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Copper status of adult male rats is not affected by feeding an AIN-93G-based diet containing high concentrations of zinc

Philip G. Reeves

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

Although previous studies have shown that copper status of rats is compromised when they consume a diet with high zinc, studies using the new AIN-93G rodent diet did not show this effect. Because the new diet formulation contains components such as L-cystine and an ultratrace element (UTE) mix that might affect copper metabolism, a study was done to determine if these components interfered with the effect of zinc. A 2 × 2 × 2 × 2 factorial study was designed with two dietary concentrations of copper, 3 and 6 mg/kg of diet; with L-cystine or DL-methionine; with or without the UTE mix; and with 2 concentrations of zinc, 35 and 350 mg/kg of diet. After 5 weeks, assessments of copper status were made. Results showed that serum ceruloplasmin amine oxidase activity, a very sensitive copper status indicator, was not affected by feeding high zinc in the diet. Other status indicators such as serum copper or liver copper concentrations also were not affected by high-zinc feeding. It was concluded that the lack of an effect of high zinc on copper status when using the AIN-93G diet was not the result of using L-cystine or UTE in the diet. Dietary UTE stimulated growth in rats fed the marginal-copper diet but not in rats fed the normal-copper diet. Rats fed diets containing DL-methionine had significantly higher concentrations of liver and intestinal copper than those fed diets with L-cystine. (J. Nutr. Biochem. 7:166–172, 1996.)

Keywords: copper; zinc; nutrient status; absorption; amino acids; ultratrace elements; rats

Introduction

It has been known for some time that a high intake of zinc interfere with copper metabolism in farm animals[1] and humans[2]. Recent studies showed that the copper status of 6-week-old male rats was significantly reduced when they were fed diets containing 350 or 560 mg of zinc/kg[3,4]. This effect was demonstrated by reductions in serum and liver copper concentrations and serum ceruloplasmin amine oxidase activities. Fischer et al.[5,6] also showed that only 120 mg of zinc/kg of diet reduced the copper status of weanling rats.

The magnitude of the effect of high dietary zinc on copper status might depend on a number of factors including the amount of zinc and/or copper in the diet, the composition of the diet, the age of the animal at the time the feeding trials begin, and the duration of the feeding trials. In this regard, Reeves[7] showed recently that 8-week-old male rats fed 350 mg of zinc/kg of a diet based on the new AIN-93G formulation[8], did not have reduced copper status after 7 weeks. In a follow-up study (unpublished), it was shown that 535 mg of zinc/kg of diet also did not influence copper status in similar rats.

Because the formulation of the AIN-93G diet is considerably different than that of diets known to initiate low copper status when they contain high zinc[4], the following study was designed to look for possible factors in the new diet that could be responsible. In the AIN-93G diet, L-cystine was substituted for DL-methionine. Recent studies[9,10] showed an interaction between dietary L-cystine

Address reprint requests to Philip G. Reeves, Ph.D., at the USDA, ARS, Grand Forks Human Nutrition Research Center, Box 9034, University Station, Grand Forks, ND 58202-9034.
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cystine(cysteine) and copper absorption and/or status. A mix of ultratrace elements (UTE) including silicon, vanadium, boron, nickel, fluoride, lithium, and chromium is part of the AIN-93G diet, and there are known interactions among various minerals in the diet. Therefore, the experiment was designed to determine whether the substitution of L-cystine for DL-methionine and/or the addition of the UTE mix was responsible for the absence of an effect of high dietary zinc on copper status in adult rats. In addition, the effect of high-zinc diets on copper absorption was determined in control rats by using ^{67}Cu and the whole body counting technique.

Methods and materials

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[11].

Experimental Design

The design was a 2 × 2 × 2 × 2 factorial with two concentrations of zinc, 35 and 350 mg/kg; two concentrations of dietary copper, 3 and 6 mg/kg; 3 g of L-cystine or DL-methionine/kg as the amino acid supplement for casein; and with or without the addition of the UTE mix. The base diet was the same as the AIN-93G[8]. Changes in copper, zinc, and UTE concentrations in the diet were made at the expense of starch. The experiment consisted of 80, 8-week-old male Sprague-Dawley rats from Sasco Inc. (Omaha, NE, USA). When the animals arrived at the laboratory, they were immediately fed the control diet that contained 6 mg of Cu and 35 mg of zinc/kg, L-cystine as the amino acid supplement, and with the UTE mix. They were acclimated to this diet for 4 days and then divided into 16 groups of 5 rats each. The makeup of the 16 diet groups can be seen in the top portion of *Table 1*.

Analytical procedures

The animals consumed their respective diets for 5 weeks and then were killed by exsanguination subsequent to anesthesia with pentobarbital sodium (50 mg/kg). Blood was collected from the abdominal aorta, allowed to clot at room temperature for 20 minutes, and then placed on ice. After 30 min, the serum was collected by centrifugation and the activity of ceruloplasmin amine oxidase (CPAO) was immediately determined by the method of Schosinsky and Lehmann[12,13]. (At 1 week into the experiment, CPAO activity was also measured in blood serum that had been collected from the tail vein.) The remaining serum was frozen for future determination of zinc and copper concentrations[7].

A 20-cm segment of the intestine, beginning at the pylorus, was excised and the contents washed out with ice-cold saline. The segment was slit open and the mucosal layer was gently scraped off with the edge of a glass slide. The scrapings were stored at −80°C until analyzed for metallothionein (MT) concentration within 1 week after collection. The method for MT analysis is described by Eaton and Cherian[14] and modified by Reeves[7]. The remaining portion of the intestinal mucosa was analyzed for zinc and copper concentrations as described by Reeves[7].

The large lobe of the liver and both kidneys were removed and stored frozen until analyzed for zinc and copper by methods described previously[7]. The right femur was removed and cleansed of adhering tissue, lyophilized to a constant weight, and ashed for zinc and copper analysis in a manner similar to that for liver and kidney.

Copper absorption by whole body counting

In a second experiment, the whole body counting technique described by Cotzias[15] and Heth and Hoekstra[16] was used to determine the apparent absorption of copper as affected by feeding a high-zinc diet. Seven rats in each of two groups were fed the diet containing 6 mg of copper/kg, L-cystine, UTE, and either 35 (35Zn) or 350 (350Zn) mg of zinc/kg. After 4 weeks of dietary exposure, the rats were fasted from 11:00 p.m. until 9:00 a.m. the next morning. Portions of each diet were thoroughly mixed with ^{67}Cu (specific activity, 18.5 GBq/g) so that 2.0 g of diet contained 185 kBq. Copper-67 was prepared from ^{67}Zn by Dr. Kurt Zinn at the Missouri University Research Reactor, Columbia, MO, USA. Four hr after the rats had consumed the 2.0 g of ^{67}Cu-containing diet, the amount of ^{67}Cu in each rat was determined by whole-body counting. The counting apparatus is described in an earlier publication by Reeves et al.[17].

After the rats were assayed for radioactivity for the first time, diet cups were returned to the cages and the rats were allowed to eat their respective ^{67}Cu-free diets ad libitum. Thereafter, the amount of radioactivity in each rat was determined each day for the next 13 days. To determine the counting efficiency of the machine, a phantom of approximately the same size as a rat was prepared by mixing ^{67}Cu in powdered sucrose, placing the sucrose in a plastic bag, and placing the bag in a polypropylene bottle. Because the half-life of ^{67}Cu is only 61 hr, the phantom was also used to determine the rate of decay of the radioisotope, and the count rate corrected accordingly.

The amount of ^{67}Cu remaining in the body each day was represented as a percentage of the amount found in the body at day 0. The apparent absorption of ^{67}Cu was calculated by fitting the data points to a double exponential equation of the form $f(t) = a_1e^{-b_1t} + a_2e^{-b_2t}$ (Fig. 1). The first component of the curve represents mostly the loss of unabsorbed ^{67}Cu in the feces. The second component represents the slow loss from the body of that portion of ^{67}Cu that was initially absorbed (a_2). The term b_2 is the rate of loss of ^{67}Cu from the body. The biological half-life (BHL) of ^{67}Cu in the body is calculated as BHL = (ln 2)/b_2.

Feces were collected daily for 7 days from each rat and the ^{67}Cu content determined by counting them in a gamma counter. The cumulative loss of ^{67}Cu in the feces was expressed as a percentage of the amount in the body at zero time. The data were fitted to an exponential equation of the form $f(t) = a(1-e^{-bt})$. The constant a represents the ^{67}Cu lost as a percentage of that consumed. The constant b represents the rate of loss of ^{67}Cu. These parameters were used to estimate the curve up to day 13 for each dietary zinc group.

Statistical analysis

Data for each group are expressed as the average for five or seven replicates. Variability is expressed as the root mean square error (RMSE), an estimate of the standard deviation across treatments. The analysis of variance for a 4-way factorial design was used to determine significant main effects and interactions (ANOVA; Crunch Statistical Software, Oakland, CA, USA). When interactions were significant, we used the post-hoc test of Tukey[18] to distinguish differences between specific means. Results of the whole body counting studies were expressed as the means ± SEM. Statistical differences were determined by Student's t test.

RESULTS

Weight gain

The average daily weight gain for all rats was 6.5 ± 1.0 g for the 5-week feeding period (data not shown). The only significant treatment effect was an interaction between copper

Table 1 Effect of various dietary variables on zinc and copper concentrations in serum, liver, kidney, femur, and intestinal mucosa, and on ceruloplasmin amine oxidase activity (CPAO) in serum, and metallothionein (MT) concentration in intestinal mucosa of adult male rats fed the diet for 5 weeks[1]

																	RMSE[2]
Dietary Variables																	
Zinc, mg/kg	35	35	35	35	35	35	35	35	350	350	350	350	350	350	350	350	
Copper, mg/kg	3	3	3	3	6	6	6	6	3	3	3	3	6	6	6	6	
Cys or Met, 3 g/kg	Cys	Cys	Met	Met	Cys	Cys	Met	Met	Cys	Cys	Met	Met	Cys	Cys	Met	Met	
Ultratrace Elem., +/−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	
Serum																	
Zinc, μmol/L	23.7	24.0	20.3	19.9	22.3	22.8	20.9	20.3	34.5	28.0	30.0	31.8	26.8	26.9	31.6	27.0	3.4
Copper, μmol/L	15.1	15.0	14.0	14.7	14.7	14.1	15.3	16.4	16.4	15.9	15.5	15.4	13.6	14.2	15.7	16.5	1.5
CPAO1, initial, U/L[3]	111	117	100	108	126	101	121	119	107	93	86	102	109	109	130	125	21
CPAO2, final, U/L	132	141	116	127	140	116	137	139	149	143	141	133	121	123	139	143	19
Liver																	
Zinc, μmol/kg	410	402	410	430	408	404	406	414	456	450	440	404	450	430	440	440	45
Copper, μmol/kg	54.2	51.5	55.6	57.6	52.2	51.4	60.7	56.0	57.5	51.9	57.8	55.9	56.8	58.5	60.1	60.7	5.4
Kidney																	
Zinc, μmol/kg	359	357	344	336	356	356	341	337	418	375	381	380	401	389	391	391	25
Copper, μmol/kg	124	125	106	115	125	109	131	128	136	106	132	96	137	144	136	118	23
Intestinal mucosa																	
Zinc, μmol/kg	262	275	257	263	259	259	270	264	497	318	376	447	403	394	636	476	125
Copper, μmol/kg	28.1	26.0	24.9	30.0	24.0	25.5	28.3	28.3	25.5	20.6	27.8	26.4	31.3	28.2	34.5	27.8	5.0
MT, μmol/kg	7.72	7.37	6.38	6.88	6.98	7.55	6.01	6.65	19.6	12.4	12.7	15.7	14.6	12.1	25.9	14.1	7.2
Femur																	
Zinc, mmol/kg	2.70	2.75	2.46	2.38	2.77	2.64	2.36	2.21	3.61	3.12	3.56	3.53	3.78	3.63	3.43	3.65	0.43
Copper, μmol/kg	23.9	24.3	24.4	25.1	23.1	24.1	25.4	22.6	23.6	22.4	23.7	23.3	25.4	24.0	25.7	21.6	2.5

[1]Each value represents the mean of 5 rats per group.
[2]Root mean square error is an estimate of the overall standard deviation and is calculated by extracting the square root of the mean sum of squares of the error term in the analysis of variance table. See Table 2 for significant *P* values calculated by the ANOVA.
[3]Measured in blood serum collected from the tail vein after 1 week of experiment.

and UTE. When dietary copper was low, the presence of UTE in the diet significantly ($P < 0.03$) stimulated weight gain by about 11%. However, when dietary copper was adequate, no effect of UTE was seen.

Serum components

The first indications of an effect of high dietary zinc on copper status are reductions in serum copper concentration and CPAO activity[19]. *Table 1* shows that in the present experiment, this did not occur. A near 10-fold increase in dietary zinc had no significant effect on either serum copper or CPAO activity. However, other dietary factors affected these parameters. After 1 week of consuming the diet, rats fed 6 mg of Cu/kg of diet had significantly ($P = 0.003$) higher (14%) CPAO activity than those fed 3 mg of Cu/kg of diet. However, by the 5th week, this difference had disappeared. There also was a significant ($P = 0.036$) interaction between dietary copper and the amino acid supplement. At marginal concentrations of dietary copper, CPAO activity was higher in rats receiving L-cystine than those receiving DL-methionine. There was a much stronger interaction between these two parameters after the rats had been on experiment for 5 weeks ($P = 0.003$), and the direction of the interaction was the same as before. A similar interaction was observed for serum copper.

There was a significant interaction between dietary amino acid supplement and zinc on serum zinc concentration. At the lower offering of dietary zinc, rats fed diets with L-cystine had higher serum zinc concentrations than those fed DL-methionine. Serum zinc concentrations were enhanced by high dietary zinc, as expected. However, the higher amount of dietary copper significantly ($P = 0.03$) depressed serum zinc. In addition, there were some complex interactions among copper, amino acids, UTE, and among all four dietary factors for serum zinc. Complex interactions of this nature often defy interpretation. This case was no exception; hence, no interpretation for the four-way interaction was attempted.

Liver components

Only main effects were observed for liver components. As expected, high dietary zinc elevated liver zinc. High dietary zinc slightly but significantly ($P = 0.043$) elevated liver copper as well. Rats consuming diets that contained L-cystine had significantly lower liver copper than those consuming diets with DL-methionine.

Kidney components

As expected, higher dietary zinc significantly ($P = 0.001$) elevated kidney zinc concentrations. Kidneys of rats consuming L-cystine had slightly but significantly higher amounts of zinc than those of rats consuming DL-methionine. The higher amount of copper in the diet resulted in a significant elevation of kidney copper over those with lower amounts of dietary copper. In addition, diets with UTE present caused a significantly higher amount of copper in the kidneys than diets without these elements. A complex interaction among zinc, copper, and UTE on kidney copper was observed. This showed that UTE elevated kidney copper in all groups except those with low dietary zinc and marginal copper, and those with high dietary zinc and normal copper.

Femur components

The only significant effect observed for femur was that high dietary zinc increased femur zinc concentration.

Intestinal mucosa components

Both zinc and MT concentrations were significantly elevated in rats consuming the high-zinc diets compared to those consuming normal-zinc diets. Both marginal dietary copper and the presence of L-cystine, significantly lowered the amount of copper in the intestinal mucosa. There was a highly significant ($P = 0.009$) interaction between dietary zinc and copper for intestinal copper concentrations, showing that when dietary zinc was high, intestinal copper was

Table 2 P values calculated by the analysis of variance for data presented in *Table 1*

	SOURCE														
	Z (Zn)	C (Cu)	M (Cys/Met)	U (UTE)	ZC	ZM	ZU	CM	CU	MU	ZCM	ZCU	ZMU	CMU	ZCMU
Serum															
Zinc	0.001	0.030				0.014								0.031	0.045
Copper								0.001							
CPAO1		0.004						0.036							
CPAO2								0.003							
Liver															
Zinc	0.001														
Copper	0.043		0.003												
Kidney															
Zinc	0.001		0.016												
Copper		0.036		0.035								0.044			
Intestinal mucosa															
Zinc	0.001														
Copper		0.041	0.041		0.009		0.027								
MT	0.001														
Femur															
Zinc	0.001														
Copper															

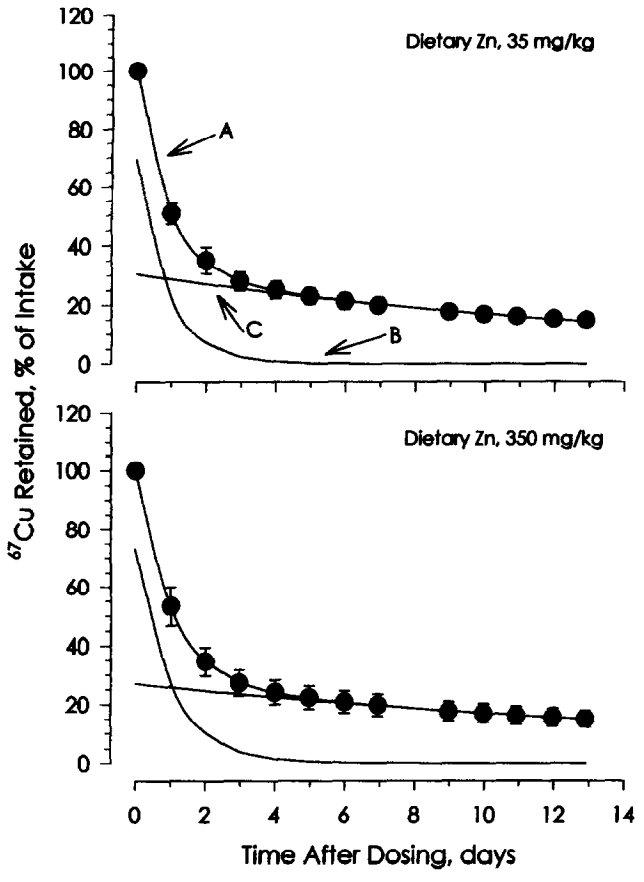

Figure 1 Whole body retention of ^{67}Cu in rats fed 35 or 350 mg of zinc/kg of diet for 4 weeks. The data are expressed as a percentage of the amount of ^{67}Cu in the body at zero time (means ± SEM of 7 replicates). The data were fitted to a double exponential equation, $f(t) = a_1e^{-b_1t} + a_2e^{-b_2t}$. The symbols represent the actual data and the lines represent the fitted equation (A), the first component of the equation (B), and the second component of the equation (C). Calculated constants are given in the results section of the text under the heading *Copper absorption.*

higher when 6 mg of copper/kg of diet were fed than when 3 mg/kg were fed. When dietary zinc was low, there was no difference. There was also a significant interaction between dietary zinc and UTE as regards intestinal copper. When dietary zinc was high, intestinal copper was higher when there was no UTE mix in the diet than when the UTE mix was present. When dietary zinc was low, there was no difference.

Copper absorption

Figure 1 shows a plot of the whole-body count data. The amount of ^{67}Cu remaining in the body each day was expressed as a percentage of the amount in the body at zero time. The symbols represent the actual data and line A represents the predicted values based on the mean constants derived by fitting the data for each animal to a double exponential equation, $f(t) = a_1e^{-b_1t} + a_2e^{-b_2t}$. Values (mean ± SEM) for rats receiving 35 mg of zinc/kg of diet (35Zn) were $a_1 = 70.0 \pm 2.9$; $b_1 = 1.12 \pm 0.10$; $a_2 = 29.7 \pm 2.8$; $b_2 = 0.059 \pm 0.003$, and for those receiving 350 mg of zinc/kg of diet (350Zn), the values were $a_1 = 74.8 \pm 4.2$; $b_1 = 1.07 \pm 0.16$; $a_2 = 25.6 \pm 4.2$; $b_2 = 0.044 \pm 0.007$. The apparent absorption for each group is predicted by the y intercept of the second component of the curve (C); 29.7 ± 2.8 and 25.6 ± 4.2 percent for 35Zn and 350Zn groups, respectively. The values are also listed in *Table 3* and the groups were found to be not significantly different ($P > 0.05$) from each other by Student's t test. The biological half-life (BHL) was calculated from the rate constant (b_2) of the second component of each curve. *Table 3* shows these values to be 12.1 ± 0.8 and 14.9 ± 1.6 days for the 35Zn and 350Zn, respectively. These values were not significantly different from each other.

Table 3 also shows the BHL calculated by another method. When unabsorbed copper had cleared the feces (approximately day 6), we determined the fractional loss of ^{67}Cu from the body through the urine and feces at days 6 and 7. The combined loss for the 35Zn group was 0.069 ± 0.006 and for the 350Zn group it was 0.061 ± 0.004/day. Using these rates, the BHL was calculated to be 10.5 ± 0.9 and 11.8 ± 0.9 days for the 35Zn and 350Zn groups, respectively. These values were not significantly different from each other, but they compare reasonably well with those derived from the curve fit analyses.

Fecal loss of ^{67}Cu

Figure 2 shows the cumulative loss of ^{67}Cu in the feces. Constants derived as a result of fitting the data to the equation $f(t) = a(1-e^{-bt})$ were $a = 71.8 \pm 2.0$; $b = 1.11 \pm 0.10$, for 35Zn and $a = 72.9 \pm 5.5$; $b = 1.01 \pm 0.14$, for 350Zn. These values were similar to those derived for the first component of the double exponential equation for ^{67}Cu loss from the body, thus confirming an earlier statement that this component represents mostly the fecal loss of the isotope. These calculated parameters were used to estimate that the total amount of ^{67}Cu lost in the feces at day 13 was approximately 73% of the copper dose. At this time, approximately 15% of the label remained in the body (*Fig. 1*), suggesting that about 12% had been lost by other means such as through excretion in the urine.

DISCUSSION

In the past, numerous studies have shown that copper status is affected when rats are fed a diet containing high zinc[4–6,20,21]. Recently, however, with the development of

Table 3 Apparent absorption (AA) and biological half-life (BHL) of ^{67}Cu are not affected by feeding high-zinc diets to adult rats for 4 weeks[1]

Dietary zinc, mg/kg	AA, %	BHL, days	
		Curve fit	Fecal/urine loss
35	29.7 ± 2.8	12.1 ± 0.8	10.5 ± 0.9
350	25.6 ± 4.2	14.9 ± 1.6	11.8 ± 0.9

[1]Values are means ± SEM of 7 replicates. ^{67}Cu retention data were fitted to a double exponential equation and the y intercept of the second component of the curve was used to estimate the AA. BHL was determined by two methods, 1) by dividing ln 2 by the rate constant of the second component of the equation (curve fit), and 2) by finding the rate of loss of copper from the body through the feces and urine, and then using this value similarly to compute the BHL.

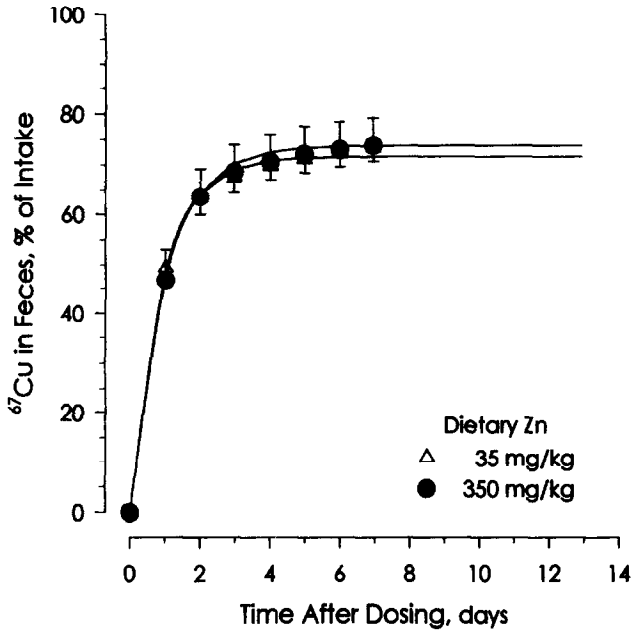

Figure 2 Cumulative loss of ^{67}Cu in the feces of rats fed 35 or 350 mg of zinc/kg of diet for 4 weeks. The data are expressed as a percentage of the amount in the body at zero time. The data were fitted to an exponential equation of the form, $f(t) = a(1-e^{-bt})$. The symbols represent the actual data and the lines represent the fitted equation for each dietary group. The points of each line are similar and the symbols overlap. Calculated constants are given in the results section of the text under the heading *Fecal loss of ^{67}Cu*. These calculated parameters were used to estimate the curve up to day 13 for each dietary zinc group.

the new AIN-93G diet, this effect seems to have been delayed, at least in studies conducted for a few weeks with adult male rats[7]. The magnitude of the zinc effect on copper status might depend on a number of factors including the amount of zinc and/or copper in the diet, the composition of the diet, sex of the animal (22), the age of the animal at the time the feeding trials begin, and the duration of the feeding trials.

The composition of the AIN-93G diet[8] is substantially different than other purified diets that have been shown to reduce copper status when they contain high concentrations of zinc. The present study was designed to determine which dietary factor(s) in the AIN-93G diet might be diminishing the high-zinc effect on copper status of adult male rats. L-cystine and UTE are components of the AIN-93G diet and both have been shown to affect copper status of rats and interact with other trace elements, respectively. Because one study showed that adding 5% L-cystine to a 10% casein diet enhanced the amount of copper in serum and liver of rats[23], we speculated that the substitution of L-cystine for DL-methionine in the AIN-93G diet might enhance copper absorption and overshadow the effects of high zinc. Other studies have shown, however, that the addition of L-cystine or L-cysteine to diets in excess of requirement tends to reduce, not enhance, copper absorption and status of chicks and rats[9,10,24–26]. Results of the present experiment showed that substituting L-cystine for DL-methionine at 0.3% of the diet had no major effect on copper status when adult male rats were fed high-zinc diets. This occurred even when the rats were fed only 3 mg of Cu/kg, about one-half the dietary requirement as recommended for rats by the NRC[27]. Overall, rats fed diets containing cystine had about 7% less copper in their livers and intestines than rats fed diets containing methionine.

The addition of the UTE mix was another possible mitigating factor that could have interacted with dietary copper and prevented the effects of high zinc. The total amount of UTE added to the diet was 8.2 mg/kg. However, of the UTE minerals, Si, Cr, F, Ni, B, Li, and V added to the diet, only nickel has been shown to reduce copper status, and this effect occurred only at very high dietary concentrations of nickel[28]. In the present study, removing the UTE mix from the diet did not significantly affect the overall copper status in rats fed high zinc. However, rats fed UTE had about 10% more copper in their kidney tissue than rats not fed UTE.

What other dietary factors could possibly account for no effect of excess dietary zinc on copper status? One of the major changes in the AIN-93G diet compared to AIN-76A was an 80% decrease in the amount of manganese. Although there are some nutritional interaction between manganese and copper, Johnson and Korynta[29] or Strause et al.[30] did not find a significant interaction between dietary manganese and copper that affected signs of copper status in rats. On the other hand, chronic exposure of rats to very high doses of manganese by injection caused an increase in bone copper[31]. It seems unlikely, therefore, that a decrease in dietary manganese could ameliorate the effect of high dietary zinc on copper status.

Other changes in the diet include the use of calcium carbonate as the source of calcium, soybean oil instead of corn oil, and the substitution of starch and a tetrasaccharide component for sucrose. Feeding sucrose or fructose as the source of carbohydrate has been shown to enhance the signs of copper deficiency[32–34]; however, rats fed diets containing only glucose as the carbohydrate source have been shown to respond to high zinc by exhibiting low copper status[3]. None of the other dietary changes mentioned has been associated with copper metabolism.

Although there was no effect of feeding high zinc on copper status, there were other interesting outcomes of this experiment. One in particular was the effect of UTE on weight gain. Although small, the weight gain in rats fed low dietary copper was depressed when UTE were left out of the diet, suggesting that one or more of the components of the UTE mix may be beneficial to the rat when dietary copper is low. It was also shown that leaving UTE out of the diet significantly depressed the concentration of copper in the kidney when compared to the effect of diets with UTE present. There was also an interaction between dietary UTE and zinc showing that when dietary zinc was high there was a depression of intestinal copper concentration in rats that did not receive UTE in their diets compared to those that did. There was a complex interaction involving copper, amino acids, and UTE relating to serum zinc, showing that when dietary copper was low and the rats were receiving L-cystine, the absence of dietary UTE caused a significant reduction in serum zinc. When copper was high and the rats were receiving DL-methionine, the absences of dietary UTE also caused a significant reduction in serum zinc. It is not known at this time which individual UTE or combinations could have caused these responses.

Conclusions

For the most part, results of the study showed that changes brought about by manipulating dietary L-cystine and DL-methionine, UTE, and copper were small and may not be physiologically relevant. Although numerous studies in the past have shown that feeding high-zinc diets to rats reduces their copper status, this phenomenon could not be demonstrated in the present study or others[7] by using the AIN-93G rodent diet. It was shown that changes in the diet, such as the substitution of L-cystine for DL-methionine and the use of an UTE mix, were not the cause. Another major difference between previous studies that showed an effect of zinc and this study that did not, is a difference in age of about 2 weeks. This difference in age may have been enough to impart resistance to copper deprivation under the conditions of this study. Overall, the results of this experiment indicate that the copper requirement of the adult rat fed the AIN-93G diet is much lower than that recommended for young, growing rats by the latest edition of the NRC Nutrient Requirements of Laboratory Animals[27].

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