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RESEARCH ARTICLE

Prior cadmium exposure improves glucoregulation in diabetic rats but exacerbates effects on metabolic dysregulation, oxidative stress, and hepatic and renal toxicity

Prem Kumar Singh, Darshee Baxi, Ruchi Diwedi, and A.V. Ramachandran

Division of Metabolic Endocrinology, Department of Zoology, Faculty of Science, The M.S. University of Baroda, Vadodara 390002, Gujarat, India

Abstract

The present study was taken up to assess the role of subchronic exposure to an environmentally relevant dosage of cadmium in type I diabetes. Female rats of the Wistar strain were treated with cadmium (5.12 mg/kg body weight) for 45 days. On day 46, rats were made diabetic by alloxan. After 7 days, diabetes (i.e., animals with serum glucose greater than 300 mg/dL) in the alloxanized animals was confirmed and further experiments were conducted for 15 days. Cadmium pretreatment showed disturbed glucose homeostasis with attendant changes in carbohydrate metabolism, coupled with decrease in food and water intake. Disturbance in carbohydrate metabolism was indicated by altered tissue metabolite load, as marked by a decrease in protein and glycogen contents and increased cholesterol store. Poor glucose clearance subsequent to a glucose challenge under the glucose tolerance test was observed in these animals (0.48/min in control vs. 0.13/min in Cd animals). There was a significantly lower glucose elevation rate in the insulin response test subsequent to an insulin-induced decrease in glucose level in Cd-exposed animals. Elevated oxidative stress was marked by increased lipid peroxidation, decreased antioxidant (both nonenzymatic and enzymatic) levels, and serum markers of hepatic and renal damage. Decreased corticosterone levels, together with increased E2 and reduced P4 levels, were some of the hallmark changes in the serum hormone profile of Cd-exposed animals. Overall, the present results are novel and interesting to open more investigations on animal models of type 1 diabetes with a history of previous Cd exposure.

Keywords: cadmium, diabetes, oxidative stress, glucoregulation

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Introduction

Cadmium (Cd), a toxic metal, is considered to be a multitarget toxicant and accumulates principally in the liver and kidney. In humans and other mammals, acute effects of Cd include injury to a number of organs and tissues, such as the liver, kidney, lungs, pancreas, testis, and bone. Divalent cadmium is also a ubiquitous industrial and environmental pollutant that accumulates in humans and animals (Waisberg et al., 2003). Cell functions are interfered with by Cd in various tissues and is ranked equal to lead and arsenic in toxicological importance with increasing environmental abundance. Chronic exposure to inorganic Cd results in the accumulation of the metal, mainly in the liver and kidney, as well as in other tissues and organs, causing many metabolic and histological changes, membrane damage, altered gene expression, and apoptosis (Waisberg et al., 2003; Shaikh et al., 1999; Casalino et al., 2002).

Recent epidemiological studies suggest a positive association between exposure to environmental Cd and the incidence and severity of diabetes (Edwards and Prozialeck 2009). Some researchers have suggested the potential mechanisms of Cd-induced diabetes. After Cd exposure, adipose, pancreas, and liver tissues, along with the adrenal gland, become injured, leading to altered glucose metabolism and/or glucose uptake that ultimately results in increased blood glucose. Elevated blood glucose levels, coupled with the direct effects of

Address for Correspondence: Prof. A.V. Ramachandran, Division of Metabolic Endocrinology, Department of Zoology, Faculty of Science, The M.S. University of Baroda, Vadodara 390002, Gujarat, India; Fax: +91-0265–2792277; E-mail: mailtoavrcn@yahoo.co.in

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Cd on renal tissue, eventually lead to kidney dysfunction and damage. Reports also suggest a relationship between Cd exposure, elevated blood glucose level, and the development of diabetes. Cd aggravates hyperglycaemia and nephrotoxicity in experimentally induced diabetic animals (Chandra et al., 1985; Bernard et al., 1991; Jin et al., 1999). In addition, human and animal studies indicate that Cd potentiates or exacerbates diabetic nephropathy. Cd elevates fasting blood glucose levels in an animal model of subchronic Cd exposure before overt signs of renal dysfunction become evident. These studies also show that Cd reduces insulin levels and has direct cytotoxic effects on the pancreas. Together, these findings indicate that Cd may be a factor in the development of some types of diabetes, and raises the possibility that Cd and diabetes-related hyperglycemia may act synergistically to damage the kidney. It has been reported that impaired glucose tolerance (IGT) in rats induced by $CdCl_2$ is accompanied by a drastic (by as much as 90%), dose-dependent reduction in GLUT 4 protein and GLUT 4 mRNA levels in adipocytes. The effect was specific to GLUT 4; neither GLUT 1 nor insulin-responsive amino peptidase in adipocytes were affected.

Findings from these multiorgan studies point toward oxidative stress as the causative agent behind the majority of organ failures. Most of these studies were conducted with Cd at potentially higher doses, based on LD_{50} values, whereas the mechanistic inferences were based on *in vitro* studies. There is much emphasis on the importance of realistic doses in assessing the mechanistic basis of heavy metal toxicity. Hence, the present study was undertaken to get a holistic picture on the alterations in carbohydrate and lipid metabolisms, levels of glucose, insulin and lipid fractions, enzymatic and nonenzymatic markers of oxidative stress, islet function, and hepatic and renal damage by previous exposure to Cd at an environmentally realistic dosage in alloxanized and nonalloxanized rats.

Further, the present study differs from other studies in that Cd exposure was before, rather than after, diabetic induction, essentially to assess the severity and manifestations of diabetes in the Cd-intoxicated state.

Methods

Animals and maintainance

Female albino rats of the Wistar strain (200–250 g, 180 days old) were obtained from Sun Pharmaceuticals Ltd. (Baroda, India) and maintained in the animal house at 20 ± 2°C with light and dark cycles of 12:12 hours, respectively. Animals were provided with a standard rodent pellet diet purchased from M/S Pranav Agro Industries Ltd. (Sangli, India). Food and water were provided *ad libitum*. Animal experiments were conducted according to the guidelines of CPCSEA (827/ac/04/CPCSEA). After the treatment schedule, animals were sacrificed and selected tissues were separated and stored at –80°C until biochemical assay. Blood was collected before sacrifice by keeping the animals under light ether anesthesia, and the separated serum obtained was used for further analysis. During the entire treatment schedule, body weight (b.w.), food, and water were monitored on a daily basis. For evaluation of parameters not needing animal sacrifice (i.e., b.w., food and water intake, and serum glucose), a sample size of 25 animals per group was used. For the rest of the parameters, 6 animals per group were employed.

Experimental groups

Nondiabetic control (NC)

Animals in this group received saline as vehicle.

Control + cadmium (Cd+NC)

Cadmium-treated rats were given 1.12 mg per animal (based on the calculated 5.1 mg/kg b.w.) of cadmium chloride salt dissolved in (0.9 N) saline, at 8:00 a.m. for 45 days.

Diabetic (DC)

Diabetic rats were treated with saline as vehicle.

Cadmium + diabetic (Cd+DC)

Cd-treated rats were given 1.1 mg/animal (based on the calculated 5.1 mg/kg b.w.) of cadmium chloride salt dissolved in (0.1 N) saline, at 8:00 a.m. for 45 days, and were made diabetic by alloxan administration.

Induction of type I diabetes

To induce diabetes, alloxan monohydrate (obtained from Sigma-Aldrich, St. Louis, Missouri, USA) was used. Animals were fasted overnight, and alloxan was administered intraperitonealy (i.p.) at a dosage of 120 mg/kg. Animals were monitored for food and water intake, b.w., and mortality for the next 6 or 7 days before analyzing their blood glucose levels. Blood was withdrawn from the orbital sinus of alloxan-treated animals after 7 days, and animals showing a glucose level above 300 mg/dL were considered diabetic and were used for treatment further (Singh et al., 2010a, 2010b).

Cadmium chloride (CdCl2)

Animals were exposed to Cd before induction of diabetes. A realistic dose of Cd (5.1 mg/kg) was given to animals by oral gavage for 45 days (Mukherjee et al., 2011). After exposure of animals to Cd, they were made diabetic by i.p. injection of alloxan (120 mg/kg b.w.) (Singh et al., 2010a, 2010b).

A separate group was given a dose of 5.12 mg/kg for 45 days at 5:00 p.m.

Oral glucose tolerance test (OGTT)

At the end of treatment schedule, animals were fasted overnight and the GTT was done by feeding orally with a glucose solution at a dose of 2 g/kg b.w. Blood was collected from the retroorbital sinus at 0, 30, 60, 90, and 120 minutes after glucose load. Serum was separated,

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and glucose was estimated in all the collected samples to get a tolerance curve for all experimental groups (Singh et al., 2010a, 2010b).

Insulin response test (IRT)

Response to insulin was checked by injecting insulin to the rats at a dose of 1 U/kg b.w. i.p. in the fed state, 1 day after the completion of treatment, and blood was collected at 0, 30, 60, 90, and 120 minutes from the retroorbital sinus under mild ether anesthesia. Serum was separated and used to estimate glucose levels, and an insulin response curve was drawn to evaluate the results (Singh et al., 2010a, 2010b).

Biochemical analysis

At the end of the 15-day treatment schedule, rats were sacrificed by cervical dislocation after an overnight fast. Liver, muscle, and kidney were excised and stored at–80°C for further analysis. Protein was estimated by Lowry et al. (1951), glycogen by Seifter et al. (1950), glycogen phoshphorylase by Cahill et al. (1957), and glucose-6-phosphatase by Harper (1965). Oxidative stress was determined by assessing the level of lipid peroxidation (LPO) and by estimating endogenous enzymatic and nonenzymatic antioxidant status. LPO was determined as per the method described by Beuge and Aust (1978), reduced glutathione (GSH) by Beutler et al. (1963), superoxide dismutase (SOD) by Marklund and Marklund (1974), catalase by Sinha (1972), and glutathione peroxidase (GPx) by Rotruck et al. (1973).

All biochemical parameters and hormones were assayed using relevant kits, as mentioned below:

Serum glucose (Agappe Diagnostics kit; Agappe Diagnostics, Ltd.), and insulin (Rat Insulin ELISA kit; Mercodia, Uppsala, Sweden), corticosterone and progesterone (Immuno-Technology & Steroid Laboratory Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi, India), estradiol (Biocheck Inc., California), serum cholesterol (Accurex Biomedical Pvt. Ltd.), serum triglyceride (Accurex), HDL (Nicolas Piramal India Ltd.), SGPT (Agappe Diagnostics Ltd.), SGOT (Crest Biosystem Ltd.), alkaline phosphatase (ALP; Rekon Diagnostics Pvt. Ltd.), acid phosphatase (ACP; Aspen Laboratories) serum glucose (Agappe Diagnostics kit), and insulin (Rat Insulin ELISA kit; Mercodia), were assayed using the above kits. Tissue cholesterol and lipids were assayed by the methods of Crawford (1959) and Folch et al. (1957), respectively.

Statistical analysis

Statistical evaluation of the data was done by one-way ANOVA, followed by Bonferroni's multiple comparison test. Results are expressed as mean ± standard error of the mean (SEM), using GraphPad Prism version 3.0 for Windows (GraphPad Software, Inc., San Diego, California, USA).

Results

Body weight, food and water intake, and relative organ weights

There was a decrement in b.w. of control and Cd-treated diabetic rats, though not statistically significant. Cd-treated normal rats showed decrement in food and water intake, measured on a per day basis. In comparison, both control and Cd-treated diabetic rats showed a significant increase in daily food and water intake. Relative organ weight of kidney and liver increased significantly in diabetic rats, as compared to the control rats (Tables 1 and 2).

Glycemic changes

There was no effect of Cd intoxication in fasted blood glucose in NC animals, but there was a significant increment

Table 1. Body weight (g), food intake (g/animal/day), and water intake (mL/animal/day) in all experimental groups.

Group	Initial body weight	Final body weight	Body weight gain/loss	Food intake g/animal/day	Water intake g/animal/day
NC	223.3 ± 15.9	263.33 ± 7.27	+40.03	16.15 ± 0.011	35.5 ± 1.156
Cd+NC	220.00 ± 5.78	211.66 ± 6.67	–8.34	10.47 ± 0.011[c]	10.5 ± 1.73[c]
DC	196.6 ± 17.65	191.66 ± 6.01	–4.94	20.89 ± 0.011[c]	73.5 ± 1.156[c]
Cd+DC	217.5 ± 18.38	207.5 ± 1 0.21	–10	23.50 ± 0.115[@]	50.9 ± 0.578[@]

$n = 25$; data are expressed as mean ± SE.
a) $P < 0.05$, c) $P < 0.01$, compared to NC; @) $P < 0.01$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Table 2. Relative weights (g/100 g of body weight) of liver, muscle, kidney, spleen, and adrenal of all experimental groups.

Group	Liver	Kidney	Muscle	Spleen	Adrenal
NC	2.22 ± 0.001	0.49 ± 0.0002	1.68 ± 0.0003	0.3126 ± 0.0007	0.0214 ± 0.006
Cd+NC	2.66 ± 0.0008[a]	0.36 ± 0.016[a]	1.327 ± 0.379	0.22 ± 0.0010[c]	0.0217 ± 0.006
DC	6.47 ± 0.000[c]	1.0069 ± 0.0002[c]	0.89 ± 0.0001[c]	0.27 ± 0.0006[c]	0.03 ± 0.002[c]
Cd+DC	3.23 ± 0.002[@]	0.88 ± 0.00036[@]	1.262 ± 0.003	0.24 ± 0.0068*	0.026 ± 0.0021*

$n = 6$; data are expressed as mean ± SE.
a) $P < 0.05$, c) $P < 0.01$, compared to NC; *$P < 0.05$, @) $P < 0.01$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

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in the fed state. Diabetic animals showed significant hypoglycemia under both fasted and fed states, and previous Cd exposure showed a significant reduction in diabetic hyperglycemia (Table 3).

Table 3. Levels of fasting and fed serum glucose (mg/dL) in cadmium-treated nondiabetic and diabetic rats.

Groups	Fasting serum glucose	Fed serum glucose
NC	93.97 ± 3.50	113.36 ± 3.18
Cd+NC	95.30 ± 1.81	133.66 ± 4.98*
DC	443.33 ± 31.21c	655.33 ± 7.60c
Cd+DC	163.67 ± 12.43@	442 ± 10.42@

n = 25; data are expressed as mean ± SE.
c) $P < 0.01$, compared to NC; *$P < 0.05$, @) $P < 0.01$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.
c) $P < 0.01$, compared to NC; *$P < 0.05$, @) $P < 0.01$, compared to DC.

OGTT

Glucose tolerance curves and area under the curve did not show any difference in NC or Cd+NC animals. Diabetic animals showed a higher positioning of the curve and greater area under the curve. Both these were greater with previous Cd exposure. The glucose clearance rate was much lower in Cd+NC animals, whereas the glucose elevation rate was much lower in Cd+DC animals (Figures 1 and 2; Table 4).

IRT (Fig 3, 4, Table 5)

Just as in GTT, diabetic animals in IRT also showed a higher positioning of insulin response curve and greater area under the curve. Though there was a slight tendency for an increase in area under the curve in Cd+NC animals, the positioning of the curve as well as the area under the curve showed an intermediate position and reduction, respectively, in Cd+DC animals. There was a significant decrement in glucose elevation in Cd+NC and significant

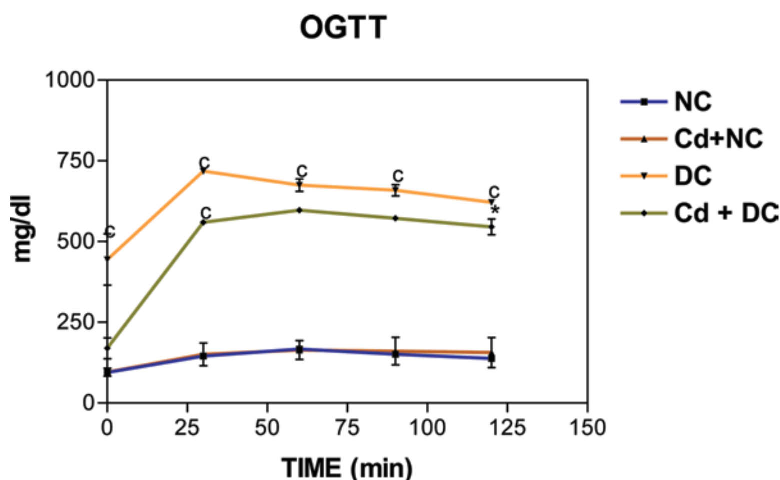

Figure 1. Serum glucose levels in response to the oral glucose tolerance test (OGTT) within a time range of 0–120 minutes in all the experimental groups. n = 6; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. c) $P < 0.01$, compared to NC; *$P < 0.05$, compared to DC. (See colour version of this figure online at www.informahealthcare.com/dct)

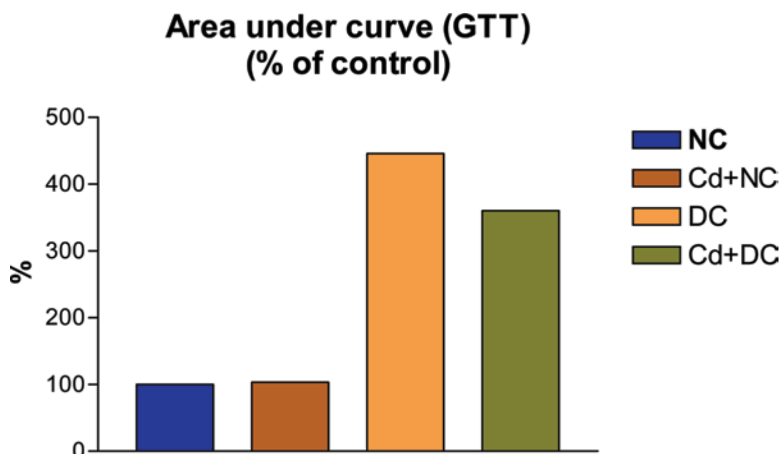

Figure 2. Area under the curve for OGTT in all experimental groups. NC, nondiabetic control; NC+CD, nondiabetic control+cadmium; DC, diabetic control; CD+DC, diabetic control+cadmium. (See colour version of this figure online at www.informahealthcare.com/dct)

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increase in elevation in Cd+DC, not noticeable in DC (Figures 3 and 4; Table 5).

Carbohydrate metabolism

There was decrement in tissue glycogen content in Cd+NC and Cd+DC animals, compared to their respective controls. Phosphorylase activity tended to show an increase in Cd+NC animals, whereas it was significantly higher in DC animals. Previous Cd exposure showed further increase in enzyme activity in DC animals. Concurrently, G6Pase activity also showed a significant increase in diabetic animals. Previous Cd exposure increased enzyme activity in both NC and DC, though more significantly in the latter (Figures 5 and 6).

Tissue protein content (Table 6)

Diabetic animals in general showed depletion in protein content of all three organs. Previous Cd exposure decreased tissue protein contents significantly from respective controls (Table 6).

Table 4. Elevation and clearance rates of glucose during OGTT in cadmium treated diabetic and non diabetic rats.

Oral glucose tolerence rate		
Groups	Rate of elevation	Rate of clearance
NC	1.21	0.50
Cd+NC	1.12	0.125
DC	9.13	0.97
Cd+DC	7.132	0.868

Table 5. Clearance and elevation rates of glucose in cadmium treated diabetic and non diabetic rats.

Insulin response test		
Groups	Rate of clearence	Rate of elevation
NC	2.1	0.56
Cd+NC	1.49	0.279
DC	2.4	0
Cd+DC	2.267	0.6

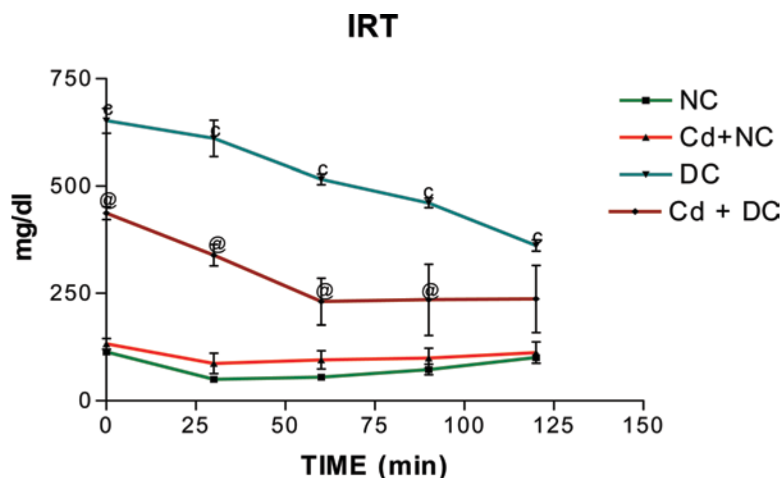

Figure 3. Serum glucose levels in response to insulin administration (IRT) within a time range of 0–120 minutes of all the experimental groups. $n=6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. c) $P<0.01$, compared to NC; and @) $P<0.01$, compared to DC. (See colour version of this figure online at www.informahealthcare.com/dct)

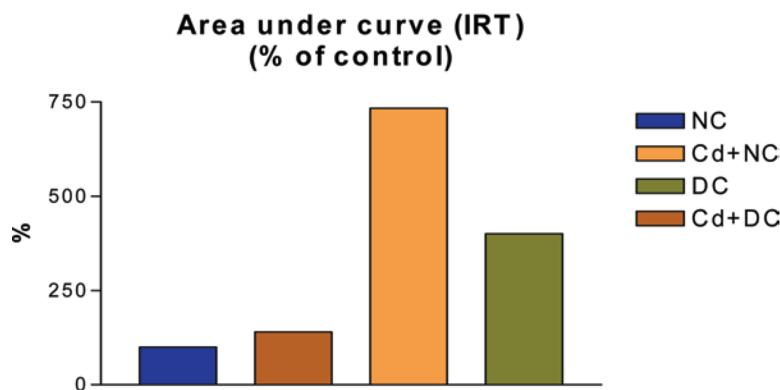

Figure 4. Area under the curve for IRT in all experimental groups. NC, nondiabetic control; NC+CD, nondiabetic control+cadmium; DC, diabetic control; CD+DC, diabetic control+cadmium. (See colour version of this figure online at www.informahealthcare.com/dct)

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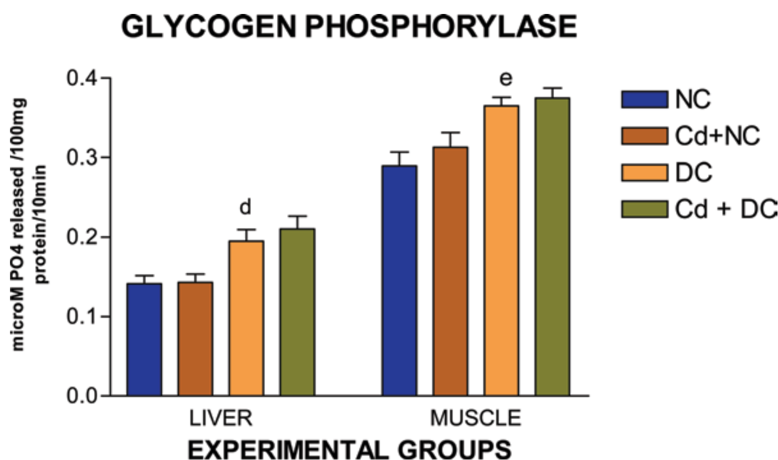

Figure 5. Hepatic and muscle glycogen phosphorylase activity in control and treated rats. $n=6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. d) $P<0.005$, e) $P<0.0005$, compared to NC. (See colour version of this figure online at www.informahealthcare.com/dct)

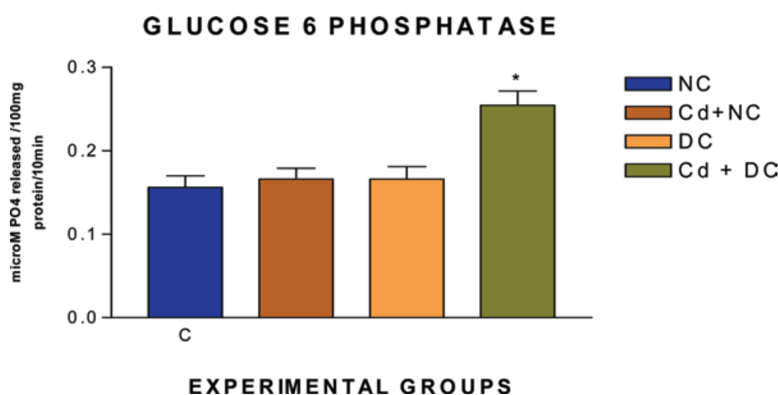

Figure 6. Hepatic glucose-6-phosphatase activity in all the experimental groups. $n=6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. e) $P<0.0005$, compared to NC. (See colour version of this figure online at www.informahealthcare.com/dct)

Table 6. Tissue protein and glycogen contents (mg/100 mg tissue) in cadmium treated diabetic and non diabetic rats.

	Protein			Glycogen	
Groups	Liver	Muscle	Kidney	Liver	Muscle
NC	16.39 ± 1.24	9.76 ± 1.42	10.39 ± 1.10	2.18 ± 0.10	0.93 ± 0.03
Cd+NC	10.69 ± 0.89[d]	5.20 ± 0.62[c]	9.25 ± 0.64	1.65 ± 0.11[d]	0.62 ± 0.05[e]
DC	13.90 ± 0.88 [c]	5.50 ± 0.73[c]	10.04 ± 1.58	1.80 ± 0.06[d]	0.58 ± 0.05[e]
Cd+DC	9.74 ± 1.29[@]	4.74 ± 0.65	8.60 ± 0.55	1.09 ± 0.08[•]	0.46 ± 0.01[#]

$n=6$; data are expressed as mean ± SE.
c) $P<0.01$, d) $P<0.005$, e) $P<0.0005$, compared to NC; #$P<0.025$, @) $P<0.01$, ·) $P<0.0005$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Tissue lipid and cholesterol

Total lipid and cholesterol contents of diabetic animals were increased significantly. Cd toxicity showed a significant lipid- and cholesterol-elevating effect in both NC and DC animals (Table 7).

Serum lipids

In general, all serum lipid fractions, except HDL, were increased in diabetic animals. Cd intoxication significantly increased all lipid fractions, except HDL, which decreased, in NC animals. However, the increase in DC animals was insignificant (Table 8).

Serum hormone profile

Corticosterone (Cort), insulin, estrogen (E_2), and progesterone (P4)

Cd exposure resulted in a significant elevation of corticosterone (cort) and E_2 levels and a decrease in insulin and P4. Diabetic animals showed a decrement in insulin and P4 levels, whereas Cort and E_2 levels were increased.

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Previous Cd exposure of diabetic animals resulted in a decrease of all hormones, except E_2 (Table 9).

Oxidative stress parameters

LPO (Fig 7)

LPO in all the three organs was increased significantly in diabetic as well as Cd-exposed NC or DC animals in the order Cd+DC>DC>Cd+NC (Figure 7).

Nonenzymatic antioxidants

GSH and ascorbic acid (AA)

In general, contents of both the antioxidants in liver, muscle, and kidney were decreased significantly in diabetic as well as Cd-intoxicated NC and DC animals in the order given for LPO, though the levels in Cd+DC were not significantly different from DC, except for kidney AA (Table 10).

Table 7. Tissue cholesterol and lipid contents (mg/100 mg tissue) in control and treated rats.

	Cholesterol			Lipid		
Groups	Liver	Muscle	Kidney	Liver	Muscle	Kidney
NC	0.28 ± 0.01	0.12 ± 0.01	0.37 ± 0.03	4.21 ± 0.71	0. 58 ± 0.0043	0.006 ± 0.0001
Cd+NC	0.45 ± 0.02^{e}	0.13 ± 0.01	0.41 ± 0.02	4.56 ± 0.52	0.71 ± 0.0023	0.0076 ± 0.0004
DC	0.60 ± 0.004^{e}	0.29 ± 0.003^{e}	0.58 ± 0.005^{e}	6.32 ± 0.81^{e}	0.45 ± 0.0002^{e}	0. 009 ± 0.0001^{e}
Cd+DC	0.69 ± 0.10	0.38 ± 0.02$^{\wedge}$	0.59 ± 0.01	6.71 ± 0.95	0.47 ± 0.0041	0.0097 ± 0.00049

$n = 6$; data are expressed as mean ± SE.
e) $P < 0.0005$, compared to NC; $P < 0.005$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Table 8. Serum lipid profile (mg/dL) of cadmium-treated nondiabetic and diabetic rats.

Groups	CHO	TG	LDL	VLDL	HDL
NC	80 ± 2.31	68.67 ± 3.45	50.67 ± 1.76	15 ± 1.73	13.11 ± 1.74
Cd+NC	88.78 ± 6.65	112.67 ± 7.90^{e}	44.67 ± 3.18^{d}	21.53 ± 2.03^{e}	23.33 ± 2.61^{d}
DC	97.33 ± 4.34^{d}	140.67 ± 2.34^{e}	45.44 ± 2.60^{d}	30.66 ± 0.87^{e}	22.22 ± 2.90^{e}
Cd+DC	108.30 ± 8.07	146.33 ± 13.36	42.20 ± 2.32	38.08 ± 4.11	28.75 ± 2.41$^{@}$

$n = 6$; sata are expressed as mean ± SE.
d) $P < 0.005$, e) $P < 0.0005$, compared to NC; @) $P < 0.01$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Table 9. Serum hormone profile of cadmium-treated nondiabetic and diabetic rats.

Groups	Insulin (pg/mL)	Corticosterone (ng/mL)	Estradiol (pg/mL)	Progesterone (ng/mL)
NC	0.35 ± 0.01	8.38 ± 0.59	0.20 ± 0.01	66.68 ± 3.48
Cd+NC	0.28 ± 0.03^{d}	9.38 ± 0.05	8.21 ± 0.13^{e}	32.35 ± 0.71^{e}
DC	0.16 ± 0.01^{e}	24.67 ± 1.45^{e}	1.99 ± 0.07^{e}	54.23 ± 1.75^{d}
Cd+DC	0.13 ± 0.01	13.33 ± 0.88$^{\bullet}$	5.30 ± 0.06$^{\bullet}$	51.37 ± 0.57

$n = 6$; data are expressed as mean ± standard error.
d) $P < 0.005$, e) $P < 0.0005$, compared to NC; •) $P < 0.0005$, compared to DC.
NC, nondiabetic control; Cd+NC, non diabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

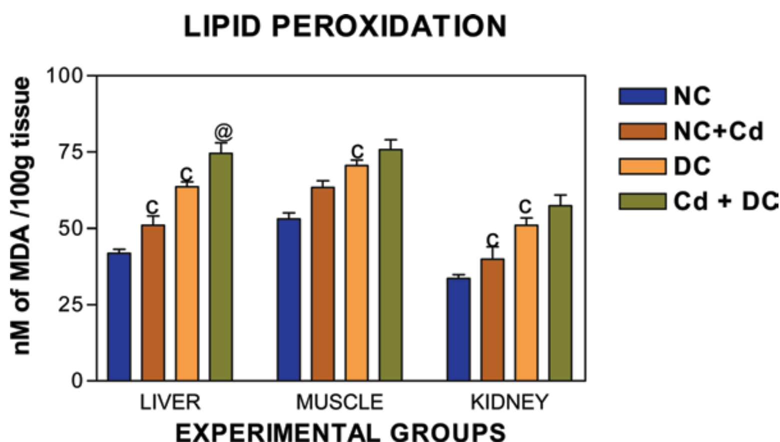

Figure 7. Levels of LPO in extract-treated diabetic and nondiabetic rats. $n = 6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. (See colour version of this figure online at www.informahealthcare.com/dct)

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Enzymatic antioxidants

Catalase (Cat), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels in Cd-exposed NC and DC animals showed a significant reduction in liver, muscle, and kidney, with the decrement in DC being more pronounced than in Cd+NC. Cd+DC animals, however, did not show any further change from DC levels (Table 11).

Serum markers of hepatic function

Serum levels of SGPT, SGOT, ALP, and ACP were increased significantly in all the experimental groups in the order Cd+DC>DC>Cd+NC (Table 12).

Serum markers of renal function

Urea and creatinine (Figs 8, 9)

Like the hepatic markers, serum levels of urea and creatinine were increased significantly in the same order as given for hepatic markers (Figures 8 and 9).

Discussion

The present study on subchronic exposure to an environmentally relevant realistic dosage of Cd for 45 days has shown disturbed glucose homeostasis, with attendant changes in carbohydrate metabolism coupled with decreased food and water intake. These results are in keeping with the results of both human and animal studies, suggesting a diabetogenic effect of Cd marked by elevated blood glucose levels (Edwards and Prozialeck, 2009). Disturbance in carbohydrate metabolism is indicated by an altered metabolic load of tissues, as marked by decreased protein and glycogen contents and increased cholesterol and lipid stores as well as increased glycogenolysis (marked by increased phosphorylase activity) and gluconeogenesis (marked by increased G-6-pase activity). Apparently, these alterations could be related to the observed islet β-cell damage and reduced serum insulin titer. Though similar changes have been noted by other workers on Cd intoxication (Edwards and Prozialeck, 2009; Merali and Singhal, 1980; Gumuslu et al., 2001; Han et al., 2003), the currently observed reduction in food and water intake tends to suggest a diabetogenic action of Cd, rather than a definitive diabetic induction. The elevated serum lipid profile and serum corticosterone are also changes suggesting this effect.

Looking to the mechanistics of the observed diabetogenic effect of Cd, the recorded poor glucose clearance

Table 10. Tissue nonenzymatic antioxidant status (mg/100 mg tissue) in cadmium-treated nondiabetic and diabetic rats.

	GSH			Ascorbic acid		
Groups	Liver	Muscle	Kidney	Liver	Muscle	Kidney
NC	31.15 ± 2.58	14.58 ± 1.52	25.03 ± 1.16	0.53 ± 0.03	0.38 ± 0.01	2.70 ± 0.19
Cd+NC	17.30 ± 2.46[d]	12.37 ± 1.53	22.69 ± 1.95	0.49 ± 0.035	0.30 ± 0.00[e]	1.24 ± 0.09[e]
DC	11.01 ± 1.29[e]	13.05 ± 1.38	13.04 ± 1.86[e]	0.31 ± 0.04[e]	0.26 ± 0.007[e]	0.48 ± 0.05[e]
Cd+DC	10.15 ± 1.14	11.16 ± 0.76	13.51 ± 1.31	0.23 ± 0.05	0.23 ± 0.02	0.31 ± 0.007[·]

$n = 6$; data are expressed as mean ± standard error.
d) $P < 0.005$, e) $P < 0.0005$, compared to NC; ·) $P < 0.0005$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Table 11. Tissue enzymatic antioxidant status of cadmium treated non-diabetic and diabetic treated rats.

	SOD			Catalase			GPX		
Groups	Liver	Muscle	Kidney	Liver	Muscle	Kidney	Liver	Muscle	Kidney
NC	8.17 ± 0.60	10.37 ± 0.60	5.38 ± 0.39	53.96 ± 2.54	73.11 ± 2.59	26.74 ± 3.10	4.60 ± 0.84	12.45 ± 1.61	2.16 ± 0.19
Cd+NC	6.51 ± 0.47[b]	8.53 ± 0.52[b]	4.42 ± 0.65	36.71 ± 3.70[d]	64.37 ± 2.61[b]	18.42 ± 2.69[b]	3.60 ± 0.36	7.69 ± 0.46[c]	1.63 ± 0.12[c]
DC	4.64 ± 0.44[e]	6.56 ± 0.48[e]	2.72 ± 0.15[e]	22.97 ± 2.09[e]	49.833 ± 2.34[e]	13.41 ± 0.87[e]	2.68 ± 0.30[b]	7.47 ± 0.90[c]	1.17 ± 0.24[d]
Cd+DC	4.31 ± 0.35	6.15 ± 0.07	2.27 ± 0.25	15.86 ± 1.07[@]	41.91 ± 2.75[#]	12.16 ± 1.58	1.3 ± 0.28[ˆ]	5.60 ± 0.66[ˆ]	1.16 ± 0.15

$n = 6$; data are expressed as mean ± SE.
b) $P < 0.025$, c) $P < 0.01$, d) $P < 0.005$, e) $P < 0.0005$, compared to NC; #$P < 0.025$, @) $P < 0.01$, ˆ$P < 0.005$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

Table 12. Serum markers of hepatic dysfunction in control and cadmium-treated rats.

Groups	SGPT U/L	SGOT U/L	ALP U/L	ACP U/L
NC	40 ± 4.05	71.33 ± 2.97	204 ± 2.65	8.5 ± 0.87
Cd+NC	60 ± 4.17[d]	128.67 ± 3.18[e]	251 ± 13.63[d]	10.77 ± 2.32
DC	125 ± 5.87[e]	290.67 ± 5.79[e]	471.67 ± 2.34[e]	12.20 ± 0.61[d]
Cd+DC	175 ± 7.78[·]	337 ± 8.90[·]	524.67 ± 9.54[·]	13.23 ± 1.18

$n = 6$; data are expressed as mean ± SE.
d) $P < 0.005$, e) $P < 0.0005$, compared to NC; ·) $P < 0.0005$, compared to DC.
NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium.

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subsequent to a glucose challenge under GTT (0.48/min in control vs. 0.13/min in Cd animals) tends to suggest a reduced glucose-induced release, rather than reduced insulin sensitivity, to be the main cause. This is supported by the concurrently observed insignificant difference in glucose clearance on insulin challenge, with the insignificant difference being explainable as being the result of a difference in absolute level of insulin (1 U/kg + 0.35 μg/L in control vs. 1 U/kg + 0.28 μg/L in Cd animals). Further, the significantly lower glucose elevation rate in IRT subsequent to insulin-induced decrease in glucose level in Cd-exposed animals suggests some dysregulation in α-cell release of glucagon as well. Taken together, both the GTT and IRT indicate a deficient insulin and glucagon release in response to glucose and insulin, respectively, with relatively greater effect on the former. This explanation is supported by the reported reduction in total GLUT-4 mRNA with no effect on GLUT-4 translocation to the adipose tissue membrane of Cd-exposed animals as well as reduced *in vitro* release of insulin to high glucose levels from islets of Cd-administered animals (Merali and Singhal, 1980; Han et al., 2003). The reduction in serum insulin levels also seems to affect lipid metabolism, as seen by the elevated tissue and serum lipid and cholesterol contents.

The reduction in circulating insulin titer observed herein, as well as the purported decrement in insulin release, could be attributed to the histologically noticeable β-cell damage in Cd-exposed animals, and, as such, there are corroborative reports on Cd-induced reduction in insulin level and its release (Edwards and Prozialeck, 2009). The apparent cause for the observed β-cell damage seems to be increased oxidative stress, as seen by the increased LPO and decreased levels of all nonenzymatic and enzymatic antioxidants. As such, Cd-induced oxidative stress (Casalino et al., 2002; Jurczuk et al., 2004; Järup and Åkesson, 2009; Liu et al., 2009; Nordberg and Nordberg, 2009; Thévenod and Friedmann, 2009; Ghafghazi and Mennear, 1975), and its role in manifestation of β-cell damage has been reported (Casalino et al., 2002; Merali and Singhal, 1980; Gumuslu et al., 2001; Ghafghazi and Mennear, 1975; Ittakissios et al., 1975; Bell et al., 1990).

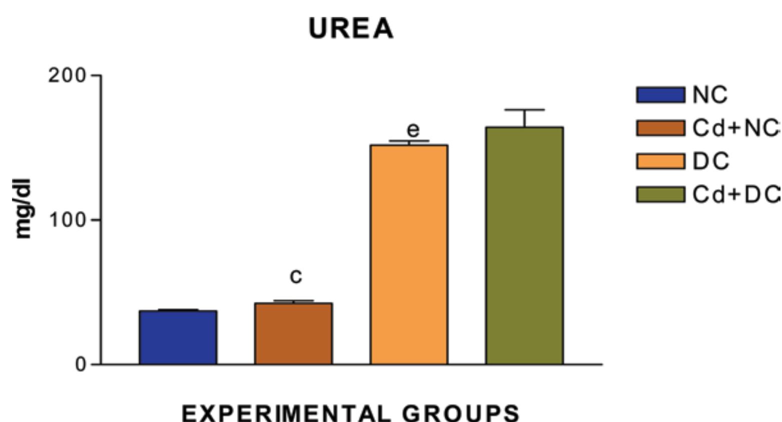

Figure 8. Serum urea level in all the experimental groups. $n=6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. c) $P<0.01$, e) $P<0.0005$, compared to NC. (See colour version of this figure online at www.informahealthcare.com/dct)

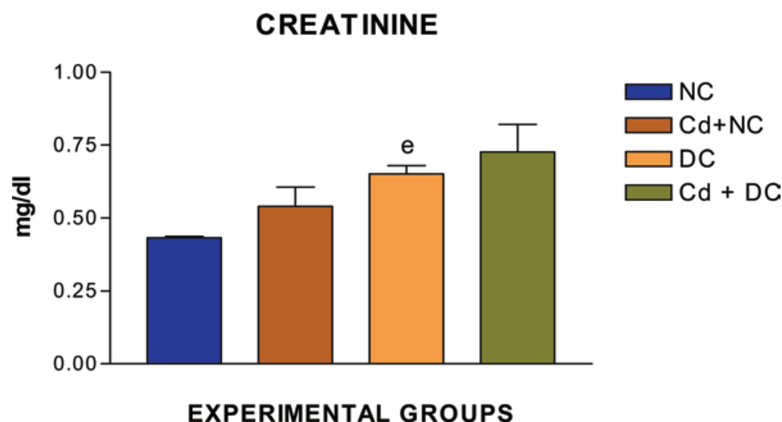

Figure 9. Serum creatinine level in all the experimental groups. $n=6$; data are expressed as mean ± SE. NC, nondiabetic control; Cd+NC, nondiabetic control+cadmium; DC, diabetic control; Cd+DC, diabetic control+cadmium. e) $P<0.0005$, compared to NC. (See colour version of this figure online at www.informahealthcare.com/dct)

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Epidemiological and animal studies of the recent past suggest a positive correlation between Cd exposure and the intensity and severity of diabetes (Edwards and Prozialeck, 2009). Many of the studies showing this link between the two have employed an experimental paradigm of Cd exposure subsequent to diabetes induction. For example, in one study, Cd was shown to exacerbate diabetic hyperglycemia and metal accumulation, with a 4-fold higher fasting blood glucose level in alloxan-induced diabetic animals treated with Cd, whereas there was only a 2-fold increase in alloxan-alone–treated animals (Bernard et al., 1991). In another study involving Cd administration to streptozotocin-induced diabetic animals, Jin et al. (1999) showed doubled urinary N-acetyl β-glucosaminidase levels and 2-fold higher metal accumulation in the kidney in these animals, compared to nondiabetic Cd-treated of animals. In contrast, the present study, with an objective of understanding the intensity or severity of diabetic complications in previously Cd-intoxicated animals, has revealed a paradoxical set of observations. Whereas oxidative stress is found to be elevated, as marked by increased LPO, decreased antioxidant (both nonenzymatic and enzymatic) levels, and serum markers of hepatic and renal damage, together with increased glycogenolysis, gluconeogenesis, and tissue cholesterol and lipid contents, but reduced protein content and decreased serum insulin titer and elevated lipid profile, changes that are suggestive of the exacerbation of diabetic effects by previous Cd exposure, glycemic and serum corticosterone levels are significantly lower than in diabetic animals, suggestive of a protective effect of previous Cd exposure in diabetic hyperglycemia. Significantly lower glucose elevation and clearance rates seen in GTT and significantly higher glucose clearance and elevation rates in IRT suggest better insulin sensitivity and glucagon response as the possible mechanism responsible for improved glycemic regulation, despite the more pronounced alteration in carbohydrate and lipid metabolism and oxidative stress. In the absence of any such study on metaboliwsms Cd exposure before diabetic induction, the present paradoxical observations defy explanation. Apparently, previous Cd exposure has a cumulative/additive effect on diabetic manifestations in terms of oxidative stress and metabolic alterations, changes that are similar to the earlier reported exacerbation of effects on diabetic induction followed by Cd exposure. The only possible explanation for the observed improved glycemic status is the probable increased glucose oxidation and channelization of products into the lipogenic pathway. Decreased corticosterone levels, together with increased E_2 and reduced P_4 levels, could, in this context, be a favorable hormonal milieu for insulin sensitivity and glucose oxidation coupled to lipogenesis. Further studies are warranted to test this hypothesis. This aberrant protective influence of previous Cd exposure against diabetic glycemic status may not be all that favorable a response, as the attendant accentuated metabolic dysregulation and higher serum lipid profile and tissue lipid/cholesterol accumulation are potent alterations that portent a possible shift toward development of type 2 diabetes.

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Conclusion

Overall, the present results are novel and could lead to further investigations on animal models of type 1 diabetes with a history of previous Cd exposure.

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Declaration of interest

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