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

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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 $>700$ 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

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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)3 (32,33) to arsenic in its trivalent state (Fig. 1). The reactions require availability of dietary methyl groups for the formation of SAM and the presence of reduced glutathione or other thiols for reduction of pentavalent arsenic (34). Full functioning of 1-carbon metabolism also requires adequate intake of folic acid, vitamin B-12, and choline to remethylate homocysteine back to methionine (35,36). 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 placental 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).

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1** One-carbon metabolism, arsenic methylation, and links to choline and folate metabolism. The methylation of arsenic occurs mainly in the liver, with other tissues having much less activity. AS3MT, arsenic(III) methyltransferase; PEMT, phosphatidylethanolamine-N-methyltransferase; CHDH, choline dehydrogenase; BHMT, betaine homocysteine methyltransferase; THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; CH3-B-12, methylcobalamin; GSH, glutathione.
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, ablepharia, 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 \(\mu g/d\) 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), 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**

1. WHO. Guidelines for drinking-water quality. 3rd edition. Geneva: World Health Organization; 2004.
2. Berg M, Stengel C, Pham TK, Pham HV, Sampson ML, Leng M, Samreth S, Fredericks D. Magnitude of arsenic pollution in the Mekong and Red River Deltas–Cambodia and Vietnam. Sci Total Environ. 2007;372:413–25.
3. IARC. Some drinking-water disinfectants and contaminants, including arsenic. Volume 84. Lyon: International Agency for Research on Cancer; 2004.
4. Chakraborti D, Sengupta MK, Rahman MM, Ahamed S, Chowdhury UK, Hossain MA, Mukherjee SC, Pari S, Saha KC, et al. Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. J Environ Monit. 2004;6:74N–83N.
5. Jakariya M, Rahman M, Chowdhury AMR, Rahman M, Yunus M, Bhuiya A, Wahed MA, Bhattacharya P, Jacks G, et al. Sustainable safe water options in Bangladeshi experiences from the Arsenic Project at Matlab (AsMat). In: Bundschuh J, editor. Natural arsenic in groundwater: occurrence, remediation and management. London: Taylor & Francis Group; 2005. p. 319–30.
6. Mehard AA, Rahman MM. Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environ Sci Technol. 2003;37:229–34.
7. Kile ML, Houseman EA, Breton CV, Smith T, Quamruzzaman Q, Rahman M, Mahiuddin G, Christians CI. Dietary arsenic exposure in Bangladesh. Environ Health Perspect. 2007;115:889–93.
8. Lindberg A, Ekstrom EC, Nermell B, Rahman M, Lonnerdal B, Persson LA, Vahlte M. Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. Environ Res Rev. 2007; in press.
9. NRC. Arsenic in drinking water: 2001 update. Washington, DC: National Academy Press; 2001.
10. WHO. Arsenic and arsenic compounds. Geneva: International Programme on Chemical Safety, World Health Organization; 2001.
11. Ahmad SA, Sayed MH, Barua S, Khan MH, Faruque MH, Jalil A, Hadi SA, Talukder HK. Arsenic in drinking water and pregnancy outcomes. Environ Health Perspect. 2001;109:629–31.
12. Milton AH, Smith W, Rahman B, Hasan Z, Kulsum U, Dear K, Rakibuddin M, Ali A. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. Epidemiology. 2005;16:82–6.
13. Kwok RK, Kaufmann RB, Jakariya M. Arsenic in drinking-water and reproductive health outcomes: a study of participants in the Bangladesh Integrated Nutrition Programme. J Health Popul Nutr. 2006;24:190–205.
14. Hopenhayn-Rich C, Browning SR, Hertz-Picciotto I, Ferreccio C, Peralta C, Gibb H. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. Environ Health Perspect. 2000;108:667–73.
15. von Ehrenstein OS, Guha Mazumder DN, Hira-Smith M, Ghosh N, Yuan Y, Windham G, Ghosh A, Haque R, Lahiri S, Kalman D, Das S, Smith AH. Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. Am J Epidemiol. 2006;163(7):662–9.
16. Hopenhayn C, Ferreccio C, Browning SR, Huang B, Peralta C, Gibb H, Hertz-Picciotto I. Arsenic exposure from drinking water and birth weight. Epidemiology. 2003;14:593–602.
17. Yang CY, Chang CC, Tsai SS, Chiang HY, Ho CK, Wu TN. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ Res. 2003;91:29–34.
18. Concha G, Vogler G, Leccano D, Nermell B, Vahlte M. Exposure to inorganic arsenic metabolites during early human development. Toxicol Sci. 1998;44:185–90.
19. Liu Z, Sanchez MA, Jiang X, Boles E, Landfear SM, Rosen BP. Mammalian glucuronide metabolism GLUT1 facilitates transport of arsenic trioxide and methylarsinous acid. Biochem Biophys Res Commun. 2006;351:424–30.
20. Leonce J, Brockton N, Robinson S, Venkatesan S, Bannister P, Raman V, Murphy K, Parker K, Pavitt D, et al. Glucose production in the human placenta. Placenta. 2006;27: Suppl A:S103–8.
21. Concha G, Vogler G, Nermell B, Vahter M. Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. Int Arch Occup Environ Health. 1998;71:42–6.

22. Vahter ME, Li L, Nermell B, Rahman A, Arifeen SE, Rahman M, Persson LA, Ekstrom EC. Arsenic exposure in pregnancy—a population based study in Matlab, Bangladesh. J Health Popul Nutr. 2006;24:236–45.

23. Rahman M, Vahter M, Wahed MA, Sohel N, Yunus M, Streafeld PK, El Arifeen S, Bhuiya A, Zaman K, et al. Prevalence of arsenic exposure and skin lesions. A population based survey in Matlab, Bangladesh. J Epidemiol Commun Health. 2006;60:242–8.

24. Rahman A, Vahter M, Ekstrom EC, Rahman M, Golam Mustafa AH, Wahed MA, Yunus M, Persson LA. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. Am J Epidemiol. 2007;165:1389–96.

25. Guha Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakraborty D, Smith AH. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. Int J Epidemiol. 1998;27:871–7.

26. Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH, Wu HY. Atherogeneity and carcinogenicity of high-arsenic artisanal well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis. 1988;8:452–60.

27. Hsuem YM, Chiou HY, Huang YL, Wu WL, Huang CC, Yang MH, Lue LC, Chen GS, Chen CJ. Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. Cancer Epidemiol Biomarkers Prev. 1997;6:389–96.

28. Hsuem YM, Wu WL, Huang YL, Chiou HY, Tseng CH, Chen CJ. Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. Atherosclerosis. 1998;141:249–57.

29. Milton AH, Hasan Z, Shahidullah SM, Sharmin S, Jakariya MD, Rahman M, Dear K, Smith W. Association between nutritional status and arsenicism due to chronic arsenic exposure in Bangladesh. Int J Environ Health Res. 2004;14:99–108.

30. Chen Y, Factor-Litvak P, Howe GR, Parvez F, Ahsan H. Nutritional influence on risk of high blood pressure in Bangladesh: a population-based cross-sectional study. Am J Clin Nutr. 2006;84:1224–32.

31. Chen Y, Hall M, Graziano JH, Slavkovich V, van Geen A, Parvez F, Ahsan H. A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions. Cancer Epidemiol Biomarkers Prev. 2007;16:207–13.

32. Marafante E, Vahter M. The effect of methyltransferase inhibition on arsenic metabolism in rat cyt19, an arsenic methyltransferase. Chem Res Toxicol. 2004;17:99–106.

33. Endogenous reductants support the catalytic function of recombinant rat cyt19, an arsenic methyltransferase. Chem Biol Interact. 1984;50:49–57.

34. Styblo M, Thomas DJ. Factors influencing in vitro methylation of arsenicals in rat liver cytosol. In: Abernathy CO, Calderon RL, Chappell WR, editors. Arsenic: exposure and health effects. London: Chapman and Hall; 1997. p. 283–95.

35. Waters SB, Devesa V, Del Razo LM, Styblo M, Thomas DJ. Endogenous reductants support the catalytic function of recombinant rat cyt19, an arsenic methyltransferase. Chem Res Toxicol. 2004;17:404–9.

36. Loenen WA. S-Adenosylmethionine: jack of all trades and master of everything? Biochem Soc Trans. 2006;34:330–3.

37. Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL. Is it time to reevaluate methyl balance in humans? Am J Clin Nutr. 2006;83:5–10.

38. Vahter ME. Mechanisms of arsenic biotransformation. Toxicology. 2002;181–182:211–7.

39. Pu YS, Yang SM, Huang YK, Chung CJ, Huang SK, Chiu AW, Yang YC, Wu MM, Hong YT, Chou MC, Faust GG, Su CL, Chang SF, Huang WL, Wang HT, Wang YH, et al. Effect of plasma homocysteine level and urinary monomethylarsonic acid on the risk of arsenic-associated carotid atherosclerosis. Toxicol Appl Pharmacol. 2006;216:168–75.

40. Maki-Paakkanen J, Kurttio P, Paldy A, Pekkanen J. Association between the clastogenic effect in peripheral lymphocytes and human exposure to arsenic through drinking water. Environ Mol Mutagen. 1998;32:301–13.

41. Cui X, Wakai T, Shirai Y, Yokoyama N, Hatakeyama K, Hirano S. Arsenic trioxide inhibits DNA methyltransferase and restores methylation-silenced genes in human liver cancer cells. Jpn Pathol. 2006;37:298–311.

42. Reichard JF, Schnekenburger M, Puga A. Long term low-dose arsenic exposure induces loss of DNA methylation. Biochem Biophys Res Commun. 2007;352:189–92.

43. Vahter ME, Li L, Nermell B, Rahman A, Arifeen SE, Rahman M, Ahsan H, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res. 2006;65:Suppl 3:50–8.

44. Langley-Evans SC. Developmental programming of health and disease. Proc Nutr Soc. 2006;65:97–105.

45. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr. 2007.

46. Szyf M, Weaver I, Meaney M. Maternal care, the epigenome and phenotypic differences in behavior. Reprod Toxicol. 2007;24:9–19.

47. Fujino Y, Guo X, Liu J, Matthews IP, Shirane K, Wu K, Kasai H, Miyatake M, Tanabe K, et al. Chronic arsenic exposure and urinary 8-hydroxy-2′-deoxyguanosine in an arsenic-affected area in Inner Mongolia, China. J Exp Anal Environ Epidemiol. 2005;15:147–52.

48. Pineda-Zavaleta AP, Garcia-Vargas G, Borja-Arjunto VH, Acosta-Saavedra LC, Vazquez E, Gomez-Munoz A, Cebrian ME, Calderon-Arauza ES. Nitric oxide and superoxide anion production in monocytes from children exposed to arsenic and lead in region Lagunera, Mexico. Toxicol Appl Pharmacol. 2004;198:283–90.

49. Wang TC, Jan KY, Wang AS, Gurr JR. Trivalent arsenicals induce lipid peroxidation, protein carboxylation, and oxidative DNA damage in human urothelial cells. Mutat Res. 2007;615:75–86.

50. Maharjan M, Watanabe C, Ahmad SA, Umezaki M, Ohtsuka M. Mutual interaction between nutritional status and chronic arsenic toxicity due to groundwater contamination in an area of Terai, lowland Nepal. J Epidemiol Community Health. 2007;61:389–94.

51. Mo J, Xia Y, Wade TJ, Schmitt M, Le XC, Dang R, Mumford JL. Chronic arsenic exposure and oxidative stress: OGG1 expression and arsenic exposure, nail selenium, and skin hyperkeratosis in Inner Mongolia. Environ Health Perspect. 2006;114:835–41.

52. Kabota R, Kunito T, Agusa T, Fujihara J, Monirith I, Iwata H, Subramanian A, Tanaka T, Tanabe S. Urinary 8-hydroxy-2′-deoxyguanosine in inhabitants chronically exposed to arsenic in groundwater in Cambodia. J Environ Monit. 2006;8:293–9.

53. Ding W, Hudson LG, Liu KJ. Inorganic arsenic compounds cause oxidative damage to DNA and protein by inducing ROS and RNS generation in human keratinocytes. Mol Cell Biochem. 2005;279:105–12.

54. Kessel M, Liu SX, Xu A, Santella R, Hei TK. Arsenic induces oxidative DNA damage in mammary cells. Mol Cell Biochem. 2002;234–235:301–8.

55. Matsui M, Nishigori C, Toyokuni S, Takada J, Ishikawa M, Imamura S, Miyachi Y. The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2′-deoxyguanosine in arsenic-related Bowen’s disease. J Invest Dermatol. 1999;113:26–31.

56. Wang RH, Kuo CY, Hsu ML, Wang TY, Chang PI, Wu TH, Huang S. Increased levels of 8-hydroxy-2′-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. Environ Health Perspect. 2005;113:1386–90.

57. Mazumder DN. Effect of chronic intake of arsenic-contaminated water on liver. Toxicol Appl Pharmacol. 2005;206:169–75.

58. Shila S, Subathra M, Devi MA, Panneerselvam C. Arsenic intoxication-induced reduction of glutathione level and of the activity of related enzymes in rat brain regions: reversal by tr-alpha-lipic acid. Arch Toxicol. 2005;79:140–6.
63. Shen ZY, Shen WY, Chen MH, Shen J, Zeng Y. Reactive oxygen species and antioxidants in apoptosis of esophageal cancer cells induced by As2O3. Int J Mol Med. 2003;11:479–84.

64. Wu MM, Chioiu HY, Wang TW, Hsieh YM, Wang IH, Chen CJ, Lee TC. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. Environ Health Perspect. 2001;109:1011–7.

65. Lin S, Del Razo LM, Styblo M, Wang C, Cullen WR, Thomas DJ. Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. Chem Res Toxicol. 2001;14:305–11.

66. Ramanathan K, Anusuyadevi M, Shila S, Panneer Selvam C. Ascorbic acid and alpha-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicol Lett. 2005;156:297–306.

67. Ramanathan K, Shila S, Kumaran S, Panneer Selvam C. Ascorbic acid and alpha-tocopherol as potent modulators on arsenic induced toxicity in mitochondria. J Nutr Biochem. 2003;14:416–20.

68. Sohini, Rana SV. Protective effect of ascorbic acid against oxidative stress induced by inorganic arsenic in liver and kidney of rat. Indian J Exp Biol. 2007;45:371–5.

69. Garcia-Chavez E, Jimenez I, Segura B, Del Razo LM. Lipid oxidative damage and distribution of inorganic arsenic and its metabolites in the rat nervous system after arsenite exposure: influence of alpha tocopherol supplementation. Neurotoxicology. 2006;27:1024–31.

70. Wei M, Arnold L, Cano M, Cohen SM. Effects of co-administration of antioxidants and arsenicals on the rat urinary bladder epithelium. Toxicol Sci. 2005;83:237–45.

71. Chattopadhyay S, Bhaumik S, Purkayastha M, Basu S, Nag Chaudhuri A, Das Gupta S. Apoptosis and necrosis in developing brain cells due to arsenic toxicity and protection with antioxidants. Toxicol Lett. 2002;136:65–76.

72. Bongiovanni GA, Soria EA, Eynard AR. Effects of the plant flavonoids Mentha spicata, M. piperita and M. arvensis on apoptosis and cell cycle distribution of K1 cells. Food Chem Toxicol. 2007;45:971–6.

73. Gupta R, Dubey DK, Kannan GM, Flora SJ. Concomitant administration of Moringa oleifera seed powder in the remediation of arsenic-induced oxidative stress in mouse. Cell Biol Int. 2007;31:44–56.

74. Mandal AK, Das S, Basu MK, Chakrabarti RN, Das N. Hepatoprotective activity of liposomal flavonoid against arsenite-induced liver fibrosis. J Pharmacol Exp Ther. 2007;320:994–1001.

75. Rabbani GH, Saha SK, Akhtar M, Marni F, Mitra AK, Ahmed S, Erum S, Khan M, Khan JI, Khan M, Khan M. Protective effect of ascorbic acid against arsenic-induced oxidative injury in rabbits: preliminary results. J Environ Sci Health A Toxic Hazard Subst Environ Eng. 2003;38:273–87.

76. Sharma A, Sharma MK, Kumar M. Protective effect of Mentha piperita against arsenic-induced toxicity in liver of Swiss albino mice. Basic Clin Pharmacol Toxicol. 2007;100:249–57.

77. Kibriya MG, Jasmine F, Argos M, Verret WJ, Rakibuz-Zaman M, Chaudhuri AK, Purkayastha M, Saha SK, Akhtar M. Methylation deficiency on the metabolism of arsenate in the rabbit. Acta Pharmacol Toxicol (Copenh). 1986;59: Suppl 7:35–8.

78. Zeng H, Uthus EO, Combs GF Jr. Mechanistic aspects of the interplay of oxidative stress and arsenic metabolism in carcinogenesis. Acta Pharmacol Toxicol (Copenh). 2001;89:121–5.

79. Steinmaus C, Carrigan K, Kalman D, Atallah R, Yuan Y, Smith AH. Diet and arsenic exposure: a workshop report. Placenta. 2007;28:52–61.

80. Heek JE, Gamble MV, Chen Y, Graziano JH, Slavkovich V, Parvez F, Barom JA, Howe GR, Ahsan H. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. Am J Clin Nutr. 2007;85:1367–74.

81. Li L, Ekstrom E-C, Goessler W, Lonnerdal B, Nermell B, Yunus M, Rahman A, El Arifeen S, Persson LÅ, et al. Nutritional status has marginal influence on the metabolism of inorganic arsenic in Bangladeshi women. Environ Health Perspect. 2007; in press.

82. Mudd SH, Brosnan JT, Brosnan ME, Jacobs RL, Stabler SP, Allen RH, Vance DE, Wagner C. Methyl balance and transmethylation fluxes in humans. Am J Clin Nutr. 2007;85:19–25.

83. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. Annu Rev Nutr. 2006;26:229–50.

84. Lindberg A-L, Kumar R, Goessler W, Thirumaran R, Gursau E, Koppova K, Rudnai P, Leonardi G, Fletcher T, et al. Metabolism of low-dose inorganic arsenic in a Central European population. Influence of sex and genetic polymorphisms. Environ Health Perspect. 2007;115:1081–6.

85. Spiegelstein O, Xu L, Le XC, Troen A, Sellub J, Melnyk S, James SJ, Finnell RH. Effects of dietary folate intake and folate binding protein-1 (Folbp1) on urinary speciation of sodium arsenate in mice. Toxicol Lett. 2003:145:167–74.

86. Gamble MV, Liu L, Ahsan H, Ries H, Slavkovich V, Parvez F, Levy D, Factor-Litvak P, et al. Folate, homocysteine, and arsenic metabolism in arsenic-exposed individuals in Bangladesh. Environ Health Perspect. 2005;113:1683–8.

87. Gamble MV, Ahsan H, Liu X, Factor-Litvak P, Illievski V, Slavkovich V, Parvez F, Graziano JH. Folate and cobalamin deficiencies and hyperhomocysteinemia in Bangladesh. Am J Clin Nutr. 2005;81:1372–7.

88. Gamble MV, Liu X, Ahsan H, Pilsner JR, Illievski V, Slavkovich V, Parvez F, Chen Y, Levy D, et al. Folate and arsenic metabolism: a double-blind, placebo-controlled folate acid-supplementation trial in Bangladesh. Am J Clin Nutr. 2006;84:1093–101.

89. Walton FS, Waters SB, Jolley SL, Clukey EL, Thomas DJ, Styblo M. Selenium compounds modulate the activity of recombinant rat ASII-methyltransferase and the methylation of arsenate by rat and human hepatocytes. Chem Res Toxicol. 2003;16:261–5.

90. Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. J Nutr. 2002;132:2333S–5S.

91. Ueland PM, Holm PI, Hustad S. Betaine: a key modulator of one-carbon metabolism and homocysteine status. Clin Chem Lab Med. 2005;43:1069–75.

92. Zeisel SH. The fetal origins of memory: the role of dietary choline in optimal brain development. J Pediatr. 2006;149:S131–6.

93. Schlawicke Engstrom K, Broberg K, Concha G, Nermell B, Warholm M, Valter M. Genetic polymorphisms influencing arsenic metabolism: evidence from Argentina. Environ Health Perspect. 2007;115:599–605.

94. Meza MM, Yu L, Rodriguez YY, Guild M, Thompson D, Gandolfi AJ, Klimecki WT. Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and CYT19 polymorphisms in children. Environ Health Perspect. 2005;113:775–81.

95. Chen H, Li S, Liu J, Diwan BA, Barrett JC, Waales MP. Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis. Carcinogenesis. 2004;25:1779–86.
106. Cooney CA. Dietary selenium and arsenic affect DNA methylation. J Nutr. 2001;131:1871–2.

107. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr. 2000;71:1344S–52S.

108. Ghoshal K, Li X, Datta J, Bai S, Pogribny I, Pogribny M, Huang Y, Young D, Jacob ST. A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. J Nutr. 2006;136:1522–7.

109. Hopenhayn-Rich C, Biggs ML, Smith AH, Kalman DA, Moore LE. Methylation study of a population environmentally exposed to arsenic in drinking water. Environ Health Perspect. 1996;104:620–8.

110. Zhang Z, Deng C, Lu Q, Richardson B. Age-dependent DNA methylation changes in the ITGAL (CD11a) promoter. Mech Ageing Dev. 2002;123:1257–68.

111. Schumacher A, Kapranov P, Kaminsky Z, Flanagan J, Assadzadeh A, Yau P, Virtanen C, Winegarden N, Cheng J, et al. Microarray-based DNA methylation profiling: technology and applications. Nucleic Acids Res. 2006;34:528–42.

112. Xie Y, Liu J, Benbrahim-Tallaa L, Ward JM, Logsdon D, Diwan BA, Waalkes MP. Aberrant DNA methylation and gene expression in livers of newborn mice transplacentally exposed to a hepatocarcinogenic dose of inorganic arsenic. Toxicology. 2007;236:7–15.

113. Anway MD, Memon MA, Uzumcu M, Skinner MK. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J Androl. 2006;27:868–79.

114. Kumar RC, Thakur MK. Androgen receptor mRNA is inversely regulated by testosterone and estradiol in adult mouse brain. Neurobiol Aging. 2004;25:925–33.

115. Jaenisch R. DNA methylation and imprinting: why bother? Trends Genet. 1997;13:323–9.