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Nickel (Ni) may have influenced on the MMA(V) reductase and DMA(V) reductase activities for inorg-As biotransformation process in humans

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ABSTRACT

Trace elements (As, Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, and Hg) were measured in blood of arsenic exposed people in Mexico by using ICP-MS to know the influence of trace elements on the MMA(V) reductase and DMA(V) reductase activities for inorganic arsenic biotransformation process.

The correlations between MMA(V) reductase and DMA(V) reductase activities in blood of both females and males were very weak (F, r= +0.06 and M, r= -0.03, respectively). The activities of MMA(V) and DMA(V) reductase were also not depended on ages of those population (r= -0.05 and r= -0.11, respectively). In this study, there were no significant differences of the MMA(V) reductase and DMA(V) reductase activities in bloods of females and males.

Nickel concentrations in blood were positively and negatively correlated with MMA(V) and DMA(V) reductase activities in blood of both females and males, respectively. These correlations were statistically significant for females. In conclusion of this study suggest that Ni may have influenced for biotransformation process of inorganic arsenic (inorg-As) in humans and it could enhance arsenic methylation, decrease urinary MMA, and increase urinary DMA.

Abbreviation: SAM, *S*-adenosyl-L-methionine; SAHC, *S*-adenosyl-L-homocysteine

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Introduction

The IARC[1] has classified arsenic as a group 1 human carcinogen. Chronic exposure to inorganic arsenic can cause cancerous[1-4] and non-cancerous health hazards[5,6] in humans. Arsenic can get entry into the human body via drinking water, eating food, inhaling dust, and/or ingesting soil.

With chronic and continuing exposure, steady-state concentrations of arsenic in blood and urine are achieved; these have been the potential to serve as biomarkers of arsenic exposure[7]. Hall et al. (2006)[7] have been suggested that with chronic exposure, blood arsenic which receives inputs not only from recent exogenous exposure but also from tissue compartments – may better reflect an individual's total internal As burden. The main organ for arsenic metabolism is the liver, but the metabolic pathway of inorganic arsenic is not yet fully clarified [8,9]. Trivalent arsenic species are more ready to cross cell membrane and inorganic pentavalent arsenate in mostly reduced to trivalent arsenite in the blood stream before entering the cells for further metabolism[10,11]. Inorganic arsenic is metabolized in the body by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine as methyl donor [8,12,13] (Fig. 1). Several studies have been shown an increasing prevalence of arsenic-related toxic effects with increasing %MMA in urine[14,15] and probably an increased concentrations of the highly toxic MMA(III)[16,17] at cellular level.

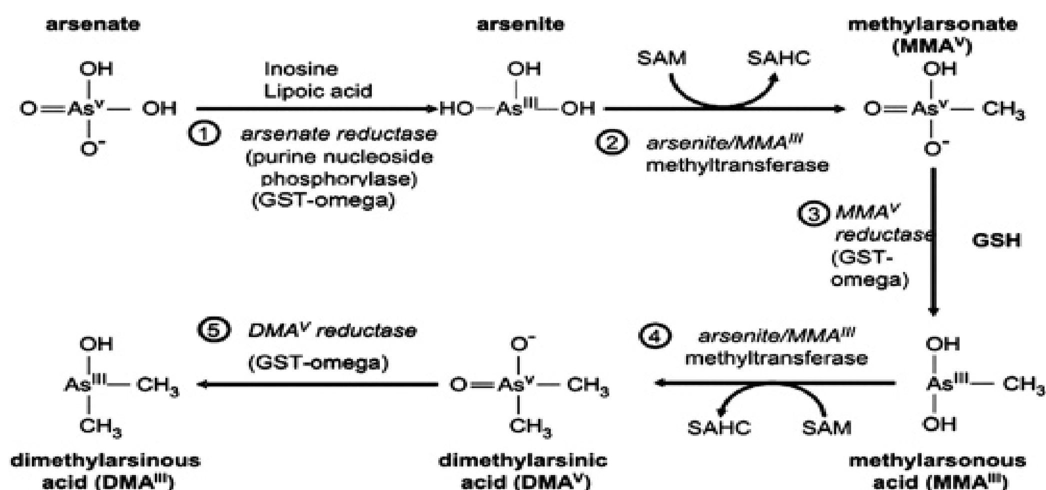


Figure 1. Biotransformation of inorganic arsenic[13].

A number of studies have shown associations between the severity of arsenic related health effects and nutritional status[18-20]. Lower Se intake is associated with enhance As toxicity[18,21-23], and lower urinary Se levels were associated with increased %inorg As and decreased %DMA in urine[23]. Another study has been reported that subjects with higher intakes of Zn had lower %MMA and higher %DMA in urine[19]. Zinc (Zn) has been linked to decrease arsenic toxicity in some studies[2,24].

To date, other metal or metalloids that may influence arsenic methylation are largely unknown. The aims of this study were to assess the influence of trace elements for biotransformation process of inorganic arsenic and the correlations between the concentrations of trace element and MMA(V) or DMA(V) reductase activities in blood among the population in Lagunera area of Mexico, who drunk water containing arsenic in range 38 to 116 µg/L. Our results suggest that Ni had influenced on arsenic methylation process in humans.

Materials And Methods

Reagents. The chemicals used and their sources are as follows: Sodium arsenate (ACS reagent grade) from MCB Reagents (Cincinnati, OH); dimethylarsinic acid (sodium salt), ammonium phosphate (dibasic), and glutathione (GSH) from Sigma Chemical Co. (St. Louis, MO); sodium m-arsenite and ammonium nitrate from Sigma-Aldrich Co. (St. Louis, MO); disodium methylarsenate from ChemService, Inc. (West Chester, PA). The arsenic and other elements standard solution was from SPEX Certiprep (Metuchen, NJ); ^{14}C-MMA(V) (0.55 µCi/nmol) and ^{14}C-DMA(V) (0.67 µCi/nmol) were synthesized by Prof. Eugene A. Mash, Jr., Department of Chemistry, The University of Arizona. Triton X-100 was from Pharmacia Biotech (Uppsala, Sweden). All other chemicals were analytical reagent grade or the highest quality obtainable. Water was doubly deionized and distilled.

Subjects. Urine and blood samples were collected from 191 subjects (98 females and 93 males), aged 18-77 years in the Lagunera area of Mexico. There were five groups, based on total arsenic concentration (38-116 µg/L) in their drinking water.

Urine and Blood Collection. All collecting containers were soaked overnight in 2% nitric acid (Baker analyzed for trace metal analysis) (J. T. Baker, Inc. Phillipsburg, NJ) and rinsed with double distilled and deionized water. All plastic measuring and collecting equipment were similarly washed, sealed in bags, placed in locked footlockers, and transported by air to the site of the study at the same time as the investigators. After collection, urine sample was immediately frozen in a portable icebox containing dry ice. Blood was collected by venous puncture, into vacutainers containing EDTA, transferred to the vial, and immediately frozen. The samples were kept frozen while being transported to the University of Arizona, Tucson where they were stored at -70°C before analysis.

Arsenic Species Analysis. Frozen urine samples were thawed at room temperature, filtered with a 0.45 µm filter (Nanosep MF Centrifugal Devices, Pall Life Sciences, Ann Arbor, MI), and diluted 5-fold using Milli Q water before injection. An HPLC-ICP-MS (High Performance Liquid Chromatography- Inductively Coupled Plasma-Mass Spectrometry) speciation method[25] was used for the measurement of arsenic species. The HPLC system consisted of a PerkinElmer

Series 200 HPLC with an anion exchange column (Gemini 5 µm C18 110A, 150 x 4.6 mm, Phenomenex, Torrance, CA). The mobile phase (pH 5.85) contained 4.7 mM tetrabutylammonium hydroxide, 2 mM malonic acid, and 4% (v/v) methanol at a flow rate of 1.2 mL/min. The column temperature was maintained at 50° C. An ELAN DRCe ICP-MS (PerkinElmer) with a cyclonic quartz spray chamber and Meinhard nebulizer was used as a detector for the analysis of arsenic species [As(V), As(III), MMA(V), MMA(III), DMA(V), and DMA(III)] in urine at 4° C (Note: The method of Gong et al. (2001)[25] could not separate AsB and AsB was overlapped with arsenite (Figs. 2B & 2D)) (Fig. 2). In this study, an HPLC-ICP-MS method[26] was modified by author for the measurement of arsenic metabolites including AsB in urine (Figs. 2C & 2D). The modified system consisted of a PerkinElmer Series 200 HPLC with an anion exchange column (Gemini PRP-X100, 10 µm, 250 x 4.6 mm, Hamilton Company, Nevada). The mobile phase (pH 8.5) contained 10 mM ammonium nitrate and 10 mM ammonium phosphate (dibasic) at a flow rate of 1 mL/min. The column temperature was maintained at 30° C. The same DRCe ICP-MS (PerkinElmer) system was used as a detector for the analysis of arsenic species [AsB, As(V), As(III), MMA(V), and DMA(V)] in urine at 4° C. The operating parameters for both were as follows: R_f power, 1400 W; plasma gas flow, 15 L/min; nebulizer gas flow, 0.82 L/min; auxiliary gas flow, 1.2 L/min; oxygen flow for DRCe, 0.87 mL/min; and arsenic was measured at m/z 91.

The working detection limits and accuracy of this analytical method were as follows: The working detection limits of arsenic metabolites were 0.80 - 1.75 µg/L. Accuracy values were calculated by spiking standard compounds of all five species (5 µg/L) in urine samples. The recoveries of the added compounds were 98-103%. Standard samples (5 µg/L) containing all five arsenic species were also analyzed after analysis the urine samples each day. The values of mean ± SE for As(V), As(III), MMA(V), and DMA(V) were found 5.09 ± 0.11, 5.16 ± 0.11, 5.02 ± 0.10, and 4.90 ± 0.05, respectively.

Measurement of Trace Elements levels in Whole Blood.

In this study, whole blood samples were analyzed for As, Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, and Hg concentrations using PerkinElmer Elan DRCe ICP-MS. Inductively coupled plasma mass spectrometry method for elements in whole blood was developed (with modifications) based on published method[27]. Whole blood samples were thawed, thoroughly mixed, diluted 50 times with diluent containing 0.65% HNO3 + 0.1% Triton X-100, and centrifuged for 10 min (3500 rpm at 4° C) with the supernatant reserved for analysis. A multi-element standard solution was used for instrument calibration. Three working mercury standard solutions, viz., 1, 2.5, and 5 µg/L were prepared from stock standard solution with 0.65% HNO3 + 0.1% Triton X-100, added gold (200 ppb), and mixed well. Five working other elements (As, Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn) standard solutions, viz., 5, 10, 20, 50, and 100 µg/L were prepared from stock standard solution with same diluent, added internal standards (Ga, In, & Re; 100 µg/L of each), and mixed well. The calibration correlation coefficients (r^2) of the elements were greater than 0.999. The rinse solution contained 2% HNO3 + 1% Triton X 100.

Analytical Validation. Frozen bovine blood reference material for toxic metals (SRM 966, NIST) was used for quality control and to validate the assay. The recoveries of the elements in the spiking, reference bovine blood samples, and QC samples were previously described [28].

MMA^V Reductase Assay. The reaction mixture contained 0.1M Tris HCL (pH 8.0), 5 mM GSH, 15 mM MMA(V), 8.1 x 10^9 cpm of [^{14}C]MMA(V), and human blood in a final volume of 250 µl and incubated for 60 min at 37° C. The MMA(III) that was produced was isolated by the extraction method previously described [13,29].

DMA^V Reductase Assay. The reaction mixture (250 µl) was the same as in MMA(V) reductase assay except that 8.2 x 10^9 cpm of [^{14}C]DMA(V) and 10 mM sodium DMA(V) replaced MMA(V). The product of the reaction that was formed was measured as previously described[13,29].

Protein Assay. Protein concentrations were determined using bovine serum albumin as the standard[30].

Statistical Analysis: The means and standard error (SE) were calculated. The unpaired t test (GraphPad Software, Inc., 2005) was used to analyze the significance difference. The correlation coefficients for different variables were tested using the Spearman Rank order correlation test (Richard Lowry, 1998-2009). *P* values less than 0.05 were considered significant.

Results

There were five groups (Gps) of participants based on total arsenic concentration in their drinking water in this study. The general characteristics of the study population have been

Table 1: Trace elements concentrations in whole bloods of females (F) and males (M) from As exposed people in the Lagunera area of Mexico. Values are the mean ± SE (F, n=98 and M, n=93).

	As	**Se**	**Zn**	**Co**	**Cu**	**Pb**	**Cd**	**Hg**	**Ni**	**Mn**
F	9.5±0.2	235±3.2	5453±61	0.87±0.04	945±16	21.3±1.1	1.2±0.1	BDL	3.6±0.3	16.3±0.3
M	11.2±0.4	233±3.0	5765±78	0.78±0.04	768±9.4	28.1±1.2	1.3±0.1	BDL	3.3±0.2	14.6±0.3
P values (F vs. M)	<0.0001*	>0.05	<0.01*	>0.05	<0.0001*	<0.0001*	>0.05		>0.05	<0.001*

BDL = Below Detection Limit; *Statistically significant

Table 2. Spearman correlation coefficients for blood element concentrations (µg/L) versus blood MMA(V) or DMA(V) reductase activities (nmol MMA(II) or DMA(III) formed/ mg protein/hr) of females and males.

	As	Se	Zn	Co	Cu	Pb	Cd	Ni	Mn
Females:									
MMA reductase	0.092	0.143	0.011	0.196	0.175	-0.044	0.175	0.258[a]	0.026
DMA reductase	-0.184	0.019	0.128	-0.141	-0.091	-0.145	-0.216[a]	-0.317[b]	0.009
Males:									
MMA reductase	-0.015	-0.138	-0.055	0.019	0.048	-0.124	0.035	0.157	0.026
DMA reductase	-0.067	-0.027	-0.049	-0.130	0.137	-0.204[a]	0.049	-0.132	-0.094

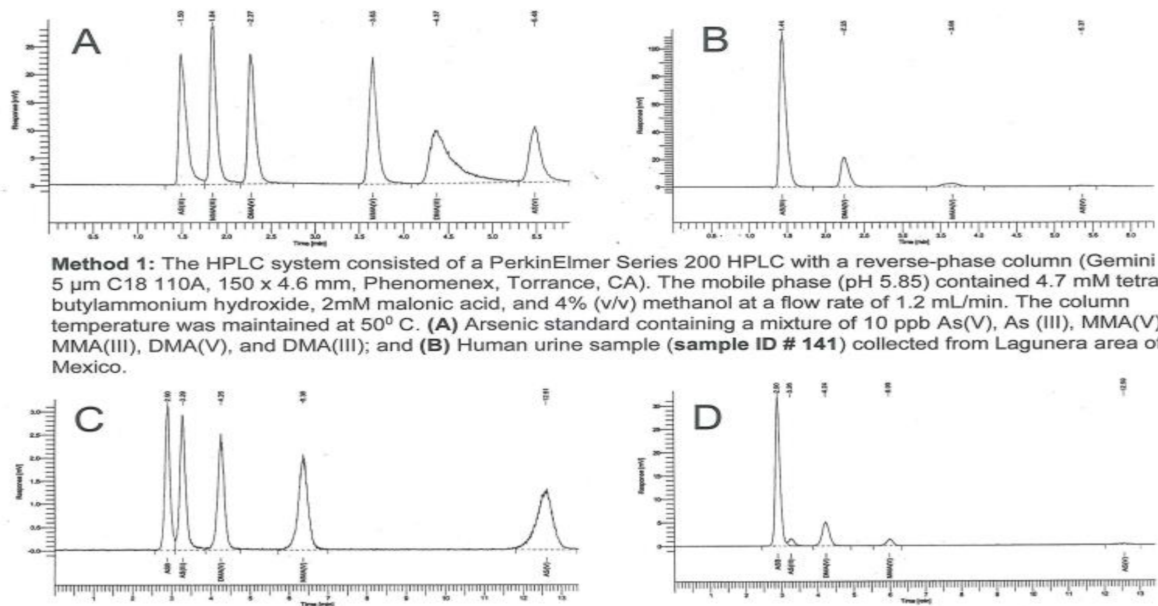
[a]p<0.05, [b]p<0.01

Table 3. Spearman correlation coefficients: (a) the ratio of Ni to As concentrations or (b) blood Ni concentrations (µg/L) versus blood MMA(V), DMA(V) reductase activities (nmol MMA(III) or DMA(III) formed/ mg protein/hr) or percentage of urinary As metabolites for females and males.

	Ratio of Ni to As	Ni levels (µg/L)
Females:		
MMA (V) reductase	0.329[b]	0.258[a]
DMA (V) reducrase	-0.282[b]	-0.317[b]
% As (V)	-0.198	-0.304[b]
% As (III)	-0.152	-0.224[a]
% Inorg As	-0.120	-0.179
% MMA	-0.090	-0.144
% DMA	0.152	0.185
% MMA/% inorgAs	0.071	0.116
% DMA/% MMA	0.154	0.186
Males:		
MMA (V) reductase	0.202	0.157
DMA (V) reducrase	-0.133	-0.132
% As (V)	-0.071	-0.244[a]
% As (III)	-0.035	-0.111
% Inorg As	-0.039	-0.152
% MMA	-0.108	-0.116
% DMA	0.067	0.217[a]
% MMA/% inorgAs	-0.026	0.100
% DMA/% MMA	0.127	0.178

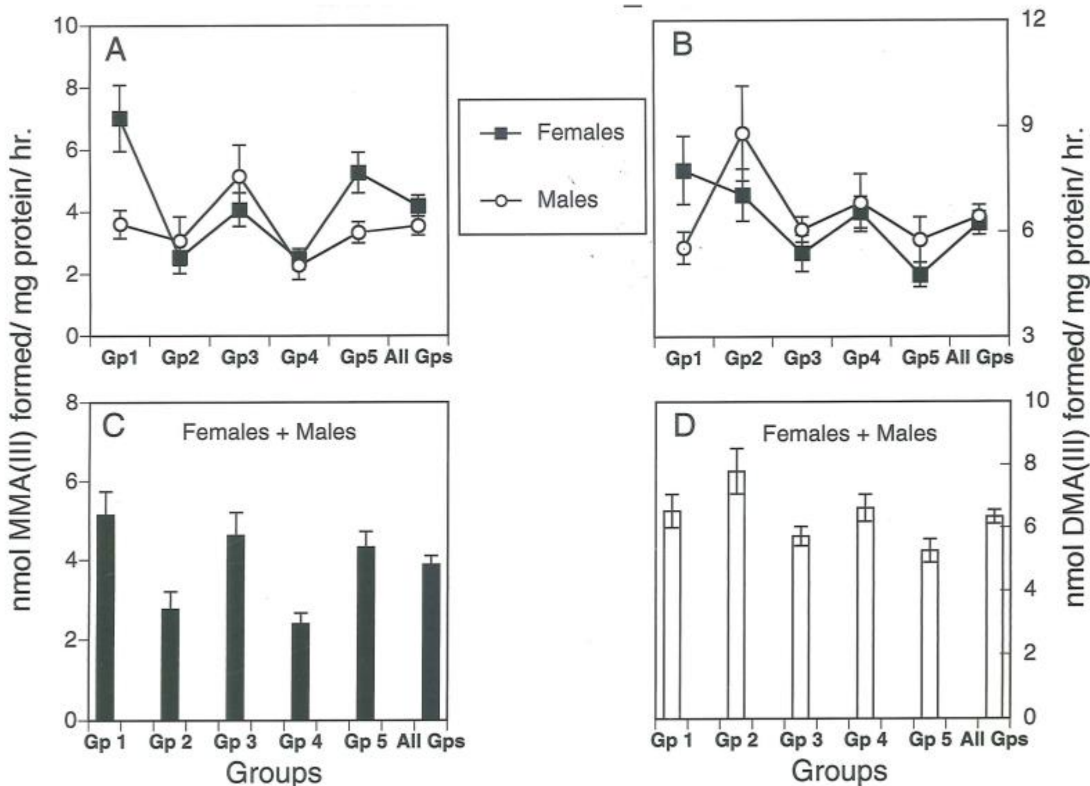
[a]p<0.05, [b]p<0.01

Figure 2. Method and chromatogram for the separation of arsenic species.



Method 2: The HPLC system consisted of a PerkinElmer Series 200 HPLC with an anion exchange column (Gemini PRP-X100, 10 µm, 250 x 4.6 mm, Hamilton Company, Nevada). The mobile phase (pH 8.5) contained 10 mM ammonium nitrate and 10 mM ammonium phosphate (dibasic) at a flow rate of 1 mL/min. The column temperature was maintained at 30^0 C. **(C)** Arsenic standard containing a mixture of 10 ppb AsB, As(V), As(III), MMA(V), and DMA(V); and **(D)** Human urine sample (**sample ID # 141**) collected from Lagunera area of Mexico.

Figure 3. Distribution of MMA(V) and DMA(V) reductase activities in bloods of females and males of different arsenic exposure groups. Values are the mean ± SE.



previously described in detail[28]. Human blood and urine samples were collected, and immediately frozen.

Trace element levels in Blood. We measured the concentrations of As, Se, Zn, Co, Cu, Pb, Cd, Ni, Mn, and Hg in human blood samples of female (F) and male (M). The mean concentrations of these elements expressed as µg/L in blood of females and males are shown in Table 1. The results show that the mean concentrations of Cu and Mn were significantly higher in blood of females compared to males (p<0.0001 and p<0.001, respectively), but the concentrations (mean) of As, Zn, and Pb were significantly lower in blood of females compared to males (p<0.0001, p<0.0001, and p<0.01, respectively). The mean concentrations of Se, Co, Cd, and Ni in bloods were not statistically significant difference between females and males. But the mean concentration of Co was higher in blood of females compared to males (0.87 +0.04 µg/L and 0.78=0.04 µg/L, respectively). There were more than 92% and 88% of the blood samples contained below working MDL concentrations of Hg (<1.10 µg/L) for females and males, respectively.

MMA(III) and DMA(III) in urines of females and males. All urine samples contained bellow detection limit of arsenic species MMA(III) (monomethylarsonous acid) and DMA(III) (dimethylarsinous acid) in the Lagunera area of Mexico.

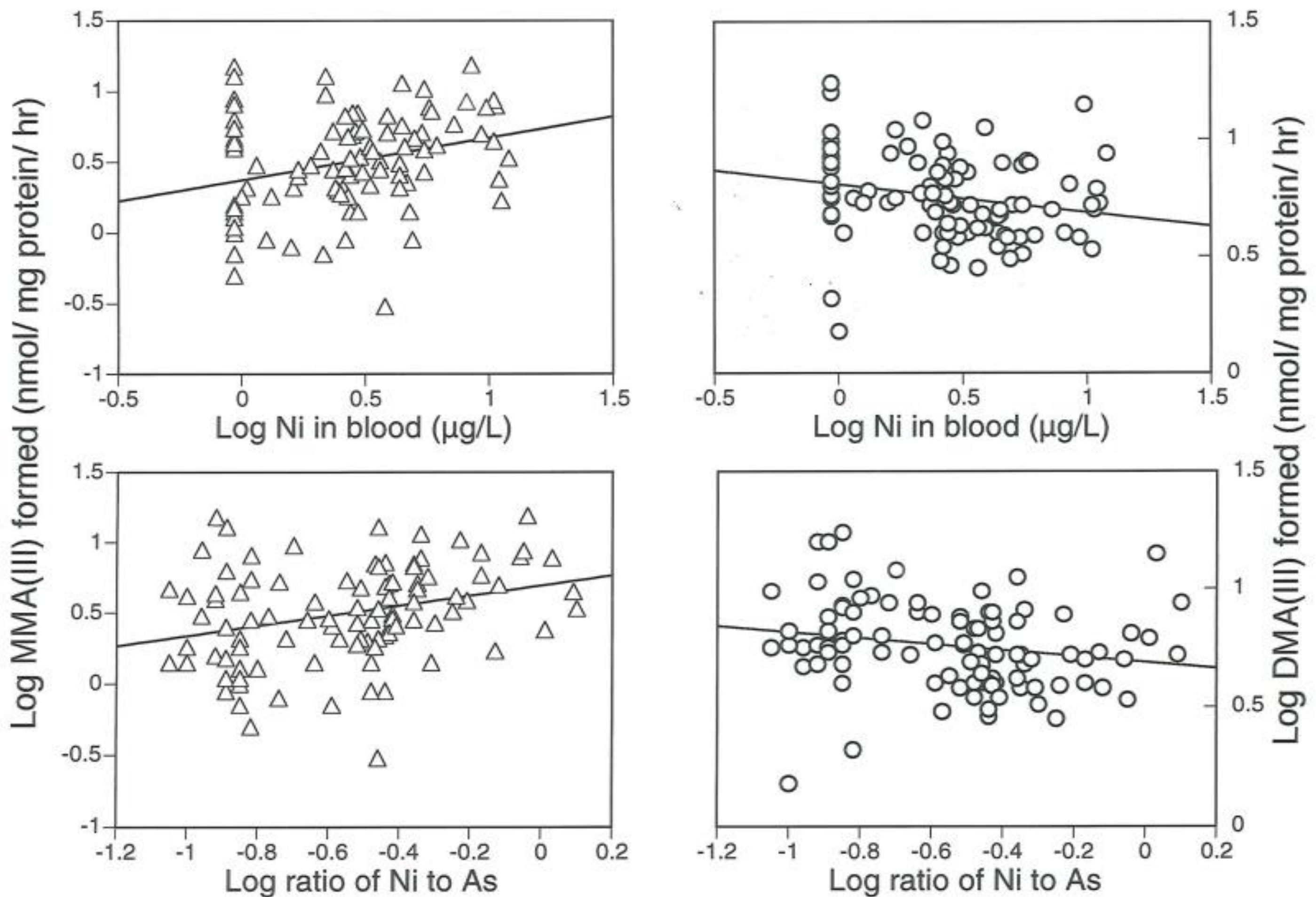
MMA(V) and DMA(V) reductase activities in bloods of females and males. The reductase activities in bloods from females and males of different arsenic exposure groups are shown in Figure 3. The activities of MMA(V) reductase were significantly lower than DMA(V) reductase activities in bloods of our study population (p<0.01; Mean values: 3.87±0.22 MMA(III) formed/ mg protein/hr versus 6.34 ±0.22 DMA(III) formed/ mg protein/ hr, respectively). The activities of MMA(V) reductase and DMA(V) reductase in blood between females and males of different arsenic exposure groups were very close (Fig. 3A and 3B, respectively) except for group 1 (Gpl). For Gpl, MMA(V) and DMA(V) reductase activities in blood were significantly higher of females compared to males (p<0.01 (Fig. 3A) and p<0.05 (Fig. 3B), respectively). We also found that MMA(V) reductase activities were significantly lower for Gp2 and Gp4 than the other groups (Gp2 vs. Gpl, p<0.01; Gp2 vs. Gp3, p<0.05; Gp2 vs. Gp5, p<0.05; Gp4 vs. Gp1, p<0.01; Gp4 vs. Gp3, p<0.01; and Gp4 vs. Gp5, p<0.01) (Fig. 3C). Overall DMA(V) reductase activities were significantly higher for Gp2 than Gp3 or Gp5 (p<0.01 and p<0.01, respectively) (Fig. 3D).

The correlations between MMA(V) reductase and DMA(V) reductase activities in blood for both females and males were very weak (r= +0.06 and r= -0.03, respectively). The activities of MMA(V) and DMA(V) reductase were not depended on ages of this population (r= -0.05 and r= -0.11, respectively) too. A weak correlation found between MMA(V) reductase activities in blood and % MMA(V) in urine of males (r= -0.14, p=0.19) but not of females (r= -0.01, p=0.93). We also found a negative and statistically significant correlation between DMA(V) reductase activities in blood and %DMA(V) in urine of males too (r= -0.23, p<0.05). This correlation was not statistically significant for females (r=-0.12, p=0.25).

Influence of element concentration on MMA(V) and DMA(V) reductase activities in human blood. The correlation coefficients between element concentrations in blood expressed as µg/L versus MMA(V) or DMA(V) reductase activities in blood [nmol MMA(III) or DMA(III) formed/ mg protein/ hr] of females and males are shown in Table 2. The concentrations of Ni in blood were positively and significantly correlated with MMA(V) reductase, and negatively and significantly correlated with DMA(V) reductase activities in blood of females (r= +0.26, p<0.05 and r=-0.32, p<0.01, respectively). We also found that MMA(V) reductase and DMA (V) reductase activities were positively and negatively correlated with Ni concentrations in blood of males, respectively. But these correlations were not statistically significant. The correlations between other element (As, Se, Zn, Co, Cu, Pb, Cd, or Mn) and MMA(V) reductase or DMA(V) reductase activities in blood were not statistically significant except for the correlation between DMA(V) reductase and Cd concentrations in blood of females, and Pb concentrations in blood of males (r= -0.22, p<0.05 and r= -0.20, p<0.05, respectively).

The ratios of the concentrations of Ni to As in blood were more positively correlated with MMA(V) reductase activities than the correlation found between the concentrations of Ni and MMA(V) reductase activities in blood of both females and males (For example, females: r= +0.33, p<0.01 versus r= +0.26, P<0.05 and males: r= +0.20, p=0.06 versus r= +0.16, p=0.15) (Table 3 and Figure 4). For total population in this study, the ratios of the concentrations of Ni to As and Ni concentrations in blood were positively and significantly correlated with MMA(V) reductase activities (r= +0.28, p<0.01 and r= +0.23, p<0.01, respectively), and negatively and significantly correlated with DMA(V) reductase activities (r=-0.21, p<0.01 and r= -0.23, p<0.01, respectively) in bloods. The ratios of the concentrations of other

Figure 4. Spearman correlation coefficients for Ni concentrations or the ratio of Ni to As concentrations versus MMA(V) or DMA(V) reductase in blood of females.



element (Se, Zn, Co, Cu, Pb, Cd, or Mn) to As in blood were not significantly correlated with MMA(V) reductase or DMA(V) reductase activities in blood for both females and males. Nickle (Ni) concentrations in blood (µg/L) were negatively and significantly correlated with %As (V) in urine for both females and males ($r= -0.30$, $p<0.01$ and $r=-0.24$, $p<0.05$, respectively) (Table 3). We also found that the %DMA in urine was significantly and positively correlated with Ni concentration in blood for males ($r= +0.22$, $p<0.05$).

Discussion

In this study, we found that the concentrations of As, Zn, and Pb were significantly lower, and the concentrations of Cu and Mn were significantly higher in blood of females compared to males.

This is the first study to provide the concentrations of trace elements (Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn) in whole blood of the population exposed to arsenic in drinking water living in the Lagunera area of Mexico. For this population in general, the mean concentrations of Se, Zn, Co, Cu, Pb, Cd, Ni, and Mn were 233.89±2.17, 5604.64±50.18, 0.83±0.03, 859.10±1.20, 24.62±0.83, 1.25±0.04, 3.45±0.19, and 15.47±0.23 µg/L, respectively. The concentrations found for Zn, Co, Cu, Pb, Cd, Ni, and Mn in bloods are in the range as in another populations[31-35]. But the concentrations of Se in blood are higher than the other studies[31,32,34]. The main reason for higher concentrations of Se in blood is that the percentage of Se recovery in blood was high (129 %, for spike value10 µg/L) and after correction the values are in the range observed in other regions of the world[32,34]. Selenium concentrations in body fluids (for example, blood) are known to be extremely susceptible to changes in dietary intake and reflect even short-term variations in input[32]. The major dietary source of selenium is plant foods, but some meats and seafood can also contribute dietary selenium.

There were significant differences of the concentrations of As, Zn, Cu, Pb, and Mn, but not Se, Co, Cd, and Ni in blood between females and males in this study. The concentrations of Cu and Mn were higher, and the concentrations of As, Zn, and Pb were lower in blood of females compared to males. Other researchers have also been reported that the concentrations of Cu were higher[31,35], and the concentrations of Zn and Pb were significantly lower[31,34-36] in blood of females compared to males. These finding could be explained by differences between females and males in dietary habits, element exposure level, and element absorption. It is also well known that the composition of whole blood from female and male is different. Due to lower concentrations of red cell in blood of females, it is expected that the concentrations of element that is bound to red cell will be lower in blood of females compared to males[32].

The concentrations of Zn in blood of females significantly increased and their Cu concentrations decreased with age, which has also been shown by Rodrigues et al. (2009) [35]. For males, these correlations were reversed and the concentrations of Cu in blood were positively and significantly correlated with age. We also found that the concentrations of Mn in blood for both females and males were negatively correlated with age and the correlation was statistically significant for females. The influence of age on blood Mn concentrations has not been demonstrated before as far as we know. The changes of lifestyle (food and drinking habits) could be a part of the explanation of the changes with age.

In this study, strong positive correlation found between BAs (blood arsenic) and BCo (blood cobalt) as well as BAs and BNi (blood nickel) of both females and males. These correlations have not been reported before. Norwood et al. (2007)[37] has been observed that arsenic bioaccumulation in the amphipod *Hyalella azteca* was enhanced with increased the concentration of metals including Co and Ni in the mixture exposure. So, we need to know that arsenic accumulation in the tissues of animal model would be enhanced or would not be enhanced with increasing the concentrations of Co and/or Ni in the mixture exposure, and this may help to know the mechanisms of arsenic toxicity in humans.

The most important findings in this study that the concentrations of Ni in blood were positively and negatively correlated with MMA(V) reductase and DMA(V) reductase activities in blood, respectively for both females and males. The reductions of arsenate to arsenite, MMA(V) to MMA(III), and DMA(V) to DMA(III) are catalyzed in vitro by GSTO1, this enzyme is crucial in the pathway for the methylation of inorganic As since only arsenic species having an oxidation state of +3 can be methylated[8,29,38,39]. Uthus and Poellot (1996)[40] have been shown that dietary Ni, folate, and their interaction could affect variables associated that Ni may be important in processes related to the vitamin B12 / folate dependent pathway in methionine metabolism, possible one-carbon metabolism. Gamble et al. have been reported that folate could facilitate arsenic methylation[41] and that folate supplementation could enhance arsenic methylation, decrease blood MMA, and increase urinary DMA[9,42]. MMA(V) reductase activities were positively correlated with the concentrations of nickel in blood and decreased urinary %MMA and increased urinary %DMA as well as the ratios of %DMA to %MMA for both females and males in our study. Therefore, the explanation of our findings that may be Ni and folate worked together and influenced arsenic reductase activities during the biotransformation process of inorganic arsenic in humans.

Results also suggest that more than one methylase may involve in the inorganic arsenic biotransformation pathway because Ni was positively correlated with MMA(V) reductase, but negatively correlated with DAM(V) reductase in bloods for both females and males in our study population. Other researchers have been suggested that more than one methylase are involved in the oxidative methylation of inorg As[8,43,44].

In conclusion, nickel may influence arsenic reductase activities in tissues for biotransformation process of inorganic arsenic in humans and it could enhance arsenic methylation, decrease urinary MMA, and increase urinary DMA.

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