

METABOLISM AND ADVERSE EFFECTS OF ARSENIC

IN GENETICALLY DIVERSE MOUSE STRAINS

by

Jinglin Ji

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Department of Nutrition

University of North Carolina

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Approved:

Advisor: Mirek Styblo, PhD

Signature:

Reader: Christelle Douillet, PhD

Signature:

Abstract

Through drinking water and food, millions of people worldwide are exposed to arsenic (As), which is a naturally occurring diabetogenic metalloid [13]. Inorganic arsenic (iAs) exposure may disrupt glucose homeostasis leading to type 2 diabetes (T2D), including: (i) insulin resistance due to inhibition of insulin signaling, (ii) inhibition of insulin secretion, and (iii) aberrant micro RNA expression and activity [1, 2, 13]. Arsenic methyltransferase (AS3MT) is the key enzyme in iAs detoxification pathway that determines disease outcome [3]. Polymorphisms in AS3MT is the single most important genetic factor affecting iAs metabolism and susceptibility to adverse effects of iAs exposure, including diabetes. This study aims to examine the relationship between *As3mt* polymorphism and the adverse outcomes of iAs exposure in two Collaborative Cross (CC) mouse strains, CC021/Unc (CC021) and CC027/TauUnc (CC027), with different genetic backgrounds. Metabolic phenotype and susceptibility to adverse effects of iAs exposure are hypothesized to differ in CC021 and CC027 due to the differences in their hepatic *As3mt* expression and iAs metabolism (in liver). The metabolic/diabetic phenotype and iAs metabolism were assessed in both strains after 12 weeks of exposure to 100 ppb or 50 ppm iAs (sodium arsenite) in drinking water. The differences we observed between phenotypes of CC021 and CC027 mice were determined mainly by the genetic backgrounds of these strains and only to smaller extent by iAs exposure. Differences due to iAs exposure were found in weight gain, glycemia and insulin response to i.p. glucose injection, as well as in total As level in urine and in the percentage of total As represented by iAs. In future studies, we aim to identify other genes that underlie the susceptibility to iAs-associated T2D and establish a better mouse model in which *As3mt* expression and iAs metabolism resemble those in humans.

Aims and Hypothesis

The overall hypothesis of the study is that *metabolic phenotype and susceptibility to adverse effects of iAs exposure differ between CC021 and CC027 mice due to the differences in their hepatic As3mt expression and iAs metabolism in liver.*

Aim 1. Evaluation of the Diabetogenic Effects of iAs Exposure

Body weight, body composition, fasting blood glucose and insulin levels, glucose tolerance, insulin resistance and β -cell function were measured in CC mice chronically exposed to iAs.

Hypothesis 1: *Metabolic phenotypes of the CC strains exposed to iAs will differ.*

Aim 2. Evaluation of the iAs Metabolism

The proportions and concentrations of iAs and its methylated (MAs) and dimethylated (DMAs) metabolites were measured in the urine of the CC mice after chronic exposures to iAs.

Hypothesis 2: *iAs metabolism will differ in CC021 and CC027 mouse strains.*

Introduction

Arsenic (As) is a naturally occurring toxic metalloid. Over 100 million people worldwide drink water with inorganic As (iAs) levels higher than the US EPA and WHO maximum level of 10 $\mu\text{g As/L}$ [4, 5]. More people may be exposed to unsafe levels of iAs in foods [6-10].

The International Agency for Research on Cancer defines iAs as a Group-I human carcinogen [11]. Exposure to iAs is also associated with a wide array of non-cancer effects such as neurological impairment, respiratory disease, cardiovascular disease, and diabetes [12]. In 2011,

the National Toxicology Program Workshop on the environmental diabetogens and obesogens found sufficient evidence for the relationship between moderate to high levels of exposure to iAs in drinking water (>150 ppb; i.e., >150 $\mu\text{g As/L}$) and diabetes [13]. Two recent meta-analyses that included studies at lower exposure levels found pooled relative risks of developing diabetes in the presence of iAs exposure equal to 1.7 and 1.23 [14,15].

According to previous studies, the insulin producing pancreatic β -cells are among the targets for iAs exposure [1, 16]. The inhibition of glucose-stimulated insulin secretion (GSIS) by low concentrations of the methylated metabolites of iAs may be the key mechanism of iAs-induced diabetes [1]. Trivalent arsenicals interfere with mechanisms regulating packaging of the insulin transport vesicles or with translocation of these vesicles to the plasma membrane [1]. Defects in insulin secretion could also be associated with aberrant microRNA expression and activity [2]. In addition, micromolar concentrations of inorganic trivalent arsenite (iAs^{III}) or its methylated trivalent metabolites, methylarsonite (MAs^{III}) and dimethylarsinite (DMAs^{III}), inhibit the insulin-activated signal transduction pathway, resulting in insulin resistance and impaired glucose metabolism in adipocytes and hepatocytes [13, 17, 18].

Arsenic methyltransferase (AS3MT) is the key enzyme in the pathway for iAs metabolism [3]. Methylation of inorganic As determines the efficiency of iAs detoxification and the disease risk. AS3MT converts iAs to mono- and dimethylated metabolites (MAs, DMAs) that are excreted mainly in urine. The efficiency of iAs methylation can be estimated using the proportions of As metabolites in urine. A low DMAs/MAs ratio and high percentage of total urinary As represented by MAs (%MAs) are indicators of an inefficient iAs methylation and known risk factors for cancers and cardiovascular diseases associated with iAs exposure [3, 19-23].

However, the methylated trivalent metabolites formed in this pathway are potent inhibitors of processes that regulate glucose metabolism, including insulin secretion by pancreatic β -cells and insulin signaling in the liver and peripheral tissues [1]. Thus, in a counter-intuitive manner, while essential for total body clearance, the metabolism/methylation of iAs generates metabolites that likely contribute to the diabetogenic effects of iAs exposure.

Many population studies have examined the association between genetic polymorphism and the pattern of iAs metabolism. Although polymorphisms in several genes that are thought to regulate various steps in iAs metabolism have been linked with differences in the capacity to methylate iAs and in iAs toxicity, polymorphisms in AS3MT are the single most important genetic factor affecting iAs metabolism and susceptibility to adverse effects of iAs exposure, including diabetes, in population studies [24-29]. Notably, results of laboratory studies using genetically modified cells and mice, including our published and preliminary studies, support the critical role for As3mt in iAs metabolism. [19, 42]

In short, according to previous studies, iAs exposure may disrupt glucose homeostasis leading to T2D, including: (i) insulin resistance due to inhibition of insulin signaling, (ii) inhibition of insulin secretion, and (iii) aberrant micro RNA expression and activity [1, 2, 13]. AS3MT is the key enzyme in As metabolism while its polymorphisms are the most important genetic factor affecting susceptibility to As-related diabetes [3]. However, the relationship between As3mt polymorphism and the adverse outcomes of iAs exposure has never been systematically examined in animal models.

The goal of the present study was to characterize the effects of iAs exposure on metabolic phenotypes of mice from two Collaborative Cross (CC) strains, CC021/Unc (CC021) and

CC027/TauUnc (CC027), that have different *As3mt* haplotypes (non-obese diabetic (NOD)/ShiLtJ and Watkins Star Line B (WSB)/EiJ, respectively) and different SNPs in *As3mt* gene. CC strains are products of an eight-way funnel breeding design involving eight genetically diverse JAX[®] mice founder strains: A/J (000646), C57BL/6J (000664), 129S1/SvImJ (002448) NOD/ShiLtJ (001976) NZO/HiLtJ (002105), CAST/EiJ (000928), PWK/PhJ (003715), and WSB/EiJ (001145) [30]. Preliminary studies carried out in our laboratory showed that the hepatic expression of *As3mt* (Fig. 1) and patterns of iAs metabolism in these two strains, specifically the proportions of total As present as iAs (%iAs) and DMAs (%DMAs) in the liver (Fig. 2), are also significantly different.

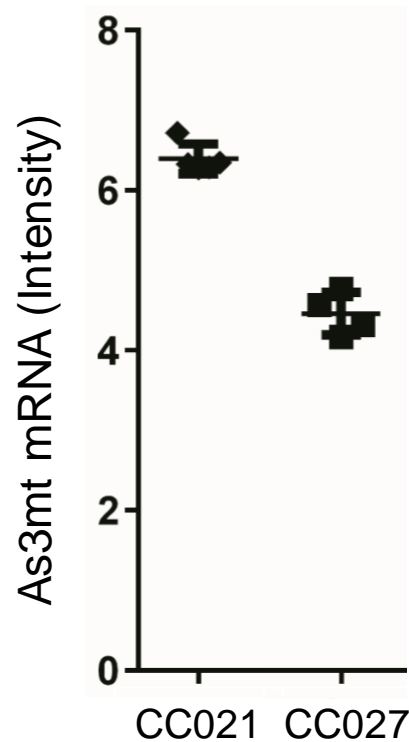


Figure 1: Baseline expression of *As3mt* in livers of male CC021 and CC027 mice. (N=4)



Figure 2: Proportions of As species in livers of male CC021 and CC027 mice. Mice were exposed to 50 ppm iAs (arsenite) for 2 weeks. (N=5). Total As (Mean \pm SD): 1342 \pm 652 ng/g (CC021) and 1609 \pm 317 ng/g (CC027).

The current project expands our knowledge of the mechanisms of iAs related diseases, specifically diabetes, and the role of As3mt genotype as factor that modifies the disease risk. This project also has a potential to move forward the entire field of iAs toxicology by providing novel mouse models for laboratory studies, and to contribute significantly to risk assessment of iAs-associated diseases based on genetic susceptibility.

Methods

Mice

All procedures involving mice were approved by the University of North Carolina (UNC) Institutional Animal Care and Use Committee. Four-week old male CC021 and CC027 mice were obtained from the UNC Systems Genetics Core Facility and allowed to acclimate for 2 weeks. During this time, mice drank deionized water (DIW) and had unlimited access to a semi-

purified AIN-93G diet. Mice were housed under controlled conditions with 12-h light/ dark cycle at 22 ± 1 °C and $50 \pm 10\%$ relative humidity (2–5 mice per cage).

Exposure

After acclimatization, mice from both strains were exposed to 100 ppb or 50 ppm iAs (sodium arsenite NaAsO_2 , $\geq 99\%$ pure; Sigma-Aldrich, St. Louis, MO, USA) in drinking water for 12 weeks; control mice continued drinking DIW. Water with sodium arsenite was prepared weekly to minimize oxidation of iAs^{III} to iAs^{V} .

Metabolic Phenotype Assessment

Body weights and water and food consumption were monitored bi-weekly during exposure. Body composition, fasting blood glucose and insulin levels, and glucose tolerance were examined at the end of exposure. Magnetic resonance imaging (MRI) (EchoMRI 3-in-1 analyzer, Echo Medical Systems, Houston, TX, USA and Labmaster version 3.2.2. Software) was used to determine body composition, specifically grams of fat mass and grams of lean mass. For glucose and insulin analysis, mice were fasted for 6 hours. Fasting blood glucose (FBG) levels were measured in blood collected from tail cuts using OneTouch Ultra 2 glucometer (LifeScan, Milpitas, CA, USA) and OneTouch Ultra Blue test strips. Fasted mice were then injected i.p. with 2 g/ kg b.w. of D-glucose (Sigma-Aldrich) dissolved in Dulbecco's phosphate-buffered saline (Mediatech, Manassas, VA, USA). Glucose levels were measured in blood collected by tail bleeds 15 min post-injection. Plasma insulin was measured in fasting and 15 min post-injection plasma using Ultra Sensitive Mouse Insulin ELISA Kit according to the manufacturer's instructions (Crystal Chem, Elk Grove Village, IL, USA).

HOMA-IR and HOMA-B were calculated from fasting blood glucose and plasma insulin values using the following formulas:

- $$\text{HOMA-IR} = \frac{\text{Fasting Blood Glucose } \left(\frac{\text{mg}}{\text{dL}}\right) \times \text{Fasting Plasma Insulin } \left(\frac{\mu\text{U}}{\text{mL}}\right)}{405} \quad [44]$$
- $$\text{HOMA-B} = \frac{360 \times \text{Fasting Plasma Insulin } \left(\frac{\mu\text{U}}{\text{mL}}\right)}{\text{Fasting Blood Glucose } \left(\frac{\text{mg}}{\text{dL}}\right) - 63} \% \quad [45]$$

$$\text{Fasting Plasma Insulin } \left(\frac{\mu\text{U}}{\text{mL}}\right) = \frac{\text{Fasting Plasma Insulin } \left(\frac{\text{ng}}{\text{mL}}\right)}{0.0347 \left(\frac{\text{ng}}{\mu\text{U}}\right)}$$

Analysis of As species in urine

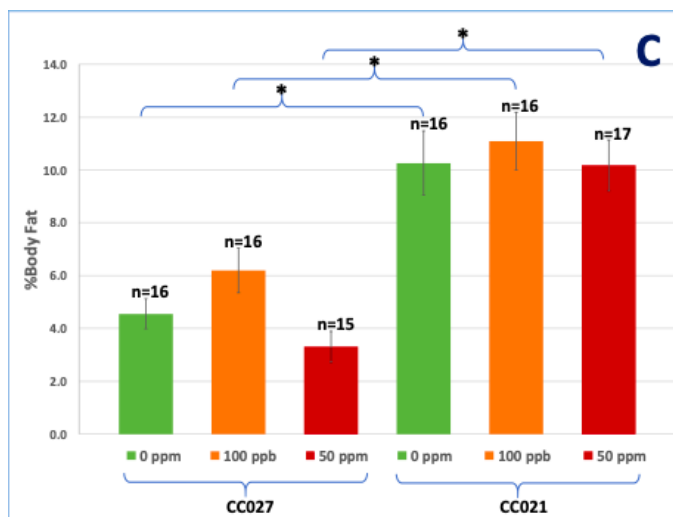
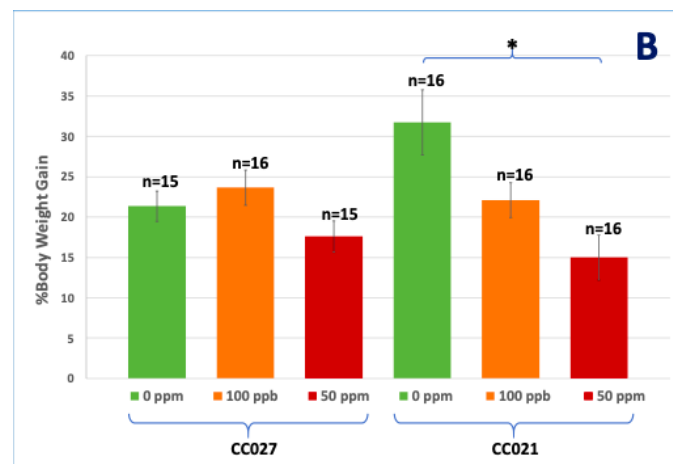
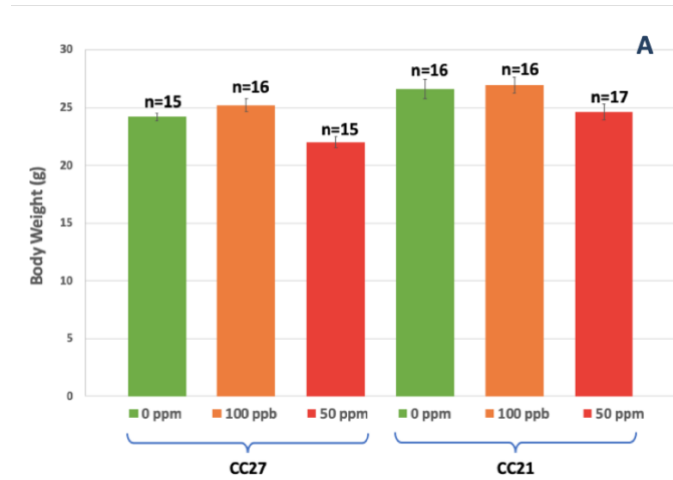
Spot urine samples were collected at the end of the exposure. The concentrations of iAs, MAs, and DMAs were measured in spot urine samples using hydride generation-atomic absorption spectrometry coupled with a cryotrap [32, 33]. The proportions of iAs and its methylated metabolites in urine (%iAs, %MAs, and %DMAs) were used to assess differences in methylation capacity of mice in both strains.

Statistical Analysis

Data were analyzed using ANOVA and Tukey-Kramer multiple comparisons test. Differences with $P < 0.05$ were regarded as statistically significant. Mean \pm SE were calculated and graphed for each outcome.

Results

Body weights were measured bi-weekly and weight gain was calculated for each mouse as a difference between body weight at the end of the exposure and body weight at the beginning of the exposure.



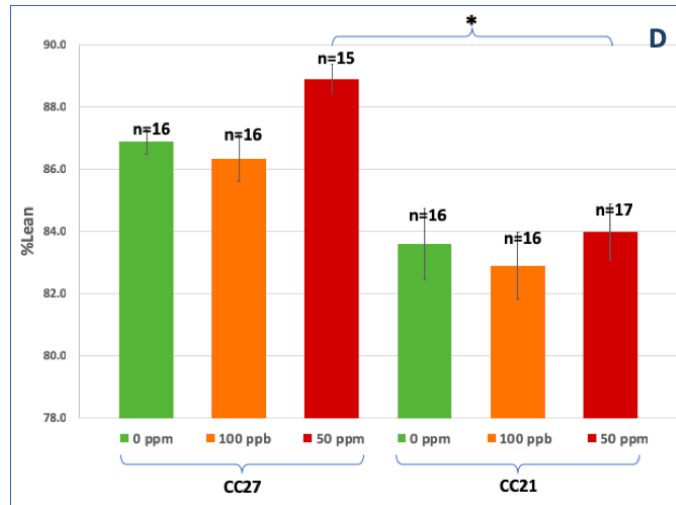


Figure 3: Average final body weight (A), weight gain (B), %body fat (C) and %lean mass (D) of CC021 and CC027 mice exposed to 100 ppb or 50 ppm iAs and the control mice (0 ppm). Mean \pm SE. * Statistically significant differences ($P < 0.05$) using ANOVA and Tukey-Kramer multiple comparisons test.

No statistically significant difference in body weight at the end of the exposure were seen between the two strains or between different exposure levels (Fig. 3A). However, CC021 mice tended to have higher body weight while mice exposed to 50 ppm iAs in both strains tended to have lower body weight (Fig. 3A). Percent weight gain decreased significantly and in a dose-dependent manner in CC021, but not CC027 mice (Fig. 3B). CC021 mice, both iAs-exposed and controls, had significantly higher %fat mass than CC027 mice (Fig. 3C). CC027 mice had higher %lean mass than CC021 mice, but this difference was statistically significant only in mice exposed to 50 ppm iAs (Fig. 3D). Exposure to 100 ppb iAs tended to reduce %lean mass while exposure to 50 ppm increased %lean mass in both strains (Fig. 3D), but these effects were not statistically significant. Exposure to 100 ppb iAs tended to increase body weight, percent weight gain and %fat mass in CC027 mice but not in CC021 mice.

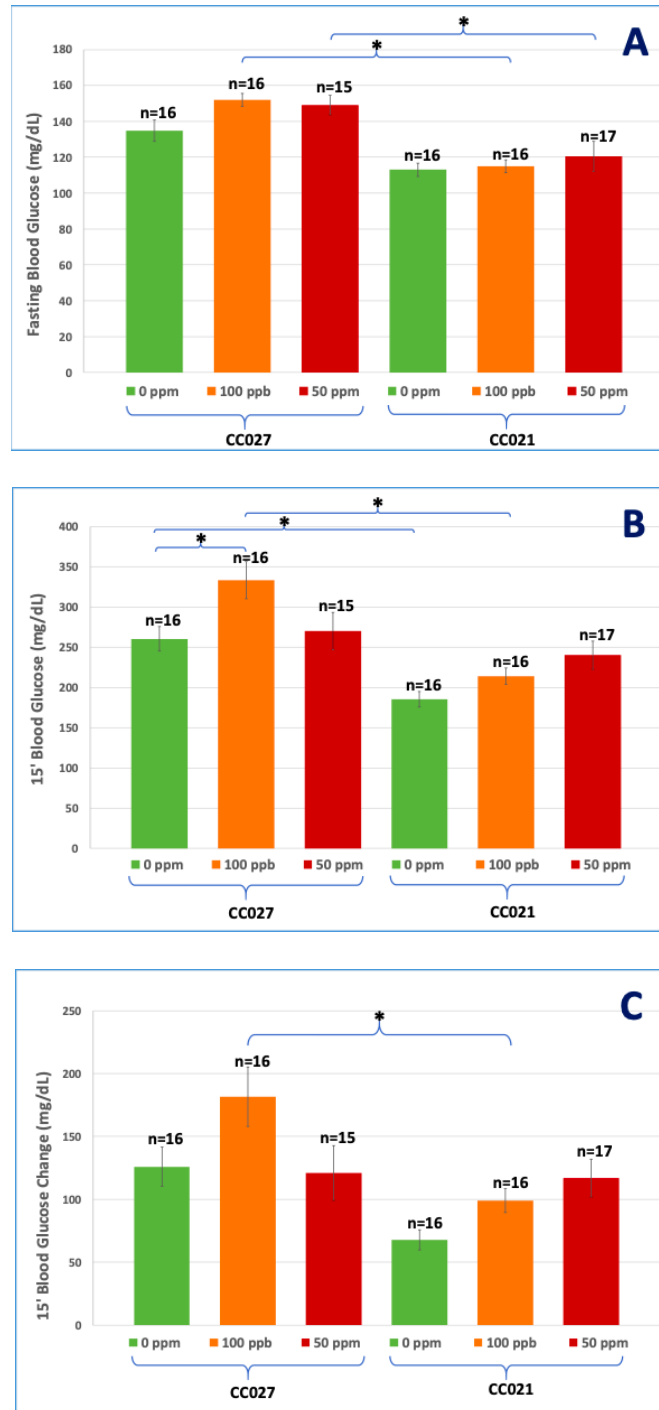
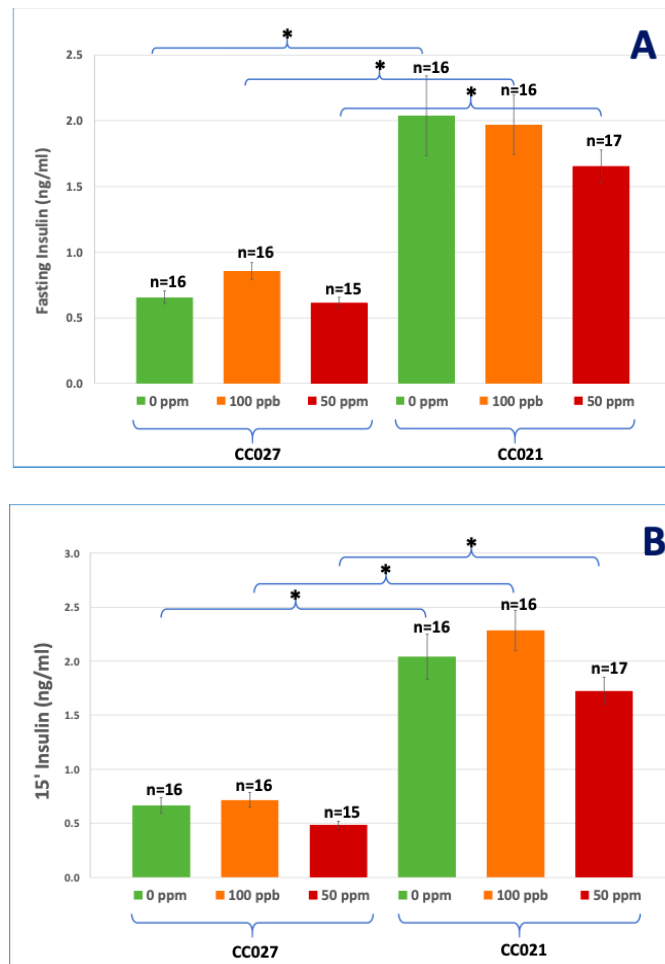


Figure 4: Blood glucose levels after fasting (A), 15 min after i.p. glucose injection (B), and the difference (change) between fasting and 15-min glucose levels (C).

Mean \pm SE. *Statistically significant differences ($P < 0.05$) using ANOVA and Tukey-Kramer multiple comparisons test.

CC027 mice exposed to 100 ppb iAs had higher blood glucose after 6-hour fasting and 15 min after i.p. glucose injection, and higher blood glucose change between fasting and 15-min glucose levels than CC021 mice (Fig. 4A, 4B, 4C). Effects of iAs exposure were relatively minor and were statistically significant only among CC027 mice. Here, mice exposed to 100 ppb iAs had significantly higher blood glucose levels 15 min after i.p. glucose injection than corresponding controls (Fig. 4A). A similar difference between control and 100 ppb mice was observed in the difference between fasting and 15-min glucose levels, but it was not statistically significant (Fig. 4C). In addition, CC027 mice exposed to 50 ppm iAs had higher blood glucose levels after 6-hour fasting than CC021 mice exposed to 50 ppm iAs (Fig. 4A). CC027 control mice had higher blood glucose levels 15 min after i.p. glucose injection than CC021 control mice (Fig. 4B).



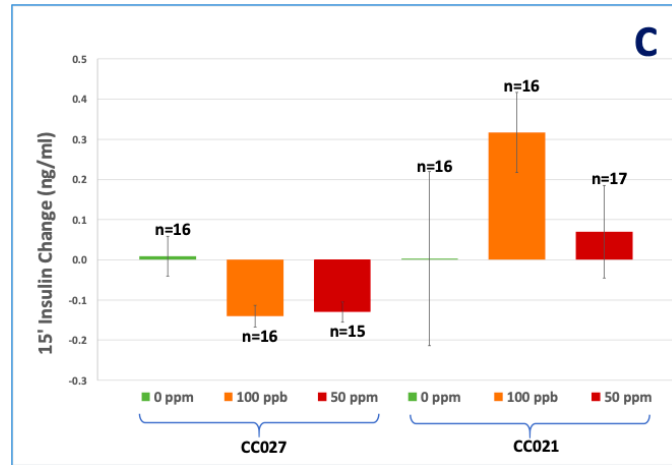


Figure 5: Plasma insulin levels after fasting (A) and 15 min after i.p. glucose injection (B), and the difference (change) in insulin levels between fasting and 15-min after glucose injection (C). Mean \pm SE. *Statistically significant differences ($P < 0.05$) using ANOVA and Tukey-Kramer multiple comparisons test.

CC021 mice, both iAs-exposed and controls, had higher plasma insulin levels after 6-hour fasting and 15 min after i.p. glucose injection than their CC027 counterparts. (Fig. 5A, 5B) Insulin response to i.p. glucose injection differed between the strains; however, this difference was not statistically significant (Fig. 5C). Fasting plasma insulin level tended to decrease in a dose dependent manner in CC021 (Fig. 5A). 100 ppb iAs exposure tended to increase fasting plasma insulin level in CC027 and 15 min plasma insulin level in CC021 (Fig. 5A, 5B).

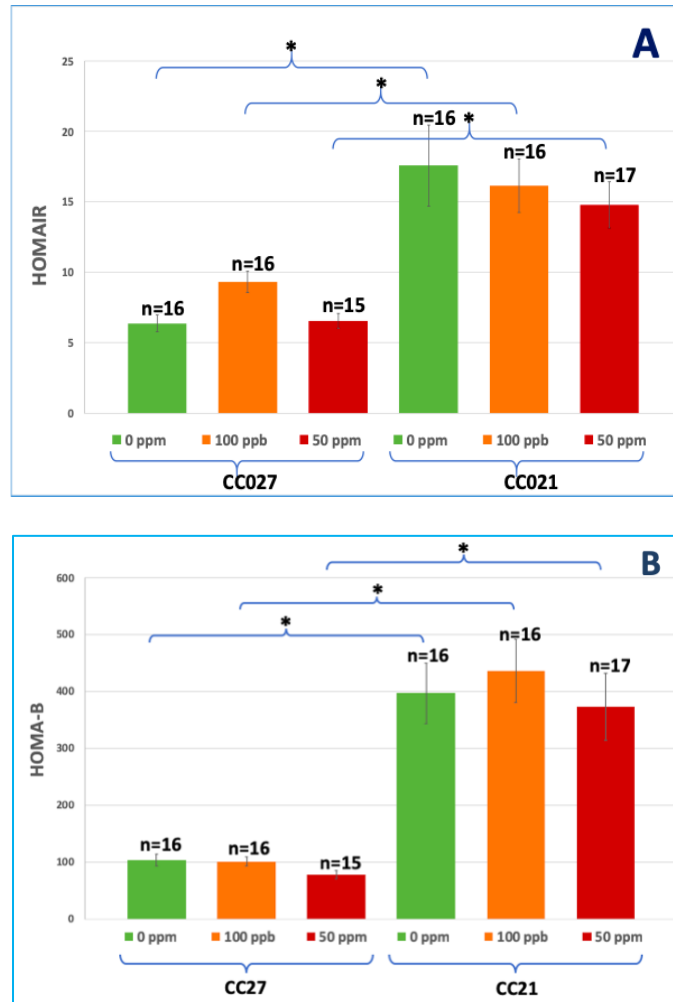


Figure 6: Insulin resistance (HOMA-IR) (A) and β -cell function (HOMA-B) (B).

*Statistically significant differences ($P < 0.05$) using ANOVA and Tukey-Kramer multiple comparisons test.

CC021 mice (with NOD background), both iAs-exposed and controls, were more insulin resistant (Fig. 6A, 6B) than their CC027 counterparts. HOMA-IR decreased in a dose dependent manner in CC021 although the differences between iAs-exposed and control mice were not statistically significant (Fig. 6A). 100 ppb iAs exposure tended to increase HOMA-IR in CC027 and HOMA-B in CC021 (Fig. 6A).

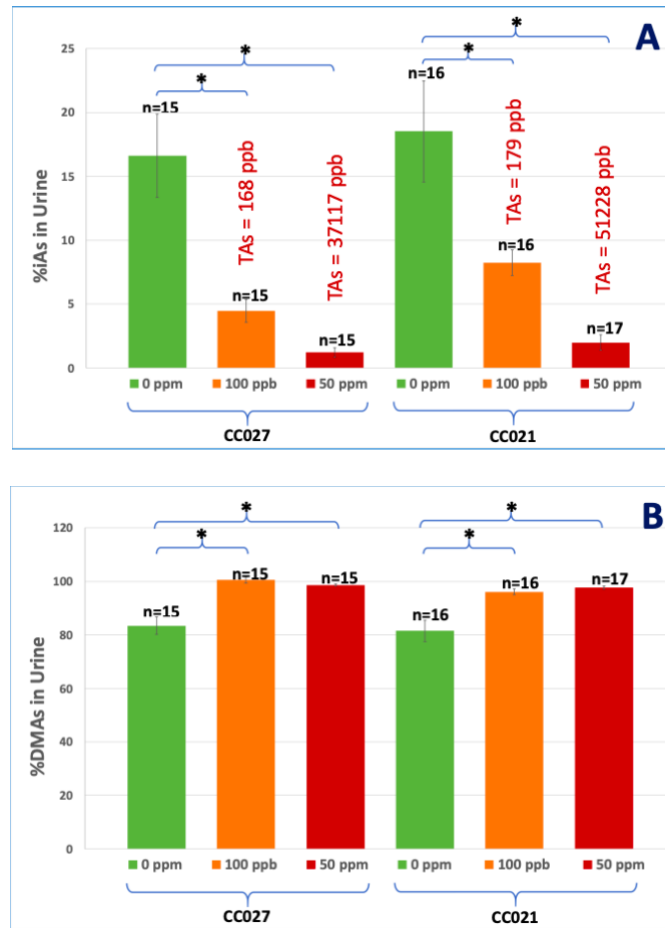


Figure 7: Proportions of As species in urine: %iAs (A) and %DMAs (B). Mean \pm SE. TAs = sum of As species. *Statistically significant differences ($P < 0.05$) using ANOVA and Tukey-Kramer multiple comparisons test.

Relatively small differences were found between the strains in As profiles in urine. Total As and %iAs were consistently higher in urine of CC021 as compared to CC027 mice, both iAs-exposed and controls, but these differences were not statistically significant. Exposure to iAs significantly decreased %iAs and increased %DMA in urine of both strains (Fig. 7A, 7B). MAs concentrations were at or below detection limit in most spot urine samples.

Discussion

Laboratory mice with varied genetic backgrounds have been extensively used in laboratory studies examining adverse effects of iAs exposure. However, it has been well documented that iAs methylation and excretion in mice is more efficient than in humans [19, 20], which likely explains the differences between these species in the responses to iAs exposure [34-36]. For instance, in all human populations studies to date, MAs and DMAs represented 10-20% and 60-70% of total urinary As, respectively, with iAs accounting for 10-30% [19, 20]. In contrast, urine of mice exposed to iAs in published studies contains more than 80% of DMAs and only traces of MAs, suggesting a more efficient conversion of MAs to DMAs [19, 20]. Thus, a better animal model is needed in which iAs metabolism would resemble the human metabolism, and in which the effects of iAs exposure found in humans can be reproduced. Strong evidence suggests that the differences in the AS3MT genotypes are in part responsible for the differences in efficiency of iAs metabolism and in susceptibility to adverse effects of iAs exposure among humans and between mice and humans. This observation provided rationale for the present study. The goal of this study was to compare effects of iAs exposure on glucose homeostasis between 2 CC mouse strains that differ in As3mt genotype and demonstrate differences in the disposition of iAs and its metabolites in the liver.

The causality of the association between iAs exposure and T2D is supported by prospective data from human cohorts and by results of laboratory studies suggesting that iAs and its metabolites impair mechanisms that regulate glucose homeostasis and are linked to T2D development [1, 16, 37-41]. Results of previous and preliminary studies point to three mechanisms underlying the diabetogenic effects of exposure to iAs, including (i) insulin resistance due to inhibition of

insulin signaling, (ii) inhibition of insulin secretion, and (iii) aberrant micro RNA expression and activity [1, 2, 13].

We have previously shown that urine and livers of control mice that drank only deionized water and were fed a regular grain-based diet (pelleted 2920X Teklad rodent chow, Envigo, Madison, WI, USA) contained relatively high levels of As, suggesting that these mice were exposed to As from diet [42]. When designing the present study, we included a two-week acclimatization period to allow for clearance of As to which the mice were exposed when fed a regular grain-based diet before delivery to UNC. The methods we used for metabolic phenotype, including measures of fasting blood glucose and insulin, are widely utilized in diagnosis of diabetes in human patients and are good approximates of the diabetogenic effect of iAs exposure in mice [40, 43]. A simplified glucose tolerance test we used in this study was to assess the physiological response of mice to a glucose challenge, specifically blood glucose and plasma insulin levels at 15 minutes after injection. The homeostasis model assessments HOMA-IR and HOMA-B, based on fasting glucose and insulin measures, have been validated and widely applied for quantifying insulin resistance and β -cell function in clinical setting, and are used here as proxies of these measure in mice [31].

The CC strains for this study were chosen based on results of our recent study that examined differences in iAs metabolism among male mice from 12 CC strains. CC21 mice had *As3mt* haplotypes from NOD founder strain while CC27 mice had *As3mt* haplotypes from WSB founder strain. However, their metabolic phenotype was more influenced by the overall genetic background. CC21 mice had lower percentage (7.16%) of NOD founder contribution in the overall genetic background that CC27 mice (18.32%) [46]. The NOD/ShiLtJ founder strain

(commonly called NOD) is a polygenic model for autoimmune type 1 diabetes, characterized by hyperglycemia and insulinitis [30].

CC027 mice exposed to 100 ppb iAs had higher blood glucose after 6-hour fasting and 15 min after i.p. glucose injection, and higher blood glucose change between fasting and 15-min glucose levels than CC021 mice (Fig. 4A, 4B, 4C). CC027 mice exposed to 50 ppm iAs had higher blood glucose levels after 6-hour fasting than CC021 mice exposed to 50 ppm iAs (Fig. 4A). CC027 control mice had higher blood glucose levels 15 min after i.p. glucose injection than CC021 control mice (Fig. 4B).

Defects in antigen presentation, T lymphocyte repertoire, natural killer cell function, macrophage cytokine production, wound healing, and C5 complement characterize immune phenotypes in the NOD background [30]. Insulinitis is a leukocytic infiltration of the pancreatic islets and should be characterized by decreased β -cell mass and insulin secretion [30]. According to our results, CC027 mice, both iAs-exposed and controls, had lower plasma insulin levels after 6-hour fasting and 15 min after i.p. glucose injection than CC021 mice (Fig. 5A, 5B). CC027 mice exposed to 100 ppb and 50 ppm iAs in drinking water had negative insulin change after glucose challenge (Fig. 5C). Lower plasma insulin level and negative insulin change in response to glucose challenge in CC027 probably represents decreased β -cell function and insulin secretion while exposure to iAs might deteriorate insulinitis.

The mechanism of developing insulinitis in CC021 is unclear for now. According to previous study, micromolar concentrations of inorganic trivalent arsenite (iAs^{III}) or its methylated

trivalent metabolites, methylarsonite (MAs^{III}) and dimethylarsinite (DMAs^{III}) inhibit the insulin-activated signal transduction pathway, resulting in insulin resistance in adipocytes and hepatocytes [13, 17, 18]. CC021 mice, both iAs-exposed and controls, had significantly higher %fat mass (Fig. 3C) and were more insulin resistant (Fig. 5, 6) than their CC027 counterparts probably due to higher %fat mass. Insulin resistance could cause a long-term overstimulation of β -cells which leads to inflammation and autoimmune attack on β -cells. Insulin resistance and lower insulin secretion could together induce diabetes.

Most of the phenotypic differences between the two strains were dependent on genetic background and independent of iAs exposure. Strain differences were pronounced in body composition (Fig. 3C, 3D), blood glucose (Fig. 4), plasma insulin (Fig. 5A, 5B) and insulin resistance (Fig. 6). iAs exposure had mostly minor effects on phenotype of mice in either strain. Dose dependent effects on the proportions of As species were seen in the urine of both strains: %iAs decreased and %DMA increased with increasing exposure level (Fig. 7). This is in contrast with data from human cohorts where %DMAs has been found to decrease with increasing exposure [22]. The differences between the strain in %iAs and %DMAs in urine, although consistent across the exposures, were not statistically significant for the given N numbers (Fig. 7). Strain-specific differences in responses to iAs exposure were also found in weight gain (Fig. 3B), 15-min blood glucose (Fig. 4B), and in insulin response to i.p. glucose injection (Fig. 5C). These differences support our hypothesis that metabolic phenotype and susceptibility to adverse effects of iAs exposure would differ in CC021 and CC027 and may be linked to the differences in their hepatic As3mt expression and iAs metabolism in the liver.

However, the fact that most of the differences between these 2 strains were independent of iAs exposure suggests that the phenotypes of CC021 and CC027 mice exposed to iAs are determined primarily by their broader genetic backgrounds rather than by *As3mt* polymorphism and expression in the liver, or by the differences in iAs metabolism. Notably, the proportions of As species in urine of both strains were consistent with those reported in previous mouse studies and differed from the proportions found in human urine [19, 20]. Thus, neither CC021 nor CC027 is an ideal model to study metabolism of iAs or adverse effects associated with iAs exposure in humans.

Although the polymorphisms in *AS3MT* have been shown to play a critical role in the efficiency of iAs metabolism in population and laboratory studies, other genes that may be involved in iAs detoxification or the regulation of glucose homeostasis are likely to contribute to T2D risk in individuals exposed to iAs [24-29]. In order to reduce variables in background genes and to isolate the effects of differences in genes affecting iAs metabolism in future studies, we need to improve our knowledge of these genes. In the next project, we will use the complementary resources of the Collaborative Cross (CC) and Diverse Outbred (DO) mouse populations to identify genes that underlie the susceptibility to iAs-associated T2D and are linked to specific diabetic phenotypes. We are also in the process of creating a humanized mouse strain. Here, we created embryonic stem cells, which express human *AS3MT* through syntenic replacement of mouse *Borcs7/As3mt* locus with the human locus. These humanized embryonic stem cells were injected into blastocysts to create mice expressing human *AS3MT*. We hypothesize that *AS3MT* expression in tissues and the pattern of iAs metabolism, and possibly the responses to iAs exposure in the humanized mice will resemble those described in human populations.

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