



EFFECT OF LIFETIME LOW DOSE EXPOSURE TO CADMIUM ON LIPID METABOLISM OF WISTAR RATS

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ABSTRACT

The aim of the study was to assess the effects of exposure to low doses of cadmium dissolved in drinking water (at a concentration 200 times higher than the maximum permissible dose) on lipid metabolism in 20 Wistar rats. Animals were divided into two groups, control and experimental. Experimental animals were exposed to low doses of cadmium chloride in concentration of 20 µM of drinking water. The biochemical parameters determined in blood plasma included lipase, triglyceride, cholesterol and HDL-cholesterol in blood of rats. Lipase decreased; triglycerides, cholesterol and HDL-cholesterol were not changed in rats exposed to cadmium. The objective assessment of potential damage should consider not only the final results but also levels of the investigated parameters throughout the trial, i.e. in its individual stages.

Keywords: cadmium, lipid metabolism, rats, toxicology

INTRODUCTION

Cadmium (Cd) is a non-essential, non-degradable and ubiquitous heavy metal widely distributed in all components of the environment (ATSDR, 2008). Toxic effects of cadmium were reported by several authors both from animal experiments and human epidemiological studies (Li et al., 2010). The most important target organs of cadmium exposure are liver and kidneys (Kramarova et al., 2005; Holovska et al., 2009), gonads (Kimakova et al., 2005; Massányi et al., 2007; Nad et al., 2007), cardiovascular and respiratory system (Molina et al., 2008; Ozturk et al., 2009), gastrointestinal system (Cigankova et al., 2010), nervous system (Sato et al., 1978) and bones (Brzóska and Moniuszko-Jakoniak, 2004). Humans are usually exposed to cadmium in the workplace or through ingestion of cadmium-contaminated food and water (Jarup and Akesson, 2009).

For the chronic, low-level patterns of exposure that are common in humans and domestic and wild animal populations, the kidneys and liver are the primary targets of toxicity. Although the exact mechanism underlying cadmium-induced tissue damage remains unclear, it is now largely accepted that the putative mechanism revolves around the ability of the metal to generate free radicals (Valko et al., 2006) causing a change in the structure of the cellular membrane in the process of lipid peroxidation. It has been reported that lipid peroxidation produced by cadmium exposure induced damage of many tissues. Toxic effects of cadmium are associated with developmental disorders and carcinogenesis (Joseph, 2009).

The purpose of this study was to evaluate by determination of physiological and biochemical parameters whether lifetime exposure to low doses of cadmium caused any alteration in lipid metabolism of rats.

The aim of the study was to observe mean lifespan, survival, body weight changes and selected parameters of sacharide metabolism in rats exposed throughout their life to low doses of cadmium via drinking water.

MATERIAL AND METHODS

Animals, breeding conditions and experimental protocol

Twenty male Wistar rats, 52 days old, mean weight 128 ± 11 g, were obtained from the SPF breed of the Central Animal Laboratory, Faculty of Medicine, Safarik University in Kosice (CAL FM ŠU) and were divided randomly into two groups, control and cadmium

exposed group. The rats were kept individually in all-glass metabolic cages with free access to drinking water and food from day 52 of age (day 0 of the experiment). Both groups were given standard feed. The control group (C; n=10) was supplied pure drinking water. The experimental group (Cd; n=10) obtained drinking water containing cadmium chloride in concentration of 20 μM ; i.e., 2.0 mg Cd/L of drinking water or 200-fold of MAC (maximum acceptable concentration) throughout the experiment.

All animals were kept in the same room at temperature of $22\pm 2^\circ\text{C}$, relative humidity 50%, and 12 h daylight/12 h darkness regimen. The experiments were performed at CAL FM ŠU, an experimental facility accredited for breeding. The experiments were approved by the Ethical commission of FM ŠU and State veterinary and food administration of SR (No. Ro-7879/04-220/3).

In 26-week intervals we determined survival, mean lifespan, body mass, intake of food, intake of water and levels of lipase, triglycerides, cholesterol and HDL-cholesterol in blood plasma. Blood was sampled always in the morning between 7 and 9 a.m.

Biochemical analysis

Blood was collected from the tail vein (500 μL) into an anticoagulant mixture with lithium heparinate, centrifuged at 1.000 g for 45 min at 4°C and stored at -24°C until analysis. In case of hemolysis the plasma was not used. We investigated the following parameters: Lipase (LPS, E.C.3.1.1.3.), triglycerides, cholesterol and HDL-cholesterol determination was performed by a commercial test from Dot Diagnostics Ltd., the Czech Republic.

Statistical analysis

The experimental data were evaluated by means of Student *t*-test and Mann–Whitney U-test or single way ANOVA with subsequent Newman-Keuls *post-hoc* test. Correlation analysis was carried out by Spearman test. A significance level of 0.05 was chosen for all statistical tests. The values are expressed as means \pm S.E.

RESULTS AND DISCUSSION

Mean lifespan and survival

Comparison of mean lifespan for both groups after 156 weeks of the experiment is shown in the figure 1A. No significant differences were observed between control and cadmium exposed rats. Survival of rats is presented in the figure 1B. Survival of the cadmium exposed rats was by 10% lower compared to the control. The most frequent mortality causes were haemorrhage into the gastrointestinal tract and tumours. Exposure levels (toxicological characteristics are presented in the Table 1.

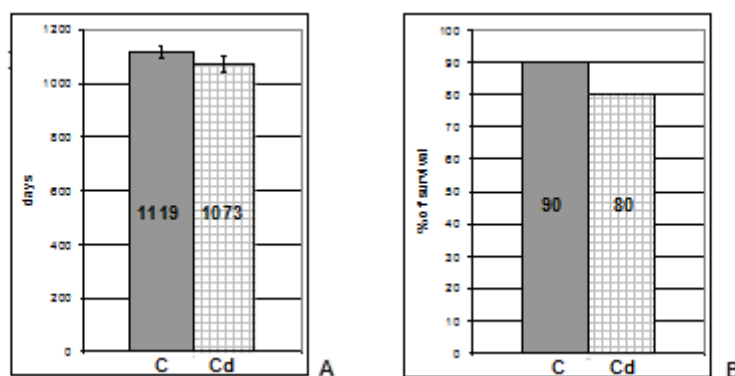


Figure 1 Mean lifespan (A) and percentage of survival (B) at week 156 of the experiment (mean \pm S. E.)

Table 1 Selected toxicological characteristic

Parameter	
Total dose received during the experiment in mg/kg b.w.	144.4
% of LD ₅₀	64.2
ADD in mg/kg/day	0.132
LADD in mg/kg/day	0.129

LD₅₀ = lethal dose 50%; ADD = mean daily dose;
LADD = longtime mean daily dose

Body weight, food and water intake

The mean body weight (Figure 2A) was significantly lower in cadmium exposed rats ($P < 0.001$). Intake of feed differed insignificantly between the groups (Figure 2B) although it

was slightly lower in the treated rats. Figure 2C shows that water intake by cadmium exposed rats was significantly lower ($P<0.001$) which is attributable to its bad taste.

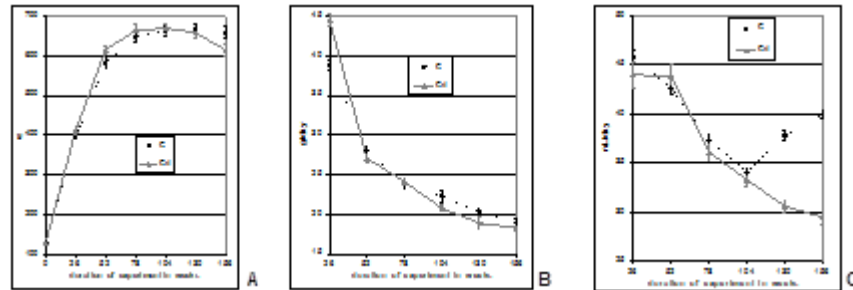


Figure 2 Changes over time in body mass (A) food (B) and water intake (C) of rats (mean \pm S. E.) during the experiment

Effects on lipid metabolism

During the experiment, we observed a significant decrease in lipase activity ($P<0.001$) in rats exposed to cadmium (Figure 3A, Table 2). No significant differences in triglyceride levels were observed between control and cadmium exposed rats (Figure 3B; Table 2). Cholesterol levels were not significantly changed after exposure to cadmium (Figure 3C, Table 2). The levels of HDL-cholesterol showed no significant differences between control and cadmium exposed animals (Figure 3D, Table 2).

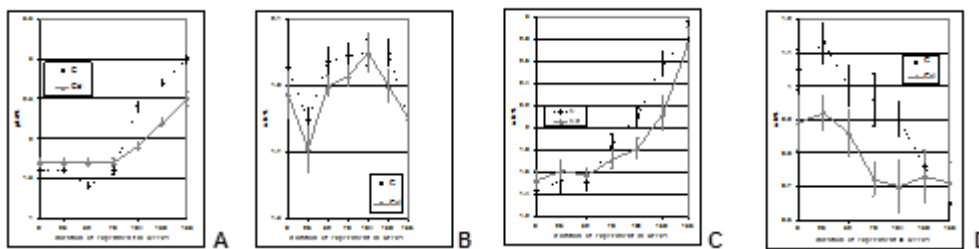


Figure 3 Changes over time in lipase (A), triglycerides (B), cholesterol (C) and HDL-cholesterol (D) in blood plasma of rats (mean \pm S. E.) during the experiment

Table 2 Comparison of parameters of lipid metabolism between control and cadmium exposed rats for wee 156 of the experiment

Group	Lipase (μ kat/L)		Triglyceride (mM/L)		Cholesterol (mM/L)		HDL-cholesterol (mM/L)	
	C	Cd	C	Cd	C	Cd	C	Cd
Mean	3.00	2.50	1.80	1.81	2.81	2.76	0.65	0.71
S.D.	0.15	0.22	0.12	0.11	0.52	0.44	0.15	0.17
S.E.	0.05	0.08	0.04	0.04	0.17	0.15	0.05	0.06
Geometric mean	3.00	2.49	1.80	1.80	2.77	2.73	0.64	0.68
Maximum	3.50	2.85	1.95	2.00	4.00	3.60	0.90	0.90
Upper quartile	3.13	2.68	1.90	1.85	3.50	3.10	0.80	0.79
Median	3.00	2.48	1.80	1.80	2.70	2.65	0.60	0.75
Lower quartile	2.90	2.36	1.68	1.76	2.50	2.43	0.55	0.64
Minimum	2.75	2.15	1.65	1.60	2.20	2.30	0.45	0.35
Range	0.45	0.70	0.30	0.40	1.80	1.30	0.45	0.55
Variance coefficient	0.05	0.09	0.07	0.06	0.19	0.16	0.23	0.23
P value	<0.001		0.912		0.839		0.474	

Almost all organ systems are involved in cadmium toxicity, although the most sensitive to damage are liver and kidney. However, few data were published about the influence of chronic exposure to cadmium on metabolism of lipids (Alvarez *et al.*, 2007). The manifestation of the toxicity of cadmium has been well documented but the details of pathogenic mechanisms causing damage to lipid metabolism at long-time low dose exposure have not yet been fully elucidated.

Based on LOAEL studies can be estimated as 7.0 mg Cd/kg/day. In our experiments these values were not exceeded. MRL (minimal risk level) for chronic duration of oral exposure to cadmium was determined as 0.1 μ g/kg/day. In our experiment this value was exceeded 1.000-fold.

The low effect of cadmium on the lifespan compared to other heavy metals can be attributed to the fact that cadmium in low doses may be an essential element (Lukacinova *et al.*, 2011; Schwarz, 1977). The potential essential role of cadmium was discussed in a review article by Anke *et al.* (2005). The authors described the effects of supplementation of very low doses of cadmium in the diets. Cadmium deficiency affected significantly the first insemination, rate of abortion and number of services per gravidity. Cadmium-deficient nutrition of mothers affected the activity of the young. In a previous study higher mean number of newborns per litter was found after exposure to cadmium (Lukacinova *et al.*, 2008). Another possible explanation of the low effect of cadmium on the lifespan may be that compared with the LD₅₀ dose, rats received only 64% of the dose throughout the trial. According to Kotsonis and Klaasen (1977) cadmium LD₅₀ dose is 225 mg/kg b.w.

There was a significant positive correlation between water and food intake ($r=0.81$, $P<0.001$). Because of that we assumed that the water intake was a major limiting factor of cadmium intake to tissues of rats. Such dependency was demonstrated in other studies (**Shibutani et al., 2001**). Comparison of body weight, food and water intake throughout the experiment is presented in the Figure 2. On the basis of body weight changes (Figure 2A) rats exposed to cadmium gained weight faster than control rats during the first 78 weeks but starting from week 104 their body weight gradually declined compared to control animals. Completely opposite trend was observed with food intake (Figure 2B) as the cadmium exposed rats showed higher intake of fed the first half of the experiment and lower in its second half in comparison with control rats. Water intake gradually decreased in cadmium exposed rats but in control animals decreased up to week 104 and then started to increase (Figure 2C).

Lipase is a secreted pancreatic enzyme with very low activity under physiological conditions. Lipase is considered to be sensitive and specific parameter in the diagnosis of pancreatitis. Digestion and absorption of long chain triglycerides, the major dietary lipids, is a very efficient process involving several steps, such as emulsification, hydrolysis by lipases to fatty acids and monoacylglycerols, dispersion of these products into the aquatic environment and intake by enterocytes. Hydrolysis by lipases begins in the stomach, where human gastric lipase breaks down about 15-20% of fatty acids and is completed in the upper small intestine, where it is mixed with pancreatic juice containing a lot of lipases. The resultant products, mixed micelles with bile salts are absorbed by enterocytes.

Pancreas excretes many lipases which break down particles of emulsion. One of these lipases, pancreatic triglyceride lipase prevails. Pancreatic lipase is a member of lipase gene family products and is closely related to two other exocrine proteins, pancreatic lipase like proteins 1 and 2 (PLRP1 and PLRP2). The role of these two similar proteins in the digestion of dietary fat, especially in adults, remains unexplained, although some data in PLRP2 deficient mice suggest that this homologue is responsible for the digestion of dietary triglycerides in the newborns.

Pancreatic triglyceride lipase, which is unbounded to an interface containing bile salts, phospholipids or proteins begins to be inhibited by binding to interface in a complex with other exocrine protein, colipase. Interaction of colipase with PLRP2 is unlikely and may differ in various species. Recently, studies were carried out dealing with molecular mechanisms that allow the function of lipases in inhospitable oil-water interface (**Reis et al., 2009**).

According to **Naito and Felts (1970)** physiological values of lipase in rats range from 1.2 to 3.1 $\mu\text{kat/L}$. Our experiments showed that the levels of lipase in the cadmium exposed rats (Figure 3A) were higher in the first 78 weeks and lower in the following weeks in comparison with control rats. This suggests that the level of lipase may be considered an indicator of chronic exposure to cadmium only after prolonged exposure.

Content of fat in the body depends on the species, nutrition, physiological state of the organism, age, gender and other factors. In rats, triglyceride values range from 1.8 to 2.1 mM/L (**During et al., 2000**). In our experiments, we found an upward trend in the level of triglycerides up to week 104, when the levels began to decrease (Figure 3B). Triglyceride levels were lower in the cadmium exposed group throughout the experiment.

Cholesterol from food is absorbed in the intestine. Before the absorption the pancreatic esterase releases cholesterol from esters. The absorption of cholesterol depends on carboxylic acids and bile which form insoluble complexes and emulsify it. Greater proportion of cholesterol is synthesized in the liver. Cholesterol is synthesized from acetyl-CoA in three stages. Serum cholesterol is transferred to low density lipoprotein (LDL) by binding to a receptor on the cell membrane which allows transfer of cholesterol into the cell by endocytosis. Cholesterol is metabolized to various substances, such as steroid hormones, bile acids, 7-dehydro-cholesterol and more. Certain proportion of cholesterol is excreted via the bile with which gets into the gut and leaves with feces, another part is converted by gut microflora to coprostanol.

Total cholesterol is one of the most famous and also most important risk factors for development of atherosclerosis. The knowledge of cholesterol, its fate in the organism and the syndromes that are associated with cholesterol biosynthesis has changed significantly in the past decades (**Herman, 2003**). Normal values in rats range from 1.2 to 2.4 mM/L (**Herman, 2003**), and increase gradually with age. In our study the levels of cholesterol (Figure 3C) were higher in cadmium exposed rats up to week 72 and after that decreased below the levels found in the control.

The levels of HDL-cholesterol were significantly lower in rats exposed to cadmium with the exception of week 156 in comparison with control animals (Figure 3D).

In order to interpret objectively the results obtained in this study it appears necessary to continue with these investigations and extend it by determination of additional biochemical, immunological and genetic parameters.

CONCLUSION

The results obtained extend the knowledge about lifetime exposure to low doses of selected heavy metals in drinking water of rats. It is particularly important to assess the changes over time in the levels of investigated parameters and compare them with those in the control rather than draw conclusion from results obtained only at the end of the experiment.

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