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Subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health

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RUNNING TITLE

Cadmium and metals essential to bone health

Keywords

Cadmium, iron, interactions, minerals, osteoporosis, risk assessment.

Abbreviations: Cd = cadmium; CdCl2 = cadmium chloride; CC16 = Clara cell protein; FAO/WHO = Food and Agriculture Organization of the united nations/ World Health Organization; GGT = gamma-glutamyltransferase; ALP = alkaline phosphatase; ICP-MS = Inductively Coupled Plasma-Mass Spectrometry; ID = iron deficiency; NAC = N-acetylcysteine;

ABSTRACT

Cadmium (Cd) was recently identified as a risk factor for osteoporosis. Skeletal damage may be the critical effect of low-level long-term exposure to Cd in the general population exposed via food, but the mechanisms behind this are not clearly understood.

We investigated the effect of dietary Cd exposure on metals involved in bone turnover. Female rats received a Cd-supplemented diet (0, 10, 50, or 200 $CdCl_2$ mg/kg diet) for 13 weeks. Cd and essential metals stored in the liver were measured by ICP-MS multianalysis. Mineral content of the livers was modified according to Cd level: iron, magnesium and selenium decreased while copper, zinc and manganese increased with increasing Cd levels. Iron was the most strikingly affected metal, falling to one-fifth of control values at high dietary Cd exposure. In this dosage group, selenium decreased to 36% of mean control concentrations while zinc increased to 168%. This mineral imbalance, especially depleted iron stores, can contribute, at least in part, to the Cd-associated risk of osteoporosis.

The association between iron metabolism and Cd exposure should be investigated in humans, as Cd and low iron stores could act synergistically as risk factors for osteoporosis.

INTRODUCTION

The heavy metal and environmental pollutant cadmium (Cd) has recently been identified as a risk factor for osteoporosis (Staessen *et al*, 1999). Cd accumulates in humans, mainly in the kidneys, via the food chain. The more Cd contaminates the agricultural environment the more it contaminates the food chain and endangers human health, and its levels in soils have increased continuously during the last century. The diet is the main source of Cd intake in non-smoking non-professionally exposed people. In France, the Cd concentration in human bones rose tenfold during the 20th century (Staessen *et al*, 1999). Today, daily Cd intake is estimated to be 10-30 µg in Europe in unpolluted areas (Nasreddine and Parent-Massin, 2002) and 4-27 µg in France (Noël *et al*, 2003). This represents 6-50% of the Provisional Tolerable Weekly Intake (PTWI) set by the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization, equivalent to 1 µg/kg/d (WHO, 1989).

Exposure to Cd is associated with renal and skeletal damage and also some cancers. Renal tubular damage is considered to be the critical health effect of Cd exposure, both in the general population and in occupationally exposed workers. Risk assessment is based on epidemiological data from populations exposed to high levels of Cd, using dose-response calculations assuming that critical biological endpoints are similar to those in populations with very low exposure. However, the effects of very low levels of ingested Cd are not well known. Skeletal damage (osteoporosis) may be a critical effect of Cd exposure, as shown in humans and in animal studies. Bone changes induced by cadmium were first described in long-term high-level exposure to cadmium, and were considered to be secondary to kidney disease (Itaï Itaï disease) (Jarüp *et al*., 1998). But primary decrease in bone mineralisation has recently been associated with low cadmium exposure in occupationally, in environmentally exposed people (Staessen *et al*, 1999; Alfven *et al*., 2002), and induced experimentally at low doses in rats (Ohta *et al*., 2000).

Osteoporosis, a major public health problem, mainly affects aging people (senile osteoporosis) and postmenopausal women, especially in elderly Caucasian women (Berglund *et al*., 2000). This population group also shows mineral deficiencies, notably in iron. The mechanisms behind cadmium-

induced bone damage are not clear, one possible mechanism implicates Cd interaction with calcium metabolism (Wu *et al*., 2001), with calciuria and/or dysfunction of tubular cells leading to decreased Ca absorption from the gut. But both experimental and epidemiological data indicate that cadmium can affect bone mineralisation as primary effect, well before the onset of kidney damage. (Ohta *et al*., 2000; Ogoshi *et al*., 1989; Wilson and Bhattacharyya, 1997).

Cadmium also interferes with trace metals acting in bone mineralisation, mainly zinc, iron, and copper. Previously described interactions involved various mineral-deficient diets in animals: for instance in both zinc and iron deficiencies, Cd uptake by tissues increased (Ohta and Cherian, 1995). The effects of essential minerals on Cd accumulation and toxicity have been investigated in vivo by feeding Cd-containing diets supplemented with various minerals: the most protective effect against cadmium accumulation was observed with a supplement combining calcium, phosphorus, iron and zinc (Groten *et al*., 1991). All these essential minerals interacting with cadmium play a role in bone turnover. Chronic exposure to environmental Cd may disturb essential mineral metabolism at low doses. Besides interfering with calcium, the effect of Cd on trace metal metabolism might help to explain the Cd-associated risk of osteoporosis. The present study was designed to answer the question: does repeated low-level Cd exposure modify mineral metabolism, before nephropathy occurs and which metal is the most affected in correlation with Cd accumulation? In female rats, we investigated the hepatic storage of essential minerals and the correlation with Cd accumulation after 13-weeks dietary exposure, that is to say the same mode of exposure as the general population.

MATERIALS AND METHODS

Animals, treatment and sampling

Three-week-old female Wistar rats (Iffa Credo, France) were housed 5/cage under standard conditions with a 12 h light/dark cycle. They were fed a standard pellet diet meeting the requirements of growing rats, composed of 26% maize, 36% wheat bran, 12% fish flour, 10% meat flour, 5% dried yeast, 5% soya, 3% alfalfa, 3% vitamin and mineral mix which was prepared by UPAE (Unité de Préparation des Aliments Expérimentaux, INRA Jouy-en-Josas, France), according to recommendations of INRA (Potier de Courcy et al., 1989). The diet was composed of 48% carbohydrate, 25% protein, 4% fat, 0.6% calcium, 0.5% phosphorus. After a 10-day adaptation period, the control rats (15) continued to be fed the baseline diet while four treatment groups received a CdCl2-supplemented diet. The four treatment groups (10 rats each) received the supplemented CdCl2 diet as follows:10 mg CdCl2/kg diet (low dosage group), 50 mg CdCl2/kg diet (medium dosage group) 200 mg CdCl2/kg diet (high dosage group) and 200 mg CdCl2/kg diet + N-acetylcysteine (NAC) by gavage (gavage group: 100 mg/kg, 5 days per week). NAC is considered a protective agent against Cd toxicity. Five rats used as positive controls for renal pathology received daily a 1 mg/kg CdCl2 injection subcutaneously for eight weeks (Lermioglu and Bernard, 1998; Liu *et al*., 1998) and were then sacrificed. All animals were examined daily for clinical signs, and the ovarian cycle was checked in treatment groups by vaginal smear from the 13th week of age. Rats were weighed weekly and their food and water consumption was measured. At weeks one, four, eight, and thirteen, blood samples were collected at the retro-orbital plexus into special heparinized vacutainer tubes, guaranteed free of any trace of metals, to measure cadmium concentrations. In treatment groups, total volume of urine was collected and measured over a 24-hour period by maintaining animals individually in metabolism cages, at weeks four, eight, eleven and thirteen. After 13 weeks of dosing, the rats were fasted for at least 4 hours, then anesthetized for blood collection before sacrifice.

At necropsy, liver, kidneys, uterus and ovaries were removed and weighed. These organs and right kidneys were preserved in 10% formaldehyde for microscopic observations while left kidneys and livers were preserved at –20°C before analysis. Hematoxylin and eosin-stained sections from right

kidneys and uterus were examined histopathologically, under light microscopy, to look for any pathological changes between control and treated rats. Renal tubular epithelium changes in cortex and medullary were specially sought.

Analytical procedures

Cd concentrations in blood and kidneys were measured by graphite furnace atomic absorption spectrophotometry (AAS) with a Perkin-Elmer Zeeman 4100 spectrophotometer (PE 4100 ZL), according to the techniques described previously (Houpert *et al*., 1997).

In the diet and livers of treated and control rats, we analysed the mineral content by ICP-MS after microwave digestion, as previously described (Noël *et al*., 2003), excepted for iron which was analised by AAS.

In the 24 h urine samples, we measured urinary pH, gammaglutamyltransferase (GGT) and alkaline phosphatase (ALP) by colorimetry (Enzyline GGT and Enzyline ALP optimisé, Biomérieux, 69280 Marcy l'Etoile, France). Tubular type proteinuria was assessed by measuring the urinary excretion of Clara cell protein (CCl6), a low-molecular-weight protein very sensitive to proximal injury (Bernard and Lauwerys, 1995). We investigated the urinary concentration of Clara cell proteins using a 1.5 ml urine sample from the high dosage treatment group and control groups in collaboration with Professor Bernard's Laboratory (Halatek *et al*., 1998).

Statistical analysis

Statistical analyses were conducted by analysis of variance (ANOVA using the general linear models procedure of the SAS system, Cary, NC). The 4 dosage groups were compared on the same treatment weeks using the Student-Newman-Keuls test following ANOVA for GGT, PAL, and urinary pH. Pearson's correlation coefficients between mineral hepatic storage and Cd concentrations were calculated using the SAS "CORR" procedure. Comparisons were considered significant at $p= 0.05$.

RESULTS

Diet composition and food consumption

Mineral composition was verified in all diets by ICP-MS (Table 1), and was within the target for Cd concentrations, and iron, zinc, and calcium were within normal ranges. Total food intake and body weights changed similarly in all the treatment groups during the 13 weeks of treatment, except for a lower weight gain from the fifth week of treatment in the high dosage group (Fig.1). In the latter, the decrease of the mean body weight on the 5^{th} week of exposure exceeded 10% of controls weights (–13.65% compared to controls). Based on food intake, the mean daily intake of Cd during the treatment period was 0.36 mg/kg of body weight/day (mg/kg/d) in the low dosage group, 2.8 mg/kg/d in the medium, and 12.4 mg/kg/d in the high dosage group. The dietary treated rats showed no treatment-related clinical signs or biological modifications and had normal sexual cycles during the last five weeks of exposure. Food intake of positive control rats treated by injection of CdCl2 was lower than that of other groups during the last three weeks (6,7,8) of treatment (Fig.1).

Pathology and urine analysis

In treated rats, there were no statistically significant between-group differences in body weight after 13 weeks, nor were there any significant differences in the weights of liver (range 8.76-9.10 g) or kidney (range 0.81-0.97 g). No treatment-related tubular lesions were noted in these animals, while in the positive controls group macroscopic and microscopic kidney lesions were detected. After 8 weeks injection of CdCl2, the kidneys appeared pale compared to normal ones. Histologically, renal toxicity was characterized by necrosis of tubular epithelia, with tubular dilatation and calcinosis. A 3.5-fold increase in CC16 protein excretion at the end of the 8 weeks treatment in the injected rats confirmed renal dysfunction while the mean level in the high dosage group was not significantly higher compared to controls.

Blood cadmium kinetics (Fig. 2)

Control blood Cd concentrations were below the limit of quantification of the analytical technique (0.8 µg/L). In the injected-group, Cd concentrations increased from 480 ± 58 µg/L after 4 weeks of administration to 821 ± 111 µg/L after 8 weeks. The mean blood concentration-time profile of cadmium during dietary administration is shown in Figure 2. Blood Cd concentrations increased to 41 ± 10 µg/L and 137 ± 26 µg/L on the 8th week of exposure in the medium and high dosage groups, respectively, before decreasing on the 13th week in all the treatment groups. In the NAC-treated group, blood concentrations paralleled those observed in the high dosage group, and showed a 15% decrease (without statistical significance). In the low dosage group, blood cadmium showed a maximum level one week after starting dietary exposure (4.2 ±1.6 µg/L) and decreased thereafter.

Cadmium in liver and kidney; minerals in the liver (Figs. 3, 4)

Cadmium concentrations in whole kidney were correlated with diet concentration in orally-treated rats, ranging from 1.7 ± 0.3 mg/kg wet weight (ww) to 42 ± 7 mg/kg ww in the low and high dosage groups respectively (Fig. 3). In the Cd-injected group, they reached 99 ± 11 mg/kg ww. Cadmium concentrations in the liver, measured by ICP-MS, ranged from 0.86 ± 0.27 to 37 ± 4 mg/kg wet weight in dietary treated rats, representing 83% to 115% of kidney concentrations. Cadmium levels in kidney and liver were both decreased by about 8% (not statistically significant) in NAC-treated rats compared to the high treatment group without NAC. Mineral content in the liver was modified in correlation to cadmium content, both positively and negatively: iron, magnesium, selenium, and sodium were decreased in correlation with Cd concentrations, while copper, zinc, manganese and molybdenum increased when Cd increased (Fig. 4). The most highly correlated minerals were iron ($r = - 0.79$, $p<0.001$), zinc ($r =0.88$, $p<0.001$), and selenium ($r = -0.77$, $p<0.001$). The most strikingly affected mineral was iron, which decreased to one-fifth the value of controls at high dosage, and to 40% of controls at medium dosage. Selenium decreased to 36% of mean control concentrations in the high dosage group while zinc increased up to 168% of control values. There were no significant variations in the hepatic storage of minerals in the high dosage group receiving NAC compared to the high dosage group without NAC.

DISCUSSION

The present results suggest that cadmium can be a risk factor for the development of osteoporosis at low doses by altering mineral metabolism before the occurrence of kidney lesions. In this study, we demonstrate that increasing dietary Cd exposure increased the related variations in the hepatic storage of various essential trace elements, in a dose-dependant manner, but did not induce nephrotoxicity. The liver is a critical organ in the homeostasis of essential metals including calcium, copper, iron and zinc. Interactions between essential minerals and Cd exposure have been reported *in vivo* in various animal species and *in vitro* (Blais *et al*., 1999), mainly involving calcium and zinc but also copper, iron, selenium. Most studies evaluated the effects of nutritional status on Cd retention using different depleted or supplemented diets (Groten *et al*., 1991; Houpert *et al*., 1997; Ohta and Cherian, 1995; 2001; Reeves and Cheney, 2000). Our study is, to our knowledge, the first to show a clear dose-dependant effect of dietary Cd on mineral metabolism, using multianalysis measurement of minerals in an *in vivo* study. We exposed young female rats subchronically to a cadmium-supplemented diet, in order to reproduce dietary exposure, the main source of environmental exposure to Cd in the general population. As often reported, blood cadmium reached a concentration corresponding to the intensity of the exposure after some weeks in rats, whereas this takes some months in humans (Jarüp *et al*., 1998). In our study, blood cadmium levels in low dosage dietary-treated rats corresponded to the upper levels reported in smokers (1 to 4 µg/L), and were higher than those induced in the rat by long-term exposure to polluted rice (Oishi *et al*., 2001). No treatment-related clinical signs or kidney lesions were observed at the end of the 13-week exposure in any of our treatment groups. ICP-MS analysis of livers revealed decreased concentrations of iron, magnesium and selenium together with increased copper, zinc, and manganese that were correlated with total Cd intake. Similar changes have already been reported: decreased iron levels and increased zinc and copper in rat livers after long-term oral exposure at non-toxic dosage (Oishi *et al*., 2001), and induced iron-deficiency state in growing rats at toxic dosage (Crowe and Morgan, 1997). We used kidney Cd levels to estimate the absorbed Cd dose and Cd accumulation in liver and kidney. Cd accumulation was in accordance with that previously described after dietary exposure in the rat (Groten *et al*., 1991) and reflected total Cd intake. Kidney

cadmium concentrations reflect total Cd intake better at low oral doses, both in the rat and in humans (Ohta *et al*, 2000; Torra et al., 1995). In our study, the low dosage group showed liver concentrations that were one-half those of kidney, while in the higher dosage groups, liver concentrations represented on average 85% of kidney concentrations. These low group liver concentrations in rats correspond to those reported in non-professionally exposed humans (0.98 ±0.5 µg/g : Torra *et al*., 1995), which were highly correlated with liver zinc, as in our present results in rats. Cadmium kidney concentrations are generally associated with tubular nephropathy. In the present study, kidney lesions were observed only in the positive control rats which showed nearly 100 mg/kg whole kidney cadmium concentrations after 8 weeks: this agrees with numerous previous reports concerning high cadmium exposure, and is consistent with critical renal concentrations of about 100 µg/g tissue in the rat (Mitsumori *et al*., 98, Groten *et al*., 1994; Liu *et al*, 1998). In the present study, dietary treated rats with kidney Cd concentrations below 50 mg/kg did not develop tubulopathy.

The general effect of Cd on mineral metabolism at low dose can help to explain the cadmium-associated risk of osteoporosis in humans. Development of osteoporosis is mainly dependant on calcium metabolism, but also upon other nutrients (Illich and Kerstetter, 2000). Our study demonstrates a dose-dependent effect on storage of essential trace elements, which could contribute to the osteoporotic changes caused by Cd, in addition to the well-known inhibition of Ca absorption. Most animal studies report effects of Cd exposure on bone, together with bone accumulation of Cd. In rats, prolonged oral exposure to Cd caused bone lesions including decreased mineral density (Berglund *et al*, 2000; Brzoska and Monuszko-Jakoniuk, 2001) osteomalacian changes (Brzoska and Monuszko-Jakoniuk, 2001), and decreased mechanical strength correlated with Cd in bones (Ogoshi *et al*., 1989). The bone demineralisation developing at low-dose prolonged oral exposure in animal studies was independent of renal damage and was observed before renal dysfunction (Ohta *et al*., 2000).The Cd-induced changes in hepatic storage of essential minerals before renal damage occurs must induce changes in homeostasis of minerals, to which bone tissue is very sensitive. A Cd-induced decrease in essential minerals in bone tissue has been reported in several studies: Cd mainly affected Ca bone content, but also Zn and iron (Brzoska and Monuszko-Jakoniuk, 2001; Ogoshi *et al*., 1989). Bone demineralisation can result indirectly from disturbed mineral homeostasis, or directly due to the *in situ*

action of cadmium on bone, or by both mechanisms. Cadmium accumulated in bone could affect activity of bone cells and enhance bone mineral loss directly (Bhattacharyya *et al*. 1988; Wilson *et al*., 1996). But metal imbalance is most likely the consequence of an indirect effect rather than an *in situ* effect, since some authors failed to show cadmium accumulation when metal balance in bone was disrupted by low-dose chronic Cd exposure (Oishi *et al*., 2001). Also, zinc co-administration prevented bone lesions without preventing Cd accumulation in bone (Brzoska and Monuszko-Jakoniuk, 2001).

Here, we suggest that Cd is a factor of osteoporosis because it alters the metabolism of several essential metals involved in mineralisation, in addition to its effect on calcium metabolism. Each of the minerals affected by Cd exposure have a role in bone formation, and their simultaneous Cd-induced changes can damage bone. Essential metals are necessary for optimal formation of bone matrix and its mineralisation. They act as cofactors for specific enzymes. The most strikingly decreased metal, iron, contributes to collagen maturation, serves as a cofactor for prolyl and lysyl hydroxylases, which catalyze an ascorbate-dependant hydroxylation. In rats, an iron-deficient diet induced reductions in bone density as does calcium restriction, and calcium restriction combined with iron deficiency results in an additive decrease in femur bone mineral density and bone strength (Medeiros *et al*., 2002). In adolescent girls, a recent four-year clinical trial of calcium supplementation showed a trend for a positive association between BMD (bone mineral density) of forearm and ferritin at baseline (Illich and Kerstetter, 2000). Zinc, copper, and magnesium imbalances can also be implicated in Cd-induced osteoporosis because of their implication in bone mineralisation and bone modeling/remodelling (Brzoska and Monuszko-Jakoniuk, 2001; Ogoshi *et al*., 1989; Klevay, 1998, Medeiros *et al*., 1997; Rude *et al*., 1999).

Iron deficiency (ID) and osteoporosis are two major health problems worldwide, so that public-health implications of cadmium exposure may be important. Risk assessment is based on renal tubular damage, which has been considered the critical health effect of cadmium exposure, both in the general population and in occupationally exposed workers. However, osteoporosis may be a critical effect after dietary cadmium exposure. The role of low-level cadmium exposure in increased risk of osteoporosis, first suggested by the correlation between decreased bone density and urinary cadmium

in postmenopausal women (Staessen *et al*., 1999), was confirmed in environmentally or occupationally exposed men and women (Alfven *et al*, 2002). Cadmium exposure in humans has been associated with changes in bone mineral content similar to those found in animals: negative correlation with calcium and magnesium, decreased calcium/zinc ratio related to the degree of osteomalacia in ribs (Honda *et al*., 1997), and positive correlation between zinc liver and Cd liver. Recent results from long-term animal studies show that renal toxicity is not induced by oral administration of low amounts of cadmium, in contrast to high-dose treatment, although tissue accumulation occurs (Shibutani *et al*., 2001). Risk assessment by the 33rd Joint FAO/WHO Expert Committee on Food Additives is based on dose/response calculations for renal toxicity, assuming that Cd-induced renal toxicity can occur below a critical level in the kidney. However, oral exposure to very low levels of cadmium for a lifetime could induce subtle metabolic changes pertinent for risk assessment. From the point of view of a re-evaluation of the critical effects of cadmium exposure, iron deficiency would seem a very sensitive indicator. The association between iron metabolism and Cd exposure needs investigation in humans, as further studies are necessary to clarify the association between osteoporosis and serum ferritin, particularly in patients who are iron deficient. Iron status itself influences absorption of cadmium so that Cd and low iron stores could act together as risk factors for osteoporosis. In conclusion, our findings show a dose-dependent mineral imbalance occurring before nephropathy. At low-dose exposure, impaired metal homeostasis may be a more sensitive indicator than renal cortical cadmium accumulation, and could thus contribute to explaining the increased risk of osteoporosis due to Cd exposure.

Ackowledgements

This work was supported by the French Ministry of Agriculture and Fisheries (DGER) for UMR N° 95025104, and by Grant INSERM N° EN98-21 from the French Ministry of Environment.

We thank Prof. F. Crespeau (Alfort Veterinary School, Pathology, Maisons-Alfort, France), and Prof. A. Bernard (Unit of Industrial Toxicology and Occupational Medicine, Louvain-La-Neuve, Belgium) for their contribution to this work. The excellent technical assistance of A. Duché and D. Huet (Toxicology unit, Alfort Veterinary School, Maisons-Alfort, France) is gratefully acknowledged.

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TABLE AND FIGURE LEGENDS

Table 1 Metal analyses of diets: basal diet (control rats) and CdCl2-supplemented diet (low dosage: 10 mg CdCl2/kg diet ; middle dosage : 50 mg CdCl2/kg diet; high dosage: 200 mg CdCl2/kg diet).

Figure 1 Food consumption (1a) and body weights (1b) during Cd exposure in female rats

Figure 2 Blood cadmium concentration during the 13-weeks dietary exposure to cadmium in female rats

Figure 3 Cadmium levels in whole kidneys and livers of rats treated orally for13-weeks with CdCl2-supplemented diets (mg/kg wet weight)

Figure 4 Changes in mineral contents in the livers of rats after 13-weeks dietary cadmium exposure (expressed as % of controls fed baseline diets). Treatment groups received 10, 50, or 200 mg CdCl2/ kg diet.

Fig. 1a

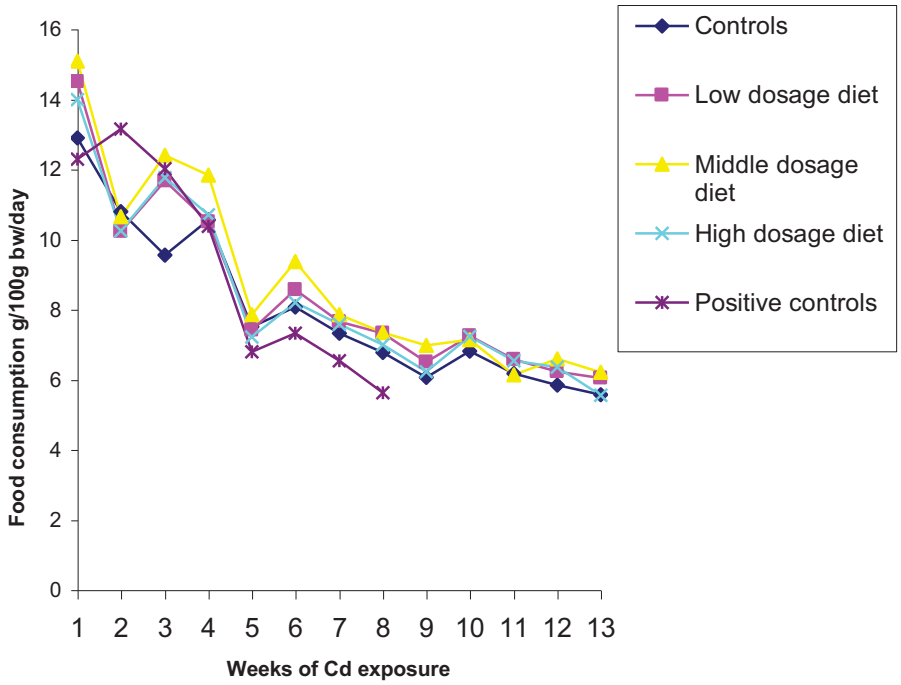
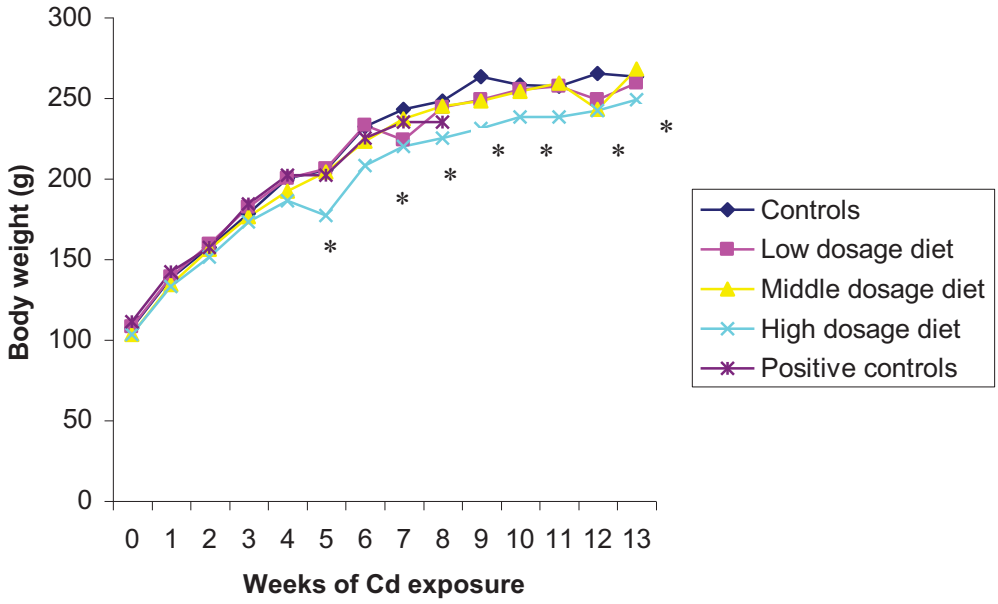


Fig.1b



(* $p<0.05$ compared to controls, from 5th week to 13th week of exposure)

Table 1 Metal analyses of diets: basal diet (control rats) and CdCl2-supplemented diet (low dosage: 10 mg CdCl2/kg diet ; middle dosage : 50 mg CdCl2/kg diet; high dosage: 200 mg CdCl2/kg diet).

Metals	Basal and cadmium supplemented diets			
	Basal diet	Low dosage diet	Middle dosage diet	High dosage diet
Cr (mg/kg)	2.24 ± 0.12	2.25 ± 0.19	2.31 ± 0.20	2.49 ± 0.25
Mn (mg/kg)	61.5 ± 1.3	66.6 ± 1.6	65.2 ± 1.5	69.4 ± 2.1
Fe (mg/kg)	28.4 ± 1.2	30.6 ± 2.1	29.8 ± 1.7	37.1 ± 2.3
Co (mg/kg)	10.7 ± 0.5	12.3 ± 0.4	12.8 ± 0.4	13.6 ± 0.6
Ni (mg/kg)	1.35 ± 0.10	1.32 ± 0.15	1.28 ± 0.12	1.40 ± 0.12
Zn (mg/kg)	72.1 ± 2.9	81.0 ± 2.2	82.7 ± 2.0	76.7 ± 2.6
Cu (mg/kg)	10.3 ± 0.4	16.9 ± 0.9	9.7 ± 1.1	10.5 ± 0.9
Se (mg/kg)	0.62 ± 0.08	0.60 ± 0.05	0.56 ± 0.07	0.61 ± 0.06
Mo (mg/kg)	7.6 ± 0.4	8.1 ± 0.7	7.5 ± 0.9	7.5 ± 0.4
Cd (mg/kg)	0.20 ± 0.01	4.30 ± 0.3	32.1 ± 1.5	149 ± 10
Pb (mg/kg)	0.18 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01

Values are mean ± SD of five determinations

Fig.2

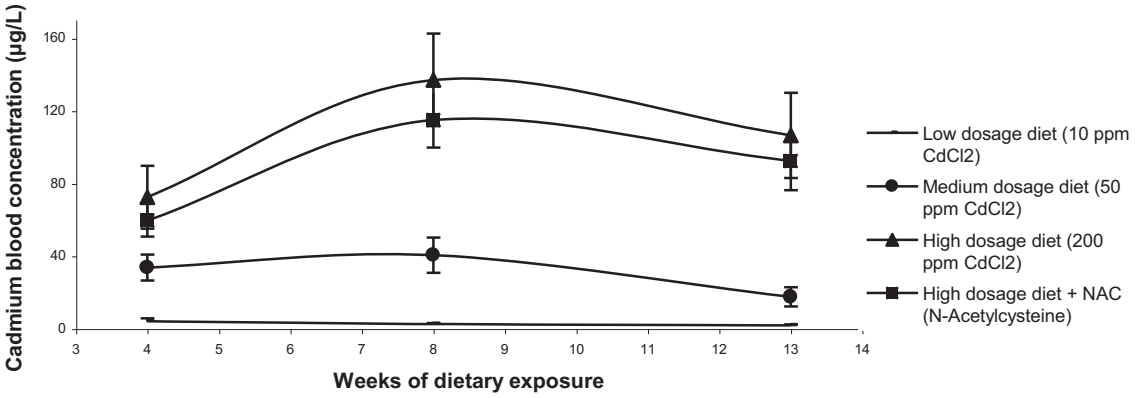

Cadmium blood concentration (µg/L)
160
120
80
40
0
3
4
5
6
7
8
9
10
11
12
13
14
Weeks of dietary exposure
Low dosage diet (10 ppm CdCl2)
Medium dosage diet (50 ppm CdCl2)
High dosage diet (200 ppm CdCl2)
High dosage diet + NAC (N-Acetylcysteine)

Fig.3

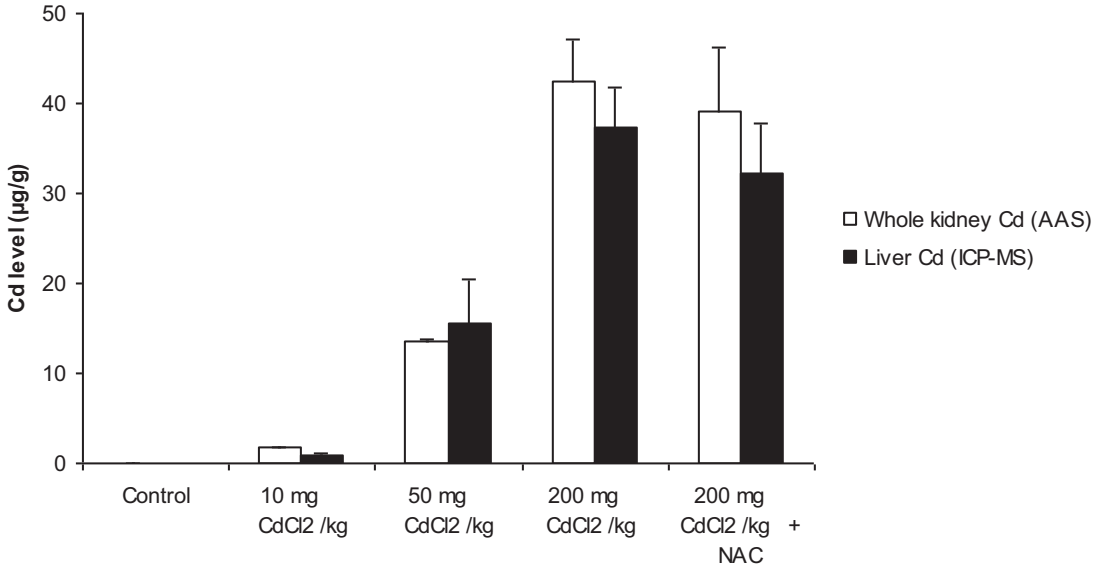
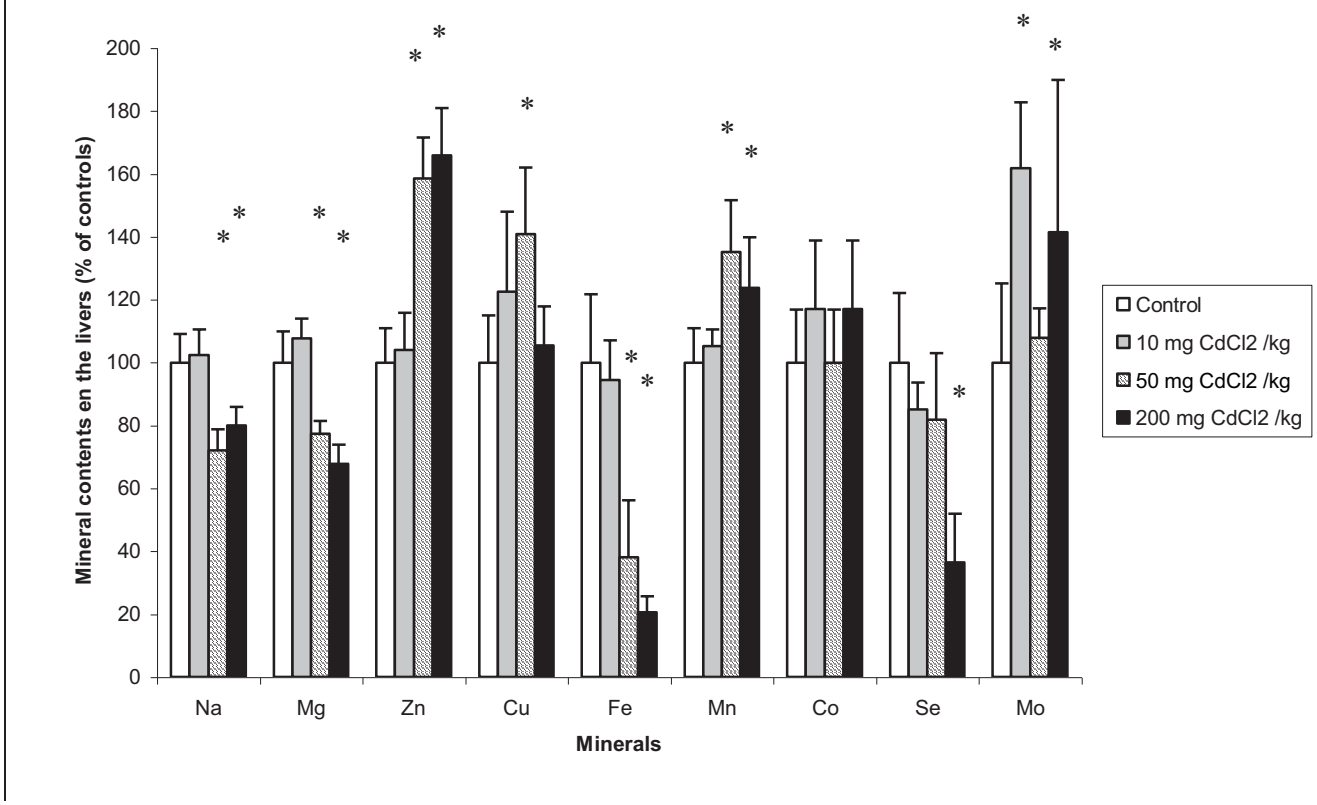

50
40
30
20
10
0
Cd level (µg/g)
Control
10 mg CdCl2 /kg
50 mg CdCl2 /kg
200 mg CdCl2 /kg
200 mg CdCl2 /kg + NAC
Whole kidney Cd (AAS)
Liver Cd (ICP-MS)

Fig.4



Significant comparisons at the 0.05 level are indicated by *