total arsenic (B-As) measured by ICP-MS. Efficiency of arsenic metabolism was evaluated by a polymorphism (rs3740393) in the main arsenic methylating gene *AS3MT* measured by TaqMan allelic discrimination, and by the relative fractions of urinary inorganic arsenic metabolites. Telomere length and mtDNAcn were determined in peripheral blood leukocytes by quantitative PCR, and urinary 8-oxo-dG and 4-HNE-MA by LC-MS/MS.

*Results:* U-As and B-As were associated with longer telomeres and higher mtDNAcn, particularly in women with a less efficient arsenic metabolism. Urinary 8-oxo-dG and 4-HNE-MA were positively associated with U-As, but only 4-HNE-MA was associated with B-As. Arsenic metabolism efficiency did not have a clear effect on the concentrations of either of these biomarkers.

*Conclusion:* Bolivian women showed indications of arsenic toxicity, measured by four different biomarkers. Telomere length, mtDNAcn, and 4-HNE-MA were positively associated with both U-As and B-As. The association of arsenic exposure with telomere length and mtDNAcn was only present in Bolivian women with a less efficient metabolism. These findings call for additional efforts to evaluate and reduce arsenic exposure in Bolivia.

## 1. Introduction

Arsenic concentrations in drinking water above the World Health Organization guidance value (10 μg/L) are widespread in Latin American countries (Khan et al., 2020). We recently investigated environmental arsenic exposure in indigenous women around Lake Poopó in the Bolivian Andes. Women from this area presented a wide variation in inorganic arsenic (iAs) exposure and an efficient arsenic metabolism (De

*Abbreviations:* AS3MT, arsenite methyltransferase; B-As, blood arsenic; DMA, dimethylarsinic acid; 8-oxo-dG, 8-oxo-2′-deoxyguanosine; 4-HNE-MA, 4-hydroxy nonenal mercapturic acid; HBB, hemoglobin beta; 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)]; LC-MS/MS,, liquid chromatography coupled with tandem mass spectrometry; LOD, limit of detection; MMA, monomethylarsonic acid; mtDNAcn, relative mitochondrial DNA copy number; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; TL, relative telomere length; U-As, urinary arsenic (sum of iAs, MMA, and DMA concentrations).

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Loma et al., 2019). While epidemiological data on arsenic exposure in Bolivia is emerging, there is yet a need to understand the health effects of arsenic exposure in this region.

Arsenic in humans is methylated into monomethylarsonic acid (MMA) and further into dimethylarsinic acid (DMA), which are excreted in urine in combination with unmethylated iAs. Arsenic metabolism in the human body is seen both as a detoxification and a bioactivation process. A higher fraction of MMA in urine is associated with more arsenic-related health effects (Lindberg et al., 2008), whereas a higher fraction of DMA in urine is associated with a lower retention in the body (Vahter, 2002). Thus, arsenic metabolism is a susceptibility factor for arsenic toxicity. AS3MT is the main arsenic methylating enzyme in humans (Pierce et al., 2012; Schlebusch et al., 2015). Variants in the *AS3MT* gene have been identified across populations as predictors of arsenic metabolism efficiency and can be used to assess the susceptibility to arsenic toxicity (Drobná et al., 2013; Engström et al., 2011).

Chronic arsenic exposure via drinking water in adults is associated with several types of cancer, such as lung, skin, and bladder cancers (Palma-Lara et al., 2020). Arsenic toxicity is not pinned down to one molecular mechanism of action. Instead, several mechanisms have been identified, including its interaction with critical sulfhydryl groups in enzymes, oxidative stress, epigenetic alterations, mitochondrial dysfunction, reduction of DNA repair capacity, and modification of telomeres (Bhattacharjee et al., 2013; Hubaux et al., 2013).

In a quest to understand arsenic toxicity in chronically exposed populations from the Bolivian Andes, we measured four toxicity biomarkers. The criteria for biomarkers of toxicity were that they were related to oxidative stress or telomere function, two main mechanisms of action for arsenic toxicity. Thus, we measured: (1) length of telomeres, nucleotide repeats at the end of eukaryotic chromosomes, where longer telomeres in peripheral blood are associated with increased risk of lung, skin or bladder cancer among others (Haycock et al., 2017); (2) mitochondrial DNA copy number (mtDNAcn), which alters in response to oxidative stress (Lee et al., 2013), and is, for example, increased in lung tumors compared to control tissues (Reznik et al., 2016); (3) 8-oxo-2′-deoxyguanosine (8-oxo-dG), a biomarker of DNA oxidative damage (Loft and Møller, 2006); and (4) 4-hydroxy nonenal mercapturic acid (4-HNE-MA), a urinary metabolite product of lipid peroxidation in relation to oxidative stress (Dalleau et al., 2013).

Longer telomeres have been repeatedly associated with arsenic exposure (Ameer et al., 2016; Gao et al., 2015a; Alegría-Torres et al., 2020). Higher mtDNAcn in blood has been identified in individuals highly exposed to arsenic (Sanyal et al., 2018) and particularly in individuals with a less efficient arsenic metabolism capacity (Ameer et al., 2016). Higher urinary 8-oxo-dG in relation to increasing urinary arsenic concentrations has been extensively described (Breton et al., 2007; Chou et al., 2014; Engström et al., 2010a, 2010b). To our knowledge, only two studies have evaluated the relationship between arsenic exposure and urinary 4-HNE-MA: one small study including Polish individuals living near copper smelting areas that showed higher HNE urinary metabolites in men highly exposed to arsenic (mean urinary total arsenic 152 μg/L) compared to those with lower exposure (17 μg/L), but not in women (Kozłowska et al., 2019); and one with Chinese men that found a positive association between urinary 4-HNE-MA and total urinary arsenic (environmental exposure, median 34 μg/L) (He et al., 2020).

The aim of this study was to evaluate the associations between arsenic exposure and a set of toxicity biomarkers related to telomere length and oxidative stress in Bolivian indigenous communities from the Andes chronically exposed to arsenic (De Loma et al., 2019). In addition, we studied how arsenic metabolism efficiency modified these associations. Our hypothesis was that arsenic exposure was positively associated with the four toxicity biomarkers, and that these associations would be stronger in individuals with an *AS3MT* genotype associated with higher %MMA, i.e., with a less efficient arsenic metabolism.

## 2. Materials and methods

### 2.1. Study participants

Between September 2015 and November 2017, 201 women were recruited from ten rural villages around Lake Poopó, in the Bolivian Andes Mountains at approximately 3700 m above sea level. Inhabitants of these villages belong to the two main ethnic groups in the Bolivian highlands: Aymara-Quechua and Uru (De Loma et al., 2019). Women were recruited on a voluntary basis as a convenience sample. Men were not included since they worked mostly outside of the village, likely resulting in a different pattern of arsenic exposure. Out of the 201 women, five did not have metal exposure data and three did not have toxicity biomarker data due to lack of blood and/or urine sample, resulting in a total of 193 participants for this study. We previously described that these women were exposed to arsenic mainly through drinking water and had a markedly efficient arsenic metabolism capacity (De Loma et al., 2019). While most of the individuals living in these communities reported drinking water from nearby wells, some reported drinking from rivers and rainwater due to the water scarcity in this arid region. The arsenic concentration in drinking water in the study area ranged between 3.3 and 571 μg/L (De Loma et al., 2019). The work was approved by the Comité Nacional de Bioética (Bolivia; Ref: CNB-CEI03/2018, Resolución Nro. 2–2018) and the Regional Ethic Committee of Karolinska Institutet (Sweden; Ref: 2016/36–31/4). Participants received written and oral information about the project before the recruitment, and they gave their signed informed consent to participate.

### 2.2. Data and sample collection

Upon recruitment, women were interviewed about their age, ethnicity, health status and use of medication, dietary habits, smoking status, alcohol consumption, and coca chewing (De Loma et al., 2019). Blood pressure (in sitting position), weight, and height were also measured during the recruitment visits.

Urine samples were collected to measure the exposure to arsenic and assess the arsenic metabolism efficiency, and to measure 8-oxo-dG and 4-HNE-MA. Mid-stream spot urine samples were collected in 20 mL trace element-free polyethylene bottles.

Blood for the assessment of arsenic exposure was collected in Trace Elements NH Sodium Heparin tubes (Vacuette, Greiner Bio, Austria), or in Lithium Heparin tubes (Vacuette) when the Sodium Heparin tubes were not available. Blood for the DNA extraction was collected in EDTA tubes (Vacuette). Venous blood samples were taken with BD Vacutainer Eclipse blood collection needles (Becton, Dickinson, USA). Hemoglobin was measured in venous blood using HemoCue201 + (HemoCue, Sweden) directly after blood sampling.

All samples were stored at −18 °C in a portable freezer (ARB, Australia) directly after obtention. Samples were stored at Universidad Mayor de San Andrés (La Paz, Bolivia) at −20 °C until further shipment on dry ice to Karolinska Institutet (Stockholm, Sweden).

### 2.3. Arsenic exposure

Exposure to arsenic was assessed by measuring arsenic concentrations in urine and whole blood. We determined the concentration of urinary arsenic (U-As) as the sum of iAs metabolite fractions in urine (iAs + MMA + DMA). The arsenic metabolites were measured by high-performance liquid chromatography online with hydride generation and inductively coupled plasma-mass spectrometry (HPLC-HG-ICP-MS) (HPLC: Agilent 1110 series, Agilent Technologies, Germany; Hamilton Column PRP-X100, US; ICP-MS: Agilent 7500ce, Agilent Technologies) as previously described (De Loma et al., 2019). Commercial urine reference materials were included in the runs to guarantee methodological accuracy as detailed elsewhere (De Loma et al., 2019). Based on

measurements of both total arsenic (including also organic arsenic forms) and as the sum of iAs metabolites, we concluded that these women were exposed only to inorganic forms of arsenic (De Loma et al., 2019). To compensate for variations in urine dilution, urinary osmolality was determined by a digital cryoscopic osmometer (OSMOMAT 030, Gonotec, Germany), and specific gravity was measured with a digital refractometer (RD712 clinical refractometer, EUROMEX, the Netherlands). Urinary osmolality (mean 727 mOsm/kg) and specific gravity (mean 1.02) were highly correlated ($r_s = 0.99$, $p$-value $< 0.001$) in this study population (De Loma et al., 2019). We adjusted U-As concentrations to the mean urinary osmolality since it has been described as an optimal correction method for urinary iAs concentrations (Middleton et al., 2019).

Arsenic concentrations in whole blood were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7900, Agilent Technologies, Japan) as described in detail elsewhere (De Loma et al., 2020). Briefly, blood samples were diluted with an alkali solution, sonicated for 5 min, and separated by centrifugation for 5 min at 694 g. Appropriate commercial whole blood reference materials were analyzed as quality controls (De Loma et al., 2020). Leach tests for the Vacuette tubes used for blood collecting were performed, and no traces of arsenic were detected.

### 2.4. Arsenic metabolism efficiency

The arsenic metabolism efficiency was assessed by two approaches: (1) using a single nucleotide polymorphism (SNP) in the *AS3MT* gene, and (2) using the relative fractions (%) of iAs metabolites (Vahter, 2002). We investigated *AS3MT* rs3740393, located in intron 6, since it has been shown to predict arsenic metabolism efficiency, including % MMA (Drobná et al., 2013; Engström et al., 2011).

To genotype the *AS3MT* SNP, DNA was extracted from peripheral blood samples with the E.Z.N.A. Blood DNA Mini kit (OMEGA Bio-Tek, USA). Genotyping was done by allelic discrimination using the LightCycler 480 II instrument with the SW 1.5. software (Roche, Switzerland). A PCR was performed with TaqMan SNP Genotyping assay for rs3740393 and master mix (Thermo Fisher Scientific, USA) in a 5 μL final volume. The PCR was done with the following thermal cycles: 95 °C for 10 min; and 45 cycles of 95 °C for 10 s, 60 °C for 1 min, and 72 °C for 1 s. Negative controls (without DNA) were included in the PCR runs.

### 2.5. Telomere length and mtDNAcn in leukocytes

Total DNA from peripheral whole blood was extracted with the E.Z.N.A. Blood DNA Mini Kit (Omega Bio-tek, USA). Telomere length and mtDNAcn were determined by real-time quantitative PCR (LightCycler 480 II, Roche) based on previously described methods (Cawthon, 2002; Xing et al., 2008). Relative telomere length and mtDNAcn values were calculated as the ratio with a single copy gene, hemoglobin beta *HBB* in this case, and therefore have arbitrary units. The ratio for each sample and biomarker was then normalized to a reference sample, repeated in all run plates, to account for inter-plate variation. In each run we included a standard curve (obtained by pooling random DNA samples and serially diluting the mix to produce DNA concentrations of 0.675 – 20 ng/μL), negative controls, and two randomly-selected samples as references to control for inter-plate variation.

Each assay, i.e., for telomere length, mtDNAcn, or *HBB*, was run in an independent plate with randomized samples in each plate. To determine the mtDNAcn, we amplified a region of the mitochondrial gene for NADH dehydrogenase 1 (*MT-ND1*) with the forward primer 5′-CCC TAA AAC CCG CCA CAT CT-3′ and reverse 5′-GAG CGA TGG TGA GAG CTA AGG T-3′. The primers for the single copy gene *HBB* were forward 5′-GTG CAC CTG ACT CCT GAG GAG A-3′ and reverse 5′-CCT TGA TAC CAA CCT GCC CAG-3′. Both the mtDNA and *HBB* were amplified using the same master mix containing 1x qPCRBIO SyGreen Blue Mix (PCR Biosystems, England), 300 nM forward primer, and 300 nM reverse primer in a final volume of 10 μL. The thermal cycling for mtDNA and *HBB* was 95 °C for 3 min, followed by 35 cycles of 95 °C for 10 s, 62 °C for 20 s, and 72 °C for 1 s

To determine the telomere length, we used the forward primer 5′-ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTGT-3′, and reverse primer 5′-TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA-3′. The master mix contained 1x HOT FIREPol Buffer B1 (Solis BioDyne, Estonia), 2 mM $MgCl_2$, 0.2 mM dNTPs, 900 nM forward primer, 900 nM reverse primer, 1.5 μM SYTO 9 fluorescent dye (Invitrogen, USA), and 1.25 U HOT FIREPol DNA polymerase (Solis BioDyne) in a final volume of 10 μL. The thermal cycling was 95 °C for 15 min; followed by two cycles of 94 °C for 15 s, and 49 °C for 15 s; and followed by 35 cycles of 94 °C for 15 s, 62 °C for 10 s, and 74 °C for 15 s

For all PCR runs, 7.5 μL of master mix and 2.5 μL of DNA (concentrations within the standard curve range) were pipetted into 384-well plates using the automated liquid handler Biomek 4000 (Beckman Coulter, USA). All samples were run in triplicates. If the standard deviation of the amplification crossing point was $\geq 0.1$, the deviating value was excluded. Calculated as the average of both reference samples, the inter-plate and intra-plate coefficient variations were 1.6% and 5.6% respectively for the telomere assay, and 3.5% and 3.3% respectively for the mtDNAcn assay.

### 2.6. 8-oxo-dG and 4-HNE-MA in urine

A volume of 0.2 mL of urine sample was transferred to glass inserts (Teknolab Sorbent, Sweden) of a 96-well Ritter plate (Teknolab Sorbent), and 0.1 mL of ammonium acetate (pH 6.5) and 0.01 mL of β-glucuronidase (*Escherichia coli*) were added. The solution was incubated for 30 min at 37 °C. Afterwards, 0.025 mL of a 50:50 (v/v) water/acetonitrile solution and 0.025 mL of isotope-labelled internal standard ($^{15}N_5$-8-oxo-dG; Cambridge Isotope Laboratories, USA) were added. The sample plates were centrifuged for 10 min at 3000 g prior to injection into the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

A 1.7 μm C18 column (2.1 mm i.d. × 50 mm; Avantor Genesis, USA) was used before the injector to reduce the interference of contaminants during the mobile phase. A Restek UltraAQ C18 column (3 μm, 4.6 mm i.d. x 100 mm, Restek, USA) was used for separation. The analysis was performed on a Shimadzu UFLC system (Shimadzu Corporation, Japan) coupled to a QTRAP5500 (triple quadrupole linear ion trap mass spectrometer) equipped with a Turbo Ion Spray source (AB Sciex, USA). For the analysis of 8-oxo-dG, 3 μL of each sample was injected. The mobile phases were MilliQ water and acetonitrile (A), both with 0.1% formic acid. The gradient was linearly increased from 5% A to 95% A within 4 min and held there for 1 min, before decreasing back to 5% A within 10 s and holding at 5% A for 1 min. Flow was 0.6 mL/min. For the measurement of 4-HNE-MA, which was present in urine at very high concentrations, only 0.2 μL of sample was injected. The gradient was linearly increased from 5% A to 95% A within 3 min and held there for 2 min, before decreasing back to 5% A within 10 s and holding at 5% A for 1 min. Flow was 1.2 mL/min. The retention time was 7.52 min for 8-oxo-dG, 7.48 min for the internal standard $^{15}N_5$-8-oxo-dG, and 2.96 min for 4-HNE-MA. Collision energy was 17 eV for 8-oxo-dG and −28 eV for 4-HNE-MA. All runs included at least ten blank samples, and background contamination was subtracted from all sample measurements. The limit of detection (LOD) was determined by analyzing ten different water blanks spiked with the internal standard and calculated as the average plus three times the standard deviation. The LOD was 0.1 ng/mL for 8-oxo-dG and 1.1 ng/mL for 4-HNE-MA. The variations in urinary dilution were compensated by adjusting 8-oxo-dG and 4-HNE-MA concentrations to the mean urinary osmolality of the study population since this correction method has been shown to be optimal for U-As (Middleton et al., 2019). However, to explore if the urinary dilution correction method could affect how the urinary toxicity biomarkers were associated with arsenic, we further compensated for urine dilution in two

ways: (1) with specific gravity- or osmolality-adjusted urinary concentrations, and (2) with specific gravity or osmolality included as a covariate in the linear regression models.

### 2.7. Statistical analyses

Analyses were performed with RStudio (version 1.1.423) using R (version 3.6.2). We made scatter plots between the four toxicity biomarkers and arsenic exposure as concentrations in urine or blood. We performed Spearman correlation tests (for continuous variables) and Wilcoxon tests (for categorical variables) between the four toxicity biomarkers, arsenic exposure (U-As and B-As), and other potential influential variables described in the literature.

We initially performed univariate linear regression analyses $\log_2$ transforming the exposure and/or the outcome variable and compared all four possible models. We selected the model with the highest adjusted $R^2$ and best residual plots in each case for further analyses in order to meet the linear regression assumptions. For telomere length and mtDNAcn, the dependent and independent variables were kept untransformed. For 8-oxo-dG and 4-HNE-MA, the dependent and independent variables were $\log_2$-transformed, although this did not result in the highest adjusted $R^2$ for the 4-HNE-MA models. We still decided to transform both variables for the 4-HNE-MA models to have similar models across toxicity biomarkers measured in the same matrix. We then performed unadjusted and adjusted linear regression models. Based on influential variables reported in the literature, we assessed whether age, hemoglobin, ethnicity, the chewing of coca leaves or smoking influenced the associations between arsenic exposure and the toxicity biomarkers. We introduced each covariate one at a time in the same order for all outcomes, and we kept the covariates that were associated with the outcome ($p$-value $< 0.1$) in the multivariable models and checked which adjusted model had the highest $R^2$. For each toxicity biomarker, if the selected covariates for the U-As and B-As models were different, we kept the highest number of covariates to facilitate the comparison between urine and blood models. As sensitivity analyses, we evaluated the associations between the toxicity biomarkers measured in urine (8-oxo-dG and 4-HNE-MA) and arsenic exposure including different methods to correct for urinary dilution (osmolality- or specific gravity-adjusted concentrations, and osmolality and specific gravity as covariates in the regression models).

We performed unadjusted and adjusted linear regression models to study the association between arsenic metabolite fractions and *AS3MT* rs3740393 genotype. To evaluate whether differences in arsenic metabolism efficiency influenced the association between arsenic exposure and the toxicity biomarkers, we performed adjusted linear regression models including an interaction term between U-As or B-As and rs3740393 genotype. Further, we stratified all models by (1) rs3740393 genotype, and (2) the median of each iAs metabolite fraction distribution. For both approaches, we used dichotomous variables as protective/non-protective genotype based on the association with %MMA, and below or equal/above the median metabolite. As sensitivity analyses, we excluded two extreme mtDNAcn values, which belonged to individuals with non-protective genotypes, to check if these values were driving the associations.

## 3. Results

### 3.1. Characteristics of the study participants

Descriptive statistics of the women are presented in Table 1. Out of the 193 women living in villages surrounding Lake Poopó in the Bolivian Andes mountains, 162 identified themselves as being Aymara-Quechua, and 31 as Uru. Smoking and consuming alcohol are uncommon behaviors among women from these villages: only five women reported smoking, and two reported drinking alcoholic beverages regularly (every other week). However, chewing coca leaves is an extended practice in the Andes, and 74% of the women reported doing so. The women had a median U-As concentration of 64 μg/L (range 12–399 μg/L), and a median B-As concentration of 2.0 ng/g (0.6–9.2 ng/g). Arsenic concentrations in both matrices were strongly correlated ($r_S = 0.85$, $p$-value $< 0.001$).

**Table 1**
Characteristics of the individuals included in the study (n = 193).

| | Mean ± SD[a] | Median (range) |
|---|---|---|
| *General characteristics* | | |
| Age (years) | 38 ± 15 | 36 (14–85) |
| Weight (kg) | 62 ± 12 | 60 (36–97) |
| Height (cm) | 149 ± 5.7 | 149 (120–162) |
| Coca chewing (yes)[b] | 74% | NA |
| Hemoglobin (g/dL) | 15 ± 1.6 | 15 (8.2–20) |
| *Exposures* | | |
| B-As (ng/g)[c] | 2.4 ± 1.6 | 2.0 (0.60–9.2) |
| U-As (μg/L)[d] | 88 ± 69 | 64 (12–399) |
| iAs (%) | 13 ± 6 | 12 (3.2–34) |
| MMA (%) | 8.0 ± 2.8 | 7.7 (2.2–18) |
| DMA (%) | 79 ± 6.8 | 80 (54–91) |
| *Toxicity biomarkers* | | |
| Telomere length[e] | 1.0 ± 0.19 | 1.0 (0.64–1.8) |
| mtDNAcn[e] | 1.5 ± 0.55 | 1.4 (0.67–4.4) |
| 8-oxo-dG (ng/mL)[f] | 6.3 ± 3.4 | 5.4 (1.3–24) |
| 4-HNE-MA (ng/mL)[f] | 63 ± 69 | 38 (1.1–390) |

Abbreviations and units: B-As, arsenic concentration in whole blood (ng/g); DMA, dimethylarsinic acid; 8-oxo-dG, 8-oxo-2′-deoxyguanosine (ng/mL, adj. osmolality); 4-HNE-MA, 4-hydroxy nonenal mercapturic acid (ng/mL, adj. osmolality); iAs, inorganic arsenic [sum of As(III) and As(V)]; MMA, monomethylarsonic acid; mtDNAcn, relative mitochondrial DNA copy number; U-As, sum of arsenic metabolite concentrations in urine (μg/L, adj. osmolality).

[a] Data presented as mean ± standard deviation (SD) unless otherwise stated.

[b] Percentage of individuals who reported chewing coca leaves regularly (n = 191, two did not answer).

[c] Measured in whole blood.

[d] Sum of urinary iAs metabolites adjusted for average urinary osmolality (727 mOsm/kg) of the total study population as described in De Loma et al. (2019).

[e] Telomere length and mitochondrial DNA copy number (mtDNAcn) are relative values without units.

[f] 8-oxo-2′-deoxyguanosine (8-oxo-dG) and 4-hydroxi nonenal mercapturic acid (4-HNE-MA) adjusted for average urinary osmolality (727 mOsm/kg) described in De Loma et al. (2019).

### 3.2. Arsenic exposure and toxicity biomarkers

We explored how the toxicity biomarkers were correlated with each other, with arsenic exposure biomarkers, and with other potential covariates (Supp. Fig. 1). Telomere length was inversely correlated with age ($r_S = -0.49$, $p = < 0.001$). B-As was inversely correlated with 8-oxo-dG ($r_S = -0.19$, $p = 0.009$), and 4-HNE-MA was positively correlated with B-As ($r_S = 0.19$, $p = 0.008$). Hemoglobin was inversely correlated with telomere length ($r_S = -0.24$, $p = 0.001$) and 4-HNE-MA ($r_S = -0.21$, $p = 0.003$), while it was positively correlated with 8-oxo-dG ($r_S = 0.29$, $p = < 0.001$). 8-oxo-dG and 4-HNE-MA were negatively correlated ($r_S = -0.28$, $p = < 0.001$). Furthermore, urinary 8-oxo-dG and 4-HNE-MA were significantly different between ethnic groups: 8-oxo-dG was higher in Aymara-Quechua women (median 5.8 ng/mL, range 1.4–24 ng/mL) compared to Uru women (median 4.1 ng/mL, range 1.3–13 ng/mL; $p = 0.003$), while 4-HNE-MA was higher in Uru (median 105 ng/mL, range 5.4–390 ng/mL) than in Aymara-Quechua women (median 34 ng/mL, range 1.1–296 ng/mL; $p < 0.001$).

We explored by linear regression how leukocyte telomere length and mtDNAcn, and urinary 8-oxo-dG and 4-HNE-MA were associated with arsenic exposure (U-As and B-As, Fig. 1). Telomere length was positively associated with U-As and B-As, and this association was strengthened when adjusting for age (Table 2). MtDNAcn was positively associated with U-As, but not B-As (Table 2). Concentrations of urinary 4-HNE-MA were positively associated with U-As and B-As, and these associations

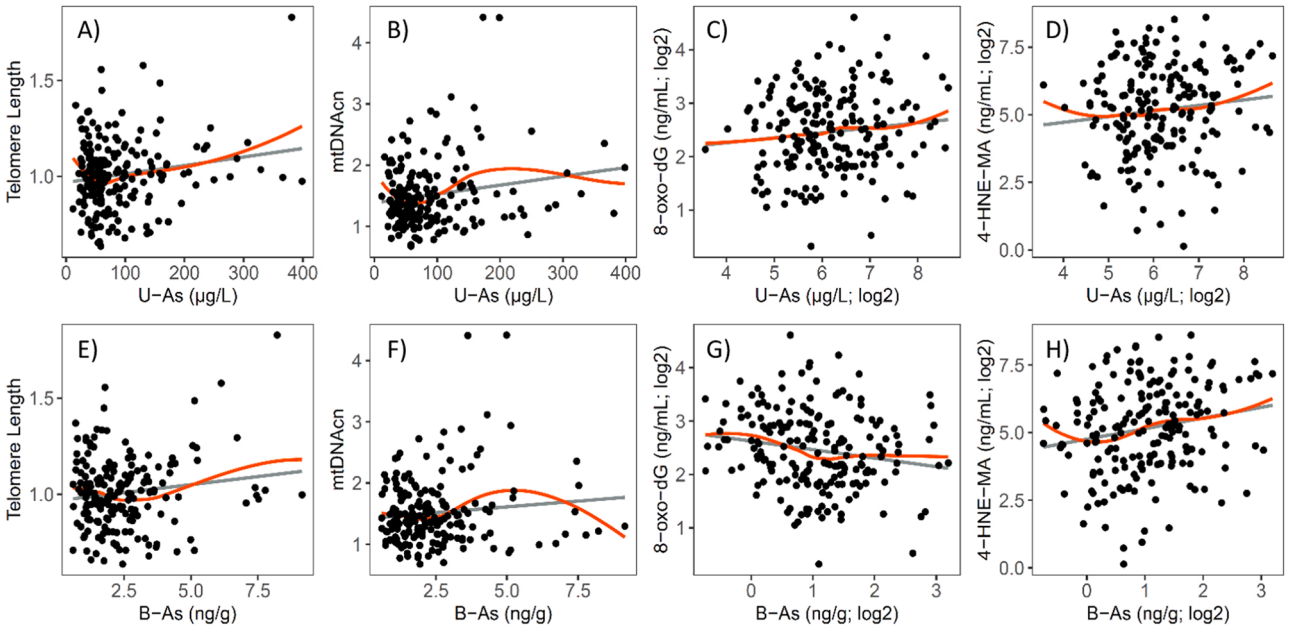


**Fig. 1.** Scatter plots of all toxicity markers against U-As (A-D) and B-As (E-H) with the linear regression model line (grey straight line) and loess line (red curved line). Abbreviations and units: B-As, arsenic concentration in whole blood (ng/g); 8-oxo-dG, 8-oxo-2′-deoxyguanosine (ng/mL, adj. osmolality); 4-HNE-MA, 4-hydroxy nonenal mercapturic acid (ng/mL, adj. osmolality); mtDNAcn, relative mitochondrial DNA copy number (no units); U-As, sum of arsenic metabolite concentrations in urine (µg/L, adj. osmolality). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

**Table 2**
Linear regression models for the associations between arsenic exposure and toxicity biomarkers.

| | | U-As | | B-As | |
|---|---|---|---|---|---|
| | n | B (95% CI) | *p*-value | B (95% CI) | *p*-value |
| **TL**[a] | | | | | |
| Unadjusted | 193 | 0.044 * (0.007, 0.082) | 0.021 | 0.017 (−0.0001, 0.033) | 0.053 |
| Adjusted | 193 | 0.048 * * (0.015, 0.082) | 0.005 | 0.017 * (0.002, 0.032) | 0.028 |
| **mtDNAcn**[b] | | | | | |
| Unadjusted | 193 | 0.144 * (0.033, 0.255) | 0.012 | 0.038 (−0.012, 0.088) | 0.139 |
| Adjusted | 193 | 0.137 * (0.026, 0.249) | 0.017 | 0.035 (−0.015, 0.085) | 0.173 |
| **8-oxo-dG**[c] | | | | | |
| Unadjusted | 193 | 0.093 (−0.016, 0.201) | 0.095 | -0.159 * (−0.284, −0.034) | 0.014 |
| Adjusted | 191 | 0.110 * (0.003, 0.217) | 0.044 | -0.149 * (−0.271, −0.027) | 0.018 |
| **4-HNE-MA**[d] | | | | | |
| Unadjusted | 193 | 0.209 (−0.038, 0.456) | 0.099 | 0.394 * * (0.111, 0.678) | 0.007 |
| Adjusted | 193 | 0.262 * (0.025, 0.500) | 0.032 | 0.422 * * (0.154, 0.691) | 0.002 |

Abbreviations and units: B-As, arsenic concentration in whole blood (ng/g); 8-oxo-dG, 8-oxo-2′-deoxyguanosine (ng/mL, adj. osmolality); 4-HNE-MA, 4-hydroxy nonenal mercapturic acid (ng/mL, adj. osmolality); mtDNAcn, relative mitochondrial DNA copy number; TL, relative telomere length; U-As, sum of arsenic metabolite concentrations in urine (µg/L, adj. osmolality).
Note: Results are presented as B coefficients (95% confidence interval) from unadjusted or multivariable-adjusted linear regression models (* *p*-value < 0.05; ** *p*-value < 0.01).
[a] $TL = \alpha + \beta1 \times$ exposure (per 100 µg/L for U-As) $+ \beta2 \times$ age
[b] mtDNAcn $= \alpha + \beta1 \times$ exposure (per 100 µg/L for U-As) $+ \beta2 \times$ hemoglobin
[c] $\log_2(\text{8-oxo-dG}) = \alpha + \beta1 \times \log_2(\text{exposure}) + \beta2 \times$ ethnicity $+ \beta3 \times$ hemoglobin $+ \beta4 \times$ coca
[d] $\log_2(\text{4-HNE-MA}) = \alpha + \beta1 \times \log_2(\text{exposure}) + \beta2 \times$ ethnicity $+ \beta3 \times$ hemoglobin

became stronger after adjusting for ethnicity and hemoglobin (Table 2). Surprisingly, the association between 8-oxo-dG and arsenic was different depending on the biomarker of exposure used. U-As concentrations were positively associated with 8-oxo-dG, while B-As was inversely associated with 8-oxo-dG, when adjusting for ethnicity, hemoglobin, and the chewing of coca leaves (Table 2). Further description of how each covariate was associated with the toxicity biomarkers is included in Supplementary Table 1. The associations between 4-HNE-MA and arsenic exposure (U-As and B-As) were concordant independent of the urinary dilution correction method used (osmolality- or specific gravity-adjusted concentrations or included as a covariate in the regression models; Supplementary Table 2). For 8-oxo-dG, the coefficient estimates varied between 0.087 and 0.144 for U-As associations, and between −0.115 and −0.154 for B-As depending on the correction method (Supplementary Table 2). Nevertheless, the discrepant associations of 8-oxo-dG with U-As and B-As were present independent of the urinary dilution correction method.

### 3.3. *Influence of arsenic metabolism efficiency*

We first evaluated whether *AS3MT* rs3740393 was associated with arsenic metabolism efficiency, measured as arsenic metabolite fractions, in this population. Genotype CC was associated with lower %MMA, i.e., a more efficient arsenic metabolism and therefore considered as protective, while genotypes GC and GG were considered non-protective (Table 3). The frequency of this protective genotype in our Bolivian study group is the highest described to date in the literature (Table 3).

A statistically significant interaction between rs3740393 and arsenic exposure on mtDNAcn was found ($p = 0.022$ for U-As and $p = 0.0064$ for B-As, Table 4). No other significant interactions between *AS3MT* genotype and arsenic exposure on the other toxicity biomarkers were found, except for a modest interaction for telomere length ($p = 0.14$ for U-As and $p = 0.19$ for B-As, Table 4). When stratifying by rs3740393 genotype, both U-As and B-As were significantly associated with longer telomeres and more mtDNAcn in those individuals with non-protective genotypes (less efficient metabolizers; Table 4, Supplementary Fig. 2). When excluding two individuals with extreme mtDNAcn (values > 4

**Table 3**
Minor allele frequencies (MAF) for *AS3MT* rs3740393 and observed mean values of arsenic metabolites by rs3740393 genotype from the current study group.

| **MAF (C < G)**[a] | | | | | |
|---|---|---|---|---|---|
| Bolivia | Chile[b] | Argentina[c] | 1000 Genomes Peru (PEL) | 1000 Genomes Colombia (CLM) | 1000 Genomes Yoruba (YRI) |
| 0.79 | 0.68 | 0.70 | 0.40 | 0.16 | 0.14 |
| | | **Observed mean values** | | | |
| **rs3740393** | | **n** | **%iAs** | **%MMA** | **%DMA** |
| CC | | 119 | 12.7 | 7.5 | 79.8 |
| GC | | 68 | 13.5 | 8.6 | 77.9 |
| GG | | 6 | 14.8 | 9.3 | 75.9 |
| *p*-value[d] | | | 0.136 | 0.001 | 0.036 |
| *p*-value adjusted[e] | | | 0.080 | 0.010 | 0.042 |

Abbreviations: DMA, dimethylarsinic acid; iAs, inorganic arsenic [sum of As(III) and As(V)]; MAF, minor allele frequency; MMA, monomethylarsonic acid.

[a] Stated first is the minor allele, based on the second most frequent allele in populations from the 1000 Genomes Project (phase 3 accessed through http://www.ensembl.org).

[b] Described in Apata et al. (2017) referring to the local population from Quebrada Camarones (Chile).

[c] Described in Engström et al. (2011).

[d] *p*-value for unadjusted linear regression models with $log_2$-transformed arsenic metabolite fractions.

[e] *p*-value for linear regression models adjusted for ethnicity, U-As (sum of metabolites, adjusted for osmolality), age, and coca chewing with $log_2$-transformed arsenic metabolite fractions.

while the rest were < 2.9), the coefficient estimate between mtDNAcn and U-As became 33% weaker (B = 0.002, $p = 0.038$), and 35% weaker with B-As (B = 0.071, $p = 0.045$), but still significant. Higher urinary 8-oxo-dG concentrations were associated only with lower B-As in the group of individuals with the protective genotype, however, the effect estimates were similar between genotype subgroups (Table 4, Supp. Fig. 3). Urinary concentrations of 4-HNE-MA were positively associated with B-As, independent of rs3740393 genotype (Table 4, Supp. Fig. 3).

We also evaluated the influence of arsenic metabolism capacity, assessed by the fractions of iAs metabolites, on the associations between arsenic exposure and toxicity biomarkers. In line with the models stratified by genotype, only individuals with less efficient arsenic metabolism, i.e., higher %iAs or %MMA, or lower %DMA, presented a significant association between arsenic exposure and longer telomeres (Supplementary Table 3). No significant associations were found between mtDNAcn and U-As or B-As when stratifying by arsenic metabolite fractions (Supplementary Table 3). For 8-oxo-dG, U-As was associated with higher 8-oxo-dG urinary concentrations in individuals with high %MMA, while lower 8-oxo-dG was associated with B-As in those individuals with lower %MMA (Supplementary Table 3). Contrary to what was expected, efficient arsenic metabolizers presented positive associations with stronger coefficient estimates between B-As and urinary 4-HNE-MA than those with less efficient arsenic metabolism (Supplementary Table 3).

## 4. Discussion

To our knowledge, this is one of the very first studies to explore the toxicity of arsenic in Bolivian populations. Arsenic exposure was associated with higher oxidative stress and longer telomeres despite the fact that these women in general have an efficient arsenic metabolism (De Loma et al., 2019). However, when stratifying for indicators of arsenic metabolism efficiency, longer telomeres and higher mtDNAcn were observed only in individuals with a less efficient arsenic metabolism capacity, stressing that they represent a particularly susceptible group. Further, the modifying effect of arsenic metabolism provides evidence that arsenic is directly associated with telomere lengthening and mitochondria DNA alterations in this population. The two other toxicity biomarkers, 8-oxo-dG and 4-HNE-MA, were both positively associated with U-As, and 4-HNE-MA with B-As, but neither of them had a clear modifying effect of arsenic metabolism. Future studies should evaluate whether the biomarkers included in this study predict later arsenic-related diseases, such as cancer, for which no data is currently available in this study group. However, during the recruitment of the study participants, none presented signs of hyperkeratosis on their palms – a premalignant sign associated with chronic inorganic arsenic exposure. As previously described, here, we showed that *AS3MT* rs3740393 CC carriers had lower %MMA. It should be noted that the Bolivian women present the highest frequency of the C-allele (79%) described in the literature to date, even when compared to other South American populations chronically exposed to iAs from Chile (68%; Apata et al., 2017) or Argentina (70%; Engström et al., 2011). Our current result of the high frequency of the protective C-allele of

**Table 4**
Effect of arsenic metabolism efficiency (assessed by *AS3MT* rs3740393 genotype stratification) on the associations between arsenic exposure and toxicity biomarkers.

| | U-As | | | | B-As | | |
|---|---|---|---|---|---|---|---|
| | n | B (95% CI) | *p*-value | *p*-value[a] | B (95% CI) | *p*-value | *p*-value[a] |
| **TL**[b] | | | | | | | |
| CC | 119 | 0.0002 (−0.0002, 0.001) | 0.29 | 0.14 | 0.007 (−0.014, 0.028) | 0.50 | 0.19 |
| GC + GG | 74 | 0.001 * * (0.0003, 0.001) | 0.002 | | 0.027 * (0.006, 0.049) | 0.015 | |
| **mtDNAcn**[c] | | | | | | | |
| CC | 119 | 0.0002 (−0.001, 0.001) | 0.73 | 0.022 | -0.030 (−0.086, 0.026) | 0.30 | 0.0064 |
| GC + GG | 74 | 0.003 * (0.001, 0.005) | 0.011 | | 0.106 * (0.015, 0.197) | 0.025 | |
| **8-oxo-dG**[d] | | | | | | | |
| CC | 118 | 0.107 (−0.035, 0.249) | 0.14 | 0.53 | -0.172 * (−0.331, −0.012) | 0.038 | 0.80 |
| GC + GG | 73 | 0.111 (−0.059, 0.281) | 0.20 | | -0.151 (−0.351, 0.048) | 0.14 | |
| **4-HNE-MA**[e] | | | | | | | |
| CC | 119 | 0.274 (−0.029, 0.577) | 0.079 | 0.36 | 0.382 * (0.039, 0.724) | 0.031 | 0.41 |
| GC + GG | 74 | 0.276 (−0.120, 0.671) | 0.18 | | 0.534 * (0.082, 0.987) | 0.024 | |

Abbreviations and units: B-As, arsenic concentration in whole blood (ng/g); 8-oxo-dG, 8-oxo-2′-deoxyguanosine (ng/mL, adj. osmolality); 4-HNE-MA, 4-hydroxy nonenal mercapturic acid (ng/mL, adj. osmolality); mtDNAcn, relative mitochondrial DNA copy number; TL, relative telomere length; U-As, sum of arsenic metabolite concentrations in urine (μg/L, adj. osmolality).

Note: Results are presented as B coefficients (95% confidence interval) from multivariable-adjusted linear regression models (* *p*-value < 0.05; ** *p*-value < 0.01). rs3740393 genotype CC was associated with lower %MMA, i.e., more efficient arsenic methylation (see Table 3).

[a] *p*-value for the interaction term between the arsenic exposure and rs3740393 genotype as dichotomous using the same covariates as described below.

[b] $TL = \alpha + \beta1 \times exposure + \beta2 \times age$

[c] $mtDNAcn = \alpha + \beta1 \times exposure + \beta2 \times hemoglobin$

[d] $log_2(8\text{-oxo-dG}) = \alpha + \beta1 \times log_2(exposure) + \beta2 \times ethnicity + \beta3 \times hemoglobin + \beta4 \times coca$

[e] $log_2(4\text{-HNE-MA}) = \alpha + \beta1 \times log_2(exposure) + \beta2 \times ethnicity + \beta3 \times hemoglobin$

rs3740393 in *AS3MT* is in line with the observation that indigenous communities of the Bolivian Andes have a very efficient arsenic metabolism phenotype. Genetic variations in *AS3MT* are strong and stable predictors of arsenic metabolism efficiency (Pierce et al., 2012; Schlebusch et al., 2015), while the fractions of iAs, MMA, and DMA in urine are also influenced, apart from *AS3MT* genetics, by other factors such as the individual's nutritional status, which may explain the less evident associations between metabolite fractions and toxicity biomarkers in this study.

### 4.1. Higher arsenic exposure resulted in longer telomeres

Longer telomeres in peripheral blood lymphocytes have been associated with increased risk for lung adenocarcinoma, bladder cancer, melanoma, and kidney cancer, among other types (Haycock et al., 2017). Arsenic exposure has been associated with longer telomeres in earlier studies including populations from the Argentinean Andes (Ameer et al., 2016; Li et al., 2012), Bangladesh (Gao et al., 2015a), and Mexico (Alegría-Torres et al., 2020). However, a study in Bangladesh showed no association (Zhang et al., 2018), and one with American Indian communities found an inverse association at low arsenic exposures (Grau-Perez et al., 2019). In the current study, we found a positive association between telomere length and arsenic exposure (with both U-As and B-As), and particularly in individuals with a less efficient arsenic metabolism. A similar susceptibility to longer telomeres via the arsenic metabolism capacity was found in women from the Argentinean Andes and lowlands (Ameer et al., 2016). Arsenic is thought to promote telomere elongation by inducing the expression of *TERT*, a subunit of the telomerase (Gao et al., 2015a; Li et al., 2012). Recently, a link between longer telomeres and arsenic-related cancer was found: 85% of arsenic-induced skin cancer tissues studied had two-fold longer telomeres compared to non-tumor tissues (Bhattacharjee et al., 2020). It must be noted, however, that they did not compare their results to other skin cancers not related to arsenic.

### 4.2. mtDNAcn was positively associated with arsenic exposure in individuals with a less efficient arsenic metabolism

In a meta-analysis of case-control studies, patients with lymphomas or breast cancers had higher mtDNAcn measured in peripheral blood than controls, while those with hepatic carcinoma presented lower mtDNAcn (Hu et al., 2016). Bladder, breast, and kidney tumor tissues had less mtDNAcn compared to normal tissues, and lung adenocarcinoma samples had higher mtDNAcn (Reznik et al., 2016). Regarding the relation between arsenic and mtDNAcn, less is known. Patients with arsenic-related Bowen's disease had mtDNA mutations linked to increased oxidative stress in skin lesions compared to surrounding healthy skin (Lee et al., 2013). Two studies, one in Mexican children (Alegría-Torres et al., 2020) and one in pregnant women from China (Song et al., 2020), showed no association between arsenic exposure and mtDNAcn. On the contrary, mtDNAcn and U-As were positively associated in adults from the Argentinean lowlands, while this association was present only in individuals from the Argentinean highlands that had a less efficient arsenic metabolism (Ameer et al., 2016). These results, in line with our current observations, highlight the importance of including arsenic metabolism capacity as a susceptibility factor. In addition, individuals exposed to arsenic, with or without skin lesions (mean U-As: 200 and 240 μg/L, respectively) had higher mtDNAcn in blood than individuals with low U-As exposure (mean 20 μg/L) (Sanyal et al., 2018). Recently, the same group confirmed that the mtDNAcn was five times higher in tissues from arsenic-induced skin tumors compared to control tissues (Sanyal et al., 2020).

### 4.3. Urinary 8-oxo-dG showed inconsistent associations with arsenic exposure biomarkers

Urinary concentrations of 8-oxo-dG have been extensively used as a biomarker of oxidative stress in relation to arsenic. We found a positive association between U-As and urinary 8-oxo-dG, an observation strongly supported by earlier studies from Bangladesh (Breton et al., 2007; Engström et al., 2010a), Taiwan (Chou et al., 2014), and the Argentinean Andes (Engström et al., 2010b), among other countries. However, despite U-As and B-As being highly correlated, B-As was negatively associated with urinary 8-oxo-dG particularly in individuals with the protective *AS3MT* genotype. A study from Bangladesh also found a positive association for U-As, but no association for arsenic in toenails (Breton et al., 2007). In another study with individuals from Arizona and Mexico, urinary 8-oxo-dG was not associated with U-As but inversely associated with arsenic in toenails in participants from some cities from Arizona (Burgess et al., 2007). The discrepancy in the association between urinary 8-oxo-dG and different matrices for arsenic exposure determination stresses that urinary 8-oxo-dG in relation to arsenic should be interpreted with caution. Several contributors to urinary 8-oxo-dG levels exist, such as DNA repair, normal cell turnover and diet (Cooke et al., 2009; Loft and Møller, 2006). A potential co-excretion between arsenic and 8-oxo-dG in urine, a phenomenon described earlier for other metals like cadmium (Wallin et al., 2014), could explain these contradictory findings. For instance, although urinary 8-oxo-dG is positively associated with urinary cadmium, it was not associated with cadmium concentrations measured in kidney biopsies (Hossain et al., 2014). More research is needed regarding the effect of urinary excretion and the best praxis to account for urinary dilution variations for this biomarker.

### 4.4. Urinary 4-HNE-MA was positively associated with arsenic exposure in individuals with a more efficient arsenic metabolism

One of the major products of lipid peroxidation is 4-hydroxy nonenal (HNE), which is further conjugated with mercapturic acid to be eliminated in urine, among other HNE metabolites, and serves as an in vivo oxidative stress marker (Dalleau et al., 2013). Rats exposed to oxidative stress-inducing compounds exhibited elevated concentrations of 4-HNE-MA in hepatic tissue and plasma (Völkel et al., 2005). There are currently few epidemiological studies on 4-HNE-MA in relation to environmental exposures, such as arsenic. A metabolomic study of Polish individuals living near copper smelting areas identified higher urinary HNE metabolites in males highly exposed to arsenic ($n = 22$) compared to those with low exposure ($n = 22$); however, these individuals were exposed mostly to arsenobetaine from seafood, and the sample size was low (Kozłowska et al., 2019). Also, U-As was positively associated with 4-HNE-MA in urine of Chinese men seeking infertility council (He et al., 2020).

Contrary to what was expected, we found that the association between 4-HNE-MA and B-As was limited to those individuals with lower %MMA or %iAs and higher %DMA, i.e., efficient arsenic metabolizers. No other study has evaluated the effect of arsenic metabolism capacity on the effect of 4-HNE-MA. A potential explanation is that there is an interaction between the mercapturic acid pathway and the one-carbon metabolism yet to be described, for example via the common glutathione pool required. The body detoxifies HNE by conjugating it to glutathione, spontaneously or by the glutathione S-transferase, and certain isoforms of this protein group have been shown to be protective against HNE toxicity (Balogh and Atkins, 2011). Furthermore, genetic variants in glutathione transferases have been previously linked to arsenic metabolism (Engström et al., 2007). This brings up the need for future studies evaluating the interaction between the metabolism of arsenic and HNE, and the potential contribution of genetics.

### 4.5. Strengths and limitations

This study combined four toxicity biomarkers in relation to arsenic exposure and evaluated the effect of arsenic metabolism efficiency by using both a genetic and a phenotypic approach. It included a novel biomarker of lipid peroxidation and assessed arsenic exposure in two biological matrices. Although arsenic is known to cause oxidative stress, no arsenic-specific biomarker of oxidative stress exists. Therefore, measuring several biomarkers increases the possibility of detecting associations in relation to arsenic exposure in this study group. However, a limitation of our study is the small sample size due to the intrinsic complexities of recruiting participants in remote Andean villages. Since only women were recruited, additional studies need to evaluate potential sex differences. Using two approaches to categorize the capacity to metabolize arsenic has each its benefits and limitations. The arsenic metabolizing phenotypes are usually based on percentage of arsenic metabolites in urine. However, although the individual metabolite percentages are relatively stable over time (Steinmaus et al., 2005), they are in our study based a one-time measurement that is susceptible to variation due to differences in exposure, nutrition, or other individual factors, such as disease. In contrast, the constitutional genotype of *AS3MT* is a stable marker that reflects the percentage of arsenic metabolites, and it is possible to compare genotype frequencies between populations. Nevertheless, genetic variants of *AS3MT*, while being the main gene involved in arsenic methylation, only explain a small percentage of the variation in arsenic metabolites fractions (Pierce et al., 2012; Schlebusch et al., 2015; Jansen et al., 2016).

## 5. Conclusions

Women from the Bolivian Andes chronically exposed to arsenic had longer telomeres and higher mtDNAcn in peripheral blood leukocytes with increasing arsenic exposure. The association of arsenic exposure with telomere length or mtDNAcn was only present in those individuals with a less efficient arsenic metabolism. The inconsistent associations between urinary 8-oxo-dG and two arsenic exposure biomarkers (positive for U-As vs. negative for B-As) may serve as a cautionary statement that more research is needed concerning the effect of urinary excretion for this biomarker. Finally, urinary 4-HNE-MA, a less studied lipid peroxidation biomarker, showed a positive association with arsenic exposure, independent of *AS3MT* genotype but modified by the fractions of iAs metabolites in urine. The identification of associations between arsenic exposure and toxicity biomarkers urges the need for further efforts to reduce and monitor arsenic levels in Bolivia.

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## CRediT authorship contribution statement

**Karin Broberg:** Conceptualization, Resources, Writing – original draft, Funding acquisition, Supervision, Writing – review & editing. Jessica De Loma: Resources, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Christian H. Lindh:** Investigation, Writing – review & editing. **Annette M. Krais:** Investigation, Writing – review & editing. **Josue Mamani:** Resources, Writing – review & editing. **Noemi Tirado:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **Jacques Gardon:** Conceptualization, Resources, Funding acquisition, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113194.

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