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Dietary Protein Effects on Cadmium and Metallothionein Accumulation in the Liver and Kidney of Rats

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The relationship of dietary protein to cadmium absorption and tissue deposition was studied in male Sprague-Dawley rats exposed to different levels of cadmium in the drinking water. In animals fed a high-protein or low-protein diet and drinking water containing 25 or 50 ppm cadmium, liver and kidney cadmium and metaliothionein were both significantly higher in rats fed the high-protein diet for 2 to 4 months. These differences may possibly be explained by the concentration of cysteine observed between these two diets. When cysteine was added to the low-protein diet to the level observed in the high-protein diet and fed to rats receiving 25 ppm cadmium in the drinking water, significant dietary differences in liver and kidney cadmium and metallothionein were not observed. The importance of dietary protein to cadmium-induced toxicity was also assessed in these studies. The activity of catechol-o-methyltransferase was used as a measure of cadmium-induced toxicity. The activity of this enzyme in the lung, liver and heart was significantly lower in rats fed a low-protein diet than those fed the high-protein diet and 50 ppm cadmium. Metallothionein concentration in the lung and liver from low protein fed rats was approximately half the level observed in rats fed the high-protein diet, which suggests a relationship between cadmium-induced toxicity and metallothionem concentrations. These results imlustrate the importance of considering dietary protein (and possibly cysteine) when studying cadmium metabolism in experimental animals.

Introduction

Several investigators have reported that dietary protein may influence the intestinal absorption of cadmium. Fitzhugh and Meiller *(1)* were the first to report that the toxicity of cadmium was increased by a low-protein diet. Since then, abnormalities of the bone, liver, and blood have been observed in animals given cadmium (orally or by injections) and fed a low-protein diet *(2,3)*. These abnormalities were not observed when the level of protein in the diet was relatively high.

In short-term studies (i.e., days), Suzuki et al. *(4)* compared the effect of low- and high-protein diets on the accumulation of cadmium in several tissues and observed higher levels of cadmium in the liver, kidney, and whole body of mice fed low protein diets for 24 hr before and after an oral dose of 115Cd. In long-term studies, rats fed low- or high-protein diets and drinking water containing 50 ppm cadmium for 3 months were found to have significantly higher concentrations of cadmium and metallothionein (MT) in the liver and kidney than those rats fed the high-protein diet *(5)*. These results suggest that the effect of dietary protein may be associated with the length of exposure.

Although Itokawa et al. *(2)* observed abnormalities of bone in rats fed cadmium and low-protein diets for 30 days, bone cadmium was higher in rats fed the high-protein diet. Thus, in relatively long-term studies, the tissue level of cadmium may be higher in animals fed a high-protein diet, but the toxicity of cadmium may be greater in animals fed a low-protein diet. These conflicting effects may be associated with the tissue level of MT. For example, MT synthesis may be reduced in animals fed a low-protein diet *(5)*, which would allow cadmium to bind to other macromolecules (i.e., enzymes), thus increasing the tissue toxicity of this element.

The present studies were performed to determine, in rats exposed to drinking water containing different levels of cadmium, the importance of dietary protein and exposure time on the tissue accumulation of cadmium and MT. The relationship of the tissue level ofMT to cadmium-induced toxicity was also assessed. Since cysteine has been suggested as a factor explaining the effect of dietary protein, studies were performed to determine its effect on cadmium absorption and tissue accumulation.

Methods

Male rats of the Sprague-Dawley strain, approximately 3 months old, and weighing an average of 150 g, were used. They were randomly divided into several groups and fed (ad libitum) drinking water and purified diets as follows. Group A, the control group, was fed various control diets [high-protein or low-protein (Table 1)] and deionized water. Groups B and C were fed, respectively, a low-protein diet or high-protein diet and drinking water containing 5, 25, or 50 ppm cadmium. Group D was fed a low-protein diet with 400 gg of L-cysteine/g diet added and drinking water containing 25 ppm cadmium. The rats were exposed to these diets and drinking water for a total period of 4 months and at 1-month intervals; 96 rats from each experimental group (i.e., 4 for each level of cadmium/group/time point) and 32 controls (i.e., 4 for each dietary level) were killed by decapitation. The urine (collected directly from the bladder), heart, kidney, and liver were then removed, and blocks of these tissues and urine were analyzed as follows.

Table 1. Chemical composition of the various diets.

Ingredient	Ingredients in diet, %		
	Low protein plus cysteine	Low protein	High protein
Casein (vitamin free)	5.5	5.5	67.5
Sucrose	27.56	27.6	9
Corn oil	5	5	5
Lard	5	5	5
L-Cysteine	0.04	0	0
Dextrin	46.55	46.55	3.15
Methionine	0.15	0.15	0.15
RP vitamin mixture[a]	2.0	2.0	2.0
Choline chloride	0.20	0.20	0.20
Mineral mixture[b]	5.0	5.0	5.0
Nonnutritive fiber (Sokka-floc)	3.0	3.0	3.0

[a]Each diet contained the following vitamins/kg of diet: thiamine HCl, 20 mg; riboflavin, 20 mg, niacin, 90 mg; pyridoxine HCl, 20 mg; D-calcium pantothenate, 60 mg; folic acid, 4 mg; D-biotin, 0.4 mg; inositol, 200 mg; metadione sodium bisulfite, 20 mg; vitamin A acetate, 22 IU/g; vitamin D_3, 2.2 IU/g and DL-α-tocopherol acetate, 50 IU/kg.

[b]Diets were adjusted to contain the following mineral nutrients: calcium, 0.75%; phosphorus, 0.45%; potassium, 0.46%; sodium, 0.29%; magnesium, 0.065%; manganese, 65 mg/kg; iron, 60 mg/kg; zinc, 20 mg/kg; copper, 15 mg/kg; fluoride, 5 mg/kg/; cobalt, 3.2 mg/kg; chromium, 3 mg/kg; iodine, 0.6 mg/kg; molybdenum, 0.8 mg/kg; and selenium, 0.2 mg/kg. The phosphorus content was 0.42% in the low-protein diet and 0.48% in the high-protein diet. Cadmium in the diets was less than 0.04 μg/g dry weight.

[redacted]

Table 2. Effect of dietary protein and drinking water cadmium on body weight at the end of 4 months of treatment.

Treatment group	Body weight, g[a]		
	Initial	Final	Difference
Controls			
Low-protein	166 ± 4	215 ± 9	49
High-protein	153 ± 3	323 ± 8	170
Low-protein + 400 μg cysteine	159 ± 4	233 ± 5	73
Experimental			
5 ppm Cd, low-protein	148 ± 4	200 ± 6	52
High-protein	152 ± 3	315 ± 11	167
25 ppm Cd, low-protein	153 ± 2	205 ± 8	52
High-protein	160 ± 6	328 ± 15	168
50 ppm Cd, low-protein	155 ± 1	195 ± 5	40
High-protein	158 ± 3	316 ± 10	158
25 ppm low-protein + 400 g cysteine	151 ± 2	229 ± 8	78

[a]Mean ± SEM for four rats per experimental group.

[redacted]

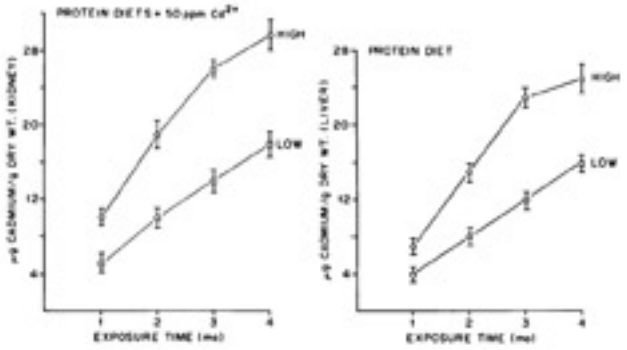

FIGURE 1. Rats were fed a high- or low-protein diet and drinking water containing 50 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

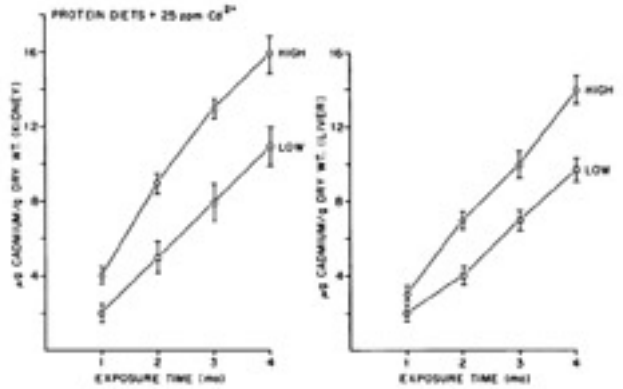

FIGURE 2. Rats were fed a high- or low-protein diet and drinking water containing 25 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

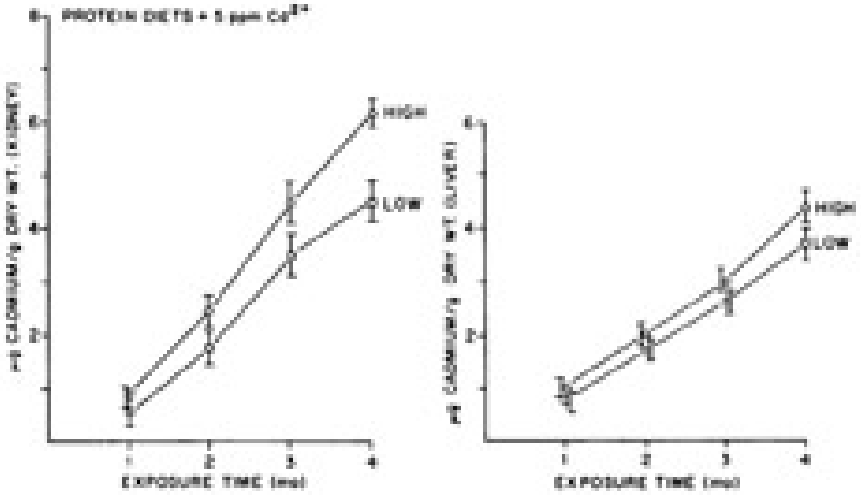

FIGURE 3. Rats were fed a high- or low-protein diet and drinking water containing 5 ppm cadmium (as cadmium chloride). At each time point, four rats from each group were killed and the concentration of cadmium determined in the kidney and liver. Results are expressed as means ± SEM.

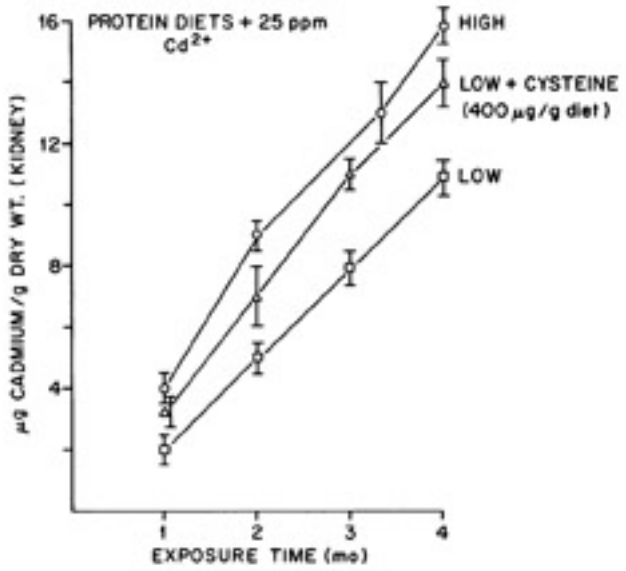

FIGURE 4. Rats were fed the low-protein diet with L-cysteine added (400 ± g/g of diet) and drinking water containing 25 ppm cadmium. At each time point, four rats from each group were killed and the concentration of cadmium was determined in the kidney. Results are expressed as means ± SEM. Data from Figure 2 were included as a comparison.

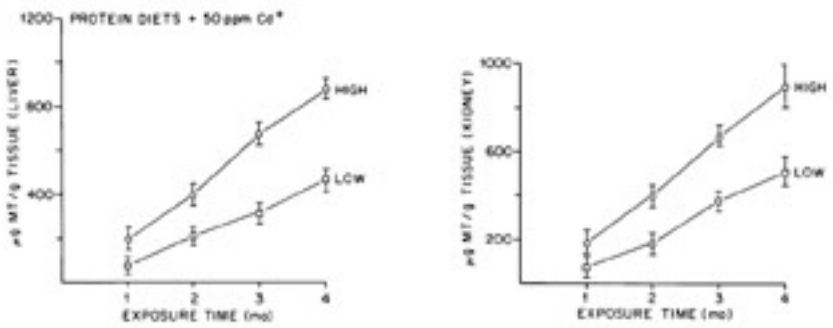

FIGURE 5. Metallothionein (MT) was determined in the liver and kidney (as discussed in method section) from rats fed the high or low protein diet and drinking water containing 25 ppm cadmium. Results are expressed as means ± SEM.

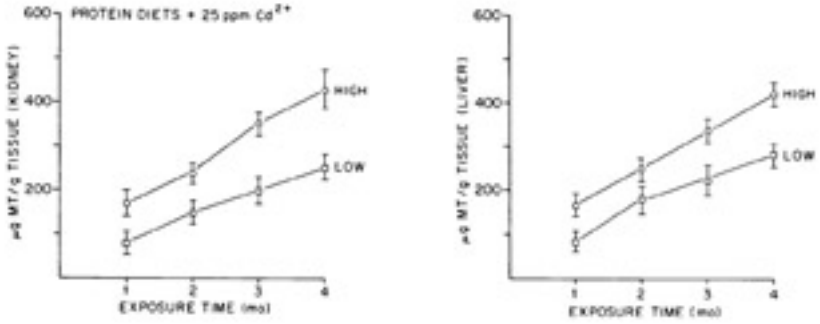

FIGURE 6. Metallothionein (MT) was determined in the liver and kidney (as discussed in text) from rats fed the high- or low-protein diet and drinking water containing 50 ppm cadmium. Results are expressed as means ± SEM.

[redacted] MT

Table 4. Effect of cadmium on catechol-*O*-methyltransferase (COMT) and metallothionein (MT) levels in the lung, heart and liver from rats given for 4 months, drinking water containing 50 ppm cadmium and a high- or low-protein diet.

		MT, μg/g wet weight[a]		COMT, μmole/mg protein/hr[a]	
Treatment group	Tissue	2 months exposure	4 months exposure	2 months exposure	4 months exposure
Controls					
Low-protein	Lung	0.09 ± 0.07	0.13 ± 0.07	15 ± 2	17 ± 3
	Liver	7 ± 2	20 ± 5	13 ± 1.6	15 ± 1.9
	Heart	—	—	2.4 ± 0.4	3.0 ± 0.2
High-protein	Lung	0.10 ± 0.08	0.17 ± 0.10	14 ± 4	18 ± 3
	Liver	10 ± 1.6	35 ± 8	14 ± 2	15 ± 1.9
	Heart	—	—	2.6 ± 0.3	3.1 ± 0.6
Experimental (50 ppm Cd)					
Low-protein	Lung	0.19 ± 0.07†	0.29 ± 0.10*	8.1 ± 0.9*	5.2 ± 1.0*
	Liver	155 ± 48†	369 ± 35‡	9.3 ± 1.3	8.7 ± 2*
	Heart	—	—	1.29 ± 0.3*	0.73 ± 0.15†
High-protein	Lung	0.66 ± 0.13†	0.89 ± 0.17†	10 ± 2	12 ± 3
	Liver	385 ± 29‡	806 ± 79‡	13 ± 4	11 ± 2
	Heart	—	—	2.0 ± 0.4	2.1 ± 0.5

[a]Means ± SE for four rats/group/time point.
*$p < 0.05$.
†$p < 0.01$.
‡$p < 0.001$.

Table 5. Retention of ^{109}Cd in various tissues expressed as a percentage of initial dose.

Experimental group	Tissue	^{109}Cd retention, % of initial dose/g dryweight[a] 24 hr exposure	48 hr exposure	72 hr exposure
High-protein diet	Liver	0.48 ± 0.14	0.48 ± 0.08	0.44 ± 0.11
	Kidney	0.98 ± 0.19	0.88 ± 0.13	0.40 ± 0.09
	GI tract	96 ± 1.5	95 ± 2.3	95 ± 1.1
Low-protein diet	Liver	0.69 ± 0.15	0.70 ± 0.14	0.68 ± 0.19
	Kidney	1.39 ± 0.21	1.20 ± 0.17	0.90 ± 0.10
	GI tract	97 ± 2.1	97 ± 1.8	97 ± 1.0
Low-protein diet plus cysteine	Liver	0.66 ± 0.13	0.60 ± 0.19	0.53 ± 0.17
	Kidney	1.63 ± 0.26	1.0 ± 0.16	0.73 ± 0.15
	GI tract	94 ± 2.3	94 ± 1.0	93 ± 1.7

[a]Rats were previously fed the respective diets for 30 days prior to intubating a solution containing ^{109}Cd and the respective diets. At the various time points, rats were killed and the liver, kidneys and gastrointestinal tract (including the esophogus, stomach and intestine, contents included) were lyophilized, weighed and radioactively determined. The value shown at each time point represent an average from six rats. The time points given are from the administration of initial dose. Values are the means ± SEM.

[illegible]

Table 6. Urinary excretion of ^{109}Cd as a percentage of the initial dose in rats previously fed diets for 30 days with different levels of protein.

Treatment group	^{109}Cd excreted, % of initial dose[a] 24 hr after exposure	48 hr after exposure	72 hr after exposure
Low-protein	0.03 ± 0.0009	0.05 ± 0.019	0.02 ± 0.010
High-protein	0.05 ± 0.010*	0.05 ± 0.007	0.03 ± 0.013
Low-protein plus cysteine	0.07 ± 0.010†	0.03 ± 0.016	0.03 ± 0.015

[a]Mean ± SEM for six rats.
*$p < 0.05$.
†$p < 0.01$.

[illegible]