Chronic cadmium exposure in rats produces pancreatic impairment and insulin resistance in multiple peripheral tissues

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A B S T R A C T

Previous studies have linked cadmium exposure to disturbances in carbohydrate and lipid metabolism. In this study we investigate the effects in Wistar rats of an oral cadmium exposure in drinking water on carbohydrates, lipids and insulin release. Also, using mathematical models we studied the effect of cadmium on insulin resistance and sensitivity in liver, muscle, adipose and cardiovascular tissue. Cadmium exposure induced hyperglycemia, increased insulin release after a glucose load, and caused increases in serum triglycerides, cholesterol, LDL-C and VLDL-C, and a decrease of HDL-C. In addition, there was an accumulation of cadmium in pancreas and an increase of insulin. After exposure, HOMA-IR was increased, while the HOMA-S%, QUICKI and Matsuda-DeFronzo indexes showed decreases. A decrease of insulin sensitivity was shown in muscle and liver. Additionally, cadmium increases insulin resistance in the liver, adipose tissue and cardiovascular system. Finally, β-cell functioning was evaluated by HOMA-B% index and insulin disposition index, which were decreased, while insulin generation index increased. In conclusion, cadmium increases insulin release, induces hyperglycemia and alters lipid metabolism. These changes likely occur as a consequence of reduced sensitivity and increased insulin resistance in multiple insulin-dependent and non-dependent tissues, producing a biochemical phenotype similar to metabolic syndrome and diabetes.

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1. Introduction

Cadmium (Cd) is a toxic transition metal, which is considered one of top five most hazardous environmental contaminants by the Agency for Toxic Substances and Disease Registry [4]. The main sources of Cd intoxication in non-occupationally exposed population are drinking water, food contaminated and tobacco smoking. Cd is considered highly toxic and is progressively bioaccumulated in organism [34,42] with a biological half-life in humans, which is estimated in decades. Acute and chronic Cd exposure in animals and humans has toxic effects in various organs and tissues, such as liver, kidney, lung, gut, central nervous system, ovaries, testes, and pancreas [6,24,25,32,45,68,71,77,79].

It has been proposed that Cd exposure can produce sustained hyperglycemia, leading to the development of a type 2 Diabetes mellitus (T2DM) [38,59,62]. Cd has been associated with a decrease in glucose tolerance and increased insulin resistance [21,26,57]. Under normal conditions, the maintenance of glucose homeostasis depends on a coordinated process involving the level of circulating glucose and the secretion of insulin by the β-cells. In the post-absorptive state, most glucose uptake takes place in insulin independent tissues, like the liver, brain, gut and kidney, and the remaining occurs in insulin-dependent tissues (muscle and adipose) [18,19]. The β-cells can sense and compensate hyperglycemic states with an increased secretion of insulin, which can lead to a state of resistance in multiple peripheral tissues [1–3,7,61]. Thus, insulin resistance can be defined as a reduced responsiveness of the tissues of an organism to high insulin concentrations while conversely a reduced sensitivity to insulin is associated with an increase of basal insulinemia [67].

A number of mathematical models are used to evaluate insulin-sensitivity and insulin-resistance, and have a good correlation with the gold standard euglycemic-hyperinsulinemic clamp, as proposed by DeFronzo et al. (1979) [17]. The homeostasis model assessment (HOMA-IR) is one of the most widely used and provides information about insulin resistance at peripheral tissue level, but other mathematic models, such as the quantitative insulin sensitivity check index (QUICKI) and the Matsuda-DeFronzo index [43] have been proposed.

On the other hand, indexes that give information about sensitivity and insulin-resistance in specific tissues include the hepatic insulin sensitivity index [43], the hepatic insulin resistance index, muscle insulin sensitivity index [2], the adipose tissue dysfunction index [19,70], and the cardiovascular resistance index [7]. Together, these indexes can be used to evaluate and characterize both systemic and organ-specific changes in the hyperglycemic states produced by different conditions or toxicants, and could be useful to determine metabolic changes during hyperglycemia caused by a cadmium exposure.

In the state of insulin resistance, the high levels of insulin can produce β-cells depletion, and eventually diabetes mellitus, therefore, evaluation of β-cells function is very important [15]. The mathematical model: homeostasis model for assessment for beta cells (HOMA-β%) [44] has been extensively described and validated to determine the function of these cells. As a complement of the complex cellular changes in hyperglycemias, Selter et al. (1967) proposed the insulinogenic index and subsequently Chung et al. (2012) added the disposition insulin index, which provides an indirect measure of the ability of different tissues to dispose or utilize circulating insulin [2,14,18,63].

In this regard, Cd has been related with the development of hyperglycemic states [27]. However, little is known about the sensitivity and/or resistance to insulin after chronic exposures to Cd. Thus, the aim of this work was study the level of Cd that produces alterations in insulin-sensitivity, and insulin-resistance in liver, muscle, cardiovascular tissue and adipose tissue, as well as determine if there is impairment in insulin secretion after a chronic exposure of Cd in rats.

2. Material and methods

2.1. Animals

Male Wistar rats (one-month age) were obtained from the animal facility shelter "Claude Bernard" from the Benemérita Universidad Autónoma de Puebla, Mexico. Animals were maintained in compliance with the requirements according to the Mexican current legislation, the NOM-062-ZOO-1999 (SAGARPA) based on the Guide for the Care and Use of Laboratory Animals and the Institutional Committee of the Benemérita Universidad Autónoma de Puebla. Rats were maintained in an environmentally controlled room under 12-h light/dark cycle. Food and water were provided *at libitum*.

2.2. Animal treatment

Ninety rats were divided randomly into control group (total n = 30 rats) and Cd exposed group (total n = 60 rats). For the groups exposed to Cd, $CdCl_2$ was added to the drinking water to yield 32.5 ppm Cd, and this water was then continuously provided to the rats until the end of the study. Because cadmium has a low absorption in gastrointestinal tract (<10%), under the conditions of this study, the amount of Cd absorbed by the rats was approximately 6.5–16.25 μg/day, level that produce an increase of Cd in pancreas without tissue injury [31–33].

Subgroups of 20 rats were sacrificed at 60, 90 and 120 days from the onset of treatment. Rats from control groups received unaltered water ad libitum, and subgroups of 10 rats were killed at 60, 90 and 120 days and acted as in time matched controls. Just prior to finish the exposure, the rats received an oral glucose load equivalent to 1.75 g-glucose/kg weight. The rats were then anesthetized intraperitoneally with xylazine/ketamine. Under anesthesia, whole blood (200 μL) was drawn via cardiac puncture at 0, 30, 60 and 90 min. Rats were then killed by exsanguination.

2.3. Animal Zoometry

Weight, percentage of fat and size of the rats were monitored weekly. The weight was measured using a digital balance (Torrey, model: LPCR-20/40), the size of each animal was obtained after measuring the length between the base of the tail to the tip of the nose and abdomen diameter was estimated using the diaphragm zone as a superior limit and the fold of the legs as the inferior limit. The body mass index (BMI) was calculated using the formula: weight/size^2, and the percent of fat was calculated according to the Lee index for rodent models, with the following formula: % fat = [(weight in g$^{(0.33)}$)/size in cm] × 100 [58]. Body mass index and percent of fat were used for the calculation of Liver Insulin Resistance Index (LIRI).

2.4. Glucose tolerance test (OGTT) and other biochemical assays

The concentrations in serum of glucose, triglycerides, cholesterol, Low Density Lipoprotein-Cholesterol (LDL-C) and High Density Lipoprotein-Cholesterol (HDL-C) in serum were determined with a semi-automatic analyzer BTS-350 (BioSystems). The level of Very Low Density Lipoprotein (VLDL) was obtained using the equation of Friedenwald [81]. Free Fatty Acid (FFA) concentration were determined according to the method described by Brunk and Swanson, 1981 [9]. Plasma insulin concentrations were determined by an ELISA immunoassay (Diagnóstica Internacional Company), with the resulting antibody–antigen complex assessed at 415 nm in a Stat fax 2600 plate reader (WinerLab Group). Insulin concentrations were obtained from a standard curve with a range of 0–20 μU/mL. The variation coefficients were: intra-assay: 1.5–2.5% and inter-assay: 6.5–8.0%. Insulin in pancreas was determined by an ELISA immunoassay (Diagnóstica Internacional Company), briefly tissue was homogenized with a solution of HCL/Ethanol (50:50) and stored overnight at −20 °C, then homogenate was centrifuged, and insulin measured in the supernatant.

2.5. Peripheral insulin sensitivity and resistance

In order to obtain information about the global function of insulin in the rats exposed to Cd, HOMA %S, HOMA-IR, QUICKI and Matsuda-DeFronzo were used as mathematical models according to the report of Wallace et al. [11,80].

2.6. Tissue-specific insulin sensitivity and resistance

Because that sensitivity and insulin resistance is dependent of the tissues, we studied the effect of the chronic Cd exposure on insulin resistance and sensitivity in specific organs and tissues, which included: liver, muscle, adipose tissue and cardiovascular system. Liver sensitivity was calculated according to the Hepatic Insulin Sensitivity index (HIS index; Matsuda and DeFronzo) [43], the Hepatic Insulin Resistance Index (HIRI; Abdul-Ghani) [2] and the Liver Insulin Resistance Index (LIRI; Vangipurapu) [76]. Insulin sensitivity in muscle was determined by the index of Muscle Sensitivity to Insulin (ISMI; Abdul-Ghani) [2]. The insulin