Zinc, Iron, Copper, and Magnesium Concentrations in Tissues of Rats Fed Various Amounts of Zinc

Han K. Kang, Phillip W. Harvey, Jane L. Valentine, and Marian E. Swendseid

An experiment was conducted with rats to determine the effects on the tissue concentrations of Zn, Fe, Cu, and Mg of feeding various amounts of zinc. The rats were pair-fed one of the following diets for four weeks: Diet A, a zinc-deficient diet; a diet containing the recommended amount of zinc (diet A plus 55 μg of zinc per gram of diet), or diet A plus 550 μg zinc per gram of diet. Concentrations of these elements in various tissues were determined by atomic absorption spectrometry after wet digestion. Feeding the rats zinc-supplemented diets resulted in increased zinc in blood, heart, kidney, and liver, and a marked decrease of iron in kidney and liver. Concentrations of the other two elements were unchanged in all tissues. Thus, the effect of zinc in decreasing iron concentrations in liver, observed by several investigators when rats were fed toxic amounts of zinc, also occurs when zinc is administered in normal or subtoxic amounts.

Additional Keyphrases: trace metals • relation between Zn and Fe • ferritin

It has long been recognized that there is a partial biological antagonism between zinc and iron and between zinc and copper (1-3). Smith and Larson (1) found that feeding young rats excess zinc, from 7000 to 10,000 μg per gram of diet, caused anemia and subnormal growth. Three-fourths of these animals died within three to five weeks when they were fed 10,000 μg of zinc per gram of diet. Cox and Harris (2) reported that zinc toxicosis of rats resulted in an accumulation of zinc in the liver with an early and marked loss of hepatic iron. Settlemire and Matrone (3) showed that high zinc intake, 7500 μg of zinc per gram of diet, decreased both the amount of hepatic ferritin and the percentage of iron bound to ferritin. The question of whether there is a similar relationship between hepatic zinc and iron content after feeding much lower amounts of dietary zinc has not been investigated. The nutritional requirement of zinc for normal growth of weanling rats is reportedly 8 to 65 μg of zinc per gram of diet (4-6).

Our study was initiated to determine the effects of low, normal, and excess (nontoxic) amounts of dietary zinc on the content of zinc and other essential elements in rat tissues.

Materials and Methods

Weanling male Sprague-Dawley rats weighing 45-60 g were maintained individually in stainless-steel cages and given free access to distilled and de-ionized water in glass bottles. The rats were randomly divided into three groups. Group A received ad libitum a commercially prepared (Bio Serv., Inc., Frenchtown, N. J. 08825) zinc-deficient diet that contained, by analysis, 1.3 μg of zinc per gram. The diet is described by Bio-Serv. as containing the following (grams per kilogram of diet): dextrose, 631; cellulose, 30; spray dried egg white solids, 200; corn oil, 100; salt mix, 37; and vitamin mix.1 We did not attempt to measure the amount of phytate in the diet because, according to information obtained from Bio-Serv., no phytate was present. Group B received the same zinc-deficient diet, but supplemented with zinc carbonate equivalent to 55 μg of zinc per gram, and group C the same diet supplemented with zinc carbonate equivalent to 550 μg of zinc per gram. Rats in group B and group C received each day an amount of diet equal to the diet intake of their pair-mates in group A.

After four weeks, the rats were decapitated and blood

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1 Salt mix, mg/kg: CaCO₃, 9944; CaHPO₄, 3149; CoCl₂·6H₂O, 2; CuSO₄·5H₂O, 10; ferric citrate, 911; MgSO₄·7H₂O, 3381; MnSO₄·H₂O, 9; KI, 27; K₂HPO₄, 14 000; NaCl, 5552. Vitamin mix, mg/kg: biotin, 4; B₆, 20; calcium pantothenate, 16; choline chloride, 1500; chlorotetracline, 250; folic acid, 0.5; menadione, 0.3; niacin, 25; pyridoxine-HCl, 4; riboflavin, 6; thiamin-HCl, 10; vitamin A palmitate, 10 × 10⁶ int. units; vitamin D₃, 1.25 × 10⁶ int. units; vitamin E, 0.11 × 10⁶ int. units.
was sampled from the head wound. Heart, lung, spleen, liver, and kidney were excised, trimmed of connective tissue, dried by blotting with filter paper, and weighed. The tissues were then placed in polyethylene bags and kept in a freezer until the analysis.

The frozen tissues were placed in an Erlenmeyer flask for wet ashing. Glass beads, prewashed with nitric acid, and 20 ml of distilled and de-ionized water were added to the flask, followed by 10 ml of concentrated nitric acid and perchloric acid (equal volumes). The samples were initially heated very gently. After foaming subsided, the temperature was increased to produce steady boiling. If charring occurred, small amounts of nitric acid were added immediately. The excess acids were boiled off to near (not complete) dryness. The digests, when cooled to room temperature, were diluted to 5 ml with distilled and de-ionized water, then diluted to bring the concentration within the range of the standard.

Metal concentrations in the digest were determined by atomic absorption spectrometry with a Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with a three-slot burner head, a hollow-cathode lamp, and air and acetylene flame. The following wavelengths were used: zinc, 213.8; copper, 324.7; iron, 248.3; and magnesium 285.2 nm. Standard solutions were purchased from Hartman-Leddon Co., Philadelphia, Pa. 19143.

For comparison of means between different groups, Student’s t-test was used.

Table 1. Weight Gain and Food Efficiency Ratio of Rats Fed Zinc-Deficient and Zinc-Supplemented Diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt. g</th>
<th>Food efficiency ratio a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At beginning</td>
<td>At end of 4 wks</td>
</tr>
<tr>
<td>A (8)b</td>
<td>50.6 ± 5.6 c</td>
<td>76.8 ± 14.9</td>
</tr>
<tr>
<td>B (7)</td>
<td>50.6 ± 4.6</td>
<td>96.0 ± 16.8</td>
</tr>
<tr>
<td>C (6)</td>
<td>50.7 ± 5.1</td>
<td>97.0 ± 25.3</td>
</tr>
</tbody>
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a Grams of body weight gain per gram of food consumed.
b Figures in parentheses: no. of animals used in each dietary group.
c Values are mean ± 1 SD.

Results

Table 1 summarizes weight gain and the food efficiency ratio of rats fed various amounts of zinc. The rats that were receiving diet A (the zinc-deficient diet) were anorexic by the fifth day of feeding. Rats fed the zinc-deficient diet ad libitum had a mean daily diet intake of 6.8 g per rat. Our preliminary study showed that rats receiving the diet supplemented with zinc carbonate equivalent to 55 μg of zinc per gram consumed a mean of 12.8 g during the same period. The food efficiency ratio, defined as grams of body weight gain per gram of food consumed, was lowest in the zinc-deficient rats. The gross appearance of the zinc-deficient rats at the end of the feeding period was similar to observations of other investigators (7–9). Body hair was sparse and coarse, especially over the abdomen and hind legs. Rats fed diet B (diet A + 55 μg of zinc per gram of diet) and diet C (diet A + 550 μg of zinc per gram of diet) showed similar weight gains and food efficiency ratios. Table 2 shows the mean organ weights at the time the rats were killed. Mean hematocrit and standard deviation for groups A, B, and C were 45 ± 2, 49 ± 2, and 47 ± 4, respectively. Table 3 shows the content of Zn, Fe, Cu, and Mg in various tissues from the rats. The zinc content of the heart, kidney, liver, and blood was lower in the zinc-deficient rats than in the zinc-supplemented rats.

Copper and magnesium content of all tissues showed no significant alteration. The magnesium content of blood from zinc-deficient rats was lower than that of the rats fed 550 μg of zinc per gram of diet. Essential-element concentrations of lung and spleen tissue were the same in all of the dietary groups, hence they are not shown in Table 3.

The relationship of zinc and iron content in the liver was evaluated by the test for independence method described by Dixon and Massey (10). The overall correlation coefficient (r) was −.485, significant at the 0.05 level.

Discussion

As mentioned, typical symptoms of zinc deficiency were observed in the rats of group A. Although the three
groups of rats were pair-fed, the rats fed zinc-supplemented diets showed greater weight gain. Rats in group B and group C showed similar weight gains and food efficiency ratios, which suggests that 550 μg of zinc per gram of diet was not toxic for them.

Macapinlac et al. (9) found a significant increase in hematocrit values in their zinc-deficient rats. We did not; our hematocrit values were within the normal range (11). A previous study by Settlémire and Matrone (3) suggests that excess dietary zinc may somehow limit the absorption of iron from the digestive tract. Conversely, Cox and Harris (2) indicate that excess zinc does not interfere with iron absorption. If concentrations of essential elements in the blood reflect absorption from the digestive tract, we are in agreement with the findings of Cox and Harris. The values for concentrations of zinc, copper, iron, and magnesium in the tissues of rats fed zinc-supplemented diets agree with those of control rats in other studies (2, 3, 5).

The zinc content in liver and kidney increased as the dietary zinc was increased, but the iron content in these organs decreased. Toxic levels of dietary zinc have been reported to result in decreased concentrations of iron in liver and kidney (2). Thus, an increase in zinc and a decrease in iron content evidently occurs not only with toxic levels of dietary zinc, as reported previously, but also when zinc is present in the diet in normal or subtoxic levels. Prasad et al. (5) found a similar relationship between zinc and iron in testes of rats fed zinc-deficient (10 μg of zinc per gram of diet) and zinc-supplemented diets (65 μg of zinc per gram of diet). The relatively high amounts of zinc in their zinc-deficient diet (10 μg zinc vs. 1.3 μg zinc per gram of diet in the present study) may explain their failure to observe similar results in other tissues. Their study failed to demonstrate any change in zinc content in the liver. Addition of phytate (to contain 10 g of phytate per kilogram of diet) to both the zinc-deficient diet and the control diet in this study may have also contributed to the different results. Chen et al. (12) have reported that the increase in liver zinc observed after feeding the rats diets supplemented with zinc acetate equivalent to either 1000 μg of zinc per gram of diet or 2000 μg of zinc per gram of diet was associated with increased amounts of zinc appearing in the liver soluble fraction. This increased zinc content in the soluble fraction was mainly attributable to an increase in the metallothionein fraction.

Settlémire and Matrone (3) have proposed a possible mechanism by which a high zinc intake could decrease hepatic iron concentration. They contend that the decreased tissue iron results from inhibition of iron incorporation into ferritin, or its increased release from ferritin, or both, rather than an increased mobilization of ferritin iron. They found that 94% of the decrease in hepatic iron in the rats fed the high zinc diet could be accounted for by decrease in the ferritin fraction.

Hepatic copper content was not altered by the amounts of dietary zinc we used. Cox and Harris (2) reported that excess zinc in the diet causes an early and marked loss of hepatic iron as well as a significant decrease in hepatic copper. This phenomenon occurs four weeks after the loss of hepatic iron. Their rats also developed an anemia. In the present study zinc-supplemented rats had normal hematocrit values and there was no significant decrease in hepatic copper. This may be attributed to subtoxic amounts of dietary zinc or that we did not allow enough time for such a reduction to become evident.
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References