Accumulation of cadmium in insulin-producing β cells

Malek El Muayed,1,* Meera R. Raja,2 Xiaomin Zhang,1 Keith W. MacRenaris,2 Surabhi Bhatt,1 Xiaojuan Chen,4 Margrit Urbanek,1 Thomas V. O’Halloran2 and William L. Lowe, Jr.1

1Division of Endocrinology; Metabolism and Molecular Medicine; Northwestern University Feinberg School of Medicine; Chicago, IL USA; 2The Chemistry of Life Processes Institute and Department of Chemistry; Northwestern University; Evanston, IL USA; 3Division of Transplant Surgery; Northwestern University; Feinberg School of Medicine; Chicago, IL USA; 4Columbia Center for Translational Immunology; Columbia University College of Physicians and Surgeons; New York, NY USA

*Correspondence to: Malek El Muayed; Email: m-muayed@northwestern.edu
Submitted: 09/10/12; Revised: 12/02/12; Accepted: 12/03/12
http://dx.doi.org/10.4161/isl.23101

Evidence suggests that chronic low level cadmium exposure impairs the function of insulin-producing β cells and may be associated with type-2 diabetes mellitus. Herein, we describe the cadmium content in primary human islets and define the uptake kinetics and effects of environmentally relevant cadmium concentrations in cultured β cells. The average cadmium content in islets from 10 non-diabetic human subjects was 29 ± 7 nmol/g protein (range 7 to 72 nmol/g protein). Exposure of the β-cell line MIN6 to CdCl2 concentrations between 0.1 and 1.0 µmol/L resulted in a dose- and time-dependent uptake of cadmium over 72 h. This uptake resulted in an induction of metallothionein expression, likely enhancing cellular cadmium accumulation. Furthermore, cadmium accumulation resulted in an inhibition of glucose stimulated insulin secretion in MIN6 cells and primary mouse islets. Our results indicate that this impairment in β-cell function is not due to an increase in cell death or due to an increase in oxidative stress. We conclude that mouse β cells accumulate cadmium in a dose- and time-dependent manner over a prolonged time course at environmentally relevant concentrations. This uptake leads to a functional impairment of β-cell function without significant alterations in cell viability, expression of genes important for β-cell function or increase in oxidative stress.

Keywords: cadmium, zinc transporters, metallothionein, insulin secretion, β cells

Introduction

Impaired function of insulin-producing pancreatic β cells in the setting of insulin resistance is the main underlying cause of type-2 diabetes. β-cell dysfunction is thought to be multifactorial, with the interaction of genetic susceptibility, age, lifestyle, insulin resistance and environmental factors leading to progressive β-cell failure. Some evidence suggests that chronic low-level exposure to cadmium (Cd) in the environment may be associated with an increased risk for developing dysglycemia and diabetes mellitus.1-3 From a biological standpoint, Cd and to a lesser degree mercury (Hg) are of interest in the biology of insulin-secreting β cells given their similarity to zinc (Zn) and the importance of Zn in β-cell physiology. The divalent metals Cd and Hg are members of the same group as Zn in the periodic table and therefore exhibit similar chemical characteristics. In multiple cell types, Cd2+, and to a lesser degree Hg2+ have been shown to compete with Zn2+ for several of its binding proteins and transporters.3-10 β cells have high intracellular concentrations of Zn, particularly within secretory vesicles, where zinc facilitates the packaging of insulin into hexamers and may play a role in the processing of pro-insulin into mature insulin.11-13 Given that Zn is co-secreted with insulin, β cells have to maintain a high turnover of Zn, shuttling large quantities of Zn through the cytoplasm into the secretory vesicle to replenish Zn co-secreted with insulin.16,17 Given the above we hypothesized that β cells may be more susceptible to the accumulation and potential toxicity of this group of divalent metals than other cell types. Therefore, our initial studies sought to determine the content of Cd and Hg in primary human islets from non-diabetic subjects. Additionally, we examined the accumulation kinetics of CdCl2 and HgCl2 in mouse β cells. These initial studies showed a significantly higher native content of Cd in human islets samples compared with Hg. Furthermore, mouse islets accumulated Cd more avidly than Hg. We therefore conducted more detailed studies to examine the accumulation kinetics and physiological effects of low level Cd exposure in insulin-producing β cells.

Results

Concentration of divalent metals in human islet. Low-level exposure to Hg and Cd is widely prevalent. At the same time, the divalent ions of these metals have been shown to compete with Zn transport and buffering mechanisms. Given this, our initial studies examined the content of the heavy metals Cd and Hg in human islets. Additional metals including Zn, copper (Cu) and nickel (Ni) were measured in order to validate our methods by comparing their expected concentration ratios with previously published reports. The concentration of Cd in islet samples was significantly higher than that of Hg (Table 1). Variations in
with lysate Cd concentrations of 422 ± 32 and 4125 ± 617 nmol/g protein respectively. For comparison, the mouse 3T3 fibroblasts accumulated Cd to a significantly lower concentration of 188 ± 16 and 1608 ± 26 nmol/g of protein under the same conditions.

We then performed studies to determine the time course of Cd accumulation. After exposure to 0.1 μmol/L CdCl₂ for 1, 2, 4, 8, 24, 48 and 72 h, MIN6 and 3T3 cells accumulated Cd in a time dependent manner (Fig. 1C). The total amount of Cd accumulated as well as the accumulation rate in 3T3 cells was significantly lower than that of MIN6 cells.

No significant changes in Zn concentration were seen in MIN6 cells following exposure to CdCl₂ except for a trend toward a higher Zn concentration following exposure to the higher CdCl₂ concentration of 1.0 μmol/L, likely related to an upregulation of the Zn buffering protein metallothionein (see below). MIN6 cell Zn concentrations following exposure to 0, 0.1, or 1.0 μmol/L CdCl₂ were 4005 ± 251, 3933 ± 676 and 7702 ± 3344 nmol/g protein, respectively (Fig. 1D).

mRNA levels of ZnT and ZIP class Zn transporters and DMT-1 in MIN6 and 3T3 cells. Cd transport into cells may be mediated, in part, by some members of the family of Zn transporters such as SLC39A8 (ZIP8), SLC39A14 (ZIP14) and divalent metal content were noted between the 10 human islet samples examined, suggesting a substantial influence of environmental exposure and other parameters such as genetic variations on islet cell metal content. Metal content in solutions used in the isolation, purification, culture and transport of islets contained no significant quantities of the metals of interest Hg and Cd (Table S1).

Accumulation kinetics of Hg and Cd. Next, we set out to confirm the higher accumulation capacity of β cells to Cd compared with Hg. To this end, the mouse β-cell line MIN6 was exposed to CdCl₂ or HgCl₂ 0.1 μmol/L for 48 h. The cells accumulated significantly more Cd compared with Hg under these circumstances to concentrations of 437 ± 69 and 139 ± 6 nmol/g protein respectively (Fig. 1A). Given this result and the higher Cd concentration in primary human islets, subsequent studies focused on examining the uptake kinetics and effects of Cd accumulation. In order to compare the uptake of Cd into β cells to that in a non-β-cell line, uptake in MIN6 cells was compared with that in the mouse fibroblast cell line 3T3. MIN6 cells and 3T3 cells were grown in MIN6 medium and exposed to final CdCl₂ concentrations of 0, 0.1 or 1 μmol/L for 72 h. Both cells lines accumulated Cd in a dose dependent manner (Fig. 1B). MIN6 cells exposed to 0.1 and 1.0 μmol/L CdCl₂ for 72 h accumulated Cd with lysate Cd concentrations of 422 ± 32 and 4125 ± 617 nmol/g protein respectively. For comparison, the mouse 3T3 fibroblasts accumulated Cd to a significantly lower concentration of 188 ± 16 and 1608 ± 26 nmol/g of protein under the same conditions.

We then performed studies to determine the time course of Cd accumulation. After exposure to 0.1 μmol/L CdCl₂ for 1, 2, 4, 8, 24, 48 and 72 h, MIN6 and 3T3 cells accumulated Cd in a time dependent manner (Fig. 1C). The total amount of Cd accumulated as well as the accumulation rate in 3T3 cells was significantly lower than that of MIN6 cells.

No significant changes in Zn concentration were seen in MIN6 cells following exposure to CdCl₂ except for a trend toward a higher Zn concentration following exposure to the higher CdCl₂ concentration of 1.0 μmol/L, likely related to an upregulation of the Zn buffering protein metallothionein (see below). MIN6 cell Zn concentrations following exposure to 0, 0.1, or 1.0 μmol/L CdCl₂ were 4005 ± 251, 3933 ± 676 and 7702 ± 3344 nmol/g protein, respectively (Fig. 1D).

mRNA levels of ZnT and ZIP class Zn transporters and DMT-1 in MIN6 and 3T3 cells. Cd transport into cells may be mediated, in part, by some members of the family of Zn transporters such as SLC39A8 (ZIP8), SLC39A14 (ZIP14) and

### Table 1. Concentration of Cd, Hg, Cu, Ni and Zn in viable human islets from 10 non-diabetic subjects

| Subject # (age, gender, BMI) | Cd         | Hg         | Cu          | Ni          | Zn          |
|-----------------------------|------------|------------|-------------|-------------|-------------|
| Human islets #1 (28 y, m, BMI 24) | 16.1 nmol/g protein | unavailable | unavailable | unavailable | unavailable |
| Human islets #2 (27 y, m, BMI 26) | 7.4 nmol/g protein | unavailable | unavailable | unavailable | unavailable |
| Human islets #3 (no data available) | 9.5 nmol/g protein | unavailable | unavailable | unavailable | unavailable |
| Human islets #4 (62 y, m, BMI 18.8) | 23.6 nmol/g protein | 5.0 nmol/g protein | 1397.0 nmol/g protein | 207.6 nmol/g protein | 4656.5 nmol/g protein |
| Human islets #5 (54 y, f, BMI 28.4) | 15.8 nmol/g protein | 4.6 nmol/g protein | 470.7 nmol/g protein | 228.6 nmol/g protein | 18061.2 nmol/g protein |
| Human islets #6 (35 y, m, BMI 46.1) | 23.8 nmol/g protein | < 4.00 nmol/g protein | 505.1 nmol/g protein | 153.5 nmol/g protein | 10981.7 nmol/g protein |
| Human islets #7 (52 y, f, BMI 29.2) | 51.90 nmol/g protein | < 4.0 nmol/g protein | 307.0 nmol/g protein | 169.7 nmol/g protein | 10432.7 nmol/g protein |
| Human islets #8 (44 y, f, BMI 22.50) | 19.1 nmol/g protein | < 4.0 nmol/g protein | 105.7 nmol/g protein | 32.8 nmol/g protein | 3645.7 nmol/g protein |
| Human islets #9 (39 y, f, BMI 29.80) | 48.5 nmol/g protein | < 4.0 nmol/g protein | 249.6 nmol/g protein | 110.7 nmol/g protein | 29861.5 nmol/g protein |
| Human islets #10 (56 y, f, BMI 25.20) | 71.9 nmol/g protein | < 4.0 nmol/g protein | 328.9 nmol/g protein | 66.6 nmol/g protein | 16916.4 nmol/g protein |
| Average human islet content | 28.8 ± 6.7 nmol/g protein | < 4.0 nmol/g protein | 480.6 ± 160.9 nmol/g protein | 138.5 ± 27.3 nmol/g protein | 13508.0 ± 3420.0 nmol/g protein |
| Negative control: Cultured MIN6 cells (n = 4) | < 4.0 nmol/g protein | < 4.0 nmol/g protein | 266.8 ± 36.6 nmol/g protein | 58.1 ± 15.5 nmol/g protein | 3813.2 ± 560.2 nmol/g protein |

Values are reported as μmol/gram lysate protein. Values in cultured MIN6 cells are listed for comparison. Values for solution used in the isolation, purification and transport of islets are reported in the supplement.
metallothionein (MT), the main known intracellular Cd buffering protein, is increased following exposure to $0.1 \mu\text{mol/L}$ CdCl$_2$. MT expression was determined using Western blot analysis following exposure of MIN6 cells to CdCl$_2$ $0.1 \mu\text{mol/L}$ for 30 min or 1, 2, 4, 8, 24, 48, or 72 h (Fig. 2A). Increased MT expression was observed beginning at the 24 h time point and remained stable over the ensuing 48 h. The increased expression of MT following exposure to CdCl$_2$ $0.1 \mu\text{mol/L}$ was confirmed in primary mouse islets by Western blot (Fig. 2B) and quantitative real time PCR (Fig. 2C) with MT protein and MT2 mRNA both showing significant increases. Contrary to prior reports showing upregulation of both MT2 and MT1 expression in various mouse tissues following Cd exposure, 27 we did not observe a significant increase in MT1 mRNA levels.

Effect of Cd exposure on glucose stimulated insulin secretion in MIN6 cells. In static incubation experiments, exposure of MIN6 cells to CdCl$_2$ concentrations of 0.1, 0.5 and 1.0 $\mu\text{mol/L}$ for 48 h only showed a trend toward a concentration dependent decrease in glucose stimulated insulin secretion (GSIS) with GSIS ratios of $5.6 \pm 0.8$, $2.8 \pm 1.0$ and $1.4 \pm 0.1$, respectively, compared with $5.6 \pm 0.6$ in the control ($p < 0.05$ for 1.0 $\mu\text{mol/L}$ CdCl$_2$ compared with control, Fig. 3A).

SLC30A1 (ZnT1). 6,8,18-20 Additionally, some reports suggest a role for the divalent metal transporter-1 (DMT-1) in cellular Cd influx in some cell types. 21,22 To begin to determine possible reasons for the difference in Cd accumulation in MIN6 and 3T3 cells, expression of the genes encoding members of the ZIP and ZnT classes of Zn transporters as well as DMT-1 in MIN6, primary mouse islets and 3T3 cells was determined. As expected, 3T3 cells had lower overall levels of mRNAs encoding Zn transporters of the SLC39 (ZIP) and SLC30 (ZnT) family compared with MIN6 and primary mouse islet cells (Table 2). Moreover, ZnT8 (encoded by slc30A8), which was previously shown to be important for Zn transport into insulin secretory vesicles in β cells11,23-26 was highly expressed in MIN6 and primary mouse islet cells but not expressed in 3T3 cells. Other transporters that were noted to have a higher expression level in MIN6 and islet cells compared with 3T3 cells included SLC39A5 (ZIP5), SLC30A3 (ZnT3), SLC30A2 (ZnT2) as well as DMT-1. Levels of SLC39A8 (ZIP8), one of the transporters thought to potentially mediate Cd transport, were higher in MIN6 cells compared with both primary islets cells and 3T3 cells.

Changes in metallothionein (MT) expression in response to exposure to Cd. Next we examined whether expression of metallothionein (MT), the main known intracellular Cd buffering protein, is increased following exposure to $0.1 \mu\text{mol/L}$ CdCl$_2$. MT expression was determined using Western blot analysis following exposure of MIN6 cells to CdCl$_2$ $0.1 \mu\text{mol/L}$ for 30 min or 1, 2, 4, 8, 24, 48 or 72 h (Fig. 2A). Increased MT expression was observed beginning at the 24 h time point and remained stable over the ensuing 48 h. The increased expression of MT following exposure to CdCl$_2$ $0.1 \mu\text{mol/L}$ was confirmed in primary mouse islets by Western blot (Fig. 2B) and quantitative real time PCR (Fig. 2C) with MT protein and MT2 mRNA both showing significant increases. Contrary to prior reports showing upregulation of both MT2 and MT1 expression in various mouse tissues following Cd exposure, 27 we did not observe a significant increase in MT1 mRNA levels.
Effect of Cd exposure in dispersed primary mouse islet cells. Having established that MIN6 cells accumulate Cd followed by inhibition of GSIS, we sought to determine whether Cd had a similar effect on primary mouse islets. We therefore examined the effect of Cd exposure on GSIS in primary dispersed mouse islets. A non-significant trend for decreased GSIS was observed following exposure of dispersed mouse islet cells to CdCl$_2$ 0.1 µmol/L for 8 h with a decrease in GSIS from 1.7 ± 0.2 to 1.4 ± 0.3 in exposed cells. However, exposure of dispersed primary mouse islets to CdCl$_2$ 0.1 µmol/L for 48 h resulted in a significant decrease in GSIS to 1.0 ± 0.04 (Fig. 3B).

Effect of Cd exposure on the viability of dispersed primary mouse islet cells. To rule out generalized cell toxicity as the cause of the Cd-induced decrease in GSIS, the viability of dispersed mouse islet cells following exposure to 0.1 µmol/L CdCl$_2$ for 8 and 48 h was examined. Significant changes in cell viability at these time points were not observed. The cell viability index at 8 and 48 h was 809 ± 86 and 849 ± 100 compared with the control (783 ± 111, Fig. 4A). There was also no change in expression of the heavy metal toxicity marker Heat Shock Protein 70 (HSP70) at 48 h as determined by Western blot analysis (Fig. 4B).

No effect of Cd exposure on the expression of genes most relevant to β-cell function in dispersed primary murine islets. To examine the effect of Cd exposure on the expression of genes central to normal β-cell function as well as Zn homeostasis in primary mouse islets, the expression of the genes encoding Insulin 1 and 2 (INS-1 and -2), MAFA, MAFB, was examined following exposure to 0.1 µmol/L CdCl$_2$ for 48 h. Given prior reports of altered regulation of genes relevant to β cell Zn and, possibly, Cd homeostasis, namely SLC39A10 (encoding ZIP10) and SLC30A1 (encoding ZnT1),$^{19,26,29}$ we also examined the expression of these genes. Additionally, we examined the effect of exposure to CdCl$_2$ 0.1 µmol/L on the mRNA level of SLC30A8 (ZnT8) and DMT-1 mRNA. All the genes examined showed no significant difference in mRNA expression levels apart from the increase in MT2 expression as noted above (Fig. S1).

Effect of Cd exposure on oxidative stress in dispersed mouse islets and MIN6 cells. To examine the influence of Cd accumulation on cellular oxidative stress, we determined the levels of GSH, GSSG and the ratio of GSH/ GSSG in MIN6 cells and primary dispersed mouse islets following exposure to various CdCl$_2$ concentrations for 48 h. In MIN6 cells, these studies

| Table 2. Relative mRNA expression levels of Zn transporters of the ZnT and ZIP class as well as DMT-1 in various cells |
|---------------------|---------------------|---------------------|
| **Mouse islets**    | **MIN6 cells**      | **3T3 cells**       |
| **Mean ± SEM**      | **Mean ± SEM**      | **Mean ± SEM**      |
| ZIP1 8.40 ± 10−03 ± 1.07 × 10−02 | 1.47 ± 10−03 ± 6.83 × 10−04 | 6.73 ± 10−03 ± 3.57 ± 10−03 |
| ZIP2 4.94 ± 10−03 ± 1.27 × 10−05 | 3.72 ± 10−05 ± 1.26 × 10−05 | 1.27 ± 10−05 ± 1.37 ± 10−05 |
| ZIP3 4.83 ± 10−03 ± 2.61 × 10−04 | 2.94 ± 10−03 ± 5.42 × 10−04 | 2.02 ± 10−03 ± 2.14 × 10−04 |
| ZIP4 7.05 ± 10−04 ± 3.31 × 10−04 | 2.21 ± 10−05 ± 2.21 × 10−05 | 1.50 ± 10−05 ± 1.05 × 10−05 |
| ZIP5 5.14 ± 10−04 ± 1.52 × 10−04 | 1.29 ± 10−05 ± 1.29 × 10−05 | 1.77 ± 10−07 ± 5.74 × 10−08 |
| ZIP6 9.84 ± 10−04 ± 1.76 × 10−04 | 1.07 ± 10−05 ± 2.27 × 10−04 | 2.76 ± 10−05 ± 1.92 ± 10−05 |
| ZIP7 2.13 ± 10−04 ± 4.76 × 10−05 | 1.04 ± 10−05 ± 1.31 × 10−02 | 4.81 ± 10−04 ± 1.80 ± 10−04 |
| ZIP8 1.33 ± 10−03 ± 2.30 × 10−04 | 1.95 ± 10−02 ± 3.06 × 10−03 | 7.15 ± 10−03 ± 3.45 × 10−04 |
| ZIP9 4.36 ± 10−03 ± 1.40 × 10−03 | 3.78 ± 10−03 ± 6.17 × 10−04 | 4.21 ± 10−03 ± 2.39 × 10−04 |
| ZIP10 5.64 ± 10−04 ± 1.37 × 10−04 | 1.28 ± 10−03 ± 1.37 × 10−04 | 4.29 ± 10−03 ± 1.17 × 10−04 |
| ZIP11 3.77 ± 10−03 ± 1.37 × 10−03 | 2.89 ± 10−04 ± 2.90 × 10−06 | 1.49 ± 10−03 ± 1.15 ± 10−04 |
| ZIP12 3.33 ± 10−03 ± 3.33 × 10−03 | 3.33 ± 10−06 ± 3.33 × 10−06 | 1.14 ± 10−07 ± 1.41 × 10−08 |
| ZIP13 8.59 ± 10−03 ± 2.68 × 10−03 | 3.56 ± 10−03 ± 4.33 × 10−04 | 6.21 ± 10−03 ± 6.68 × 10−04 |
| ZIP14 2.35 ± 10−03 ± 2.58 × 10−05 | 1.49 ± 10−03 ± 2.81 × 10−04 | 3.01 ± 10−03 ± 1.71 × 10−04 |
| ZnT1 4.38 ± 10−03 ± 1.71 × 10−03 | 2.79 ± 10−03 ± 4.10 × 10−04 | 1.34 ± 10−03 ± 7.04 × 10−04 |
| ZnT2 4.88 ± 10−04 ± 7.66 × 10−05 | 1.29 ± 10−05 ± 1.08 × 10−05 | 2.34 ± 10−06 ± 1.92 × 10−06 |
| ZnT3 1.82 ± 10−03 ± 1.41 × 10−03 | 5.68 ± 10−06 ± 3.17 × 10−06 | 6.95 ± 10−06 ± 8.89 × 10−03 |
| ZnT4 1.75 ± 10−03 ± 1.34 × 10−04 | 2.27 ± 10−03 ± 1.14 × 10−04 | 1.68 ± 10−02 ± 5.13 × 10−03 |
| ZnT5 1.37 ± 10−03 ± 2.28 × 10−03 | 2.60 ± 10−03 ± 3.27 × 10−03 | 7.75 ± 10−03 ± 3.61 × 10−04 |
| ZnT6 5.64 ± 10−03 ± 8.13 × 10−04 | 5.20 ± 10−03 ± 7.52 × 10−04 | 9.87 ± 10−03 ± 3.96 × 10−04 |
| ZnT7 1.01 ± 10−03 ± 5.94 × 10−04 | 5.82 ± 10−03 ± 8.71 × 10−04 | 1.62 ± 10−02 ± 2.79 × 10−04 |
| ZnT8 5.76 ± 10−02 ± 2.33 × 10−02 | 1.16 ± 10−01 ± 1.89 × 10−02 | 0.00 ± 0.00 |
| ZnT9 2.59 ± 10−03 ± 6.62 × 10−04 | 2.86 ± 10−03 ± 2.01 × 10−04 | 8.00 ± 10−03 ± 6.33 × 10−05 |
| ZnT10 0.00 ± 0.00 | 0.00 ± 0.00 | 4.52 ± 10−07 ± 3.06 × 10−07 |
| DMT-1 2.56 ± 10−02 ± 6.98 × 10−03 | 3.38 ± 10−02 ± 1.90 × 10−02 | 9.56 ± 10−03 ± 9.22 × 10−05 |

mRNA levels of Zn transporters of the ZnT and ZIP families as well as DMT-1 in primary mouse islets, MIN6 cells and 3T3 relative to the house keeping genes β actin and 18S (mean ± SEM, n = 3).
showed evidence for mild oxidative stress only following exposure to a CdCl₂ concentration of 1.0 μmol/L, but not at the lower CdCl₂ concentrations of 0.1 and 0.5 μmol/L. This increase in oxidative stress was evident from a significant decrease of GSH in cells exposed to 1.0 μmol/L to 64.7 ± 9% of the GSH level in untreated control cells (p < 0.05, Fig. 5A). There was also a non-significant trend toward an increase in the GSSG/GSH ratio from 0.05 ± 0.003 in the untreated control to 0.08 ± 0.006 under the same conditions. In contrast, there was no evidence for an increase in oxidative stress markers in dispersed primary mouse islet cells following exposure to CdCl₂ at concentrations of 0.1 and 0.5 μmol/L. Surprisingly, the GSSG/GSH ratio in these cells decreased significantly at both CdCl₂ concentrations from 0.31 ± 0.015 in the control cells to 0.22 ± 0.005 and 0.19 ± 0.001 in cells exposed to 0.1 and 0.5 μmol/L CdCl₂, respectively, likely indicating an upregulation of cellular protective mechanisms (p < 0.05, Fig. 5B). Measurement of mRNA levels of the cellular oxidative response factors GCLC, GCLM and HMOX1 showed an increase of GCLM and HMOX1 mRNA levels only in MIN6 cells exposed to 1.0 μmol/L of CdCl₂ (p < 0.05, Fig. 5C). No changes were observed in dispersed primary mouse islet cells exposed to 0.1 μmol/L CdCl₂ for 48 h (Fig. 5D).

**Discussion**

Zinc (Zn) is the second most abundant transition metal in mammals and is utilized in numerous cellular processes. Zn is of particular importance in the physiology of insulin-producing β cells given its unique role in this cell type. β Cells have high intracellular concentrations of Zn, particularly within secretory vesicles, where zinc facilitates the packaging of insulin into hexamers. Given that Zn is co-secreted with insulin, β cells are required to maintain a high Zn turnover rate. In order to achieve this, β cells require the trafficking of Zn across cell and vesicular membranes. This is thought to occur mainly through members of the Zinc-Transporter (ZnT, SLC30A) and Zrt-Irt-like Protein (ZIP, SLC39A) transporter families. ZnTs and ZIPs facilitate Zn diffusion out of or into the cytoplasm, respectively. Within the cell, Zn is mostly bound to buffering proteins, of which metallothionein (MT) is thought to have the highest capacity, thereby acting as a buffer and reservoir for intracellular Zn. Zn, Cd and Hg belong to the same group of divalent metals in the periodic table and have similar chemical properties. It is therefore not surprising that Cd and—to a lesser extent—Hg have been shown to compete with Zn for transporters of the ZnT and ZIP families as well as for binding to MT. Kinetic and competition studies by He et al. showed that ZIP8 has a high capacity to transport Cd but a lower capacity for Hg transport. Although we do not have conclusive data from knockout or knockdown models, it is conceivable that Zn transporters play a significant role in β-cell heavy metal accumulation in β cells analogous to tubular renal cells and testicular cells where ZIP8 has been showing to play a significant role in Cd accumulation and toxicity. Another potential mechanism for Cd accumulation in β cells is DMT-1, a transporter previously reported to have a relatively high capacity for Cd transport but—to our knowledge—no prior reports of Hg transport capacity. Our current study shows a relatively high abundance of ZIP8 and DMT-1 mRNA in β cells. Under this assumption, our results showing a less avid accumulation of Hg compared with Cd are expected given the lower affinity of Hg for MT, DMT-1 and ZIP class transporters. To our knowledge, our report is the first description of human islet content of the metals measured in the current study.

Given this difference in the accumulation of Cd and Hg, our subsequent studies focused on the accumulation kinetics and effects of Cd. These studies showed that the mouse β-cell line MIN6 accumulates Cd in a time- and dose-dependent manner over 72 h. To the best of our knowledge, these studies are the first report exploring the accumulation kinetics of Cd in insulin-producing β cells. For comparison, we show that a mouse fibroblast cell line accumulated Cd less avidly with a lower rate of
seven Zn binding sites are donated to other acceptor proteins in MT binding sites. It has been described that Zn bound to MT's all Zn concentration despite displacement of Zn from some of its binding sites by Cd, thereby maintaining a stable over-increase in MT levels compensated for any displacement of Zn concentrations approaching those of Zn. It is likely that the observed upregulation of MT observed in our studies. To our knowledge, no prior studies on Cd accumulation and its effects on β-cell function in native islets comparable to our current studies have been reported.

on the intracellular distribution and availability of Zn will be. It has been reported that Zn is displaced by Cd from MT in a similar, predetermined order, with the lower affinity Zn atoms displaced first. It is therefore likely that the Zn distribution and fluxes within the cell are altered by Cd accumulation. One of the important findings in this study was the inhibition of GSIS by Cd in β cells. Further studies will have to clarify the underlying mechanism for this effect. Importantly, a general cytotoxic effect or an increase in general oxidative stress as an underlying factor is unlikely, as the expression of Heat Shock Protein-70 (HSP70), which has been reported as a marker of β-cell toxicity, was unaltered following exposure to 0.1 μmol/L Cd. Moreover, Cd did not have a negative effect on islet cell viability. Additionally, the expression of several genes relevant for β-cell function examined in our studies was unaltered. This suggests that the observed impairment in the ability of primary mouse β cells to secrete insulin in response to Cd exposure is a result of a functional disturbance of a cellular process relevant for normal β-cell function. Our studies showed no clear evidence for an increase in oxidative stress in response to Cd accumulation except at the relatively high CdCl₂ concentration of 1.0 μmol/L. In contrast, we observed a decrease in GSSG/GSH ratio in dispersed primary mouse islets exposed to CdCl₂, likely due to an upregulation of cellular protective mechanisms other than GCLC, GCLM and HMOX1. It is possible that the observed induction of MT expression contributed to this effect, but it is unlikely to be the only responsible mechanism. The difference in oxidative stress levels between primary mouse islet cells and MIN6 cells following Cd accumulation suggests a substantial difference in their response to Cd-induced oxidative stress. Given these results, it is possible that oxidative stress contributed to the observed decrease in MIN6 GSIS at the highest Cd exposure concentration of 1.0 μmol/L. However, it is unlikely to be a significant factor in the reduced GSIS in dispersed primary mouse islet cells following exposure to the much lower CdCl₂ concentration of 0.1 μmol/L.

The impairment in β-cell function observed in our studies following exposure to a relatively low concentration of Cd is consistent with prior reports of a diabetogenic effect of Cd in vivo. Edwards et al. reported that exposure of adult Sprague-Dawley rats to Cd at a dose of 0.6 mg/kg/day, 5 d a week for 12 weeks resulted in an increase in fasting glucose and HbA1c and a decrease in serum insulin levels at week 12 but not earlier than week 11, suggesting a gradual accumulation of Cd and subsequent β-cell dysfunction. Whether Cd accumulation in β cells plays a role in promoting β-cell dysfunction or the development of frank diabetes mellitus under normal environmental Cd exposure is unclear. Nilsson et al. had reported the acute accumulation and effect of Cd following exposure to 2.5 or 5 μmol/L for 1 h. These studies are not comparable given the substantially higher concentration and shorter exposure duration, especially given the gradual accumulation of Cd and upregulation of MT observed in our studies. To our knowledge, no prior studies on Cd accumulation and its effects on β-cell function in native islets comparable to our current studies have been reported.

In our experiments, Cd accumulation did not alter the total cell Zn concentration. Remarkably, this was also true following exposure of MIN6 cells to a high concentration of CdCl₂, 1.0 μmol/L, which resulted in accumulation of Cd to concentrations approaching those of Zn. It is likely that the observed increase in MT levels compensated for any displacement of Zn from MT binding sites by Cd, thereby maintaining a stable overall Zn concentration despite displacement of Zn from some of its MT binding sites. It has been described that Zn bound to MT’s seven Zn binding sites are donated to other acceptor proteins in a fixed, predetermined order. Given the likely role of MT as a Zn reservoir, it is uncertain what the net effect of Zn displacement from MT in combination with increased MT expression...
Human Cd exposure in the general population below the threshold generally considered as toxic is highly prevalent. Reports of serum or blood Cd concentrations in the population range from 0.0009 to 0.087 μmol/L,26,60-67 which is consistent with the lower concentration of Cd used in our studies. These exposure levels are mostly considered to be below the toxic exposure levels, which are defined based on the nephrotoxic effect of Cd. We consider our findings of significant β-cell accumulation of Cd and the measureable quantity of Cd in primary human islets as being of potential relevance to the pathophysiology of diabetes mellitus. Indeed, some epidemiologic reports in humans suggest that chronic low-level Cd exposure may be linked to altered glucose homeostasis, impaired β-cell function and an increased risk for type 2 diabetes. A sub analysis on participants in the NHANESIII observational cohort study reported an association between elevated urinary Cd levels and impaired fasting glucose levels as well as frank diabetes mellitus.1 Swaddiwudhipong et al. reported a 5-y observational study in a cohort of 436 persons exposed to high environmental Cd concentrations. They reported an increase incidence of diabetes in the 217 persons with continued high Cd exposure compared with the 219 persons who lowered their Cd intake through dietary interventions.68 Furthermore, Afridi et al. reported increased Cd levels in scalp hair of subjects with diabetes mellitus compared with non-diabetic controls.2 The causal relationship of these observations to diabetes mellitus remains to be explored. Our data provide a basis for further investigating the effect of Cd on insulin-producing β cells. It is unclear if the toxic effect of Cd on β cells has a threshold level below which Cd does not exert an adverse effect on β cells or whether the effect follows a continuous dose response curve. Assuming that the effect of Cd on β cells has a threshold, the "lowest observed adverse effect level" (LOAEL) for the inhibition of GSIS was between 0.1 and 1.0 μmol/L in our experiments for primary mouse islets and MIN6 cells respectively. Formulas that are commonly employed to extrapolate acceptable reference concentrations (RIC) used to define a tolerable environmental exposure levels have traditionally incorporated safety margins between 100 and 1,000.69 The concentration of Cd observed in MIN6 cells following exposure to CdCl2 0.1 and 1.0 μmol/L was 14.5 and 145 times higher respectively than the average Cd concentration observed in human islet samples from non-diabetic subjects. It was also approximately 6 and 60 times higher than the highest Cd concentration observed in subject 10, the subject with the highest islet Cd concentration in our cohort. These concentrations fall well within the commonly employed safety margins discussed above. Given this, further studies to investigate whether the Cd concentrations found in human islets have a significant impact on normal human β-cell physiology are warranted. We acknowledge limitations of our studies. First, using short-term studies in cultured cells does not fully overcome the challenges associated with attempting to recapitulate the impact of chronic exposure in vivo. Furthermore, we were unable to compare the degree of Cd accumulation in primary mouse islets with that in MIN6 cells due to technical limitations. Also, our study was limited to islets from non-diabetic subjects and limited demographic and geographical data are available. Despite these limitations, the study provides important new information on Cd accumulation in islets under normal environmental conditions and its impact on islet function.

We conclude that primary human islets from individuals exposed to normal environmental conditions contain measurable quantities of Cd. Cultured mouse β cells accumulate Cd gradually over more than 72 h. This accumulation leads to the selective activation of only some of the known protective cellular response mechanisms, namely the upregulation of the metal buffering protein metallothionein, but not others such as ZnT-1, thereby prolonging the intracellular half-life of Cd. Cd accumulation led to an alteration of β-cell function in mouse islet cells without inducing general cell toxicity or oxidative stress at environmentally relevant Cd concentrations. Further studies will be required to elucidate whether this effect is relevant under normal environmental exposure levels and to elucidate the underlying mechanism.

Materials and Methods

Accrualment of human islets. Viable human islets where provided through the NIH/NIDDK sponsored Integrated Islet Distribution Program (IIDP) via overnight shipment from IIDP centers in the United States. Islets were processed immediately upon arrival. Additional samples of freshly isolated islets were
cultured cells, culture medium was changed 48 h after plating the cells. Cells were harvested 72 h later. Prior to cell harvest, CdCl₂ 100 μmol dissolved in PBS was added to the medium at appropriate volumes to achieve the intended end concentration 1, 2, 4, 8, 24, 48 or 72 h prior to cell harvest. Control cells were treated with a corresponding volume of vehicle (PBS). Accurate exposure concentrations were verified by analyzing medium samples by ICP-MS.

Western blot analysis. Western blot analysis of cell lysates was performed as described previously, with modifications as described herein. For Heat Shock Protein 70 (HSP70), lysates containing 20 μg of protein were suspended in βME-containing Laemmli buffer, heated to 95°C for 10 min and cooled to room temperature. 10% SDS-PAGE gel electrophoresis was performed followed by detection per standard protocol using antibodies against mouse HSP70 (Cell Signaling) at a dilution of 1:1,000 in TBS-T with 5% bovine serum albumin (BSA). For metallothionein immunoblotting, 40 μg of protein (20 μg for dispersed mouse islets) was supplemented with 0.1 mol/l DTT and heated to 95°C for 10 min in Kimura buffer (0.04M Tris, pH 8.8, 1.6% SDS, 10% glycerol final concentration), cooled to 50°C, supplemented with iodoacetamide (final concentration 0.1 mol/l),...
incubated at 50°C for 15 min and subjected to 15% SDS-PAGE followed by immunoblotting and detection as previously described using a 1:500 dilution of monoclonal mouse-anti metallothionein antibody (Dako).

Glucose stimulated insulin secretion (GSIS) studies. Glucose stimulated insulin secretion was performed following incubation without or with CdCl₂ as previously described.²⁴ Briefly, cells were grown in tissue culture plates. The appropriate volume of a 100 μmol/l CdCl₂ solution in PBS was added to the medium either 8 or 48 h prior to GSIS to achieve the desired concentration and exposure duration prior to GSIS at 48 h. At 48 h, culture medium was removed and the cells washed with PBS twice. Next, the cells were equilibrated in HEPES-buffered Krebs solution (HK: 130 mmol/l NaCl, 3.6 mmol/l KCl, 0.5 mmol/l NaH₂PO₄, 0.5 mmol/l MgSO₄, 1.5 mmol/l CaCl₂, 1% HEPES, 0.1% BSA, pH 7.4) containing 5.6 mmol/l glucose for 30 min. This was followed by baseline incubation in HK containing 2.8 mmol/l glucose for 30 min and then stimulation by incubation in HK containing 16.7 mmol/l glucose for 30 min. After each of the incubation periods in baseline or stimulated conditions, 200 μL of the supernatant were collected. Samples were stored at −80°C for later measurement of insulin content.

Analysis of Zn and Cd concentration in cell lysates. Cultured MIN6 and 3T3 cells were harvested by washing the plates with PBS twice. Cells were incubated in 0.05% trypsin + EDTA (Invitrogen) at 37°C × 4 min for cell separation and removal of diverant metals from the cell surface. After neutralization with DME medium (Invitrogen), cells were transferred to acid-washed 600 μl plastic tubes. Cells were pelleted by centrifugation at 500 RCF for 3 min and with washed with PBS. The pelleting and washing in PBS was repeated twice. Hydrolysis was performed by adding trace metal grade 1 N NaOH (Fisher), incubation at 99°C for 15 min and neutralized with trace metal grade 1 N HCl (Fisher). The protein concentration of the lysates was measured using the micro-BCA assay (Pierce/Thermo Fisher Scientific). Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure the content of the elements reported.

Quantification of Ni, Cu, Zn, Cd and Hg was accomplished using ICP-MS of cell lysates. Specifically, lysates were diluted in metal-free 15-mL polypropylene conical tubes (VWR Scientific) with ultra-pure trace metal grade water (Fisher Scientific), trace element concentrated nitric acid (TraceSelect 70%, Fisher Scientific), trace element hydrochloric acid (TraceSelect ≥ 37%, Fisher Scientific) and multi-element internal standard containing 208Bi, 160Ho, 81²In, 6²Li, 51Sc, 159²Tb and 89²Y (Inorganic Ventures) to produce a final solution of 2.0% nitric acid (v/v), 2.0% hydrochloric acid (v/v) and 5.0 ng/g internal standard. Fully quantitative element standards were prepared starting from a custom mixed multi-element standard (Inorganic Ventures) containing 10 μg/mL Ca, Mg, Co, Cr, Ni, Cu, Zn, Cd, Hg and 50 μg/mL Au (to bind Hg) using eight fully quantitave concentrations ranging from 0.1 to 90 ng/g standard concentrations containing 2.0% nitric acid (v/v), 2.0% hydrochloric acid (v/v) and 5.0 ng/g internal standard. All samples and standards were run within 24 h of preparation due to the low stability of Hg in solution.

ICP-MS was performed on a computer-controlled (Plasmalab Software) Thermo X series II ICP-MS (Thermo Fisher Scientific) operating in standard (Xt) mode equipped with Xt sample (nickel) and skimmer (platinum) cones and a CETAC 260 autosampler (Omaha). Instrument performance was optimized and checked daily using a multi-element tune solution, autotune sequence and performance report analysis meeting manufacturer’s specifications. Each sample was acquired using one survey run (10 sweeps) and three main (peak jumping) runs (100 sweeps). The isotopes selected for analysis were 60Ni, 63,65 Cu, 66,68 Zn, 111,113Cd and 200,202Hg (using 45Sc, 89Y, 115In and 165Ho as internal standards for data interpolation). Fully quantitative standards were run prior to and following unknown sample runs for quality control purposes.

Cell viability measurement using the Multitox assay. Cell viability was measured using the double fluorescence Multitox® assay (Promega) as according to the manufacturers recommendations and as previously described.²⁴ Briefly, dispersed mouse islet cells were seeded and cultured in black, opaque fluorescence 96-well tissue culture plates (BD-Falcon) in 100 μl medium containing CdCl₂, at the specified concentration. Cell viability was assessed 48 h later by adding 100 μL of the assay mix to each well and incubated at 37°C for 2 h. Fluorescence was measured on a fluorescence plate reader at excitation wavelength (Ex) 400 nm/emission wavelength (Em) 505 nm and Ex 485/Em 520, which measured fluorescence from proteases in viable and dead cells, respectively. Ratios of the fluorescence readouts were calculated. Changes in the cell viability index correspond to changes in the ratio of live to dead cells.

Real time PCR. Previously-reported primers for house keeping genes and Zn transporters of the ZnT and ZIP family were used.²⁴ Additionally, the following mouse primer pairs were used: insulin-I F: 5’-GCA AGC AGG TCA TTT CAA CCA C-3’ and R: 5’-AAG CCT GGG TGG GTT TGG-3’; insulin-II F: 5’-CCA CCC AGG CTT TGG CAA AA-3’ and R: 5’-CCC AGC TCC AGT TTG TCC AC-3’; insulin-MaF F: 5’-CTT GTA GAG AAG GAA GCC GAG GAA-3’ and R: 5’-AACT TGA AGT CCA GGT TCA TAT AGT CA-3’; DMT-1 F: 5’-AGG AAG TGC GGG AAG CAC ATG AAT-3’ and R: 5’-ACA CGA AAA CAT TGA TGA A-3’; HMOX1 F: 5’-ACC CGC GAT CCT GAT TTG TGG-3’; MafB F: 5’-CAA CAG CTA CCC ACT AGC CA-3’ and R: 5’-GTC AGC GCC GAG ATT GGC-3’; IQS-2 F: 5’-GCC AGC ACT CTC CTT CAA CAA GTC GAG TAT AGC-3’; IRS-2 F: 5’-GCC TCA TGT TCT TCA CT-3’ and R: 5’-AAC TGA AGT CCA GGT TCA TAT AGT CA-3’; and R: 5’-ACC CGC AGA CAT TGA TGA TGA A-3’; HMOX2 F: 5’-AAC AAG AAC AGC CCA GTC GAG TAT GC-3’; and R: 5’-AGC TAC TGT TGT AGC GAT GT-3’; GCLC F: 5’-ATG TGG ACA CCC GAT GCA GTA TT-3’ and R: 5’-TGT CCT GGT TGT AGC GAT GT-3’; and R: 5’-CAG GGA TGG TTT CTA GAA GAG-3’.
achieve similar 18S and β-actin Ct values within one cycle in order to minimize the effect of differences in primer efficiencies.

Measurement of cellular oxidized and reduced glutathione.

Reduced glutathione (GSH) and oxidized glutathione (GSSG) as well as the ratio of GSH to GSSG were measured using the GSH/GSSG-Glo™ Assay (Promega). Briefly, dispersed mouse islet cells or MIN6 cells were seeded and cultured in white, opaque well, clear bottom 96-well tissue culture plates (Greiner Bio-One) in 90 μL medium containing CdCl₂ at the specified concentration. GSH and GSSG were measured 48 h later by following the manufacturer’s instructions. GSH and GSSG values were normalized to the values from the non-treated control within each experiment to adjust for variations in cell density between experiments.

Statistical analysis.

Results are reported as means ± SEM unless otherwise stated. The “n” represents the number of independent experiments performed. Two-sided unpaired Student’s t-test was used to compare parameters to control. The Bonferroni correction was used where appropriate. Two-way ANOVA was used to compare the time course of Cd accumulation in 3T3 and MIN6 cells. p values ≤ 0.05 and 0.005 were considered statistically significant and highly significant respectively. (STATA-IC V.10.1 and Graphpad Prism V6.0b).

For simplification, the symbols Cd and Zn were used throughout the article when referring to their respective ions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This study was funded by a Northwestern Memorial Foundation MD-Scientist Fellowship in Genetic Medicine award and the Northwestern Memorial Foundation/NUCATS Draxon Young Investigator Award and a NIEHS/NIH grant IK08ES020880-01 for M.E. The study was also funded by two NIH grants: R37GM038784 and U01CA151461 for TVO, P50HD044405 (M.U. and S.B.) and RO1HD057450 (M.U.). Human islets where provided through the NIH sponsored Integrated Islet Distribution Program (IIDP). ICP-MS Metal analysis was performed at the Northwestern University Quantitative Bioelemental Imaging Center generously supported by NASA Ames Research Center NNA04CC36G. We thank the following individuals and institutions for providing samples of the solutions used in the process of isolating human islets for meta analysis: City of Hope: Ismail H. Al-Abdullah, PhD, Foud Kandeel, MD, PhD, Noe Gonzales; the University of Wisconsin: Laura Zitur, Luis A. Fernandez, MD; The University of Miami: Omaima Malik MD, Aisha Khan PhD, Camillo Ricordi, MD.

Supplemental Materials

Supplemental materials may be found here: www.landesbioscience.com/journals/islets/article/23101

References

1. Schwartz GG, Ilyasova D, Ivanova A. Urinary cadmium, impaired fasting glucose, and diabetes in the NHANES III. Diabetes Care 2003; 26:468-70; PMID:12547882; http://dx.doi.org/10.2373/diabetes.26.2.468

2. Afkizi H, Kazi TG, Kazi N, Jamal MI, Azain MB, Jalbani N, et al. Evaluation of status of toxic metals in biological samples of diabetes mellitus patients. Bio Res Clin Pract 2008; 80:280-8; PMID:18275629; http://dx.doi.org/10.1016/j.biures.2007.12.021

3. Edins D, Petro A, Pollard N, Freeman JD, Levin ED. Mercury-induced cognitive impairment in metallothionein-1/2 null mice. Neurotox Res 2008; 508-95; PMID:18218264; http://dx.doi.org/10.1016/j.nire.2007.12.005

4. Gerson RJ, Shaikh ZA. Uptake and binding of cadmium and mercury to metallothionein in rat hepato- cyte primary cultures. Biochem J 1982; 208:465-72; PMID:7159412

5. Schulze P, Weigert E, Braun W, Wagner G, Vaik S, M. Kagi JH, et al. Conformation of (Cd7)metallothionein-2 from rat liver in aqueous solution determined by nuclear magnetic resonance spectroscopy. J Mol Biol 1988; 203:251-68; PMID:2814190; http://dx.doi.org/10.1016/0022-2836(88)90178-0

6. Dallon TP, He L, Wang B, Miller ML, Lin L, Stringer KF, et al. Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. Proc Natl Acad Sci U S A 2005; 102:3401-6; PMID:15722412; http://dx.doi.org/10.1073/ pnas.0406051102

7. Elinder CG, Nordberg M, Palm B, Björk L, Jonsson L. Cadmium, zinc, and copper in rabbit kidney metallothionein–relation to kidney toxicity. Environ Res 1987; 42:53-62; PMID:3552562; http://dx.doi.org/10.1016/0013-9351(87)80222-0

8. He L, Girijashanker K, Dallon TP, Reed J, Li H, Soleimani M, et al. ZIP8, member of the solute-carri-

9. Wisniewski JM, Trojanowksa B, Piotrowski J, Jakowski B. Binding of mercury in the rat kidney by metallothionein. Toxicol Appl Pharmcol 1970; 16:754-63; PMID:5422217; http://dx.doi.org/10.1016/0041-000X(70)90801-5

10. Antal A, Dempski RE. The human ZIP4 transporter has two distinct binding affinities and mediates transport of multiple transition metals. Biochemistry 2012; 51:963-73; PMID:22242765; http://dx.doi.org/10.1021/bi201553p

11. Lemaire K, Ravier MA, Schraenen A, Cremer CW, Van de Plas R, Gravnik M, et al. Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. Proc Natl Acad Sci U S A 2009; 106:14872-7; PMID:19706465; http://dx.doi.org/10.1073/pnas.0906587106

12. Dodson G, Steiner D. The role of assembly in insulin’s biosynthesis. Curr Opin Struct Biol 1998; 8:189-94; PMID:9633292; http://dx.doi.org/10.1016/S0959-440X(98)80037-7

13. Chimenti F, Devergasia S, Patuocchi F, Schuit E, Garcia-Cuenca R, Vandewalle B, et al. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci 2006; 119:4199-206; PMID:16984975; http://dx.doi.org/10.1242/jcs.03164

14. Figlewicz DP, Forhan SE, Hodgson AT, Grodsky GM. Zinc and endogenous zinc content and distribution in islets in relationship to insulin content. Endocrinology 1984; 115:877-81; PMID:6378606; http://dx.doi.org/10.1210/endo-115-3-877

15. Huang XF, Arvan P. Intracellular transport of proin-

16. Liuozzi JP, Cousins RJ, Mammalian zinc transporters. Annu Rev Nutr 2004; 24:151-72; PMID:15388710; http://dx.doi.org/10.1146/annurev.nutr.24.120203.132402

17. Liuozzi JP, Boba JA, Lichten LA, Samuelon DA, Cousins RJ. Responsive transporter genes within the murine intestinal-pancreatic axis form a basis of zinc homeostasis. Proc Natl Acad Sci U S A 2004; 101:14555-60; PMID:15538176; doi:http://dx.doi.org/10.1073/pnas.0406216101

18. Wang B, Schneider SN, Dragin N, Girijashanker K, Dallon TP, He L, et al. Enhanced cadmium-induced testicular necrosis and renal proximal tubule damage caused by gene-dose increase in a Slc39A8- transgenic mouse line. Am J Physiol Cell Physiol 2006; 292:C1523-35; PMID:17108809; http://dx.doi.org/10.1152/ajpcell.00409.2006

19. Ohana E, Sekler I, Kaismann T, Kahn N, Cove J, Silverman WF, et al. Silencing of ZnT-1 expression enhances heavy metal influx and toxicity. J Mol Med (Berl) 2006; 84:753-63; PMID:16741752; http://dx.doi.org/10.1007/s00109-006-0024-2

20. Girijashanker K, He L, Soleimani M, Reed JM, Li H, Liu Z, et al. Slc39A4 gene encodes ZIP14, a metal/bicarbonate symporter: similarities to the ZIP8 transporter. Mol Pharmacol 2008; 73:1413-3; PMID:18270315; http://dx.doi.org/10.1124/mol.107.045588

21. Garrick MD, Dolan KG, Horbinski C, Ghio AJ, Higgins D, Porubcin M, et al. DMT1, a mam-

22. Kim DW, Kim KY, Choi BS, Youn P, Ryu DY, Klaassen CD, et al. Regulation of metal transporters by dietary iron, and the relationship between body iron levels and cadmium uptake. Arch Toxicol 2007; 81:327-34; PMID:17031680; http://dx.doi.org/10.1007/s00204-006-0160-7

23. Chimenti F, Favier A, Seve M. ZnT8, a pancre-

24. Volume 4 Issue 6

Islets
38. Al Mgamed M, Billings LG, Raja MR, Zhang X, Park PJ, Newman MV, et al. Acute cytokine-mediated downregulation of the zinc transporter ZnT8 alters pancreatic beta-cell function. J Endocrinol 2010; 206:159-69; PMID:20508080; http://dx.doi.org/10.1677/JOE-09-0551

39. Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, Baldwin JM, Gyulkyhandanav AV, et al. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes 2009; 58:2070-83; PMID:19154220; http://dx.doi.org/10.2373/db09-0551

40. Wijesekara N, Dai FF, Hardy AB, Giglou PR, Bhattacharje A, Koshkin V, et al. Beta cell-specific ZnT9 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia 2010; 53:1656-68; PMID:20424817; http://dx.doi.org/10.1007/s00125-010-1733-9

41. Andrews GK. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol 2000; 59:95-104; PMID:10609598; http://dx.doi.org/10.1016/S0006-2952(99)00319-9

42. Haasui M, Suzuki K, Matsui H, Koike H, Ito K, Yamanaka H. Regulation of metallothionein and zinc transporter expression in human prostate cancer cells. Cancer Lett 2003; 200:187-97; PMID:14568174; http://dx.doi.org/10.1016/S0304-3835(03)00441-5

43. Günter V, Lindert U, Schaffner W. The taste of heavy metals: gene regulation by MTF-1. Biochim Biophys Acta 2012; 1823:1416-25; PMID:22289350; http://dx.doi.org/10.1016/j.bbadis.2012.01.005

44. Milne DB, Ralston NV, Wallwork JC. Zinc content of blood cellular components and lymph node and spleen lymphocytes in severely zinc-deficient rats. J Nutr 1985; 115:1073-8; PMID:4020486

45. Vickers JW, Green G, McNab K, Milner DB, Sandstead HH. The effect of interactions between dietary egg white processing, crystallisation and secretion. Diabetologia 1985; 115:1073-8; PMID:4020486

46. Nordberg GF. Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol 2009; 238:192-200; PMID:19341754; http://dx.doi.org/10.1016/j.taap.2009.03.015

47. Masters BA, Kelly EJ, Quaife CJ, Britstler RL, Palmieri RD. Targeted disruption of metallothionein I and II genes increases plasma copper. Proc Natl Acad Sci U S A 1994; 91:5984-8; PMID:8290567; http://dx.doi.org/10.1073/pnas.91.12.5984

48. Waisberg M, Harvey MJ, Klaassen CD. Relative in vivo affinity of hepatic metallothionein for metals. Toxicol Lett 1994; 203:39-46; PMID:6605994; http://dx.doi.org/10.1016/0378-4274(94)90179-6

49. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003; 192:95-117; PMID:12841588; http://dx.doi.org/10.1016/S0300-5777(03)00035-6

50. Zanger K, Ot G, Orvos JD, Armitage IM. Three-dimensional solution structure of mouse [C(7)-]metallothionein-1 by homonuclear and heteronuclear 2H MR spectroscopy. Protein Sci 1999; 8:2630-8; PMID:10179178; http://dx.doi.org/10.1110/ps.8.12.2630

51. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem 2005; 12:1161-208; PMID:15892631; http://dx.doi.org/10.2174/0929867053764635

52. Jiang LJ, Marot W, Valle BL. The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proc Natl Acad Sci U S A 1995; 92:3483-8; PMID:25502392; http://dx.doi.org/10.1073/pnas.92.9.3483

53. Jiang LJ, Vasak M, Valle BL, Marot W. Zinc transfer potentials of the alpha- and beta-clusters of metallothionein are affected by domain interactions in the whole molecule. Proc Natl Acad Sci U S A 2000; 97:2503-8; PMID:10716985; http://dx.doi.org/10.1073/pnas.97.6.2503

54. Feng W, Cai J, Pierce WM, Franklin RB, Marot W, Benz FW, et al. Metallothionein transfers zinc to mitochondrial aconitase through a direct interaction in mouse hearts. Biochem Biophys Res Commun 2005; 323:853-8; PMID:15913554; http://dx.doi.org/10.1016/j.bbrc.2005.04.170

55. Mason AZ, Perico N, Moeller R, Thrippleton K. Blood and urine metallothionein expression in nickel-cadmium battery workers in Serbia. Toxicol Ind Health 2009; 25:129-35; PMID:19458135; http://dx.doi.org/10.1177/0748246X09346148

56. Link B, Gabrio T, Piechotowski I, Zöller I, Schwenk M. Baden-Württemberg Environmental Health Survey (BW-EHS) from 1996 to 2003: toxic metals in blood and urine of children. Int J Environ Res Public Health 2010; 7:357-71; PMID:21375318; http://dx.doi.org/10.3390/ijerph.7.1.035

57. Marot W, Perico N, Moeller R, Thrippleton K, Asaoma E, Konstantinidis K, Zilmer K, Merkl C, Tempel J, et al. Blood and urine cadmium and bioelements profile in artificial insemination donors, and unselected volunteers. Clin Chem 2009; 55:223-30; PMID:19185595; http://dx.doi.org/10.1093/clinchem/hvp084

58. Ruiz P, Muntau M, Osterloh J, Fisher J, Fowler BA. Interpreting NHANES biomonitoring data, cadmium. Toxicol Lett 2010; 198:44-8; PMID:20447450; http://dx.doi.org/10.1016/j.toxlet.2010.04.022

59. Swadhirudhipong W, Limparachanat P, Mahasupak P, Khamprasit P, Bornschein T, Teng P, Swadhirudhipong S. The comparison of cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: a five-year follow-up. Environ Health 2012; 11:2014-8; PMID:22031668; http://dx.doi.org/10.1186/1476-069X-11-0094

60. Casaretto LJ, Doudl J, Klaassen CD, Casaretto and Doudl’s toxicology: the basic science of poisons. New York: McGraw-Hill, 2008:116-28
70. Miyazaki J, Araki K, Yamato E, Ikekami H, Asano T, Shibasaki Y, et al. Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 1990; 127:126-32; PMID:2163307; http://dx.doi.org/10.1210/endo-127-1-126

71. Artner I, Le Lay J, Hang Y, Elghazi L, Schisler JC, Henderson E, et al. MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. Diabetes 2006; 55:297-304; PMID:16643760; http://dx.doi.org/10.2337/diabetes.55.02.06.db05-0946

72. Raum JC, Hunter CS, Artner I, Henderson E, Guo M, Elghazi L, et al. Islet beta-cell-specific MafA transcription requires the 5'-flanking conserved region 3 control domain. Mol Cell Biol 2010; 30:4234-44; PMID:20584984; http://dx.doi.org/10.1128/MCB.01396-09

73. Hussain MA, Porras DL, Rowe MH, West JR, Song WJ, Schreiber WE, et al. Increased pancreatic beta-cell proliferation mediated by CREB binding protein gene activation, Mol Cell Biol 2006; 26:7747-59; PMID:16908541; http://dx.doi.org/10.1128/MCB.02353-05

74. Zhang H, Liu H, Davies KJ, Sioutas C, Finch CE, Morgan TE, et al. Nrf2-regulated phase II enzymes are induced by chronic ambient nanoparticle exposure in young mice with age-related impairments. Free Radic Biol Med 2012; 52:2038-46; PMID:22401859; http://dx.doi.org/10.1016/j.freeradbiomed.2012.02.042