Interactions between Arsenic-Induced Toxicity and Nutrition in Early Life\textsuperscript{1,2}

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Abstract

Exposure to arsenic through drinking water is a major public health problem affecting most countries, although the situation is particularly severe in low-income nations. The health consequences of chronic arsenic exposure include increased risk for various forms of cancer and numerous noncancer effects, including diabetes, skin diseases, chronic cough, and toxic effects on liver, kidney, cardiovascular system, and peripheral and central nervous systems. In recent years increasing reports of effects on fetal and child development have appeared. There seems to be a wide variation in susceptibility to arsenic toxicity, which is likely to be related to factors such as variation in arsenic metabolism, nutrition, host-related defense mechanisms, and genetic predisposition. The main mechanisms of arsenic-nutrition interactions include arsenic-induced oxidative stress, which requires nutrient-dependent defense systems, and arsenic metabolism (methylation) via 1-carbon metabolism, which requires methyl groups, folic acid, vitamin B-12, and betaine for the remethylation of homocysteine to methionine. An efficient first methylation step in combination with a slow second methylation step seems to be most critical from a toxicological point of view. A third mode of arsenic-nutrition interaction involves epigenetic effects and fetal programming via DNA methylation. J. Nutr. 137: 2798–2804, 2007.

Arsenic exposure and health effects

Millions of people worldwide, mainly in the developing countries, are exposed to arsenic because of emissions from mining activities, industrial or pesticide use, or contaminated well water. Arsenic in the bedrock or soil is easily dissolved in the surrounding ground water, and elevated concentrations of arsenic, i.e., above the WHO guideline level of 10 \(\mu\)g \(L^{-1}\) (1), are present in most countries, although the prevalence and concentrations vary considerably. South-East Asia is among the most severely affected regions (2–4), and in Bangladesh about half of the 10 million tube wells installed during the last 30 y produce water above the guideline value (5). In addition, the use of arsenic-containing ground water for irrigation leads to widespread contamination of land and additional exposure via food (6–8).

Arsenic is a well-documented potent human carcinogen causing cancer of the bladder, lung, skin, and possibly also kidney and liver (3). A large number of reports show associations between arsenic exposure and multiple noncancer health effects, e.g., diabetes, skin diseases, chronic cough, and toxic effects on liver, kidney, cardiovascular system, and peripheral and central nervous systems (9,10). In recent years, a few reports on adverse effects of arsenic on fetal growth and development in populations exposed to arsenic from drinking water have appeared (11–17). Because several of the studies are ecological in design or include few subjects, more research is needed for firm conclusions on dose-response relations. However, it is quite likely that arsenic has adverse effects on the fetus because it readily crosses the placenta (18), possibly by Glut1, which has been shown to catalyze the cellular uptake of both arsenite and its monomethylated metabolite (19), and to be the main transplacental glucose transporter (20). Arsenic also accumulates in the placenta (18), possibly producing toxic effects in placental tissues, mediated via oxidative stress, and interfering with nutrient transport to the fetus, thereby affecting fetal growth.

In contrast to the extensive fetal exposure in women exposed to arsenic during pregnancy, the breast-fed infant is protected against arsenic exposure because the excretion of arsenic in breast milk is limited (21). Still, fetal exposure may give rise to long-lasting effects. Our ongoing studies on the effects of arsenic exposure early in life are carried out in Matlab, a rural area located \(\sim 53\) km southeast of Dhaka, where elevated concentrations of inorganic arsenic in tube-well water as well as poor nutrition are prevalent (22). Construction of tube-wells during the past few decades has given 95% of the population access to ground water. However, screening for arsenic in all the 13,200 tube-wells in Matlab revealed a wide range of arsenic concentrations, from below 1 \(\mu\)g \(L^{-1}\) to \(>3000\) \(\mu\)g \(L^{-1}\) (23), with \(>70\%\) of the tube-wells exceeding the WHO guideline of 10 \(\mu\)g \(L^{-1}\). The initial study, comprising 29,000 pregnancies, showed an association between arsenic exposure in pregnancy and increased infant mortality, particularly from infectious diseases (24). Because most women breast-feed their infants in Bangladesh, the results indicate that the intrauterine exposure affected the immune function, either...
directly or via inhibition of fetal growth (16,17), and that this caused increased morbidity and mortality during infancy.

**Arsenic-nutrition interactions**

There is wide variation in susceptibility to arsenic-induced toxicity, and there is reason to believe that nutrition is an important susceptibility factor. A number of studies have shown associations between the prevalence or severity of arsenic-related health effects and indicators of food and nutritional status (25–31), suggesting that people with poor nutrition are particularly susceptible. Although these studies mainly concern health effects in adult life, it seems likely that nutrition also may modify the effects of arsenic induced early in life.

There are several plausible mechanisms by which arsenic toxicity can be affected by nutrition, which are discussed in detail individually in the following sections. First, arsenic induces oxidative stress, an effect that is further compounded by arsenic-induced inhibition of several of the antioxidant systems. Second, a number of studies have shown an association between a low degree of arsenic metabolism and the risk of various toxic effects. Arsenic is metabolized by a series of reduction and methylation reactions via 1-carbon metabolism, in which methyl groups are transferred from S-adenosylmethionine (SAM) to arsenic in its trivalent form (32,33) to arsenic in the liver, with some unmethylated inorganic arsenic (37). The main metabolites of inorganic arsenic are methylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are excreted in urine together with some unmethylated inorganic arsenic (37). However, highly reactive intermediate metabolites, such as MMA(III) and DMA(III), with arsenic in its trivalent form, may also be formed. The trivalent forms of arsenic are the most toxic forms, reacting with essential groups, mainly sulfhydryl groups, in, e.g., enzymes and transcription factors (3).

Consistently, a higher proportion of MMA in urine has been associated with a higher prevalence of bladder (38,39) and skin cancers (27,40), other skin effects (41), cardiovascular effects (42), and chromosomal aberrations (43). Probably, a higher proportion of MMA in urine reflects a lower capacity for optimal methylation to DMA and higher retention of arsenic in the body (37). Therefore, a high percentage of MMA in urine may be considered a risk factor for arsenic-induced health effects.

A third mechanism of arsenic-nutrition interaction is related to the recent findings that arsenic exerts epigenetic effects, probably by interfering with DNA methylation (44–46), which is essential for fetal development and fetal programming and developmental origins of health and disease (47,48). The fetal programming theory has largely focused on fetal nutrition, but there is increasing evidence for effects of chemical exposure early in life (49,50).

**Arsenic-induced oxidative stress and interaction with nutrition**

Oxidative stress has been identified as an important mechanism of arsenic toxicity and carcinogenicity. In particular, arsenic induces oxidative DNA damage and lipid peroxidation (51–55). A number of studies have shown arsenic-induced formation of reactive oxygen and nitrogen species as well as elevated DNA oxidation (51,56–60). The toxic effects of such events are highly dependent on defense mechanisms in the body, i.e., the status and dietary intake of antioxidants. It is becoming increasingly evident that arsenic not only induces reactive oxygen and nitrogen species but also affects the defense against those species. Inorganic arsenic has been shown to inhibit several of the antioxidant systems in the body, such as glutathione, glutathione peroxidase, thioredoxin reductase, and superoxide dismutase (61–65). Thus, increasing the antioxidant levels in the body may protect against arsenic-induced toxicity. Indeed, the administration of ascorbic acid, α-tocopherol (66–71), plant extracts, flavonoids, polyphenols (72–76), or selenium (75,77–81) has been shown to decrease arsenic-induced toxicity.

Most experimental and epidemiological studies on arsenic-induced oxidative stress have been carried out on adults, and little is known about the effects of arsenic-induced oxidative stress and antioxidant defense on early development. Obviously, antioxidant status is also likely to be critical for protection against arsenic-induced effects early in life. Oxidative stress and disrupted antioxidant systems have been shown to be involved in a wide range of pregnancy complications such as impaired fetal growth, preeclampsia, and miscarriages (82,83). For example, the fact that arsenic has been shown to inhibit thioredoxin reductase (65) and glutathione peroxidase (84) may be of importance for the developing organism, as both thioredoxin reductase and glutathione peroxidase are major stress protection systems in the placenta (82,85). Arsenic-induced decrease in the placenta concentrations of these selenoenzymes was indicated in experiments with selenium-deficient pregnant mice but not in mice with adequate selenium intake (85). Selenium deficiency also enhanced accumulation of arsenic in maternal liver and fetal brain compared with mice with adequate selenium. In other studies, N-acetylcysteine was found to effectively prevent arsenic-induced oxidative stress, telomere erosion, chromosome instability, and apoptosis in mouse early embryos (86). Similarly, vitamins C and E reversed arsenic-induced oxidative stress and apoptosis in the developing rat brain (71). Further, rat neonatal brain explants from 1-d-old litters exposed to 0.3 mg L−1 arsenic

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2 Abbreviations used: DMA, dimethylarsinic acid; MMA, methylarsonic acid; PEMT, phosphatidylethanolamine-N-methyltransferase; SAM, S-adenosylmethionine.
in drinking water during gestation showed increased generation of NO and ROS, loss of glutathione content, increased lipid peroxidation, and decreased superoxide dismutase levels (71). Vitamins C and E partially reversed the effects, indicating possible protection from arsenic toxicity.

**Arsenic metabolism and interaction with nutritional status**

Because the methylation of arsenic occurs by transfer of methyl groups from SAM (Fig. 1), it seems reasonable to assume that arsenic methylation is influenced by availability of dietary methyl groups as well as enzymes and cofactors involved in 1-carbon metabolism. The first studies demonstrating the critical involvement of SAM-dependent methylation and nutrition in arsenic methylation showed a significant decrease in arsenic methylation and increased body retention of arsenic following inhibition of SAM (32) or by feeding diets low in protein, methionine, or choline (87). More recent studies showed that arsenic exposure of pregnant mice fed a protein-deficient diet decreased maternal weight gain and increased the incidences of exencephaly, abelepharia, and skeletal defects compared with mice fed a protein-adequate diet, possibly by impairment of arsenic methylation (88). There are also a few indications of lowered arsenic methylation in people with low protein intake. Assessment of dietary intakes and urinary arsenic methylation patterns in 87 subjects from 2 arsenic-exposed regions in the western United States showed that subjects in the lower quartile of protein intake had a higher proportion of MMA and a lower proportion as DMA in urine than did subjects in the upper quartile of protein intake (89). Similarly, higher estimated intakes of protein, methionine, and choline were associated with slightly higher percentages of the methylated metabolites in urine of more than 1000 Bangladeshi adults exposed to arsenic in drinking water (90). Our studies in rural Bangladesh, involving several hundreds of individuals with generally low energy intake and a mean BMI of ~20 kg m⁻², showed remarkably efficient arsenic methylation (8,91). Apparently, the intake of methyl groups with the diet, in combination with methionine recycling and endogenously produced choline, provides enough SAM to maintain an efficient 1-carbon metabolism (92,93), including methylation of arsenic. Interestingly, premenopausal Bangladeshi women showed particularly efficient methylation of arsenic (8), an observation also made in East European population groups (94). This may be related to the enhanced capacity to produce choline endogenously through de novo synthesis of phosphatidylcholine catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) in the female liver (93). PEMT is induced by estrogen, allowing premenopausal women to synthesize more choline, which can be used for remethylation of homocysteine to methionine and further to SAM.

There are also several mechanisms by which poor micronutrient intake can affect the metabolism of arsenic. In particular, low intakes of folic acid and vitamin B-12, which are involved in the remethylation of homocysteine to methionine, may decrease the efficiency of 1-carbon metabolism (35,36). Indeed, associations between these micronutrients and arsenic methylation have been reported in both experimental and epidemiological studies (90,95–97). In arsenic-exposed people in rural Bangladesh, plasma folate was positively associated with the percentage of DMA and negatively associated with the percentage of inorganic arsenic and MMA in urine, although the effect sizes were small (96). Furthermore, higher dietary intakes of cysteine, methionine, calcium, protein, and vitamin B-12 were associated with slightly lower percentages of inorganic arsenic and higher ratios of MMA to inorganic arsenic in urine, whereas higher intakes of choline, which also is involved in the remethylation of methionine from homocysteine, were associated with higher DMA-to-MMA ratio (90). Supplementation of 100 of the Bangladeshi individuals previously found to have low plasma concentrations of folate with folic acid at a dose of 400 µg/d for 12 wk was found to reduce the urinary MMA from 13% to 10% and inorganic arsenic from 15% to 11%, indicating that folic acid supplementation to participants with low plasma folate enhances arsenic methylation (98). Other studies have indicated associations between iron, zinc, or selenium status and arsenic methylation efficiency (89,99).

Our studies in Bangladesh showed that the arsenic metabolism of women in early pregnancy was only marginally influenced by micronutrient status (91). We evaluated the effects of measured biomarkers of folate, vitamin B-12, zinc, iron, and selenium status on arsenic metabolism in 442 women in early pregnancy, controlling for arsenic exposure, which was the main factor influencing arsenic methylation. Despite poor micronutrient status and high arsenic exposure, the women showed a remarkably efficient methylation of arsenic (91). The median percentage of urinary DMA (74%) is in the upper range, and that of MMA (11%) in the lower range, of what is commonly seen in urine of individuals in developed countries with much better nutrition (37). Only at very high arsenic exposure levels were low folate levels associated with the methylation of arsenic to DMA, and even then the effect size was small. Even women with a combination of low folate, vitamin B-12, and zinc showed almost as good methylation capacity as the better-nourished group. Apparently, the methylation capacity is sufficient at low to moderate exposure levels despite low status of folate and vitamin B-12. Human methylation pathways closely interconnect choline, methionine, methyltetrahydrofolate, and vitamins B-12 and B-6 because the regeneration of methionine from homocysteine is essential for maintaining the numerous methylation reactions required for DNA functioning and for the biosynthesis of key components such as creatine and phospholipids (100,101). A change in one of these pathways results in compensatory changes in the others. In addition, the enhanced capacity of endogenous production of choline, catalyzed by PEMT, in women (93) might have rendered the Bangladeshi women less sensitive to poor micronutrient intake for efficient arsenic methylation. In particular, the rise in estradiol during pregnancy is associated with enhanced endogenous production of choline to support fetal development (102), partly by providing an efficient 1-carbon metabolism. In fact, arsenic methylation to DMA has been shown to be particularly efficient in pregnant women (18,91).

It remains to be seen whether gene-nutrition interactions have a role in the interindividual variation in arsenic methylation. Polymorphisms in the As(III) and MMA(III) methyltransferases (e.g., AS3MT) and, to a lesser extent, the As(V) and MMA(V) reductases (e.g., hGSTO1) have been shown to affect arsenic metabolism in populations in Central Europe (94), Argentina (103), and Mexico (104). Possibly, individuals with a genotype associated with less efficient methylation of arsenic may be more sensitive to interactions with poor nutrition.

**Epigenetic effects of arsenic**

Another potentially critical interaction between arsenic and nutrition is in the epigenetic effects of arsenic that result from interference with DNA methylation (44,45,105,106). DNA methylation is an important mechanism of fetal programming, largely discussed in terms of nutrition during fetal development and disease later in life, the so-called Barker effect (47,48,107).
The epigenome of the developing fetus is sensitive not only to maternal nutrition but also to environmental toxicants and stress (50). Arsenic-induced changes in DNA methylation, particularly in combination with poor nutrition, may have severe consequences for the development of health effects both before and after birth.

Although there is no indication of a common methyltransferase for both arsenic and DNA methylation, there are several parallels between the modifying factors in arsenic and DNA methylation. Some of these may be related to the fact that both arsenic and DNA are methylated via 1-carbon metabolism. A combined folate and methyl deficiency was found to alter components of the DNA methylation machinery (108). Generally, children and adolescents have more efficient arsenic methylation than adults (8,109), and it has been shown that the expression of DNA methylation genes decreases significantly with age (110). Possibly, a high rate of methylation during periods of growth, in combination with increasing exposure to environmental pollutants, known to inhibit methylation of both arsenic (8,91,109) and DNA (111), with increasing age contributes to decreasing methylation efficiency with increasing age. However, there are more specific similarities. As discussed above, arsenic inhibits As(III)-methyltransferase(s) at very low levels (8,91). Similarly, arsenic causes hypomethylation of DNA by inhibiting DNA methyltransferases (44). Of particular interest is the finding that transplacental exposure to arsenic induced alterations in DNA methylation in the newborn liver that were related to cancer development later in life (112). Further, the methylation of both arsenic and DNA differs by gender. We recently reported that women, particularly at childbearing ages, are more efficient at methylating arsenic than in men, suggesting an effect of sex hormones (8,94). Estrogens and testosterone also interact with DNA methylation (111,113,114). Our previous findings of induction of arsenic methylation in pregnancy (18), interact with DNA methylation (111,113,114). Our previous findings of induction of arsenic methylation in pregnancy (18), similar to that of DNA (115), support the role of steroid hormones for arsenic methylation. Interestingly, the enhanced capacity of endogenous production of choline in women, in particularly during pregnancy, seems to be estrogen dependent (93). Apparently, estrogen induces the PEMT gene, which regulates the de novo synthesis of phosphatidylcholine, allowing pregnant women to make more of their needed choline, so critical for fetal development, endogenously (93,102).

Conclusions
Identified risk-modifying factors in arsenic-related health effects include both nutrition and arsenic metabolism. One of the prevailing mechanisms of arsenic toxicity is oxidative stress, which requires nutrient-dependent defense systems. Some antioxidant systems are also inhibited by arsenic, thus aggravating arsenic toxicity, particularly in individuals with poor nutrition and dietary intakes of antioxidants. Although most studies involve adults, it is likely that arsenic also induces oxidative stress and inhibition of antioxidant systems in early life exposure. Arsenic is metabolized by methylation via 1-carbon metabolism. An efficient high first methylation step in combination with a slow second methylation step seems to be most critical from a toxicological point of view. A large number of studies have shown that a higher proportion of MMA in urine is associated with increased risk for cancer and other effects of arsenic. Recent studies found that arsenic methylation is influenced by protein intake and micronutrient status. However, women, especially during pregnancy, seem to be remarkably insensitive to poor nutrition for efficient methylation. Arsenic-induced inhibition of 1-carbon metabolism results in epigenetic effects because of impaired DNA methylation, which might influence early life programming and diseases later in life.

Literature Cited
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