Genetic and epigenetic mechanisms underlying arsenic-associated diabetes mellitus: a perspective of the current evidence

Chronic exposure to arsenic has been associated with the development of diabetes mellitus (DM), a disease characterized by hyperglycemia resulting from dysregulation of glucose homeostasis. This review summarizes four major mechanisms by which arsenic induces diabetes, namely inhibition of insulin-dependent glucose uptake, pancreatic β -cell damage, pancreatic β -cell dysfunction and stimulation of liver gluconeogenesis that are supported by both *in vivo* and *in vitro* studies. Additionally, the role of polymorphic variants associated with arsenic toxicity and disease susceptibility, as well as epigenetic modifications associated with arsenic exposure, are considered in the context of arsenic-associated DM. Taken together, *in vitro*, *in vivo* and human genetic/epigenetic studies support that arsenic has the potential to induce DM phenotypes and impair key pathways involved in the regulation of glucose homeostasis.

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Type 2 diabetes mellitus (DM) is a metabolic disorder characterized by disruption of the insulin-signaling pathway resulting in insulin resistance and pancreatic β -cell dysfunction and in limited uptake of glucose by cells. More than 640 million people are affected by Type 2 DM globally, with up to 40% unexplained by rises in obesity and other associated lifestyle factors [1]. Numerous studies have investigated the role of environmental contaminants in the development of DM [2,3]. Of the chemicals investigated, the 2011 National Toxicology Program Workshop on DM and the Environment found sufficient evidence for the relationship between exposure to moderate and high ($\geq 150 \mu\text{g As/l}$) levels of inorganic arsenic in drinking water and DM [4]. At the time, the Workshop deemed the evidence for association between moderate-to-low level ($< 150 \mu\text{g As/l}$) exposure to be insufficient [4]. More recently, two meta-analyses that include 19 new studies have been conducted substanti-

ating the association between chronic arsenic exposure at levels less than $150 \mu\text{g As/l}$ and DM [5,6]. These meta-analyses found pooled relative risks of developing DM in the presence of arsenic equal to 1.7 and 1.23, respectively [5,6].

Globally, more than 200 million people are exposed to inorganic arsenic at levels that exceed $10 \mu\text{g As/l}$ [7]. Arsenic is associated with numerous health endpoints including, including cancer of the skin and bladder, cardiovascular disease, stroke, neurological effects, peripheral vascular disease, and DM [8]. Several plausible mechanisms underlying DM have been proposed based upon *in vivo* and *in vitro* laboratory studies [8,9]. The data support the impact of arsenic on processes that govern glucose homeostasis, supporting that arsenic impairs insulin-dependent glucose uptake by inhibiting insulin-activated signal transduction pathway, upregulates gluconeogenesis and inhibits glucose-stimulated insulin secretion by

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pancreas [10]. These mechanisms are further supported by epidemiological studies in arsenic-exposed human populations where arsenic is associated with biomarkers of pathological processes typically associated with DM. Specifically these biomarkers represent renal damage, oxidative stress, low-grade inflammation and endothelial damage [11]. There are also provocative data suggesting a role for epigenetic alterations underlying arsenic-associated DM [12–17]. To date, a limited number of studies have examined the role of the epigenome in the context of arsenic-associated DM. As arsenic is known to modify various components of the epigenetic machinery [18], it is likely that study of the epigenome could provide key insight into mechanisms by which arsenic induces DM in humans.

In the present review, we summarize the molecular mechanisms proposed to underlie arsenic-associated DM. Additionally, existing studies focusing on arsenic-associated DM including those that assess genetic variants linked to DM as well as arsenic-associated modification of the epigenome are detailed. These genetic variants and epigenetic alterations could play a critical role in driving molecular changes that induce DM. Finally, gaps in the literature are noted and potential directions for future research are discussed.

Mechanisms of arsenic-associated DM

DM is a group of metabolic diseases characterized by hyperglycemia that results from disruption of key processes involved in the regulation of glucose homeostasis. The sustained hyperglycemia is the result of the failure to produce or secrete insulin in the pancreatic β -cells and/or the failure of peripheral tissues to respond to insulin signaling. Type 1 DM is the result of an autoimmune condition where pancreatic β -cells are destroyed resulting in a loss of insulin and the subsequent inability of tissues to utilize blood glucose. In contrast, Type 2 DM is characterized by impaired insulin signaling (i.e., insulin resistance) in peripheral

tissues, and subsequent β -cell dysfunction. Both Type 1 and 2 DM are characterized by hyperglycemia. Interestingly, it has been demonstrated that laboratory animals exposed to inorganic arsenic develop phenotypes consistent with DM, namely increased fasting blood glucose, impaired glucose tolerance and/or insulin resistance (Supplementary Box 1) [19–25]. Additionally, prenatal exposure to inorganic arsenic predisposes offspring to the development of DM in laboratory studies [26,27]. In 11 of the 13 currently published rodent studies, the doses used to promote DM in laboratory animals were much higher than those typically associated with the environmental exposures (Supplementary Box 1). It is important to note that these higher doses may be necessary as mice are less susceptible to arsenic-induced health effects than humans due to more efficient metabolism and clearance [28].

In vitro and *in vivo* laboratory studies support four major mechanisms by which inorganic arsenic and its methylated metabolites are able to influence DM-related processes (Box 1, Supplementary Box 1 & Supplementary Table 2). The first mechanism is the inhibition of insulin signaling, resulting in insulin resistance and in an impaired glucose uptake and utilization. The second mechanism is the induction of apoptosis of pancreatic β -cells, the primary site of insulin synthesis. The third mechanism is the inhibition of glucose-stimulated insulin secretion by pancreatic β -cells at noncytotoxic concentrations. The fourth mechanism is the induction of gluconeogenesis. While these processes are also involved in Type 2 DM [25], the ability of arsenic to dysregulate these processes supports that it is a diabetogen (Box 1).

In addition to inorganic arsenic exposure, different methylated metabolites of arsenic influence processes involved in the development of DM to different degrees, suggesting that metabolism of arsenic is a determinant of DM development. Inorganic arsenic is metabolized by the enzyme arsenic (+3-oxidation state)

Box 1. Major mechanisms underlying arsenic-associated diabetes.

Mechanism 1: arsenic inhibits insulin-dependent glucose uptake

- Inhibition of GLUT4 localization to the plasma membrane
- Inhibition of Akt signaling to mobilize GLUT4
- Altered gene expression of the Akt pathway
- Upregulation of antioxidant pathways leading to decreased secondary messengers
- Altered development of the glucose transport system

Mechanism 2: β -cell damage

- Induction of apoptosis due to production of ROS
- Induction of chronic inflammation due to production of ROS

Mechanism 3: β -cell dysfunction

- Failed glucose-stimulated insulin secretion due to increased ROS

Mechanism 4: stimulation of gluconeogenesis

- Induced expression of phosphoenolpyruvate carboxykinase

Akt: Protein kinase B; GLUT4: Glucose transporter type 4; ROS: Reactive oxygen species.

methyltransferase (*AS3MT*) into different arsenicals. *AS3MT* first methylates and reduces inorganic arsenic to produce monomethyl arsenicals (MAsIII, MAsV). This process is repeated to produce the dimethyl arsenicals (DMAsIII, DMAsV). Importantly, it has been shown that the trivalent arsenicals are much more potent than the pentavalent counterparts as inhibitors of insulin signaling and insulin secretion by β -cells, suggesting that the trivalent species may be the main drivers of arsenic-associated DM [29]. However, it is important to note that limited evidence on pentavalent arsenical species hinders the ability to understand their roles in the induction of arsenic-associated DM. The use of biomarkers of cell injury and altered glucose signaling provides support that arsenic-induced injuries resulting in DM occur in humans. Taken together, the following studies of the effect of arsenic on glucose homeostasis pathways provide substantive evidence that arsenic is an etiologic factor in the development of DM.

Mechanisms of arsenic-associated DM: inhibition of insulin-dependent glucose uptake (insulin resistance)

Arsenic and its metabolites can alter glucose homeostasis through insulin resistance and impaired glucose uptake [28–30]. Arsenite (AsIII), and its trivalent metabolites, methylarsonous acid (MAsIII) and dimethylarsinous acid (DMAsIII), inhibit glucose transporter type 4 (GLUT4) recruitment to the plasma membrane despite insulin stimulation *in vitro* (Figure 1) [29]. Furthermore, *in vitro* studies have demonstrated that AsIII and MAsIII inhibit the insulin-dependent activation of protein Akt, a key signaling step required for GLUT4 translocation to the plasma membrane, by preventing its phosphorylation by PDK-1 (Figure 1) [23,31]. DMAsIII works downstream of Akt to prevent the recruitment of GLUT4, but the exact mechanism remains unclear (Figure 1). The process of Akt inhibition is compounded by the fact that arsenic can influence the expression of the PI3K transcription factor that is upstream of Akt, and that regulates other important steps in insulin signaling *in vivo* and *in vitro* [31,32]. Additionally, the expression of many of the genes in this pathway, including *GLUT4*, and *Akt* are decreased in response to arsenic exposure *in vivo* and *in vitro* [30,32–33]. In mice, arsenic exposure is associated with generation of reactive oxygen species (ROS) and upregulation of cellular antioxidant pathways including Nrf2 [34,35]. The constitutive upregulation of Nrf2 and the consequent increase in antioxidant enzyme expression could inhibit insulin-stimulated glucose uptake as the insulin pathway may also require ROS as signaling molecules [33].

In addition to directly inhibiting the process of insulin-dependent glucose uptake, arsenic can interfere with adipogenesis, the differentiation of pre-adipocytes to adipocytes, as well as differentiation of myoblast into myotubes in skeletal muscle at doses between 0.5 and 5 μ M [30,36–37] (Supplementary Table 2). Adipocytes in white adipose tissue and myotubes in skeletal muscle tissue play critical roles in glucose utilization and in the maintenance of blood glucose levels. Arsenic inhibits adipocyte differentiation by altering the expression of key genes and transcription factors involved in adipogenesis, specifically *PPAR- γ* and *CEBP- α* [31,36,38–39]. This indirectly affects numerous other proteins involved in adipocyte differentiation including p21 and A-FABP [40]. Alterations of adipogenesis likely lead to decreased lipid-storage capacity and insulin resistance, as it has been shown that defects in adipogenesis can result in insulin impairment and Type 2 diabetes [36,41].

Arsenic may also influence the development of the insulin-responsive glucose transport system [30] by decreasing the phosphorylation of mechanistic target of mTOR and p70, key regulators in this process [42]. Notably, in humans, urinary total arsenic (U-tAs; a biomarker measure of arsenic exposure) is inversely associated with insulin sensitivity levels [43]. However, it has also been shown that arsenic exposure is inversely related to fasting plasma insulin and to the measure of insulin resistance (HOMA-IR) [44]. Similarly, mice that were exposed to arsenic and fed a high-fat diet displayed impaired glucose tolerance and a dose-dependent decrease in HOMA-IR, likely due to lower fasting insulin and higher fasting blood glucose than in mice fed a low-fat diet [25]. Insulin response to glucose challenge was also impaired in these mice. Thus, both impaired insulin sensitivity and impaired insulin secretion in response to high blood glucose may underlie the diabetogenic effects of arsenic exposure.

Mechanisms of arsenic-associated DM: β -cell damage

There is a growing body of evidence that arsenic can damage pancreatic β -cells. It has been shown that arsenic metabolites accumulate in the pancreata of mice exposed to arsenic [19,24,28]. Arsenic accumulation induces apoptosis of pancreatic β -cells *in vitro*, likely through the production of ROS, and induction of PARP and Caspase-3, as well as through other mechanisms [37,45–47]. In addition to causing cell death, this accumulation of ROS has been shown to result in chronic inflammation. Such chronic inflammation can result in pancreatitis *in vivo*, which can lead to the development of DM [47]. Moreover, excessive levels of ROS result in upregulation of antioxidant pathways. It

Figure 1. Arsenic (iAsIII) and its metabolites inhibit insulin-dependent glucose uptake by disrupting the insulin-activated signaling cascade. Specifically, iAsIII and MAAsIII have been shown to inhibit the phosphorylation of Akt by PDK, resulting in inhibition of GLUT4 translocation to plasma membrane; DMAAsIII inhibits signaling downstream of PDK/Akt, but the exact target has not been identified. Figure adapted from [23].

has been proposed that a persistent activation of Nrf2, a key transcription factor in the antioxidant system, reduces glucose-triggered ROS-mediated signaling and thus inhibits glucose-stimulated insulin secretion *in vitro* [48]. Exposure to AsIII and MAAsIII have also been shown to lead to inhibition of TXNRD1 *in vivo*, a key member of the cellular anti-oxidant defense, thus resulting in apoptosis [19,49].

Mechanisms of arsenic-associated DM: β -cell dysfunction

Arsenic and its methylated metabolites can inhibit the gene transcription of insulin and/or secretion in the pancreas. Some studies suggested that AsIII may decrease the levels of insulin mRNA produced by pancreatic β -cells. Decreased insulin mRNA production would produce the same effect as destruction of these cells. Still, there have been conflicting data on the subject, likely due to dose differences employed across studies [50,51]. The destruction of cells by ROS-mediated mechanisms, as well as other cellular targets of AsIII, directly impacts the ability of the pancreas to produce insulin. Recent *in vitro* research has demonstrated that pancreatic islets exposed to low arsenic concentrations continue to produce insulin, but fail to

secrete it. The result is a blunted glucose-stimulated insulin secretion [50,51]. Consistent with these findings are results of a recent population study that showed arsenic exposure to be associated with a decrease in the insulin secretion index, HOMA2%B [52]. An additional *in vitro* study using a β -cell line suggested that this is likely due to the ability of AsIII to inhibit calcium oscillation, which is necessary for glucose-stimulated insulin secretion [53]. Interestingly, both MAAsIII and DMAAsIII are more potent inhibitors of glucose-stimulated insulin secretion by pancreatic islets than AsIII, though the mechanisms for this are unclear [51]. It has also been shown *in vitro* that the cellular adaptation to chronic oxidative stress caused by AsIII exposure lowers ROS production and impairs glucose-stimulated insulin secretion, which may require low-level ROS as one of the regulatory mechanisms [54].

Mechanisms of arsenic-associated DM: stimulation of gluconeogenesis

In addition to inhibiting insulin secretion, gene expression changes induced by arsenic *in vivo* can also induce hepatic gluconeogenesis. Hepatic gluconeogenesis is the pathway for synthesis of glucose from noncarbohydrate sources during fasting. Arsenic induces increased expression of phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in gluconeogenesis resulting in fasting hyperglycemia [55,56]. In summary, these data detail the manner by which arsenic is able to regulate glucose homeostasis resulting in hyperglycemia and DM.

Genetic & epigenetic underpinnings for arsenic-associated DM

Multiple studies have found that differences in the metabolism of inorganic arsenic and the distribution and excretion of arsenic metabolites are associated with likelihood of diabetes development [12,57–60]. As detailed previously, inorganic arsenic is methylated primarily by *AS3MT* to form the monomethylated and dimethylated arsenic metabolites, MAs and DMAs. Studies of arsenic-exposed populations have found that higher inorganic arsenic and MAs in urothelial cells and higher DMAs in urine are more strongly associated with DM than drinking water measures of arsenic or U-tAs [44,59,61–62]. These differences in arsenic metabolite profiles represent a potential indicator of the risk of developing DM and could be mediated by differences in genotypes, specifically single nucleotide polymorphisms (SNPs), of critical genes such as *AS3MT* [63], *CAPN-10* – a calcium-dependent protease that plays a key role in exocytosis of insulin-containing vesicles in β -cells [64], *GSTO1* – an enzyme that contributes to the reduction of arsenic from the pentava-

lent to trivalent form [57] and *NOTCH2* – a member of a signaling cascade involved in cell differentiation [57–58,64–65]. Interestingly, the majority of SNPs associated with differences in arsenic metabolism occur in non-coding regions of the genes, suggesting a potential role for regulatory factors or splice variants.

In addition to the role of polymorphic variants of key genes, the processes of glucose homeostasis, as well as arsenic metabolism, may also be altered by epigenetic regulation. In support of this, exposure to arsenic induces alterations to the epigenome, including changes in CpG methylation and miRNA expression [18,66–67]. The epigenome constitutes potentially heritable changes that influence gene expression but are not contained within the DNA sequence [18]. These changes result in altered gene expression and subsequent changes in protein levels. In contrast to SNPs or other DNA sequence variations, epigenetic alterations can influence gene and protein expression, but do not alter amino acid sequence. Of the epigenetic modifications studied in relationship to arsenic, DNA methylation has been the best characterized in human populations [18].

Results of epigenetic studies of arsenic exposure suggest that the epigenome could play a key role in the development of arsenic-associated DM. Four studies of arsenic-exposed non-diabetic individuals have shown that arsenic disrupts gene expression, miRNA expression and DNA methylation profiles in a manner consistent with DM [12–16]. Andrew *et al.* assessed gene expression in the blood of individuals exposed to arsenic in New Hampshire, USA, where the highest level of exposure was 74.4 µg/l of inorganic arsenic in drinking water [14]. Importantly, many of the genes identified as dysregulated in association with arsenic were involved in Type 1 DM signaling [14]. These genes included members of the *HLA* cluster that account for 40–50% of familial aggregation of Type 1 DM, as well as other genes associated with hereditary Type 1 DM. These genes are responsible for regulation of the immune response, a primary mechanism of β-cell destruction in Type 1 DM. In addition to changes in mRNA expression, it has been shown that arsenic is associated with altered miRNA expression. Rager *et al.* analyzed miRNA profiles from the Biomarkers of Exposure to ARsenic pregnancy cohort in Gómez Palacio, Mexico, where arsenic ranged from 0 to 240 µg/l. It was found that miRNAs with altered expression in response to prenatal arsenic exposure were enriched for roles in DM [16]. Specifically, arsenic-associated changes in miR-107 and miR-20b were observed, both of which have previously been associated with DM. Furthermore, ten downstream targets of these miRNAs were identified as differentially

expressed in response to arsenic exposure. In addition to these data in a different study, miR-25, a regulator of glycemic control, was altered in rats exposed to arsenic at 0.1–100 mg/l [17]. These data highlight that arsenic influences the altered expression of both mRNA and miRNAs associated with DM signaling.

In addition to expression of miRNAs and mRNAs, arsenic has been shown to perturb DNA (CpG) methylation. Smeester *et al.* assessed blood-based DNA methylation patterns associated with arsenicosis in adults from Zimapán, Mexico. The exposure of these individuals was characterized by urinary As levels (27.5–90.8 µg/l) and arsenicosis by the presence of skin lesions [13]. Using a genome-wide assay with a methyl-binding protein bound to methylated DNA followed by array hybridization, a total of 183 genes displayed methylation levels that were significantly associated with U-tAs levels. Among these genes are those that are involved in DM including *CENPE*, which was previously identified through a genome-wide association study as being associated with risk of DM development [68]. Subsequent analysis of these data performed in Bailey *et al.* assessed DNA methylation changes as they related to arsenic metabolism (i.e., proportions of the methylated metabolites in urine) [12]. Specifically, enrichment for DM-associated genes was observed in methylated probes associated with the dimethylated fraction of arsenic (%DMAs) in urine. Notably, these genes were associated with pancreatic β-cell function and apoptosis, as well as with insulin signaling and insulin resistance. Genes regulating insulin production (*PDX1*, *INS*), and those involved in glucose-stimulated insulin secretion (*VAMP2*) were among those that were differentially methylated in relationship to arsenic exposure. A limitation of the studies of the Zimapán population was the lack of gene expression measures, thus it is unclear what the specific impact of these CpG alterations were on transcript level. Lastly, DNA methylation changes that resulted in functional gene expression changes in cord blood were assessed in the Biomarkers of Exposure to ARsenic cohort [15]. Among the set of genes that was identified with altered methylation in relation to arsenic exposure was the imprinted gene, *KCNQ1*. *KCNQ1* is a key protein in the glucose-stimulated cascade that leads to insulin secretion by β-cells [69]. Interestingly, SNPs in this gene have been associated with increased odds of development of Type 2 DM, gestational diabetes and glucose tolerance [70–72]. Furthermore, previous studies have linked hypomethylation of this gene to increased odds of DM development [70,72]. Taken together, these studies suggest that changes to the epigenome, specifically CpG methylation and miRNA expression, are tied to altered gene expression of DM-associated genes. The genes

identified in these studies are involved in multiple DM processes, including destruction of β -cells, glucose-stimulated insulin secretion and insulin secretion by β -cells. In future research, the precise role of the epigenome in the development of arsenic-associated DM should be further explored.

In addition to determining associations of arsenic exposure and DM, population-based studies have demonstrated sex-dependent differences in the development of arsenic-associated disease [73]. In particular, some studies have found that women have higher standardized mortality ratios and hazard ratios for DM-related death and disease development in association with arsenic [74–76]. These findings have been substantiated by *in vivo* animal studies where female rodents are more susceptible than male rodents to the diabetogenic effects of arsenic exposure [20,56]. While these data suggest that sex influences the development of arsenic-associated DM, further research is needed to fully elucidate the underpinnings of this relationship. Interestingly, this could further support the role of epigenetic alterations as a potential driver of arse-

nic-associated DM as multiple studies have shown sex-dependent DNA methylation patterns [77–79].

The summarized studies support the role for genetic variation and epigenetic modification as susceptibility factors for the development of arsenic-associated DM. Moving forward, future studies on the role of the epigenome should include assessments of functional changes in gene and protein expression to elucidate causal mechanisms important to the development of arsenic-associated DM. As some epigenetic marks are stable over time [80], they could provide evidence for the temporality of arsenic exposure and the development of DM in later life.

Conclusion & future perspective

There is strong evidence that arsenic is a diabetogen. This evidence comes from epidemiologic studies with a mechanistic basis supported by both *in vitro* and *in vivo* studies. The data demonstrate that arsenic triggers the development of DM phenotypes in laboratory animals, and impairs key pathways involved in glucose homeostasis. Some observations from *in vivo* studies, such as

Executive summary

Background

- Increased incidence of diabetes has been associated with arsenic exposure in numerous epidemiological studies.
- Arsenic has been shown to induce DM phenotypes *in vivo* and disrupt key processes responsible for glucose homeostasis *in vitro*.

Mechanisms of arsenic associated-diabetes mellitus

- There are four major mechanisms by which arsenic is hypothesized to induce DM.
- First, arsenic has been shown to inhibit insulin-dependent glucose uptake particularly through inhibition the glucose transporter, GLUT4.
- Second, arsenic has been shown to induce pancreatic β -cell damage as it accumulates in the pancreata of exposed laboratory animals and stimulates the production of ROS.
- Third, arsenic results in pancreatic β -cell dysfunction, specifically chronic arsenic exposure leads to inhibition of glucose-stimulated insulin secretion.
- Lastly, arsenic is thought to stimulate liver gluconeogenesis, resulting in increased glucose entering the blood stream.

Genetic & epigenetic underpinnings for arsenic-associated diabetes mellitus

- Variants in the genes *AS3MT*, *CAPN-10*, *GSTO1* and *NOTCH2* have been linked to increased likelihood of developing DM in the presence of inorganic arsenic.
- Epigenetic mechanisms, specifically DNA methylation and miRNA expression, likely play a key role in arsenic-associated DM.
- In human populations exposed to arsenic, genes associated with Type 1 DM display altered expression at the mRNA level.
- Arsenic has been shown to alter miRNAs associated with DM and glycemic regulation in humans and *in vivo*.
- Three studies have identified arsenic-associated changes in DNA methylation of DM-related genes. Two of these studies were in adult populations and one was in infants exposed to arsenic prenatally.

Conclusions & future outlook

- *In vivo* and *in vitro* studies suggest that arsenic is a diabetogen, and data from epidemiological studies of human populations support this.
- The epigenome remains understudied in the context of arsenic-associated DM and could provide valuable insight into mechanism underlying the ability of arsenic to affect glucose homeostasis in humans.
- *In vivo* and *in vitro* models that can better assess low-dose effects of glucose homeostasis should be developed.

the sex-dependent nature of the relationship between arsenic and DM, and *in vitro* studies, such as dysfunction of β -cells, are substantiated by human population-based research. Importantly, evidence suggests a potential role for the epigenome (e.g., DNA methylation, miRNAs) as a means by which arsenic could influence the development of DM. Research that focuses on molecular measurements, such as DNA methylation, gene expression and protein expression, could provide valuable insight into how arsenic exposure affects glucose homeostasis and which of the proposed mechanisms is most likely driving the association observed in human populations. Moving forward, we foresee environmental DM research will incorporate biomarker-based DM indicators, such as measures of insulin resistance and β -cell function, to be used in conjunction with arsenic exposure biomarkers in human studies. Furthermore, researchers should focus on three primary areas. First, there is a need to develop *in vivo* and *in vitro* models to better assess low-dose effects on

glucose homeostasis. Second, it is important to characterize key developmental windows of susceptibility related to DM phenotypes and assess susceptibility factors such as genetic variation and sex. Lastly, the use of standardized methods for the assessment of arsenic exposure and metabolism and for DM diagnosis should be implemented across studies to ensure comparability. These directions of study will further elucidate the mechanistic basis underlying arsenic-associated DM.

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References

- 1 Flegal KM, Panagiotou OA, Graubard BI. Estimating population attributable fractions to quantify the health burden of obesity. *Ann. Epidemiol.* 25(3), 201–207 (2015).
- 2 Thayer KA, Heindel JJ, Bucher JR, Gallo MA. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environ. Health Perspect.* 120(6), 779–789 (2012).
- 3 Longnecker MP, Daniels JL. Environmental contaminants as etiologic factors for diabetes. *Environ. Health Perspect.* 109(Suppl. 6), 871–876 (2001).
- 4 Maull EA, Ahsan H, Edwards J *et al.* Evaluation of the association between arsenic and diabetes: a National Toxicology Program workshop review. *Environ. Health Perspect.* 120(12), 1658–1670 (2012).
- Provided a detailed review of studies through 2012 detailing the associations between arsenic and DM.
- 5 Sung TC, Huang JW, Guo HR. Association between arsenic exposure and diabetes: a meta-analysis. *Biomed. Res. Int.* 2015, 368087 (2015).
- Provided a comprehensive list of human population-based studies that have examined the association between arsenic exposure and DM.
- 6 Wang W, Xie Z, Lin Y, Zhang D. Association of inorganic arsenic exposure with Type 2 diabetes mellitus: a meta-analysis. *J. Epidemiol. Community Health* 68(2), 176–184 (2014).
- 7 Naujokas MF, Anderson B, Ahsan H *et al.* The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ. Health Perspect.* 121(3), 295–302 (2013).
- 8 Mohammed Abdul KS, Jayasinghe SS, Chandana EP, Jayasumana C, De Silva PM. Arsenic and human health effects: a review. *Environ. Toxicol. Pharmacol.* 40(3), 828–846 (2015).
- 9 Tseng CH. The potential biological mechanisms of arsenic-induced diabetes mellitus. *Toxicol. Appl. Pharmacol.* 197(2), 67–83 (2004).
- 10 Huang CF, Chen YW, Yang CY, Tsai KS, Yang RS, Liu SH. Arsenic and diabetes: current perspectives. *Kaohsiung J. Med. Sci.* 27(9), 402–410 (2011).
- 11 Andra SS, Makris KC, Christophi CA, Ettinger AS. Delineating the degree of association between biomarkers of arsenic exposure and Type-2 diabetes mellitus. *Int. J. Hyg. Environ. Health* 216(1), 35–49 (2013).
- 12 Bailey KA, Wu MC, Ward WO *et al.* Arsenic and the epigenome: interindividual differences in arsenic metabolism related to distinct patterns of DNA methylation. *J. Biochem. Mol. Toxicol.* 27(2), 106–115 (2013).
- Examined the relationship between inter-individual differences in arsenic metabolism and DNA methylation and demonstrated that these genes are involved in DM.
- 13 Smeester L, Rager JE, Bailey KA *et al.* Epigenetic changes in individuals with arsenicosis. *Chem. Res. Toxicol.* 24(2), 165–167 (2011).
- Examined the relationship between skin lesions and DNA methylation profiles and highlighted DM-associated genes.
- 14 Andrew AS, Jewell DA, Mason RA, Whitfield ML, Moore JH, Karagas MR. Drinking-water arsenic exposure modulates gene expression in human lymphocytes from a U.S. population. *Environ. Health Perspect.* 116(4), 524–531 (2008).
- Determined that prenatal arsenic exposure resulted in altered gene expression changes and highlighted an enrichment for genes involved in DM.

- 15 Rojas D, Rager JE, Smeester L *et al.* Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. *Toxicol. Sci.* 143(1), 97–106 (2015).
- **Established functional DNA methylation changes in DM-associated genes, specifically the imprinted gene, *KCNQ1*, in relation to prenatal arsenic exposure.**
- 16 Rager JE, Bailey KA, Smeester L *et al.* Prenatal arsenic exposure and the epigenome: altered microRNAs associated with innate and adaptive immune signaling in newborn cord blood. *Environ. Mol. Mutagen.* 55(3), 196–208 (2014).
- **Observed that prenatal arsenic exposure was associated with the expression of DM-associated miRNAs and further demonstrated functional changes in miRNA target gene expression profiles.**
- 17 Ren X, Gaile DP, Gong Z *et al.* Arsenic responsive microRNAs *in vivo* and their potential involvement in arsenic-induced oxidative stress. *Toxicol. Appl. Pharmacol.* 283(3), 198–209 (2015).
- **Demonstrated that arsenic exposure in rodents resulted in altered expression of miR-25, a regulator of glycemic control.**
- 18 Bailey KA, Fry RC. Arsenic-associated changes to the epigenome: what are the functional consequences? *Curr. Environ. Health Rep.* 1, 22–34 (2014).
- 19 Izquierdo-Vega JA, Soto CA, Sanchez-Pena LC, De Vizcaya-Ruiz A, Del Razo LM. Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite. *Toxicol. Lett.* 160(2), 135–142 (2006).
- 20 Palacios J, Roman D, Cifuentes F. Exposure to low level of arsenic and lead in drinking water from Antofagasta city induces gender differences in glucose homeostasis in rats. *Biol. Trace. Elem. Res.* 148(2), 224–231 (2012).
- 21 Patel HV, Kalia K. Role of hepatic and pancreatic oxidative stress in arsenic induced diabetic condition in Wistar rats. *J. Environ. Biol.* 34(2), 231–236 (2013).
- 22 Singh N, Rana SV. Effect of insulin on arsenic toxicity in diabetic rats-liver function studies. *Biol. Trace. Elem. Res.* 132(1–3), 215–226 (2009).
- 23 Paul DS, Harmon AW, Devesa V, Thomas DJ, Styblo M. Molecular mechanisms of the diabetogenic effects of arsenic: inhibition of insulin signaling by arsenite and methylarsonous acid. *Environ. Health Perspect.* 115(5), 734–742 (2007).
- 24 Paul DS, Hernandez-Zavala A, Walton FS *et al.* Examination of the effects of arsenic on glucose homeostasis in cell culture and animal studies: development of a mouse model for arsenic-induced diabetes. *Toxicol. Appl. Pharmacol.* 222(3), 305–314 (2007).
- 25 Paul DS, Walton FS, Saunders RJ, Styblo M. Characterization of the impaired glucose homeostasis produced in C57BL/6 mice by chronic exposure to arsenic and high-fat diet. *Environ. Health Perspect.* 119(8), 1104–1109 (2011).
- 26 Davila-Esqueda ME, Morales JM, Jimenez-Capdeville ME *et al.* Low-level subchronic arsenic exposure from prenatal developmental stages to adult life results in an impaired glucose homeostasis. *Exp. Clin. Endocrinol. Diabetes* 119(10), 613–617 (2011).
- 27 States JC, Singh AV, Knudsen TB *et al.* Prenatal arsenic exposure alters gene expression in the adult liver to a proinflammatory state contributing to accelerated atherosclerosis. *PLoS ONE* 7(6), e38713 (2012).
- 28 Paul DS, Devesa V, Hernandez-Zavala A *et al.* Environmental arsenic as a disruptor of insulin signaling. *Met. Ions Biol. Med.* 10, 1–7 (2008).
- 29 Walton FS, Harmon AW, Paul DS, Drobná Z, Patel YM, Styblo M. Inhibition of insulin-dependent glucose uptake by trivalent arsenicals: possible mechanism of arsenic-induced diabetes. *Toxicol. Appl. Pharmacol.* 198(3), 424–433 (2004).
- 30 Padmaja Divya S, Pratheeshkumar P, Son YO *et al.* Arsenic induces insulin resistance in mouse adipocytes and myotubes via oxidative stress-regulated mitochondrial Sirt3-FOXO3a signaling pathway. *Toxicol. Sci.* 146(2), 290–300 (2015).
- 31 Hamann I, Petroll K, Hou X, Anwar-Mohamed A, El-Kadi AO, Klotz LO. Acute and long-term effects of arsenite in HepG2 cells: modulation of insulin signaling. *Biomaterials* 27(2), 317–332 (2014).
- 32 Chakraborty D, Mukherjee A, Sikdar S, Paul A, Ghosh S, Khuda-Bukhsh AR. [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice. *Toxicol. Lett.* 210(1), 34–43 (2012).
- 33 Xue P, Hou Y, Zhang Q *et al.* Prolonged inorganic arsenite exposure suppresses insulin-stimulated AKT S473 phosphorylation and glucose uptake in 3T3-L1 adipocytes: involvement of the adaptive antioxidant response. *Biochem. Biophys. Res. Commun.* 407(2), 360–365 (2011).
- 34 Duan X, Li J, Zhang Y *et al.* Activation of NRF2 pathway in spleen, thymus as well as peripheral blood mononuclear cells by acute arsenic exposure in mice. *Int. Immunopharmacol.* 28(2), 1059–1067 (2015).
- 35 Li J, Duan X, Dong D *et al.* Hepatic and nephric NRF2 pathway up-regulation, an early antioxidant response, in acute arsenic-exposed mice. *Int. J. Environ. Res. Public Health* 12(10), 12628–12642 (2015).
- 36 Hou Y, Xue P, Woods CG *et al.* Association between arsenic suppression of adipogenesis and induction of CHOP10 via the endoplasmic reticulum stress response. *Environ. Health Perspect.* 121(2), 237–243 (2013).
- 37 Zhu XX, Yao XF, Jiang LP *et al.* Sodium arsenite induces ROS-dependent autophagic cell death in pancreatic beta-cells. *Food Chem. Toxicol.* 70, 144–150 (2014).
- 38 Wauson EM, Langan AS, Vorce RL. Sodium arsenite inhibits and reverses expression of adipogenic and fat cell-specific genes during *in vitro* adipogenesis. *Toxicol. Sci.* 65(2), 211–219 (2002).
- 39 Yadav S, Anbalagan M, Shi Y, Wang F, Wang H. Arsenic inhibits the adipogenic differentiation of mesenchymal stem cells by down-regulating peroxisome proliferator-activated receptor gamma and CCAAT enhancer-binding proteins. *Toxicol. In vitro* 27(1), 211–219 (2013).

- 40 Wang ZX, Jiang CS, Liu L *et al.* The role of Akt on arsenic trioxide suppression of 3T3-L1 preadipocyte differentiation. *Cell Res.* 15(5), 379–386 (2005).
- 41 Garg A, Agarwal AK. Lipodystrophies: disorders of adipose tissue biology. *Biochim. Biophys. Acta* 1791(6), 507–513 (2009).
- 42 Yen YP, Tsai KS, Chen YW, Huang CF, Yang RS, Liu SH. Arsenic inhibits myogenic differentiation and muscle regeneration. *Environ. Health Perspect.* 118(7), 949–956 (2010).
- 43 Park SK, Peng Q, Bielak LF, Silver KD, Peyser PA, Mitchell BD. Urinary arsenic and measures of insulin sensitivity and beta-cell function in non-diabetic Amish adults. *Diabetes Metab. Res. Rev.* 32(6), 565–571 (2015).
- 44 Del Razo LM, Garcia-Vargas GG, Valenzuela OL *et al.* Exposure to arsenic in drinking water is associated with increased prevalence of diabetes: a cross-sectional study in the Zimapan and Lagunera regions in Mexico. *Environ. Health* 10, 73 (2011).
- 45 Lu TH, Su CC, Chen YW *et al.* Arsenic induces pancreatic beta-cell apoptosis via the oxidative stress-regulated mitochondria-dependent and endoplasmic reticulum stress-triggered signaling pathways. *Toxicol. Lett.* 201(1), 15–26 (2011).
- 46 Pan X, Jiang L, Zhong L *et al.* Arsenic induces apoptosis by the lysosomal-mitochondrial pathway in INS-1 cells. *Environ. Toxicol.* 31(2), 133–141 (2016).
- 47 Yen CC, Lu FJ, Huang CF, Chen WK, Liu SH, Lin-Shiau SY. The diabetogenic effects of the combination of humic acid and arsenic: *in vitro* and *in vivo* studies. *Toxicol. Lett.* 172(3), 91–105 (2007).
- 48 Yang B, Fu J, Zheng H *et al.* Deficiency in the nuclear factor E2-related factor 2 renders pancreatic beta-cells vulnerable to arsenic-induced cell damage. *Toxicol. Appl. Pharmacol.* 264(3), 315–323 (2012).
- 49 Yao XF, Zheng BL, Bai J *et al.* Low-level sodium arsenite induces apoptosis through inhibiting TrxR activity in pancreatic beta-cells. *Environ. Toxicol. Pharmacol.* 40(2), 486–491 (2015).
- 50 Diaz-Villasenor A, Sanchez-Soto MC, Cebrian ME, Ostrosky-Wegman P, Hiriart M. Sodium arsenite impairs insulin secretion and transcription in pancreatic beta-cells. *Toxicol. Appl. Pharmacol.* 214(1), 30–34 (2006).
- 51 Douillet C, Currier J, Saunders J, Bodnar WM, Matousek T, Styblo M. Methylated trivalent arsenicals are potent inhibitors of glucose stimulated insulin secretion by murine pancreatic islets. *Toxicol. Appl. Pharmacol.* 267(1), 11–15 (2013).
- 52 Rhee SY, Hwang YC, Woo JT, Chin SO, Chon S, Kim YS. Arsenic exposure and prevalence of diabetes mellitus in Korean adults. *J. Korean Med. Sci.* 28(6), 861–868 (2013).
- 53 Diaz-Villasenor A, Burns AL, Salazar AM *et al.* Arsenite reduces insulin secretion in rat pancreatic beta-cells by decreasing the calcium-dependent calpain-10 proteolysis of SNAP-25. *Toxicol. Appl. Pharmacol.* 231(3), 291–299 (2008).
- 54 Fu J, Woods CG, Yehuda-Shnaidman E *et al.* Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: involvement of cellular adaptive response to oxidative stress. *Environ. Health Perspect.* 118(6), 864–870 (2010).
- 55 Liu S, Guo X, Wu B, Yu H, Zhang X, Li M. Arsenic induces diabetic effects through beta-cell dysfunction and increased gluconeogenesis in mice. *Sci. Rep.* 4, 6894 (2014).
- 56 Huang CF, Yang CY, Chan DC *et al.* Arsenic exposure and glucose intolerance/insulin resistance in estrogen-deficient female mice. *Environ. Health Perspect.* 123(11), 1138–1144 (2015).
- 57 Chen JW, Wang SL, Wang YH *et al.* Arsenic methylation, GSTO1 polymorphisms, and metabolic syndrome in an arseniasis endemic area of southwestern Taiwan. *Chemosphere* 88(4), 432–438 (2012).
- 58 Drobná Z, Del Razo LM, Garcia-Vargas GG *et al.* Environmental exposure to arsenic, AS3MT polymorphism and prevalence of diabetes in Mexico. *J. Expo. Sci. Environ. Epidemiol.* 23(2), 151–155 (2013).
- 59 Kuo CC, Howard BV, Umans JG *et al.* Arsenic exposure, arsenic metabolism, and incident diabetes in the strong heart study. *Diabetes Care* 38(4), 620–627 (2015).
- 60 Mendez MA, Gonzalez-Horta C, Sanchez-Ramirez B *et al.* Chronic exposure to arsenic and markers of cardiometabolic risk: a cross-sectional study in Chihuahua, Mexico. *Environ. Health Perspect.* 124(1), 104–111 (2016).
- 61 Nizam S, Kato M, Yatsuya H *et al.* Differences in urinary arsenic metabolites between diabetic and non-diabetic subjects in Bangladesh. *Int. J. Environ. Res. Public Health* 10(3), 1006–1019 (2013).
- 62 Currier JM, Ishida MC, Gonzalez-Horta C *et al.* Associations between arsenic species in exfoliated urothelial cells and prevalence of diabetes among residents of Chihuahua, Mexico. *Environ. Health Perspect.* 122(10), 1088–1094 (2014).
- 63 Dheeman DS, Packianathan C, Pillai JK, Rosen BP. Pathway of human AS3MT arsenic methylation. *Chem. Res. Toxicol.* 27(11), 1979–1989 (2014).
- 64 Diaz-Villasenor A, Cruz L, Cebrian A *et al.* Arsenic exposure and calpain-10 polymorphisms impair the function of pancreatic beta-cells in humans: a pilot study of risk factors for T2DM. *PLoS ONE* 8(1), e51642 (2013).
- 65 Pan WC, Kile ML, Seow WJ *et al.* Genetic susceptible locus in *NOTCH2* interacts with arsenic in drinking water on risk of type 2 diabetes. *PLoS ONE* 8(8), e70792 (2013).
- 66 Argos M. Arsenic exposure and epigenetic alterations: recent findings based on the Illumina 450K DNA methylation array. *Curr. Environ. Health Rep.* 2(2), 137–144 (2015).
- 67 Michailidi C, Hayashi M, Datta S *et al.* Involvement of epigenetics and EMT-related miRNA in arsenic-induced neoplastic transformation and their potential clinical use. *Cancer Prev. Res. (Phila.)* 8(3), 208–221 (2015).
- 68 Zheng XH, Watts GS, Vaught S, Gandolfi AJ. Low-level arsenite induced gene expression in HEK293 cells. *Toxicology* 187(1), 39–48 (2003).
- 69 Robbins J. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. *Pharmacol. Ther.* 90(1), 1–19 (2001).

- 70 Travers ME, Mackay DJ, Dekker Nitert M *et al.* Insights into the molecular mechanism for Type 2 diabetes susceptibility at the *KCNQ1* locus from temporal changes in imprinting status in human islets. *Diabetes* 62(3), 987–992 (2013).
- 71 Ao D, Wang HJ, Wang LF, Song JY, Yang HX, Wang Y. The rs2237892 polymorphism in *KCNQ1* influences gestational diabetes mellitus and glucose levels: a case-control study and meta-analysis. *PLoS ONE* 10(6), e0128901 (2015).
- 72 Ligthart S, Steenaard RV, Peters MJ *et al.* Tobacco smoking is associated with DNA methylation of diabetes susceptibility genes. *Diabetologia* 59(5), 998–1006 (2016).
- 73 Smith AH, Goycoalea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. *Am. J. Epidemiol.* 147(7), 660–669 (1998).
- 74 D’ippoliti D, Santelli E, De Sario M, Scortichini M, Davoli M, Michelozzi P. Arsenic in drinking water and mortality for cancer and chronic diseases in Central Italy, 1990–2010. *PLoS ONE* 10(9), e0138182 (2015).
- 75 Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J, Calderon RL. Drinking water arsenic in Utah: a cohort mortality study. *Environ. Health Perspect.* 107(5), 359–365 (1999).
- 76 Chiu HF, Chang CC, Tsai SS, Yang CY. Does arsenic exposure increase the risk for diabetes mellitus? *J. Occup. Environ. Med.* 48(1), 63–67 (2006).
- 77 Broberg K, Ahmed S, Engstrom K *et al.* Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. *J. Dev. Orig. Health Dis.* 5(4), 288–298 (2014).
- 78 Niedzwiecki MM, Liu X, Hall MN *et al.* Sex-specific associations of arsenic exposure with global DNA methylation and hydroxymethylation in leukocytes: results from two studies in Bangladesh. *Cancer Epidemiol. Biomarkers Prev.* 24(11), 1748–1757 (2015).
- 79 Pilsner JR, Hall MN, Liu X *et al.* Influence of prenatal arsenic exposure and newborn sex on global methylation of cord blood DNA. *PLoS ONE* 7(5), e37147 (2012).
- 80 Kanherkar RR, Bhatia-Dey N, Csoka AB. Epigenetics across the human lifespan. *Front. Cell. Dev. Biol.* 2, 49 (2014).