Bull. Environ. Contam. Toxicol. (1995) 54:760–767

Environmental Contamination and Toxicology

Effect of Dietary Phytic Acid and Cadmium on the Availability of Cadmium, Zinc, Copper, Iron, and Manganese to Rats

T. Turecki,[1] R. C. Ewan,[1] H. M. Stahr[2]

[1]Department of Animal Science, Iowa State University, Ames, Iowa 50010, USA
[2]Department of Veterinary Pathology, Iowa State University, Ames, Iowa 50010, USA

Received: 21 September 1993/Accepted: 15 September 1994

The main route of cadmium intake for general population, both human and animal, is via ingestion (Camner et al., 1986). The intestinal absorption of cadmium is relatively low, 6% of a single oral dose for humans (Rahola et al., 1972) and less than 2% for various animal species (Kostial, 1986). However, due to poor excretion, accumulation of cadmium occurs, primarily in kidney. The chronic exposure even to low levels of dietary cadmium can lead to the development of renal disturbances (Piscator, 1982).

Fox (1988) suggests that phytic acid might be a dietary component capable to influence the intestinal absorption of cadmium. Phytic acid naturally occurs as the major phosphorus storage constituent of most cereals, legumes, and oilseeds (Reddy et al., 1982). At physiological pH, phytic acid is ionized and has a strong affinity for divalent cations. The potential of phytic acid to decrease the availability of Zn (Oberleas et al., 1962; Forbes et al. 1983) has been for long time of concern for nutritionists. Phytic acid has also been reported to decrease the availability of other trace metals (Davies and Nightingale, 1975). For nonessential elements, reduced availability of lead has been observed (Wise, 1982; Rose and Quarterman, 1984). The experimental data concerning the effect of dietary phytic acid on the availability of dietary cadmium are limited to the work of Rose and Quarterman (1984).

The objective of this experiment was to examine: (1) the effect of dietary phytic acid on the availability of cadmium under conditions of chronic dietary exposure of rats to cadmium, and (2) the effect of dietary phytic acid and of chronic dietary exposure to cadmium on the availability of zinc, copper, iron, and manganese to rats.

MATERIAL AND METHODS

Sixty three, male Holtzman rats were used in a randomized complete block design with 7 littermates per block. Animals within a block

Correspondence to: R. Turecki

were randomly assigned to treatments and one animal was assigned to an initial slaughter group to establish the trace metal status of duodenum, liver, and kidney at the beginning of the experiment.

Experimental diets were fed for 91 days. During the experiment animals were kept at 22 to 26°C with 12 hour light, 12 hour dark cycle, and had ad libitum access to water and feed. Animals were weighed weekly. The average initial weight of the animals was 91 g.

The composition of diets was 64.8% ground corn, 26% ground soybean meal, 5% corn oil, 3% mineral premix, 1% vitamin premix, and 0.2% choline chloride. A two x three factorial arrangement of dietary treatments was used with two levels of cadmium (0 and 3 ppm) and three levels of phytic acid (low, medium, high). Cadmium was added to the diets in the form of $CdCl_2$. 2 1/2 H_2O. The low phytic acid diets were achieved by the addition of 1000 units of phytase (crude, from *Aspergillus ficuum*, Sigma Chemical Company, St. Louis, MO) per kilogram of the diet. The medium level of phytic acid was represented by the natural phytic acid level in basal corn-soybean diet. The high phytic acid diet was achieved by the addition of dodecasodium hydrate of phytic acid (Aldrich Chemical Company, Inc., Milwaukee, WI) to provide 10 g of phytic acid per kg of the diet.

At the end of the experiment, animals were killed by decapitation and samples of duodenum, kidney, and liver were removed. Duodenum was specified as the proximal 7 cm of small intestine, just distal to the pyloric sphincter. The contents of each duodenum were flushed with milipore water and the mesenterial attachment was removed from the surface of the duodenum.

Tissue and diet samples were analyzed for Cd, Zn, Cu, Fe, and Mn using the flame-atomic absorption spectrophotometry after dry ashing of the sample (Stahr, 1991). Recoveries from spiked samples were 91% for Cd, and Zn, 92% for Cu, 90% for Fe, and 98% for Mn. Total livers were homogenized and aliquots of homogenate were used for trace metal analysis. The whole duodenum and kidney samples were analyzed. Dietary concentrations of Ca and Mg were measured by flame-AAS after addition of $LaCl_3$ to provide a final concentration of .75% of $LaCl_3$ to reduce the formation of refractory compounds with phosphates and aluminum (Stahr, 1991). The flame-AAS analysis were performed on AA-spectrophotometer IL 251 (Instrumentation Laboratory, Inc., Wilmington, MA).

Phytic acid contents of the diets were measured colorimetrically as described by Latta and Eskin (1980) on a spectrophotometer Cary 219 (Varian Associates, Inc., Palo Alto, CA) at the wavelength of 500 nm. The measurement was performed after the extraction of dietary samples with 2% HCl and purification by ion-exchange chromatography (AG1-X10, chloride form, 100 - 200 mesh, .5 g, Bio-Rad Laboratories, Hercules, CA), based on the procedure described by Latta and Eskin (1980).

The results were analyzed by ANOVA as a randomized complete block design using the GLM procedure of SAS with the effect of block, cadmium, phytic acid, and cadmium x phytic acid interaction in the model statement. Least square means are reported.

RESULTS AND DISCUSSION

Trace element, Ca, Mg, and phytic acid contents measured in the experimental diets are reported in Table 1.

Cadmium supplementation or different levels of phytic acid did not affect weight gain ($P > 0.77$, and $P > 0.86$) over the experimental period (data not shown).

Table 1. Analyzed mineral, and phytic acid content of diets

	Diet					
	Cd, 0 ppm			Cd, 3 ppm		
	Phytic acid			Phytic acid		
Item	Low	Medium	High	Low	Medium	High
	ppm of air-dry weight					
Cd	0.38	0.38	0.48	3.12	3.49	2.76
Zn	48	51	53	48	50	51
Cu	12.3	12.3	11.2	13.1	12.1	13.0
Fe	103	103	84	101	93	90
Mn	114	103	107	87	87	105
	%					
Ca	0.53	0.61	0.51	0.54	0.57	0.53
Mg	0.23	0.21	0.18	0.19	0.20	0.21
Phyt.[a]	-	0.85	1.98	-	0.86	1.66
Phyt.[b]	0.17	0.45	1.03	0.11	0.55	1.45

[a]Phytic acid content in diets with phytase addition was not measured because the measurement would not reflect the decrease of phytic acid content due to phytase action.
[b]To test the effect of phytase on phytic acid concentration, the diets were preincubated in deionized water (pH 5.0) for 1 hour at 55°C before the measurement of phytic acid concentration.

The addition of cadmium to the diet increased the cadmium concentration in duodenum ($P < 0.0001$), liver ($P < 0.0001$), and kidney ($P < 0.0001$). Also the total cadmium contents in liver ($P < 0.0001$), and kidney ($P < 0.0001$) were increased with addition of cadmium to the diet. Phytic acid did not affect cadmium concentration in duodenum ($P > 0.30$), liver ($P > 0.39$), or kidney ($P > 0.71$). It also did not affect the total cadmium content of liver ($P > 0.36$), or kidney ($P > 0.51$) (Table 2). Similarly Rose

Table 2. Effect of phytic acid and cadmium on cadmium concentration of tissues, and on cadmium content of liver and kidney

	Treatment						
	Cd, 0 ppm			Cd, 3 ppm[a]			
	Phytic acid			Phytic acid			
Item[b]	Low	Medium	High	Low	Medium	High	S.D.
n	9	6	9	8	8	8	
	ppm of wet weight						
D	0.122	0.132	0.131	2.837	2.264	2.057	0.731
L	0.126	0.098	0.113	0.373	0.331	0.351	0.067
K	0.115	0.145	0.175	1.006	0.998	1.021	0.133
	μg						
TL	1.670	1.382	1.461	4.917	4.563	4.386	0.789
TK	0.310	0.384	0.474	2.792	2.580	2.819	0.365

[a]Effect of cadmium on cadmium concentration of duodenum ($P < 0.0001$), liver ($P < 0.0001$), kidney ($P < 0.0001$), and cadmium content of liver ($P < 0.0001$), and kidney ($P < 0.0001$).
[b]D = Duodenum; L = liver; K = kidney; TL = total liver; TK = total kidney.

and Quarterman (1984) failed to observe a decrease in cadmium concentration in liver and kidneys of rats fed diet supplemented with phytic acid. They used 5 ppm of cadmium added to the diet as $Cd(OH)_2$. However, their study lasted for only four weeks which may not be sufficient time period for exposure to low cadmium levels to allow observation of differences in cadmium levels in organs due to different dietary treatments. Nevertheless, they reported that phytic acid prevented the increase in liver and kidney cadmium concentration promoted by calcium supplementation to the diet.

Although phytic acid did not affect the concentration of cadmium in liver, kidney, and duodenum, it significantly affected the liver concentrations of zinc ($P < 0.02$), copper ($P < 0.06$), iron ($P < 0.03$) and manganese ($P < 0.01$), all in a similar way (Table 3 and 4). The lowest concentrations of trace elements were observed in groups fed diets containing medium levels of dietary phytic acid (corn-soybean meal diet with natural phytic acid level). Liver concentrations of zinc, copper, iron, and manganese were greater in groups fed low phytic acid diet (phytase addition) and high phytic acid diet (addition of phytic acid as dodecasodium hydrate), with the highest concentrations often observed for the latter treatment. Although cadmium concentrations in liver were

Table 3. Effect of phytic acid and cadmium on liver concentrations of zinc, copper, and manganese

	Treatment						
	Cd, 0 ppm			Cd, 3 ppm			
	Phytic acid			Phytic acid			
Element	Low	Medium	High	Low	Medium	High	S.D.
n	9	6	9	8	8	8	
	ppm of wet weight						
Zn[a]	27.5	26.3	29.4	27.7	26.7	29.9	2.8
Cu[b]	4.60	4.83	5.28	5.07	4.76	5.39	0.67
Mn[c]	2.38	2.12	2.45	2.41	2.22	2.40	0.18

[a]Effect of phytic acid on zinc concentration of liver ($P < 0.02$).
[b]Effect of phytic acid on copper concentration of liver ($P < 0.06$).
[c]Effect of phytic acid on manganese concentration of liver ($P < 0.01$)

not significantly affected by phytic acid, they show similar pattern as the elements which concentrations in liver were significantly affected by phytic acid (Table 2). While the observation of higher trace element concentrations in livers of animals fed diets with phytase addition (low phytic acid diets) was expected, the elevated concentration of trace metals in livers of animals fed diets with phytate addition (high phytic acid diets) was surprising. These results suggest that synthetic phytic acid added to the diet could facilitate intestinal absorption of trace elements and thus behave differently from phytic acid naturally present in dietary components. Recently, Sakamoto et al. (1993) observed rapid absorption of synthetic phytic acid when this was intragastrically administered to rats in drinking water. Such observations support the need to reexamine the use of additions of synthetic phytic acid to the diet as an experimental tool to model high phytic acid diets in experiments designed to observe the effect of dietary phytic acid on the availability of trace elements.

Zinc, copper, and manganese concentrations in duodenum, and kidney as well as total liver and kidney contents of these metals were not affected by dietary cadmium or phytic acid (data not shown).

Phytic acid did not affect iron concentrations in duodenum ($P > 0.30$), and kidney ($P > 0.96$) as well as total iron content in liver ($P > 0.28$), and kidney ($P > 0.58$)(Table 4).

Increased dietary cadmium did not affect liver concentrations of zinc ($P > 0.65$), copper ($P > 0.39$), and manganese ($P > 0.59$).

Table 4. Effect of phytic acid and cadmium on iron concentration of tissues, and on iron content of liver and kidney

	Treatment						
	Cd, 0 ppm			Cd, 3 ppm[a]			
	Phytic acid			Phytic acid			
Item[b]	Low	Medium	High	Low	Medium	High	S.D.
n	9	6	9	8	8	8	
	ppm of wet weight						
D	31.9	34.0	32.0	23.4	30.9	20.4	11.1
L[c]	101.9	90.2	102.8	90.1	80.2	96.7	13.5
K	64.5	60.9	60.1	58.9	60.8	62.3	8.5
	μg						
TL	1348	1251	1331	1201	1105	1220	183
TK	163.1	159.9	167.0	160.1	154.1	168.0	25.8

[a]Effect of cadmium on the iron concentration of duodenum ($P < 0.03$), and liver ($P < 0.03$), and iron content of liver ($P < 0.02$).
[b]D = Duodenum; L = liver; K = kidney; TL = total liver; TK = total kidney.
[c]Effect of phytic acid on iron concentration of liver ($P < 0.03$).

Increased dietary cadmium decreased iron concentration in duodenum ($P < 0.03$), and liver ($P < 0.03$) as well as total iron content of liver ($P < 0.02$). It did not affect iron concentration in kidney ($P > 0.66$) and total iron content in kidney ($P > 0.75$). The observation of decreased iron concentrations in duodenum and liver due to increased dietary cadmium is in agreement with Maji and Yoshida (1974), who reported growth retardation and depression of hematocrit and hemoglobin caused by increased dietary cadmium (50 and 75 ppm). Dietary supplementation of $FeSO_4$ and ascorbic acid prevented these signs. Also, Webster (1979a) observed in pregnant mice, maternal and fetal anemia and fetal growth retardation caused by exposure to 40 ppm of Cd in drinking water. He suggested that increased cadmium reduced the intestinal absorption of iron. When pregnant mice were fed iron supplemented diets, cadmium induced fetal growth retardation was partially prevented (Webster, 1979b). While Maji and Yoshida (1974) and Webster (1979a) studied the effect of high levels of cadmium (40 to 75 ppm) over a relatively short period of time (13 to 19 days), our experiment was a long exposure to a low level of cadmium (3 ppm for three months). The findings suggest that chronic cadmium exposure could lead to the development of anemia as stated by Friberg et al. (1986).

Previously performed *in vitro* experiments allowed evaluation of the effect of phytic acid, and calcium on the intestinal absorption of cadmium (*Turecki et al., 1993*). The results supported the hypothesis that phytic acid decreased the intestinal absorption of cadmium. The *in vivo* experiment failed to demonstrate a similar effect of phytic acid. This difference between the results of *in vitro* experiments and *in vivo* experiment could be explained by the complexity of *in vivo* experimental conditions. Diets used in *in vivo* experiment contained relatively high levels of essential trace minerals (two to three times the NRC dietary requirement for rat for Zn, Cu, Fe, and Mn; NRC, 1980). The higher levels of essential trace minerals were used to prevent a potential deficiency due to dietary phytic acid. As most of these trace metals were present in diets in concentrations considerably higher than were the concentrations of cadmium, they could compete with cadmium for binding sites on calcium-phytate complexes and in this way influence the interaction between phytic acid and cadmium.

Acknowledgments. Supported in part by Mary Jane Brinton Czechoslovakian Scholarship Program, Iowa State University.

REFERENCES

Camner P, Clarkson TW, Nordberg GF (1986) Routes of exposure, dose and metabolism of metals. In: Friberg L, Nordberg GF, Vouk V (eds) Handbook on the toxicology of metals, 2nd edition, Elsevier Science Publishers, Amsterdam, Holland. p 85

Davies NT, Nightingale R (1975) The effects of phytate on intestinal absorption and secretion of zinc and whole-body retention of Zn, copper, iron, and manganese in rats. Brit J Nutr 34:243-258

Forbes RM, Endman JW, Parker HM, Kondo H, Ketelsen SM (1983) Bioavailability of zinc in coagulated soy protein (Tofu) to rats and effect of dietary calcium at a constant phytate:zinc ratio. J Nutr 113:205-210

Fox S (1988) Nutritional factors that may influence bioavailability of cadmium. J of Environ Quality 2:175-179

Friberg L, Kjellstrom T, Nordberg GF (1986) Cadmium. In: Friberg L, Nordberg GF, Vouk V (eds) Handbook on the toxicology of metals, 2nd edition, Elsevier Science Publishers, Amsterdam, Holland. p 130

Kostial K (1986) Cadmium. In: Mertz W (ed) Trace elements in human and animal nutrition, 5th edition, vol 2. Academic Press, Orlando, FL, p 319

Latta M, Eskin M (1980) A simple and rapid colorimetric method for phytate determination. J Agric Food Chem 28:1315-1317

Maji T, Yoshida A (1974) Therapeutic effect of dietary iron and ascorbic acid on cadmium toxicity of rats. Nutr Rep Int 10:139-149

National Research Council (1980) Mineral tolerance of domestic animals, National Academy of Sciences, Washington, D.C.

Oberleas D, Muhrer ME, O'Dell BL (1962) Effects of phytic acid on zinc availability and paraketosis in swine. J Anim Sci 21:57-61

Piscator M (1982) Cadmium - exposure and effects in the general population and in occupationally exposed workers. In: Prasad AS (ed) Clinical, biochemical, and nutritional aspects of trace elements. Alan R. Liss, Inc., New York, NY. p 521
Rahola T, Aaran RK, Miettinen JK (1972) Assessment of radioactive contamination in man. IAEA Proceedings Series, SM-150/12, Unipublishers, New York, NY.
Reddy NR, Sathe SK, Salunke DK (1982) Phytates in legumes and cereals. Adv Food Res 28:1-92
Rose HE, Quarterman J (1984) Effects of dietary phytic acid on lead and cadmium uptake and depletion in rats. Environ Res 35:482-489
Sakamoto K, Vucenik I, Shamsuddin AM (1993) [^{3}H] Phytic acid (inositol hexaphosphate) is absorbed and distributed to various tissues in rats. J Nutr 123:713-720
Stahr HM (1991) Analytical methods in toxicology. John Wiley, New York, NY
Webster WS (1979a) Iron deficiency and its role in cadmium-induced fetal growth retardation. J Nutr 109:1640-1645
Webster WS (1979b) Cadmium-induced fetal growth retardation in mice and the effects of dietary supplements of zinc, copper, iron, and selenium. J Nutr 109:1646-1651
Wise A (1982) Blood lead levels after chronic feeding to mice of lead acetate with calcium phytate in the diet. Bull Environ Contam Toxicol 29:550-553