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Effect of dietary calcium on cadmium absorption and retention in suckling rats

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Abstract

The effect of calcium supplementation on absorption and retention of cadmium in the suckling period was evaluated in Wistar rat pups of both sexes. Animals were maintained in the litters with the mother rats and supplemented with 1%, 3% or 6% calcium (as $CaHPO_4 \times 2H_2O$) in cow's milk by artificial feeding from day of birth 6 through 14. All rats were exposed to cadmium (as $CdCl_2 \times H_2O$) either orally or parenterally. Oral cadmium dose of 0.5 mg/kg body weight a day was administered through nine-day period of calcium supplementation and parenteral cadmium dose was injected subcutaneously in a single dose of 0.5 mg Cd/kg body weight prior to calcium supplementation. On experimental day 10 (at the age of pups of 15 days) all animals were killed and the liver, kidneys, brain and carcass (body without organs and skin) were removed for element analyses. Cadmium and essential elements calcium, zinc and iron were analysed in the tissues by atomic absorption spectrometry. Results showed that after oral exposure cadmium concentrations in all calcium-supplemented groups were significantly decreased in the organs and carcass and that the effect was dose-related. No such effect of calcium was found after parenteral cadmium exposure. Calcium supplementation *per se* significantly increased calcium concentration in the carcass and had no effect on iron in organs and zinc in carcass. It was concluded that calcium supplementation during the suckling period could be an efficient way of reducing oral cadmium absorption and retention without affecting tissue essential trace element concentrations.

Infants and young children are orally exposed to cadmium from food, water, household dust and/or garden soil (hand-to-mouth route of exposure) (IPCS 1992; Järup *et al.* 1998). Due to specific features of metal metabolism, the very young organism is at a higher risk for health effects of toxic metals such as cadmium than adults at the same level of environmental exposure (Kello & Kostial 1977; Kostial 1983; Kostial *et al.* 1991; Oskarsson *et al.* 1998).

Beside age, intestinal absorption of cadmium is influenced by a variety of factors including its chemical form, dose and route of exposure, environmental matrix in which it is contained, intestinal content, diet composition, nutritional status, and interactions of cadmium with other nutrients (Kello *et al.* 1979; IPCS 1992; WHO 1996; Diamond *et al.* 1998; Bhattacharyya *et al.* 2000). Recent work of Eklund & Oskarsson (1999) confirms that the composition of food has a great influence on the estimated mean weekly intake of dietary cadmium in infants. Soya-based formulas and diets with cereal products have a significantly higher content of cadmium than cow's milk formulas. It has been shown that essential minerals such as iron, calcium, phosphorus and zinc alone or in combination interact with intestinal (mainly duodenal) cadmium absorption and toxicity (Groten *et al.* 1991; IPCS 1992; Goyer 1995; Peraza *et al.* 1998). By increasing the calcium content in the diet, de-

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creased cadmium absorption and retention in the body has been found. On the other hand, deficient dietary intake of calcium causes increased intestinal absorption of cadmium and its increased organ accumulation finally leading to enhancement of cadmium toxicity. Protective effects of calcium against cadmium absorption, accumulation and toxicity have been documented (reviewed by Brzóska & Moniuszko-Jakoniuk 1998).

Data on calcium and cadmium interactions in very young are not available. It is known that during the suckling period gastrointestinal absorption of mineral is very high due to increased nutritional requirements during the period of rapid growth and development (Kostial 1983; WHO 1996). This applies not only to the essential minerals such as calcium, but also to the toxic metals such as cadmium or lead. In our previous investigation we evaluated the influence of calcium supplementation on lead absorption, retention and elimination in suckling rats. It was found that supplementation with calcium hydrogen phosphate by artificial feeding during concomitant oral lead exposure significantly reduced body burden of lead (Varnai 1999; Varnai *et al.* 2000). In the present investigation we applied the same experimental model to assess cadmium absorption and retention in suckling rats exposed to cadmium either orally or parenterally under condition of oral calcium supplementation.

Materials and methods

Animals

Suckling Wistar rats of both sexes (from the Institute's breeding farm), six-day-old, with average weight of 14 g were used. Two days after birth five litters were reduced to eight pups each. During experiments pups were kept in litters with their mothers in individual policarbonate cages (26 × 20 × 14 cm; Ehret, Germany). Pups' body weights were recorded daily. The pine shaving beddings were changed every day.

The research procedures were performed according to the national law on the care and use of laboratory animals and were approved by the Croatian Ministry of Agriculture and Forestry.

Experimental design

Two experiments were performed. Experiment 1 was carried out to assess whether calcium supplementation influences intestinal cadmium absorption and/or incorporated cadmium elimination. Experiment 2 was carried out to evaluate dose-relationship of the effect of calcium supplementation during oral cadmium exposure. In both experiments pups were exposed to cadmium and randomly designated to either control (unsupplemented) or calcium-supplemented group within each litter.

Calcium supplementation.
Calcium (as $CaHPO_4 \times 2H_2O$, p.a. grade, Kemika Co., Zagreb, Croatia) was supplemented to cow's milk (commercial dairy product with 2.8% fat, 1200 mg/l calcium, 0.3 μg/l cadmium, 1 mg/l iron and 5 mg/l zinc) with addition of 6% calcium (Experiment 1) or increasing doses of 1%, 3% or 6% calcium (Experiment 2). Control (unsupplemented) group received cow's milk. Milk and calcium suspensions were administered to the pups during nine consecutive days starting on day of birth 6. Artificial feeding with a dropper was applied. The method closely resembles bottle-feeding of the infants and it has been originally introduced by Kostial *et al.* (1971). Drop-by-drop feeding was carried out with an automatic pipettor (25 μl) during 7 h (9:00 a.m. till 4:00 p.m. with pauses). Number of drops of milk (in control, unsupplemented pups) or milk calcium-suspension (in supplemented pups) was increasing gradually by pups' age starting with 10 and ending with 45 drops per day. After 4:00 p.m. pups were returned to the mother rats where they remained the rest of the day and over night.

Cadmium exposure.
In both experiments pups were exposed to cadmium as cadmium chloride solution dissolved in distilled water when given orally and in saline when given subcutaneously ($CdCl_2 \times H_2O$, p.a. grade, Kemika Co., Zagreb, Croatia).

Experiment 1
There were four groups with 10 pups per group (2 pups per group in each of 5 litters):

Cd p.o.	- orally Cd-exposed control (unsupplemented) group;
Cd p.o. + Ca 6%	- orally Cd-exposed and 6% Ca-supplemented group;
Cd s.c.	- subcutaneously Cd-exposed control (unsupplemented) group;
Cd s.c + Ca 6%	- subcutaneously Cd-exposed and 6% Ca-supplemented group.

In the first two groups cadmium was administered orally during the nine-day period of artificial feeding

(with cow's milk or with 6% calcium suspension in cow's milk) at a daily dose of 0.5 mg Cd/kg body weight. Total dose of cadmium was 4.5 mg Cd/kg body weight. Other two groups were administered a single subcutaneous injection of 0.5 mg Cd/kg body weight on 5th day of life, 24 h prior to beginning of calcium supplementation.

Experiment 2

There were four groups with 10 pups per group (2 pups per group in each of 5 litters):

Cd p.o.	- orally Cd-exposed control (unsupplemented) group;
Cd p.o. + Ca 1%	- orally Cd-exposed and 1% Ca-supplemented group;
Cd p.o. + Ca 3%	- orally Cd-exposed and 3% Ca-supplemented group;
Cd p.o. + Ca 6%	- orally Cd-exposed and 6% Ca-supplemented group.

In all groups cadmium was administered orally in increasing doses (as 1%, 3% or 6% calcium suspension) during the nine-day period of artificial feeding in cow's milk at a daily dose of 0.5 mg/kg Cd kg body weight (total dose 4.5 mg Cd/kg body weight).

Sampling and element analysis

On the 10th day of the experiment (day of birth 15), rats were weighed and killed by exsanguination from the abdominal aorta in ether anaesthesia. In both experiments, the liver, both kidneys and brain, and in Experiment 1 also carcass (whole body without internal organs, entire gastrointestinal tract and skin) were removed for element analyses.

Fresh organ and carcass weights were measured. Samples were dried at 105 °C for 24 h, weighted and ashed at 450 °C in muffle furnace (Gallenkamp, UK) for 24 h (Blanuša & Breški 1981). After cooling down the samples were weighed again and adjusted to 10 ml with 10% HNO_3. All organ weights and carcass wet, dry and ash weights were recorded. Cadmium in the liver, iron in soft tissues and calcium and zinc in the carcass were analysed by flame atomic absorption spectrometry (AAS) on Varian instrument AA-375 (Varian; Australia). Cadmium in kidney and brain was analysed by electrothermal AAS on Varian AA300 with GTA 96. Deuterium correction was applied during all measurements.

To verify our chemical analysis Certified Standard Reference Material Bovine Liver 1577b (NIST, USA) and Animal Bone H-5 (IAEA, Austria) were analysed by the same procedure as the samples from the experiments. For cadmium, iron and zinc in Bovine Liver obtained values were 0.55, 184 and 127 μg/g (certified values are 0.50, 184 and 127), respectively. For calcium and cadmium in Animal Bone obtained values were 217 mg/g and 0.026 μg/g (reference values 212 and 0.023), respectively.

Statistical analysis

Results are presented as arithmetic means and standard errors of the means. Trace elements cadmium, iron and zinc were expressed as μg/g tissue wet weight, whereas calcium in carcass was presented in mg g wet weight. In the first experiment statistical difference between the control and supplemented group was evaluated by Student's *t*-test (at the level of significance $P < 0.05$). The statistical differences between groups in the second experiment were analysed by general ANOVA/MANOVA with calcium supplementation as an independent variable. Where main effect of calcium supplementation was found, *post hoc* Duncan's multiple range test (at the level of significance $P < 0.05$) was applied. Statistica for Windows program (StatSoft release 5) was used for all statistical analyses.

Results

General effects

In both experiments body weight gain was about 2 g a day. At the end of both experiments average pup body weight was 33.0 g with no significant differences between the groups. No changes due to calcium supplementation were observed either in organ weights (wet or dry) or carcass weights (wet, dry or ash weight) (Table 1).

Effects on tissue cadmium concentration

We found that calcium supplementation during ongoing oral cadmium exposure caused a statistically significant reduction in tissue cadmium concentrations (Experiment 1). Cadmium concentration in the carcass was reduced to 57% of control values (Table 2), and the liver, kidneys and brain cadmium concentrations were reduced to 30%, 52% and 42% of control values (Table 3). By parenteral cadmium administration, calcium supplementation had no effect on cadmium

Table 1. Whole body weights, fresh and dry organ weights and wet, dry and ash carcass weights (g) in suckling rats[a].

Group	Body weight	Liver weight		Kidney weight		Brain weight		Carcass weight		
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Ash
Experiment 1										
Cd p.o.	34.5 ± 1.39	1.13 ± 0.046	0.271 ± 0.011	0.408 ± 0.021	0.075 ± 0.004	1.33 ± 0.030	0.198 ± 0.005	14.7 ± 0.649	3.71 ± 0.158	0.643 ± 0.032
Cd p.o. + Ca 6%	33.4 ± 1.17	1.07 ± 0.041	0.259 ± 0.010	0.375 ± 0.015	0.070 ± 0.003	1.26 ± 0.037	0.188 ± 0.006	13.9 ± 0.362	3.57 ± 0.095	0.669 ± 0.025
Cd s.c.	34.1 ± 1.16	1.09 ± 0.052	0.256 ± 0.012	0.384 ± 0.018	0.070 ± 0.003	1.29 ± 0.035	0.194 ± 0.006	14.3 ± 0.600	3.61 ± 0.156	0.640 ± 0.030
Cd s.c. + Ca 6%	32.7 ± 1.14	1.10 ± 0.041	0.258 ± 0.010	0.389 ± 0.015	0.074 ± 0.003	1.29 ± 0.018	0.198 ± 0.003	13.8 ± 0.520	3.58 ± 0.142	0.679 ± 0.025
Experiment 2										
Cd p.o.	33.7 ± 0.559	1.09 ± 0.031	0.269 ± 0.022	0.337 ± 0.008	0.065 ± 0.002	1.22 ± 0.032	0.190 ± 0.005	14.7 ± 0.289	3.81 ± 0.122	0.600 ± 0.009
Cd p.o. + Ca 1%	32.9 ± 0.607	1.07 ± 0.032	0.257 ± 0.027	0.340 ± 0.008	0.063 ± 0.002	1.20 ± 0.021	0.188 ± 0.003	14.4 ± 0.393	3.78 ± 0.152	0.610 ± 0.003
Cd p.o. + Ca 3%	32.5 ± 0.515	1.05 ± 0.019	0.259 ± 0.020	0.330 ± 0.007	0.062 ± 0.002	1.24 ± 0.024	0.193 ± 0.004	13.9 ± 0.275	3.60 ± 0.096	0.621 ± 0.010
Cd p.o. + Ca 6%	32.7 ± 0.565	1.07 ± 0.026	0.266 ± 0.017	0.333 ± 0.008	0.062 ± 0.001	1.21 ± 0.025	0.188 ± 0.004	14.1 ± 0.271	3.67 ± 0.083	0.637 ± 0.009

[a] Six-day-old suckling rats received cadmium (as $CdCl_2 \times H_2O$ in distilled water) either *per os* (Cd p.o.) in totalamount of 4.5 mg Cd/kg body weight during 9 days with daily amount of 0.5 mg Cd/kg body weight divided into two drops per day (Experiment 1 and Experiment 2) or subcutaneously (as $CdCl_2 \times H_2O$ in saline) as a single dose of 0.5 mg Cd/kg body weight (Cd s.c.) prior to the 9-day experiment (Experiment 1).

Calcium supplement (as $CaHPO_4x2H_2O$) was given in 6% Ca suspension (Experiment 1) or in increasing concentrations of 1%, 3% or 6% Ca (Experiment 2) in cow's milk by artificial feeding during 7 h a day over 9 days.

Results are presented as arithmetic means ± SEM of 10 animals per group.

Table 2. Concentrations of cadmium, zinc and calcium in carcass of suckling rats orally supplemented with 6% calcium in cow's milk and orally or subcutaneously exposed to cadmium[#].

Group	Element concentrations in carcass				
	Cadmium (μg/g wet wt)		Zinc	Calcium (mg/g wet wt)	
Experiment 1					
Cd p.o.	0.044 ± 0.006		28.1 ± 0.202	14.6 ± 0.195	
Cd p.o. + Ca 6%	0.024 ± 0.001*	(57)	29.1 ± 0.554	16.1 ± 0.440*	(110)
Cd s.c.	0.102 ± 0.010		29.8 ± 0.464	13.8 ± 0.674	
Cd s.c. + Ca 6%	0.090 ± 0.008	(88)	30.0 ± 0.669	16.9 ± 0.347*	(123)

[#]Experimental protocol same as described under Table 1.
Results are presented as arithmetic means ± SEM of 10 animals per group (percentage of value in respective unsupplemented control group in parentheses). *Significantly different from respective control group at $P < 0.05$ by Student's *t*-test

Table 3. Concentrations of cadmium and iron in the liver, kidneys and brain of suckling rats (μg/g wet organ weight) orally supplemented with 1%, 3% or 6% calcium in cow's milk and orally or subcutaneously exposed to cadmium[#].

Group	Liver		Kidneys		Brain	
			Cadmium concentrations (μg/g wet wt)			
Experiment 1						
Cd p.o.	1.37 ± 0.192		1.04 ± 0.110		0.024 ± 0.004	
Cd p.o. + Ca 6%	0.406 ± 0.033*	(30)	0.544 ± 0.037*	(52)	0.010 ± 0.002*	(42)
Cd s.c.	3.42 ± 0.323		0.745 ± 0.060		0.033 ± 0.005	
Cd s.c. + Ca 6%	2.95 ± 0.231	(86)	0.588 ± 0.059	(79)	0.037 ± 0.006	(119)
Experiment 2						
Cd p.o.	1.17 ± 0.067^{a}		1.09 ± 0.063^{a}		0.014 ± 0.002^{ab}	
Cd p.o. + Ca 1%	0.974 ± 0.069^{b}	(83)	0.910 ± 0.057^{b}	(84)	0.019 ± 0.004^{a}	(136)
Cd p.o. + Ca 3%	0.582 ± 0.026^{c}	(50)	0.679 ± 0.033^{c}	(63)	0.011 ± 0.003^{bc}	(79)
Cd p.o. + Ca 6%	0.403 ± 0.034^{d}	(34)	0.565 ± 0.056^{c}	(52)	0.005 ± 0.001^{c}	(36)
			Iron concentrations (μg/g wet wt)			
Experiment 1						
Cd p.o.	42.8 ± 2.22		27.9 ± 1.62		11.1 ± 0.766	
Cd p.o. + Ca 6%	43.1 ± 2.76		24.7 ± 1.56		9.76 ± 0.733	
Cd s.c.	47.9 ± 5.00		26.9 ± 0.80		10.4 ± 0.485	
Cd s.c. + Ca 6%	46.2 ± 3.66		26.6 ± 1.20		9.69 ± 0.806	
Experiment 2						
Cd p.o.	46.9 ± 4.45		26.7 ± 1.14		10.6 ± 0.59	
Cd p.o. + Ca 1%	40.6 ± 1.45		24.9 ± 0.80		10.5 ± 0.44	
Cd p.o. + Ca 3%	40.4 ± 3.68		25.6 ± 0.80		10.2 ± 0.37	
Cd p.o. + Ca 6%	41.5 ± 2.70		24.4 ± 0.88		9.94 ± 0.25	

[#]Experimental protocol same as described under Table 1.
Results are presented as arithmetic means ± SEM of 10 animals per group (percentage of value in respective unsupplemented control group in parentheses).
*Significantly different from respective control value at P<0.05 by Student's *t*-test (Experiment 1).
[abcd]Significant differences between groups indicated by different superscript letters at $P < 0.05$ by Duncan's multiple range test (Experiment 2).

tissue concentrations either in carcass (Table 2) or in organs (Table 3).

In Experiment 2, by oral cadmium exposure and the highest dose of calcium (6% Ca) supplementation, cadmium concentrations in organs were the same as in Experiment 1 at the same dose of calcium supplementation (carcass was not analysed). At the 3% calcium supplementation, cadmium concentrations in the liver, kidneys and brain were significantly reduced to 50%, 63% and 79% of control values (Table 3). At the lowest level of calcium (1% Ca) supplementation, significant reduction in cadmium concentrations were observed in the liver and kidneys (to about 80% of control values) and there was no effect in the brain.

Effects on tissue essential elements

By calcium supplementation no change in carcass zinc concentration was found. Carcass calcium concentrations were significantly increased in all calcium-supplemented pups (Experiment 1, Table 2).

In both experiments calcium supplementation caused no changes in iron concentrations in the liver, kidneys or brain irrespective of the route of cadmium exposure (Table 3).

Discussion

Several authors (e.g., Kozlowska *et al.* 1993; Rimbach *et al.* 1995) have found adverse effect of oral cadmium exposure on growth and survival in rats. Oral and subcutaneous cadmium doses chosen in our experiments were much lower than respective LD_{50} doses reported for suckling animals (Kostial *et al.* 1979). Daily oral dose was 3% of LD_{50} determined for oral exposure and subcutaneous dose was 10% of LD_{50} determined for intraperitoneal exposure to cadmium in suckling rats. At these cadmium doses pups gained about 2 g body weight per day and their general appearance (mobility, reaction to mechanical stimuli) and expected developmental changes (fur growth, eye and ear opening, development of motoric abilities and increase of the ability to swallow drops) were not affected.

The quantity of calcium supplementation in our experiments was chosen after literature search of data on calcium concentration in cow's and rat's milk. Most of the reported results for calcium concentration in mature cow's milk are between 1.1 and 1.3 g/kg (IAEA 1982). Taking an average of the reported data for rat's milk intake and its calcium concentration, we have estimated the quantity of ingested calcium from mother rat's milk during experiment to be about 100 mg plus 8 mg calcium through cow's milk (Luckey *et al.* 1954; Kametaka *et al.* 1974; Yagil *et al.* 1976; Keen *et al.* 1981; Auestad *et al.* 1989; Nicholas & Hartmann 1991). Calcium supplementations of 1%, 3% and 6% calcium suspension in cow's milk during 9 days contained 76, 211 and 415 mg calcium. That is, by giving 6% calcium supplementation we increased calcium intake about 4 times.

It has been previously found that calcium-deficient diet enhances cadmium absorption and that cadmium has a direct effect on bone in mice and rats (Wang & Bhattacharyya 1993; Whelton *et al.* 1997a, b; Piasek *et al.* 1997). In our previous investigations we found that adult rats on lower calcium diet and concomitantly exposed to cadmium in drinking water had reduced mineral content in the bone, increased cadmium concentration in the liver, and changed zinc and iron tissue concentrations in comparison with the rats on higher calcium diet (Blanuša *et al.* 1983; Piasek *et al.* 1997). Studies with calcium supplementation in adult rats showed lower whole body retention of cadmium with increasing dietary calcium level (Kello *et al.* 1979). In growing rats the best reduction of cadmium accumulation and toxicity has been obtained with mineral composition supplement containing iron, calcium/phosphorus and zinc (Groten *et al.* 1991; Walter *et al.* 2000). Results of our present investigation on suckling rats show that calcium supplementation primarily influences absorption and retention of orally administered cadmium and has no effect on elimination of incorporated cadmium. In addition, our results show that the effect of calcium supplementation on decreasing tissue concentration of toxic metal in young organism is dose-related. That is, after oral cadmium exposure, even the lowest level of calcium supplementation caused a significant reduction of cadmium retention in pups' liver and kidneys.

From presented results it might be concluded that calcium supplemented milk could be a way to reduce cadmium tissue levels early in life. However, before any final conclusions on the effectiveness of such preventive treatment, more data are needed to evaluate the health benefits or risks of calcium supplementation at this early period of life.

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