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Arsenite in drinking water produces glucose intolerance in pregnant rats and their female offspring

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ABSTRACT

Drinking water is the main source of arsenic exposure. Chronic exposure has been associated with metabolic disorders. Here we studied the effects of arsenic on glucose metabolism, in pregnant and postpartum of dams and their offspring.

We administered 5 (A5) or 50 (A50) mg/L of sodium arsenite in drinking water to rats from gestational day 1 (GD1) until two months postpartum (2MPP), and to their offspring from weaning until 8 weeks old.

Liver arsenic dose-dependently increased in arsenite-treated rats to levels similar to exposed population. Pregnant A50 rats gained less weight than controls and recovered normal weight at 2MPP. Arsenite-treated pregnant animals showed glucose intolerance on GD16-17, with impaired insulin secretion but normal insulin sensitivity; they showed dose-dependent increased pancreas insulin on GD18. All alterations reverted at 2MPP. Offspring from A50-treated mothers showed lower body weight at birth, 4 and 8 weeks of age, and glucose intolerance in adult females, probably due to insulin secretion and sensitivity alterations.

Arsenic alters glucose homeostasis during pregnancy by altering beta-cell function, increasing risk of developing gestational diabetes. In pups, it induces low body weight from birth to 8 weeks of age, and glucose intolerance in females, demonstrating a sex specific response.

1. Introduction

The effects of exposure to chemicals that act as endocrine disruptors on the general population are a growing worldwide concern. Endocrine disruptors are synthetic or natural compounds that have the ability to interfere with, mimic or antagonize the function and/or production of hormones. These compounds are widely distributed in our environment and include pesticides, pharmaceutical drugs and several chemicals, including metals. Metals that possess endocrine disrupting effects are varied, affecting one or more targets. For example Cadmium can induce endometriosis (Smarr et al., 2016), alterations in gonadotropin levels and in testicular or ovarian structure and activity (Lafuente, 2013), breast cancer (De Coster and van Larebeke, 2012) and also cardiovascular disease by increasing atherosclerotic plaque formation (Kirkley and Sargis, 2014). Metals, such as iron, arsenic, mercury, lead, cadmium and nickel, can also have effects of glycemic control (Gonzalez-Villalva et al., 2016). Among them, arsenic has been described as an endocrine disruptor and is currently being studied from this new perspective (Davey et al., 2008).

Arsenic is a naturally occurring metalloid released into the environment by natural events and human activities. The largest sources of exposure to inorganic arsenic are drinking water, crops such as rice and meals (Charnley, 2014; Nacano et al., 2014; Turra et al., 2010). Millions of people worldwide are exposed to

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contaminated water, mostly from natural mineral deposits. High levels of arsenic in water, up to 200 mg/L, have been reported around the world (Concha et al., 1998; Akter et al., 2005; National Research Council, 2001; Gonzalez-Villalva et al., 2016). The WHO's recommended safety limits for arsenic in drinking water is 10 μg/L, and it is calculated based on the effects of arsenic on cancer incidence. According to these standards, there is a huge concern in India, Bangladesh, Chile, Mexico, Taiwan and Argentina, among other parts of the world. An extended zone in Argentina contains ground water with relatively high levels of arsenic, with an estimated population exposed of approximately 7% of country population. (Ministerio de Educación de la Nación-Argentina, 2009) Although the effects of arsenic exposure increasing cancer incidence in the Argentine population have been described (Bardach et al., 2015), little is known on the effects of this metal on glucose metabolism in our country.

Inorganic arsenic is highly toxic and carcinogenic to humans; numerous studies have associated chronic exposure to inorganic arsenic in drinking water with increased prevalence of several cancers (Steinmaus et al., 2014; Cheng et al., 2016; Yang et al., 2008). Regarding the non-cancer health effects, chronic exposure to inorganic arsenic has been associated with loss of body weight (Nandi et al., 2005), metabolic disorders such as diabetes (Brauner et al., 2014; Longnecker and Daniels, 2001; Gonzalez-Villalva et al., 2016), cardiovascular disease (Moon et al., 2013; Li et al., 2013), chronic respiratory symptoms (Smith et al., 2013), and reproductive system alterations (Singh et al., 2007).

Epidemiological and experimental data indicate a diabetogenic role of arsenic. *In vitro* and *in vivo* experiments sustain this hypothesis (Paul et al., 2007a, 2007b; Brauner et al., 2014; James et al., 2013; Islam et al., 2012; Wang et al., 2014), but the biological mechanism for an association between chronic arsenic exposure and increased diabetes risk is not completely understood. Several studies suggest that arsenic might increase the risk for type 2 diabetes via multiple mechanisms, affecting a cluster of regulated events, which in conjunction trigger the disease (Diaz-Villasenor et al., 2007). Furthermore, gestational diabetes is similar to type 2 diabetes regarding its pathogenesis and clinical symptoms; however, it occurs in women during pregnancy and usually improves or disappears after childbirth. Epidemiological data suggest that arsenic may also increase the risk of developing gestational diabetes (Shapiro et al., 2015).

During the gestational period, exposure to As may cause alterations to the host and fetus in rats and humans at fairly low exposure levels (Chattopadhyay et al., 2001; DeSesso, 2001; Holson et al., 2000; Ahmad et al., 2001; Hopenhayn-Rich et al., 2000; Hopenhayn et al., 2003; Kile et al., 2014). Arsenic crosses the placental barrier in both animals and humans, and experimental studies support a role for arsenic as a developmental toxicant, e.g. exposure from drinking water has been related with increased rates of fetal loss, congenital malformation, pre-term births, and neonatal and young adult mortality, as well as decreased birth weight (Hopenhayn et al., 2003; Nandi et al., 2005; Concha et al., 1998; Devesa et al., 2006; Smith et al., 2012).

The goal of this study was to elucidate the deleterious effects of arsenic exposure through drinking water on rat glucose metabolism in particular physiological conditions that have not been thoroughly addressed such as during pregnancy and postpartum in dams, as well as on their offspring.

2. Materials and methods

2.1. Animals

We used young, virgin female Sprague-Dawley rats from the IBYME colony. Animals were housed in air-conditioned rooms, with lights on from 0700 to 1900, and given free access to laboratory chow and water. Studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of the IBYME-CONICET (in accordance with the Division of Animal Welfare, Office for Protection from Research Risks, National Institutes of Health, Animal Welfare Assurance for the Institute of Biology and Experimental Medicine A#5072-01). Animals were treated humanely and with regard for alleviation of suffering.

Rats were given sodium arsenite in drinking water at doses previously described (Paul et al., 2007b): A5: 5 mg/L or A50: 50 mg/L dissolved in distilled water, or distilled water as Control. Beverages were given ad libitum and changed every 2–3 days to avoid oxidation of As (III); standard chow was given ad libitum. Rats were exposed to sodium arsenite from gestation day 1 (GD1), confirmed by presence of vaginal sperm plug, until sacrifice. The offspring received the same treatments as their mother from weaning until sacrifice.

Water consumption did not show statistical differences among groups and the mean consumption per day was 53 ± 4 ml/animal; mean body weight (BW) of adult Sprague Dawley rats at GD1 was 229 ± 4 g. Thus, exposure levels expressed as mg/kg BW/day were 1.15 mg/kg BW/day and 11.5 mg/kg BW/day for A5 and A50 animals respectively.

Pregnant dams were housed singly until weaning; litter size was determined at birth and then reduced to eight pups when necessary; at weaning, offspring were separated by sex. Additionally, duration of pregnancy and male/female pup proportion was recorded.

We evaluated the effects of arsenic exposure in three experimental groups: pregnant dams, postpartum dams and their offspring.

2.1.1. Pregnant dams

Body weight was determined along pregnancy. Glucose metabolism (glucose tolerance test, insulin secretion test and HOMA of insulin resistance) was evaluated on GD16-17 and litter parameters at birth. One group of pregnant rats was sacrificed on GD18 and insulin content was determined in their pancreases.

2.1.2. Postpartum dams

Body weight was determined on the day after parturition (0 month) and at 1 and 2 months postpartum. Glucose metabolism, liver arsenic content and liver oxidative stress parameters were evaluated at 2 months postpartum.

2.1.3. Offspring

Body weight was determined on postnatal day 1 and at 4 and 8 weeks of age. At 8 weeks of age glucose metabolism was evaluated. Animals were sacrificed at two months of age and liver arsenic content and liver oxidative stress parameters were determined.

2.2. Arsenic tissue levels

We determined arsenic content in liver samples from dams sacrificed at two months postpartum (2MPP), and from offspring at two months of age. After a wet-digested mineralization, total As was determined by the silver diethyldithiocarbamate (AgDDTC) method that follows ISO 2590 guidelines. (ISO TC 47 SC1, 1973).

2.3. Glucose tolerance test (GTT) and insulin secretion test (IST)

We performed GTT/IST on GD16-17 and 2MPP in the same dams; GD16-17 was chosen to ensure that the animals were in a condition equivalent to the human third trimester for the first GTT (Petry

et al., 2011). Additionally, GTT/IST were performed on offspring at 8 weeks of age. Intraperitoneal glucose (2 g/kg BW) was injected to overnight fasted rats (15–18 h), blood glucose levels were evaluated at 0, 30, 60, 90 and 120 min post injection and serum insulin levels were determined at 0, 10, 30 and 60 min. Blood glucose was measured with a One touch® Ultra™ glucose-meter (Lifescan, Scotland Ltd) from tail blood with reactive strips (a gift from Johnson & Johnson, Argentina). Serum insulin was measured with an Ultrasensitive rat insulin ELISA kit (Crystal Chem, Chicago, Il).

2.4. HOMA of insulin resistance

We calculated HOMA of insulin resistance (HOMA-IR) with blood glucose and serum insulin measured after overnight fasting, on females during pregnancy (GD16-17) and at 2MPP and also on the offspring at adulthood (8 weeks age).

HOMA-IR= Fasting insulin (μU/ml) x Fasting glucose (mmol/L)/22.5 (Bonaventura et al., 2008).

2.5. Pancreatic insulin quantification

To measure pancreas insulin content, rats were sacrificed in the morning of GD18 and insulin was extracted from pancreas homogenates with acid-ethanol, as previously described (Bonaventura et al., 2008). Insulin was measured by RIA using human insulin for iodination and standard, kindly provided by Laboratorios Beta (Buenos Aires, Argentina) and anti-bovine insulin antibody (Sigma, Missouri, USA) (Bonaventura et al., 2008). Minimum detectable concentrations were 2 ng. Intra-assay coefficient of variation was 6.8%.

2.6. Oxidative stress measurements

Oxidative stress measurements were performed as described (Ventura et al., 2015) in livers from dams at 2MPP and in livers from two month-old offspring. Sample preparation: at sacrifice one part of a lobe of liver per rat was dissected, weighed and homogenized in phosphate buffer (KH_2PO_4/K_2HPO_4 50 mM pH 7.4) for catalase and lipid peroxidation determinations. For GSH content another part of the lobe was homogenized in 1 ml $HClO_4$ 0.5 N.

2.6.1. CAT activity

Liver homogenates were centrifuged at 10,000×*g* for 10 min at 4 °C and the supernatant kept for CAT activity determination. CAT activity was measured spectrophotometrically by monitoring the disappearance of H_2O_2 at 240 nm. The reaction mixture for the assay contained 50 mM phosphate buffer (pH 7.8), 25 mM H_2O_2 (MERK, Darmstadt, Germany) and 50 μl of CAT-containing samples, in a total volume of 1 ml. One unit of CAT was defined as the disappearance of 1 μmol of H_2O_2/min ($\varepsilon = 43.6\ M^{-1}cm^{-1}$). Results are indicated as International Units of CAT per g of tissue.

2.6.2. Lipid peroxidation

The formation of lipid oxidation products was evaluated by determination of 2-thiobarbituric acid reactant substances (TBARS). Liver homogenates were mixed with reaction buffer [15% (v/v) trichloroacetic acid, 0.25 N hydrochloric acid and 0.375% (w/v) 2-thiobarbituric acid] and heated for 15 min at 90 °C. The complex formed with 2-thiobarbituric acid was extracted with 3 ml of butanol and quantified fluorimetrically (λ_{ex} = 515 nm; λ_{em} = 555 nm). TBARS were expressed as μmol of malondialdehyde (MDA) per gram of tissue. MDA standard was prepared from 16.4 μM 1,1,3,3-tetraethoxy propane.

2.6.3. GSH content

Liver homogenates in $HClO_4$ acid were centrifuged at 600×*g* for 10 min at 4 °C and were neutralized with 0.44 M Na_3PO_4 to pH = 7.20 μl of each sample was mixed with 0.9 ml of reaction buffer (100 mM phosphate buffer, 10 mM EDTA, pH 7.4) and the absorbance at 412 nm was measured. Thereafter, 100 μl of DTNB solution [6 mM DTNB (Sigma Chemical Co., MO, USA) in sodium bicarbonate 0.5% w/v] was added. Samples were incubated for 1 min and absorbance at 412 nm was measured for a second time. GSH content was calculated as: GSH content = $(Abs_2 - Abs_1)/\varepsilon$ x V_s x C_p, were Abs_2: Absorbance after addition of DTNB solution and Abs_1: Absorbance before addition of DTNB solution, ε = 13.6 $mM^{-1}cm^{-1}$, V_s: sample volume and C_p: protein concentration.

2.7. DNA isolation and fragmentation analysis

DNA was extracted from pancreas of two month-old offspring as described (Parborell et al., 2005). Briefly, tissues were homogenized in a buffer containing 100 mM NaCl, 4 mM EDTA, 50 mM Tris-HCl, 0.5% SDS pH 8, and proteinase K (100 mg/ml) at 55 °C for 4 h to facilitate membrane and protein disruption. After incubation, samples were cooled for 30 min on ice in 1 M potassium acetate and 50% chloroform to initiate protein precipitation, and centrifuged at 9000xg for 8 min at 4 °C. Supernatants were then precipitated for 30 min in 2.5 vol of ethanol and centrifuged for 20 min at 5000xg at 4 °C. Finally, samples were extracted in 70% ethanol and resuspended in water. DNA content was measured by reading the absorbance at 260 nm, and incubated for 1 h with RNase (10 mg/ml) at 37 °C. DNA samples (4 mg) containing SyberGreen were electrophoretically separated on 1.7% agarose gels in Tris-borate-EDTA buffer. DNA was visualized with a UV (302 nm) transilluminator and image captured with G-Box.

2.8. Statistical analysis

Data are expressed as means ± SEM. Statistical analysis was performed with STATISTICA (Data analysis software system), version 8.0, StatSoft, Inc. (2008, OK, USA). The differences between means were analyzed by one- or two-way ANOVA, followed by Newman-Keuls test or Tukey HSD test for unequal N. For multiple determinations in the same animal, we used two–way ANOVA with repeated measures design. $P < 0.05$ was considered significant.

3. Results

3.1. Liver arsenic accumulation

Chronic treatment of dams with sodium arsenite produced an increase in total liver arsenic content in a dose dependent manner, exhibiting a 3.4- and 17-fold increase in A5 and A50 groups (1.7 and 8.7 mg/kg), respectively. These differences respect to control are, indeed, underestimated, since all control samples contained arsenic levels below the detection limit of the technique (Fig. 1a).

A50 treated offspring showed increased liver arsenic content compared to Control or A5, in both sexes. In this case, arsenic levels were lower than those of dams, suggesting that arsenic metabolism and/or excretion may be augmented, this could also be the reason why A5 treated animals presented levels similar to Controls (Fig. 1b).

3.2. Arsenite effect on body weight and glucose metabolism in pregnant rats

BW was monitored from GD1 to GD21 in pregnant rats. During pregnancy, A50 treated rats gained less weight than Control or A5

animals, showing at GD21 a significantly lower weight than the other groups (Fig. 2). Nevertheless, pregnancy duration, litter size and male/female pup proportion did not differ among treatment groups (Table 1).

Arsenite did not alter fasted glucose levels in pregnant rats although it induced glucose intolerance in a dose dependent manner at GD16-17 (Fig. 3a). The impairment in glucose clearance was more pronounced in A50 rats, attaining statistical significance at 30 and 60 min, while the A5 group differed from Control only at 30 min post glucose overload. The Area Under the Curve (AUC) was significantly increased for A50-treated animals compared to Controls.

Likewise, although fasted insulin did not differ among groups, insulin secretion in response to a glucose overload was greatly impaired in both A5 and A50 treated pregnant rats at this stage of pregnancy, with lower AUC in A50 treated animals (Fig. 3b). The HOMA–IR index is a parameter that accounts for insulin resistance, as the higher this index, the higher peripheral insulin resistance. No significant differences in the HOMA-IR were observed among treatment groups (Fig. 3d), suggesting that a decrease in peripheral glucose uptake does not contribute to the altered GTT. On GD18 (when one group of dams of each treatment group was sacrificed), insulin pancreatic content was augmented in A5 and A50 groups compared to Controls (Fig. 3c).

When taking into account the decreased IST and the increased insulin pancreatic content, impaired insulin secretion by beta cells, as a consequence of arsenite exposure, may be suggested.

Despite glucose tolerance alterations on GD16-17 and the increased pancreatic insulin content on GD18, we observed no significant differences in fasted blood glucose or fasted serum insulin between treatment groups on GD16-17 (Fig. 3a and b).

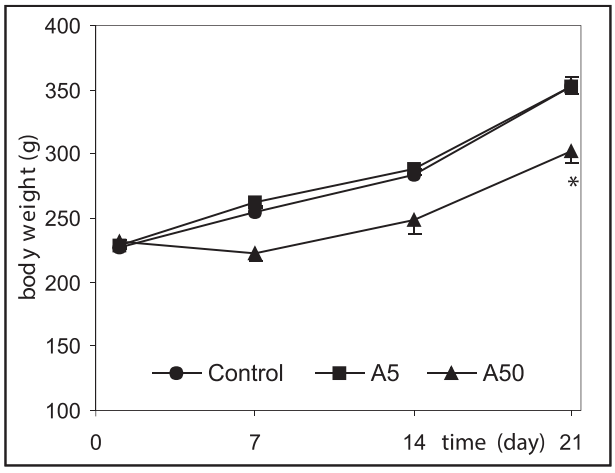

Fig. 2. Effect of arsenic treatment on BW increase during pregnancy. Body weight was checked on days 1 (determined by presence of sperm in vaginal smears), 7, 14 and 21 of pregnancy. The A50 group showed a significant impairment in body weight increase. Two-way ANOVA with repeated-measures design, factors: time and treatment: interaction: $p < 0.05$. * = A50 different from Control and A5 at 21 days, $p < 0.05$.

Table 1
Litter parameters.

	Control	A5	A50
Pregnancy duration (days)	23 ± 0	23 ± 0	23 ± 0
Litter size (number pups)	12,1 ± 0,4	11,3 ± 0,8	11,8 ± 0,9
Percentage male/female	44 ± 4/56 ± 4	50 ± 5/50 ± 5	51 ± 5/49 ± 5

One-way ANOVA: ns.

3.3. Arsenite effect on body weight and glucose metabolism during postpartum in dams

On postpartum day 1 (PPD1), A50 dams maintained the lower BW present during pregnancy, which was totally normalized after two months, even though arsenite exposure continued up to the day of sacrifice (Fig. 4).

Dams were reassessed two months post-partum. Normal glucose tolerance and insulin secretion had been reinstalled. Moreover, no significant differences in fasted blood glucose or fasted serum insulin were observed among groups. When evaluating peripheral insulin sensitivity, HOMA-IR index showed no alterations in treated animals compared to Controls (Table 2).

Oxidative stress parameters were evaluated in liver of dams at 2MPP. No significant differences were observed between experimental groups (not shown), despite high arsenic liver contents in exposed animals, as shown in Fig. 1.

3.4. Arsenite effect on body weight and glucose metabolism in offspring

Offspring from arsenite treated dams also showed diminished body weight. A50 treated animals weighed less than A5 or Control animals evaluated at three time points during their development, i.e. PND1 and 4 and 8 weeks of age (Fig. 5), indicating that the decrease in weight gain persists from birth to adulthood and

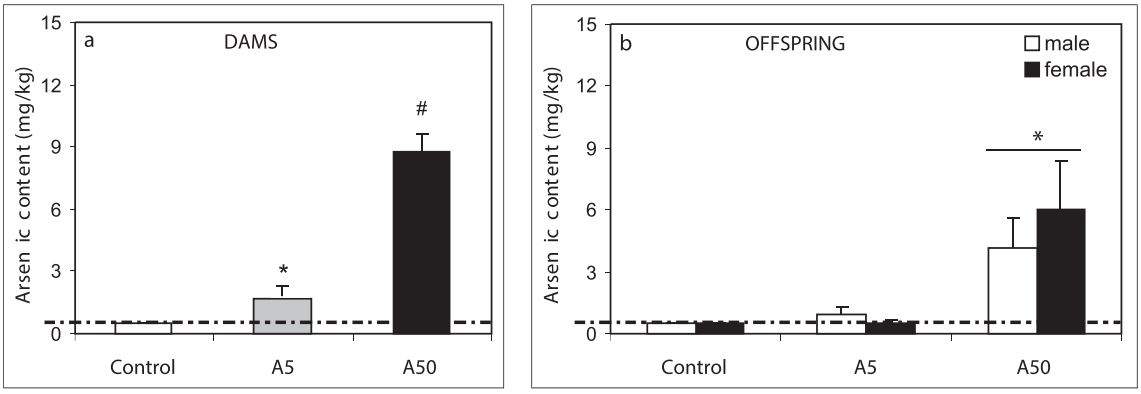

Fig. 1. a) Liver arsenic content in dams sacrificed two months postpartum (2MPP). One-way ANOVA: $p < 0.05$. * = A5 different from Control ($p < 0.05$),# = A50 different from Control and A5 ($p < 0.05$). **b)** Liver arsenic content in offspring at 8 weeks of age. Two-way ANOVA: interaction: ns, main effect: sex: ns, main effect: treatment $p < 0.05$. * = A50 different from Control ($p < 0.05$) and from A5 ($p < 0.05$). = Technique lower detection limit 0.5 mg/kg.

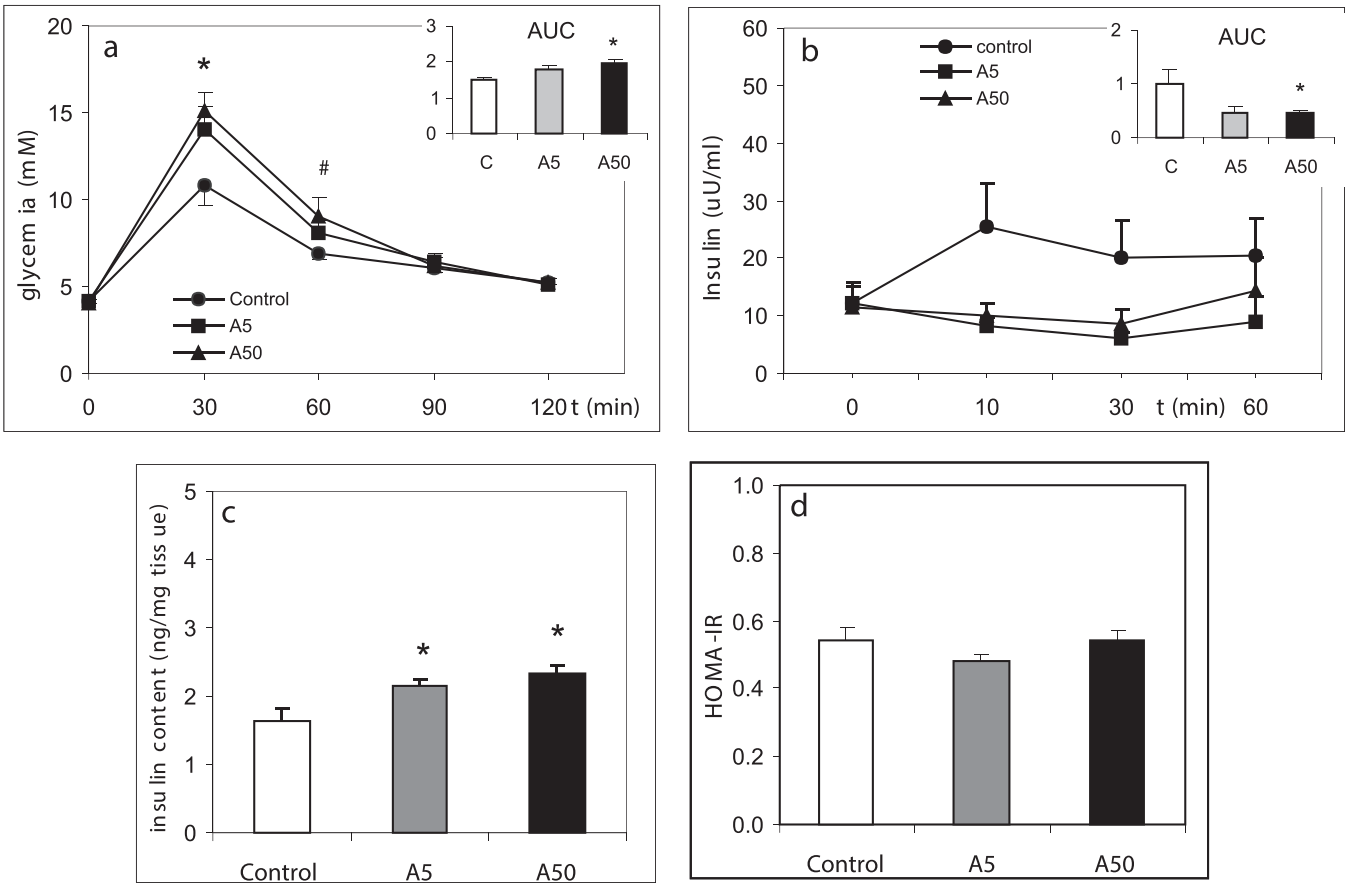

Fig. 3. Effect of arsenic on glucose homeostasis in pregnant rats. Glucose tolerance tests (GTT) and insulin secretion tests (IST) were performed on GD16-17. **a)** Arsenic exposure induced dose-dependent glucose intolerance. Two-way ANOVA with repeated-measures design: interaction,$p < 0.05$, * = A50 and A5 different from Control at 30 min ($p < 0.05$). # = A50 different from Control at 60 min ($p < 0.05$), (n = 11-12); AUC: one-way ANOVA, $p < 0.05$, * = A50 different from Controls: $p < 0.05$. **b)** Insulin section test (IST) was performed during the GTT. Insulin secretion was significantly impaired by arsenic exposure. Two-way ANOVA with repeated-measures design: interaction: ns; time: ns; treatment: $p < 0.05$; A50 and A5 different from Control, $p < 0.05$; AUC: one-way ANOVA, $p < 0.05$, * = A50 different from Controls: $p < 0.05$. **c)** Pancreatic insulin contents in dams sacrificed on GD18. Arsenic exposure induced a significant increase in pancreas insulin content. One-way ANOVA: $p < 0.05$: * = A5 and A50 different from Control, $p < 0.05$. **d)** HOMA-IR index calculated with fasting glucose and fasting insulin. Arsenic did not affect insulin resistance. One-way ANOVA: ns.

depends on the dose of exposure.

Despite their decreased weight, no significant differences in fasted blood glucose or fasted serum insulin were observed between groups (Fig. 6a and b). In contrast, the HOMA-IR index was altered in both males and females, but in opposite ways; while A50 females showed an increased HOMA-IR index compared to Controls, indicating some degree of insulin resistance, A50 males presented decreased HOMA-IR index, showing increased sensitivity to insulin (Fig. 6c). When evaluating Glucose Tolerance, females showed mild glucose intolerance in an i.p. GTT, being A50 treated animals more affected than A5 (Fig. 6d). This was also evident when comparing the area under the glucose curve (AUC), since only A50 treated females differed from Controls (Fig. 6f). Male offspring showed no glucose intolerance at adulthood, since i.p. GTT and the corresponding AUC were unaffected (Fig. 6e and f). We also determined insulin secretion during the GTT (IST) in control and A50 offspring. In arsenic-exposed offspring insulin secretion tended to be lower than in controls but did not reach statistical significance (Fig. 6g–h). Nevertheless, when the area under the insulin secretion curve was calculated a significant decrease was observed in both male and female offspring (Fig. 6i), similar to what we had observed in arsenic-exposed pregnant rats.

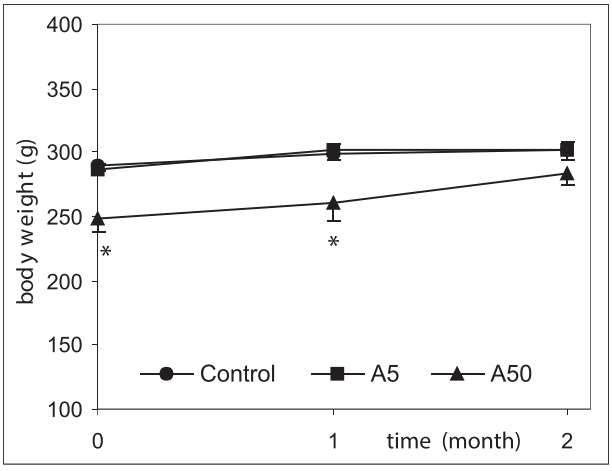

Fig. 4. Post-partum restoration of BW in dams. BW was monitored the day after parturition and at one and two months postpartum. A50-treated females recovered normal BW at two months postpartum. Two-way ANOVA with repeated-measures design, factors: time and treatment: interaction: $p < 0.05$. * = A50 different from Control and A5 on the day of parturition (0 months, $p < 0.05$) and one month later ($p < 0.05$).

Table 2
Glucose tolerance evaluation on dams, two month post-partum.

	Control	A5	A50
Glycemia (mM)	4,7 ± 0,2	4,6 ± 0,1	4,3 ± 0,2
Serum insulin (μU/ml)	4,0 ± 0,9	3,5 ± 0,7	4,4 ± 1,0
AUC (GTT) (A.U. × 10^{-4})	1,48 ± 0,06	1,79 ± 0,11	1,94 ± 0,13
AUC (IST) (A.U. × 10^{-3})	0,99 ± 0,27	0,48 ± 0,09	0,45 ± 0,06
HOMA-IR	0,62 ± 0,04	0,72 ± 0,15	0,81 ± 0,14

One-way ANOVA: ns.

respectively; these titers correlate with levels found by other authors at similar exposure levels (Nandi et al., 2005). Hepatic arsenic contents determined in exposed rats are similar to those found in population exposed to arsenic in drinking water, e.g. arsenic content in liver of people in West Bengal, India, was 6 mg/kg (Guha Mazumder, 2000). This may indicate that our experimental conditions mimic the exposure levels of specific populations. Regarding arsenic concentrations in liver of offspring, we found levels comparable to those reported by others in similar experimental models (Pineda et al., 2013). Liver arsenic levels in offspring

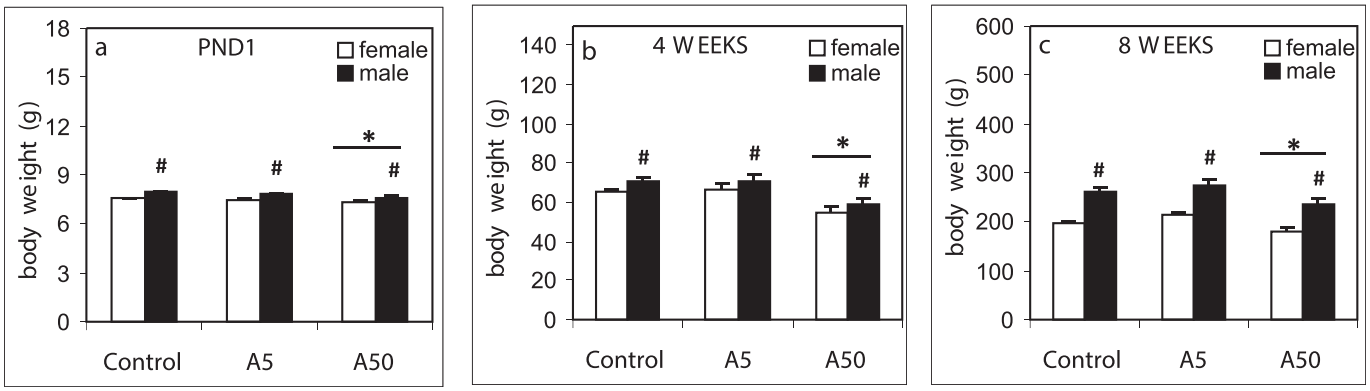

Fig. 5. Body weight (BW) of pups from control and arsenic-treated dams was evaluated on PND1 and at 4 and 8 weeks of age. Arsenic induced a persistent decrease in BW. **a)** PND1. Two-way ANOVA: $p < 0.05$, interaction: ns, main effect sex: #: males > females, $p < 0.05$, main effect treatment: $p < 0.05$, * = A50 different from Control and A5, $p < 0.05$; **b)** 4 weeks of age. Two-way ANOVA: $p < 0.05$, interaction: ns, main effect sex: #:males > females, $p < 0.05$, main effect treatment: $p < 0.05$, * = A50 different from Control and A5; **c)** 8 weeks of age. Two-way ANOVA: $p < 0.05$, interaction: ns, main effect sex: #: males > females, $p < 0.05$, main effect treatment: $p < 0.05$, * = A50 different from Control and A5.

3.5. Arsenite effect on liver oxidative stress parameters in offspring

The effect of arsenite exposure on oxidative stress parameters was also evaluated in livers from adult offspring at two months of age. Interestingly, in A50–exposed female offspring lipid peroxidation (TBARS, measured as MDA content) and GSH content were increased, while catalase activity was not altered (Fig. 7a–c). No alterations were observed in livers from male offspring in any of the parameters evaluated (Fig. 7d–f).

3.6. Arsenite effect on pancreas DNA fragmentation in offspring

Arsenite exposure did not induce apoptosis, estimated as DNA fragmentation, in whole pancreases from male or female offspring (not shown), although we cannot discard that the effect of arsenite on islet cells may vary from what is observed in whole pancreas, as islets only accounts for two percent of the tissue.

4. Discussion

Arsenic, widely distributed through drinking water, is considered a serious, worldwide environmental health threat (Akram et al., 2010). The present study investigated adverse effects of arsenic on glucose metabolism in dams, during pregnancy and one month after weaning of their litters, and in their offspring at adulthood. Our results suggest a specific association between arsenic exposure and glucose clearance, insulin dysregulation and liver toxicity as also suggested by other authors (Heindel et al., 2016).

Arsenic content in liver samples of dams was significantly augmented in a concentration-dependent manner, attaining levels of 1.7 and 8.7 mg/kg in the 5 and 50 mg/L exposed groups, were lower than those in 2MPP dams although also relevant in A50 exposed animals (approximately 5 mg/kg), suggesting differences in metabolism and/or excretion with regard to dams; this could explain why A5 treated offspring presented levels similar to Controls.

Pregnant rats exposed to 50 mg/L of sodium arsenite showed diminished BW increase during pregnancy and this difference was not attributable to differences in offspring number or body weight of pups at birth. This difference persisted after delivery (PPD1 and 1MPP), and was totally normalized after 2 months. Effects of chronic intoxication with arsenic on body weight varies according to the experimental model but there is general consensus on the effects at developmental stages, where arsenic induces diminished weight gain compared to controls (Izquierdo-Vega et al., 2006; Guan et al., 2012; Hopenhayn et al., 2003; Xi et al., 2009; Nordstrom et al., 1978, 1979). We found no reports evaluating this parameter in pregnant rats, but our results are in agreement with this general observation, since A50-treated rats gained less weight than Controls during pregnancy and lactation, although they eventually recovered normal weight, mainly after weaning.

These alterations in body weight were also present in the offspring, where A50 treated pups of both sexes showed lower weight at birth than Controls or A5 treated pups, maintaining this difference until at least 8 weeks of age, demonstrating no catch-up growth. Our findings are consistent with previous studies showing reduced weight of pups exposed to arsenic *in utero* (Petrick et al., 2009; Izquierdo-Vega et al., 2006; Guan et al., 2012; Hopenhayn et al., 2003; Gutierrez-Torres et al., 2015). In addition, an increased rate of low birth weights was observed in children from women exposed to high levels of As compared with women from non-contaminated areas (Ahmad et al., 2001; Hopenhayn-Rich et al., 2000; Kapaj et al., 2006; Rahman et al., 2009). Although no clear

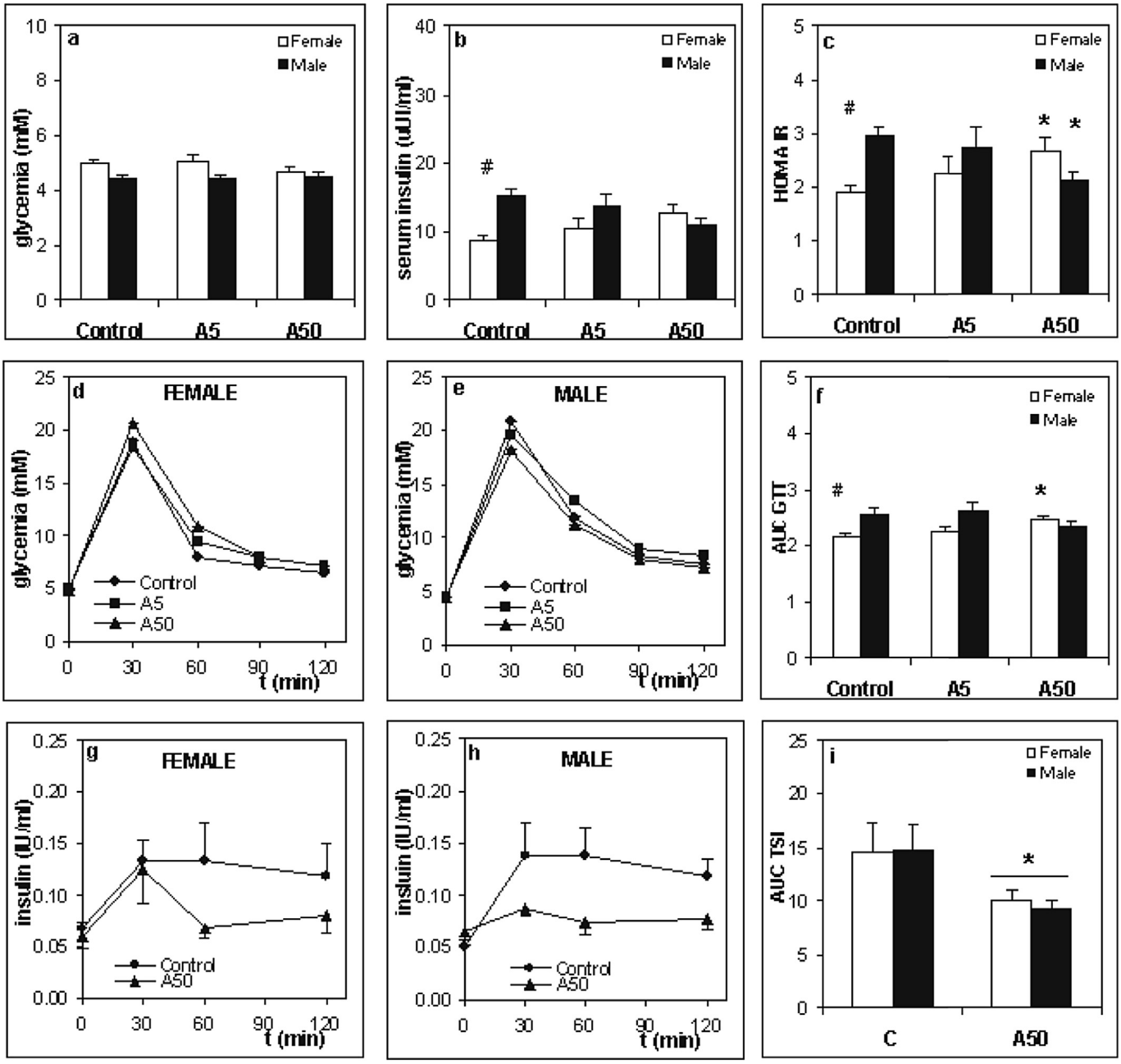

Fig. 6. Effect of arsenic on glucose homeostasis in offspring at 8 weeks of age. **a)** Glycemia in fasted animals was not affected by arsenic exposure. Two-way ANOVA, interaction: ns, main effect treatment: ns; main effect sex: females > males, $p < 0.05$. **b)** Serum insulin levels in fasted animals were not affected by arsenic exposure. The typical sex difference (males > females) in basal insulin was observed in control animals. Two-way ANOVA, interaction: $p < 0.05$. # = Control female different from Control male, $p < 0.05$. **c)** HOMA-IR index, calculated with fasted blood glucose and fasted insulin levels. Arsenic exposure induced insulin resistance in female offspring and insulin sensitivity in male offspring. Two-way ANOVA: interaction: $p < 0.05$: # = Control female different from Control male, $p < 0.05$, * = A50 female different from Control female, $p < 0.05$, * = A50 male different from Control male, $p < 0.05$. **d)** GTTin females. Arsenic exposure induced mild glucose intolerance in female offspring. Two-way ANOVA with repeated-measures design: interaction: ns, main effect time: $p < 0.05$; main effect treatment: $p < 0.05$, A50 different from Control. **e)** GTT in males. Two-way ANOVA with repeated-measures design: interaction: ns, main effect time: $p < 0.05$; main effect treatment: ns. **f)** AUC of GTT. Mild glucose intolerance was observed in arsenic-exposed female offspring, but not in males. Two-way ANOVA: interaction: $p < 0.05$: # = Control female different from Control male, $p < 0.05$, * = A50 female different from Control female, $p < 0.05$. **g)** IST in females. Two-way ANOVA with repeated-measures design: interaction: ns, main effect treatment: ns, main effect time: $p < 0.05$; 30′ different from basal. **h)** IST in males.Two-way ANOVA with repeated-measures design: interaction: ns, main effect treatment: ns, main effect time: $p < 0.05$; 30′, 60′ and 120′ different from basal. **i)** AUC of IST. Although the ISTs did not show statistical differences, the area under the insulin secretion curve was significantly reduced in both male and female arsenic-exposed offspring. Two-way ANOVA: interaction: ns, main effect sex: ns, main effect treatment: $p < 0.05$; A50 different from Control.

biologic mechanism by which arsenic could affect birth weight has been proposed, decreased blood flow through umbilical cord (Hopenhayn et al., 2003) or decreased provision of adequate fetal nutrition because of decreased placental GLUT4 glucose transporter expression (Gutierrez-Torres et al., 2015) may be some of the underlying causes. In view of our results in arsenite exposed dams, in which we demonstrate clear impairment in insulin secretion, but not synthesis (see below), if this effect of arsenite can be assumed to also occur at the fetal pancreas, then decreased fetal insulin could also contribute to the decreased pup weight, as fetal insulin

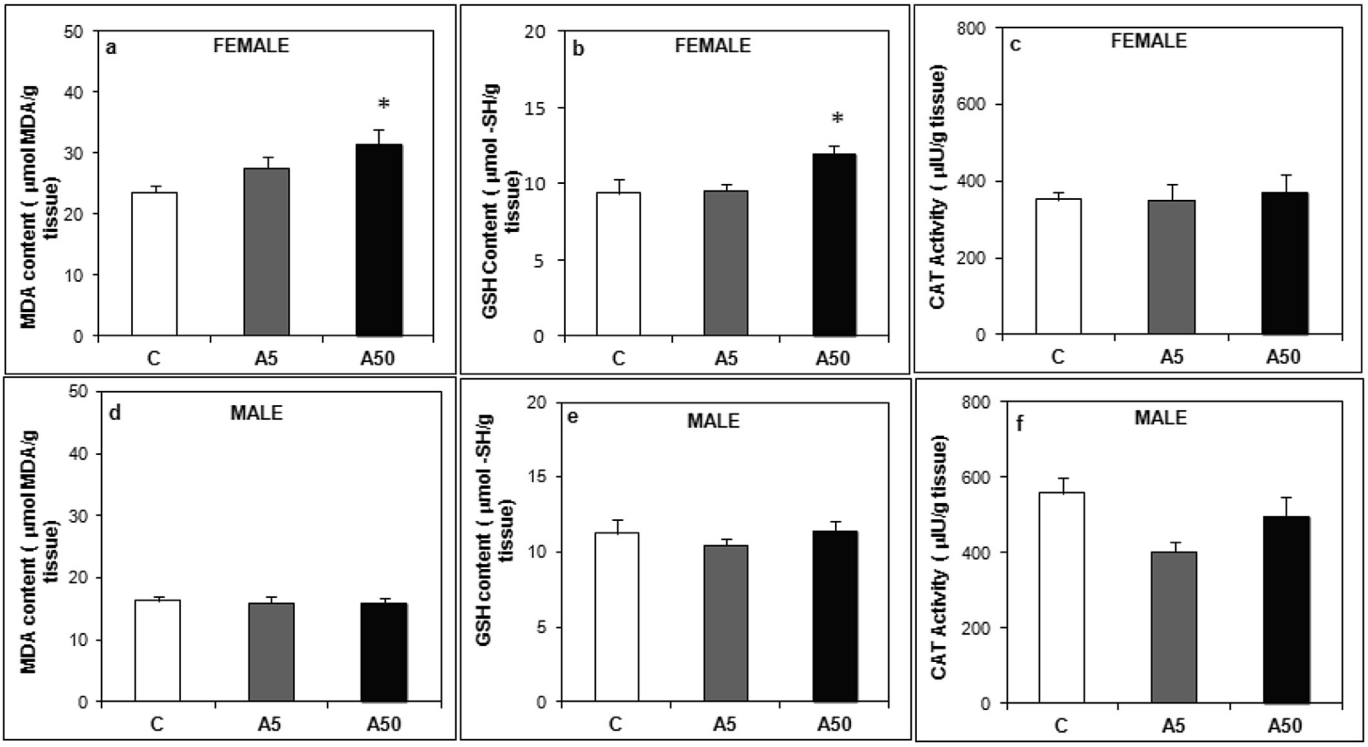

Fig. 7. Effect of arsenite exposure on oxidative stress parameters in liver from offspring at 8 weeks of age. **a)** TBARS, expressed as MDA content in females. The high dose of arsenic exposure induced an increase in MDA content. One-way ANOVA: $p < 0.05$. * = A50 different from Control ($p < 0.05$). **b)** GSH content in females. Arsenic exposure induced an increase in GSH contentin A50 rats. One-way ANOVA: $p < 0.05$. * = A50 different from Control ($p < 0.05$). **c)** Catalase Activity was unaffected by arsenic exposure in females, One-way ANOVA: ns. **d)** MDA content in males was not affected by arsenic exposure. One-way ANOVA: ns. **e)** GSH content in males was unaffected by arsenic exposure. One-way ANOVA: ns. **f)** Catalase Activity in males was unaffected by arsenic exposure. One-way ANOVA: ns.

has been shown to be a key hormone determining intrauterine fetal growth (Kurjak, 2015).

In our experimental model, arsenite induced marked glucose intolerance in dams that was evident on a GTT, being this impairment in glycemia restoration more pronounced in A50-treated animals, in agreement with observations in male mice exposed to 50 mg/L of sodium arsenite (Paul et al., 2007a). Diminished clearance of glucose during a GTT could be explained by impairment in glucose-stimulated insulin secretion by beta cells, a reduction of peripheral insulin sensitivity, or a combination of both. In our model, glucose intolerance can be attributed to impaired insulin secretion, as seen on the IST, with normal peripheral sensitivity, since HOMA-IR in treated animals was similar to Controls. Taking these results together, we can infer that arsenite induces alterations in glucose homeostasis primarily by altering beta cell function. In this regard, several *in vivo* and *in vitro* studies describe impaired insulin secretion in relation to oxidative stress, a phenomenon widely described to be induced by arsenite (Fu et al., 2010; Nandi et al., 2005). However, oxidative stress evaluation in livers of exposed dams at two months postpartum, when liver arsenic content was high, did not show differences among groups, although we cannot discard that this may have been different during pregnancy. We have also found an increase in insulin content in arsenite-treated animals, consistent with the hypothesis of impaired insulin secretion but not its synthesis. We therefore suppose that the insulin secreting mechanism may be affected by As and this will be matter of further studies.

Our findings reveal a detrimental effect of arsenite on glucose homeostasis in dams that could lead to an increased risk of developing gestational diabetes. Despite several experimental models evaluating arsenic intoxication during pregnancy, most of them study the detrimental effects on the offspring but provide very little information about the effects on the mothers. Furthermore, although there is accumulating evidence showing an increased risk of type 2 diabetes in general population exposed to arsenic (Maull et al., 2012; Brauner et al., 2014; James et al., 2013; Islam et al., 2012; Wang et al., 2014), less is known about exposure during pregnancy and its association with gestational diabetes. Concerning this, Ettinger et al. demonstrated an association between arsenic exposure via drinking water and higher odds of impaired GTT in pregnant women from Oklahoma, USA (Ettinger et al., 2009), and from Canada (Shapiro et al., 2015), in agreement with our observations.

Interestingly, at the arsenite exposure levels tested, the glucose intolerance observed during pregnancy completely reverted one month after weaning, even in the presence of continuous arsenite exposure. In agreement with these results, no alterations in hepatic oxidative stress parameters were observed at this time point. In view of these results we can postulate that the effects of arsenite on glucose metabolism are only revealed in the particular physiological status of pregnancy, although we cannot discard that alterations may appear in these dams later in life.

Offspring exposed to 50 mg/L of arsenite from conception to adulthood showed decreased body weight, and females also showed glucose intolerance at 8 weeks of age. Insulin secretion (AUC), determined during the GTT, was significantly impaired in both male and female A50 offspring, similar to what we had observed in arsenite-exposed pregnant rats. Interestingly, we observed a sex difference regarding peripheral tissue insulin sensitivity in arsenite–exposed offspring. While the HOMA-IR was increased in A50 females, it was decreased in A50 males with regard to their respective controls. The increased insulin sensitivity in

males may be the cause of lack of GTT alteration even in the presence of diminished insulin secretion. Increased HOMA-IR was also determined by Dávila-Esqueda et al. in female Wistar rats subjected to a similar arsenic exposure (Davila-Esqueda et al., 2011). In this regard, Rodriguez et al. found glucose intolerance in mouse female offspring of CD-1 mothers exposed to As, but not decreased body weight (Rodriguez et al., 2015). In contrast, Kozul-Horvarth et al. found decreased body weight in male and female offspring of C57 mice at birth, which persisted only in females at PND42 (Kozul-Horvath et al., 2012). Taking these data together, it is evident that arsenite negative effects vary with the experimental model, but females seem to be more sensitive than males. In agreement with this hypothesis, oxidative stress parameters were found altered in females but not in males. We observed an increase in liver lipid peroxidation in A50 female offspring, as also described by other authors (Muthumani and Miltonprabu, 2015; Nandi et al., 2005; Jalaludeen et al., 2016; Xu et al., 2013). Several works have shown decreased glutathione levels in the presence of oxidative stress (Jalaludeen et al., 2016; Muthumani and Miltonprabu, 2015). In contrast, we observed an increase in glutathione levels in A50-exposed females, concomitant with the increase in lipid peroxidation; the increase in glutathione may be a reaction of the liver to try to compensate the increase in oxidative stress; GSH-related compensatory mechanisms have also been proposed by others (Tong et al., 2016; Raza et al., 2012). No differences were observed in liver catalase activity, as also observed by others (Nandi et al., 2005). Regarding sex differences, catalase levels were double as high in males than in females and this may protect the cells of males against oxidative stress. In addition, we cannot discard the possibility that males may develop other alterations on glucose homeostasis due to arsenite exposure later in life.

It is also interesting to speculate on why oxidative stress was increased in liver of female offspring and not in liver of their mothers. The fact that offspring were exposed to arsenite from conception onwards may be the clue to this observation, a fact that warrants further studies. In this regard, it is widely accepted that increased susceptibility to disease in adulthood could have a fetal basis if the exposure to endocrine disruptors occurs during vulnerable windows of developmental stages (Heindel et al., 2016).

5. Conclusions

Our findings demonstrate that arsenic acts as an endocrine disruptor, since it alters normal insulin function/release. Arsenite exposure provokes diminished body weight in dams and offspring and glucose intolerance in pregnant dams and in their female offspring.

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References

Ahmad, S.A., Sayed, M.H., Barua, S., Khan, M.H., Faruquee, M.H., Jalil, A., et al., 2001. Arsenic in drinking water and pregnancy outcomes. Environ. Health Perspect. 109, 629–631.
Akram, Z., Jalali, S., Shami, S.A., Ahmad, L., Batool, S., Kalsoom, O., 2010. Adverse effects of arsenic exposure on uterine function and structure in female rat. Exp. Toxicol. Pathol. 62, 451–459.
Akter, K.F., Owens, G., Davey, D.E., Naidu, R., 2005. Arsenic speciation and toxicity in biological systems. Rev. Environ. Contam. Toxicol. 184, 97–149.
Bardach, A.E., Ciapponi, A., Soto, N., Chaparro, M.R., Calderon, M., Briatore, A., et al., 2015. Epidemiology of chronic disease related to arsenic in Argentina: a systematic review. Sci. Total Environ. 538, 802–816.
Bonaventura, M.M., Catalano, P.N., Chamson-Reig, A., Arany, E., Hill, D., Bettler, B., et al., 2008. GABAB receptors and glucose homeostasis: evaluation in GABAB receptor knockout mice. Am. J. Physiol. Endocrinol. Metab. 294, E157–E167.
Brauner, E.V., Nordsborg, R.B., Andersen, Z.J., Tjonneland, A., Loft, S., Raaschou-Nielsen, O., 2014. Long-term exposure to low-level arsenic in drinking water and diabetes incidence: a prospective study of the diet, cancer and health cohort. Environ. Health Perspect. 122, 1059–1065.
Charnley, G., 2014. Arsenic in rice and other foods. JAMA Intern. Med. 174, 298.
Chattopadhyay, S., Ghosh, S., Debnath, J., Ghosh, D., 2001. Protection of sodium arsenite-induced ovarian toxicity by coadministration of L-ascorbate (vitamin C) in mature wistar strain rat. Arch. Environ. Contam. Toxicol. 41, 83–89.
Cheng, P.S., Weng, S.F., Chiang, C.H., Lai, F.J., 2016 Feb. Relationship between arsenic-containing drinking water and skin cancers in the arseniasis endemic areas in Taiwan. J. Dermatol 43 (2), 181–186.
Concha, G., Vogler, G., Lezcano, D., Nermell, B., Vahter, M., 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicol. Sci. 44, 185–190.
Davey, J.C., Nomikos, A.P., Wungjiranirun, M., Sherman, J.R., Ingram, L., Batki, C., et al., 2008. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor-and thyroid hormone receptor-mediated gene regulation and thyroid hormone-mediated amphibian tail metamorphosis. Environ. Health Perspect. 116, 165–172.
Davila-Esqueda, M.E., Morales, J.M., Jimenez-Capdeville, M.E., De la, C.E., Falcon-Escobedo, R., Chi-Ahumada, E., et al., 2011. Low-level subchronic arsenic exposure from prenatal developmental stages to adult life results in an impaired glucose homeostasis 1. Exp. Clin. Endocrinol. Diabetes 119, 613–617.
De Coster, S., van Larebeke, N., 2012. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J. Environ. Public Health 2012, 713696.
DeSesso, J.M., 2001. Teratogen update: inorganic arsenic. Teratology 64, 170–173.
Devesa, V., Adair, B.M., Liu, J., Waalkes, M.P., Diwan, B.A., Styblo, M., et al., 2006. Arsenicals in maternal and fetal mouse tissues after gestational exposure to arsenite. Toxicology 224, 147–155.
Diaz-Villasenor, A., Burns, A.L., Hiriart, M., Cebrian, M.E., Ostrosky-Wegman, P., 2007. Arsenic-induced alteration in the expression of genes related to type 2 diabetes mellitus. Toxicol. Appl. Pharmacol. 225, 123–133.
Ettinger, A.S., Zota, A.R., Amarasiriwardena, C.J., Hopkins, M.R., Schwartz, J., Hu, H., et al., 2009. Maternal arsenic exposure and impaired glucose tolerance during pregnancy. Environ. Health Perspect. 117, 1059–1064.
Fu, J., Woods, C.G., Yehuda-Shnaidman, E., Zhang, Q., Wong, V., Collins, S., et al., 2010. Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: involvement of cellular adaptive response to oxidative stress. Environ. Health Perspect. 118, 864–870.
Gonzalez-Villalva, A., Colin-Barenque, L., Bizarro-Nevares, P., Rojas-Lemus, M., Rodriguez-Lara, V., Garcia-Pelaez, I., et al., 2016. Pollution by metals: is there a relationship in glycemic control? Environ. Toxicol. Pharmacol. 46, 337–343.
Guan, H., Piao, F., Zhang, X., Li, X., Li, Q., Xu, L., et al., 2012. Prenatal exposure to arsenic and its effects on fetal development in the general population of. Dalian. Biol. Trace Elem. Res. 149, 10–15.
Guha Mazumder, D.N., 2000. Diagnosis and Treatment of Chronic Arsenic Poisoning, pp. 2–34. http://www.who.int/water_sanitation_health/dwq/arsenicun4.pdf.
Gutierrez-Torres, D.S., Gonzalez-Horta, C., Del Razo, L.M., Infante-Ramirez, R., Ramos-Martinez, E., Levario-Carrillo, M., et al., 2015. Prenatal exposure to sodium arsenite alters placental glucose 1, 3, and 4 transporters in balb/c mice, 2015 Biomed. Res. Int. 175025.
Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., et al., 2016 Oct 17. Metabolism disrupting chemicals and metabolic disorders. Reprod. Toxicol. http://dx.doi.org/10.1016/j.reprotox.2016.10.001 pii: S0890-6238(16)30363-X. [Epub ahead of print].
Holson, J.F., DeSesso, J.M., Jacobson, C.F., Farr, C.H., 2000. Appropriate use of animal models in the assessment of risk during prenatal development: an illustration using inorganic arsenic. Teratology 62, 51–71.
Hopenhayn, C., Ferreccio, C., Browning, S.R., Huang, B., Peralta, C., Gibb, H., et al., 2003. Arsenic exposure from drinking water and birth weight. Epidemiology 14, 593–602.
Hopenhayn-Rich, C., Browning, S.R., Hertz-Picciotto, I., Ferreccio, C., Peralta, C., Gibb, H., 2000. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. Environ. Health Perspect. 108, 667–673.
Islam, R., Khan, I., Hassan, S.N., McEvoy, M., D'Este, C., Attia, J., et al., 2012. Association between type 2 diabetes and chronic arsenic exposure in drinking water: a cross sectional study in Bangladesh. Environ. Health 11, 38.
ISO TC 47 SC1, 1973. ISO 2590:1973, General Method for the Determination of

Arsenic - Silver Diethyldithiocarbamate Photometric Method, first ed. American National Standards Institute (ANSI).
Izquierdo-Vega, J.A., Soto, C.A., Sanchez-Pena, L.C., Vizcaya-Ruiz, A., Del Razo, L.M., 2006. Diabetogenic effects and pancreatic oxidative damage in rats subchronically exposed to arsenite. Toxicol. Lett. 160, 135–142.
Jalaludeen, A.M., Ha, W.T., Lee, R., Kim, J.H., Do, J.T., Park, C., et al., 2016. Biochanin a ameliorates arsenic-induced hepato- and hematotoxicity in rats. Molecules 21, 69.
James, K.A., Marshall, J.A., Hokanson, J.E., Meliker, J.R., Zerbe, G.O., Byers, T.E., 2013. A case-cohort study examining lifetime exposure to inorganic arsenic in drinking water and diabetes mellitus. Environ. Res. 123, 33–38.
Kapaj, S., Peterson, H., Liber, K., Bhattacharya, P., 2006. Human health effects from chronic arsenic poisoning–a review. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 41, 2399–2428.
Kile, M.L., Rodrigues, E.G., Mazumdar, M., Dobson, C.B., Diao, N., Golam, M., et al., 2014. A prospective cohort study of the association between drinking water arsenic exposure and self-reported maternal health symptoms during pregnancy in Bangladesh. Environ. Health 13, 29.
Kirkley, A.G., Sargis, R.M., 2014. Environmental endocrine disruption of energy metabolism and cardiovascular risk. Curr. Diab. Rep. 14, 494.
Kozul-Horvath, C.D., Zandbergen, F., Jackson, B.P., Enelow, R.I., Hamilton, J.W., 2012. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. PLoS. One 7, e38249.
Kurjak, A., 2015. Textbook of Perinatal Medicine: 2 Volume Set. Jaypee Brothers Medical Publishers Ltd.
Lafuente, A., 2013. The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. An update of recent studies and potential therapeutic approaches. Food Chem. Toxicol. 59, 395–404.
Li, X., Li, B., Xi, S., Zheng, Q., Lv, X., Sun, G., 2013. Prolonged environmental exposure of arsenic through drinking water on the risk of hypertension and type 2 diabetes. Environ. Sci. Pollut. Res. Int. 20, 8151–8161.
Longnecker, M.P., Daniels, J.L., 2001. Environmental contaminants as etiologic factors for diabetes. Environ. Health Perspect. 109 (Suppl. 6), 871–876.
Maull, E.A., Ahsan, H., Edwards, J., Longnecker, M.P., Navas-Acien, A., Pi, J., et al., 2012. Evaluation of the association between arsenic and diabetes: a national toxicology program workshop review. Environ. Health Perspect. 120, 1658–1670.
Ministerio de Educación de la Nación-Argentina 2009. mapaeducativo.edu.ar/Atlas/Arsenico.
Moon, K.A., Guallar, E., Umans, J.G., Devereux, R.B., Best, L.G., Francesconi, K.A., et al., 2013. Association between exposure to low to moderate arsenic levels and incident cardiovascular disease. A Prospect. cohort study. Ann. Intern. Med. 159, 649–659.
Muthumani, M., Miltonprabu, S., 2015. Ameliorative efficacy of tetrahydrocurcumin against arsenic induced oxidative damage, dyslipidemia and hepatic mitochondrial toxicity in rats. Chem. Biol. Interact. 235, 95–105.
Nacano, L.R., de Freitas, R., Barbosa Jr., F., 2014. Evaluation of seasonal dietary exposure to arsenic, cadmium and lead in schoolchildren through the analysis of meals served by public schools of Ribeirao Preto, Brazil. J. Toxicol. Environ. Health A 77, 367–374.
Nandi, D., Patra, R.C., Swarup, D., 2005. Effect of cysteine, methionine, ascorbic acid and thiamine on arsenic-induced oxidative stress and biochemical alterations in rats. Toxicology 211, 26–35.
National Research Council, 2001. Arsenic in Drinking Water 2001 Update.
Nordstrom, S., Beckman, L., Nordenson, I., 1978. Occupational and environmental risks in and around a smelter in northern Sweden. I. Var. birth weight. Hered. 88, 43–46.
Nordstrom, S., Beckman, L., Nordenson, I., 1979. Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous Abort. among female employees decreased birth weight their offspring. Hered. 90, 291–296.
Parborell, F., Irusta, G., Vitale, A., Gonzalez, O., Pecci, A., Tesone, M., 2005. Gonadotropin-releasing hormone antagonist antide inhibits apoptosis of preovulatory follicle cells in rat ovary. Biol. Reprod. 72, 659–666.
Paul, D.S., Harmon, A.W., Devesa, V., Thomas, D.J., Styblo, M., 2007a. Molecular mechanisms of the diabetogenic effects of arsenic: inhibition of insulin signaling by arsenite and methylarsonous acid. Environ. Health Perspect. 115, 734–742.
Paul, D.S., Hernandez-Zavala, A., Walton, F.S., Adair, B.M., Dedina, J., Matousek, T., et al., 2007b. 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, 305–314.
Petrick, J.S., Blachere, F.M., Selmin, O., Lantz, R.C., 2009. Inorganic arsenic as a developmental toxicant: in utero exposure and alterations in the developing rat lungs. Mol. Nutr. Food Res. 53, 583–591.
Petry, C.J., Evans, M.L., Wingate, D.L., Ong, K.K., Reik, W., Constancia, M., et al., 2011. Increased placental glucose transport rates in pregnant mice carrying fetuses with targeted disruption of their placental-specific Igf2 transcripts are not associated with raised circulating glucose concentrations, 2011 Exp. Diabetes Res. 171376.
Pineda, J., Herrera, A., Antonio, M.T., 2013. Comparison between hepatic and renal effects in rats treated with arsenic and/or antioxidants during gestation and lactation. J. Trace Elem. Med. Biol. 27, 236–241.
Rahman, A., Vahter, M., Smith, A.H., Nermell, B., Yunus, M., El Arifeen, S., et al., 2009. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. Am. J. Epidemiol. 169, 304–312.
Raza, H., John, A., Howarth, F.C., 2012. Alterations in glutathione redox metabolism, oxidative stress, and mitochondrial function in the left ventricle of elderly Zucker diabetic fatty rat heart. Int. J. Mol. Sci. 13, 16241–16254.
Rodriguez, K.F., Ungewitter, E.K., Crespo-Mejias, Y., Liu, C., Nicol, B., Kissling, G.E., et al., 2015. Effects of exposure to arsenic during the second half of gestation on reproductive end points and metabolic parameters in female CD-1 mice. Environ. Health Perspect. http://dx.doi.org/10.1289/ehp.1509703 (in press).
Shapiro, G.D., Dodds, L., Arbuckle, T.E., Ashley-Martin, J., Fraser, W., Fisher, M., et al., 2015. Exposure to phthalates, bisphenol A and metals in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: the MIREC study. Environ. Int. 83, 63–71.
Singh, N., Kumar, D., Sahu, A.P., 2007. Arsenic in the environment: effects on human health and possible prevention. J. Environ. Biol. 28, 359–365.
Smarr, M.M., Kannan, K., Buck Louis, G.M., 2016. Endocrine disrupting chemicals and endometriosis. Fertil. Steril. 106, 959–966.
Smith, A.H., Marshall, G., Liaw, J., Yuan, Y., Ferreccio, C., Steinmaus, C., 2012. Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. Environ. Health Perspect. 120, 1527–1531.
Smith, A.H., Yunus, M., Khan, A.F., Ercumen, A., Yuan, Y., Smith, M.H., et al., 2013. Chronic respiratory symptoms in children following in utero and early life exposure to arsenic in drinking water in Bangladesh. Int. J. Epidemiol. 42, 1077–1086.
Steinmaus, C., Ferreccio, C., Acevedo, J., Yuan, Y., Liaw, J., Duran, V., et al., 2014. Increased lung and bladder cancer incidence in adults after in utero and early-life arsenic exposure. Cancer Epidemiol. Biomarkers Prev. 23, 1529–1538.
Tong, J., Fitzmaurice, P.S., Moszczynska, A., Mattina, K., Ang, L.C., Boileau, I., et al., 2016. Do glutathione levels decline in aging human brain? Free Radic. Biol. Med. 93, 110–117.
Turra, C., Nadai Fernandes, E.A., Antonio, S.V., Arruda, B.M., Adrian, S.G., Enrique Lai, R.A., 2010. Arsenic and chromium in Brazilian agricultural supplies. J. Toxicol. Environ. Health A 73, 910–915.
Ventura, C., Venturino, A., Miret, N., Randi, A., Rivera, E., Nunez, M., et al., 2015. Chlorpyrifos inhibits cell proliferation through ERK1/2 phosphorylation in breast cancer cell lines. Chemosphere 120, 343–350.
Wang, W., Xie, Z., Lin, Y., Zhang, D., 2014. Association of inorganic arsenic exposure with type 2 diabetes mellitus: a meta-analysis. J. Epidemiol. Community Health 68, 176–184.
Xi, S., Sun, W., Wang, F., Jin, Y., Sun, G., 2009. Transplacental and early life exposure to inorganic arsenic affected development and behavior in offspring rats. Arch. Toxicol. 83, 549–556.
Xu, Z., Wang, Z., Li, J.J., Chen, C., Zhang, P.C., Dong, L., et al., 2013. Protective effects of selenium on oxidative damage and oxidative stress related gene expression in rat liver under chronic poisoning of arsenic. Food Chem. Toxicol. 58, 1–7.
Yang, C.Y., Chang, C.C., Chiu, H.F., 2008. Does arsenic exposure increase the risk for prostate cancer.? J. Toxicol. Environ. Health A 71, 1559–1563.