A review on environmental factors regulating arsenic methylation in humans

Chin-Hsiao Tseng

National Taiwan University College of Medicine, Taipei, Taiwan
Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
Department of Medical Research and Development, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan
School of Public Health, Taipei Medical University, Taipei, Taiwan
Division of Environmental Health and Occupational Medicine of the National Health Research Institutes, Taipei, Taiwan

Abstract

Subjects exposed to arsenic show significant inter-individual variation in urinary patterns of arsenic metabolites but insignificant day-to-day intra-individual variation. The inter-individual variation in arsenic methylation can be partly responsible for the variation in susceptibility to arsenic toxicity. Wide inter-ethnic variation and family correlation in urinary arsenic profile suggest a genetic effect on arsenic metabolism. In this paper the environmental factors affecting arsenic metabolism are reviewed. Methylation capacity might reduce with increasing dosage of arsenic exposure. Furthermore, women, especially at pregnancy, have better methylation capacity than their men counterparts, probably due to the effect of estrogen. Children might have better methylation capacity than adults and age shows inconsistent relevance in adults. Smoking and alcohol consumption might be associated with a poorer methylation capacity. Nutritional status is important in the methylation capacity and folate may facilitate the methylation and excretion of arsenic. Besides, general health conditions and medications might influence the arsenic methylation capacity; and technical problems can cause biased estimates. The consumption of seafood, seaweed, rice and other food with high arsenic contents and the extent of cooking and arsenic-containing water used in food preparation may also interfere with the presentation of the urinary arsenic profile. Future studies are necessary to clarify the effects of the various arsenic metabolites including the trivalent methylated forms on the development of arsenic-induced human diseases with the consideration of the effects of confounding factors and the interactions with other effect modifiers.

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Introduction

It is well established that chronic exposure to arsenic can cause cancerous and non-cancerous health hazards in a dose-responsive pattern, and environmental exposure to inorganic arsenic, especially from drinking water or occupational settings, has caused calamity in several countries (Alam et al., 2002; Centeno et al., 2007; Mandal and Suzuki, 2002; Tseng, 2005). However, recent studies suggest that the development of human diseases associated with chronic arsenic exposure including peripheral arterial disease, hypertension, arsenic-induced skin lesions, skin cancer and urothelial cancer (mainly bladder cancer) is not only determined by the dosage of exposure, but also by the individual methylation capacity and the metabolic patterns (Tseng et al., 2005; Tseng, 2007a, 2007b; Steinmaus et al., 2006). The World Health Organization (WHO) recommended a maximal level of 50 μg/L of arsenic in drinking water since 1963. With increasing evidence showing a link between exposure to low arsenic concentrations and cancer risk, the WHO lowered the recommendation level to 10 μg/L in 1992 (WHO, 1993). Currently the maximal allowable concentration of arsenic in drinking water has been lowered to 10 μg/L in Taiwan since 2000, in the European Union since 2003 and in the USA since 2006. However the national standard for arsenic in drinking water in some countries such as Bangladesh, Chile, China and India remains at 50 μg/L.

In surface water, pentavalent arsenate is the most common inorganic species and trivalent arsenite is more likely to exist in anaerobic ground water. The main organ for arsenic metabolism is the liver, but the metabolic pathway of inorganic arsenic is not yet fully clarified. In the pH range of 4 to 10, trivalent arsenic compounds are neutral in charge and pentavalent arsenicals are negatively charged. Therefore trivalent arsenic species are more ready to cross cell membrane and pentavalent species cannot easily enter the cells at physiologic pH (Cohen et al., 2006). Inorganic pentavalent arsenate is mostly reduced to trivalent arsenite in the blood stream before entering the cells for further metabolism (Cohen et al., 2006; Vahle, 2002). It is also now known that both arsenite and arsenate can be actively transported into cells via aquaglyceroporins and phosphate transporters, respectively (Rossman, 2003).

If monomethylarsonic acid (MMAV) or dimethylarsinic acid (DMAV) are administered orally to humans, they are excreted in the urine largely in the unchanged forms and only a minor proportion of MMAV is converted to DMAV and no further metabolism of DMAV is observed [but a study reported 4% of trimethylated form of DMAV are administered orally to humans, they are excreted in the urine largely in the unchanged forms and only a minor proportion of MMAV is converted to DMAV and no further metabolism of DMAV is observed (Francesconi et al., 2002; Raml et al., 2005)].

Fig. 1 shows the generally accepted classical pathway (Fig. 1A) and a newly proposed pathway (Fig. 1B) for arsenic metabolism. In the generally accepted classical pathway, inorganic trivalent arsenite is a preferential substrate for As(+3 oxidation state)-methyltransferase (As3MT, or previously known as cyt19) and the metabolism involves a series of reduction and oxidative methylation, in which the pentavalent arsenic species are formed before the respective trivalent species, and mono- and dimethylated metabolites are generated sequentially (Fig. 1A) (Tseng, 2007a). The reduction of MMAV to monomethylarsonous acid (MMAIII) may be the rate-limiting step in the metabolism of inorganic arsenic (Cohen et al., 2006; Zakaryan et al., 2001). In humans such metabolic process is not complete and inorganic arsenic along with mono- and dimethylated metabolites are excreted in urine. Determination of the concentration and the proportional distribution of the various arsenic species including the inorganic arsenic and the methylated metabolites in urine can give a reflection of the capacity to methylate inorganic arsenic in human body. Some investigators used ratio expressions of the urinary arsenic metabolites as surrogate markers for methylation capacity. For example, primary methylation index (PMI) defined as the ratio between MMAV and inorganic arsenic (arsenate+arsenite) level, and secondary methylation index (SMI) as the ratio between DMAV and MMAV are used to assess the arsenic methylation capacity of the first and second methylation step.
respectively (Tseng, 2007a). Others calculate the percentage ratio between MMA\textsuperscript{V} plus DMA\textsuperscript{V} and total arsenic for assessing the methylation capacity (Tseng, 2007a).

The newly proposed pathway suggested that trivalent methylated arsenic species might be formed before the respective end-products of pentavalent species (Fig. 1B) (Hayakawa et al., 2005). This newly proposed pathway is compatible with the concept that “oxidation is detoxification of arsenic” (Aposhian et al., 2003) because trivalent methylated arsenic species are more toxic than the pentavalent methylated arsenic species and the inorganic species (Bredfeldt et al., 2006). However, this hypothetical pathway requires further confirmation.

Trivalent dimethylarsinous acid (DMA\textsuperscript{III}) has neutral charge and can diffuse easily into cells (up to 16%); but the negatively charged pentavalent methylated species can poorly enter the cells (0–2%) (Dopp et al., 2005). The arsenic methylation process, which is regulated by As3MT, primarily occurs in human hepatocytes (Dopp et al., 2008). Although fibroblasts and urothelial cells do not express As3MT and are deemed as non-methylating tissues, the uptake or accumulation of organic and inorganic arsenuic acids in these cell types are more efficient than in the methylating hepatocytes (Dopp et al., 2008). However, cytotoxicity and genotoxicity are more distinct in hepatocytes because the arsenic species preferentially accumulate in the nuclei and mitochondria of the hepatocytes, in contrast to in the ribosome of urothelial cells (Dopp et al., 2008).

Inter-individual variation in arsenic metabolism has been observed (Concha et al., 2006). This could be one of the possible explanations for the inter-individual susceptibility to arsenic-induced health hazards (Tseng, 2007a). In case of long-term exposure to arsenic from drinking water, urinary arsenic concentration seems to be a better marker than concentrations in drinking water (due to spatial and temporal variation) or hair (due to external contamination) (Concha et al., 2006). Increased urinary MMA\textsuperscript{V} in arsenic-exposed subjects suggests an inefficient methylation and probably an increased concentration of the highly toxic MMA\textsuperscript{V} at cellular level. Epidemiologic studies have confirmed a significant association between increased levels of urinary MMA\textsuperscript{V} or less efficiency in arsenic methylation and arsenic-related health hazards including bladder cancer, skin cancer or other skin lesions, structural chromosomal aberrations, peripheral arterial disease and hypertension (Huang et al., 2007; Steinmaus et al., 2006; Tseng, 2007a).

Therefore, the toxicity of arsenic is closely related to its metabolism and is highly dependent on the methylation status and the valence state of the metabolites. Growing epidemiologic evidence also suggests that some factors are related to its metabolism and can be important predictors for arsenic-related health hazards. Although the association between arsenic methylation capacity and human diseases (Tseng, 2007a, 2007b) and the association between genetic factors regulating arsenic metabolism and human diseases (Ghosh et al., 2008; Hernández and Marcos, 2008) have been extensively reviewed recently, a general review on genetic traits than the other two pairs, compared to the lowest

arsenic metabolism is not the aim of this article because interested readers can refer to other review articles (Ghosh et al., 2008; Hernández and Marcos, 2008).

**Ethnicities**

Arsenic metabolites in the urine of various population groups have been reported to be fairly constant initially, i.e. 10–30% inorganic arsenic, 10–20% MMA\textsuperscript{V}, and 60–80% DMA\textsuperscript{V} (Vahter, 1999). However, ethnic differences have clearly been shown recently. Indigenous people living in the Andes in Northern Argentina (mainly Atacamenos) and exposed to arsenic concentration of about 200 μg/L excreted only a few percent of MMA\textsuperscript{V} (2.1% in women to 3.6% in children) (Concha et al., 1998a). In the Yacu Valley, Sonora, Mexico, adults exposed to mean arsenic concentrations of 5.5–43.3 μg/L in well water excreted 7.5–9.7% MMA\textsuperscript{V} in the urine (Meza et al., 2004). Adults in Nevada, USA with a high arsenic exposure dose of 1300 μg/L excreted 23% of MMA\textsuperscript{V} (Warner et al., 1994). The Taiwanese exposed to arsenic concentration of a wide range from <1 to ≥3000 μg/L seemed to have an unusually high percentage of MMA\textsuperscript{V} in urine, with an average of 20–30% (Chiou et al., 1997).

The ethnic difference in the distribution of urinary arsenic species cannot be explained by the arsenic concentrations of the water and is further confirmed by a study that showed significant differences in the distribution of urinary arsenic species among different ethnicities exposed to comparable dosages of arsenic in high and low arsenic exposure groups in the people of China, Mexico and Chile (Loffredo et al., 2003). Although this study suffered from limitations such as small sample sizes from the populations of Inner Mongolia and Chile, the population in Mexico composing a mixture of Native American and European Ancestry, and the lack of adjustment for important covariates, the differences were remarkable and highly suggestive of an ethnic difference.

Another study by Hopenhayn-Rich et al. (1996b) also suggested an ethnic difference in arsenic methylation capacity. By comparing the urinary arsenic metabolites in people of Atacameno and European ethnic origin living in arsenic-exposure areas in Chile, the Atacamenos were better methylators of arsenic than the Europeans in both univariate and multivariate analyses. It is interesting to note that the indigenous people in Chile have lived in the Andes with exposure to the arsenic-containing drinking water for thousands of years (Smith et al., 2000). Therefore, a natural selection for those with efficient methylation of arsenic is highly possible, which is also responsible for the less severe clinical manifestations in these people.

It is still unclear whether the different capacity for arsenic methylation among different ethnicities could be due to the differences in genetic factors, duration and dosage of exposure, diet, lifestyle or other unknown confounders. The answer to this question awaits some comparative studies derived from multi-ethnic comparisons based on adequate sample sizes, standard study protocols and consideration of potential confounders.

**Family aggregation**

Another evidence for genetic determination of arsenic methylation capacity can be demonstrated by the study by Chung et al. (2002), which compared the urinary arsenic profile of a father, a mother and 2 children in 11 families of inhabitants of the small Chiu Chiu village with about 250 inhabitants in northern Chile, where the inhabitants drank water containing arsenic of 735–762 μg/L. Methylation patterns were compared between father and mother, parent and child, and sibling and sibling. The strongest correlations were observed between siblings who shared most of the similarities in genetic traits than the other two pairs, compared to the lowest
correlations between father–mother pairs who shared the least similarities in genetic traits. These correlation patterns did not change after adjustment for age, sex, total urinary arsenic, or blood micronutrients potentially related to methylation.

**Intra-individual variation**

Although inter-individual variation in arsenic metabolism is observed, the intra-individual variation is small and intra-individual patterns of arsenic methylation are fairly stable over time (Concha et al., 2002; Steinmaus et al., 2005b). A study investigating the two aspects of intra-individual variation of day-to-day variation (n = 15) and within-day variation (n = 7) of urinary arsenic species in Andean healthy women exposed to inorganic arsenic from drinking water (150–170 μg/L) in Argentina disclosed no significant day-to-day variation, but slightly higher MMA\textsuperscript{V} percentage (5.0%) and lower inorganic arsenic percentage (5.8%) during the morning/day (03:00–15:00) period than during the evening/night (15:00–03:00) period. MMA\textsuperscript{V} percentage did not show similar diurnal change (Concha et al., 2002). The lower inorganic arsenic during the day might reflect the lack of arsenic exposure during the night, whereas the increased DMA\textsuperscript{V} during this period might be a result of methylation over the night. However, because the sample sizes are small and the changes are minimal, these diurnal changes require further confirmation. The lack of day-to-day variation was also reported in an earlier study carried out in 96 individuals chronically exposed to drinking water contaminated with arsenic of 8–620 μg/L in Millard County, Utah, USA (Calderon et al., 1999). The stability of intra-individual methylation patterns is also confirmed in a recent study conducted in the USA, which examined the urinary arsenic profile in 81 subjects with low to moderate arsenic exposures from drinking water by collecting multiple urine samples (2 to 3 samples) from each subject over a 1-year period (Steinmaus et al., 2005b).

**Arsenic exposure dosage**

A methylation threshold hypothesis for inorganic arsenic has been proposed, suggesting that after a certain threshold of exposure to inorganic arsenic, the methylation capacity is saturated and the toxic effects of inorganic arsenic will increase (Buchet et al., 1980). Hopenhayn-Rich et al. (1993) analyzed the urinary arsenic species in 10 subjects living in Mojave Desert of California with arsenic exposure from drinking water containing arsenic 300–400 μg/L, together with the urinary arsenic profile reported in arsenic-exposed subjects from various sources and various levels of exposure obtained from other studies. Their results suggested that there was no threshold effect for arsenic methylation as proposed earlier, because a proportion of the inorganic arsenic will remain unmethylated even at a very low dose of exposure, and there is no evidence for a rise in this percentage with increasing dose of exposure. Later studies also did not support this methylation threshold hypothesis in studies carried out in Chile (Hopenhayn-Rich et al., 1993, 1996a, 1996b) and in Finland (Kurttio et al., 1998).

Urinary MMA\textsuperscript{V} percentage might increase and DMA\textsuperscript{V} percentage might decrease with increasing arsenic exposure dosage (Del Razo et al., 1997; Hopenhayn-Rich et al., 1996a; Kurttio et al., 1998; Li et al., 2008; Lindberg et al., 2008a; Styblo et al., 1999). A recent study observed that MMA\textsuperscript{V} percentage started to increase at a very low arsenic exposure level of 50 μg/L in urine (Li et al., 2008). The decrease in methylation efficiency associated with increased arsenic exposure levels could possibly be a result of the inhibition of the second methylation step by excessive inorganic arsenic (De Kimpe et al., 1999; Styblo et al., 2000).

In a prospective follow-up study evaluating the association between arsenic exposure, urinary arsenic metabolites and risk of urothelial cancer in the southwest arseniasis-endemic areas of Taiwan, increased PMI was noted in subgroups with a higher cumulative exposure to arsenic expressed by the following indicators: duration of consuming high-arsenic artesian well water (years), average concentration of arsenic in artesian well water (mg/L), and cumulative arsenic exposure (mg/L-year) (Huang et al., 2008a). It is interesting to observe that the MMA\textsuperscript{V} percentage (16.3% in men and 11.6% in women) in subjects living in the blackfoot disease area of the southwest coast of Taiwan (Tseng et al., 2005) was much lower than the reported 20–30% in the northeast coast of Taiwan (Chiou et al., 1997). One of the possible explanations is that the exposure has been terminated for about 15–20 years when the study in the southwest coast of Taiwan was conducted, but the exposure was continuous at the time when the study at the northeast coast of Taiwan was performed, because MMA\textsuperscript{V} percentage increases with increasing dosage of exposure (Vahter, 1999) and decreases after exposure dosage is reduced (Hopenhayn-Rich et al., 1996a).

However, it should be noted that not all studies agree with such a trend of increasing MMA\textsuperscript{V} percentage and decreasing DMA\textsuperscript{V} percentage in corresponding to increasing dosage of arsenic exposure. In an earlier study comparing the urinary arsenic profile before and after work in six arsenic acid plant workers, except for a borderline significance with increased total urinary arsenic (p = 0.08, Wilcoxon signed rank test, re-analyzed by the author of the present paper), the changes in inorganic arsenic percentage, MMA\textsuperscript{V} percentage and DMA\textsuperscript{V} percentage were not statistically significant (Yamamura and Yamauchi, 1980). In the study by Chieu et al. (1997) arsenic methylation capacity was not associated with the arsenic contents in either drinking water or urine. In the Finnish study, the urinary arsenic profile also did not correlate with the daily dose or the cumulative dose of exposure, especially when the effect of age was adjusted (Kurttio et al., 1998).

**Age**

A slight increase of DMA\textsuperscript{V} with age in adults exposed to arsenic was observed in Finland (Kurttio et al., 1998) but no age or sex differences in children were observed in Argentina (Buchet et al., 1980; Kalman et al., 1990). The effect of age seemed to be independent of exposure dose in the Finnish study (Kurttio et al., 1998). On the contrary, MMA\textsuperscript{V} percentage increased and DMA\textsuperscript{V} percentage and SMI decreased with age significantly in the study by Tseng et al. (2005) and in a recent prospective follow-up study in Taiwan (Huang et al., 2008a), suggesting that decreasing second methylation capacity is associated with increasing age. Similar diminishing capacity with age was also observed by other investigators (Hsu et al., 1997, 1998).

In the study by Hopenhayn-Rich et al. (1996b) conducted in northern Chile, although aging was significantly associated with the different distribution of arsenic metabolites in urine in univariate analyses, characterized by decreasing inorganic arsenic percentage, MMA\textsuperscript{V} percentage and MMA\textsuperscript{V}/DMA\textsuperscript{V} (1/SMI) and increasing DMA\textsuperscript{V} percentage, this aging effect was probably explainable by other confounding factors such as the length of residence (as an indicator of duration of exposure) because age was not a significant associate with any of the urinary arsenic metabolites in multivariate regression analyses.

In a recent hospital-based case-control study evaluating the association between exposure to low arsenic levels and urothelial cancer in Taiwan, age was not associated with any of the urinary arsenic parameters in patients with urothelial cancer (Chung et al., 2008). Similarly in a recent study conducted in Argentina, although aging was associated with a significantly decreasing percentage of inorganic arsenic in urine, the percent distributions of MMA\textsuperscript{V} and DMA\textsuperscript{V} and SMI did not differ significantly among different age groups (Steinmaus et al., 2007). On the other hand, an age of 63 years or older was significantly associated with a lower percentage of urinary inorganic arsenic and a higher percentage of DMA\textsuperscript{V} than those with an
age < 63 years in healthy controls, while the percentage of MMA\(^\gamma\), PMI and SMI were not significantly different between the two age groups (Chung et al., 2008).

Because of the inconsistent results associated with age and the lack of control for potential confounders, the age effect on arsenic metabolism needs to be confirmed. Furthermore, aging can probably be associated with a variety of functional changes in the organs involved in the metabolism or retention of the metabolites of arsenic. Most of the studies did not consider the multi-organ functions and therefore a confounding effect from such functional changes of the organs is possible.

**Adults vs. children**

Children in Bangladesh seemed to have more active second methylation capacity than adults, with lower urinary inorganic arsenic and MMA\(^\gamma\), and higher DMA\(^\gamma\) along with a higher SMI than adults (Chowdhury et al., 2003). A study of 42 spot urine samples collected from Datterhat (South) village of Madaripur district of Bangladesh showed an average arsenic concentration of 376 (118–620) μg/L, in comparison to 27 samples taken from residents of an unexposed area in the Badhadam village of Medinipur district of West Bengal, India where drinking water contained arsenic < 3 μg/L (Chowdhury et al., 2003). While comparing the urinary arsenic parameters between adults and children below 11 years of age, the children seemed to retain less arsenic in body tissue such as hair and nail, and more efficiently excreted arsenic in urine. However, this study gave only the average and range of the arsenic species in different subgroups and fell short of a small sample size and not giving any statistical analyses throughout the text. Therefore it is not known whether the differences between adults and children were statistically significant or not. The comparison of the urinary arsenic species of four pairs of one adult and one child from a single family using the same tubewell water seemed to give evidence that children are better methylators and excrete more total urinary arsenic, lower inorganic arsenic percentage, lower MMA\(^\gamma\) percentage, higher DMA\(^\gamma\) percentage and higher SMI. But when the author of the present paper re-analyzed the data (replacing the ND in inorganic arsenic percentage with 0.5%) with Wilcoxon signed ranks test, only the differences for total urinary arsenic and SMI showed borderline significance (2-tailed p values=0.068), while the other parameters did not differ (p=0.1) between adults and children. The reason for a lack of significant difference could possibly be due to the small number of cases, which did not provide sufficient power for detecting statistical differences. The authors suggested that the more efficient second methylation of arsenic in children could possibly explain the less incident of skin lesions in children exposed to arsenic in Bangladesh. However, this hypothesis requires further investigation because children tend to have shorter durations of exposure than the average latency for skin lesions, which has been estimated as 23 years from first exposure to arsenic in a nested case-control study conducted in West Bengal, India (Haque et al., 2003).

Another study investigating the urinary arsenic species in 51 subjects from 12 arsenic-affected families using water from 7 tubewells in Mushidabad district in West Bengal, India listed the individual data including the presence of skin lesions such as melanosis or keratosis in 24 subjects (Tokunaga et al., 2002). The study did not evaluate the association between skin lesions and the urinary arsenic species. The author of the present paper re-analyzed the data using the individual data of age, sex, symptoms, arsenic concentrations in drinking water, urinary arsenic species listed in the paper and calculated the PMI and SMI. However, the author of the present paper could not find a better arsenic methylation capacity in the children as indicated by SMI or PMI from the crude data listed.

Not all studies agree with a better methylation in children. In indigenous people (mainly Atacamenos) living in San Antonio de los Cobres in northeast Argentina and exposed to drinking water containing arsenic of about 200 μg/L, the urinary MMA\(^\gamma\) percentage in children was slightly higher than in adult women (3.6% vs. 2.1%) (Concha et al., 1998a). Similarly in another area with comparable arsenic exposure in Taco Pozo, where most of the inhabitants were descendants of Spanish immigrants, the children also had slightly higher urinary MMA\(^\gamma\) percentage than adult women (3.4% vs. 2.2%) (Concha et al., 1998a). In both of the above areas, children also had significantly higher level of urinary inorganic arsenic percentage, especially in San Antonio (49% vs. 25% for children vs. women). These results suggested that the methylation capacity of children was poorer or not better than adult women and might be more sensitive to arsenic toxicity. The data presented by Chung et al. (2002) in 11 Chilean families also did not support a better methylation capacity in the children.

Therefore current studies seem to be contradictory and inconsistent in the comparison of the methylation capacity between adults and children. The case numbers of most studies are relatively small and some suffer from the limitation of making a conclusion without adequate statistical analyses.

**Sex**

Most studies suggest that women have a better methylation capacity than men. This sexual difference in arsenic methylation capacity could probably be explained in part by the effect of estrogen as discussed later (Fig. 2). In the study by Tseng et al. (2005) carried out in the BFD area, women had a higher DMA\(^\gamma\) percentage and SMI, and a lower urinary total arsenic and MMA\(^\gamma\) percentages than men had, indicating that women possessed a higher capacity of arsenic methylation than their men counterparts did. This sexual difference was not only observed in univariate analyses, but also in multiple regression analyses. Similar sexual difference was observed in many other studies indicating a more efficient methylation capacity in women (Chung et al., 2008; Gamble et al., 2005; Huang et al., 2008a, 2008b; Lindberg et al., 2008a, 2008b; Pu et al., 2007; Steinmaus et al., 2005b, 2007). In the study by Hopenhayn-Rich et al. (1996b) conducted in northern Chile, in the presence of similar urinary total arsenic, women were better methylators of arsenic with significantly lower MMA\(^\gamma\) percentage and MMA\(^\gamma\)/DMA\(^\gamma\) (1/SMI) and higher DMA\(^\gamma\) percentage in both the univariate and multivariate analyses. A recent study conducted in Bangladesh also confirmed a less efficiency of arsenic methylation in men, which explained the higher risk of arsenic-induced skin lesions in men than in women (Lindberg et al., 2008b). Similarly women had a significantly lower percentage of inorganic arsenic in urine, a higher percentage of DMA\(^\gamma\) and higher SMI with borderline significance but insignificant difference in percentage of MMA\(^\gamma\) in a recent study in Argentina (Steinmaus et al., 2007).

However, sexual difference in arsenic methylation capacity was not observed by some other investigators (Chen et al., 2003; Chiu et al., 1997; Kurttio et al., 1998). The sexual difference in arsenic methylation capacity in the Finnish study observed in univariate analyses became insignificant after adjustment for the effect of age (Kurttio et al., 1998).

An opposite result was observed by Chen et al. (2003) showing that women had significantly higher PMI than men did. But it is not known whether selection bias existed and whether there was significant difference in age distribution between men and women in that study, because there were more men in the cases than in the controls (70.6% vs. 58.5%).

**Pregnancy and breast feeding**

Pregnant women seem to have more efficient methylation of arsenic than non-pregnant women, and the methylation capacity increases with increasing duration of pregnancy. Although the etiology
for an increased arsenic methylation capacity during pregnancy is not known, it is possible that estrogen may play a role (Fig. 2, to be discussed later). The influence of pregnancy on methylation capacity mainly comes from 2 studies in Argentina and Chile, respectively.

Concha et al. (1998b) studied the urinary arsenic profile in a group of pregnant women of native Andeans living in the village San Antonio de los Cobres in the northwest of Argentina, where the drinking water contained arsenic of about 200 μg/L. The urinary arsenic profile was determined in 11 pregnant women before delivery, 2.8 weeks postpartum, 2.5 months postpartum and 4.4 months postpartum. It was evidenced that before delivery the inorganic arsenic percentage and MMAV percentage were lowest, and DMAV percentage the highest; after delivery the inorganic arsenic percentage and MMAV percentage increased and DMAV percentage decreased gradually to the levels comparable to adult women without pregnancy. The levels of DMAV in urine and blood were >90% before delivery (Concha et al., 1998b).

Another recent study measuring a sequence of 4 samples of the urinary arsenic profile during the course of pregnancy in 26 women living in Antofagasta, Chile, who drank tap water containing inorganic arsenic of 40 μg/L has found that total urinary arsenic increased with increasing weeks of gestation, from an initial 36.1 μg/L to a final 54.3 μg/L (Hopenhayn et al., 2003b). Although the cause of the increase of the total urinary arsenic during pregnancy is not clear (may be due to an increase of water intake or due to increased release of tissue-bound arsenic), the urinary arsenic profile showed decreasing inorganic arsenic percentage, MMAV percentage and MMAV/ DMAV (1/SMI), and increasing DMAV percentage, all of them were statistically significant (Hopenhayn et al., 2003b). Although the toxicologic significance of these changes in urinary arsenic profile during pregnancy is not known, the results were consistent with the earlier study by Concha et al. (1998b) showing more efficient methylation of arsenic during pregnancy.

Arsenic can also efficiently pass through the human placenta (Concha et al., 1998b) and may possibly have an effect on pregnancy outcomes (Ahmad et al., 2001; Hopenhayn et al., 2003a; Milton et al., 2005; Rahman et al., 2007; Yang et al., 2003) and early human development (Rosado et al., 2007; von Ehrenstein et al., 2007; Wang et al., 2007; Wasserman et al., 2004; Wasserman et al., 2007). Arsenic exposure in early life may also affect the development of arsenic-induced diseases later in life (Vaher, 2007). DMA is the main form of arsenic metabolite transported from mother to the fetus in late gestation because newborn of arsenic-exposed mother excretes mainly DMA in the urine (Concha et al., 1998b). This could be resulted from an induced methylation by estrogen in mother during pregnancy.

However, in Andean women who were exposed to high levels of arsenic of about 200 μg/L in drinking water, the concentration of arsenic in breast milk was actually very low, with an average of 2.3 (0.83–7.6) μg/kg fresh weight, suggesting that arsenic is not efficiently excreted from human breast milk (Concha et al., 1998c). It has also been shown in a recent study in Matlab, Bangladesh that breastfed infants at 3 months of age had remarkably low percent MMAV (mean 3.8%) and high percent DMAV (mean 84%) in urine, implying an efficient methylation of arsenic during infancy (Fångström et al., 2008). The percent MMAV was only about one third of that seen in their mothers (mean 9.8%) (Fångström et al., 2008) and was much lower than that seen in children aged 5–12 years in the same study area (Lindberg et al., 2008a).

The efficient methylation of arsenic during infancy can be explained by the presence of high circulating concentrations of folate and choline required for the methylation of homocysteine to methionine (Fig. 2). According to studies conducted in Bangladesh, despite a prevalent deficiency of folate in the mothers (Li et al., 2008), fetus can store sufficient folate in the liver (Maloney et al., 2007; Wallace et al., 2008) and additional folate is provided from breast milk (Hay et al., 2008). Similarly choline levels in neonates are elevated due to the estrogen-dependent up-regulation of endogenous synthesis of phosphatidylcholine during pregnancy and lactation to meet the demand of the growing child (Zeisel, 2006a). Breast milk contains the highest levels of free choline during the first 3 months and the child choline levels will decrease slowly with age until they approach adult levels at the age of 5–10 years (Ilicol et al., 2005). Therefore breast feeding protects the infants from arsenic toxicity during early life.

**Estrogen**

The metabolic pathways linking estrogen, choline, betaine, vitamin B12, folate and arsenic methylation are shown in Fig. 2. Choline...
synthesis is increased during pregnancy for fetal tissue growth and brain development (Zeisel, 2006b). Estrogen can up-regulate phosphatidylethanolamine methyltransferase leading to de novo synthesis of choline (Fischer et al., 2007; Vahter, 2007). The betaine formed under the oxidation of choline can donate its methyl group to homocysteine, which is catalyzed by betaine-homocysteine methyltransferase (Fig. 2) (Ueland et al., 2005). This provides an alternative pathway for remethylation of homocysteine to methionine (Li et al., 2008). If this estrogen effect does occur, then it is reasonable to observe a better methylation in women before menopause than in age-comparable men, and probably a similar or even poorer methylation in women after menopause, as shown by the contradictory results in sexual differences in methylation capacity by different observers. Should future studies be conducted, the effect of age, menstruation cycle, the status of menopause and hormone replacement therapy should all be taken into account because of the change of estrogen levels with regards to these physiological or medicinal effects. However, the confounding effects of age, exposure dosage (women tend to drink less water per day than men) or other unidentified factors cannot be completely excluded for the sexual difference in arsenic methylation capacity and the observed change in methylation capacity during the course of pregnancy.

Smoking

Some studies suggest that smoking is associated with a poorer methylation capacity. In a study by Tseng et al. (2005), cigarette smokers had significantly higher urinary total arsenic and MMA\(^\text{V}\) percentage and lower SMI than non-smokers had, implying that smoking could exert an effect on the second methylation phase. This observation was also in conformity to the study by Hopenhayn-Rich et al. (1996b). However, the effect of smoking was observed only in univariate analyses and not in multivariate analyses in the study by Tseng et al. (2005), but remained significant in multivariate analyses in the study by Hopenhayn-Rich et al. (1996b). Similarly, smoking was associated with a higher MMA\(^\text{V}\) percentage and a lower DMA\(^\text{V}\) percentage in the non-cancerous control subjects in a recent hospital-based case-control study evaluating the association between low arsenic exposure and urothelial cancer by recruiting cases and controls from non-arseniasis endemic areas in Taiwan (Pu et al., 2007) and in a prospective follow-up study conducted in the southwest arseniasis-endemic areas in Taiwan (Huang et al., 2008a).

On the other hand, some other studies suggest that smoking is not associated with arsenic methylation capacity in both univariate and multivariate analyses (Kurttio et al., 1998). In a recent study conducted in Argentina, smoking was not associated with any of the urinary arsenic profile including percentage of inorganic arsenic, MMA\(^\text{V}\), DMA\(^\text{V}\) and SMI (Steinmaus et al., 2007).

It is possible that some chemicals in cigarettes compete for some enzymes or co-factors involved in the methylation processes, particularly those involved in the second methylation phase. Cigarettes may also contain some arsenic and it has been estimated that smoking one cigarette will inhale about 0.25 μg of arsenic (ATSDR, 1993). It is possible that some of the urinary arsenic metabolites could be ascribed to arsenic exposure from cigarette smoking. But taking into account the small amount of arsenic content in cigarette, the exposure dosage from cigarette smoking might be negligible when compared to the exposure dosage from contaminated drinking water. Confounding factors (for example, male sex) might also contribute partly to the explanation of a poorer methylation capacity associated with smoking because a much higher proportion of men than women are smokers.

Alcohol, tea and coffee

Comparing with non-drinkers, alcohol drinkers had urinary arsenic profile indicating a poorer methylation capacity with significantly higher inorganic arsenic percentage, MMA\(^\text{V}\) percentage, MMA\(^\text{V}\)/DMA\(^\text{V}\) (1/SMI) and lower DMA\(^\text{V}\) (Hopenhayn-Rich et al., 1996b). But the alcohol effect was not independent in multivariate analyses (Hopenhayn-Rich et al., 1996b).

The alcohol effect on arsenic methylation cannot be confirmed in recent Taiwanese hospital-based case-control studies evaluating the association between exposure to low arsenic level and urothelial cancer (Huang et al., 2008b; Pu et al., 2007) and in a 12-year prospective follow-up study conducted in the southwest arseniasis-endemic areas (Huang et al., 2008a).

In addition to smoking and alcohol, some investigators considered tea and coffee consumption as potential confounders (Paiva et al., 2008). However, their effect on urinary arsenic profile seemed to be irrelevant in analysis. Therefore, it is inconclusive whether these daily common beverages could exert an effect on arsenic metabolism and more research works are required to provide an answer.

Nutrition and dietary factors

Nutritional status is highly associated with the clinical manifestations of toxic effects of metal ions (Peraza et al., 1998). Lower intake of dietary methyl groups (i.e., low dietary content of methionine or protein) can result in lower arsenic methylation (Vahter and Marafante, 1987). Choline-deficient diet may limit the availability of S-adenosylmethionine in mice, leading to significant decrease in total urinary excretion of orally administered sodium arsenite and marked modulation of arsenic-induced DNA damage in target organs (Tice et al., 1997). Dietary intake and serum concentration of cysteine, methionine, choline, selenium, zinc, folate, niacin, vitamin B\(_2\), ferritin, α-tocopherol and some other antioxidants can also modify the metabolism, retention and toxicity of arsenic (Fig. 2) (Heck et al., 2007; Hong et al., 2000; Peraza et al., 1998; Spiegelstein et al., 2003; Steinmaus et al., 2005a; Walton et al., 2003).

There is growing evidence that nutritional status may play an important role in the regulation of arsenic methylation and arsenic-related health hazards in humans. In Taiwan, it has been reported that serum β-carotene level can modify the extent of risk association between methylated arsenic species and skin cancer (Hsieh et al., 1997) and ischemic heart disease (Hsueh et al., 1998). Although a lack of association between nutritional status and arsenic-induced skin lesions was observed in a study carried out in a small village called Chiu Chiu in northern Chile (Smith et al., 2000), a more recent study in West Bengal, India showed that the prevalence of skin lesions in people with the lowest intake of certain nutrients was approximately two times higher (Mitra et al., 2004).

Folate is a water-soluble vitamin B that can provide one-carbon groups to DNA methylation and DNA synthesis (Stanger, 2002; Suh et al., 2001) and can also be involved in arsenic methylation via one-carbon metabolism (Fig. 2) (Li et al., 2008). Mitra et al. (2004) reported in a case-control study conducted in West Bengal, India that arsenic-related skin lesions were more commonly seen in arsenic-exposed subjects with low dietary folate intake. Gamble et al. reported in studies conducted in Bangladesh that folate could facilitate arsenic methylation (Gamble and Liu, 2005) and that folate supplementation could enhance arsenic methylation, decrease blood MMA and increase urinary DMA (Gamble et al., 2007). A recent study in Taiwan confirmed that high plasma folate level was associated with a lower risk of urothelial cancer (Huang et al., 2008b).

Urinary creatinine (as a proxy indicator of nutritional status because of its close association with muscle mass and meat intake) has been found to be positively associated with DMA percentage in studies conducted in Bangladesh (Gamble and Liu, 2005; Li et al., 2008). However, because creatinine, a precursor of creatinine, is also formed during arsenic methylation while using S-adenosylmethionine as methyl donor (Fig. 2), the interpretation of such a proxy indicator should be cautious (Li et al., 2008).
Although not intensively studied, the interactions between nutritional status and other minerals, trace elements or medications consumed in our daily life such as coffee, vitamin, tea and diuretic etc. are worthy of further investigation. The nutritional status should also be considered by using more validated measures such as serum albumin level, waist and hip circumferences and skin-fold thickness.

**Body mass index**

Body fat content may have an effect on the storage of arsenic and therefore may exert an impact on the metabolism of arsenic. Body mass index has been used as an indicator for obesity or nutritional status. Most studies did not consider the effect of body mass index on the urinary arsenic species. In the study by Tseng et al. (2005) evaluating the association between urinary arsenic metabolites and peripheral arterial disease, body mass index was treated as a potential confounder in multivariate analyses, which showed significant association with DMA³ positively and MMA³ negatively (Tseng et al., 2005). This was also demonstrated in men but not in women in another study by Lindberg et al. (2007). Therefore, the effect of anthropometric factors should be clarified in future studies.

**Arsenosugars**

Although arsenobetaine found abundant in crustacean seafood is not metabolized in humans and is excreted unchanged into urine (Ma and Le, 1998), arsenobetaine abundant in seaweeds, mussels, clams and oysters are metabolized in humans with substantial increase in DMA³ and at least five unidentified urinary arsenic species are found (Ma and Le, 1998). Synthetic forms of arsenosugars may also increase the excretion of DMA³ in urine (Francesconi et al., 2002). Therefore, for the measurement of urinary arsenic species for the assessment of exposure to inorganic arsenic, it is suggested that the subjects should not take seafood for at least 3 days before collection of urine (Ma and Le, 1998).

**Use of chelating agents**

Administration of the chelating agent 2,3-dimercaptopropane sulfonic acid (DMPS, Na salt, DIMAVAL®) has been shown to increase the urinary amount of inorganic arsenic and the methylated arsenic metabolites in arsenic exposed people in both the San Pedro de Atacama and Toconao of Chile (Aposhian, 1997). The increase of MMA³ percentage was most remarkable with a change from 12–15% to 41–42%, indicating the conversion of MMA³ to DMA³ may be inhibited by DMPS specifically or by the resulting DMPS-cellular reducing environment (Aposhian, 1997).

**Concurrent diseases**

None of the currently published papers on the association between urinary arsenic profile and human diseases considered the effect of concurrent diseases. Because liver is the main organ involved in arsenic methylation and the arsenic metabolites are mainly excreted via the kidney, theoretically, abnormal functioning of these two organs can directly lead to disturbed arsenic metabolism. However, the health condition of the individuals not directly related to liver or kidney can also exert an effect on the function of these two organs. For example, heart failure can impair blood perfusion to liver and kidney can also exert an effect on the function of these two organs. For heart failure can impair blood perfusion to liver and kidney can also exert an effect on the function of these two organs. Therefore, the uptake and metabolism of arsenic is highly dependent on cell types and the health condition of the individuals is probably important and should be considered in our future investigations.

**Cooking**

The concentration and the speciation of arsenic in seafood can be changed by cooking, especially when the food is overcooked (Devesa et al., 2001a, 2001b, 2001c). For example, arsenobetaine can be transformed to TMAO and the tetrathymethylarsion ion (TMA³); but this does not occur if the food is just barely cooked (Devesa et al., 2001c; Lai et al., 2004). Inorganic arsenic in water probably does not undergo biotransformation during boiling because it was reported that there was no transformation of arsenic species even after heating up to 120 °C (Devesa et al., 2001c). However, it is not known whether the inorganic arsenicals in water or the arsenic species in food will undergo biochemical changes as a result of boiling or cooking with certain food constituents (e.g., proteins containing sulfhydryl groups). In a study that examined the change of arsenic content in rice after boiling with arsenic-containing water in Bangladesh, the arsenic in cooked rice was higher than in raw rice, suggesting a chelating effect of rice grains or a concentration of arsenic because of water evaporation during cooking (Bae et al., 2002).

Rice is a staple food in Asia and DMA is the main arsenic species in rice (Smith et al., 2008). Therefore the content and species of arsenic in rice irrigated by using the arsenic-contaminated water would have an effect on the urinary pattern of arsenic metabolites in humans consuming such grain. Studies from Bangladesh have shown that intake of arsenic via food contributes to 20–50 μg/L of urinary arsenic in adults (Vahter et al., 2006; Lindberg et al., 2008a). Therefore the arsenic exposure mainly comes from contaminated rice, the urinary arsenic level will be in the low concentration range with a high percent of DMA.

A study conducted in Mexico suggested that arsenic content in food including pinto beans, potatoes, sauce and pork or beef meat (all boiled); fried eggs and the corn-based flat-bread ‘tortillas’ was dependent on the water amount taken up by the specific food during preparation and the cooking time (Del Razo et al., 2002). Therefore pinto beans and pasta soup showed the highest arsenic content and tortillas which had lower water content showed the lowest arsenic content (Del Razo et al., 2002).

**Technical problems**

**Trivalent methylated arsenicals**

Although the validity of analytical methods were questioned, urinary MMA⁴ and DMA⁴ are claimed to be detectable in arsenic-exposed subjects with or without the treatment of DMPS (Cohen et al., 2006). It is not until recently that the methodology for measurement of trivalent methylated arsenic metabolites is developed (Del Razo et al., 2001). However, the validity of the methods used for storage and pre-treatment and the standards used in assay were questioned for the earlier studies which claimed to identify the trivalent methylated arsenicals in human urine (Francesconi and Kuehnelt, 2004). In a recent study evaluating the urinary arsenic profile in 147 women in Argentina, MMA⁴ was not detected in any of the urine samples (Schlärwicke Engström et al., 2007). Therefore, a more rigorous identification method is necessary to confirm the existence of trivalent methylated metabolites in human urine.
Table 1  
Factors affecting arsenic metabolism and contributing to variations in urinary arsenic species

<table>
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<th>Factors</th>
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| **Ethnicity**                  | 1. Inter-ethnic differences in arsenic metabolism have been observed.  
3. Differences in genetic factors and arsenic exposure history, diet, nutrition and lifestyle may be responsible for inter-ethnic differences.  
4. Genetic variations in enzymatic activity involved in arsenic metabolism between different ethnicities may be due to natural selection through generations of exposure. | 1. Concha et al., 1998a.  
| **Family aggregation**         | 1. A Chilean study shows correlation of arsenic methylation pattern among family members.  
2. Correlation of methylation pattern is strongest in siblings.  
| **Intra-individual variation** | 1. Day-to-day variation of arsenic methylation is not significant, but within-day variation is observed: higher DMAV and lower inorganic arsenic in the morning and no change with MMAV in Andean women in Argentina.  
2. Diurnal variation in the intake of water amount might be responsible for the within-day variation. | 1. Concha et al., 2002.  
2. Steinmaus et al., 2005b |
| **Arsenic exposure dosage**    | 1. Higher exposure dosage might lower methylation capacity (increased MMAV and decreased DMAV); but adaptation might occur with increasing duration of exposure.  
2. Excessive inorganic arsenic might inhibit second methylation step.  
3. Note: some early studies did not suggest such impairment in arsenic methylation with increasing dosage of arsenic exposure. | 1. Del Razo et al., 1997.  
4. Li et al., 2008.  
5. Lindberg et al., 2008a.  
| **Age**                        | 1. Controversial results reported regarding age-related changes in methylation capacity.  
2. Older age might indicate longer duration of arsenic exposure or higher rates of comorbidities.  
3. Most of the studies did not consider the change of organic functions involved in arsenic metabolism associated with ageing. | 1. Tseng et al., 2005.  
4. Huang et al., 2008a.  
6. Chung et al., 2008.  
7. Steinmaus et al., 2007. |
| **Adults vs. children**        | 1. Studies comparing methylation capacity between adults and children showed inconclusive results.  
2. The study by Chowdhury et al. (2003) in Bangladesh suggested a more active second methylation capacity in children than in adults. However, the differences did not reach statistical significance when data were analyzed by the author of the present review article.  
3. Data from West Bengal, India presented by Tokunaga et al. (2002) and statistically analyzed by the author of the present review article did not show a better arsenic methylation capacity in the children.  
4. A study by Concha et al. (1998a) in Argentina showed that methylation capacity in children was poorer or not better than adult women. | 1. Chowdhury et al., 2003.  
2. Tokunaga et al., 2002.  
3. Concha et al., 1998a. |
| **Sex**                        | 1. Women have better methylation capacity than men in most studies.  
2. Some studies showed no significant difference in arsenic methylation capacity between men and women and one study by Chen et al. (2003) showed better methylation capacity in men.  
3. Menopausal status and estrogen replacement therapy should be considered in future clarification of sexual difference. | 1. Tseng et al., 2005.  
3. Lindberg et al., 2008b.  
4. Steinmaus et al., 2007.  
5. Chen et al., 2003. |
| **Pregnancy and breast feeding** | 1. Methylation capacity increases during pregnancy in women. Estrogen effect might be responsible.  
2. Arsenic can pass through placenta (mainly DMA) and affect pregnancy outcomes and early human development.  
3. Arsenic is not efficiently excreted from human breast milk.  
4. Arsenic methylation is efficient during infancy, probably due to sufficient storage of folate in fetal liver and additional provision from breast milk.  
5. Breast feeding may protect infants from arsenic toxicity during early life.  
2. Hopenhayn et al., 2003b.  
4. Rosado et al., 2007.  
5. von Ehrenstein et al., 2007.  
7. Wasserman et al., 2007 |
| **Estrogen**                    | 1. Methionine is required for arsenic methylation. Estrogen stimulates choline synthesis, which in turn remethylate homocysteine to methionine. Therefore estrogen may facilitate arsenic methylation. | 1. Fischer et al., 2007.  
| **Smoking**                    | 1. Smoking is associated with decreased methylation capacity in some but not all studies.  
2. Cigarettes contain arsenic and other chemicals that might interfere with analyses. | 1. Hopenhayn-Rich et al., 1996b.  
2. Tseng et al., 2005.  
3. Steinmaus et al., 2007. |
| **Alcohol, tea and coffee**    | 1. Studies evaluating the effects of these beverages are scarce.  
2. Alcohol might decrease methylation capacity in univariate analyses which became statistically insignificant after multivariate adjustment in the study by Hopenhayn-Rich et al. (1996b).  
3. Alcohol effect was not observed in Taiwanese studies.  
2. Huang et al., 2008a.  
3. Paiva et al., 2008. |
| **Nutrition and dietary factors** | 1. Sufficient protein intake may improve methylation capacity.  
2. Low dietary folate intake may increase the risk of arsenic-related skin lesions and folate supplementation may enhance arsenic methylation. | 1. Mitra et al., 2004.  
2. Gamble et al., 2007. |
Storage

A study reported that arsenic species were stable for up to 2 months if urinary samples were stored without any additives at temperature of 4 °C and −20 °C; and some urinary samples were stable for a period of even up to 8 months (Feldmann et al., 1999). Chen et al. (2002) showed that arsenic species in human urine is stable for at least 6 months preserved at −20 °C. Le et al. (2000) reported that approximately 60% of DMA III and 95% of DMA III were oxidized to MMA IV and DMA IV, respectively, after the sample was stored at 4 °C for 2 weeks. A later report suggested that both MMA III and DMA III are unstable in urine and DMA III in urine is completely converted to DMA V within 17 h even stored at −20 °C (Francesconi and Kuehnelt, 2004).

The storage and manipulation of urinary samples in polyethylene or glass containers can exert a different effect on the determination of arsenic species (Feldmann et al., 1999). Because arsenic can be adsorbed onto glass, it is recommended that polyethylene containers should be used. Furthermore, strong acidification or additives are not recommended for urinary storage for the purpose of arsenic speciation (Feldmann et al., 1999).

Renal function and urinary volume

To take into account the uncertainty due to the change of urinary volume, some studies used urinary creatinine level for adjustment while expressing urinary arsenic concentration (Chung et al., 2008). However, creatinine level may vary with meat intake, muscle mass, age and physical activity (Suwazono et al., 2005) and this adjustment seems unnecessary in population studies (Hinwood et al., 2002). Actually, when the urinary arsenic parameters are expressed as ratios between arsenic species, these calculations have already eliminated the potential influence of renal function or volume change because dividing the nominator and denominator simultaneously by a similar factor of urinary creatinine would not give a different ratio. Therefore, when the parameters are ratios of arsenic metabolites, adjustment for urinary creatinine is not necessary. A recent study showed that urinary concentrations of arsenic and creatinine were significantly correlated, indicating adjustment for creatinine might lead to underestimation of exposure (Nermell et al., 2008).

To compensate for the variation in the dilution of urinary samples, some investigators adjusted the urinary arsenic concentration by a certain specific gravity of urine (e.g., 1.0242) and excluded very dilute urinary samples (e.g., <1.010) from analyses (Kurtto et al., 1999; Makipaakkanne et al., 1998); while the others used the average of all urinary samples (e.g., 1.019, 1.014 or 1.016 g/ml) for adjustment (Concha et al., 1998a, 2006; Schläwicke Engström et al., 2007; Vahter et al., 2003). A recent study showed that adjustment by specific gravity was less affected by body size, age, sex and season than adjustment by creatinine (Nermell et al., 2008). Therefore, adjustment for specific gravity rather than urinary creatinine should be considered during data analysis.

Methods and timing of urinary collection

Some investigators used spot urine (Chung et al., 2002; Chowdhury et al., 2003; Concha et al., 1998a) and others collected urine over a period of time either overnight (Hsueh et al., 1997; Tseng et al., 2005) or for 24 h (Chiou et al., 1997; Yu et al., 2000) for the determination of arsenic species. Although it is theoretically possible that different methods will yield different concentrations of urinary arsenic species, there has not been any study comparing the effects of different methods of urinary collection.

Discussion

Inter-individual variation in arsenic metabolism can be due to environmental or genetic factors. The genetic factors associated with arsenic metabolism have been recently reviewed (Ghosh et al., 2008; Hernández and Marcos, 2008), but the environmental factors remain unattended. Genetic factors are mostly deemed as unmodifiable but many of the environmental factors are probably modifiable. So far, not many studies focused primarily on these environmental factors, but a knowledge of these factors is clinically important because their clarification may help to identify subjects at a higher risk of developing arsenic-induced health hazards and may render a chance of clinical intervention. These factors are summarized in Table 1 and should be considered for adjustment while analyzing the association between diseases and urinary arsenic species.
Wide inter-individual variation in arsenic methylation capacity is observed in different ethnicities, which is probably genetically determined in part. However, demographic characteristics and environmental factors such as dosage and duration of arsenic exposure, termination of exposure, lifestyle, dietary factors, nutritional status and general health conditions can influence the arsenic methylation capacity. Although their effects were not consistently shown in different studies, these potential factors should be considered and adjusted for while evaluating the association between arsenic methylation capacity and human health in our future studies. Technical problems can cause biased estimates and there is also a need to clarify the storage conditions for the stability of the urinary arsenic species. The detection of the recently identified trivalent methylated metabolites in urine awaits further confirmation. Furthermore, the interactions among the potential regulating factors are worthy of exploration.

Because the urinary arsenic profile will change after termination of exposure to arsenic, this exposure change should also be considered while evaluating the association between urinary arsenic profile and human diseases, especially when heterogeneity exists among the subjects in the duration of termination for exposure.

If the hormone hypothesis for the explanation of the more efficiency of arsenic methylation in women than in men and during the course of pregnancy is correct, there is implication for the use of estrogen to diminish the toxic effect of arsenic for more rapid removal of inorganic arsenic in selected cases. There is also clinical implication that improvement in nutritional status by providing folate or other micronutrients may help to reduce arsenic-related diseases.

It should also be pointed out that the metabolic pathways of arsenic may be different between humans and animals (especially rats) and between different cell types in the same species (Cohen et al., 2006; Csanaky and Gregus, 2002; Vahter, 1999). The carcinogenic effects and non-cancerous diseases observed in humans in epidemiologic studies cannot always similarly be shown in animals. Therefore, we still do not have a good animal model for investigating the mechanisms of arsenic-induced human diseases and epidemiologic studies should be done with a more careful and stringent study design.

Except for a few prospective follow-up studies (Huang et al., 2008a), most studies are cross-sectional and tend to have inherent selection or information bias and unknown correctness in temporality. Patients with clinical manifestations of arsenic-induced health hazards might also have changed their dietary habits, withdrawn from smoking, increased consumption of vitamins or taken some kinds of medications. All of these might have an impact on the methylation of arsenic leading to a change of the urinary arsenic profile. Therefore prospective and longitudinal follow-up studies are required to confirm the association between arsenic methylation and human diseases and to elucidate the factors associated with the capacity to metabolize arsenic.

Co-contamination is commonly seen in water containing high arsenic levels. For example, iron level is as high as 0.8 mg/L in arsenic-containing water in Argentina, which might precipitate arsenic if kept in tubing or containers for a long period before consumption (Concha et al., 2006). The effects of co-contamination are rarely considered in the literature and should be attended in future research.

Inter-ethnic comparison studies with adequate sample size and standard protocols are required. Small sample size increases the probability of a positive or negative association by chance and publication bias for positive results might exist. Gender differences in the association with genetic factors (Steinmaus et al., 2007) is interesting and should be reconfirmed in future studies.

Conclusions

Susceptibility to arsenic-induced health hazards differs among individuals. Part of this inter-individual susceptibility could be ascribed to the inter-individual differences in arsenic metabolism. Understanding the carcinogenic pathway of arsenic, the different cellular effects of the metabolites and the factors affecting its metabolism are of clinical importance. Wide inter-ethnic variation and family differences in urinary arsenic metabolites indicate a genetic effect on arsenic metabolism. Arsenic methylation pattern can also be influenced by a variety of environmental factors such as age, sex, pregnancy, arsenic exposure level, smoking habits, nutritional status and dietary factors. The findings of a close interaction between plasma folate, urinary arsenic metabolites and cancer risk and the increased excretion of arsenic after folate supplementation provide a potential intervention to manage and reduce the arsenic-related health hazards in most endemic areas over the world.

Conflict of interest statement

There are no conflicts of interest.

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