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Zinc-and/or cadmium-induced intestinal metallothionein and copper metabolism in adult rats

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Feeding diets with high zinc or cadmium concentrations to animals compromises their copper status. Previous work suggested that metallothionein (MT), induced in the intestinal mucosa by zinc or cadmium, binds copper and inhibits its absorption. More recent studies showed that this mechanism may not be operative for zinc and that intestinal MT adapts to long-term feeding of high-zinc diets. The present study was designed to determine the effects of feeding high-zinc and/or high-cadmium diets on the induction of intestinal MT and its subsequent effect on copper status. Six-week-old male rats were placed in a 2 × 2 × 3 factorial experiment with two concentrations of dietary zinc (60 and 350 mg/kg), two concentrations of dietary copper (3 and 9 mg/kg), and three concentrations of dietary cadmium (0, 1, and 5 mg/kg). After 3 weeks, the difference in the MT concentration between rats fed high- and normal-zinc diets was only 1.5 times. However, rats fed the highest amount of cadmium had MT concentrations about five time higher than those not fed cadmium. In both the zinc and cadmium groups, the concentration of intestinal zinc and cadmium followed that of MT; however, the copper concentrations were not changed. Although intestinal MT was not elevated appreciably in zinc-fed rats, the copper status of these rats fed 3 mg of copper/kg of diet was severely depressed. Rats fed 9 mg of copper/kg of diet were not affected. The copper status in rats fed high-cadmium and 3 mg of copper/kg of diet was depressed even more than with a high-zinc diet. This study suggests that the effects of high dietary zinc or cadmium on copper status are not the result of induced intestinal MT binding of copper thus preventing its passage into the circulation. (J. Nutr. Biochem. 7: 128–134, 1996.)

Keywords: zinc; copper; cadmium; metallothionein; intestine; kidney; liver; absorption; rat

Introduction

Previous studies have shown that feeding high-zinc diets to animals depresses their copper status.[1–4] It is believed that the major site for this interaction is at the level of the intestinal mucosa where zinc interferes with copper absorption.[2,5,6] The general dogma has been that high zinc levels in the intestinal lumen, as a result of consuming a high-zinc diet, induces mucosal metallothionein (MT) which in turn binds copper and reduces its movement from the cell to the circulation.[4–6] This theory implies that the amount of copper bound to MT increases with the amount of MT present in the mucosal cells. This does not occur, however. Reeves et al.[7] could not find a positive correlation between the amount of zinc-induced MT and the concentration of copper in the intestinal mucosa. In most cases, there was an inverse relationship between the amounts of copper and MT in the mucosa. Ogiso et al.[4] also showed that the copper concentration in the intestine was 50% lower in rats fed high-zinc diets than in those fed normal dietary zinc. These studies suggest that the induction of intestinal MT by high dietary

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zinc is not related to the effects of zinc on copper absorption and/or status.

High intakes of cadmium also affect copper absorption and status[8–10] in the rat. Using a dietary Cd:Cu molar ratio of one (4.4 mg of Cd and 2.6 mg of Cu/kg of diet), Davies and Campbell[9] found no effect of cadmium on ^{64}Cu absorption in rats fed for 7 days. However, a dietary ratio of four significantly reduced copper absorption during this period. Because cadmium is a highly effective inducer of intestinal MT,[11,12] the same mechanism proposed for the effect of zinc-induced MT on copper absorption also could be proposed for cadmium-induced MT. To explore this possibility, we performed experiments to determine if there were associations among the dietary cadmium concentration, intestinal MT concentrations, and copper status of rats. Because we wanted to confirm previous studies,[7] we incorporated a third variable, normal and high amounts of dietary zinc, and used a three-way factorial experimental design.

Methods and materials

Animals and diet

This study was approved by the Animal Use Committee of the USDA, ARS, Grand Forks Human Nutrition Research Center and was in accordance with guidelines of the National Institutes of Health on the experimental use of laboratory animals.[13]

One hundred and twenty 6-week-old male Sprague–Dawley rats were purchased from Sasco, Inc. (Omaha, NE USA).* Upon arrival at the laboratory, they were housed individually in stainless-steel cages with wire-mesh floors and fed a diet similar to the basal diet outlined in *Table 1* except that it contained adequate copper (6 mg/kg) and zinc (60 mg/kg). The rats were provided with fresh deionized water daily. After 1 week of consuming this diet, the animals were randomly divided into 12 groups of 10 rats each. The experimental design was a 2 × 2 × 3 factorial with two concentrations of dietary zinc (60 and 350 mg/kg), two concentrations of dietary copper (3 and 9 mg/kg), and 3 concentrations of dietary cadmium (0, 1, and 5 mg/kg). The analyzed concentrations of each dietary mineral were (mean ± SD) zinc, 57 ± 1 and 337 ± 7; copper, 2.9 ± 0.1 and 8.8 ±± 0.2; cadmium, 0.1 ± 0.02, 1.1 ± 0.1; and 4.8 ± 0.5 mg/kg of diet, respectively.

Analytical procedures

After 3 weeks of consuming these various regimens, the rats were anesthetized (without fasting) with 50 mg of pentobarbital sodium/kg of body weight, and blood was withdrawn from the abdominal aorta until they expired. Blood was centrifuged at 1,500*g* for 20 min and the serum was collected and kept frozen at −20°C until it was analyzed for copper and zinc concentrations and ceruloplasmin amine oxidase (CPAO) activity. A 20-cm section of the upper intestine, beginning at the pylorus, was excised, and the lumen contents were washed out with ice-cold saline. The segment was slit open, and the mucosal lining was gently scraped off with the edge of a glass slide. Intestinal scrapings were kept frozen at −80°C until analysis for copper, zinc, cadmium, and MT concentrations. The liver and both kidneys of each rat were removed and frozen.

*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1 Composition of basal diet

Dietary ingredients	g/kg
Cornstarch*	300
Glucose†	313
Casein‡	150
Soybean oil§	100
Egg white solids‖	50
Zn/Cu free mineral mix#	35
Cellulose¶	30
Biotin premix**	10
AIN-76 vitamin mix††	10
Choline bitartrate	2

*Argo, CPC International, Englewood Cliffs, NJ USA.
†ICN Biochemicals, Cleveland, OH USA.
‡High protein, Teklad, Madison, WI USA.
§Crisco Oil, Procter & Gamble, Cincinnati, OH USA.
‖Supplies the required amount of sulfur amino acids, Teklad, Madison, WI USA.
#Contains in g/kg of mix: calcium phosphate (dibasic), 500; sodium chloride, 74; potassium citrate (monohydrate), 220; potassium sulfate, 52; magnesium oxide, 24; manganous carbonate, 3.5; ferric citrate, 6; potassium iodate, 0.01; sodium selenite (5 hydrate), 0.01; chromium potassium sulfate (12 hydrate), 0.55; sucrose (powdered), 119.93. To vary the zinc (zinc carbonate), copper (copper carbonate), and/or cadmium (cadmium chloride) concentrations in the diet, premixes of each were prepared in glucose and added at 10 g/kg of diet. The amount of dietary glucose was adjusted to account for the premix additions.
¶Teklad, Madison, WI USA.
**Eighty milligrams of D-biotin/kg in powdered sucrose.
††Teklad, Madison, WI USA.

The copper and zinc concentrations were determined in serum by precipitating serum proteins from 0.5 mL of serum with 1.0 mL of deionized water and 0.5 mL of 5-sulfosalicylic acid in water (0.45 mol/L). The mixture was allowed to sit at room temperature for 24 hr and then centrifuged at 2,000*g* for 20 min. The supernatant was analyzed for zinc and copper by flame atomic absorption spectroscopy (AAS) according to standard procedures. The serum cadmium level was also determined, but because the values were near or below the detection limits of the instrument the values were not reported. Fresh serum was analyzed for CPAO activity by the method of Schosinsky et al.[14]

Concentrations of MT were determined in each tissue. Intestinal scrapings were each divided into two portions. One portion was homogenized in buffer containing 50 mmol Tris-HCl/L and 1 mmol 2-mercaptoethanol/L, pH 7.4. One milliliter of the homogenate was heated for 10 min at 95°C and centrifuged for 5 min at 10,000*g*. The anterior half of the kidney and a portion of the right lobe of the liver each were treated in a manner similar to that for the intestinal scrapings. Aliquots of the supernatants from each tissue were analyzed for MT concentration by adapting the ^{109}Cd displacement assay of Eaton and Cherian.[15]

For mineral analyses, the remaining portion of the intestinal scrapings, the posterior portion of the kidney, and a portion of the right lobe of the liver were lyophilized to a constant weight and ashed in a muffle furnace at 450°C for 48 hr. The residue from each sample was diluted to 5 mL in 0.1 mol HCl/L and analyzed for zinc, copper, and cadmium by AAS. For quality control, samples of liver standard reference material (1577b, National Institute of Standards and Technology, Gaithersburg, MD USA) were analyzed with each batch. Values for minerals in the standards were within the specified range. The recovery of cadmium by this procedure was greater than 90%.

Statistical analysis

Data for each group are expressed as the average for 10 replicates. Variability is expressed as the root mean square error (RMSE), an estimate of the standard deviation across treatments. The analysis of variance (ANOVA; Crunch Statistical Software, Oakland, CA USA) for a 3-way factorial design was used to determine the significant main effects and interactions. When interactions were significant, we used the post hoc test of Tukey[16] to distinguish differences between specific means. For the ANOVA to be valid, the operating assumption is that the variances are homogeneous. A test for homogeneity was done for each ANOVA by the method of Hartley.[17] When the test was significant, i.e., variances were not homogeneous, the data were transformed to achieve or to approach homogeneity and analyzed again. For those variables treated in this fashion, the transformed means are listed in the tables. Although the ANOVAs were run on transformed data, those means are in units that may not be familiar to the reader. Thus the means were back-transformed into the usual units and recorded in parentheses.

RESULTS

Weight gain

Treatment of young adult rats with high dietary zinc or cadmium or marginal copper had no significant effect on growth. *Table 2* shows that the daily gain for the 3-week period on experiment was not different among groups.

Serum components

However, there were marked effects on some serum components (*Table 2*). Increasing dietary zinc from 60 to 350 mg/kg caused a 20% increase in the concentration of zinc in serum ($P < 0.0001$). Serum copper concentrations and CPAO activities of rats fed 3 mg of Cu/kg of diet were markedly depressed in rats fed the high-zinc diets. However, when rats were fed 9 mg of Cu/kg, feeding high zinc had no effect. This resulted in a highly significant Zn x Cu interaction ($P < 0.0005$) for both parameters.

There were significant effects of dietary cadmium on serum zinc, copper, and CPAO activity (*Table 2*). An interaction between zinc and cadmium significantly affected the serum zinc concentrations ($P < 0.009$). In rats fed 60 mg of Zn/kg of diet, serum zinc decreased as the concentration of dietary cadmium increased. However, in rats fed the higher amount of zinc, there was no effect of increasing dietary cadmium on the serum zinc concentration. The concentration of serum copper and the activity of CPAO were significantly ($P < 0.005$) lower in rats fed dietary cadmium than in those not fed cadmium. The percent decrease in these values was greater in rats fed 60 mg of Zn/kg of diet than in those fed 350 mg/kg. As expected, increasing dietary copper from 3 to 9 mg/kg significantly increased serum copper concentrations and CPAO activity ($P < 0.0001$).

Intestinal mucosa components

When dietary copper was increased from 3 to 9 mg/kg, there was a significant increase ($P < 0.0001$) in the amount of copper in the intestinal mucosa (*Table 3*). When dietary copper was elevated there was a significant decrease ($P <$

Table 2 Effect of dietary zinc, copper, and cadmium on weight gain, and zinc and copper concentrations, and CPAO activity in serum of male rats*

Dietary minerals				Serum minerals		
Zn (mg/kg of diet)	Cu (mg/kg of diet)	Cd (mg/kg of diet)	Weight gain (g/day)	Zn (μmol/L)	Cu (μmol/L)	Serum CPAO activity (U/L)
60	3	0	5.1	19.5	12.4	117.5
60	3	1	5.2	18.2	11.1	92.9
60	3	5	4.9	18.0	7.8	48.6
60	9	0	4.5	20.1	16.0	174.8
60	9	1	4.8	18.7	15.9	162.0
60	9	5	5.3	17.7	16.4	180.5
350	3	0	5.1	23.2	7.2	44.3
350	3	1	5.5	22.3	4.1	27.7
350	3	5	5.4	23.3	5.5	9.2
350	9	0	4.8	20.4	16.1	178.2
350	9	1	5.0	20.8	15.3	163.7
350	9	5	5.1	23.4	14.2	143.5
RMSE†			1.1	2.5	2.5	36.6
ANOVA				*P* values		
Zinc				0.0001	0.0001	0.0001
Copper					0.0001	0.0001
Cadmium					0.0010	0.0005
Zinc x copper					0.0001	0.0005
Zinc x cadmium				0.0091		
Copper x cadmium					0.0412	
Zinc x copper x cadmium					0.0320	

*Values represent the mean of 10 replicates per group.
†RMSE = root mean square error and is an estimate of the overall variance and is calculated by taking the square root of the mean square error term from the ANOVA calculation.

Table 3 Effect of dietary zinc, copper, and cadmium on the concentration of zinc, copper, cadmium, and MT in intestinal mucosa of male rats*

Dietary minerals			Intestinal mucosa			
				Cu	Cd	
				U/kg wet weight		
Zn (mg/kg of diet)	Cu (mg/kg of diet)	Cd (mg/kg of diet)	Zn (ln μmol [μmol])	(μmol)	($\sqrt{\mu mol}$ [μmol])	MT (ln μmol [μmol])
60	3	0	5.64 (281)†	26.1	0.66 (0.44)‡	1.10 (3.00)†
60	3	1	5.71 (302)	24.2	5.84 (34.1)	1.99 (7.32)
60	3	5	5.72 (305)	26.0	11.63 (135)	2.72 (15.2)
60	9	0	5.57 (262)	26.7	0.74 (0.55)	0.95 (2.59)
60	9	1	5.71 (302)	30.9	6.03 (36.4)	1.64 (5.16)
60	9	5	5.71 (302)	31.2	10.35 (107)	2.42 (11.2)
350	3	0	5.95 (384)	19.1	1.02 (1.04)	1.46 (4.31)
350	3	1	6.06 (428)	18.9	3.87 (15.0)	2.55 (12.8)
350	3	5	6.18 (483)	20.2	11.11 (123)	3.15 (23.3)
350	9	0	6.02 (412)	28.8	0.25 (0.06)	1.19 (3.29)
350	9	1	6.05 (424)	30.4	4.37 (19.1)	2.32 (10.2)
350	9	5	5.98 (395)	26.2	6.36 (40.4)	2.68 (14.6)
RMSE§			0.18	5.9	1.73	0.30
ANOVA			*P* values			
Zinc			0.0001	0.0004	0.0001	0.0001
Copper				0.0001	0.0020	0.0001
Cadmium			0.0244		0.0001	0.000
Zinc × copper				0.0123	0.0375	
Zinc × cadmium					0.0135	0.0353
Copper × cadmium					0.0001	
Zinc × copper × cadmium					0.0485	

*Values represent the mean of 10 replicates per group.
†ANOVA was performed on the ln transformed data. The values in parenthesis represent the back transformations of the means.
‡ANOVA was performed on the square root transformed data. The values in parenthesis represent the back transformations of the mean.
§RMSE = root mean square error and is an estimate of the overall standard deviation. It is calculated by taking the square root of the mean square error term from the ANOVA calculation.

0.0002) in the amount of MT in the mucosa. This occurred in rats fed either the normal or high-zinc diets. Dietary copper had no effect on the amount of cadmium in the intestinal mucosa. A significant interaction between zinc and copper ($P < 0.02$) affected intestinal copper. When dietary zinc was high, intestinal copper was low in rats fed low-copper diets but not when the rats were fed a normal-zinc low-copper diet.

There was a very complex interaction among zinc, copper, and cadmium that significantly affected intestinal cadmium. Elevating cadmium in the diet markedly elevated the amount of cadmium in the intestinal mucosa (*Table 3*). In addition, cadmium caused on average about a 5 fold increase in the intestinal MT. An increase in dietary cadmium slightly elevated the intestinal zinc ($P < 0.03$) but had no effect on the intestinal copper concentrations.

Liver components

Feeding high-zinc diets to rats had no effect on the liver zinc concentration (*Table 4*). However, rats consuming diets high in zinc had lower liver copper ($P < 0.0001$) and cadmium ($P < 0.002$) concentrations than rats fed normal-zinc diets. There was an interaction between copper and cadmium ($P < 0.04$) indicating that when dietary copper was low, high dietary cadmium lowered liver copper (*Table 4*). Liver MT concentrations were increased about 25% in rats fed the high-zinc diets compared with those fed normal-zinc diets. Rats fed high-cadmium diets also had significantly higher ($P < 0.0001$) liver MT than rats not fed cadmium. A significant ($P < 0.02$) interaction between zinc and cadmium for MT suggested a greater relative induction of MT by cadmium in rats receiving normal dietary zinc than in those receiving high zinc.

Because the concentrations of cadmium in the livers of rats fed the lowest amount of cadmium were undetectable, it was not appropriate to do a 3-way ANOVA. However, when a 2-way ANOVA was done with the 0-cadmium group missing, there was a significant increase in liver cadmium as the dietary cadmium was increased. Feeding high-zinc diets significantly lowered the amount of cadmium in the liver.

Kidney components

A significant ($P < 0.011$) interaction between dietary zinc and copper affected the kidney zinc concentration (*Table 5*). When dietary zinc was normal, kidney zinc concentrations were not affected by increasing dietary copper from 3 to 9 mg/kg, but when dietary zinc was high, kidney zinc concentrations were elevated ($P < 0.05$) when dietary copper was raised from 3 to 9 mg/kg. Increasing dietary zinc 7 fold had no effect on the kidney concentration of MT.

Table 4 Effect of dietary zinc, copper, and cadmium on the concentration of zinc, copper, cadmium, and MT in liver of male rats*

Dietary minerals			Liver			
Zn (mg/kg of diet)	Cu (mg/kg of diet)	Cd (mg/kg of diet)	Zn (μmol/kg of wet wt)	Cu (μmol/kg of wet wt)	Cd (μmol/kg of wet wt)	MT (μmol/kg of wet wt)
60	3	0	424	59.5	ND†	1.20
60	3	1	414	56.0	0.16	1.20
60	3	5	443	53.0	4.4	1.63
60	9	0	436	68.1	ND	1.25
60	9	1	431	68.5	0.07	1.35
60	9	5	428	70.1	3.7	1.82
350	3	0	432	53.1	ND	1.70
350	3	1	405	41.5	0.04	1.65
350	3	5	427	46.2	1.7	1.87
350	9	0	443	63.4	ND	1.42
350	9	1	447	65.4	ND	1.93
350	9	5	424	62.2	2.4	1.84
RMSE‡			48	7.8	1.3	0.29
ANOVA				*P* values		
Zinc				0.0001	0.0500	0.0001
Copper				0.0001		
Cadmium					0.0020	0.0001
Zinc × copper						
Zinc × cadmium						0.0140
Copper × cadmium				0.0333		0.0403
Zinc × copper × cadmium						

*Values represent the mean of 10 replicates per group.
†Not detectable.
‡RMSE = root mean square error and is an estimate of the overall standard deviation. It is calculated by taking the square root of the mean square error term from the ANOVA calculation.

Overall, increasing dietary zinc did not significantly affect the kidney copper concentrations; the apparent reduction was only about 10%. However, increasing dietary copper 3 fold increased the kidney copper levels by as much as 85% ($P < 0.0001$). Feeding rats increasing amounts of dietary cadmium significantly ($P < 0.025$) reduced the amount of copper in the kidneys. High concentrations of either dietary copper or cadmium significantly ($P < 0.0001$) increased the amount of MT in the kidneys. The concentration of cadmium in the kidney was directly proportional to the amount in the diet.

Because the concentrations of cadmium in the kidneys of rats fed the lowest amount of cadmium were undetectable, it was not appropriate to do a 3-way ANOVA. However, when a 2-way ANOVA was done with the 0-cadmium group missing, there was a significant increase in kidney cadmium as the dietary cadmium was increased. Feeding high-zinc diets significantly lowered the amount of cadmium in the kidney.

Discussion

It has been known for years that feeding rats high-zinc or high-cadmium diets compromises their copper status. The results of this experiment corroborated these earlier findings when rats were fed marginal amounts but not when they were fed a surplus of dietary copper. The widely accepted theory to explain this phenomenon is that an increase in intestinal MT induced by zinc sequesters copper in the intestinal mucosa and prevents its passage into the body. The sequestered copper is eventually lost as the mucosal cells slough off and are eliminated through the feces.

Although the theory sounds convincing, the evidence to support it is not. To suggest that copper binds to induced intestinal MT implies that as the concentration of MT increases the copper concentration also increases. This clearly does not happen when rats are fed high-zinc diets[4,7] and apparently not when they are fed high-cadmium diets, as shown in the present experiment. Short-term studies by Davies and Campbell[9] suggested that copper binds to cadmium-induced intestinal MT when MT is isolated by gel filtration. However, the present study showed no change in the copper concentration in the intestinal mucosa even though MT concentrations increased 5-fold as dietary cadmium was raised from near zero to 5 mg/kg of diet. This strongly suggests that the intestinal MT induced by zinc or cadmium did not bind copper.

The demonstration that copper binds to Zn/Cd-induced MT during isolation by gel filtration may only reflect the redistribution of copper from low affinity ligands to MT, a ligand with a high affinity for copper.[7,18] Farrell et al.[18] isolated MT by gel filtration and showed that the addition of MT to cell extracts containing low endogenous MT caused a shift of copper to the MT peak from other copper-binding ligands. This suggests that using similar procedures for cell extracts containing high endogenous concentrations of MT

Table 5 Effect of dietary zinc, copper, and cadmium on the concentration of zinc, copper, cadmium, and MT in the kidney of male rats*

Dietary minerals			Kidney			
			Zn	Cu	Cd	MT
				u/kg wet weight		
Zn (mg/kg of diet)	Cu (mg/kg of diet)	Cd (mg/kg of diet)	(μmol)	(ln μmol [μmol])	(μmol)	(μmol)
60	3	0	372	4.16 (64.1)†	ND‡	5.2
60	3	1	363	4.06 (58.1)	3.7	5.3
60	3	5	376	3.95 (51.9)	15.6	6.2
60	9	0	370	4.87 (130.3)	ND	6.6
60	9	1	342	4.66 (105.6)	2.7	6.9
60	9	5	372	4.69 (108.9)	12.0	7.6
350	3	0	355	4.05 (57.4)	ND	4.9
350	3	1	341	4.03 (56.3)	2.4	4.7
350	3	5	363	3.93 (50.9)	10.3	7.6
350	9	0	362	4.72 (112.2)	ND	6.3
350	9	1	387	4.55 (94.6)	2.2	6.7
350	9	5	388	4.65 (104.6)	10.9	7.8
RMSE§			37	0.25	2.3	1.6
ANOVA				*P* values		
Zinc					0.0014	
Copper				0.0001		0.0001
Cadmium				0.0232	0.0001	0.0001
Zinc × copper			0.0109			
Zinc × cadmium						
Copper × cadmium						
Zinc × copper × cadmium						

*Values represent the mean of 10 replicates per group.
†ANOVA was performed on the ln transformed data. The values in parenthesis represent the back transformations of the means.
‡Not detectable.
§RMSE = root mean square error and is an estimate of the overall standard deviation. It is calculated by taking the square root of the mean square error term from the ANOVA calculation.

might produce artifactual results. Consequently, demonstrating that the distribution of copper is shifted to isolated MT does not necessarily mean that it is also bound to MT in the intact cell.

Other lines of evidence suggest that MT may not play a role in the lowering of the copper status in rats fed high dietary concentrations of zinc. The initial response of the intestinal mucosa within 24 hr after the introduction of a high-zinc diet is to produce a large amount of MT.[7] This can reach 5 fold depending on the amount of zinc fed. However, Reeves[19] showed that when the high-zinc diet is continually fed for up to 7 weeks, MT concentrations in the intestinal mucosa gradually decline to near normal values, only 1.5 fold higher than in rats fed a normal-zinc diet. Data from the present study suggest that this adaptation response also occurred here. Initial values for intestinal MT were not determined, but after 3 weeks of feeding the high-zinc diet, the MT values were only about 1.5 times higher in rats fed high-zinc than in those fed normal zinc. Although there was only a small difference in the MT concentrations between the two dietary regimens, feeding high zinc caused a severe depression of copper status. It could be argued that the initial increase in intestinal MT upon feeding high-zinc diets affected copper absorption, and thus the copper status. The effect was still present after 3 weeks, even though MT had adapted, because the experiment had not been carried out long enough for the animals to recover. It is more likely that the high zinc concentration in the intestinal lumen directly interfered with the transport of copper across the mucosal cells.[20]

The results of this study on the effect of excess dietary zinc on copper status corroborated some of the earlier findings[5-7] but not others.[19] For example, Reeves[19] fed adult male rats 350 mg of zinc and 4 mg of copper/kg of diet for up to 7 weeks and saw no effect on copper status. A follow-up to this study (data not published) showed similar results. Fischer et al.,[5,6] however, found that weanling rats fed 120 mg of zinc and 6 mg of copper/kg of diet for 5 weeks had significantly reduced concentrations of copper in serum. In the present study, we showed an effect when feeding 350 mg of zinc and 3 mg of copper/kg of diet but not when feeding 9 mg of copper/kg. The magnitude of the effect could depend on a number of factors including the amounts of zinc and copper in the diet, the age of the animal, the duration of feeding, and the composition of the diet. When feeding rats the AIN-93G diet,[19] which contains L-cystine as the amino acid supplement for casein, we observe that control values for the concentration of serum zinc are about 30% higher than when a similar diet that contains DL-methionine is fed (data not shown). Furthermore, we have observed a negative effect on the copper status of rats fed high zinc only when their diets contained casein and DL-

methionine or a small amount of dried egg white as the sulfur amino acid supplement. Further studies are needed to determine if dietary L-cystine protects rats from low copper status when fed high-zinc diets.

Van Campen[21] and Starcher[22] were the first to demonstrate that dietary cadmium reduced the absorption of copper from rat intestine. The Cd:Cu ratios used by these investigators were very high: 150:1 and 20:1, respectively. Later, Davies and Campbell[9] showed that a 4:1 ratio, but not a 1:1 or a 2:1 ratio, was effective in reducing ^{64}Cu absorption in rats fed cadmium for 1 week. From our own study it is obvious that a much smaller dietary Cd:Cu molar ratio also will reduce the amount of copper in serum and in some other tissues if the rats are fed for a long enough time. It was shown that when the dietary molar ratio of Cd:Cu went from 0.02 to 0.96 there was a 35% reduction ($P < 0.001$) in serum copper and a 20% reduction ($P < 0.008$) in kidney copper of rats fed 60 mg of zinc and 3 mg of copper/kg of diet. These data suggest that under certain conditions even low dietary Cd:Cu ratios will reduce the amount of copper crossing the intestinal mucosa cells. Interestingly, the copper concentrations in the intestinal mucosa and liver were not affected by dietary cadmium under these short-term feeding conditions. It is unclear why the kidney is more reflective of the copper status than the liver in Cd-fed rats. The kidney also was more responsive to changes in dietary Cu than either the serum or liver.

Earlier in this report we suggested that intestinal mucosa MT had possibly adapted to the high-zinc diet. The ratio of MT concentrations between rats fed 60 and 350 mg of zinc/kg of diet for 3 weeks was much lower than was previously found after a shorter period of feeding these amounts of zinc.[18] There is no direct evidence that the concentration of intestinal MT also adapts to increased amounts of cadmium in the diet. However, the possibility is intriguing given the strong indication that the primary role for MT in the intestinal mucosa is for protection against the intake of heavy metals that could be detrimental to health.[23] The ramifications of such an adaptation response to long-term intakes of either high zinc or cadmium are equally intriguing.

Conclusions

In a previous report,[7] we suggested that the negative effect of chronic feeding of high-zinc diets on copper status was not related to sequestration of copper by zinc-induced MT in the intestinal mucosa. Results from the present study support those findings and suggest that similar effects of cadmium on copper status are not related to reduced copper absorption by cadmium-induced MT and copper sequestration.

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