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Comparative effect of water and food-chain mediated cadmium exposure in rats

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Abstract This study sets out to compare the absorption and toxicity of Cadmium (Cd) administered via the food-chain and inorganic Cd administered in drinking water after 1 and 3 months exposure using rats as animal model. The food-chain was mimicked by exposing rats to diet containing Cd pre-exposed fish. The uptake of Cd by the rats after both mode of exposure was calculated by summing up the Cd burden in the liver and kidneys and was expressed in terms of % intake. The toxicity of Cd was assessed by monitoring biochemical indices of liver function in the plasma and liver. Regardless of the mode of exposure of the rats, the Cd load in the liver and kidney was significantly ($P < 0.05$) higher than the respective controls with the kidney having a significantly higher load than the liver after both periods of exposure. However irrespective of the mode of exposure, more Cd was accumulated in the liver and kidney of the 3 months exposed rats relative to those exposed for 1 month. The uptake of Cd by rats exposed to Cd via the food-chain for 1 and 3 months was significantly ($P < 0.05$) lower when compared to the corresponding water mediated Cd exposed rats, except for the liver after 3 months of exposure. The liver L-ALT activity of rats administered inorganic Cd in drinking water for 1 and 3 months was significantly ($P < 0.05$) lower as compared to controls. Parallel analysis of the plasma showed no significant ($P > 0.05$) difference in L-ALT activity between both groups after the same periods of exposure. The L-AST activity in the plasma of rats similarly exposed to Cd for 1 and 3 months was significantly ($P < 0.05$) higher as compared to controls with a corresponding reduction in the liver. Conversely no significant ($P > 0.05$) change was observed in plasma and liver L-ALT and L-AST activities after food-chain mediated exposure to Cd for 1 and 3 months in relation to their respective controls. These findings indicate that Cd incorporated in fish is more easily bioavailable, but less toxic relative to inorganic Cd salts at the end of 3 months of exposure in rats.

Keywords Cadmium · Food-chain · Bioavailability · Liver function · Rats

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Introduction

Cadmium (Cd) is an environmental and industrial pollutant that poses a serious health risk to humans and animals. The metal accumulates mainly in the liver and kidney where it has multiple cytotoxic and metabolic effects. Some of these effects include the interference with the normal action of essential

metals (Chmielnicka and Sowa 1996; Oishi et al. 2000) and induction of oxidative stress (Sarkar et al. 1995; Zikic et al. 1996; Bagchi et al. 1996; Asagba et al. 2004; Roqalska et al. 2009; Chater et al. 2009). Cd induces oxidative stress by disturbing the antioxidant defence system thus causing oxidative damage in erythrocytes and various tissues (Patra et al. 1999; Bagchi et al. 2000). Cd induced oxidative damage in the liver can cause the release of abnormal quantities of L-aspartate aminotransferases (L-AST) and L-alanine aminotransferases (L-ALT) into the blood (Guilhermino et al. 1998; Asagba and Eriyamremu 2007).

Contamination of our water bodies and soil by cadmium may result in the uptake of the metal by aquatic and terrestrial organisms. Hence in the absence of such adverse habits as smoking, the main route of exposure to Cd in humans appears to be through the food or drinking water. Foods such as wheat, rice, snails, crabs, oysters and mushrooms obtained from contaminated areas have been shown to have high Cd concentrations (WHO 1992). Cd may get into the body when these foods are eaten, which makes the food chain an important source of exposure to the metal. Bioavailability of Cd from some of these foods have been the subject of numerous studies in literature (Sullivan et al. 1984; Groten et al. 1990; Lind et al. 1995; Kikuchi et al. 2003; Reeves et al. 2005). Available evidence in man and animals indicate that the intestinal absorption of Cd from shellfish is lower than that of inorganic Cd (Siewicki et al. 1987; Vahter et al. 1996), although the study by Sullivan et al. (1984) found no difference in bioavailability of Cd from shellfish in relation to inorganic Cd. The differences in bioavailability of Cd have been linked to the chemical forms of Cd. It has been reported that Cd in foods exists mainly as Cd-MT or MT-like binding proteins (Ohta et al. 1993). It has been observed that the intestinal absorption and organ distribution of Cd-MT is lower or comparable to inorganic Cd in rodents (Muller et al. 1986; Sugawara and Sugawara 1991; Ohta and Cherian 1991; Groten et al. 1994). The differences in the absorption and distribution of these different forms of Cd underscore the need to compare the toxicity of inorganic Cd and Cd from foods.

Fishes are exposed to varying Cd concentrations in their habitats and like oysters they have an ability to accumulate Cd burdens at levels which exceed aquatic concentrations (Shukla et al. 2007; Asagba et al. 2007). Fish may therefore be an important vector of Cd transfer to higher levels of the food chain, including humans when consumed in foods. Few studies exist on the bioavailability and toxicity of Cd incorporated into fish. Fewer still exist on the comparative bioavailability and toxicity of Cd from Cd-exposed fish and inorganic Cd in drinking water. In this study the bioavailability and toxicity of Cd from Cd exposed fish was compared with almost an equivalent concentration of inorganic Cd provided in drinking water. Specifically the absorption of Cd was assessed by estimating the uptake of the metal in the liver and kidneys while the toxicity of the metal was assessed by monitoring biochemical indices of liver function in the plasma and liver.

Materials and methods

Experimental animals

Two experiments were performed in this study. One water-mediated exposure to Cd and the other a food-chain mediated exposure to Cd. Forty-four male adult albino rats of the Wistar strain were used for the experiments. The animals were obtained from the animal unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. They were allowed to acclimatize to Laboratory conditions for 2 weeks before the commencement of the study.

Experimental design

For food-chain mediated Cd exposure rats with a mean weight of 186 ± 2 g were distributed into two groups with ten rats each. In order to simulate exposure to Cd through the food chain two diets (control and test diets) that differed in terms of the nature of the protein were formulated. The test diet contained milled Cd exposed fish as a source of protein, while the control diet contained milled non-Cd exposed fish. Other components of the diets were cornstarch (Livestock Feed, Plc, Lagos), ABC multivitamin/minerals mix (Vitamin World, New York, USA), palm oil and peanut husk (obtained locally in Benin-City, Nigeria). The percentage composition of the control and test diets and their respective Cd

content are presented in Table 1. The Cd content of the control and test diets was found to be statistically significant. Five of the rats in each group were fed the test diet, while the other five were fed the control diet. They were allowed free access to water but rats fed the control diet and test diet were weight matched and pair-fed (i.e. the feed intake of the control was restricted to the ad libitum intake of the test). The animals were housed individually in wire bottomed cages and the food consumption of the test animals was measured daily. While one group was given this treatment for 1 month, the other received the treatment for 3 months.

The experimental protocol employed for the rats exposed to Cd via drinking water was similar to that of rats which received Cd exposed fish in diet. For the water-mediated exposure to Cd 24 male albino rats with a mean weight of 185 ± 5 g were also distributed into two groups with twelve rats each. Six of the rats in each group were given Cd-free deionised water as drinking water and served as controls, while the other six were exposed ad libitum to aqueous solution of $CdSO_4$ containing the equivalent of 0.3 mg Cd/l. The rats were also housed individually in wire bottomed cages and their water consumption was measured daily. The first group of rats was treated for 1 month, while the second was for 3 months. During the exposure periods, the concentration of cadmium in treated water was measured to ensure conformity with the specified dose. All rats used for the water-mediated Cd exposure were allowed free access to grower's mash (Product of Bendel Feeds & Flour Mills, BFFM Ltd., Ewu, Edo State, Nigeria) containing 1.1% calcium, 0.7% phosphorus and 1 I.U. vitamin D3/g. Cd concentration in the diet was measured in our laboratory to be 0.12 ± 0.04 μg/g.

Table 1 Composition of control and test diets

Ingredients	Control diet (%)	Test diet (%)
Milled Cd-exposed fish	0.00	22.00
Milled Cd-free fish	22.00	0.00
Corn starch	53.00	53.00
Palm oil	07.00	07.00
Dried pea nut husk	08.00	08.00
ABC multivitamin/minerals	05.00	05.00
Total	100.00	100.00

AAS analysis revealed that the Cd contents of the control and test diets were 0.1 ± 0.02 and 0.4 ± 0.03 mg per Kg, respectively

Regardless of their method of exposure, all rats were treated in accordance with the guidelines of the NIH on the experimental use of laboratory animals (National Research Council 1996). At the end of the specified duration of exposure, all the rats in both experiments were weighed and subsequently anesthetized (in chloroform saturated chamber). While under anaesthesia each rat was exsanguinated by heart puncture by means of hypodermic syringe and needle. The blood collected from the heart was transferred to heparinised tubes which were carefully swirled. Plasma was later obtained by centrifugation at 3000*g* for 10 min. The livers and kidneys were also quickly excised, placed on ice and subsequently weighed. Portions of the liver of each rat was homogenized to give 20% homogenates and centrifuged at 10,000*g* for 15 min to obtain clear supernatants for biochemical analysis.

Digestion of samples

Weighed samples of the liver and kidney of each rat as well as the compounded feeds were digested separately in beakers with 20 ml of acid mixture ($HNO_3/HClO_4$; 4:1 v/v). The digestion was facilitated by heating at 100°C after which the samples were allowed to cool and then diluted with deionised water to give a final volume of 100 ml. Before use, all glass and plastic utensils were washed in dilute nitric acid and rinsed with deionised water.

Cadmium analysis

The cadmium concentrations in the digests were measured by atomic absorption spectrophotometry (Varian AA 1475 Spectrophotometer). The test metal was dissolved in deionised water and used as standard. The analytical methodologies on the samples were calibrated with the analysis of International Atomic Energy Agency (IAEA) reference biological sample V-10 (Hays). The cadmium concentration obtained for the reference sample was in agreement with the certified value. In all the determinations, blanks were prepared to determine the effect of reagent purity on the metal levels.

Biochemical assays

L-Alanine aminotransferases (L-ALT) and L-aspartate aminotransferases (L-AST) activities in the plasma and liver homogenate supernatants were estimated by the methods of Reitman and Frankel (1957) and their activities were expressed in units/ml.

Statistical analysis

Data are expressed as means ± SEM. Differences between the experimental groups of both exposure modes were evaluated using the non-parametric Mann–Whitney *U*-test. A $P < 0.05$ was considered significant. All statistical calculations were done using STATISTICA version 5.0 computer program.

Results

The body weight gain, liver and kidney weights, tissue Cd concentration and tissue Cd load of rats after water and food-chain mediated Cd exposure are shown in Table 2. The results obtained show that the body weight gain of rats administered inorganic Cd in drinking water for 1 and 3 months was not significantly ($P > 0.05$) different as compared to the controls. Conversely a body weight loss was observed in rats fed Cd-exposed fish for the same periods and their pair-fed controls. However the body weight loss of rats fed Cd-exposed fish for 3 months was significantly ($P < 0.05$) more pronounced relative to the pair fed controls. Comparatively there was a significant ($P < 0.05$) difference in the body weight gain of rats fed Cd in diet and corresponding rats administered Cd in drinking water.

Irrespective of the mode of Cd exposure, no significant ($P > 0.05$) change was observed in the liver and kidney (1 month excluded) weight of Cd exposed rats relative to controls after both periods of exposure. The kidney weight of rats exposed to inorganic Cd in drinking water for 1 month was significantly ($P < 0.05$) higher than the controls. However the liver and kidney weights were significantly ($P < 0.05$) lower in rats exposed to Cd in diet as compared to rats offered inorganic Cd in drinking water after both exposure periods.

Regardless of the mode of exposure of the rats, the Cd load in the liver and kidney was significantly ($P < 0.05$) higher than the respective controls with the kidney having a higher load than the liver after both periods of exposure. More Cd was also accumulated in these organs in the 3 months exposed rats. However the Cd load in the liver and kidney of rats exposed to Cd via the experimental food-chain was significantly ($P < 0.05$) less than those exposed via drinking water, except for the liver after 3 months of exposure. Thus the study shows that weight gain, organ weights and Cd load in organs are influenced by the mode of exposure to Cd.

The food consumption pattern of rats fed Cd via the food-chain for 1 and 3 months is shown in Fig. 1. There was a time dependent decrease in food consumption of the rats during both periods of exposure.

Figure 2 presents data for the water and food intakes, Cd consumption and sum of liver and kidney Cd uptake of rats after water and food-chain-mediated Cd exposure. The water intake of rats administered Cd in drinking water was significantly ($P < 0.05$) less after both periods of exposure. In contrast the food consumption of rats exposed to Cd via feed was similar to their controls as rats exposed to Cd by this mode were pair-fed.

The intake of Cd was calculated based on the water consumed by rats exposed to the metal in drinking water and the feed consumed by those exposed via the diet. The amount of Cd consumed by rats exposed to the Cd-tainted diet was not significantly ($P > 0.05$) different from that of rats exposed to the metal in drinking water after both periods of exposure. The data obtained also indicate that more Cd was consumed by the 3 months exposed rats after both modes of exposure to the metal. The intake of Cd by the controls of rats exposed to Cd in drinking water was assumed to be zero as these rats consumed deionised water. The uptake of Cd by the rats after both mode of exposure was also calculated by summing up the Cd burden in the liver and kidneys and was expressed in terms of % intake. The uptake of Cd by rats exposed to Cd via the food-chain for 1 and 3 months was significantly ($P < 0.05$) lower than the corresponding rats treated with inorganic Cd in drinking water. Thus the study reveals that the Cd body burden of rats offered Cd in the diet was lower in relation to that of rats administered the metal in drinking water despite the consumption of similar amounts of Cd.

Table 2 Body weight gain, organ weight and organ Cd load of rats after water and food-chain mediated Cd exposure

Parameters	Mode of exposure			
	Water-mediated ($n = 6$)		Food-chain mediated ($n = 5$)	
	Control (−Cd)	Test (+Cd)	Control (−Cd)	Test (+Cd)
Body weight gain (g)				
One month exposure	37.9 ± 1.9	42.6 ± 2.7	−2.6 ± 1.6*†	−2.3 ± 3.8*†
Three month exposure	75.4 ± 9.9	78.2 ± 7.1	−9.6 ± 4.6*†	−19.8 ± 3.9*†‡
Organ weights (g)				
Liver				
One month exposure	7.6 ± 0.3	7.5 ± 0.3	6.9 ± 0.2*†	7.1 ± 0.1*†
Three months exposure	11.5 ± 0.2	11.7 ± 0.6	9.2 ± 0.3*†	9.6 ± 0.5*†
Kidney				
One month exposure	0.67 ± 0.02	0.71 ± 0.02*	0.61 ± 0.01†	0.63 ± 0.03†
Three months exposure	0.90 ± 0.02	0.87 ± 0.02	0.57 ± 0.02*†	0.59 ± 0.02*†
Tissue Cd load (ng/g)				
Liver				
One month exposure	25.0 ± 1.2	57.0 ± 2.9*	30.0 ± 2.2*†	42.0 ± 1.8*†‡
Three months exposure	36.0 ± 1.6	145 ± 20.4*	74.0 ± 4.9*†	160.0 ± 31.3*†‡
Kidney				
One month exposure	21.0 ± 2.9	67.0 ± 2.0*	35.0 ± 2.7*†	51.0 ± 3.6*†‡
Three months exposure	110.0 ± 24.5	560.0 ± 32.7*	58.0 ± 3.1*†	190.0 ± 33.5*†‡
Total Cd load (ng Cd)				
Liver				
One month exposure	190.0 ± 6.1	430.0 ± 11.0*	210.0 ± 11.2†	300.0 ± 15.2*†‡
Three months exposure	410.0 ± 19.6	1700.0 ± 200.0*	680.0 ± 58.0*†	1530.0 ± 75.9*†‡
Kidney				
One month exposure	14.0 ± 1.6	47.0 ± 1.6*	21.0 ± 1.3*†	32.0 ± 2.2*†‡
Three months exposure	98.0 ± 19.2	480.0 ± 32.7*	33.0 ± 2.2*†	110.0 ± 17.4*†‡

Values are means ± SEM. $P < 0.05$ (Mann–Whitney U-test) compared to control of water-mediated Cd exposure (*), test of water-mediated Cd exposure (†) and control of food-chain mediated Cd exposure (‡) groups

The activities of plasma and liver aminotransferases of the rats after both modes of exposure to Cd are presented in Fig. 3. The liver L-ALT activity was significantly ($P < 0.05$) lower in rats exposed to Cd in drinking water for 1 and 3 months relative to the respective controls, but parallel analysis in the plasma revealed no significant ($P > 0.05$) change in the activity of this enzyme after both duration of exposure. The plasma L-ALT activity of rats exposed to Cd in diet for 1 month was significantly ($P < 0.05$) less, but rats similarly exposed for 3 months had no significant ($P > 0.05$) change in plasma L-ALT activity relative to the pair-fed controls. The liver L-ALT activity of rats fed Cd in diet was also not significantly ($P > 0.05$) altered after both durations of exposure. In relation to rats exposed to inorganic Cd in drinking water, the plasma L-ALT activity of rats fed Cd in diet was significantly ($P < 0.05$) lower after 1 and 3 months but parallel analysis in the liver revealed that the activity of the enzyme was significantly ($P < 0.05$) higher after both periods of exposure.

There was a significant ($P < 0.05$) rise in L-AST activity in the plasma of rats offered Cd in drinking water for 1 and 3 months with a corresponding reduction in the liver as compared to the controls. In contrast no significant ($P > 0.05$) change in the activity of this enzyme was observed in the plasma and liver of rats exposed to Cd-incorporated fish in diet after both periods of exposure. However the liver

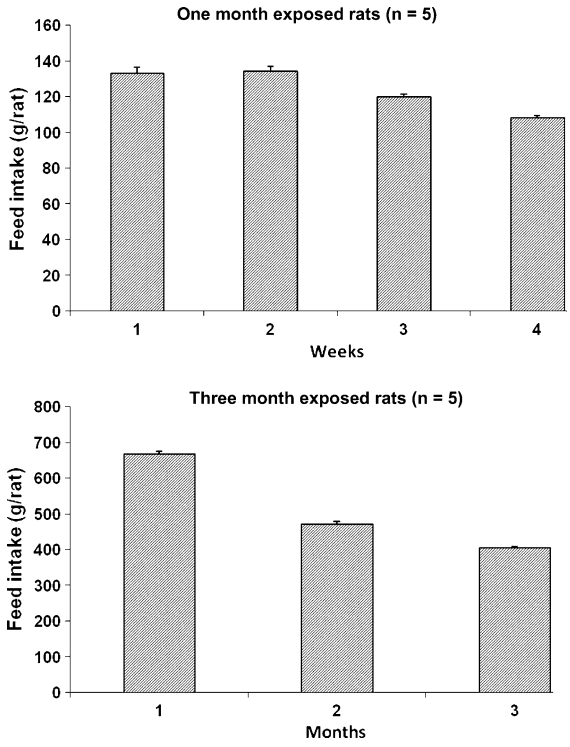

Fig. 1 Feed consumption pattern of rats after food-chain mediated Cd exposure

L-AST activity was significantly ($P < 0.05$) greater in rats fed Cd in diet relative to those administered the metal in drinking water after both periods of exposure. Also, the plasma L-AST activity of rats exposed to Cd in diet for 1 month was significantly ($P < 0.05$) greater, while those exposed for 3 months had a significantly ($P < 0.05$) smaller plasma L-AST activity as compared to that of corresponding test rats in the water-mediated Cd exposure. The results suggest that L-ALT and L-AST activities in the plasma and liver are influenced by the mode of exposure to Cd.

Discussion

The aim of this study was to compare the accumulation of Cd over time and its effect in rats after water and food-chain mediated Cd exposure. The results obtained in the study indicate that the level of Cd in the drinking water offered to the rats (0.3 mg Cd/l) did not alter the mean body weight gain of rats significantly ($P > 0.05$) in all exposure periods relative to their respective controls (Table 2). Significant ($P < 0.05$) body weight loss was observed by Horiguchi et al. (1996) after Cd administration in rats. All the same the result of the present study agrees with that of a previous study by Brzóska et al. (2000). Of particular interest is the difference in route of administration in these experiments. Brzóska et al. (2000) exposed the rats to 5 mg Cd/l via drinking water; While Horiguchi et al. (1996) exposed them to Cd (2.0 mg Cd/Kg bodyweight) subcutaneously once a week. Thus the present result adds credence to the current view that the route of administration is one of the factors that influence Cd toxicity. Gastrointestinal absorption of Cd is reported to be low (WHO 1992). Hence meaningful effects on body weight gain may only occur after exposure to very high doses of Cd in drinking water.

The lack of significant ($P > 0.05$) body weight change (Table 2) in rats fed Cd supplemented diet for 1 month relative to the pair-fed controls is in harmony with the reports of Haouem et al. (2007). These workers results indicate that rats given diet containing Cd-polluted radish bulb (1.1 microgram Cd/g of diet) had identical weight gain relative to controls. The significantly ($P < 0.05$) increased body weight loss observed in test rats fed Cd in diet for 3 months relative to the controls (Table 2) is an indication of Cd toxicity. The reduction in food intake by rats exposed to the Cd supplemented diet (Fig. 1) over time after both periods of exposure may be due to taste aversion occasioned by the presence of Cd and this may have led to a state of starvation in the rats. It could therefore be concluded that the observed body weight loss of these rats may also be due to starvation stress rather than Cd alone since it was observed in the pair-fed controls. These results suggest that Cd causes an additional stress over and above the starvation stress, which may be responsible for the differential changes in weight gain of these rats. Similar decrease in food consumption and consequent body weight loss has been reported in TCCD-treated rats and their pair-fed controls (Gorski et al. 1988). These findings underscore the importance of using pair-fed controls to fully control for toxicological changes in animal studies.

The marked accumulation of Cd in the liver and kidneys over time after both methods of exposures (Table 2) is not surprising as experimental evidence indicates that metallothionein (MT) aids the transport and distribution of Cd from the intestine to the tissues

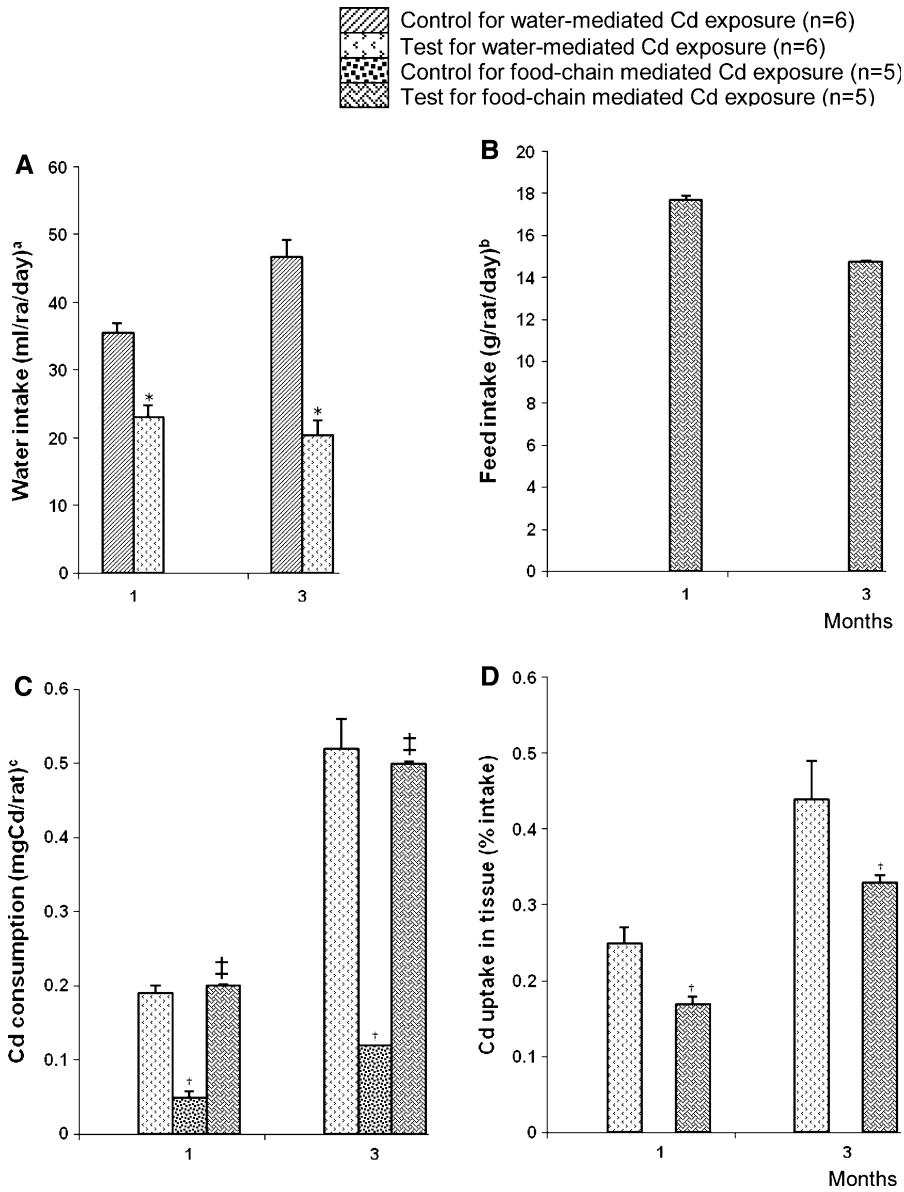

Fig. 2 Water intake (**a**), Feed intake (**b**), Cd consumption (**c**) and sum of Cd uptake by kidney and liver (**d**) of rats after water and food-chain mediated Cd exposure. Values are means ± SEM. $P < 0.05$ (Mann–Whitney *U*-test) compared to control of water mediated Cd exposure (*), test of water mediated Cd exposure (†) and control of food-chain mediated Cd exposure (‡) groups. [a] Water intake of rats was measured only for rats exposed to inorganic Cd in drinking water. [b] Feed intake was measured only for rats exposed to Cd in diet (controls were pair-fed) [c] Cd intake of rats exposed to Cd in water and diet was calculated on the basis of the concentration of Cd in drinking water (0.3 mg Cd/l) and diet (0.4 mg Cd/kg), respectively

(Ohta et al. 1993; Chang et al. 2009). MTs are a family of low molecular weight heavy metal binding proteins, unique in their high cysteine (Cys) content. These proteins are widespread in eukaryotes and plants and are also found in prokaryotes (Manuel et al. 1992; Klaassen et al. 2009). The higher concentration of Cd in the kidney relative to the liver in Cd exposed rats (Table 2) is consistent with previous reports (Cherian et al. 1978; Sabbioni et al. 1978; Min et al. 1992; Elsenhans et al. 1997). However the liver, which is much larger than kidneys, contained more total Cd (Table 2) after both methods of exposure.

Certainly it would have been ideal to have the data on the Cd levels in the urine and faeces of the rats, since it would give an idea of the % uptake in the body of the amount taken via either diet or drinking water and the difference between both modes of exposure. However, it has been reported that almost all Cd (about 99.3–99.6) accumulated in internal organs of rats exposed orally to 50 mg Cd/l was retained in the liver and kidneys (Brzóska et al.

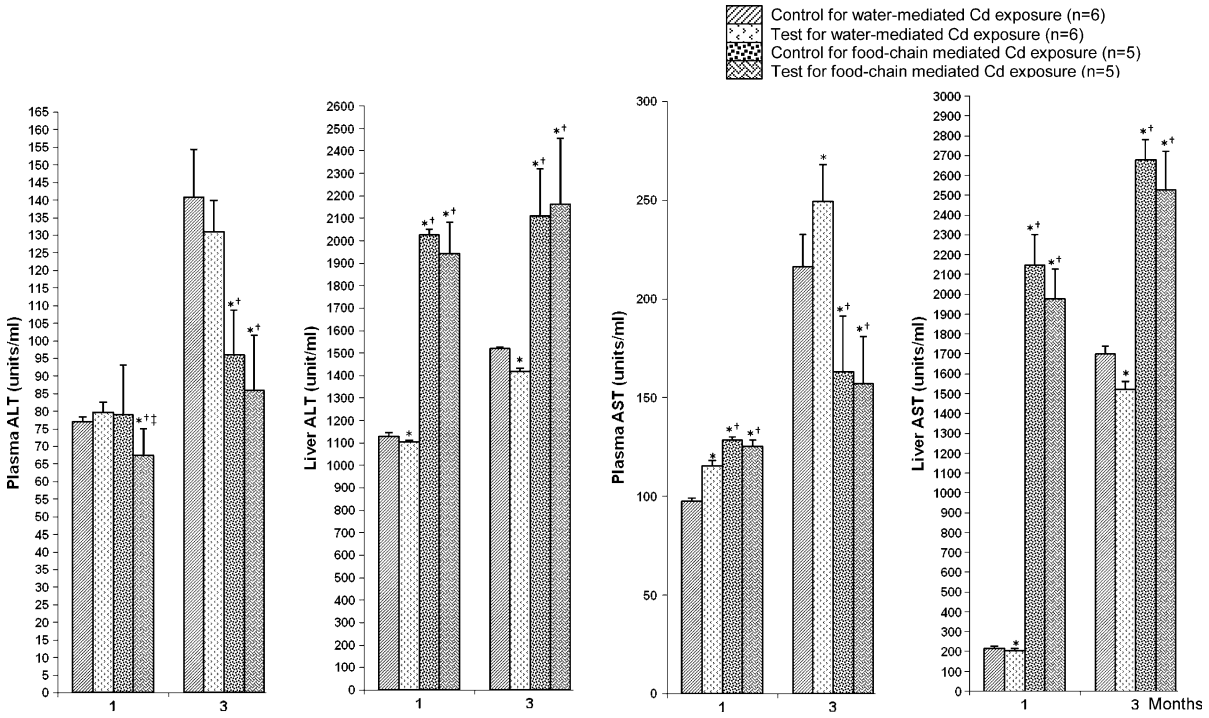

Fig. 3 Activities of plasma and liver aminotransferases of rats after water and food-chain mediated Cd exposure. Values are means ± SEM. $P < 0.05$ (Mann–Whitney U-test) compared to control of water mediated Cd exposure (*), test of water mediated Cd exposure (†) and control of food-chain mediated Cd exposure (‡) groups

2002). This accounts for the use of Cd load in the liver and kidneys in the estimation of Cd uptake in the present study which is also in harmony with previous reports (Andersen et al. 1992; Crowe and Morgan, 1997; Reeves and Vanderpool 1998; Lind et al. 1995; Lind et al. 1998). Thus the significantly ($P < 0.05$) smaller combined Cd load of the liver plus that of the kidney of rats exposed to Cd via the experimental food-chain relative to those exposed to the element in drinking water for the same exposure periods (Fig. 2) is not surprising as the same trend was observed for the Cd burden in the liver and kidney of rats fed Cd diet except for the liver after 3 months of exposure which was significantly higher (Table 2). The Cd load of the kidney of rats fed Cd in diet for 3 months was expected to be significantly higher like in the liver having passed via this organ. However for reasons presently unclear, the kidney Cd load remained substantially less after 3 months in rats where Cd was administered via the food versus drinking water. However it is conceivable that the kidney Cd load may also increase with a longer exposure time. As the liver is the first organ to receive Cd taken up from the gastro-intestinal system via the portal system, the significantly higher liver Cd load of rats fed Cd in diet for 3 months is a likely indication that Cd is more easily available via the food than via the drinking water. Contrary to the present findings Lind et al. (1995) who also used Cd accumulation in the liver and kidney as a measure of Cd absorption, demonstrated that bioavailability of Cd from boiled crab hepatopancreas is slightly lower than that of Cd from mushroom and inorganic Cd. This interpretation is based on the results they obtained when female Balb/C mice were fed a diet containing 0.4 ppm Cd from either boiled crab (*Cancer pagurus*) hepatopancreas or dried mushroom (*Agaricus augustus*) or as inorganic Cd ($CdCl_2$). Fractionation of Cd in boiled crab hepatopancreas and mushroom revealed that Cd in crab hepatopancreas is mainly associated with denatured proteins with low solubility, whereas a large fraction of Cd in dried mushroom is associated with soluble ligands. No attempt was made to fractionate the fish proteins in the present study, so

the reason for the greater bioavailability of Cd in rats exposed to Cd via the experimental food-chain for 3 months cannot be offered with certainty.

Elevations in plasma L-alanine aminotransferases (L-ALT) and L-aspartate aminotransferases (L-AST) have been reported to accompany damage to the liver (L-ALT in particular) and other organs of the body (Timbrell 2000). Thus the significantly higher plasma and lower liver L-AST (Fig. 3) of rats administered Cd in drinking water for 1 and 3 months is a likely indication of liver damage. Generally, the liver is the most important target organ when considering Cd-induced toxicity because Cd primarily accumulates in this organ (Guilhermino et al. 1998; Kuester et al. 2002). Also, Cd induced necrosis in the liver can cause the release of abnormal quantities of L-AST and L-ALT into the blood (Guilhermino et al. 1998; Asagba and Eriyamremu 2007; Borges et al. 2008; Chater et al. 2009). However, it is noteworthy that the significantly ($P < 0.05$) lower liver L-ALT activity observed in rats administered Cd in drinking water after both exposure periods was not accompanied by a corresponding rise in the plasma. This lack of significant ($P > 0.05$) variation in the plasma L-ALT activity of rats after water-mediated Cd exposure relative to controls, and the fact that the decrease in liver ALT activity, although significant is small (Fig. 3) may be an indication that the damage to the liver (if any) was not severe enough to elicit a rise in the plasma activity of this enzyme. On the other hand the lack of significant ($P > 0.05$) differences in the activities of L-AST and L-ALT in the plasma and liver of rats offered Cd via the food chain as compared to the pair-fed controls (Fig. 3) suggests that liver damage may not have occurred in this group of rats. The lack of liver damage observed in this study is an indication of tolerance to Cd exposure by the liver of rats fed Cd via the food chain despite the significantly higher Cd uptake in this organ. However liver damage was reported in a similar study by Groten et al. (1990) in which rats were fed diets containing either tissue-incorporated cadmium or cadmium salt for 4 weeks. The test diets contained 30 mg Cd/Kg either as $CdCl_2$, or as Cd incorporated in pigs' livers; the control group was fed a diet containing liver from a pig not treated with cadmium. In addition analysis of the diet and determination of the food consumption revealed that both Cd fed groups were exposed to similar dietary Cd levels. It was observed that over 90% of the Cd present in the pigs' livers was bound to MT. Also, both cadmium-treated groups showed clear signs of anaemia and increased plasma L-AST and L-ALT activities, but these effects were more pronounced for the group fed $CdCl_2$ than for the group fed Cd incorporated in liver. The feeding of Cd incorporated in pigs' livers resulted in about half the accumulation of Cd in the rats' livers that took place after intake of a diet containing $CdCl_2$. It was suggested that the differences in the extent of the toxic effects between the inorganic and the tissue-incorporated Cd was due to differences in the Cd concentrations in liver.

One of the most striking results from this study was the enormous increase in the activity of the aminotransferases in the liver of test animals who received Cd in fish diet relative to those treated with inorganic Cd in drinking water, although no corresponding increase was observed in the plasma except the plasma AST of the 1 month exposed rats (Fig. 3). The reason for this enormous increase in the level of liver aminotransferases of the test rats fed the fish diet is not clear. But it is reasonable to assume that it may be due to increased metabolism of amino acid due to increased tissue breakdown occasioned by starvation stress. This assumption is corroborated by the fact that these enzymes were also increased in the pair-fed controls.

In summary this study sets out to compare the absorption and toxicity of Cd administered via the food-chain and inorganic Cd administered in drinking water. The food-chain was mimicked by exposing rats to diet containing Cd pre-exposed fish. The findings of the study indicate that Cd incorporated in fish is more easily bioavailable but less toxic than inorganic Cd salts after 3 months of exposure in rats.

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