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Impact of *In Vitro* Heavy Metal Exposure on Pancreatic β -cell Function

E.N. Dover^{*}, Naishal Y Patel[†], and Miroslav Styblo[†]

^{*}Curriculum in Toxicology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA

[†]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, USA

Abstract

Susceptibility to type-2 diabetes mellitus (DM) is determined, in part, by a variety of environmental factors, including exposure to metals. Heavy metals including inorganic arsenic (iAs), zinc (Zn), manganese (Mn), and cadmium (Cd) have been reported to affect glucose homeostasis or DM risk in population-based and/or laboratory studies. Previous evidence from our lab has shown that iAs can increase DM risk by impairing mitochondrial metabolism, one of the key steps in the regulation of glucose-stimulated insulin secretion (GSIS) in pancreatic β -cells. The goal of the current study was to compare the effects of iAs on GSIS and mitochondrial function in INS-1 832/13 β -cells with those of Cd, Mn, and Zn, and to evaluate effects of binary mixtures of these metals. As expected, 24-hour exposure to iAs (arsenite, 1 μ M) significantly inhibited GSIS as did Cd (5 μ M) and Mn (12.5, 25, or 50 μ M). Zn had no effects on GSIS at concentrations up to 50 μ M. Mitochondrial function was assessed by measuring oxygen consumption rate (OCR) after glucose stimulation and during simulated mitochondrial stress. While both iAs and Mn impaired mitochondrial function (inhibiting OCR, maximal respiration, and/or spare respiratory capacity of mitochondria), no significant effects were found in cells exposed to Cd. Interestingly, no additive or synergistic effects on GSIS or OCR were observed in binary mixtures of iAs with either Mn or Cd. These data suggest that Mn, like iAs, may inhibit GSIS by impairing mitochondrial function, whereas Cd may target other mechanisms that regulate GSIS in β -cells.

Keywords

arsenic; diabetes; mixtures; mitochondria; cadmium; manganese

Corresponding author: Miroslav Styblo, PhD, Department of Nutrition, CB# 74612, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7461, USA, Phone: (919) 966-5721, Fax:(919) 843-0776, styblo@med.unc.edu.

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1. Introduction

The increasing prevalence of metabolic diseases, specifically type-2 diabetes mellitus (DM), has been attributed to a multitude of risk factors including genetic susceptibility, obesity, high-caloric diet, lack of physical activity, and high-blood pressure (Singh et al., 2010). Emerging studies have suggested that environmental exposure to naturally occurring or synthetic chemicals, which can act as obesogens or diabetogens, may also be a contributing factor (Heindel et al., 2017; Longnecker and Daniels, 2001; Sargis et al., 2012; Thayer et al., 2012). The classification of a diabetogen has recently expanded to include heavy metals like inorganic arsenic (iAs) and cadmium (Cd), naturally occurring and well-studied toxicants (Fénichel and Chevalier, 2017). A 2011 review panel assembled by the National Toxicology Program (NTP) established that there was sufficient evidence to link chronic, high-level exposure inorganic arsenic (iAs) (>150 ppb) with increased risk of DM (Maull et al., 2012). In human populations, exposure to iAs commonly occurs through drinking water and foods, which have been shown to also contain other heavy metals, including zinc (Zn), manganese (Mn), and Cd (Alam et al., 2003; Cherry et al., 2010; Sanders et al., 2014). Zn and Mn, are essential trace elements and micronutrients; however, both are toxic at high exposure levels (Goldhaber, 2003). Unlike Zn and Mn, iAs and Cd have no known physiological functions and are regulated by the Environmental Protection Agency with maximum contaminant level (MCL) values in drinking water of 0.01 mg/l and 0.005 mg/l, respectively (US EPA, 2015). Previous studies carried out by our laboratory and by others have demonstrated that iAs is a potent diabetogen, and growing evidence suggests that exposures to Zn, Cd, or Mn can also effect glucose metabolism and homeostasis (Khan and Awan, 2014).

Mn and Zn are critical for normal biological processes serving as cofactors of multiple metalloproteins and playing a role in insulin secretion (Bleackley and Macgillivray, 2011). For example, supplementation with Mn was shown to increase insulin secretion in mice fed a diabetogenic, high-fat diet (Lee et al., 2013), and Mn deficiency has been associated with hyperglycemia in rats (Baly et al., 1984). *In vitro* studies using pancreatic islets and β -cell lines have suggested that Zn plays an important role in the packaging and storage of insulin granules, and Zn supplementation has been shown to increase insulin secretion in a dose dependent manner (Li, 2014; Nygaard et al., 2014). In contrast, Cd can accumulate in human and mouse β -cells causing a significant reduction in insulin secretion (Muayed et al., 2012). Laboratory and epidemiologic studies have linked exposure to Cd with hypoinsulinemia, hyperglycemia, insulin resistance, and DM risk (Edwards and Ackerman, 2016; Tinkov et al., 2017). Like Cd, iAs exposure has been strongly correlated with the development of dysglycemia and DM in population studies (Maull et al., 2012). In laboratory studies, we have shown that iAs and its metabolites inhibit insulin signaling in adipose and liver (Paul et al., 2008, 2007; Walton et al., 2004; Zhang et al., 2017), but are even more potent as inhibitors of glucose-stimulated insulin secretion (GSIS) and mitochondrial function in pancreatic islets and INS-1 832/13 cells (Douillet et al., 2013; Dover et al., 2018). While the mechanisms underlying the diabetogenic effects of iAs have been extensively studied, little is known about the mechanism by which Zn, and particularly Cd and Mn alters glucose and insulin metabolism and action.

GSIS is a highly complex process that begins with glucose entry into the β -cell followed by metabolism of glucose in cytosolic and mitochondrial pathways. Many metals, including iAs, Cd, and Mn accumulate in mitochondria, and may thus impair the processes that are essential for GSIS (Meyer et al., 2013). Mitochondrial function, specifically mitochondrial oxidative phosphorylation paired with respiration (oxygen consumption), is essential for generating ATP in response to rising blood glucose levels (Maechler, 2013). The increase in ATP levels results in closure of ATP-sensitive potassium channels, membrane depolarization, calcium influx, and formation and exocytosis of insulin-containing vesicles (Rorsman et al., 2000). Thus, any impairment of mitochondrial function, as a result of heavy metal exposure, may also impact GSIS in β -cells. Notably, heavy metals are known to target mitochondrial metabolism even at low exposure levels. For example, Fu and colleagues observed a minor, but statistically significant, reduction in oxygen consumption rate (OCR) in glucose-stimulated rat insulinoma INS-1 832/13 β -cells exposed to sub-micromolar concentrations of arsenite (iAs^{III}), which was associated with inhibition of GSIS (Fu et al., 2010a). We have recently shown that sub-chronic, low-dose iAs^{III} and methylarsonite (MAs^{III}), a toxic methylated metabolite of iAs, inhibits OCR and GSIS in INS-1 832/13 cells (Dover et al., 2018). The other metals examined in the current study, Mn and Zn, are naturally found in mitochondria as stable co-factors in enzymes and proteins, but also in labile low-molecular-mass complexes that are involved in the metal trafficking and metalation of the apometalloproteins (Lindahl and Moore, 2016). Furthermore, Cd has been shown to accumulate preferentially in the mitochondria (Meyer et al., 2013), triggering a multitude of adverse events, inducing oxidative stress, lipid peroxidation, mitochondrial DNA mutation, aberrant gene expression, and ultimately causing apoptosis (Cannino et al., 2009). Therefore, the mitochondria are a likely target of heavy metal exposure, which could impact GSIS in β -cells. The goal of the present study was to examine the effects of sub-chronic, low-dose Cd, Mn, and Zn exposure on indicators of β -cell function and assess the effects of these metals alone and in binary mixtures with iAs^{III} .

2. Materials and Methods

2.1 Cell Culture and Treatment

Rat insulinoma cells expressing human pre-proinsulin, INS-1 832/13 (Hohmeier et al., 2000), passage numbers 45-60, were cultured at 5% CO_2 , 37°C in RPMI 1640 medium supplemented with 10% FBS, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin (all from Gibco, Waltham, MA), and 0.05 mM β -mercaptoethanol (Sigma, St. Louis, MO). INS-1 832/13 cells were exposed to iAs^{III} (sodium arsenite, >99% pure), ZnCl_2 (98% Pure), CdCl_2 (100% Pure), or MnCl_2 (99% Pure) (all from Sigma-Aldrich, St. Louis, MO) for 24 hours either alone or in binary mixtures as indicated in figure legends.

2.2 GSIS Assay

Twenty-four hours prior to treatment, INS-1 832/13 cells were seeded at a density of 1,000,000 cells/well in a 12-well plate. The cells were then exposed to iAs^{III} , Mn, Zn, or Cd, or to a mixture of iAs^{III} and Mn, iAs^{III} and Zn, or iAs^{III} and Cd for 24 hours prior to and during the GSIS assay. During the GSIS assay, cells were first incubated in secretion assay

buffer (SAB), (114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.16 mM MgSCo_4 , 20 mM HEPES, 2.5 mM CaCl_2 , 0.2% bovine serum albumin, and 25.5 mM NaHCCO_3), containing 0 mM glucose for 40 minutes, followed by incubation in 2.5 mM glucose SAB for 1 hour, and by a final incubation in 16.7 mM glucose SAB for 2 hours. The culture medium was collected at each time point. Insulin secreted into the medium was measured using a Rat/Mouse Insulin ELISA (Millipore, Billerica, MA). The amount of insulin was normalized for intracellular protein content determined using a bicinchoninic acid assay (Sigma, St. Louis, MO). Data are further normalized to control and expressed as percent of control in the high glucose condition.

2.3 Mitochondrial Respiration

INS-1 832/13 cells were seeded at a density of 50,000 cells/well in a 96 well Seahorse XFe plate 24 hours prior to treatment. Cells were then treated for 24 hours with iAs^{III} , Cd, and Mn alone or in combination, and treatment was continued throughout the respiration measurements. Following 24-hour metal treatment, cells were incubated in 0 mM glucose SAB for 40 minutes in a 5% CO_2 , 37°C incubator, followed by a 1 hour incubation in 2.5 mM glucose SAB (lacking NaHCO_3) at 37°C without CO_2 supplementation. Following an incubation in 2.5 mM glucose SAB, the culture plate was transferred to the Seahorse XFe96 machine (Agilent, Santa Clara, CA) and glucose (16.7 mM), oligomycin (2 μM), FCCP (Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone) (4 μM), and a rotenone/antimycin A mix (1 μM) were added sequentially. OCR was recorded for 30 minutes following glucose injection, and 18 minutes following injection of each of the other compounds. All compounds were purchased from Sigma-Aldrich (St. Louis, MO). This assay monitors the OCR increase in response to increased glucose levels in the culture, as well as respiration parameters under mitochondrial stress conditions, including basal respiration, maximal respiration, spare respiratory capacity, and non-mitochondrial respiration.

2.4 Cell Viability Assay

INS-1 832/13 cells were seeded at a density of 1,000,000 cells/well in a 12-well plate, 24 hours prior to treatment with iAs^{III} , Zn, Mn, or Cd. Following 24-hour metal treatment, cells were incubated in phenol red free RPMI medium containing 0.5 mg/ml MTT (3-(4,5-Dimethylthiazol,2-yl)-2,5-Deiphyltetrazolium Bromide) for 1 hour at 37°C, 5% CO_2 . Medium was then removed, cells were lysed by addition of 1 ml DMSO, and absorbance was recorded at 570 nM using a Synergy HT plate reader (Biotek, Winooski, VT).

2.5 Statistical analysis

All experiments were run with 2-3 biological replicates with up to 20 technical replicates (as specified in figure legends). Data generated by GSIS and MTT assays are expressed as mean + standard deviation (SD) for technical replicates and OCR data as mean + SD for biological replicates. Data were analyzed using a one (GSIS and MTT data) or two-way (OCR data) ANOVA with Dunnet's post-test comparison as indicated. Differences between treatments with $P < 0.05$ were considered statistically significant. Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software Inc., California).

3. Results

3.1 Effects of Heavy Metals on GSIS

GSIS was assessed in INS-1 832/13 cells following 24 hour exposure to Cd, Mn, iAs^{III}, or Zn. Exposure to 1 or 2 μ M Cd had no significant effects on insulin secretion, whereas 5 μ M Cd resulted in a significant, 47% decline in GSIS (Figure 1A). Exposure to 12.5, 25, or 50 μ M Mn also resulted in a significant, 57-64% decrease in insulin secretion (Figure 1B). As seen previously (Dover et al., 2018), 1 or 2 μ M iAs^{III} inhibited GSIS by more than 50% (Figure 1C). Surprisingly, exposures to up to 50 μ M Zn had no significant on GSIS (Figure 1D). The effects of iAs^{III} in binary mixture with Cd, Mn, and Zn was also examined, however the effects of mixtures were not significantly different from the effects of iAs^{III} exposure alone (Supplemental Figure 1).

3.2 Effects of Metal Exposures on OCR

OCR was examined after glucose stimulation following the GSIS assay conditions. OCR in cells exposed to Cd, Mn, or iAs^{III} was continuously measured in the presence of low glucose (2.5 mM) followed by measurements in high glucose (16.7 mM). Twenty-four hour treatment with Cd revealed minor effects on OCR; in cells exposed to 5 μ M Cd, OCR slightly decreased at both the 2.5 and 16.7 mM glucose levels, however this reduction did not reach statistical significance (Figure 2 A, B). INS-1 832/13 cells exposed to Mn for 24 hours exhibited no significant changes in OCR during the 2.5 mM glucose incubation, regardless of Mn concentration (Figure 2 C,D). However, exposure to 12.5, 25, or 50 μ M Mn resulted in significant reductions of OCR during the incubation with 16.7 mM glucose (Figure 2 C). In contrast, 24-hour exposure to 2 μ M iAs^{III} resulted in a significant reduction in OCR during both the 2.5 and 16.7 mM glucose incubations (Figure 2 E,F). In agreement with the GSIS data, effects of the binary mixtures of iAs^{III} with Cd or Mn on OCR did not differ from the effects of iAs^{III} alone (Supplemental Figure 2).

3.3 Effects of Metal Exposures on OCR During the Mitochondrial Stress Test

OCR was measured in control and metal-exposed, glucose-stimulated cells following sequential addition of oligomycin (inhibitor of ATP synthase), FCCP (an uncoupler), and rotenone/antimycin A (inhibitors of the electron transport chain complexes I and III, respectively). This design was used to assess additional parameters of mitochondrial respiration, including basal respiration, maximal respiration, spare respiratory capacity, and non-mitochondrial respiration (Supplemental Figure 3 and Supplemental Table 1). Exposure to Cd had no significant effect on any of these parameters (Figure 3). Exposure to Mn slightly decreased basal, maximal, and spare respiratory capacities, but these effects did not reach statistical significance (Figure 4). As observed in our previous study (Dover et al., 2018), exposure to 1 and 2 μ M iAs^{III} resulted in a significant reduction in maximal respiration and spare respiratory capacity (Figure 5 B,C). In accordance with GSIS and OCR data, effects of co-exposures to iAs^{III} with either Cd or Mn resembled those of iAs^{III} exposure alone (Supplemental Figure 4 and Supplemental Figure 5).

3.4 Effects of Metal Exposure on Cell Viability

To determine if the effects of metals on GSIS and OCR in INS-1 832/13 cells were due to reduced cell fitness or viability, an MTT assay was used which measures the reduction of a tetrazolium dye by cellular dehydrogenases (Berridge et al., 2005; Janjic and Wollheim, 1992). Exposure to Cd or Mn alone, at all concentrations examined, did not decrease cell viability (Figure 6 A, B). In contrast, exposure to 1 or 2 μM iAs^{III} alone reduced cell viability, but only by ~17-25% (Figure 6 C) while the same concentration of iAs^{III} inhibited GSIS by >50% (Figure 1C). A small, but statistically significant, reduction in cell viability was found only in cells exposed to 200 μM Zn (Figure 6 D). Significant decreases in viability were observed when Cd, Mn, or Zn were combined with iAs^{III} , but these effects were driven by iAs^{III} (Supplemental Figure 6).

4. Discussion

The rise of DM has been linked to many contributing factors; most recently, the impact of environmental toxicants on β -cell function and glucose homeostasis regulation has been a growing concern. Epidemiological studies have pointed to iAs^{III} as a diabetogen, showing that populations with moderate to high levels of iAs^{III} in drinking water have an overall higher prevalence or incidence of DM (Kuo et al., 2013; Maull et al., 2012; Sung et al., 2015; Wang et al., 2014). *In vitro* studies performed in our lab previously using the fast expanding cell line, INS-1 832/13, have shown that sub-chronic (24 hours or less) exposure to iAs^{III} results in inhibition of GSIS without causing a difference in cell viability (Dover et al., 2018). Other *in vitro* studies published by Fu et al. (Fu et al., 2010b) and our lab (Douillet et al., 2013) have shown that sub-chronic, low-level exposure to iAs^{III} inhibits GSIS in INS-1 832/13 cells and in isolated pancreatic islets without causing a deficit in insulin biosynthesis or impairing cell/islet viability. The results of these studies suggest that short-term, sub-chronic exposure is sufficient to illicit significant deleterious effects on insulin secretory pathways in these cell types. The previously mentioned laboratory studies are consistent with epidemiologic data, which suggest that exposure to iAs in drinking water is associated with impaired β -cell function rather than with insulin resistance (Del Razo et al., 2011; Gribble et al., 2012; Rhee et al., 2013). Taken together, population-based and laboratory studies point to β -cells as a target in iAs-induced DM, and a potential target of other heavy metal exposures as well.

In 2012, a study from Muayed and associates described inhibition of GSIS in murine pancreatic islets exposed for 48 hours to 0.1 μM Cd and in murine MIN6 pancreatic β -cells exposed to 1 μM Cd (Muayed et al., 2012). However, in the present study, we observed inhibition of GSIS in rat insulinoma INS-1 832/13 cells after 24-hour exposure to 5 μM Cd, while lower concentrations (1 or 2 μM) had no significant effects. This data suggests that the effects of Cd on β -cell function in *in vitro* studies depends on the timing of exposure and dosage, as well as on a cell type. While the mechanism for the inhibition of GSIS by Cd has not been systematically studied, there have been multiple reports that heavy metals, including Cd, accumulate in the mitochondria (Meyer et al., 2013). Mitochondrial metabolism is essential for insulin secretion since it generates the ATP necessary to close ATP-sensitive potassium channels, thus triggering membrane depolarization in response to

rising glucose levels. Dysregulation of energy metabolism in mitochondria has been proposed as one of the possible mechanisms by which Cd inhibits GSIS (Edwards and Ackerman, 2016); however, the exact target has not been identified. The present study found that while 5 μM Cd inhibited GSIS, it had no significant effects on either basal or glucose stimulated OCR or on other parameters of cellular/mitochondrial respiration in INS-1 832/13 cells. Thus, Cd must target other components of the pathway regulating GSIS in β -cells. These targets may include transporters involved in glucose uptake, calcium channels that mediate calcium influx after membrane depolarization, or structures involved in insulin exocytosis (Edwards and Ackerman, 2016). Notably, co-exposure to Cd did not modify the inhibition of GSIS or OCR by iAs^{III} , suggesting that iAs^{III} drives the effects of the binary mixture.

Manganese is a well-characterized essential metal and micronutrient, and the pancreas is among the organs with the highest Mn concentrations (Watts, 1990). However, while it is necessary for proper function of multiple enzymes, overexposure to Mn can result in serious health consequences (Crossgrove and Zheng, 2004). Interestingly, Mn deficiency has been associated with impaired glucose homeostasis and a DM-like phenotype (Kazi et al., 2008). Additional evidence has shown that Mn supplementation can protect against high fat diet-induced DM in mice by increasing insulin secretion and improving glucose tolerance, possibly through metalation of Mn-superoxide dismutase and suppression of oxidative stress in the mitochondria (Lee et al., 2013). However, in the present study, exposure to Mn at concentrations as low as 12.5 μM resulted in a significant inhibition of GSIS in INS-1 832/13 cells. Notably, a similar Mn concentration (80 $\mu\text{mol Mn/kg}$ dry weight; i.e., $\sim 16 \mu\text{M}$ in intact tissue) was found in pancreatic islets of *ob/ob* mice, the laboratory model for obesity-linked DM (Rorsman et al., 1982). Since Mn accumulates in the mitochondria (Brown and Taylor, 1999; Gunter and Puskin, 1975; Zhang et al., 2003), and mitochondria are critically important to the insulin secretory pathway, we tested the effects of Mn on OCR as a proxy for mitochondrial function. Mn caused significant deficits in GSIS, and also inhibited OCR during incubation with 16.7 mM glucose, but did not affect other respiratory parameters. In addition, there was no apparent interaction between Mn and iAs^{III} during exposures to the binary mixture in any parameter examined. The extent and dose-response pattern for GSIS inhibition in cells exposed to the binary mixtures of Mn and iAs^{III} suggest that, unlike for the mixture of Cd and iAs^{III} , the inhibition of GSIS was driven primarily by Mn. Conversely, the inhibition of mitochondrial respiration in cells exposed to Mn and iAs^{III} mixtures was driven by iAs^{III} . Overall, it appears that although Mn impairs mitochondrial respiration, it may also target other mechanisms that regulate GSIS in INS-1 832/13 cells. Thus, future studies should investigate other potential mechanisms, including the effects of Mn on insulin biosynthesis or on steps involved in insulin packaging and exocytosis.

Pancreatic β -cells have exceptionally high Zn content with the highest, millimolar concentrations found specifically in insulin secretory granules (Davidson et al., 2014). Thus, Zn has been suggested to play a significant role in insulin production, packaging, translocation, and/or secretion (Li, 2014; Nygaard et al., 2014). However, in the present study, exposures to up to 50 μM Zn did not stimulate GSIS in INS-1 832/13 cells and did not alleviate the inhibition of GSIS or mitochondrial respiration caused by iAs^{III} exposure. It is possible that even higher concentrations of Zn are needed to reach the critical intracellular

Zn concentrations that stimulate GSIS in this type of β -cell, or that these cells lack the islet-specific Zn transporters, which have been positively correlated with GSIS in previous studies (Huang et al., 2017; Liu et al., 2015).

While iAs appears to have a significant impact on the development of diabetes, it is not the only metal contaminant found in drinking water or food worldwide. Multiple studies have pointed to the co-occurrence of iAs and other heavy metals, some of which have also been linked to altered glucose homeostasis, including the three metals examined in this study Cd, Zn, and Mn (Cobbina et al., 2015; Jadhav et al., 2007; Sanders et al., 2014). Thus, examining the diabetogenic effects of metal mixtures, rather than effects of a single metal, is needed to properly characterize the DM risk associated with real-world metal exposures. The present study was the first step in this direction, but found no significant effect of the examined metal mixtures on GSIS or mitochondrial function.

In summary, results of the present study contribute to the ongoing effort to examine the diabetogenic effects (and other adverse effects) of metal mixtures that are commonly found in the environment. This study is the first to examine side-by-side effects of *in vitro* exposures to iAs^{III}, Cd, Zn, and Mn, alone and in binary mixtures on GSIS in INS-1 832/13 cells, a β -cell line that has been frequently used in studies examining regulation of insulin secretion. Results indicate that, like iAs^{III} and Mn, non-cytotoxic concentrations of Cd inhibit GSIS; however, unlike iAs^{III} and Mn, Cd does not significantly interfere with the mitochondrial respiration pathway, which plays a key role in GSIS regulation. Thus, Cd is likely to target other mechanisms or structures that are involved in insulin secretion from glucose-stimulated β -cells. Notably, no additive or synergistic effects on GSIS were found in INS-1 832/13 cells exposed to binary mixtures of iAs^{III}/Cd or iAs^{III}/Mn at the tested concentrations. Future studies should examine the metal content of the cells exposed to Cd, Mn, Zn, and iAs to determine intracellular metal concentration in both single and binary mixtures, which could explain the lack of Zn effect. Additionally, further research on mechanisms underlying GSIS inhibition by Cd and Mn will be needed to explain this observation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Heavy metal exposure (Cd, Mn and iAs) inhibits glucose stimulated insulin secretion
- Oxygen consumption rate is inhibited by iAs and Mn in INS-1 832/13 cells
- Cd has no significant effect on oxygen consumption rate in INS-1 832/13 cells
- Zn exposure doesn't inhibit either GSIS or oxygen consumption rate at levels tested
- Cd, Mn, and iAs impair β -cell function, but appear to do so by different mechanisms

Figure 1: Glucose Stimulated Insulin Secretion in INS-1 832/13 Cells after 24-hour Exposures to Cd (A), Mn (B), iAs^{III} (C) or Zn (D).

Insulin secretion was measured following stimulation with 2.5 mM or 16.7 mM glucose.

Data are represented as Mean + SD for 3 or more technical replicates. ++ $p < 0.01$ treatment versus control high glucose.

Figure 2: Oxygen Consumption Rate (OCR) in INS-1 832/13 cells after 24-hour exposure to Cd, Mn, or iAs^{III}.

OCR was measured in INS-1 832/13 cells stimulated with 2.5 or 16.7 mM glucose following 24 hour exposure to Cd (A,B), Mn (C,D) or iAs^{III} (E,F). OCR was recorded in cells stimulated with low glucose (2.5 mM) followed by high glucose (16.7 mM) (line charts). OCR values were averaged to quantify the overall effects of exposure (bar charts). Mean + SD is shown for 3 biological replicates. * $p < 0.05$ for treatment versus control, ++ $p < 0.01$ for treatment versus control.

Figure 3: Mitochondrial Respiration Parameters of INS-1 832/13 cells after 24-hour exposure to Cd.

OCR was measured in INS-1 832/13 cells following 24-hour exposure to Cd. Basal respiration (A), Maximal Respiration (B), Spare Respiratory Capacity (C), and Non-Mitochondrial Respiration (D) were measured following sequential addition of 16.7 mM glucose, oligomycin, FCCP, and a rotenone/Anitmycin A mix. Mean + SD is shown for 3 biological replicates.

Figure 4: Mitochondrial Respiration Parameters of INS-1 832/13 cells after 24-hour exposure to Mn.

OCR was measured in INS-1 832/13 cells following 24-hour exposure to Mn. Basal respiration (A), Maximal Respiration (B), Spare Respiratory Capacity (C), and Non-Mitochondrial Respiration (D) were measured following sequential addition of 16.7 mM glucose, oligomycin, FCCP, and a rotenone/Anitmycin A mix. Mean + SD is shown for 3 biological replicates.

Figure 5: Mitochondrial Respiration Parameters of INS-1 832/13 cells after 24-hour exposure to iAs^{III}.

OCR was measured in INS-1 832/13 cells following 24-hour exposure to iAs^{III}. Basal respiration (A), Maximal Respiration (B), Spare Respiratory Capacity (C), and Non-Mitochondrial Respiration (D) were measured following sequential addition of 16.7 mM glucose, oligomycin, FCCP, and a rotenone/Anitmycin A mix. Mean + SD is shown for 3 biological replicates. + $p < 0.05$ treatment vs control; ++ $p < 0.01$ treatment vs control.

Figure 6: Cell Viability in INS-1 832/13 cells after 24-hour exposure to Cd, Mn, iAs^{III}, or Zn. Cell viability was measured in INS-1 832/13 cells following 24-hour exposure to Cd (A), Mn (B), iAs^{III} (C), or Zn (D) using an MTT assay. Mean + SD is shown for 4 or more technical replicates. + $p < 0.05$ treatment versus control, ++ $p < 0.05$ treatment versus control.