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Effect of Phytic Acid and Calcium on the Intestinal Absorption of Cadmium *In Vitro*

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

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Various animal experiments have shown that intestinal absorption of cadmium is less than 2% (Kostial, 1986). However, because it is excreted slowly and tends to accumulate, especially in kidney (Nordberg et al., 1985), even the trace amounts of cadmium in the diet represent a potential hazard. Among the nutrients with potential to affect Cd uptake and toxicity, Fox (1988) mentions Zn, Cu, Ca, Fe, ascorbic acid and protein. She also suggests that phytic acid could be of significance.

Phytic acid is 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. Phytic acid has been reported to reduce intestinal absorption of Zn (Likuski and Forbes, 1965; Oberleas et al., 1966) as well as Fe, Cu and Mg (Davies and Nightingale, 1975; Brink et al, 1991). The formation of phytate-calcium-trace metal complexes has been suggested as the mechanism responsible for decreased intestinal absorption of trace metals (Wise, 1986). Only limited experimental data concerning the effect of phytic acid on the availability of dietary cadmium (Rose and Quarterman, 1984) have been reported.

The objective of this project was to evaluate the effect of phytic acid on *in vitro* intestinal absorption of cadmium (Exp. 1) and to determine how the effect of phytic acid was modified by calcium concentration (Exp. 2).

MATERIAL AND METHODS

Experiment 1: Thirty-six, male Holtzman rats with average weight of 270 g were used as the donors of intestines for *in vitro* incubation of everted intestinal sacs. Prior to 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 commercial rat chow (7002 rodent diet, Harlan Teklad, Madison, WI). Animals were not

* *Present address:* Department of Animal Physiology and Zoology, University of Agriculture, Praha 6-Suchdol, 165 21, Czech Republic
Correspondence to: T. Turecki

intentionally exposed to cadmium.

Feed was removed 18 hours before the animals were killed by decapitation. From the duodenum, just distal to the pyloric sphincter, two segments of intestine, each 5-cm long, were used for the everted intestinal sacs technique. The original method (Wilson and Wiseman, 1954) was modified: the serosal layer was removed from the intestine and pH of the incubation medium used was 7.4 on the serosal side and 6.0 on the mucosal side. The pH of the incubation medium was adjusted just prior to the incubation.

The composition of the incubation medium (Krebs-Ringer phosphate buffer) was 125 mM NaCl; 5.4 mM KCl; 2.7 mM $CaCl_2$; 1.2 mM $MgSO_4.7H_2O$; 1.5 mM KH_2PO_4; 3.6 mM $NaHCO_3$; 9 mM phosphate buffer. D-glucose was added to the incubation medium to provide the concentration of 5 mM.

A completely randomized design with 2 x 3 factorial arrangement of treatments was used with 2 levels of phytic acid (0 and 10 mM) and three levels of cadmium (0.5, 5, and 25 ppm). Six animals per treatment group were used and two segments of small intestine were used from each animal.

Cadmium was added to the incubation medium at the mucosal side in the form of $CdCl_2$. 2 1/2 H_2O. The radioisotope, ^{109}Cd, (0.1 μCi/mL of incubation medium, specific radioactivity 2.65 Ci per mg of Cd, NEN, Boston, MA) was added to the incubation medium at the mucosal side. Phytic acid was added to the incubation medium at the mucosal side in the form of dodecasodium hydrate (Aldrich Chemical Company, Milwaukee, WI) to provide the concentration of 10 mM. Incubation medium (0.6 mL) inserted inside (serosal side) the everted intestinal sac did not contain cadmium or phytic acid. Everted intestinal sacs were incubated for 45 min in 15 mL of aerated (95% O_2 + 5% CO_2) incubation medium at 37°C.

Experiment 2: Thirty-six, male Holtzman rats of average weight 370 g were used as the donors of intestines for *in vitro* incubation of everted intestinal sacs. A completely randomized design with 2 x 3 factorial arrangement of treatments was used with 2 levels of phytic acid (0 and 10 mM) and three levels of calcium (2.7, 50 and 125 mM). Six animals per treatment group were used and two segments of small intestine were used from each animal.

Cadmium was added to the incubation medium at the mucosal side in the form of $CdCl_2$. 2 1/2 H_2O to provide the cadmium concentration of 5 ppm. Calcium was added to the incubation medium at the mucosal side in the form of $CaCl_2$.

In Exp. 2, lactate dehydrogenase (LDH) activity in the intestinal tissue was measured after tissue homogenization. Tissue homogenate (0.05 mL) was mixed with LDH substrate (2.950 mL, pH 8.8, 25°C) and placed in a 3 mL cuvette of 1 cm pathway in spectrometer Spectronic 600 (Bausch & Lomb, Rochester, NY). Absorbance increase

at 340 nm was observed. One unit of LDH activity was defined as an increase in absorbance of 0.001 per min per mL of sample under the indicated conditions.

All other procedures involved were the same as in Exp. 1.

After the incubation, the radioactivity in the incubation medium at the serosal and mucosal side and in the intestinal tissue was measured by liquid scintillation counter (Packard 2425, Packard Instrument Company, Inc., Downers Growe, IL). The results were adjusted for background, for matrix effects using standards and for radioactive decay.

Cadmium in rat chow was measured using flame-atomic absorption spectrophotometry after the dry ashing of the samples (Stahr, 1991). Recovery from spiked samples was 91%. Cadmium in the incubation medium was measured directly by flame-AAS. Calcium in the incubation medium was measured by flame-AAS after addition of $LaCl_3$ to provide a final concentration of 0.75 % of $LaCl_3$ to reduce the formation of refractory compounds with phosphates and aluminum (Stahr, 1991). All the flame-AAS analysis were performed on AA-spectrophotometer IL 251 (Instrumentation Laboratory, Inc., Wilmington, MA).

For histological examination, samples of intestinal tissue were fixed in 10% neutral buffered formalin, processed on a Shandon Hypercenter tissue processor and embedded. The parafilm blocks were cut at 4 microns. Tissue sections were then stained with hemotoxylin and eosin and examined under the light microscope.

Data were expressed as the percentage of total recovered cadmium (Exp. 1. - 93%; Exp. 2. - 102% recovery). Calculations of recovered cadmium were based on radioactivity measurements. Data were analyzed using the GLM procedure of SAS. Split-plot analysis of variance was used, with average value for the 2 sacs representing an animal and with the variance among animals treated alike used as the error term to test the effect of main effects and their interaction. Least square means ± SE are reported.

RESULTS AND DISCUSSION

Experiment 1: The chow that was fed from weaning until the rats were used as donors of intestines for everted intestinal sac procedure contained 0.125 ± 0.100 ppm of cadmium (air-dry weight). The cadmium concentrations measured in incubation mediums were 0.44, 4.87 and 26.14 ppm (the calculated concentrations were 0.5, 5, and 25 ppm). Histological examination of the intestinal tissue incubated as everted intestinal sacs indicated that tissue segments were viable after the incubation. However, varying degrees of damage, including loss of epithelium from about 15% of the surface area of intestinal villi, were observed.

Although phytic acid did not affect the transport of cadmium

across the intestinal wall ($P > 0.15$), phytic acid tended to decrease cadmium transport across the intestinal wall at cadmium levels of 5 and 25 ppm (Table 1). Phytic acid increased the relative amount of unabsorbed cadmium ($P < 0.01$, Table 1) and decreased the percentage of total recovered cadmium retained in the intestinal tissue ($P < 0.01$, Table 1).

Table 1. Experiment 1. The effect of phytic acid and cadmium concentration on the cadmium transport across intestinal wall, unabsorbed cadmium, and cadmium retained per 0.1 g of wet intestinal tissue (expressed as percentage of total recovered cadmium)

Cadmium, ppm	Phytic acid, mM	
	0	10
	Cadmium transport across intestinal wall	
.5	0.53 ± 0.07 (n=11)	0.66 ± 0.06 (n=12)
5.0	0.52 ± 0.06 (n=12)	0.26 ± 0.07 (n=11)
25.0	0.66 ± 0.07 (n=11)	0.46 ± 0.07 (n=11)
	Unabsorbed cadmium[a]	
.5	86.61 ± 0.72 (n=11)	93.87 ± 0.66 (n=12)
5.0	87.38 ± 0.66 (n=12)	93.82 ± 0.72 (n=11)
25.0	89.92 ± 0.72 (n=11)	95.10 ± 0.72 (n=11)
	Cadmium retained per 0.1 g of wet intestinal tissue[ab]	
.5	3.50 ± 0.23 (n=11)	1.66 ± 0.21 (n=12)
5.0	3.26 ± 0.21 (n=12)	1.52 ± 0.23 (n=11)
25.0	2.43 ± 0.23 (n=11)	1.06 ± 0.23 (n=11)

[a]Effect of phytic acid ($P < 0.0001$).
[b]Effect of cadmium concentration ($P < 0.04$).

When phytic acid was not present in the incubation medium, the greatest percentage of cadmium transported across the wall was observed for the greatest concentration of cadmium (Table 1). Lehman and Klaassen (1986) and Scheuhammer (1988) also observed increased relative absorption of cadmium in response to increased dose of cadmium. Increasing concentration of cadmium tended to increase the percentage of unabsorbed cadmium ($P < 0.08$, Table 1) and decrease the percentage of cadmium retained by the tissue ($P < 0.04$, Table 1). Koo et al. (1978) reported similar results for intestinal absorption of cadmium with concentrations of stable cadmium ranging from 1 to 112 ppm.

Experiment 2: The chow that was fed from weaning until the rats were used as donors of intestines for everted intestinal sac procedure contained 0.177 ± 0.054 ppm of cadmium (air-dry weight).

Cadmium concentration measured in the incubation medium was 5.08 ppm (the calculated concentration was 5 ppm). Calcium concentrations measured in the media were 108, 2104 and 5061 ppm. The calculated concentrations were 108, 2004 and 5010 ppm (2.7, 50 and 125 mM). Histological examination of the intestinal tissue incubated as everted intestinal sacs indicated that tissue segments were viable after the incubation. However, varying degrees of damage, including loss of epithelium from about 20% of the surface area of intestinal villi, were observed. In addition, the LDH activity (units/mg of wet tissue) in intestinal segments decreased after incubation (50 for first segment, and 70 for second segment, relative to 126 for nonincubated tissue) indicating that tissue damage occurred during the processing of tissue and its incubation.

Phytic acid decreased the transport of cadmium across the intestinal wall ($P < 0.01$, Table 2). Consistently with its effect on cadmium transport across the intestinal wall, phytic acid increased the relative amount of unabsorbed cadmium ($P < 0.01$, Table 2) and decreased the percentage of cadmium retained by the intestinal tissue ($P < 0.01$, Table 2).

The greatest decrease in cadmium absorption due to phytic acid was observed at calcium:phytic acid molar ratio of 5:1 (50 mM Ca, 10 mM phytic acid). This is approximately the ratio of maximal precipitation of phytic acid by calcium (Nolan et al., 1987). Such finding supports the theory that trace metals are coprecipitated on the Ca-phytate complexes and thus are less available for intestinal absorption (Wise, 1986). The effect of phytic acid-calcium precipitation on cadmium absorption persisted at the level of 125 mM of calcium and this is of importance, because 125 mM is the level of rat dietary requirement for calcium (NRC, 1980).

Increasing concentrations of calcium in the absence of phytic acid resulted in a linear increase in the cadmium transport across the intestinal wall ($P < 0.06$, Table 2) and in the relative amount of unabsorbed cadmium ($P < 0.01$, Table 2), resulting in the linear decrease in the cadmium in the intestinal tissue ($P < 0.02$, Table 2). These relationships were more complex when phytic acid was present in the incubation medium. These findings do not agree with the results of *in vivo* studies that linked calcium deficient diets with increased absorption of cadmium (Koo et al., 1978; Van Barneveld and Van den Hamer, 1985). Only Rose and Quarterman (1984) reported increased cadmium accumulation in liver and kidney of rats fed high calcium diets (1.2% Ca). However, this level is more than twice as high as the highest calcium level used (125 mM = 0.5%).

Unlike the results of *in vivo* study performed by Rose and Quarterman (1984), the results of our *in vitro* experiments suggest the potential ability of phytic acid to decrease the intestinal absorption of cadmium. Phytic acid decreased not only the transport across the intestinal wall but also the amount of

Table 2. Experiment 2. The effect of phytic acid and calcium concentration on the cadmium transport across the intestinal wall, unabsorbed cadmium, and cadmium retained per 0.1 g of wet intestinal tissue (expressed as percentage of total recovered cadmium)

Calcium, mM	Phytic acid, mM	
	0[a]	10[b]
	Cadmium transport across the intestinal wall[c]	
2.7	0.19 ± 0.03 (n=10)	0.22 ± 0.03 (n=12)
50	0.23 ± 0.03 (n=12)	0.01 ± 0.03 (n=11)
125	0.28 ± 0.03 (n=12)	0.08 ± 0.03 (n=12)
	Unabsorbed cadmium[c]	
2.7	72.50 ± 1.57 (n=10)	83.68 ± 1.36 (n=12)
50	77.01 ± 1.36 (n=12)	97.50 ± 1.49 (n=11)
125	81.93 ± 1.36 (n=12)	93.69 ± 1.36 (n=12)
	Cadmium retained per 0.1 g of wet intestinal tissue[c]	
2.7	6.16 ± 0.33 (n=10)	3.92 ± 0.28 (n=12)
50	5.87 ± 0.28 (n=12)	0.59 ± 0.31 (n=11)
125	4.73 ± 0.28 (n=12)	1.63 ± 0.28 (n=12)

[a]Linear effect of calcium within phytic acid concentration on cadmium transported across the intestinal wall ($P < 0.06$), unabsorbed cadmium ($P < 0.0001$) and cadmium retained by intestinal tissue ($P < 0.02$)
[b]Quadratic effect of calcium within phytic acid concentration on cadmium transported across the intestinal wall ($P < 0.001$), unabsorbed cadmium ($P < 0.0001$) and cadmium retained by intestinal tissue ($P < 0.0001$)
[c]Effect of phytic acid ($P < 0.0001$).

cadmium retained by the tissue, which suggests that its action prevents the initial step of cadmium absorption, i.e. the binding of cadmium to the anionic sites on mucosal surface and subsequent transport through the brush border membrane. The probable explanation for this action is formation of insoluble complexes involving phytic acid and calcium with cadmium coprecipitated on these complexes.

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