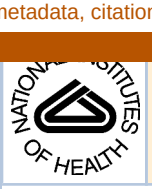

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Examination of the Effects of Arsenic on Glucose Homeostasis in Cell Culture and Animal Studies: Development of a Mouse Model for Arsenic-Induced Diabetes

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Abstract

Previous epidemiologic studies found increased prevalences of type 2 diabetes mellitus in populations exposed to high levels of inorganic arsenic (iAs) in drinking water. Although results of epidemiologic studies in low-exposure areas or occupational settings have been inconclusive, laboratory research has shown that exposures to iAs can produce effects that are consistent with type 2 diabetes. The current paper reviews the results of laboratory studies that examined the effects of iAs on glucose metabolism and describes new experiments in which the diabetogenic effects of iAs exposure were reproduced in a mouse model. Here, weanling male C57BL/6 mice drank deionized water with or without the addition of arsenite (25 or 50 ppm As) for 8 weeks. Intraperitoneal glucose tolerance tests revealed impaired glucose tolerance in mice exposed to 50 ppm As, but not to 25 ppm As. Exposure to 25 and 50 ppm As in drinking-water resulted in proportional increases in the concentration of iAs and its metabolites in the liver and in organs targeted by type 2 diabetes, including pancreas, skeletal muscle and adipose tissue. Dimethylarsenic was the predominant form of As in the tissues of mice in both 25 and 50 ppm groups. Notably, the average concentration of total speciated arsenic in livers from mice in the 50 ppm group was comparable to the highest concentration of total arsenic reported in the livers of Bangladeshi residents who had consumed water with an order of magnitude lower level of iAs. These data suggest that mice are less susceptible than humans to the diabetogenic effects of chronic exposure to iAs due to a more efficient clearance of iAs or its metabolites from target tissues.

Keywords

arsenic; arsenite; speciation; diabetes; C57BL/6; glucose tolerance

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Introduction

Arsenic (As) is a naturally occurring toxic metalloid found in the environment in both inorganic and organic forms. Inorganic As (iAs) is the predominant form of As in surface and underground water reservoirs. Drinking-water containing high levels of iAs and industrial pollution are major sources of iAs exposure for millions of people throughout the world. Countries where As levels in drinking-water have been found to exceed the World Health Organization Standard of 10 μg/L include Argentina, Australia, Bangladesh, Chile, China, Hungary, India, Mexico, Peru, Taiwan, and the United States of America (WHO, 2001). Adverse health effects associated with chronic exposure to iAs have been documented in most of these countries. It is estimated that more than 50 million people in Bangladesh (BGS, 2001) and 13 million residents of the U.S. (Focazio *et al.*, 1999) are exposed to drinking-water with iAs concentrations at or above 10 μg/L. iAs is classified as a human carcinogen (IARC, 1987). Numerous epidemiologic studies have associated chronic exposure to iAs with increased prevalence of cancers of the skin, bladder, liver, lung, and stomach (Bates *et al.*, 1995; Bates *et al.*, 1992; Chen *et al.*, 1992; Chen *et al.*, 1985; Chiang *et al.*, 1988; Chiou *et al.*, 1995; Guo *et al.*, 1997; Hopenhayn-Rich *et al.*, 1996; Hopenhayn-Rich *et al.*, 1998; Lewis *et al.*, 1999; Smith *et al.*, 1998; Smith *et al.*, 1992; Tseng *et al.*, 1968; Tsuda *et al.*, 1995; Wu *et al.*, 1989). Previous research of the effects of chronic exposure to iAs has focused primarily on its carcinogenic potential. However, chronic exposures to iAs from the environment or in occupational settings have also been linked to non-carcinogenic diseases, including peripheral vascular disease, cardiovascular and cerebrovascular diseases (Chen *et al.*, 1995; Chen *et al.*, 1996; Chiou *et al.*, 1997; Engel *et al.*, 1994; Thomas and Goyer, 1995; Tseng *et al.*, 1995; Tseng *et al.*, 1997), hypertension (Chen *et al.*, 1995), goiter (Chang *et al.*, 1991), hepatomegaly (Santra *et al.*, 1999), respiratory system dysfunction (Mazumder *et al.*, 2000), nervous system dysfunction (Bencko *et al.*, 1977; Chisolm Jr. and Thomas, 1983; Masahiko and Hideyasu, 1973), and diabetes mellitus (Lai *et al.*, 1994; Rahman *et al.*, 1998; Rahman *et al.*, 1999; Tseng *et al.*, 2000).

Epidemiologic Evidence of the Association Between Chronic Exposure to iAs and Diabetes Mellitus

Studies examining the role of iAs exposure on the development of diabetes represent a growing area of research. Diabetes refers to a group of metabolic diseases characterized by systemic disruption of glucose homeostasis. Insulin-dependent (type 1) diabetes is caused by the autoimmune destruction of insulin-producing pancreatic β-cells, resulting in a severe deficiency of circulating insulin (hypoinsulinemia) and the concomitant elevation of blood glucose (hyperglycemia). Non-insulin-dependent (type 2) diabetes is characterized by hyperglycemia due to the resistance of peripheral tissues such as adipose tissue, skeletal muscle and the liver to insulin stimulus and a relative insufficiency of insulin production by pancreatic β-cells. Gestational diabetes (type 3) is similar to type 2 diabetes with respect to its pathogenesis and clinical symptoms; however, it occurs in women during pregnancy and usually improves or disappears after child birth. The pathogenesis of arsenic-induced diabetes is unknown. Diagnosis of diabetes in populations exposed to iAs has relied primarily on measurements of fasting blood glucose, glucosuria, glycosylated hemoglobin (HbA1c), oral glucose tolerance tests, clinical history of the disease or mortality linked to diabetes. The results of these diagnoses are suggestive of insulin resistance and type 2 diabetes; however, β-cell dysfunction cannot be ruled out.

The association between iAs exposure via drinking-water and increased risk of developing type 2 diabetes was first reported in Taiwan by Lai and associates (1994). These authors found a twofold increase in the prevalence of diabetes mellitus among residents of arseniasis-endemic

areas as compared to residents in non-endemic areas. Furthermore, a significant dose-response relationship was found between cumulative exposure to iAs and the prevalence of diabetes. The link between diabetes mellitus and consumption of water containing iAs was later confirmed by several cross-sectional and cohort studies from Taiwan (Tseng *et al.*, 2000) and Bangladesh (Rahman *et al.*, 1998; Rahman *et al.*, 1999). Other studies have examined the association between exposure to iAs in occupational settings and the occurrence of symptoms that are consistent with diabetes (Jensen and Hansen, 1998; Rahman and Axelson, 1995; Rahman *et al.*, 1996). These studies were retrospective in nature and primarily used death certificates as their diagnostic criteria. It should be noted that some of the epidemiologic studies linking chronic exposures to iAs with type 2 diabetes suffer serious problems associated mainly with insufficient assessment of exposure to iAs, inadequate diagnosis of diabetes or lack of dose-response data. A critical review of these studies has recently been published by Navas-Acien and associates (2006). These authors concluded that methodological problems limit the causal interpretation of the moderately strong association between iAs exposure and diabetes in Taiwan and Bangladesh. Overall, the epidemiologic evidence was regarded as insufficient and inadequate to establish causality.

Laboratory Studies of the Effects of As on Glucose Metabolism

The effects of As on glucose metabolism have been examined by numerous laboratory studies. However, it should be noted that As has traditionally been used, along with other chemical or physical agents, as an acute stressor in research of stress-mediated cell signaling or stress-induced responses in various metabolic pathways, including the pathways of carbohydrate metabolism. Studies in this area of research have examined insulin secretion and glucose metabolism in cultured cells or laboratory animals exposed to highly toxic concentrations of As, which are incompatible with chronic exposures in humans. Therefore, data from these studies cannot be evaluated with respect to possible mechanisms of diabetes induced by environmental or occupational exposures and attempts to do so would inevitably yield confusing results (Navas-Acien *et al.*, 2006). The following is a brief contextual review of laboratory studies that have examined the effects of As on processes directly related to glucose homeostasis.

Effects on glucose uptake—Insulin-stimulated glucose uptake (ISGU) by skeletal muscle and adipose tissue is a key process responsible for the normalization of postprandial blood glucose levels. Results of early laboratory studies suggest that disruption of ISGU is a potential mechanism responsible for the development of type 2 diabetes in response to chronic exposures to iAs. In 1985, Frost and associates reported that phenylarsine oxide (PAO), a aromatic derivative of trivalent arsenic (As^{III}), is a potent inhibitor (K_i = 7 μM) of ISGU by murine 3T3-L1 adipocytes (Frost and Lane, 1985). Micromolar concentrations of PAO have also been reported to inhibit ISGU by isolated rat skeletal muscle (Henriksen and Holloszy, 1990; Sowell *et al.*, 1988). Later reports have shown that the inhibition of ISGU in 3T3-L1 adipocytes treated with PAO is associated with hypophosphorylation of two endogenous phosphoproteins (p24 and p240), possible components of the insulin-stimulated signal transduction pathway (Frost and Lane, 1985). Notably, PAO does not interfere with the insulin-dependent phosphorylation of the insulin receptor or interact directly with glucose transporters (Frost *et al.*, 1987; Frost and Lane, 1985).

The effects of PAO and other arsenicals on basal (insulin independent) glucose uptake have also been examined by several studies that focused mostly on stress-induced responses in cultured cells. These studies used high concentrations of arsenicals that are cytotoxic for most cell types. Here, PAO (50 μM) or iAs^{III} (200–1000 μM) have been shown to stimulate basal, insulin-independent, glucose uptake in baby hamster kidney (BHK) cells (Pasternak *et al.*, 1991; Sviderskaya *et al.*, 1996; Warren *et al.*, 1986; Widnell *et al.*, 1990), bovine chromaffin

cells (Fladeby and Serck-Hanssen, 1999), 3T3-L1 adipocytes (Bazuine *et al.*, 2004; Bazuine *et al.*, 2003; Gould *et al.*, 1989) and in L6 myotubes (McDowell *et al.*, 1997). A modest 1–3 fold increase in basal glucose uptake was typically found with no effect on the translocation of GLUT4, an insulin-sensitive glucose transporter, to the plasma membrane. Only one study showed an increased presence of GLUT4 at the plasma membrane of BHK cells in response to treatment with PAO (35 μM) (Widnell *et al.*, 1990). In contrast, two studies reported inhibition of basal glucose uptake in Madin-Darby canine kidney (MDCK) cells treated with 25 μM PAO or 1000 μM iAs^{III} (Liebl *et al.*, 1992) and in rat tubule kidney (RTK) cells treated with 2 μM PAO (Liebl *et al.*, 1995). The increase in basal glucose uptake by toxic concentrations of PAO or iAs^{III} was associated with the activation of a stress response, p38 MAPK-mediated signal transduction pathway in several studies (Bazuine *et al.*, 2004; Bazuine *et al.*, 2003; Fladeby and Serck-Hanssen, 1999). Phosphorylation of p38 MAPK has been shown to increase intrinsic activity of GLUT4 transporters already present at the plasma membrane, resulting in increased glucose uptake by cells (Somwar *et al.*, 2002; Sweeney *et al.*, 1999). In several studies, toxic concentrations of iAs^{III} were used explicitly for their capacity to activate the p38 MAPK pathway and to induce acute stress.

Data from previous studies indicate that an alternative mechanism for increased basal glucose uptake by cells exposed to high concentrations of trivalent arsenicals may involve the activation of phosphatidylinositol-3-kinase (PI-3K) and PI-3K-dependent phosphorylation of protein kinase B (PKB/Akt). Increased PI-3K-mediated PKB/Akt phosphorylation has been reported in cells exposed to toxic concentrations (200–500 μM) of iAs^{III} (McDowell *et al.*, 1997; Souza *et al.*, 2001). Stress-induced phosphorylation of PKB/Akt is associated with the activation of pro-survival mechanisms aimed at preventing apoptosis and promoting cell proliferation (Dudek *et al.*, 1997; Ibuki and Goto, 2000; Zhou *et al.*, 2000). However, PKB/Akt phosphorylation is also one of the key steps in the activation of GLUT4 transporters by insulin (Kohn *et al.*, 1996; Tanti *et al.*, 1997). Thus, exposure to toxic concentrations of trivalent arsenicals may mimic the action of insulin by activating the p-PKB/Akt-mediated glucose transport in cells expressing GLUT4.

Taken together, studies examining glucose transport in cell culture systems suggest that acute stress induced by exposures to toxic concentrations of trivalent arsenicals is associated with p38- or p-PKB/Akt-mediated increase in basal, insulin-independent, glucose uptake. In contrast, exposures to low micromolar concentrations of PAO inhibit ISGU. It should be noted that PAO is not a metabolite of iAs and its chemical properties and metabolic fates differ from those of iAs or its methylated metabolites. Therefore, while studies using subtoxic concentrations of PAO may provide valuable insights into the diabetogenic effects of chronic exposures to iAs, the significance of these studies for evaluation of the molecular mechanisms underlying effects of iAs or its metabolites is limited.

The effects of physiologically relevant trivalent arsenicals, the known metabolites of iAs, on ISGU and insulin-activated signal transduction have been examined in this laboratory. Here, dose-dependent decreases in ISGU were observed in 3T3-L1 adipocytes exposed for 4 or 24 h to subtoxic concentrations of iAs^{III}, or the methylated metabolites of iAs, methylarsonite (MAs^{III}) and dimethylarsinite ($DMAs^{III}$) (Walton *et al.*, 2004). We have recently shown that two of these arsenicals, iAs^{III} or MAs^{III}, inhibit the activity of 3-phosphoinositide dependent kinase 1 (PDK-1) and the subsequent PDK-1-catalyzed phosphorylation of PKB/Akt (Paul *et al.*, submitted). Notably, $DMAs^{III}$ inhibited GLUT4 translocation in insulin-activated adipocytes by a mechanism independent of PKB/Akt phosphorylation. Results from other laboratories suggest that metabolites of iAs may also interfere with glucose metabolism by inhibiting α-ketoglutarate dehydrogenase or pyruvate dehydrogenase (Boquist *et al.*, 1988; Petrick *et al.*, 2001), two rate limiting enzymes involved in the oxidative metabolism of

carbohydrates. However, it is unclear whether concentrations of iAs metabolites in tissues of chronically exposed individuals can reach levels necessary for inhibition of these enzymes.

Effects on β-cell function—Compared with data on glucose metabolism and insulin signaling in mammalian cells exposed to trivalent arsenicals, much less information is available on the effects of arsenicals on insulin production by β-cells. Insulin is a metabolic hormone produced and secreted by β-cells in response to elevated blood glucose concentrations and is responsible for the stimulation of glucose uptake by peripheral adipose and skeletal muscle tissues as well as the suppression of gluconeogenesis in the liver. Insulin insufficiency has deleterious effects on glucose homeostasis and contributes to the pathogenesis of type 1 and type 2 diabetes. Several studies have examined the effects of arsenicals on pancreatic/duodenal homeobox-1 (PDX-1, also identified as insulin upstream factor 1), a transcription factor that binds to the promoter of the preproinsulin gene in response to elevated blood glucose concentrations. PDX-1 DNA binding was found to be increased in isolated human islets and MIN6 cells, a mouse β-cell line, treated with iAs^{III} for 30 min (Macfarlane *et al.*, 1997). In addition, iAs^{III} was found to promote PDX-1 activation and translocation to the nucleus, which is a critical step in the stimulation of preproinsulin mRNA transcription (Elrick and Docherty, 2001; Macfarlane *et al.*, 1999). However, both these studies used a toxic (1 mM) concentration of iAs^{III} to induce stress-activated p38 MAPK, which is believed to play a role in regulating insulin production in response to glucose stimulus. In contrast with the stimulatory effects of millimolar iAs^{III}, toxic (5 mM) concentration of a pentavalent iAs, arsenate (iAs^{V}), has been shown to disrupt insulin secretion by interfering with pancreatic islet respiration (Ortsater *et al.*, 2002). Because of the acute nature of the exposure, the significance of these findings for chronic environmental exposures is questionable. The effects of low micromolar concentrations of iAs^{III} on β-cell function have recently been examined (Diaz-Villasenor *et al.*, 2006). The authors reported that exposures to a subtoxic (5 μM) concentration of iAs^{III} for 72 h inhibits glucose-stimulated expression of preproinsulin and insulin secretion by isolated rat pancreatic islets. However, because DNA damage was used in this study as an indicator of cytotoxicity, the actual effect of this exposure on cell viability is unclear.

Laboratory Studies in Animals

The potential mechanisms responsible for the diabetogenic effects associated with chronic exposures to iAs provided by *in vitro* studies have not yet been validated by *in vivo* experiments. Previous *in vivo* studies have examined blood glucose or insulin levels in goats, rats, or mice after exposures to iAs^{V} or iAs^{III} via food, drinking water or intraperitoneal injection (Biswas *et al.*, 2000; Cobo and Castineira, 1997; Ghafghazi *et al.*, 1980; Hughes and Thompson, 1996; Izquierdo-Vega *et al.*, 2006; Pal and Chatterjee, 2005, 2004a, b). The dose, duration and form of As used in these studies have varied greatly, producing conflicting results and confusing the interpretation of data with respect to environmental exposures to iAs in humans. The effects of iAs exposure on glucose tolerance in mice have recently been examined in this laboratory. The experimental section of this report describes the development of a mouse model for diabetes induced by chronic exposure to iAs in drinking water. The diabetogenic effects of iAs exposure are evaluated with respect to tissue retention and distribution of iAs metabolites.

Materials and methods

Chemicals

Sodium arsenite (99% pure) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium borohydride ($NaBH_4$) was from EM Science (Gibbstown, NJ). Ultrapure phosphoric acid was obtained from J.T. Baker (Phillipsburg, NJ). Sodium arsenate (96%, Sigma), monomethylarsonate (MAs^{V}), disodium salt (98%, Chem Service, West Chester, PA), dimethylarsinic acid ($DMAs^{V}$) (98%, Strem Chemicals, Inc., Newburyport, MA) and

trimethylarsine oxide ($TMAs^VO$, gift from Dr. William Cullen, UBC, Vancouver) were used as standards for speciation analysis of As in mouse tissues. All other chemicals used were the highest grade commercially available.

Animals

Four-week-old male weanling C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and housed five per cage with free access to food and drinking water (see below). All mice were housed in polycarbonate cages with corn cob bedding in the University of North Carolina Animal Facility (12 h light/dark cycle, 22 ± 1°C and humidity of 50 ± 10%), which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Animals were allowed free access to food (Lab Diet 5058, Nutrition International, Brentwood, MO) and deionized water (dH_2O). Mice in two groups (5 animals each) drank dH_2O with the addition of iAs^{III} (25 or 50 ppm As). The third group (n = 5) drank pure dH_2O. Water containing iAs^{III} was freshly prepared every 3–4 days to minimize oxidation to iAs^V. Water consumption and body weights were monitored in all exposure groups every week or two weeks, respectively.

Intraperitoneal Glucose Tolerance Test (IPGTT)

Mice were fasted 5 h prior to administration of the IPGTT. D-glucose (Sigma) was dissolved in phosphate buffered saline and administered to mice via i.p. injection (2 g/kg). Samples of whole-blood (2–3 μl each) were collected from a tail clip bleed immediately before and 15, 30, 60, 90, and 120 min after glucose injection. Blood glucose levels were measured using a Freestyle Glucose Monitoring System (Abbott Laboratories, Abbott Park, IL.)

Speciation Analysis of As

Freshly dissected tissues were aliquoted into 2.0 ml cryotubes, snap frozen in liquid nitrogen and stored at −70°C until analysis. Speciated arsenicals in tissues were determined by automated hydride generation-atomic absorption spectrometry (HG-AAS) coupled with a cryotrap. Tissues were digested overnight in 2 M ultrapure phosphoric acid (90°C) (Hughes *et al.*, 2005). The digestion converts all trivalent arsenicals to pentavalency. Phosphoric acid in digested samples was neutralized by NaOH. L-cysteine was added to each sample at a final concentration of 2%. For analysis of $TMAs^VO$, samples were analyzed without cysteine (Devesa, 2004). Arsines were generated from a 500 μl aliquot of the digested tissue, which was injected into a flow of deionized water continuously mixed with a flow of 0.75M TRIS-HCl buffer (pH 6) and a flow of 1% $NaBH_4$ in 0.1% NaOH/0.02% antifoam B silicone emulsion, all at the rate of 1 ml/min. Arsines were cryotrapped and separated by boiling points, as previously described (Devesa, 2004). The content of As in arsines was determined using a Perkin-Elmer model 5100 atomic absorption spectrometer equipped with a quartz multiatomizer (Matousek *et al.*, 2002). Under these conditions, this method routinely resolves arsines generated from iAs^V, MAs^V, $DMAs^V$, and $TMAs^VO$. Five concentrations (0.05, 0.25, 0.5, 1 and 2.5 ng/ml) of each of these arsenicals were used to prepare calibration curves. Arsenicals in tissue samples were identified by spiking with appropriate As standards at several concentrations. The concentration of total speciated As for each tissue sample was calculated as the sum of concentrations of iAs^V, MAs^V, $DMAs^V$, and $TMAs^VO$. In order to determine the recovery of As during the HG-AAS analysis, the total As in selected tissues was analyzed by graphite furnace (GF) AAS using a Perkin-Elmer model 5100 atomic absorption spectrometer with an autosampler. For this analysis, tissues were microwave digested (CEM, Model MARS 5) following the 3052 EPA method with some modifications. Briefly, 0.1 g of tissue was completely digested in 70% nitric acid in a total volume of 10 ml. A 1-ml aliquot of each digestate was diluted with 20% nitric acid to a final volume of 5 ml. A 40 μl aliquot of this solution was injected into the GF along with 10 μl of a chemical modifier (5 μg of

palladium and 5 μg of magnesium nitrate in 2% nitric acid). The GF program included a drying step at 130° C for 40s, ashing step at 1300° C for 40s, and atomization step at 2300° C for 3s. The recovery of As was calculated as the total speciated As concentration (determined by HG-AAS) divided by the total As concentration (determined by GF-AAS) for each tissue. HG-AAS was also used for analysis of As species in all lots of the laboratory diet used in this study.

Results

Water Consumption and Body Weights

Water consumption by mice in each exposure group was measured twice a week throughout the course of the study. An initial decline in water intake, possibly indicative of an acclimation period, was noted for all groups (Fig 1A). However, water consumption stabilized by week 2 for the 25 ppm and 50 ppm groups, and by week 3 for the control group. Control mice consumed an average of 5.0 ml of water per day (ml/d). Mice in the 25 ppm and 50 ppm group consumed significantly less water: 3.8 ml and 2.5 ml per day, respectively (Fig 1B). Average daily As intake in drinking water per mouse was estimated based on the water consumption. Mice in the 25 ppm group ingested 94.7 μg of As/day via drinking water while mice in the 50 ppm group ingested 125.3 μg of As/day (Fig 1C). The laboratory diet was a minor source of As. The total As concentration in several lots of the diet ranged from 19.5 to 28.6 ng/g. Notably, iAs was the main As species, accounting for 70 to 80% of the total As in the diet. Body weights of mice in each group were measured prior to exposure and every two weeks thereafter until the conclusion of the study (Fig 2A). Although weight gains were not significantly different between groups at 8 weeks, mice in the 50 ppm group appeared to stop gaining weight between weeks 4 and 6. However, this trend did not reach statistical significance. Mice in the control group gained in average 7.3 g through week 8 (Fig 2B) as compared to 7.5 g and 5.3 g for mice in the 25 ppm and 50 ppm groups, respectively (Fig 2B). Additionally, no significant differences were noted in liver weights between experimental groups (data not shown). No obvious signs of pathology were noted in dissected tissues.

Effect of iAs exposure on glucose tolerance

To determine the effects of iAs^{III} ingestion on glucose tolerance, mice in all exposure groups were subjected to IPGTT (Fig 3). No significant differences in fasting blood glucose concentrations were noted prior to glucose administration. All groups exhibited the characteristic rapid rise in blood glucose within 15–30 min of glucose challenge, followed by a gradual decrease in blood glucose concentrations that began 30 min after injection and approached baseline levels by 120 min. The 50 ppm group experienced the greatest increase in blood glucose concentration, reaching 24.6 mmol/L 15 min post injection, which was significantly higher than the peak blood glucose concentration of 16.9 mmol/L in the control group. Blood glucose levels in the 50 ppm group remained significantly higher than those of control mice at 30 and 60 min after injection. No significant differences were noted between control and 50 ppm groups at 90 and 120 min post injection or between control and 25 ppm groups anytime during the IPGTT.

Concentrations of As species in mouse tissues

Traces of arsenicals were detected in tissues of control mice that were exposed only to As from the diet. The concentrations of total speciated As in adipose tissue, pancreas, skeletal muscle, and liver increased proportionally with the intake of iAs from drinking water (Fig 4). The concentrations of total speciated As were 1.8- to 3.7-fold higher in tissues of mice in the 50 ppm group as compared to mice in the 25 ppm group. For both groups, the highest concentrations of total speciated As were found in the liver and the lowest in adipose tissue. The results of As speciation in tissues from mice in all three experimental groups are shown in Figure 5. Notably, tissues of control mice contained almost exclusively iAs (Fig 5A). In this

group of mice, the highest levels of iAs^V were found in adipose tissue, followed by skeletal muscle, liver and pancreas. In contrast, tissues of mice from the 25 ppm (Fig 5B) and 50 ppm groups (Fig 5C) contained iAs^V, MAs^V and $DMAs^V$. $TMAs^VO$ was not detected in tissues of mice in either the 25 or 50 ppm groups. $DMAs^V$ was the predominant As species in all tissues collected from mice in these groups. However, in the liver a significant fraction of As was represented by iAs^V: 39.8 and 29.4% for the 25 and 50 ppm groups, respectively. To examine the recovery of As during the HG-AAS analysis, total As content was determined in livers and skeletal muscle from mice in the 50 ppm group, using GF-AAS. Based on the comparison of the HG-AAS and GF-AAS data, the average recovery of As was 110% for skeletal muscle and 105% for the liver. Because of limited access to GF-AAS, total As concentration was not measured in pancreas or adipose tissue.

Discussion

Impaired glucose tolerance, an early indicator of insulin resistance and diabetes mellitus, signifies the inability of peripheral tissues to perform glucose uptake at rates that are sufficient to prevent excessive post-prandial blood glucose elevations. Previous reports on the effects of As exposure on glucose homeostasis and *in vivo* insulin and carbohydrate metabolism in laboratory animals have been inconsistent due in part to variations in choice of animal species (mice, rats, goats) and arsenicals (iAs^{III}, iAs^V, MAs^{III}, and MAs^V), as well as the route (i.p. vs. p.o.), concentration (0.025 ppm – 1,300 ppm), and duration (7 days – 2 years) of exposure. The present study introduces a viable mouse model to investigate *in vivo* diabetogenic effects of chronic exposures to iAs in drinking-water. We chose the C57BL/6 mouse strain for this study because of its low baseline occurrence of type 2 diabetes but high susceptibility to the development of diet-induced type 2 diabetes (Petro *et al.*, 2004; Surwit *et al.*, 1995; Surwit *et al.*, 1988). Our results show that 8-week exposure of C57BL/6 mice to 50 ppm iAs^{III} in drinking-water promotes impaired glucose tolerance, which is consistent with diabetes mellitus. However, the concentration of iAs in drinking water needed to produce this effect is an order of magnitude higher than iAs concentrations shown to produce arseniasis symptoms, including diabetes, in humans. For example, in arseniasis-endemic areas of Bangladesh, the concentration of iAs in drinking water can reach 3.4 ppm (Alam *et al.*, 2002). Liver samples from local residents who developed hepatomegaly as a result of drinking water with 0.22 to 2 ppm iAs contained from 500 to 6,000 μg As/kg dry weight (Mazumder, 2005). This corresponds to approximately 100 to 1,200 μg As/kg of intact liver. In our studies, similar concentrations of total speciated As were found in livers of mice drinking water with 25 and 50 ppm iAs^{III}: 423 and 1165 μg As/kg, respectively. Results of an independent study carried out in this laboratory showed that livers of mice exposed to 1 or 10 ppm iAs^{III} in drinking water for 8 weeks contained on average only 11 and 155 μg As/kg, respectively (Paul *et al.*, unpublished data). These data suggest that mice metabolize iAs and clear iAs metabolites from tissues more efficiently than humans and that significantly higher exposure levels or longer exposure times are needed in mice to produce symptoms of chronic As toxicity found in humans.

It should be noted that the concentrations of iAs metabolites in tissues of mice in the 25 ppm and 50 ppm groups (Fig. 5B,C) were not in proportion with the corresponding estimated intakes of iAs from drinking water (Fig. 1C). The tissue concentrations of iAs metabolites in the 50 ppm group were several fold greater than in the 25 ppm group, despite a relatively small difference in iAs intake. This may be, in part, due to significantly lower water consumption by mice in the 50 ppm as compared to the 25 ppm group. (Fig. 1A,B). The smaller amount of water consumed daily by mice in the 50 ppm group would result in a decreased urine production and possibly, in a less efficient excretion of iAs metabolites in the urinary tract. Thus, the profound increase in tissue retention of iAs metabolites in the 50 ppm as compared to the 25 ppm group may be a combined effect of the increased iAs intake and the impaired clearance

of iAs metabolites due to lower water consumption. The disproportional increase in the tissue concentrations of iAs metabolites in the 50 ppm group may explain why mice in this group developed impaired glucose tolerance while mice in the 25 ppm group exhibit normal pattern for glucose utilization.

As shown in Fig. 1, mice in both 25 and 50 ppm groups consumed significantly less water than did control mice. Although mice are generally resistant to a prolonged dehydration (Haines *et al.*, 1978), it is unclear if the decreased water intake could directly contribute to the impaired glucose tolerance in mice exposed to 50 ppm iAs. The potential role of dehydration in modulating glucose metabolism and insulin sensitivity has been the subject of several studies. *In vitro* studies have suggested that dehydration on the cellular level may disrupt insulin signaling and glucose metabolism via mechanisms related to cell volume (Schliess and Haussinger, 2003, 2000). However, a recent study suggests that dehydration does not significantly impair insulin sensitivity or glucose metabolism in human subjects (Keller *et al.*, 2003). With no clear consensus regarding the effects of dehydration on glucose tolerance, future studies should address this potential confounding factor when examining the effects of chronic exposures to iAs or other arsenicals in drinking water.

Although the present study shows that mice exposed to iAs in drinking water develop impaired glucose tolerance, the mechanisms underlying this outcome remain unclear. We have previously shown that trivalent arsenicals, the metabolites of iAs, are potent inhibitors of insulin-stimulated glucose uptake in cultured adipocytes (Paul *et al.*, submitted; Walton *et al.*, 2004). Subtoxic concentrations of iAs^{III} and MAs^{III} inhibited the insulin-dependent phosphorylation of PKB/Akt by PDK-1 and p-PKB/Akt-dependent translocation of GLUT4 to the plasma membrane. In contrast, $DMAs^{III}$ inhibited GLUT4 translocation by a PKB/Akt-independent mechanism. Thus, it is plausible that the same mechanisms are responsible for impaired glucose tolerance in mice exposed to iAs in drinking water. However, inhibition of insulin production in pancreatic β-cells by iAs or its metabolites may contribute to the overall diabetogenic effects of iAs exposure. Future laboratory studies will clarify whether the impaired glucose tolerance results from the inhibition of insulin signaling and/or decreased insulin production by β-cells in this mouse model. Evaluation of these mechanisms with respect to tissue concentrations and the level of exposure to iAs and its metabolites will help to improve designs for future epidemiologic studies examining the association between the exposure to iAs in drinking water, the individual pattern of iAs metabolism, and the risk of developing diabetes mellitus.

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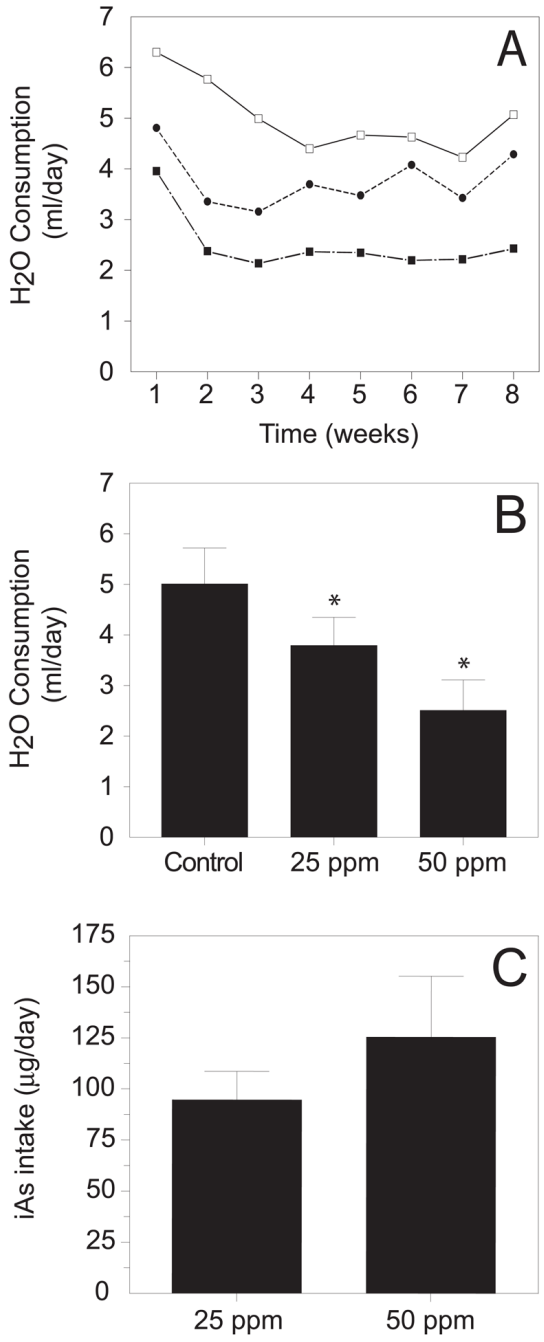

Fig. 1.

Water consumption by mice in the treatment groups: (A) Changes in the daily water consumption by mice exposed to 25 ppm As (●) and 50 ppm As (■) and by control mice (□). (B) The average daily consumption of water by mice in the control, 25 ppm, and 50 ppm groups. (C) Estimated average intake of As by mice in the 25 ppm and 50 ppm groups. (Mean and SD, n = 5.) *Value is significantly different ($P < 0.05$) from that in the control group.

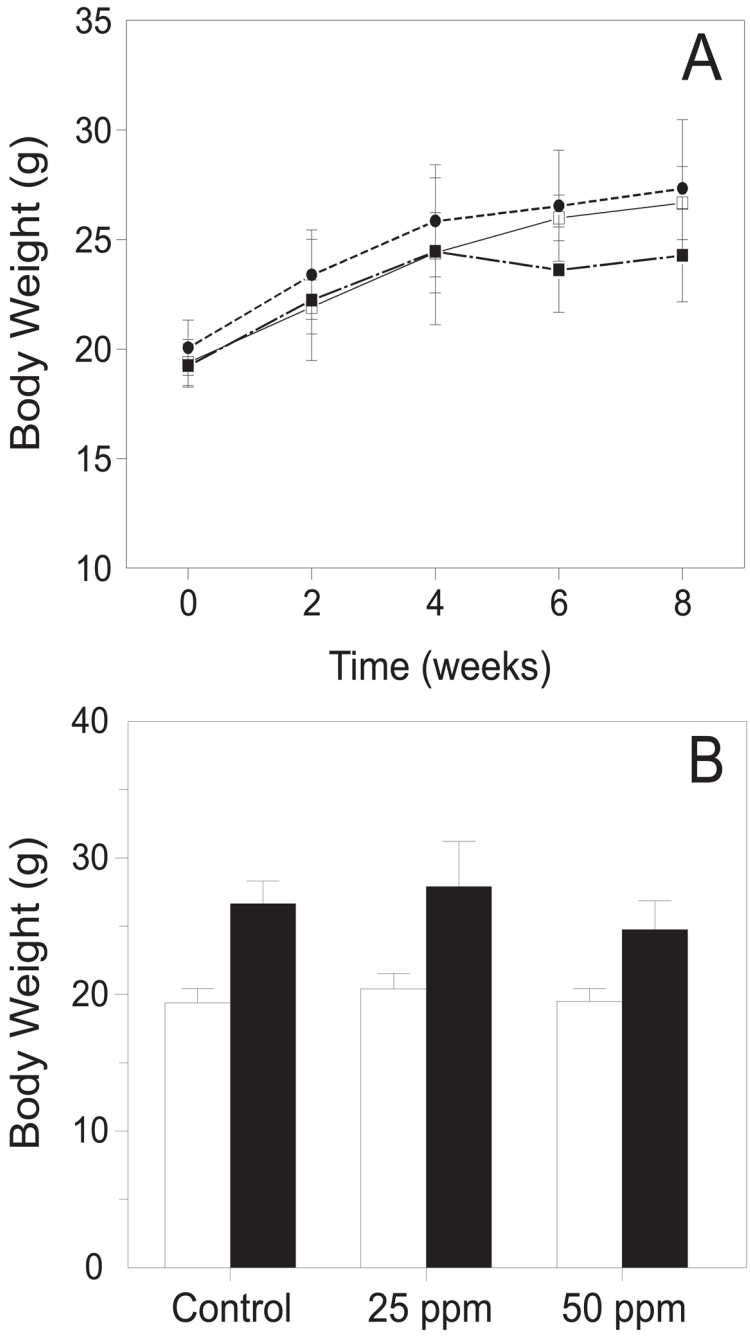

Fig. 2.

Body weights of mice in the treatment groups: (A) Changes in the body weights of mice exposed to 25 ppm As (●) and 50 ppm As (■) and control mice (□) (Mean ± SD, n = 5). (B) The average body weights of mice in the control, 25 ppm, and 50 ppm groups at the beginning (□) and the end (■) of the study. (Mean and SD, n = 5.)

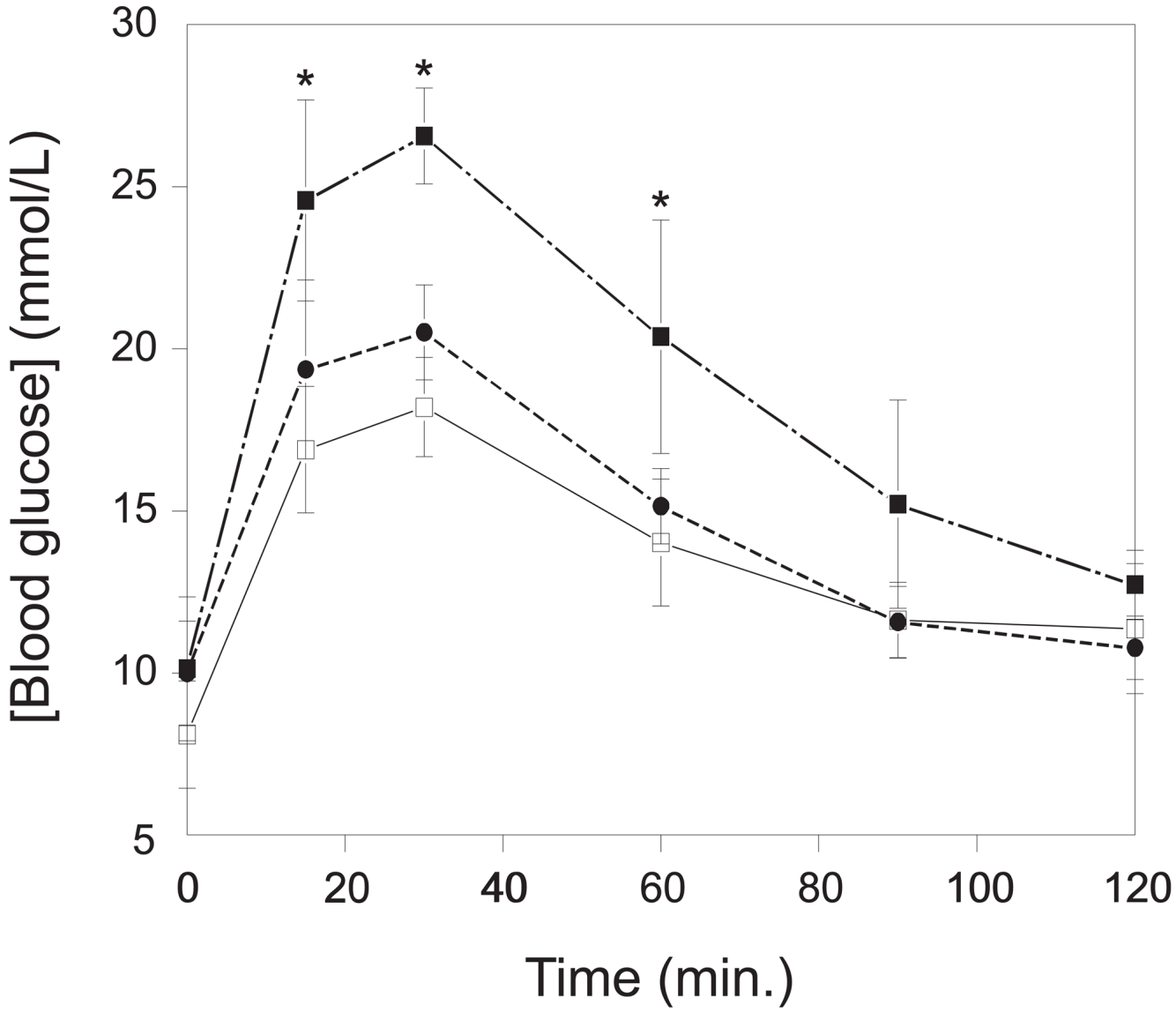

Fig. 3.

Glucose concentrations in the blood of mice before and during the intraperitoneal glucose tolerance test: Mice exposed to 25 ppm As (●) and 50 ppm As (■) and control mice (□). (Mean ± SD, n = 5.) *Value is significantly different ($P < 0.05$) from that in the control group.

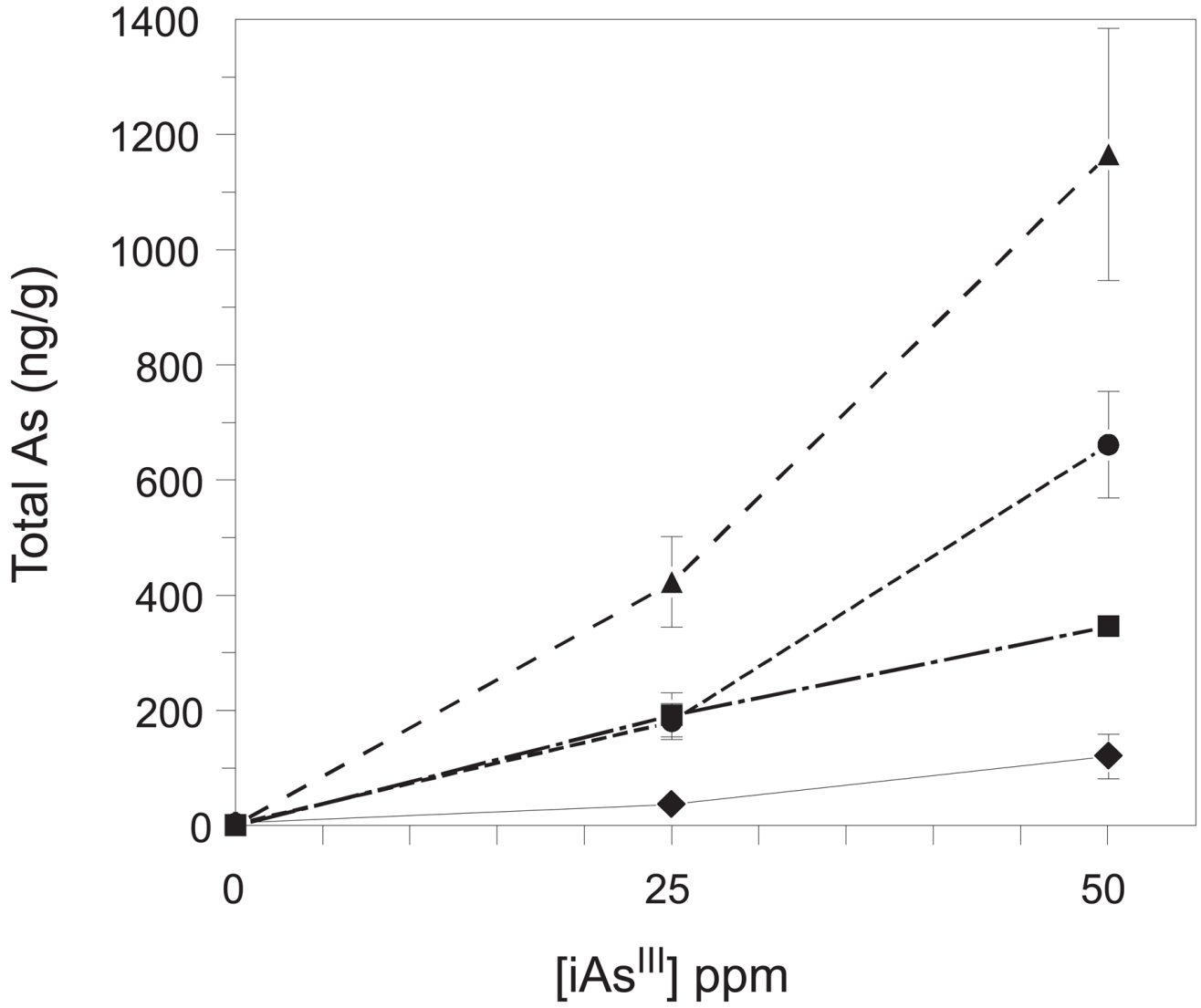

Fig. 4.

Dose-dependent increases in the total speciated As ($iAs^V + MAs^V + DMAs^V$) levels in adipose tissue (◆), pancreas (■), skeletal muscle (●), and liver (▲) of mice exposed to 25 ppm and 50 ppm As. (Mean ± SD, n = 5.)

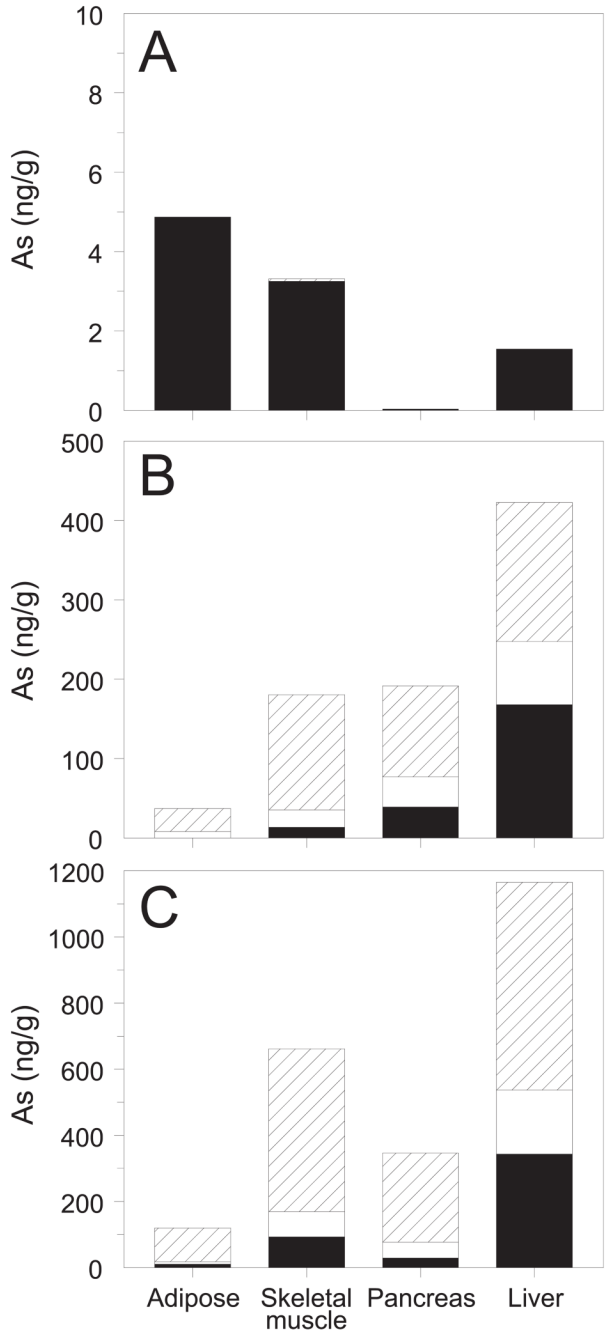

Fig. 5.

Arsenic species in adipose tissue, skeletal muscle, pancreas, and livers of control mice (A) and mice exposed to 25 ppm As (B) and 50 ppm As (C): iAs^V (■), MAs^V (□) and $DMAs^V$ (▨). (Mean; n = 5 for adipose tissue, skeletal muscle, and liver; n = 3 for pancreas.)