Review Articles

Arsenic and Diabetes: Current Perspectives

Short running title: Arsenic and Diabetes

Chun Fa Huang[1], Ya Wen Chen[2], Ching Yao Yang[3], Keh Sung Tsai[4], Rong Sen Yang[5], Shing Hwa Liu[6,*]

[1]School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan; [2]Department of Physiology and Graduate Institute of Basic Medical Science, College of Medicine, China Medical University, Taichung, Taiwan; [3]Department of Laboratory Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; [4]Department of Surgery, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; [5]Department of Orthopaedics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan; [6]Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan

黃俊發[1]、陳雅雯[2]、楊卿堯[3]、蔡克嵩[4]、楊榮森[5]、劉興華[6,*]

[1]中國醫藥大學中醫學院中醫系；[2]中國醫藥大學醫學院生理學科及基礎醫學科學研究所；[3]國立台灣大學醫學院檢驗醫學科；[4]國立台灣大學醫學院外科；[5]國立台灣大學醫學院骨科；[6]國立台灣大學醫學院毒理學研究所

* Author to whom correspondence: Shing-Hwa Liu, Ph.D., Institute of Toxicology, College of Medicine, National Taiwan University, Taipei 10051, TAIWAN. Tel: 886-2-23123456ext.88605; Fax:886-2-23410217; E-mail:shinghwaliu@ntu.edu.tw

* 通訊作者：劉興華教授，國立台灣大學醫學院毒理學研究所，10051 台北市仁愛路一段一號 5F，電話：(02) 23123456 ext. 88605；傳真：(02) 23410217; E-mail: shinghwaliu@ntu.edu.tw

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Abstract

Arsenic is a naturally occurring toxic metalloid of global concern. Many studies have indicated a dose-response relationship between accumulative arsenic exposure and the prevalence of diabetes mellitus (DM) in arseniasis-endemic areas in Taiwan and Bangladesh, where arsenic exposure occurs via drinking water. Epidemiological researches have suggested that the characteristics of arsenic-induced diabetes mellitus observed in arseniasis-endemic areas in Taiwan and Mexico are similar to those of non-insulin-dependent diabetes mellitus (type 2 DM). These studies analyzed the association between high and chronic exposure to inorganic arsenic in drinking water and the development of diabetes, but the effect of exposure to low to moderate levels of inorganic arsenic on the risk of diabetes is unclear. Navas-Acien and colleagues recently proposed that a positive association between total urine arsenic and the prevalence of type 2 DM in peoples exposed to low to moderate levels of arsenic. However, the diabetogenic role played by arsenic is still debated upon. An increase in the prevalence of diabetes mellitus has been observed among residents of highly arsenic-contaminated areas, whereas the findings from community-based and occupational studies in low-arsenic-exposure areas have been inconsistent. Recently, a population-based cross-sectional study showed that current findings did not support an association between arsenic exposure from drinking water at levels < 300 μg/L and a significantly increased risk of diabetes mellitus. Moreover, although the precise mechanisms for the arsenic-induced diabetogenic effect are still largely undefined, recent *in vitro* experimental studies indicated that inorganic arsenic or its metabolites impair insulin-dependent glucose uptake or glucose-stimulated insulin secretion. Nevertheless, the dose, form of arsenic used, and the experimental duration in the *in vivo* studies varied greatly, leading to conflicting results and ambiguous interpretation of these data with respect to human exposure to arsenic in the environment. Moreover, the experimental

studies were limited to the use of arsenic concentrations much higher than those relevant to human exposure. Further prospective epidemiologic studies may help to clarify this controversy. The issues about environmental exposure assessment and appropriate biomarkers should also be considered. Here, we discuss the currently available evidence and conditions for the association between environmental arsenic exposure and the development of diabetes.

Keywords: Arsenic, Diabetes, Epidemiologic studies, Experimental studies

中文摘要

砷是一種天然存在而為全球關注的有毒類金屬。許多研究指出，在台灣及孟加拉砷中毒流行地區，經由飲用水而累積暴露到砷的情形與糖尿病患病率間具有一種劑量反應關係存在。流行病學研究曾建議，在台灣和墨西哥砷中毒流行地區觀察到的砷誘導的糖尿病性狀是類似於非胰島素依賴型糖尿病（2 型糖尿病）。這些流行病學研究分析了長期接觸高量無機砷的飲用水和糖尿病發生之間的關係，但對於暴露於低到中等量無機砷對糖尿病發生的風險則是不清楚。最近 Navas-Acien 等人的研究曾建議，對於人們暴露到低至中度砷含量時，總尿砷量與 2 型糖尿病患病率之間有正向的關聯。然而，流行病學和科學性研究結果顯示砷在糖尿病發生上的角色仍有爭議。糖尿病患病率的增加已經可在高砷污染地區的居民上觀察到，但對於在低砷暴露地區的基於社區性和職業性的研究上，則有不一致的結果。最近，在一項基於人群的橫斷面的研究上，研究結果顯示並不支持飲用水砷暴露濃度在 300 μg/L 以下時，砷暴露和發生糖尿病風險之間有關聯。此外，雖然砷誘導糖尿病發生的確切機制仍然很不確定，但最近在體外實驗研究顯示無機砷及其代謝產物會損害胰島素依賴性葡萄糖吸收或葡萄糖刺激的胰島素分泌情形。然而，在嘗試以動物體內試驗來反映人類暴露於砷環境中的情形時，許多研究在劑量、砷的形式及實驗期上具有種類繁多、矛盾的結果及數據解釋上的混亂等方面的問題。體外或體內實驗性研究可

能會受限於砷濃度的使用，因為劑量的使用上明顯高於相關的人類暴露情形。進一步前瞻性流行病學研究的進行將有助於問題的釐清。關於環境暴露評估和適當的生物標誌物的問題也應考慮。在此綜論性論文，我們討論了在環境砷暴露與糖尿病發生之間的關聯性上最近有用的證據和條件。

關鍵字：砷、糖尿病、流行病學研究、實驗性研究

Introduction

Arsenic is a naturally occurring toxic metalloid of global concern. It can be found as inorganic and organic forms in the environment. Inorganic forms of arsenic, which are the predominant forms in surface and groundwater reservoirs, are more toxic than the organic forms. Arsenic can be easily solubilized in ground waters, depending on pH, redox conditions, temperature, and solution composition. Many geothermal waters contain high concentrations of arsenic. Natural arsenic in ground water at concentrations above the drinking water standard of 10 μg/L is not uncommon. Man-made sources of arsenic, such as mineral extraction and processing wastes, poultry and swine feed additives, pesticides, and highly soluble arsenic trioxide stockpiles are also not uncommon and have contaminated soil and drinking water [1, 2]. Arsenic-contaminated food is also a widespread problem worldwide [3]. It has been described that data derived from population-based studies, clinical case series, and case reports relating to ingestion of inorganic arsenic in drinking water, medication, or contaminated food or beverages show the capacity of arsenate and arsenite to adversely affect multiple organ systems [3]. An estimated 36 million people in the Bengal Delta are at a risk because of consumption of arsenic-contaminated water. The occurrence of arsenic contamination in ground water in Taiwan has been recognized for several decades [1]. Epidemiological studies have demonstrated that it was associated with chronic exposure to arsenic in drinking water and increased rates of various chronic diseases, including cancers, diseases of the nervous system, peripheral vascular disease (blackfoot disease, a peripheral artery disease), and endocrine dysfunction in the United States and other countries [4, 5]. Therefore, the United States Environmental Protection Agency (US EPA) recommended a reduction in the maximum contaminant level (MCL) from 50 μg/L to 10 μg/L for arsenic in public drinking water supplies. In Taiwan, the areas along the southwestern coast are known to have arsenic contamination in drinking wells or underground water, and hyper-endemic

occurrence of peripheral vascular disease (as blackfoot disease) is observed in the villages of these areas [5-7]. In these areas, arsenic concentrations in drinking water are measured and in the range 0.35-1.14 mg/L, with a median concentration of 0.78 mg/L, in the early 1960s [8].

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion by pancreatic β-cells and/or insulin action on peripheral tissues. From the multivariable diabetes risk score, it has been analyzed that the number of adults at a high risk of diabetes was 38.4 million in 1991 and 49.9 million in 2001 [9]. The authors also predicted the total diabetes burden to be 11.5% (25.4 million) in 2011, 13.5% (32.6 million) in 2021, and 14.5% (37.7 million) in 2031 [9]. Insulin-dependent DM (IDDM, type 1 DM) is caused by autoimmune or idiopathic destruction of the insulin-producing pancreatic β-cells, leading to a severe deficiency of insulin (hypoinsulinemia) and the elevation of blood glucose levels (hyperglycemia) [10]. Various pro-inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor α (TNF-α), interferon (IFN)-γ, and reactive oxygen species (ROS), have been found to play important roles in islet β-cell destruction. A key role played by NFκB signaling in cytokine-induced β-cell dysfunction and death was also shown [11, 12]. In addition, non-insulin-dependent DM (NIDDM, type 2 DM) is a multiorgan disease with an unknown specific etiology (although hereditary factors, ageing, and obesity are important risk factors) that involves both peripheral insulin resistance in adipose, liver, and muscle cells, and insufficient of insulin production because of pancreatic β-cell dysfunction [13]. It is estimated that approximately 90-95 % of diabetes cases are type 2 DM, whereas <10 % cases are type 1 DM and other specific types.

Many studies have indicated that there is a dose-response relationship between accumulative arsenic exposure and the prevalence of DM in the villages along the south-western coast of Taiwan, where the inhabitants are exposed to arsenic through

drinking water (0.1-15 and ＞15 mg/L every year). The incidence of diabetes in these villages was 2 to 5 times higher than that in other areas where arseniasis is non-endemic [14, 15]. Moreover, similar studies have been reported in Bangladesh, Sweden, and the United States [16-18]. Therefore, chronic exposure to arsenic implies a risk factor for diabetes mellitus in the arsenic-contaminated environments. However, the detailed effects and molecular mechanisms of arsenic-related diabetes mellitus remain unclear.

Epidemiological Research

Positive Suggestions

In 1994, Lai and colleagues first reported that chronic exposure to inorganic arsenic from drinking water may be associated with the prevalence of diabetes mellitus in the blackfoot disease-hyperendemic villages of Taiwan [14]. The authors further suggested is the presence of a dose-response relationship between cumulative arsenic exposure and the prevalence of DM; the multivariate-adjusted odds ratio was 6.61 and 10.05, respectively, for those who had a cumulative arsenic exposure of 0.1-15.0 ppm/year and greater than 15.0 ppm/year, respectively, compared with those who were unexposed [14]. Other researches showed a link between the prevalence of diabetes mellitus and the chronic consumption of ground water, which contains high levels of inorganic arsenic, and this finding was later confirmed by several cross-sectional studies from Taiwan [7, 19-21]. In Bangladesh, the crude prevalence ratio for diabetes mellitus among keratotic subjects exposed to arsenic was evaluated to be 4.4 (with a 95% confidence interval of 2.5-7.7) [17]. Nabi and colleagues further found that the prevalence of diabetes mellitus among chronic arsenic-exposed subjects in Bangladesh, where the average levels of arsenic in the drinking water and spot urine samples were 218.1 ppb and 234.6 ppb, respectively, was approximately 2.8 times higher than in the unexposed subjects [22]. In case-control data from a Swedish copper smelter, the odds ratios found for DM with

increasing arsenic exposure categories (<0.5 mg/m^3, 0.5 mg/m^3, and > 0.5 mg/m^3) were 2.0, 4.2, and 7.0 (the unstratified test for trend was weakly significant, p = 0.03), respectively [16]. In Mexico, a case-control study in an arseniasis-endemic region also found that subjects with intermediate total urinary arsenic levels (63.5-104 μg/g creatinine) were at a 2-fold higher risk of diabetes (odds ratio=2.16; 95% confidence interval 1.23-3.79), but the risk was almost 3 times greater in subjects with higher levels of total urinary arsenic (odds ratio=2.84; 95% confidence interval 1.64-4.92) [23]. Moreover, epidemiological researches have suggested that the characteristics of arsenic-induced DM in arseniasis-endemic areas in Taiwan and Mexico are similar to those of type-2 DM [19-21, 23]. These findings suggest that the ingestion of arsenic may predispose the development of diabetes mellitus in arsenic-endemic areas.

The above epidemiologic studies analyzed the association between high chronic exposure to inorganic arsenic in drinking water and the development of diabetes. However, the effect of exposure to low to moderate levels of inorganic arsenic on the risk of diabetes is unclear. Recently, Navas-Acien and colleagues reported that the odds ratio for type 2 DM was 3.58 (95% confidence interval, 1.18-10.83), when they compared participants at the 80th percentile with those at the 20th percentile for the level of total urinary arsenic (16.5 vs. 3.0 μg As/L). Therefore, the authors suggested a positive association between total urine arsenic, which reflected the inorganic arsenic exposure from drinking water and food, and the prevalence of type 2 DM in peoples with low to moderate arsenic exposure [24].

The studies of Lai et al. and Tsai et al. have indicated that an increased prevalence of diabetes in women compared with men occurred after 40 years of age in areas with high levels of inorganic arsenic in drinking water. [7, 14]. For both men and women, the prevalence of diabetes increased with age. The prevalence was slightly higher among men than among women before 40 years of age. However, the prevalence was higher

among women than among men thereafter (the age-adjusted prevalence was significantly higher in women), especially in the postmenopausal phase (> 50 years of age, i.e., women who had low or deficient estrogen levels) in areas with high levels of arsenic in drinking water [7, 14]. The study of Wang and colleagues also showed that the prevalence odds ratios of diabetes in the arseniasis-endemic areas in Taiwan, in comparison with the non-endemic areas, were consistently greater for women than for men [21]. It was also suggested that the association between arsenic exposure (in a Blackfoot disease endemic area) and DM was likely to be causal in women but not in men [25]. Chiou and colleagues showed that the prevalence of microvascular diseases significantly increased with arsenic exposure, especially at higher levels, and that the relationship was stronger in diabetic than in non-diabetic subjects. For diabetic patients, the prevalence of microvascular diseases among female subjects was greater than male subjects for all categories of the arsenic levels [5].

Weak Points and Negative Suggestions

Epidemiological and scientific results indicate that the diabetogenic role of arsenic is still debated upon. An increased prevalence of diabetes mellitus has been observed among residents of highly arsenic-contaminated areas, whereas the findings from have community-based and occupational studies in low-arsenic-exposure areas have been inconsistent [15, 26-28]. A case-reference analysis on the death records of Swedish art glass workers, who were regarded as potentially exposed to arsenic, showed that a slightly elevated risk (Mantel-Haenszel odds ratio 1.2, 95% confidence interval 0.82-1.8) for DM. This study provided limited support for the possibility that occupational arsenic exposure could play a role in the development of diabetes mellitus [26]. The reviewed article by Tseng et al. (2002) also stated that the use of weak cross-sectional or case-control study designs, the use of glucosuria or diabetes death as diagnostic criteria,

and the lack of adjustment for possible confounding variables in some studies, are major limitations that weaken the evidence for an association between arsenic exposure and diabetes mellitus in studies from Taiwan, Bangladesh, and Sweden [15]. Similarly, a systematic review by Navas-Acien et al. (2006) mentioned that the available evidence was inadequate to establish a causal role played by arsenic in diabetes. They suspected that methodologic issues limit the interpretation of the association in the studies from Taiwan and Bangladesh, and the evidence from occupational studies and from the general populations in countries other than Taiwan or Bangladesh, was inconsistent [27]. Chen and colleagues also commented that the reason for inconsistent findings of arsenic and diabetes in occupational studies may be related to the "healthy worker effect" and the variation in exposure measurement, age composition, patient number, accuracy in diagnosis, and classification of underlying causes of death, competing causes of death, and diabetes detection methods [28]. Moreover, the recent study by Kile et al. (2008) indicated that 1 of the limitations in the analysis of an association between arsenic exposure and DM has been the use of total urinary arsenic as the exposure metric. They further explained that the use of urinary arsenic as a biomarker may cause difficulty in ascertaining historical exposures that may be more relevant for the pathogenesis of type 2 DM, because urinary arsenic is a biomarker of short-term exposure with a half-life of approximately 3 days [29].

There have been no reports concerning of diabetes in populations known to be exposed to high levels of arsenic in drinking water in Chile and Argentina, although this could reflect a lack of research or be related to a publication bias [30]. Recently, Chen et al. (2010) conducted a population-based cross-sectional study using baseline data of 11,319 participants in Bangladesh to evaluate the association between well water arsenic and total urinary arsenic concentration and the prevalence of diabetes mellitus and glucosuria. The authors found that more than 90% of the cohort members were exposed

to drinking water with an arsenic concentration < 300 μg/L and observed no association between arsenic exposure and the prevalence of diabetes and glucosuria: there is no evidence of an association between well water arsenic concentration, total urinary arsenic, or the composition of urinary arsenic metabolites with glycosylated hemoglobin (HbA1c) levels [31].

Basic Research

In Vitro Experimental Studies

Insulin, a metabolic hormone produced and secreted by the pancreatic islet β-cells, triggers the principal responses to lower blood glucose level by stimulating the uptake of glucose into skeletal muscle and peripheral adipose tissue as well as suppressing gluconeogenesis and glycogenolysis in the liver. Insulin insufficiency causes deleterious effects on glucose homeostasis, involved in the pathophysiological processes of type 1 and type 2 DM [32]. In physiologically, glucose transport into the pancreatic β-cells could induce insulin secretion. The signaling transduction pathway begins with the entrance of glucose into the cell via a transporter followed by glycolysis and leads to the production of ATP production, which, in turn, closes the ATP-sensitive potassium channel and depolarizes and opens the voltage-dependent calcium channel present in the cell membrane. A calcium flux through the opened channels finally triggers exocytosis of insulin from the β-cells [33, 34]. On the other hand, oxidative stress is induced under diabetic conditions through various pathways, including the electron transport chain in mitochondria and the nonenzymatic glycosylation reaction, and is likely to be involved in the progression of the pancreatic β-cell dysfunction that develops in diabetes [35]. Pancreatic β-cells are the most vulnerable to oxidative stress-induced damage because they have lower levels of anti-oxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase [36]. Superoxide has been suggested to impair of

glucose-stimulated insulin secretion in pancreatic β-cells in which endogenous superoxide was released from the mitochondria [37]. If excess oxidative stress is produced in the pancreatic β-cells, it may be expected to impair insulin secretion.

Although the cellular and molecular mechanisms by which arsenic induces its diabetogenic effect are still largely undefined, recent *in vitro* experimental studies indicate that inorganic arsenic or its metabolites impair insulin-dependent glucose uptake or glucose-stimulated insulin secretion. Table 2 presents several *in vitro* studies regarding the effects of arsenic on insulin-dependent glucose uptake or glucose-stimulated insulin secretion published in past 5 years. The study by Diaz-Villasenor et al. (2006) showed that the incubation of isolated rat pancreatic islets with a subtoxic concentration of arsenite (5 μM) for 72 hours significantly inhibited glucose-stimulated insulin secretion and mRNA expression [38]. Diaz-Villasenor et al. (2008) further demonstrated that subchronic low levels of arsenite (0.5-2 μM) impaired insulin secretion by decreasing the oscillation of intracellular free Ca^{2+}, thus reducing calcium-dependent calpain-10 partial proteolysis of the synaptosomal-associated protein of 25 kDa (SNAP-25, a member of the insulin secretory machinery) [39]. Yen et al. (2007) also showed that arsenic trioxide (As_2O_3, 1-10 μM) induced the dysfunction of insulin secretion, which may be mediated by oxidative stress in pancreatic β-cells [40]. Interestly, a recent study by Fu et al. (2010) showed that the exposure of pancreatic β-cells to low levels of arsenite (0.05-0.5 μM) impaired glucose-stimulated insulin secretion [41]. The authors further suggested that nuclear factor-erythroid 2-related factor2 (Nrf2) activation and the induction of antioxidant enzymes in response to arsenic exposure impedes ROS signaling involved in glucose-stimulated insulin secretion and thus disturbed β-cell function [41]. These findings suggested that arsenic contributes to the development of diabetes mellitus by impairing pancreatic β-cell functions, particularly insulin synthesis and secretion.

Insulin-stimulated glucose uptake by peripheral tissues is a crucial process

responsible for the regulation of postprandial blood glucose levels. Disruption of glucose homeostasis can involve impaired glucose utilization and/or insulin resistance by adipose tissue and skeletal muscle [42]. It has been indicated that arsenicals can alter signal transduction factors, including p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K) and its downstream signals 3-phosphoinositide-dependent kinase-1 (PDK-1) and PI3K-dependent phosphorylation of protein kinase B (PKB/Akt), tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and NFκB, to affect insulin-stimulated glucose uptake in adipocytes or skeletal muscle cells [43, 44]. Disruption of insulin-stimulated glucose uptake has been suggested to be the response to chronic arsenic exposure for potential mechanism to develop the type 2 DM. Phenylarsine oxide, an aromatic derivative of trivalent arsenic, has been shown to inhibit insulin-stimulated glucose transport in adipocytes, which may be associated with inhibition of phosphorylation of endogenous phosphoproteins (p24 and p240) [45-47]. Later reports demonstrated that phenylarsine oxide inhibits the insulin-stimulated glucose transporter (GLUT) 4 translocation and triggers GLUT4 degradation in adipocytes [48, 49]. Moreover, the recent study by Scott et al. (2009) found that phenylarsine oxide stimulates glucose uptake at low concentrations (3 μM, 30 min), but inhibits glucose uptake at higher concentration (40 μM) in L929 fibroblast cells, which express only GLUT1 [50]. On the other hand, insulin-stimulated p38 MAPK phosphorylation has been shown to increase GLUT4 translocation, resulting in an increase in glucose uptake [51, 52]. Akt (PKB) signaling expression is also one of the key steps in the activation of GLUT4 and its translocation to the cellular membrane in response to insulin [53, 54]. Several studies have found that exposure to high levels of arsenicals (phenylarsine oxide or arsenite) can stimulate basal glucose uptake (insulin-independent) in adipocytes and skeletal muscle cells [55-58]. These effects of toxic concentrations of arsenicals were associated with the activation of p38 MAPK or PI3K/Akt-mediated signal pathways [55,

56, 58-60]. A study by Paul et al. (2007) demonstrated that short-term exposure to arsenite and methylarsonous acid significantly inhibited insulin-stimulated glucose uptake in adipocytes via a PDK-1/PKB/Akt-mediated transduction pathway [61]. Yen et al. (2010) also found that low doses of arsenic (As_2O_3, 0.1-0.5 μM) inhibited myogenic differentiation and muscle regeneration via a PKB/Akt-related signaling pathway [62]. These findings suggested that arsenic contributes to the development of diabetes mellitus or insulin resistance by impairing insulin-stimulated glucose uptake.

In Vivo Experimental Studies

Previous studies have investigated the alterations of blood glucose and insulin levels in goats, rats, and mice treated with arsenite or arsenate via food, drinking water, or intraperitoneal injection [63-67]. However, the dose, form of arsenic used, and the experimental duration in these studies varied greatly, leading to conflicting results and ambiguous interpretation of the data with respect to human exposure to arsenic present in the environment. Table 1 shows the potential responses for diabetogenic effect associated with chronic exposure to arsenic in animals published in the past 5 years. These studies examined blood glucose or insulin levels in rats or mice after exposure to inorganic arsenic via drinking water, oral gavage, or intraperitoneal injection. Blood glucose and liver glycogen level were decreased in rats exposed to 5.55 ppm arsenite by intraperitoneal injection for 30 consecutive days [68, 69]. Izuierdo-Vega et al. (2006) found that hyperglycemia, hyperinsulinemia, low insulin sensitivity, elevated homeostasis model assessment of insulin resistant (HOMA-IR), and increased pancreatic lipid peroxidation were induced by oral administration of sodium arsenite to rats at 1.7 mg/kg for 90 days [70]. The authors also suggested that subchronic exposure to inorganic arsenic induced oxidative stress and oxidative damage in the pancreas, and this could be implicated as a cause of insulin resistance [70]. Moreover, the expression of hexokinase

II in the renal cortical glomeruli was significantly up-regulated in C57BL/6 mice exposed to low levels of arsenic (10 ppb and 50 ppb) via the drinking water for 21 days; altered hexokinase II expression in the renal cortex has been demonstrated to be associated with a variety of pathological conditions, including diabetes [71]. An impaired glucose tolerance was also observed in C57BL/6 mice exposed to a high level of arsenite (50 ppm) for 8 weeks; the authors further suggested that mice are less susceptible than are humans to the arsenic-induced diabetogenic effect because of their ability to more efficiently clear arsenic or its metabolites from target tissues [72 ,73]. Yen et al. (2007) reported that plasma insulin levels were significantly decreased in ICR mice exposed to arsenic trioxide (10 ppm) in drinking water for 5-12 weeks [74]. Recently, Hill et al. (2009) showed that arsenate (9.6 mg/kg), which was administrated by intraperitoneal injection to maternal LM/Bc/Fnn mice on gestational days 7.5 and 8.5, significantly increased fasting plasma glucose and insulin levels, glucose intolerance, and HOMA-IR [75].

Limitations and Conclusions

Many epidemiological studies have indicated that exposure to arsenic from drinking water in arsenic-contaminated areas can induce DM, suggesting a possible role played by high arsenic exposure in DM, whereas the effects of exposure to lower concentrations of arsenic on diabetes are unclear. A recent study by Ana Navas-Acien et al. (2008) found a positive association between total urinary arsenic and the prevalence of type 2 DM in a population in the United States exposed to moderate levels of arsenic [24]. However, urinary arsenic may not be an appropriate biomarker to ascertain historical exposures for the pathogenesis of type 2 DM3. Moreover, the events of arsenic toxicity and arsenic-induced altered xenobiotic metabolism and excretion may also be important limitations for this analysis [29]. Recently, the findings by Chen et al. (2010) did not support an association between arsenic exposure through drinking water at levels of <

300 μg/L and a significantly increased risk of DM [31]. Therefore, further prospective epidemiologic studies may help to clarify the controversy. The issues about environmental exposure assessment and appropriate biomarkers should also be considered.

The experimental studies were limited to the use of arsenic concentrations much higher than those relevant to human exposure. The current US EPA recommended standard for arsenic in drinking water is 10 ppb. The concentrations of inorganic arsenic (arsenite) used in studies of glucose uptake in cultured cells were 400-750,000 ppb, and the concentrations of arsenite in *in vivo* studies of glucose metabolism were 5,000-100,000 ppb [27]. Nevertheless, several recent studies used low levels of arsenic in the *in vitro* (0.05-0.5 μM) and *in vivo* (10 and 50 ppb) experiments (Table 1). Moreover, whether arsenic through the generation of oxidative stress causes β-cell dysfunction and glucose metabolism/homeostasis, and whether chronic arsenic exposure affects the expression of the β-cell-related or glucose metabolism/homeostasis-related signaling transduction molecules, and then alters blood glucose regulation and induces diabetes is unknown. These doubts therefore also need to be clarified. Taken together, although the data from some laboratory studies support the incidence and clinical symptoms of arsenic-induced diabetes, many experimental data are presented insufficiently and inadequately to explain the epidemiologic findings. It is important to identify the appropriate cell and animal models that can mimic human-exposed conditions in arsenic-contaminated areas, and thus can clearly link arsenic exposure and diabetes.

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Disclosure of Conflicts of Interest

The authors have no relevant financial interests.

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Table 1. Arsenic: *in vitro* and *in vivo* experiments.

Main Refs.	Experimental cells/animals	Arsenic exposure	Exposure dose and duration	Results
		***In Vitro* Experiments**		
[38]	isolated islets	Arsenite	0.5-10 μM 72 or 144 h	GSIS (5 μM, 72 h) ↓ Insulin mRNA levels ↓
[39]	β-cells	Arsenite	0.5-2 μM 72 h	GSIS ↓ Intracellular free $[Ca^{2+}]i$ ↓ SNAP-25 proteolysis ↓
[40]	β-cells	Arsenic trioxide (As_2O_3)	1-10 μM 2-8 h	Insulin secretion ↓ ROS generation ↑ ATP depletion and cell apoptosis
[41]	β-cells	Arsenite	0.05-0.5 μM 96 h	GSIS ↓ Nrf2 activity ↑ Intracellular GSH ↑ Glucose-stimulated intracellular peroxide production ↓
[50]	fibroblasts	Phenylarsine oxide (PAO)	1-40 μM 30 min	Glucose uptake at low-dose PAO ↑ Glucose uptake at high-dose PAO↓
[61]	adipocytes	Arsenite MAs^{III}	50 μM 2 μM 4h	ISGU ↓ PDK-1 and Akt phosphorylation ↓
[62]	myoblasts	Arsenic trioxide	0.1-0.5 μM	Inhibition of myogenesis

				Akt phosphorylation ↓

In Vivo Experiments

[69]	Wistar rat (♂)	Arsenite (ip)	5.55 ppm 30 days	Blood glucose levels ↓ Liver glycogen ↓
[70]	Wistar rat (♂)	Arsenite (og)	1.7 mg/kg 90 days	Blood glucose levels ↑ Plasma insulin levels ↑ HOMA-IR index ↑ Low insulin sensitivity
[71]	C57BL/6 mice (♂)	Arsenite (po)	10, 50 ppb 21 days	Altered hexokinase II expression
[72]	C57BL/6 mice (♂)	Arsenite (po)	25, 50 ppm 56 days	Impaired glucose tolerance
[73]	C57BL/6 mice (♂)	Arsenite (po)	1-50 ppm 8 weeks	Impaired glucose tolerance
[74]	ICR mice (♂)	Arsenic trioxide (As_2O_3) (po)	10 ppm 5-12 weeks	decreased plasma insulin
[75]	LM/Bc/Fnn mice (♀)	Arsenate (ip)	9.6 mg/kg 2 days	Fasting plasma glucose ↑ Fasting plasma insulin ↑ HOMA-IR index ↑ Impaired glucose tolerance

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Abbreviations: ip, intraperitoneal; og, oral by gavaged; po, per oral in drinking water; HOMA-IR, the homeostasis model assessment of insulin resistant; HKII, hexokinase II; GSIS, glucose-stimulated insulin secretion; SNAP-25, synaptosomal-associated protein of 25 kDa; Nrf2, nuclear factor-erythroid 2-related factor2; GSH, glutathione; H_2O_2, hydrogen peroxide; ISGU, Insulin-stimulated glucose uptake; PDK-1, 3-phosphoinositide

dependent kinase 1.