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Effects of Preconception and in Utero Inorganic Arsenic Exposure on the Metabolic Phenotype of Genetically Diverse Collaborative Cross Mice

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Abstract

In humans and mice, in utero exposure to inorganic arsenic (iAs) is associated with adverse health outcomes later in life. The contribution of preconception exposure to the adverse outcomes in offspring has never been studied. Here combined in utero and postnatal exposures produce insulin resistance in two collaborative cross strains. Furthermore, combined preconception and in utero exposure resulted in increased birth weight and developed insulin resistance in one strain. Thus, preconception exposure to arsenic may contribute to the metabolic disorders later in life, but the susceptibility to the effects of this exposure is determined, at least in part, by genetics.

Graphical Abstract

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Supporting Information

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Details of experimental design and reagents (PDF)

The authors declare no competing financial interest.

Contamination of drinking water with inorganic arsenic (iAs) is a global health crisis. Millions of people worldwide are exposed to iAs in their drinking water.¹ While the World Health Organization (WHO) has established a maximum accepted exposure limit of 10 μg As/L (10 ppb) for iAs in drinking water, iAs contamination exceeds this limit in numerous countries, including the United States,² with levels up to >5000 ppb having been observed.³ Elevated levels of iAs in drinking water are of concern because iAs has been associated with several diseases including type 2 diabetes (T2D).⁴

T2D is a metabolic disorder characterized by disruption of the insulin-signaling pathway, resulting in insulin resistance, pancreatic β -cell dysfunction, impaired glucose utilization, and high fasting blood glucose.⁵ Although genetic factors and obesity are among the strongest risk factors for T2D,⁶ a review by the National Toxicology Program has identified a strong association between high levels of iAs exposure in drinking water (150 μg /L) and T2D.⁴ More recently, a meta-analysis of 17 publications found that arsenic in drinking water and urine was associated with T2D with a 13% increased risk for every 100 μg As/L increase in drinking water.⁷ While the mechanism underlying the association between iAs and T2D is not fully understood, in vivo and in vitro studies have suggested that iAs and its metabolites interfere directly with both the insulin producing pancreatic β -cells^{8,9} and insulin signaling.¹⁵

Interestingly, exposure to iAs not only occurs after birth but also before and during gestation. Prenatal iAs exposure has been associated with adverse birth outcomes such as reduced gestation age.¹⁰ In addition to adverse birth outcomes, prenatal iAs exposure has been associated with chronic adult diseases.¹¹ Regarding a potential mechanism by which iAs exposure increases risk of later life diseases, in utero iAs exposure has been associated with alterations in DNA methylation and mRNA expression in cord blood leukocytes.¹² While association between adult iAs exposure and T2D development have been established, is not known whether exposure during the developmental window increases risk of T2D.

Another factor that can influence the development of diabetes and other health outcomes is iAs metabolism. Ingested iAs is biotransformed through a cascade of methylation reactions, catalyzed by arsenic (+3 oxidation state) methyl transferase (AS3MT).¹³ Interindividual variation in iAs metabolism has been reported.¹⁴ Thus, some individuals are able to methylate and excrete iAs more efficiently than others. Genome-wide studies have associated these interindividual variations with SNPs identified in *AS3MT* and in other genes.^{14,15}

Results of human studies suggest a link between *AS3MT* SNPs and the susceptibility to adverse effects of iAs exposure. Supportive of this, mice that are deficient for *AS3MT* display differential metabolism of iAs.^{16,17} Nevertheless, the genetic contribution in mice to iAs-induced disease is understudied. The goals of this study were to assess genetic variability within the collaborative cross (CC), as a novel tool to understand interindividual susceptibility, and to determine whether the timing of iAs exposure influences the risk of metabolic disorders in later life of G1 progeny.

The CC has been established to model in mice the genetic diversity found in human populations. The CC is a highly diverse mouse population composed of a large panel of recombinant inbred strains, which were derived from eight inbred founder strains.¹⁸ Gene expression in CC is driven in large part by cis regulatory variants that differentiate three of the founders of the CC, CAST/EiJ, PWK/PhJ, and WSJ/EiJ.¹⁹ This is true for the *As3mt* gene, where the expression of the CAST allele is significantly higher in liver, kidney, and brain than expression of the PWK allele.¹⁹ For the present study, we selected two CC strains (CC004/TauUnc and CC032/GeniUnc, CC004 and CC032 hereafter, respectively) because of the presence of SNPs and cis regulatory variation in their genetic makeup that leads to haplotype dependent gene expression differences in *As3mt* levels in the liver such that the CC004 has the PWK allele while the CC032 has the CAST allele and thus the CC032 has greater levels of *AS3MT* expression than the CC004.^{19,20}

Female CC mice born in 2016 were purchased from the UNC Systems Genetics Core Facility and mated to FVB/NJ males purchased from The Jackson Laboratory. A total of 3–4 dams were exposed to 10 ppb iAs (NaAsO₂) in drinking (deionized) water at two different exposure windows: gestation day (GD) 0 through postnatal day (PND) 21 (Exposure 1), or at least 14 days prior to conception and throughout gestation (Exposure 2). The phenotype assessment was carried at PND 35.

To control for background exposure to iAs in laboratory chow, the mice were fed a purified diet called AIN-93G (Dyets Inc.) that contained low levels of iAs (10–11 ppb). Birth weight was determined on PND 0, or as soon as possible after birth, and blood samples were collected from the mice at PND 35 after 6-h fasting. To characterize metabolic phenotypes, fasting plasma insulin (FPI) levels and fasting blood glucose (FBG) were measured, and homeostatic model assessment of insulin resistance (HOMA-IR) was calculated by combining data from male and female offspring. HOMA-IR, an indicator of insulin resistance is calculated as $HOMAIR = [(FPI \text{ (ng/mL)} \times FBG \text{ (mg/dL)})/190.35]$.

We observed significant, strain-dependent, effects of the iAs exposure window in three phenotypes: birthweight, FPI, and HOMA-IR. CC004 progeny who experienced Exposure 2 showed a significant increase in birth weight compared to their respective control. In contrast to strain CC004, in strain CC032, neither Exposure 1 nor 2 resulted in a change in birthweight (Figure 1A,B). These findings indicate strain-dependence and exposure time-specific response to iAs exposure.

FBG levels were not affected by iAs exposure in either strain or exposure window (Figure 1C,D). FBG measures in relation to iAs have shown variability. For example, a previous study in adult C57BL/6J and *As3mt* knockout (KO) mice, which were exposed to iAs (0.1 or 1.0 ppm) in drinking water for 24 weeks. Only male *As3mt* KO mice were insulin resistant and showed high FBG.²¹ In another study where C57BL/6J male mice were exposed for 20 weeks to high iAs (25 or 50 ppm) alone or combined with high fat diet (HFD), HFD diet alone increased FBG and insulin resistance. In contrast, mice exposed to both iAs and HFD had reduced FBG and were less insulin resistance than mice exposed to HFD alone.⁹ Taken together, these results suggest that FBG modulation is likely impacted by sex, diet, and dose.

Future research should investigate the interaction between iAs and FBG in relation to strain, sex, diet, and duration of exposure.

FPI levels were significantly higher in iAs-exposed CC004 mice compared to the control in both exposure windows. In contrast, neither exposure 1 or 2 significantly affected FPI levels in CC032 strain (Figure 1E,F), highlighting strain-specific responses to iAs exposure. Similarly, insulin resistance (HOMA-IR) was observed in the iAs-exposed CC004 mice after both exposure windows (Figure 1G). However, in the CC032 strain, effects on HOMA-IR were limited to exposure 2 only (Figure 1H).

Both FPI and HOMA-IR are biomarkers of insulin resistance in humans; high FPI or HOMA-IR indicate increased risk for T2D. It was interesting to note that strain CC004 that displayed higher HOMA-IR and higher FPI at PND 35 in response to iAs exposure also displayed increased birth weight.

In the present study, the dietary contribution of iAs was controlled. This is critical as it has been suggested that exposure scenario where low iAs (10 ppb) in drinking water is the nominal route of exposure, exposure through diet can significantly contribute to the overall accumulation of iAs as diet composed of natural ingredients typically contain 60–400 ppb total iAs levels.²² To minimize the contribution of diet which may potentially aggregate iAs exposure, all mice in this study were fed a purified diet that contained 10–11 ppb iAs for 2 weeks prior to exposure and throughout the study.

In summary, in this pilot study, we have shown two major findings that are fundamental to the iAs toxicology field. First, we have established an association between in utero/postnatal and preconception/in utero iAs exposure with adverse metabolic phenotypes in progeny. Specifically, the exposure window was critical for iAs dependent variation in phenotypic outcomes. Preconception plus in utero iAs exposure (Exposure 2) was associated with a significant increase in birth weight in CC004 pups and increase in HOMA-IR in CC032. However, in utero plus postnatal iAs exposure (Exposure 1) did not affect both outcomes in their respective strains. Together these data highlight the novel role of preconception exposure as a potential driver influencing later life disease risks. Second, the effects of iAs from early life iAs exposure vary by CC strain. In this study, the CC004 strain was more susceptible to early iAs exposure than the CC032 strain, resulting in increase of FPI and HOMA-IR, the indicators of T2D risk. The variability in responses to iAs exposure in the two strains may be tied to differences in *As3mt* expression and emphasizes the importance of the inclusion of mouse strain diversity as an essential component of iAs toxicity testing paradigms. Future studies should focus on expanding the sample size and the number of strains included in testing to more adequately predict iAs exposure effects in a diverse human population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

iAs	arsenic
T2D	Type 2 Diabetes
FPI	fasting plasma insulin
FBG	fasting blood glucose
CC	collaborative cross

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Figure 1. Differential response of CC004 and CC032 mice to iAs exposure in drinking water (10 ppb) at two different exposure windows: Exposure 1 (GD0–PND 21), Exposure 2 (14 days prior to conception–GD21). (A, B) Birthweights, (C, D) FBG in blood collected after 6 h of fasting at PND 35, (E, F) fasting plasma insulin, (G, H) HOMA-IR (ANOVA, means $\pm$ SD for $n = 7$ –26 pups, NS = not significant) *, $p < 0.05$ significantly different from control. Models were adjusted by offspring sex and litter size. The upper p -value bar represents the difference between the effects of 10 ppb iAs for Exposure 1 and Exposure 2. The within exposure p -value bars are comparing the effects between control and mice treated with 10 ppb iAs.