


# Relationships among Inorganic Arsenic, Nutritional Status CpG Methylation and microRNAs: A Review of the Literature

Epigenetics Insights  
Volume 14: 1–9  
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DOI: 10.1177/2516865721989719



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**ABSTRACT:** Inorganic arsenic is a naturally occurring toxicant that poses a significant and persistent challenge to public health. The World Health Organization has identified many geographical regions where inorganic arsenic levels exceed safe limits in drinking water. Numerous epidemiological studies have associated exposure to inorganic arsenic with increased risk of adverse health outcomes. Randomized clinical trials have shown that nutritional supplementation can mitigate or reduce exacerbation of exposure-related effects. Although a growing body of evidence suggests that epigenetic status influences toxicity, the relationships among environmental exposure to arsenic, nutrition, and the epigenome are not well detailed. This review provides a comprehensive summary of findings from human, rodent, and in vitro studies highlighting these interactive relationships.

**KEYWORDS:** arsenic, nutrition, epigenetics, CpG methylation, miRNAs

**RECEIVED:** September 2, 2020. **ACCEPTED:** December 31, 2020.

**TYPE:** Environmental and Nutritional Epigenetics - Review

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded in part by the National Institute of Environmental Health Sciences (P42-ES031007).

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

Nutritional status is one of the key determinants of health or disease and thus has broad implications for public health.<sup>1</sup> In support of this, a suboptimal diet and resultant nutritional status is strongly associated with an increased risk of chronic illness and other conditions.<sup>2–4</sup> The physiological processes that are influenced by nutrients are broad and include the modulation of hormones, oxidative stress levels, and inflammatory responses.<sup>5–7</sup> Nutritional status is also associated with the modulation of machinery that is critical for the detoxification of chemicals, thereby influencing contaminant-induced health effects.<sup>8</sup> With relevance to this review, nutrients can also serve as modifiers of the epigenomic landscape.<sup>9</sup> Epigenetic alterations are increasingly recognized as mechanisms that are associated with disease states, exposure-related effects, and altered toxicant metabolism.

The focus of this review is on exposure to the environmental toxicant inorganic arsenic (iAs) and its relationships with both nutritional status and the epigenome. As a toxicant, iAs is important to study as it is a naturally occurring metalloid abundant across the globe and its presence is associated with health effects world-wide.<sup>10</sup> The current drinking water standard of iAs as regulated by the United States Environmental Protection Agency (US

EPA) is 10 parts per billion. Of public health concern, the World Health Organization (WHO) has determined that groundwater in several countries have much higher levels of iAs with millions exposed to harmful levels.<sup>11</sup> In humans, a major route of exposure to iAs is from drinking water while occupational exposures can also occur from industrial activities such as coal combustion and smelting operations.<sup>12</sup> In addition, consumption of food contaminated with iAs such as fish, poultry, and rice, is an additional source of exposure and iAs-associated toxicity.<sup>10,13</sup> The International Agency for Cancer (IARC) has classified iAs as Group 1 substance that is a known carcinogen to humans.<sup>14</sup> Specifically, long term exposure to iAs from drinking water and food is associated with cancer of the lungs, bladder, kidney, and skin.<sup>15–17</sup> In addition, skin lesions and pigmentations are also symptoms of arsenicosis.<sup>10</sup> Although a single mechanism underlying iAs-associated disease is unlikely, alterations to the epigenome such as CpG methylation or microRNA (miRNA) expression are hypothesized to contribute to its carcinogenic properties.<sup>18</sup> Detoxification of iAs is dependent on the availability of cellular methyl donors, S-adenosylmethionine (SAM).<sup>19</sup> In addition, iAs has been observed to alter DNA methylation machinery that may impact genome-wide DNA methylation levels.<sup>20</sup> This review highlights the relationships among exposure to iAs, nutrition, the epigenome and health effects based on evidence from humans, rodents, and in vitro studies.

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## IAAs-Nutrition Relationships in Humans

In the human studies portion of this review, we highlight pregnancy and birth cohorts that investigate the relationships among iAs exposure, nutritional factors, and where available epigenetic measurements. These cohorts include the Health Effects of Arsenic Longitudinal Study (HEALS), the Nutritional Influences on Arsenic Toxicity (NIAT) cohort, the US National Health and Nutrition Examination Survey (NHANES), Biomarkers of Exposure to ARsenic (BEAR) cohort, and the New Hampshire Birth cohort study (NHBCS). In addition to these cohort studies, we will discuss others that directly address the toxicity and susceptibility of chronic iAs exposure such as the Folate and Oxidative Stress Study (FOX). This research covers a range of exposure sources from drinking water, to other biological markers of exposure such as: maternal urine, child urine, blood, cord blood, toe nail samples, lymphoblasts, and placenta samples. Other outcome measurements of the human studies aside from epigenetic modification include: cognitive development, mortality, pregnancy outcomes, skin lesions, and birth outcomes. Acute, chronic, and ongoing iAs exposure will be reviewed in terms of its known associations and relationships with potentially increased risk and susceptibility for illnesses and diseases.

Several studies have examined the role of nutritional factors in mitigating the adverse effects of iAs.<sup>21-23</sup> As an example, the HEALS cohort is a large epidemiologic investigation in Bangladesh, focusing on the adverse health effects of iAs in relation to infant cognitive development, mortality, and pregnancy outcomes.<sup>24</sup> In the HEALS study, investigators focused on several communities that were chronically-exposed to varying concentrations of iAs through their drinking water. Personal information, including diet, education, age, gender, and marital status, was collected from all participants.<sup>24</sup> Clinical evaluation was conducted in blood and spot urine samples.<sup>24</sup> The HEALS cohort study revealed a positive correlation between plasma folate concentrations and urine creatinine levels in the adults.<sup>25</sup> A large cross-sectional study nested within HEALS, known as the NIAT cohort, was designed to uncover potential nutritional influences on iAs metabolism.<sup>21</sup> Research from the NIAT cohort examined the relationship between hyperhomocysteinemia with folate and cobalamin levels.<sup>25</sup> The iAs exposure ranged up to 860 µg/L which far exceeds the 10 µg/L maximum contaminant level.<sup>26,27</sup> The investigators found that plasma folate levels were lower among males than female study participants, while plasma cobalamin levels were higher in males than females. The investigators also reported a significantly higher prevalence of hyperhomocysteinemia in males (63%) compared to females (26%).<sup>25</sup> A significant negative association was observed between plasma total homocysteine and folate levels in both male and female participants. The authors concluded that hyperhomocysteinemia in male participants may be attributable to folate deficiency. Two follow up investigations from the same group investigated the relationship between folate and iAs metabolism.<sup>22,23</sup> One of these was a double-blind, randomized trial to test whether folic acid

supplementation would increase iAs methylation. Specifically, 130 participants consumed folic acid supplements (400 µg/d) for 12 weeks.<sup>23</sup> This folic acid supplementation dosage used is consistent with the recommendation of US Public Health Service for all women capable of childbearing to reduce adverse outcomes such as neural tube defects (NTDs) and specifically spina bifida.<sup>28</sup> The placebo-controlled trial revealed statistically significantly lower blood iAs levels by 13.62% with folic acid supplementation in the treatment group and 2.49% in the placebo group. Because iAs is methylated to dimethylarsinic acid (DMAs) and methylarsonic acid (MMAs) in a folate-dependent manner via the one-carbon pathway, the authors sought to examine the relationships between these metabolites and the protective effects of folic acid. They identified decreased blood MMAs in the treatment group (22.24%) relative to the placebo group (1.24%). No significant changes in blood DMAs were seen between the two groups, but urinary DMAs were found to be significantly elevated in the treatment group. The authors concluded that folic acid supplementation may potentially increase iAs methylation.<sup>21-23,25</sup>

In another large-scale study, the US NHANES investigated the association between serum folate levels and iAs methylation.<sup>29</sup> Here, iAs was measured in urine with a >0.25 ml inclusion cutoff. Percent urinary monomethylarsonic acid (MMA<sup>V</sup>) was positively associated with serum folate levels in adults, and percent dimethylarsinic acid (DMA<sup>V</sup>) was positively associated with serum folate levels in children.<sup>29</sup> Thus, the conclusion was that folic acid supplementation promotes iAs methylation differently in adults than in children.<sup>29</sup>

Developmental delay in preschool children has been linked to inefficient iAs methylation capacity.<sup>30</sup> This association led investigators to explore the potential nutritional influence of vitamin B12 and plasma folate intake on the relationship on iAs methylation capacity and effects in a cross-sectional study among Uruguayan school children.<sup>31</sup> To assess this, the researchers tested B12 intake, B12 status, and iAs methylation capacity as potential effect modifiers of the relationship between urinary arsenic (U-As) and academic achievement. IAAs<sup>III</sup> and iAs<sup>V</sup>, along with the methylated metabolites, were measured in urine. Serum samples were collected from children to assay folate and B12 intake. IAAs methylation capacity was measured as the proportion of urinary monomethylarsonic acid (%MMA) and the Woodcock-Johnson Achievement Battery was used to assess academic achievement. The investigators found no association between urinary iAs, reading scores and broad math skills.<sup>31</sup> Moreover, iAs methylation capacity was also not modified by B12.<sup>31,32</sup> The role of methylation capacity in iAs-associated neurotoxicity among children was inconclusive. Further, no evidence of effect modification was observed in the two studies on iAs and neurodevelopment that also included stratification by %MMA.<sup>32,33</sup> Complicating these analyses is the fact that the high and low cutoffs for %MMA have yet to be standardized due to the many factors influencing iAs methylation.<sup>31</sup> The efficiency of iAs methylation capacity was identified by percentages

of low MMA<sup>V</sup>, low iAs, and high DMA<sup>V</sup> and the average urinary arsenic levels were 11.7 µg/L<sup>31</sup> and 11.9 µg/L.<sup>32</sup>

Hamadani et al. set out to elucidate the critical window of exposure for iAs-associated cognitive impairment within the Matlab, Bangladesh longitudinal population-based cohort where >95% of the population was exposed to water arsenic levels >10 µg/l.<sup>33,34</sup> The researchers assessed the effects of nutrient supplementation during pregnancy as part of a maternal and infant nutrition intervention in Matlab (MINIMat), and children born in the study were recruited to measure child development.<sup>33</sup> The data supported a significant negative association between urinary iAs in girls and cognitive measurements (verbal IQ and full-scale IQ). No significant association was found between urinary iAs and cognitive measurements in boys. The effect size calculation showed a decrement of 1–3 intelligence quotient (IQ) points in both cognitive measures at 100 µg/L in girls.<sup>33</sup> In another study, the effect of niacin, thiamin, vitamin B-6, and vitamin B12 intake on iAs methylation was assessed in children with low-level iAs exposure (<50 µg/L in water; urinary arsenic 5–50 µg/L).<sup>35</sup> Although the researchers found an inverse association between B-6 levels and urinary MMA percentage, there was no significant relationship observed between iAs methylation and B-6 levels.<sup>35</sup>

Several studies have investigated the relationships among iAs exposure, nutritional status, pregnancy, and health outcomes.<sup>36–38</sup> For example, within the BEAR cohort in Mexico, the relationship between the one-carbon metabolic pathway and iAs methylation has been investigated.<sup>36</sup> The BEAR cohort aimed to identify the underlying mechanisms linking prenatal iAs exposure with detrimental health effects in offspring.<sup>39</sup> Specifically, iAs exposure from drinking water ranging from 0.456–236 µg/l was examined in newborns from the BEAR cohort.<sup>40</sup> Folate, homocysteine, and B12 levels were evaluated as one-carbon metabolism indicators (OCM) in maternal serum obtained from study participants. The cohort consisted of 200 mother-newborn pairs in Gómez Palacio, Mexico, with iAs exposure information derived from maternal urine samples and drinking water measurements. iAs and its metabolites were measured in neonatal cord serum as well as maternal urine. The study revealed that 74% of the pregnant women in the cohort exhibited deficient B12 serum levels, which were defined as serum levels <148 pmol/L. The study also suggested that defined OCM indicators may influence iAs metabolism within the BEAR cohort since a strong correlation between maternal women with elevated % MMA in cord serum and low folate levels was identified.<sup>36</sup> To summarize, these studies collectively show that nutritional status is associated with iAs-associated health outcomes.

### **iAs-Nutrition-DNA Methylation Relationships in Humans**

DNA methylation is a post-replication modification as well as one of the main epigenetic mechanisms used to control gene expression, especially during early development.<sup>41,42</sup> DNA

methylation is the addition of methyl groups to the 5' position of cytosines which impacts gene transcription. Specifically, methyl groups are capable of either repressing or activating gene expression and can be derived from certain dietary sources.<sup>41</sup> DNA methylation is an important potentially heritable phenotypic measurement because it is essential for cell differentiation and embryonic development. Alterations in the early embryonic reprogramming can lead to changes in DNA methylation patterns, inducing permanent impairments to the developmental program which may result in adverse health outcome.<sup>43</sup> Changes in DNA methylation can occur as a result of maternal environmental exposure influencing embryonic development as well as lasting epigenetic alterations in offspring.<sup>43</sup>

In relation to iAs exposure, researchers have identified a suite of genes with altered methylation status in association with arsenicosis.<sup>44</sup> The identified genes encode proteins that are involved in cancer, diabetes, and heart disease including SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily d, member 2 (*SMARCD2*), homeobox B9 (*HOXB9*); msh homeobox 1, also known as HOX7 (*MSX1*) and matrix metalloproteinase 15 (membrane inserted; *MMP15*).<sup>44</sup>

Several studies have expanded analysis beyond maternal urine and cord serum, to examine the association between iAs exposures and DNA methylation status in target tissue using placenta samples.<sup>45–47</sup> As a result of this change in utilizing the placenta as an investigative model, recent studies have successfully found an association between changes in DNA methylation in the placenta with environmental exposures on newborn growth and neurodevelopment outcomes.<sup>45–47</sup> As an example, the NHBCS is a prospective birth cohort consisting of pregnant women and their corresponding placenta samples. An inclusion criterion for study participation was that the pregnant women's primary source of drinking water be from an unregulated private well. Placental samples were used to carry out genetic, epigenetic, and environmental exposure assays.<sup>48</sup> The analysis of placental DNA methylation in the NHBCS revealed differential methylation in LYR Motif Containing 2 (*LYRM2*) gene and genome regions following iAs exposure.<sup>49</sup> A separate study focused on the association between prenatal iAs exposure and DNA methylation in infant cord blood. The analysis revealed 2919 differentially methylated genes, and a subset that displayed correlation between both DNA methylation and mRNA expression. Among these were protein tyrosine phosphatase receptor type E (*PTPRE*), WD repeat domain 55 (*WDR55*), ring finger protein 213 (*RNF213*), cyclin G associated kinase (*GAK*), potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*), protein phosphatase targeting COQ7 (*PPTC7*), and post-GPI attachment to proteins 2 (*PGAP2*) which were associated with changes birth outcomes such as gestational age and head circumference. These findings support the need for additional studies relating to functional outcomes and changes in coupled epigenetic modifications as a result of iAs exposure.<sup>40</sup>

In a separate study, a Taiwanese maternal-infant and birth cohort reported an association between methylated genes and long-term low-density lipoproteins (LDL) in children following DNA methylation analysis from cord blood samples.<sup>50</sup> After initial discoveries identified various CpG sites associated with prenatal iAs exposure, a follow-up validation study confirmed DNA methylation changes were driven by iAs.<sup>50</sup> Having both discovery and validation phases within a study will help determine biological pathways that underlie epigenetic changes as a result of in utero iAs exposure. In a separate study population in Bangladesh, researchers evaluated the relationship between in utero iAs exposure, birth outcomes such as gestational age and birth weight, and DNA methyltransferase 3 alpha (*DNMT3A*) DNA methylation.<sup>51</sup> In utero iAs exposure was assessed in maternal toenail samples at birth, and cord blood was used to measure DNA methylation of *DNMT3A* of three CpGs. The limit of detection for iAs exposure in the toenail clippings ranged from 0.004 to 0.85 µg As/g, and iAs-contaminated drinking water exposure range was 0.5 to 545.0 µg/L. The investigators identified iAs-associated genes that were involved in DNA methylation, RNA polymerase binding and transcription, genetic imprinting, mitotic cell cycle, protein binding, aging, and regulation of cell death. This study supported *DNMT3A* as a candidate mediator gene between prenatal exposure and birth outcomes, as *DNMT3A* was found to partially mediate prenatal As exposure and gestational age and birth weight. This suggests that DNMT3A may play a critical role in fetal epigenetic programming, at least in the context of DNA methylation following in utero iAs exposure.<sup>51</sup> In the same cohort, iAs exposure was examined in intrauterine and maternal peripheral blood metabolites, and they were both found to influence the association between iAs toxicity and low birth weight.<sup>52</sup> Two pathways were identified from the mediation analysis between cord blood arsenic, metabolites, and birthweight, namely medium-chain fatty acid, and fatty acid, branched benzoate metabolism.<sup>52</sup> In summary, these studies support the relationship between altered gene DNA methylation and disease outcomes with iAs exposure.

Studies detailing the effects of iAs exposure on epigenetic modifications to DNA are becoming more prevalent in the literature.<sup>20,53,54</sup> The effects of both iAs exposure levels and folic acid on DNA methylation in the NIAT cohort have been reported.<sup>55</sup> The researchers sought to determine if iAs exposure was linked to genomic hypomethylation of peripheral blood leukocyte (PBL) DNA among chronically exposed adults. They also investigated how folate nutritional status might mediate iAs-induced changes in DNA methylation. In order to examine chronic exposure in the study, an inclusion criterion was applied to drinking water with >50g As/L (70% of the participants). Plasma folate concentrations, PBL DNA methylation, urine arsenic, and plasma arsenic were also assessed. Folate was an effect modifier of the positive correlation between arsenic levels (urinary arsenic:  $P=0.009$ , plasma arsenic:  $P=0.03$ , and plasma

folate:  $P=0.03$ ) and genomic DNA methylation in PBL in a dose-dependent manner. Age and [3H]-methyl incorporation of PBL DNA was significantly higher in the low-folate group (participants with plasma folate concentrations <9nmol/L) than in the high-folate group (participants with plasma folate concentrations  $\geq 9$ nmol/L). Age was the greatest predictor of genomic methylation of PBL DNA.<sup>55</sup> These findings suggest that adequate folate may lead to iAs-induced increases in DNA methylation.<sup>55</sup>

The FOX cohort study sought to address the chronic iAs exposure that persists in drinking water in Bangladesh across 5 exposure categories [ $<10$  mg/L ( $n=76$ ), 10-100 mg/L ( $n=104$ ), 101-200 mg/L ( $n=86$ ), 201-300 mg/L ( $n=67$ ), and  $>300$  mg/L ( $n=45$ )].<sup>56</sup> A null association was found between S-adenosylhomocysteine (SAH) and iAs metabolites measured in blood and urine samples. A positive association was found between SAM and the percentage of blood MMA in folate- and cobalamin-deficient individuals. In contrast, log(SAM) was negatively associated with log percent urinary iAs. These findings suggest that interindividual differences in iAs metabolism may play a role in iAs toxicity susceptibility. This was evidenced by folate and cobalamin nutritional status modifying the associations between SAM and the percentage of both arsenic metabolites (MMA and DMA) in an inverse manner.<sup>57</sup>

### iAs-Nutrition-miRNA Relationships in Humans

In addition to DNA methylation detailed previously, microRNAs (MiRNAs) represent another form of an epigenetic modifier. miRNAs are epigenetic modifications in the form of short (ie, 22 nucleotides in length) non-coding endogenous RNA molecules that bind to target mRNAs and silence expression. MiRNAs regulate cellular functions such as development, growth, and metabolism, as well as differentiation.<sup>58</sup> The expression of miRNAs can be influenced by nutritional and environmental factors.<sup>59</sup> As mentioned before, the BEAR cohort aimed to identify the underlying etiologies of health effects associated with prenatal inorganic arsenic exposures.<sup>39</sup> iAs exposure concentrations ranged up to 236 µg As/L, and 53% of the women had drinking water measurements that exceeded the recommended maximum contaminant level.<sup>26,27</sup> In a subset of the BEAR cohort, newborn cord blood samples were examined to assess genome-wide changes in microRNA (miRNA) relative to in utero iAs exposure.<sup>60</sup> Associations were identified between microRNAs and immune signaling pathways produced by immune cell-specific response gene expression data from newborn cord blood samples in the BEAR cohort that were altered by arsenic exposure.<sup>60</sup> Rager et al. analyzed miRNA profiles from the BEAR pregnancy cohort with arsenic exposure ranging up to 240 µg/l. They found 12 miRNAs in response to maternal urine arsenic exposure involved in diabetes mellitus (DM)-related signaling pathways (ie, insulin-signaling pathway).<sup>60</sup> DM is a metabolic disorder characterized by insulin

resistance and pancreatic  $\beta$ -cell dysfunction and limited uptake of glucose by cells as a result of alterations in the insulin-signaling pathway.<sup>61</sup> Of the 12 identified miRNAs, arsenic-associated changes in miR-107 and miR-20b have previously been associated with DM.<sup>60</sup> In a separate study, the relationship between miRNAs, iAs, and an increase in the level of albumin in the urine also known as microalbuminuria, was assessed in adolescents in a cross-sectional, population-recruited study.<sup>62</sup> A negative association was found between urinary arsenic levels and two miRNAs (miR-21 and MiR-221), which suggests that these miRNAs may be involved in the pathological mechanism between iAs exposure and albuminuria.<sup>62</sup>

### iAs-Nutrition-DNA Methylation Relationships in Rodent Models

Several animal studies have been conducted to understand the effect of iAs, in various forms, on DNA methylation and gene expression.<sup>63-65</sup> For example, chronic exposure to iAs results in tumor formation similar to tumors caused by altered estrogen receptor (ER) signaling.<sup>63</sup> As an example, in mice exposed to iAs, estrogen receptor-alpha (*ER $\alpha$* ), cyclin D1 and many cytochrome P450 (*Cyp*) genes were investigated for altered estrogen signaling where quantitative real-time RT-PCR and methylation-specific PCR were used to study DNA methylation status of the target genes. Mice exposed to iAs showed a 90% reduction in methylation of the *ER $\alpha$*  gene, indicating that estrogen signaling may play a role in hepatocellular Carcinoma (HCC) by arsenic exposure.<sup>63</sup> Similar to the mouse studies, Uthus et al. exposed rats to 0.5 or 50  $\mu$ g iAs in the diet for 12 weeks and showed dimethylhydrazine-induced aberrant crypts within the colon.<sup>64</sup> When global DNA methylation and DNMT activity was measured in the liver, relative DNA hypomethylation and increased DNMT activity were observed. The authors concluded that there is a threshold for iAs toxicity on dimethylhydrazine-induced aberrant crypt formation in colon.<sup>64</sup> Reproductive and developmental studies have also been conducted to investigate the effects of iAs.<sup>65</sup> For instance, pregnant mice were exposed to iAs at carcinogenic levels (85 ppm) in drinking water. Following birth, the livers of the pups were analyzed for iAs content which was found in relatively high levels. This indicates that iAs was able to pass the placental barrier and the pups were exposed to iAs. However, the global methylation status of the hepatic DNA of the pups was relatively unchanged. Over 600 genes were analyzed and at least 40 showed alterations in gene expression in newborn livers. Expression of genes in the glutathione system such as Glutathione S-Transferase (*GST*) alpha were found to be significantly increased. There was also increased expression in the stress-associated gene Metallothionein-1 (*MT1*). In addition, the expression of the cyclin-dependent kinase inhibitor (*p57kip2*) was increased with iAs, while the expression of cyclin-dependent kinase inhibitor (*p16*) decreased. Furthermore, *CYP2A4* gene expression was increased, but in general, the

expression of cytochrome P450 enzyme genes (*CYP3A25*, *CYP2F2*, *CYP2J5*, *CYP7B1*) was decreased. Exposure to iAs also altered genes in the insulin-like growth factor signaling system (*IGF-2*, *IGF-1*, *IGFBP1* and *IGFBP3*). Additional aberrant expression was observed, indicating that gene expression changes exists in the newborn liver is exposed to transplacental hepatocarcinogenic exposures to arsenic.<sup>65</sup>

In relation to diet, supplementation of folate has been shown to modify iAs metabolism and the adverse effects of iAs exposure. Studies have been conducted both in adult mice and in the context of prenatal exposures.<sup>66-68</sup> Specifically, dams were exposed to iAs in drinking water and supplemented with folic acid in their diet.<sup>66</sup> The metabolic phenotype of the offspring was assessed, and it was observed that the male offspring from iAs-exposed dams fed with folic acid diet developed insulin resistance and minimal effect on female offspring. These results demonstrate that prenatal iAs exposure impairs glucose metabolism in a sex-specific manner. It was also suggested that folate supplementation may improve the metabolic phenotype in offspring.<sup>66</sup> On the contrary, another study demonstrated that a combined in utero exposure to iAs and a high folic acid intake could affect DNA methylation profiles and weight of fetuses.<sup>69</sup> This study showed that iAs-folate exposure altered the methylation of nearly 3000 CpG island genes and also suggested that imprinting genes were part of the genomic targets. A few examples of these imprinted genes include Guanine nucleotide-binding protein, alpha stimulating (*Gnas*), *Neuronatin (Nnat)*, Potassium Two Pore Domain Channel Subfamily K Member 9 (*Kcnk9*), Insulin Like Growth Factor 2 Receptor (*Igf2r*), Delta Like Non-Canonical Notch Ligand 1 (*Dlk1*), Tumor Suppressing Subtransferable Candidate 4 (*Tssc4*), Growth Factor Receptor Bound Protein 10 (*Grb10*), and Mesoderm-specific transcript homolog protein coding gene (*Mest*). Most of these genes were associated with neurodevelopment, cancer, cell cycle, and signaling networks.<sup>69</sup> The authors also suggested an increased risk for early disease onset in the offspring. In addition, the role of a methyl-deficient diet and iAs exposure was studied in mice administered via drinking water.<sup>70</sup> Exposure to iAs increased genomic hypomethylation in a dose-dependent manner at several cytosine sites within the promoter regions of *Ha-ras*. This decreased methylation in *Ha-ras* region might inform perturbation of methylation patterns of cellular growth genes. This study confirms that methyl-deficient diets reduce DNA methylation and establishes the role of iAs in reducing genomic DNA methylation. It is speculated that the reduction in DNA methylation could occur because of either depletion in the methyl-donor pool or by inhibition of the DNA methyltransferase.<sup>70</sup> These studies highlight the importance of dose and duration of exposure, and also the time of exposure of both inorganic arsenic and folate supplementation.

The effect of dietary folate deficiency has been studied on iAs-induced genotoxicity in mice.<sup>68</sup> Exposure to iAs resulted in a significant increase in mouse peripheral blood micronucleus,

particularly in polychromatic erythrocytes (PCEs). Significant alterations in MN-PCEs were observed with both folic acid-deficient and folic acid-sufficient diets in mice. Overall, folic acid deficiency increased iAs-induced clastogenesis at certain doses.<sup>68</sup> In another study, wild type and arsenic 3 methyltransferase (As3mt) knock out mice were fed a low fat and high-fat diet supplemented with folate.<sup>67</sup> The mice were exposed to two different concentrations of arsenic. The knock out mice gained more fat than the wild type mice and were also seen to be more insulin resistant. A high folic acid diet reduced the effect of insulin resistance.<sup>67</sup>

### iAs-Selenium Relationships in Humans and Rodent Models

Other nutritional factors beyond folate have been studied in the context of iAs toxicity. Selenium is an essential mineral found in shellfish, red meat, grains, eggs, chicken, among other foods.<sup>71</sup> It is known for its antioxidant properties has been shown to modulate disease risk by altering the epigenome.<sup>71</sup> Selenium has been shown to have antagonistic effects on iAs toxicity. In addition to several studies in human populations, the relationship between selenium and iAs has been investigated using animal models.<sup>72,73</sup> It is speculated that since the toxicity of either selenium or iAs will reduce the other, selenium status in human populations will have an effect on iAs-exposed populations. As an example, a Bangladeshi case-control study reported plasma selenium levels from blood and urine samples inversely proportional to genomic DNA methylation in leukocytes from iAs-exposed adults.<sup>74</sup> This study is a continuation of the NIAT study as a follow-up to the folic acid supplementation study.<sup>25</sup> Plasma selenium ranged between 70 and 90 µg/L, with a plasma deficiency defined as <70 µg/L. The plasma selenium levels detected were positively correlated with [<sup>3</sup>H] methyl incorporation, affecting iAs metabolism.<sup>74</sup> In contrast to a previous study finding where iAs exposure was found to be associated with increased DNA methylation, the present analysis was unchanged by the inclusion of water iAs.<sup>56,74</sup>

Among its many relative adverse health outcomes in humans, iAs exposure has also been linked to skin lesions.<sup>75</sup> In a randomized, double-blind placebo-control trial, urinary arsenic levels of men and women chronically exposed to iAs contaminated drinking water in Bangladesh received one of four treatments.<sup>76</sup> The four treatments consisted of vitamin E (400 mg racemic-tocopherol), selenium (200 µg/L-selenomethionine), vitamin E, and selenium (combination), or placebo. The investigators found that vitamin E and selenium supplementation together or alone were able to improve the status of the participants' skin lesions, but not enough to be considered significant.<sup>76</sup> A large longitudinal study from the HEALS cohort investigated the association of the incidence of iAs-associated skin lesions and blood selenium levels.<sup>77</sup> The study concluded that dietary selenium intake could be beneficial in reducing the incidence of iAs-related skin lesions among populations exposed to iAs-contaminated drinking water.<sup>77</sup>

In a separate study, the authors assessed the effects of selenium supplementation on blood-based molecular responses with iAs exposures on skin lesions as well as the effects of exposure to iAs-contaminated drinking water.<sup>78</sup> Whole-genome transcriptional changes in individuals exposed to iAs with premalignant skin lesions were monitored via peripheral blood mononuclear cells. The individuals received supplemental selenium for six months. Gene expression profiles of tumor necrosis factor (*TNF*), interleukin beta (*IL*β), interleukin 8 (*IL*8), superoxide dismutase 2 (*SOD*2), Chemokine ligand 2 (*CXCL*2) and several other immunological and stress-related genes were generated from mononuclear cells were assessed before and after selenium supplementation. For individuals with known iAs exposures and arsenic-skin lesions, several inflammatory and anti-oxidative stress genes were identified as up-regulated post selenium supplementation that were previously down-regulated prior to selenium supplementation. The authors suggested that long-term supplemental selenium may give rise to a reversible effect on gene expression relative to iAs-driven skin lesions. This human evidence collectively suggests that selenium supplementation offers protective effects to iAs-driven responses, but the potential underlying epigenetic mechanisms are not well characterized.<sup>78</sup>

As seen with folate and cobalamin studies relative to iAs exposure where nutritional status modified iAs metabolism, the Uthus et al.,<sup>64</sup> study focused on selenium status and arsenic-associated skin lesions in populations exposed to iAs-contaminated drinking water using total urine and serum for iAs and selenium respectively.<sup>79</sup> Lower selenium status (<50 µg/l) was significantly correlated with iAs-associated skin lesions. The investigators also found an accumulation of iAs due to chronic exposure when exposed to low selenium.<sup>79</sup>

Other investigations have looked into the effects of selenium-enriched foods following chronic iAs exposure in Bangladesh populations.<sup>80</sup> Researchers have aimed to reduce the risk of iAs-induced toxicity and health problems that have been linked to low blood selenium levels. In a randomized, double-blind, placebo-control trial, a selenium-rich lentil diet was administered to the intervention group (55 µg Se/day), and a low Se lentil diet was given to the control group (1.5 µg Se/day). Total iAs and iAs metabolite measurements were assessed in blood, urine, and stool samples. Consuming a selenium-rich lentil diet significantly improved the health indicators such as iAs metabolite excretion in urine with continued iAs exposure.<sup>80</sup> Specifically, selenium has had an effect on health outcome such as cancer, specifically on *p*53 gene, cardiovascular, and auto-immune diseases.<sup>81,82</sup> Selenium modifies the epigenome through one-carbon metabolism which provides a methyl donor for DNA.<sup>83</sup>

Messarah et al. evaluated the protective effect of selenium against iAs-induced oxidative damage in rats.<sup>73</sup> Male ApoE(-/-) mice were fed 3 diets: selenium deficient, selenium adequate, and a selenium fortified diet.<sup>73</sup> These mice were exposed to 200 ppb of arsenic through drinking water, and the lesion formation in

the aortic arch was assessed. In the selenium fortified diet, there were no lesions on the aortic arc; however, lesions increased in mice exposed to iAs on the selenium-deficient and adequate diets. These results indicate that there is merit to diets high in selenium and may prove as an effective intervention in human populations.<sup>73,84</sup> Weanling female B6C3F1 mice were given either a control diet of powdered rodent meal or a Torula yeast-based meal. The time course for the elimination of arsenic and its metabolites were measured over a 48 hours period.<sup>85</sup> This study found that significantly less iAs was excreted in feces from the powdered rodent meal mice compared to that of the Torula yeast-based meal. However, it was observed that significantly more iAs was excreted in urine from the powdered rodent meal. Overall, the total arsenic excreted through both urine and feces was similar across both groups.

Another study investigated the effectiveness of selenium on a timescale in comparison to an iAs exposure.<sup>86</sup> The study exposed Swiss albino mice to equal amounts of sodium arsenite and sodium selenite. The administration of the selenium occurred in 3 fashions: 1 h before iAs, coexposure, and following iAs exposure. The study found that when sodium selenite was given prior to sodium arsenite, there was a significant reduction in clastogenic effects. The protection was to a lesser effect during the co-treatment and showed a negative effect when given after sodium arsenite exposure.<sup>86</sup>

### **iAs-Folate and iAs-Selenium Relationships in vitro**

Various human cell lines have been used as in vitro models to study the impacts of iAs.<sup>87-89</sup> Exposure to human lung adenocarcinoma cells to iAs resulted in significant dose-response in hypermethylation which occurred specifically in the promoter region of the p53 gene.<sup>87</sup> In addition, genomic DNA methylation, specifically DNA methyltransferase (DNMT) activity, was significantly reduced in CAsE-PE cells.<sup>88</sup> In contrast to the investigators' hypothesis, this study showed that the K-ras promoter region did not display altered methylation levels. Human hepatocellular carcinoma cells and liver cancer cell lines have also been used to examine the methylation status of the CpG islands of tumor suppressor genes.<sup>88</sup> In particular, promoter regions of cyclin-dependent kinase inhibitor 2A locus (*p16INK4a*), Ras association domain family 1 isoform A (*RASSF1A*), E cadherin, and Glutathione S-Transferase Pi 1 (*GSTP1*) were examined. Hypomethylation was observed in the promoter region of all the genes to various degrees.<sup>89</sup> In vitro studies have also been conducted using animal cells such as rat liver epithelial cells where iAs exposure was associated with DNA hypomethylation and changes in gene expression.<sup>90</sup>

To understand the mechanism(s) of folic acid protection against iAs-induced toxicity, several in vitro studies have been conducted.<sup>91-93</sup> Folic acid protected embryo fibroblasts from dimethylarsinic acid cytotoxicity in a dose-dependent manner.<sup>91</sup> It has been proposed that iAs exerts its effect, in part, by perturbing cellular methyl metabolism. It is also known that dietary folic acid can generate the methyl donor SAM. To test this hypothesis, the

authors used folate binding protein (Folbp2) positive (+/+) and null (-/-) fibroblasts to understand iAs-induced toxicity.<sup>92</sup> Folic acid supplementation resulted in partial rescue of wildtype/positive cells from iAs-induced toxicity, supporting the possibility that toxicity could occur, at least in part, by perturbing cellular methyl biochemical reaction. Furthermore, the authors investigated the folic acid on iAs-induced toxicity in human hepatocytes.<sup>93</sup> Cells were cultured under three different conditions: folic acid-deficient medium, normal folic acid medium, folic acid supplemented medium. These cells were then co-treated with iAs in the form of sodium arsenite. The results showed that folic acid deficiency significantly increased iAs-induced apoptosis. On the other hand, folic acid supplementation decreased this effect.<sup>93</sup> In addition, folic acid deficiency significantly reduced the arsenic methylation capacity in the hepatocytes. These results suggest folate plays an essential role in mitigating iAs-induced toxicity.

In vitro models are generally used for rapid screening, understanding mechanisms of disease, and investigating complex cellular interactions. As an example, a study was conducted to evaluate the protective effect of selenium on arsenic trioxide-induced cytotoxicity, DNA damage, and apoptosis.<sup>94</sup> This study pretreated hepatocellular carcinoma line 1 (PLHC-1) cells with selenium at various concentrations and then exposed the cells to 100uM of arsenic trioxide. The cells that were pretreated with selenium displayed improved cell survival against arsenic trioxide. The increased survival rate is likely due to increased glutathione peroxidase activity. It was concluded that arsenic-induced damage and apoptosis are mediated by oxidative stress, which selenium can protect against.<sup>94</sup> Primary rat hepatocytes were exposed to iAs, and the effect of selenite on cellular retention, methylation and cytotoxicity was examined.<sup>95</sup> Coexposure to selenium and arsenic resulted in inhibition of methylation and an increase in cellular arsenic retention. Similar effects, but to a lesser extent, were observed when the cells were pre-exposed to selenium. It is suggested that these results, increasing cellular retention, and decrease in methylation may be a pathway for detoxification of inorganic arsenic.<sup>95</sup> Co-treatment exposure to sodium selenite and arsenite was shown to inhibit methylation in cultured rat hepatocytes.<sup>96</sup> It was found that sodium selenite was the most potent selenium metabolite in inhibiting methylation.<sup>96</sup>

### **Conclusion**


This review provides a survey of the literature that supports associations among iAs, nutritional factors, epigenetic modifications and health outcomes. The studies include those carried out in human populations, in vivo and in vitro that together provide sufficient evidence to support that nutritional status can influence iAs metabolism, as well as varied health effects. The data also support that there is a complex interplay between iAs exposure and epigenetic alterations. Because exposure to iAs is a persistent and global public health challenge, the need to promote nutri-epigenetic approaches is essential for better preventive and therapeutic approaches. For example, with an eye on disease prevention,

researchers should consider the role of diet as a modifier of epigenetic machinery and a potentially useful therapeutic approach to modify disease risk.<sup>59</sup> Where human population-based research helps to identify associations, toxicological experiments in rodents and using in vitro models can better assist in filling mechanistic gaps on the effects of iAs in humans. Together these studies aim to elucidate the biological pathways that may serve as therapeutic targets to prevent iAs-induced toxicity.

### Author Contribution

RCF and AV conceived of the review. AV, CAM, AMK and RCF contributed to the writing of the article.

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### Supplemental material

Supplemental material for this article is available online.

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