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Oxidation state specific analysis of arsenic species in tissues of wild-type and arsenic (+3 oxidation state) methyltransferase-knockout mice

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ABSTRACT

Arsenic methyltransferase (As3mt) catalyzes the conversion of inorganic arsenic (iAs) to its methylated metabolites, including toxic methylarsonite (MAS^{III}) and dimethylarsinite (DMAs^{III}). Knockout (KO) of As3mt was shown to reduce the capacity to methylate iAs in mice. However, no data are available on the oxidation states of As species in tissues of these mice. Here, we compare the oxidation states of As species in tissues of male C57BL/6 As3mt-KO and wild-type (WT) mice exposed to arsenite (iAs^{III}) in drinking water. WT mice were exposed to 50 mg/L As and As3mt-KO mice that cannot tolerate 50 mg/L As were exposed to 0, 15, 20, 25 or 30 mg/L As. iAs^{III} accounted for 53% to 74% of total As in liver, pancreas, adipose, lung, heart, and kidney of As3mt-KO mice; tri- and pentavalent methylated arsenicals did not exceed 10% of total As. Tissues of WT mice retained iAs and methylated arsenicals: iAs^{III}, MAS^{III} and DMAs^{III} represented 55%–68% of the total As in the liver, pancreas, and brain. High levels of methylated species, particularly MAS^{III}, were found in the intestine of WT, but not As3mt-KO mice, suggesting that intestinal bacteria are not a major source of methylated As. Blood of WT mice contained significantly higher levels of As than blood of As3mt-KO mice. This study is the first to determine oxidation states of As species in tissues of As3mt-KO mice. Results will help to design studies using WT and As3mt-KO mice to examine the role of iAs methylation in adverse effects of iAs exposure.

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Introduction

Inorganic arsenic (iAs), a potent human carcinogen, is ubiquitous in the environment and accumulates in aquifers naturally and through anthropogenic activities. The ingestion of iAs through contaminated drinking water, most commonly as arsenite (iAs^{III}) and arsenate (iAs^V), has been associated with numerous adverse effects, including peripheral vascular disease, hypertension, and cancer of the lungs, liver, and bladder (Tseng et al., 2007; Wang et al., 2007a; IARC Working Group on the Evaluation of Carcinogenic

Risks to Humans et al., 2004). A recent National Toxicology Program workshop examining the effects of environmental chemicals on the development of diabetes and obesity concluded that there was sufficient evidence to link iAs exposures to an increased risk of diabetes in populations exposed to high levels of iAs in drinking water (Maull et al., 2012).

The enzyme, arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the S-adenosylmethionine (SAM)-dependent methylation of iAs to tri- and pentavalent methylated metabolites (Thomas, 2004). AS3MT mRNA has been found in several

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human and rodent tissues, including, liver, kidney, urinary bladder, heart, lung, testes, and adrenal gland (Lin et al., 2002). Once ingested, iAs is sequentially methylated by AS3MT producing methylarsonite (MAS^{III}), methylarsonate (MAS^{V}), dimethylarsinite (DMAs^{III}), dimethylarsinate (DMAs^{V}), and trimethylarsine oxide (TMA^{VO}). Growing evidence suggests that the methylated trivalent As (As^{III}) species, MAS^{III} and DMAs^{III} , produced in the course of iAs metabolism, are more toxic than iAs or their pentavalent counterparts (Thomas et al., 2001; Lin et al., 2001; Drobna et al., 2003; Wang et al., 2007b; Douillet et al., 2013).

Laboratory-based studies have shown that iAs exposure alters glucose homeostasis and several mechanisms regulating glucose metabolism. Specifically, in our studies, exposure to subtoxic concentrations of iAs^{III} , MAS^{III} or, DMAs^{III} inhibited glucose-stimulated insulin secretion by isolated murine pancreatic islets without affecting basal insulin secretion or insulin content and expression, suggesting that As^{III} species inhibit insulin transport vesicle packaging or translocation to the plasma membrane (Douillet et al., 2013). In β -cell lines exposed to iAs, an impairment of glucose-stimulated insulin secretion has been associated with reduced insulin expression (Díaz-Villaseñor et al., 2006) alterations in Ca^{2+} oscillations (Díaz-Villaseñor et al., 2008), or with an Nrf2-mediated antioxidant response suppressing endogenous reactive oxygen species (Yen et al., 2007; Fu et al., 2010) that may be required for insulin secretion (Pi and Collins, 2010). In other cell culture models, iAs^{III} has been shown to inhibit differentiation of adipocytes (Trouba et al., 2000; Wauson et al., 2002) and myotubes (Steffens et al., 2011), the cell types that are involved in glucose utilization *in vivo*. Moreover, we have shown that As^{III} species inhibit insulin signaling and insulin-stimulated glucose uptake in cultured differentiated adipocytes (Paul et al., 2007a; Walton et al., 2004). We have also shown that in C57BL/6 mice exposure to 50 mg/L As as iAs^{III} in drinking water resulted in impaired glucose tolerance (Paul et al., 2007b, 2011). Notably, mice chronically exposed to iAs^{III} in combination with high-fat diet produced a unique diabetic phenotype characterized by impaired glucose tolerance in the absence of significant obesity and insulin resistance (Paul et al., 2011), suggesting that the mechanisms underlying As-induced diabetes differ from those responsible for development of the obesity-associated type 2 diabetes.

Genetically altered, C57BL/6 *As3mt*-knockout (KO) mice have been recently developed and partially characterized (Drobna et al., 2009). When exposed to iAs these mice retained significantly more As than WT mice (Chen et al., 2011; Drobna et al., 2009; Hughes et al., 2010) and exhibited increased sensitivity to iAs toxicity (Yokohira et al., 2010, 2011). Chemical analyses have shown that iAs was the predominant species in tissues of *As3mt*-KO mice exposed to iAs; however, methylated As metabolites were detected in liver and plasma, suggesting the methylation of iAs by other methyltransferases or by intestinal microbiota (Drobna et al., 2009; Naranmandura et al., 2012). The oxidation states of iAs or the methylated As species found in tissues of *As3mt*-KO mice have never been determined. In spite of this information gap, the *As3mt*-KO mice have been used as a laboratory model to explore the role of iAs methylation and the contribution of trivalent methylated arsenicals in the development of iAs-induced diseases.

Hydride generation-cryotrapping-atomic absorption spectrometry (HG-CT-AAS) is uniquely suited for the oxidation state specific speciation analysis of As in complex biological matrices. The analysis using HG-CT-AAS does not require sample pretreatment or extraction, thus preserving the methylation state of unstable MAS^{III} and DMAs^{III} (Matoušek et al., 2008; Hernández-Zavala et al., 2008; Currier et al., 2011a, 2011b). This method has been successfully used to determine concentrations of the methylated trivalent arsenicals, MAS^{III} and DMAs^{III} , in human urine (Del Razo et al., 2001, 2011; Valenzuela et al., 2004, 2009), mouse tissues (Currier et al., 2011a, 2011b), *in vitro* cell cultures (Del Razo et al., 2001; Hernández-Zavala et al., 2008) and *in vitro* mixtures for methylation of iAs by recombinant AS3MT (Hernández-Zavala et al., 2008; Ding et al., 2012). In this study, we used HG-CT-AAS to characterize the retention of tri- and pentavalent arsenicals in tissues of wild-type (WT) and *As3mt*-KO C57/BL6 mice after exposure to iAs^{III} .

1. Materials and methods

1.1. Arsenicals

The following pentavalent arsenicals were used for calibration during the HG-CT-AAS analysis: sodium arsenite ($\text{NaAs}^{\text{III}}\text{O}_2$) and sodium arsenate ($\text{Na}_2\text{HAS}^{\text{V}}\text{O}_4$) (both $\geq 99\%$ pure) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylarsonic acid, disodium salt ($\text{CH}_3\text{As}^{\text{V}}\text{O}(\text{ONa})_2$), and dimethylarsinic acid ($(\text{CH}_3)_2\text{As}^{\text{V}}\text{O}(\text{OH})$), both better than 98% pure, were purchased from Chem Service (West Chester, PA, USA). The As content in each of the standards was determined by graphite furnace-AAS (Matoušek et al., 2008).

1.2. Mice and treatments

All procedures involving mice were approved by the University of North Carolina Institutional Animal Care and Use Committee. The *As3mt*-KO mouse colony has been established at UNC using breeding pairs provided by Dr. David Thomas, U.S. EPA. In this study, 13- to 18-week old male C57BL/6 *As3mt*-KO mice were used along with 13–17 week old male WT C57BL/6J mice from Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed in polycarbonate cages (5 per cage) with corn cob bedding at the University of North Carolina Animal Facility (12 hr light/dark cycle, $22 \pm 1^\circ\text{C}$ and humidity $50\% \pm 10\%$). Mice were fed Lab Diet 5058 (Nutrition International, Brentwood, MO, USA) and drank pure deionized water (DIW) or DIW containing iAs^{III} *ad libitum* for the 4-week study period. *As3mt*-KO mice were exposed to 0, 15, 20, 25, or 30 mg/L As as iAs^{III} ($n = 5$ per treatment group). WT mice were exposed to 50 mg/L As ($n = 10$), the dose that previously produced a diabetic phenotype in this mouse strain (Paul et al., 2007b). The concentration of iAs in water in all treatment groups was verified by HG-CT-AAS analysis. Mice were euthanized by cervical dislocation. Freshly dissected tissues were processed as previously described, and tissue homogenates were prepared in ice cold DIW (10%, W/V) using an electrical overhead stirrer equipped with a Teflon pestle tissue homogenizer (Wheaton Industries, Inc., Millville, NJ, USA) (Currier et al., 2011b).

1.3. Speciation analysis of As by HG-CT-AAS

Arsenic species were analyzed by an optimized method for the oxidation state specific analysis of As species in biological matrices (Matoušek et al., 2008; Hernández-Zavala et al., 2008; Currier et al., 2011a, 2011b). Briefly, HG-CT-AAS analysis was performed using a FIAS 400 flow injection accessory (Perkin-Elmer, Norwalk, CT, USA) coupled to a cryotrapping unit and an AAnalyst 800 spectrometer (Perkin-Elmer) equipped with a multiple microflame quartz tube atomizer (multiatomizer) (Matoušek et al., 2008; Hernández-Zavala et al., 2008). To measure the oxidation state of As species, two aliquots of each sample are analyzed; arsine gases from trivalent arsenicals (iAs^{III} , MA^{III} and $DMAs^{III}$) and from TMA^{VO} are generated directly at pH 6, while arsines from both tri- and pentavalent arsenicals (iAs^{III+V} , MA^{III+V} and $DMAs^{III+V}$) are measured after pre-reduction for 1 hr at room temperature with 2% L-cysteine (EMD Chemicals Inc., Gibbstown, NJ, USA) (Matoušek et al., 2008; Hernández-Zavala et al., 2008). The pre-reduction, however, results in loss of TMA^{VO} . The concentrations of the pentavalent iAs^V , MA^V , and $DMAs^V$ are calculated by subtracting the AAS peak area signals obtained for L-cysteine-treated and directly analyzed, untreated sample aliquots. Calibration curves for quantification of tri- and pentavalent As species were generated using aqueous solutions of the pentavalent standards (iAs^V , MA^V , and $DMAs^V$) pre-treated with 2% L-cysteine. Notably, results of our published work shows that HG-CT-AAS analyses of crude tissue homogenates result in high (~80%–100%) recoveries of As (Currier et al., 2011b).

1.4. Statistical analysis

All statistical analyses were performed using GraphPad InStat software package (GraphPad Software Inc., San Diego, CA, USA). Linear regression and correlation analyses were employed to characterize the calibration curves using aqueous pentavalent As standards. ANOVA followed by Bonferroni's multiple comparison post-test was used to determine significant differences

between *As3mt*-KO and WT mice for each of the measured endpoints. Statistical significance was considered at the level of $p < 0.05$.

2. Results and discussion

2.1. Water consumption and body weights

The consumption of water for each exposure group and individual body weights were measured weekly throughout the study. Fig. 1 depicts the estimated daily water consumption and the corresponding iAs intake for each exposure group over the 4-week study period. Water intake increased after the first week and then plateaued for the remaining study period except in *As3mt*-KO mice exposed to 25 mg/L As, which exhibited decreased water consumption in weeks 3 and 4 (Fig. 1a). The *As3mt*-KO mice exposed to pure DIW consumed significantly more water than all iAs-treated groups (Fig. 1a and b). *As3mt*-KO mice exposed to 15, 20, 25, or 30 mg/L As ingested approximately 27.1, 34.9, 43.4, and 42.7 μ g of iAs/day, respectively, while WT mice exposed to 50 mg/L As ingested approximately 107.6 μ g of iAs/day (Fig. 1c). There was no significant difference in iAs/day ingestion among the *As3mt*-KO groups, but the WT group ingested significantly more iAs than all other groups. We have previously shown that WT mice exposed to 50 mg/L As could taste the high concentration As and drank less water than WT mice at lower exposure levels or unexposed mice (Paul et al., 2007b, 2011). The current data suggest that KO mice are even more sensitive to As and further limit the water consumption.

The body weight of each mouse was measured prior to iAs exposure and weekly throughout the study. On average, all mice gained weight except for *As3mt*-KO mice exposed to 30 mg/L As (Fig. 2). Unexposed *As3mt*-KO mice gained an average of 3.2 g over the 4-week study period, while *As3mt*-KO mice exposed to 15, 20, or 25 mg/L As gained an average of 1.2, 1.6, and 1.1 g, respectively. *As3mt*-KO mice exposed to 30 mg/L As lost an average of 1.2 g over the study period. However, no

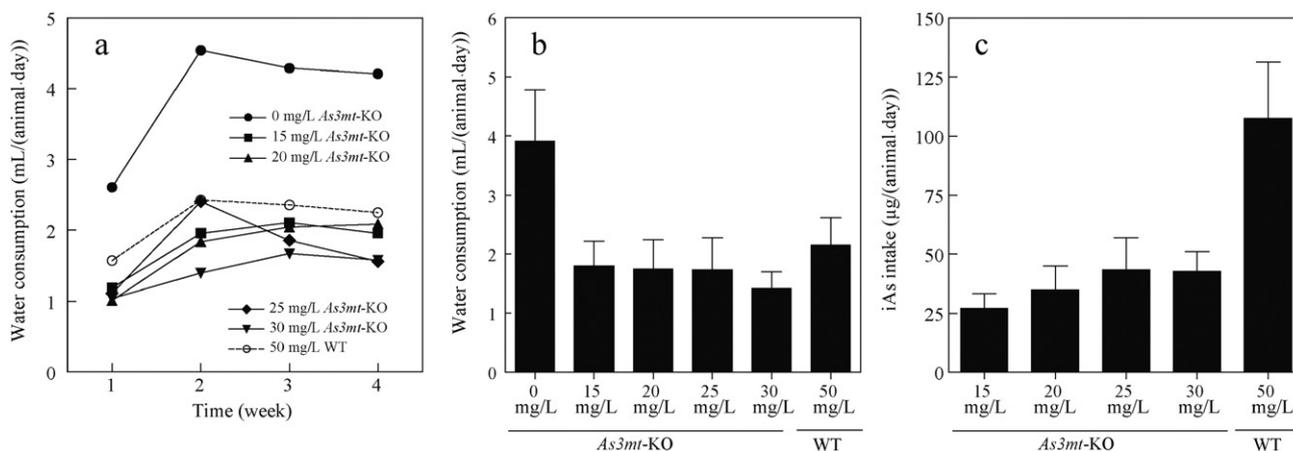


Fig. 1 – Water consumption and iAs intake by *As3mt*-KO and WT mice. (a) Estimated daily water consumption by week for *As3mt*-KO mice exposed to 0, 15, 20, 25, or 30 mg/L As and wild-type (WT) mice exposed to 50 mg/L As. (b) Average daily water consumption per mouse for each treatment group. (c) Estimated daily iAs intake per mouse for each treatment group. Mean and SD values are shown; $n = 5$ for each of the *As3mt*-KO group and $n = 10$ for WT group. iAs: inorganic arsenic; *As3mt*: arsenic methyltransferase; KO: knockout; WT: wild-type.

other signs of toxicity were observed during the study or in dissected tissues. This is in contrast with previous studies reporting lethality and histopathological abnormalities in As3mt-KO mice exposed to 25 mg/L for 4 weeks (Yokohira et al., 2011; Chen et al., 2011).

2.2. Effect of genotype on As speciation in tissues

The speciation of As in tissues of As3mt-KO mice has previously been examined. However, the analysis was limited to plasma, red blood cells, liver, kidney, lung, and bladder, and did not determine the oxidation state of As in the detected As species (Drobna et al., 2009; Hughes et al., 2010; Chen et al., 2011). Only recent optimization of the HG-CT-AAS method has allowed for the analysis of tri- and pentavalent As species in tissue homogenates without sample extractions or digestions that could affect the oxidation state of As. This method was used in the present study to compare the speciation of As and to measure the concentration of As^{III} and As^V species in tissues of As3mt-KO mice and WT mice. As expected, iAs^{III} and iAs^V were the main species retained in tissues of As3mt-KO mice, while methylated As metabolites predominated in most tissues from WT mice. Fig. 3 describes the distribution of As species in tissues involved in regulation of glucose homeostasis (i.e., liver, adipose tissue, pancreas, and skeletal muscle). As3mt-KO mice exposed to 0 mg/L As retained small amounts of iAs, likely due to the presence of iAs in standard rodent chow (Paul et al., 2007b). In As3mt-KO mice exposed to iAs in drinking water, iAs^{III} was the predominate species in

liver, adipose tissue, and pancreas, representing 57% to 74% of total speciated As (Fig. 3a-c). In skeletal muscle, iAs^{III} and iAs^V ranged from 42% to 53% and 45% to 57% of total speciated As, respectively (Fig. 3d). The concentrations of As species in As3mt-KO mice exposed to 15, 20, 25, or 30 mg/L As increased in a dose-dependent manner in liver, skeletal muscle and pancreas. In adipose tissue, the amount of iAs increased only between 15 and 25 mg/L iAs, but decreased at 30 mg/L. It is possible that this decrease was associated with the overall toxicity that was manifested by decrease in body weight of the As3mt-KO mice exposed to 30 mg/L As. DMAs^{III} was the major As species in the liver and pancreas of WT mice, accounting for 29% and 32% of total speciated As respectively. DMAs^V represented 45% and 47% of As in adipose and skeletal muscle tissues, respectively. Notably, the sum of trivalent species (iAs^{III} + MAs^{III} + DMAs^{III}) accounted for 55% and 68% of As in pancreas and liver of WT mice, respectively.

In a previous study of iAs metabolism in As3mt-KO mice, methylated species accounted for 28% to 32% of total As in liver and 22% to 28% in urine of As3mt-KO mice exposed to a single dose of iAs^V, suggesting the role of alternative As methylation mechanisms (Drobna et al., 2009). However, a more recent study in As3mt-KO mice exposed to iAs^{III} through drinking water (Chen et al., 2011) supports our finding that iAs^{III} and iAs^V are exclusive As species retained in liver, with no detectable levels of the methylated metabolites. The observed differences may be explained by the dosing (single vs. chronic) or by the fact that mice were exposed to iAs^{III} in one study but to iAs^V in the other. Notably, in the present study, the methylated arsenicals in tissues of As3mt-KO mice never exceeded 10% of the total speciated As and no methylated As species were detected in liver, pancreas, or adipose tissue.

In addition to the analysis of As^{III} and As^V species in the glucose regulating tissues, we also performed the oxidation-state analysis of As in the blood. Fig. 4 shows As species in blood plasma and blood cells of WT and As3mt-KO mice. Plasma and blood cells of WT mice exposed to 50 mg/L As contained significantly more As as compared to any As3mt-KO treatment group. In plasma and blood cells of As3mt-KO mice, iAs^{III} and iAs^V were present at approximately equal concentrations. An average of 348 ng As/mL was present in plasma of WT mice exposed to 50 mg/L As compared with only 6, 17, 21, 33, and 22 ng As/mL in As3mt-KO mice exposed to 0, 15, 20, 25 and 30 mg/L As, a greater than 10-fold difference (Fig. 4a). In the blood cells of As3mt-KO mice, a dose-dependent increase in As from 15 to 317 ng As/g tissue was observed, while an average of 506 ng As/g tissue was retained in blood cells of WT mice (Fig. 4b). Our finding that WT mice retain more total As in blood cells than As3mt-KO mice conflicts with a previous study where WT and As3mt-KO mice were exposed to 1, 10, and 25 mg/L As as iAs^{III} in drinking water for 33 days (Chen et al., 2011). Here, the authors reported that greater levels of total As were retained by erythrocytes of As3mt-KO mice than WT mice. This difference could be associated with differences in sample preparation, analytical speciation technique, differences in As recovery, or with higher exposures in the present study for WT mice. Notably, the total As level in blood cells from As3mt-KO mice exposed to 25 mg/L As in our study (245 ng As/g tissue) is in good agreement with the level (~300 µg/L As) reported by Chen et al. (2011).

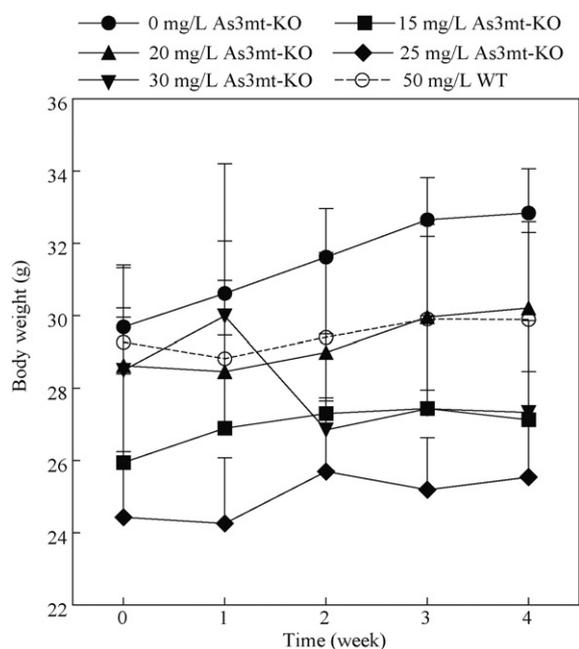


Fig. 2 – Change in body weights of As3mt-KO mice exposed to 0, 15, 20, 25, or 30 mg/L As and WT mice exposed to 50 mg/L As. Mean and SD, n = 5 for each of the As3mt-KO group and n = 10 for the WT group. There were no statistically significant differences in weekly average weights between the treatment groups.

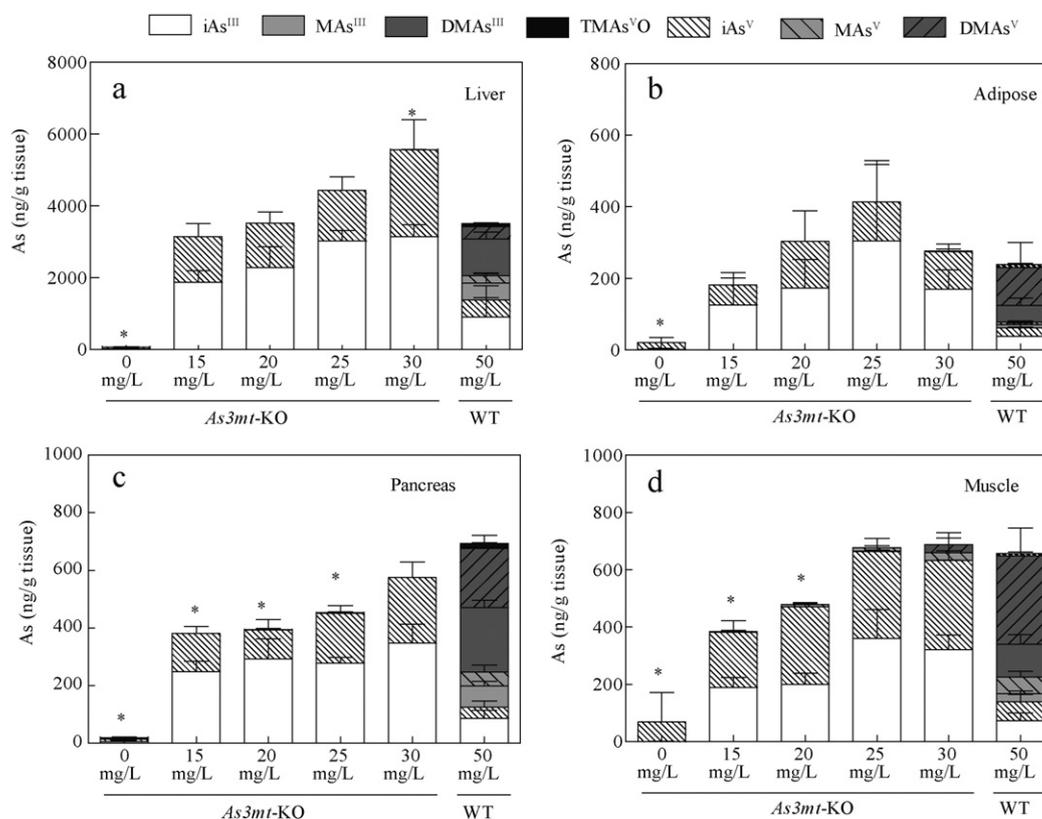


Fig. 3 – Oxidation state specific analysis of As in tissues critical for glucose homeostasis. The concentration (ng As/g wet tissue) of seven As species in liver (a), adipose tissue (b), pancreas (c), and skeletal muscle (d) of *As3mt*-KO mice exposed to 0, 15, 20, 25, and 30 mg/L and WT mice exposed to 50 mg/L As as iAs^{III}. Data is represented as means with SD for each arsenical (*As3mt*-KO, $n = 5$; WT, $n = 10$). *Statistically significant difference in total speciated As compared to 50 mg/L WT group ($p > 0.05$).

The concentrations of As^{III} and As^v species in several other tissues from WT and *As3mt*-KO mice, including intestine, kidney, lung, heart, brain, and testes were also examined (Fig. 5). Notably, MAs^{III} accounted for 62% of As in the intestine (with intestinal content included) of WT mice exposed to 50 mg/L As (Fig. 5a). In

contrast, iAs^{III} was the predominant species in the intestine from *As3mt*-KO mice while the methylated arsenicals represented less than 10% of total speciated As. It has been previously suggested that iAs methylation by intestinal bacteria is responsible for the formation of methylated As species in *As3mt*-KO mice (Drobna et

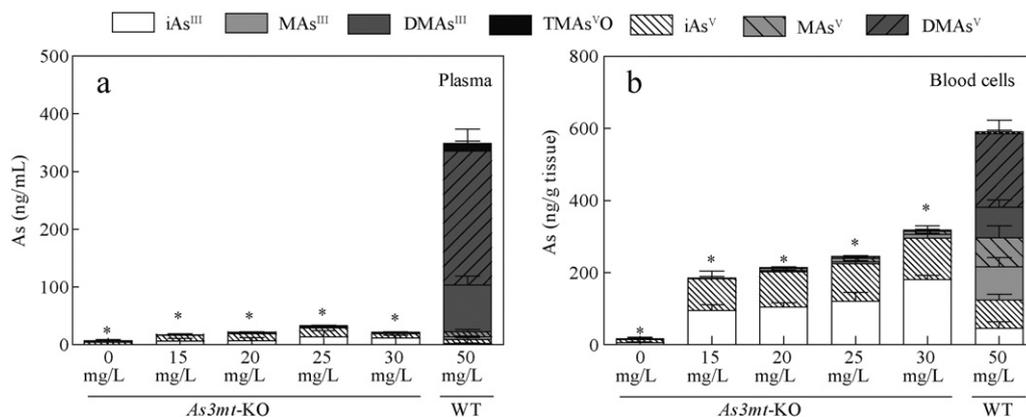


Fig. 4 – Oxidation state specific analysis of circulating As in *As3mt*-KO mice exposed to 0, 15, 20, 25, and 30 mg/L As and WT mice exposed to 50 mg/L As as iAs^{III}. The concentration of As species in plasma (a, ng As/mL) and blood cells (b, ng As/g wet tissue). Data is represented as means with SD for each arsenical. (*As3mt*-KO, $n = 5$; WT, $n = 10$). * Statistically significant difference in total speciated As compared to 50 mg/L WT group ($p > 0.05$).

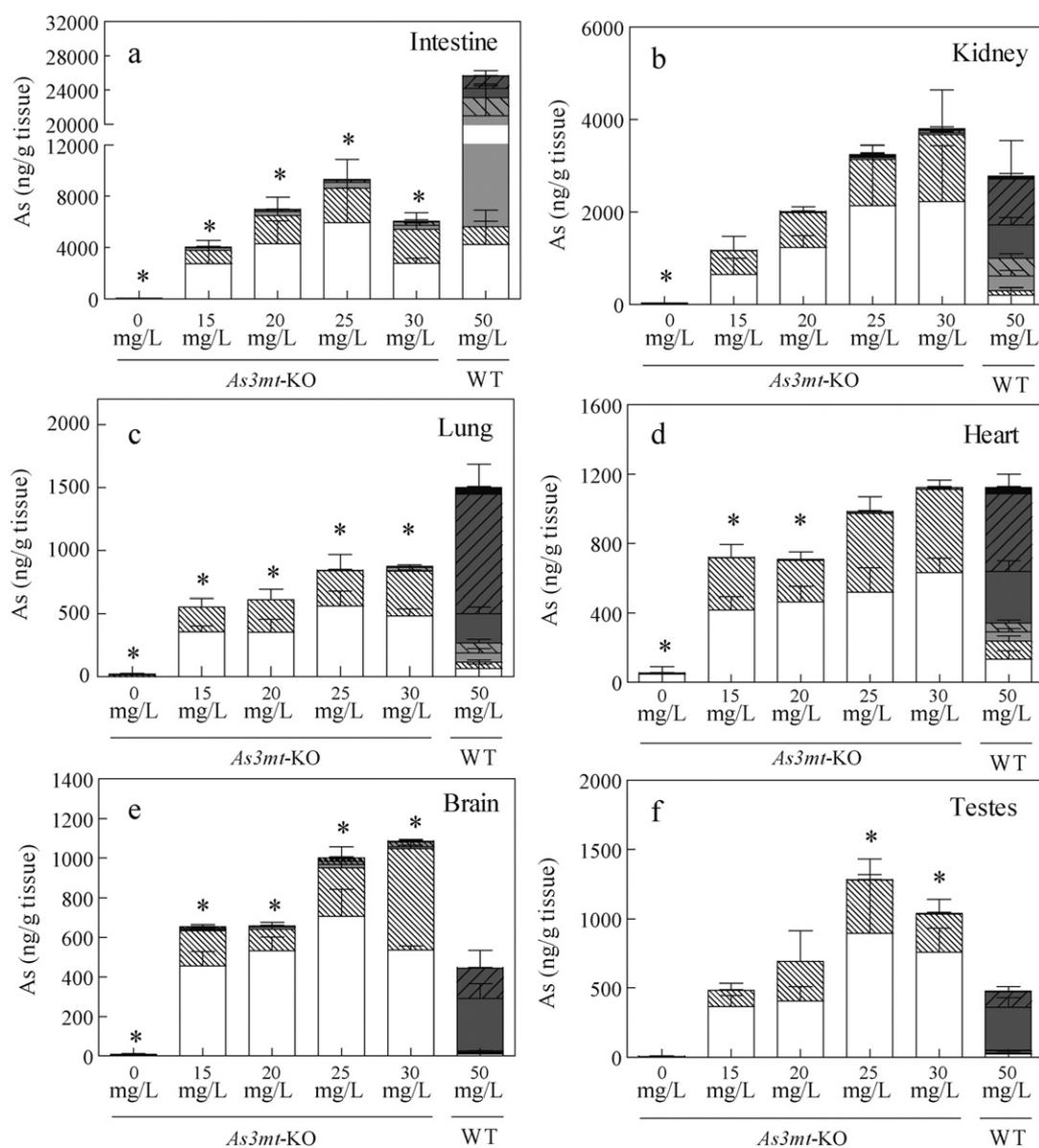


Fig. 5 – Oxidation state specific analysis of As in tissues from *As3mt-KO* mice exposed to 0, 15, 20, 25, and 30 mg/L As and WT mice exposed to 50 mg/L As as *iAs*^{III}. The concentration of seven As species (ng As/g wet tissue) in intestine with intestinal content (a), kidney (b), lung (c), heart (d), brain (e), and testes (f). Data is represented as means with SD for each arsenical. (*As3mt-KO*: *n* = 5; WT, *n* = 10).

al., 2009). Studies in other laboratories have shown that *iAs* can also be methylated by N-6 adenine-specific DNA methyltransferase 1 (Ren et al., 2011). Thus, methylation by intestinal microbiota or other methyltransferases may explain the presence of small amounts of the methylated As species in the intestine and in other tissues of *As3mt-KO* mice examined in the present study. Interestingly, total speciated As in the intestinal samples averaged between 4040 and 9273 ng As/g in *As3mt-KO* mice, but 24,879 ng As/g in WT mice. This data suggests that methylation increases intestinal content and/or excretion of As via feces. In a previous study where WT C57/BL6 and *As3mt-KO* mice were repeatedly dosed with 0.5 mg/kg *iAs*^V, whole body As burden and daily excretion analysis revealed that WT mice excreted As at least 10 to 20 times faster than *As3mt-KO* mice (Hughes et al., 2010). Our results show that in addition to urine, excretion in

feces contributes significantly to the clearance of methylated As species from the mouse body.

In the kidney, lung, brain, and testes of *As3mt-KO* mice, *iAs*^{III} accounted for 49% to 81% of total speciated As (Fig. 5). In kidney, heart, lung of WT mice, DMAs^V accounted for 36%, 40%, and 63% of speciated As, respectively, while DMAs^{III} predominated in brain (61%) and testes (67%). Notably, total speciated As level in lung from WT mice exposed to 50 mg/L As was significantly higher than in *As3mt-KO* mice in any treatment group. A recent report indicates significantly higher retention in lungs of *As3mt-KO* mice exposed to 1 and 10 mg/L *iAs*^{III} compared to WT mice at the same exposures, but not 25 mg/L (Chen et al., 2011). Our results suggest that at higher exposures, methylated arsenicals are more preferentially retained in lung tissue. Overall, these data indicate that trivalent

As species are highly retained in most tissues of WT and As3mt-KO mice exposed to As and likely play a significant role in the development of iAs-induced diseases.

2.3. Comparison of the tissue levels of As

In the present study, we exposed WT C57BL/6J mice to 50 mg/L As in drinking water, the dose that produced diabetes in these mice in our previous studies (Paul et al., 2007b, 2011). Human exposures are order of magnitude lower. However, mice metabolize iAs more efficiently than humans, thus higher doses may be needed in studies using mice to produce the effects described in human studies. In fact, total As levels in the livers of mice exposed to 50 mg/L in our previous studies (Paul et al., 2007b, 2011) were in the range of the concentrations reported in livers of Bangladeshi residents exposed to 0.2–2 mg/L As (Mazumder, 2005), indicating that this concentration may be relevant for human exposures.

The As3mt-KO mouse model provides a unique platform to study the adverse effects of individual arsenicals because little internal methylation occurs. However, the As3mt genotype affects As retention and susceptibility to As-induced toxicity; studies in As3mt-KO mice have reported lethality at 50 mg/L As exposures and toxic effects at exposures as low as 25 mg/L, concentrations that are well tolerated by WT mice (Yokohira et al., 2010, 2011). Thus, to determine which As exposures in As3mt-KO mice yield equivalent internal As doses compared to WT mice, As retention in tissues critical for glucose homeostasis (i.e., pancreas, liver, skeletal muscle, and adipose tissue; Fig. 3) was examined. Here, differences between the sum of iAs^{III+V}, MAs^{III+V}, DMAs^{III+V} and TMAs^{VO} for each As3mt-KO group and the WT group exposed to 50 mg/L As were evaluated by one-way ANOVA and Bonferroni's multiple comparison test (Table 1). Total speciated As values for As3mt-KO exposure groups that were not significantly different from the 50 mg/L WT group were considered to produce approximately equivalent internal doses. In the pancreas, equivalent internal doses to WT mice were only achieved in As3mt-KO mice exposed to 30 mg/L As. Equivalent internal As doses in the liver were seen in As3mt-KO mice exposed to 15, 20, and 25 mg/L As and for all exposures in adipose tissue. In skeletal muscle, 25 and 30 mg/L

As exposure in As3mt-KO mice resulted in equivalent internal As doses compared to WT mice exposed to 50 mg/L As.

While exposure to 30 mg/L in As3mt-KO mice for 4 weeks produced equivalent total As tissue retention in the pancreas, skeletal muscle and adipose, decreased body weights were observed in this treatment group after 4 weeks of exposure (Fig. 2). In contrast to other studies, no signs of toxicity were observed in any of the other As3mt-KO treatment groups (Yokohira et al., 2010, 2011). However, study duration and potential toxicity should be carefully considered when planning experiments involving As3mt-KO mice. Therefore, exposure to both 25 and 30 mg/L would produce equivalent internal As doses in tissues critical for glucose homeostasis and will be used in future studies examining the effects of As on the development of diabetes.

3. Conclusions

In As3mt-KO mice exposed to 15, 20, 25, or 30 mg/L As, iAs^{III} is the most prevalent species in liver, pancreas, and adipose tissues. The majority of iAs and methylated As species retained in liver and pancreas of WT mice exposed to 50 mg/L As are in the trivalent form. DMAs^V is the most prevalent species retained in skeletal muscle and adipose tissue of WT mice. For tissues critical to glucose homeostasis, doses of 25 and 30 mg/L As as iAs^{III} will produce in As3mt-KO mice total As levels approximately equivalent to those in WT mice exposed to the diabetogenic level of 50 mg/L As. These results should be considered when designing future studies using As3mt-KO mice to examine then role of iAs metabolism in adverse effects associated with iAs exposure.

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Table 1 – Determination of equivalent internal As doses in tissues critical for glucose homeostasis.

Treatment group	Total speciated As ^a (ng As/g tissue)			
	Pancreas	Liver	Skeletal muscle	Adipose
0 mg/L As3mt-KO	16 (7)	67 (10)	69 (102)	21 (14)
15 mg/L As3mt-KO	381 (38)	3137 (476) [#]	386 (55)	181 (107) [#]
20 mg/L As3mt-KO	394 (86)	3525 (787) [#]	479 (49)	304 (112) [#]
25 mg/L As3mt-KO	452 (26)	4497 (300) [#]	677 (114) [#]	413 (329) [#]
30 mg/L As3mt-KO	576 (83) [#]	5564 (1024)	688 (147) [#]	277 (61) ^{#y}
50 mg/L WT	693 (141)	3511 (1174)	658 (185)	239 (118)

^a Total speciated As includes the sum of iAs^{III+V}, MAs^{III+V}, DMAs^{III+V} and TMAs^{VO} determined by HG-CT-AAS. Mean (SD); As3mt-KO, n = 5 and WT, n = 10.

[#] Statistically non-significant difference in total speciated As compared to 50 mg/L WT group (p > 0.05).

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