Ultrastructural Changes in the Intestine of Rats Fed High-Zinc Diets

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The feeding of high-zinc diets to humans is often used as therapy for patients with Wilson’s disease, an autosomal recessive disorder of copper accumulation. There seem to be no outward adverse effects of this treatment; however, preliminary studies in our laboratory have shown apparent weaknesses in the intestinal wall of rats fed high-zinc diets. As a consequence, this study was carried out to determine if feeding high-zinc diets to rats would affect the ultrastructural morphology of the small intestine. The effects of treatment on copper status of the rats also were determined. Weanling male rats were fed diets containing either 35 or 350 mg of zinc/kg. After 7 weeks, blood and various tissues were collected to measure copper status indicators, and portions of the upper duodenum were excised and prepared for light and electron microscopy. Results showed that rats fed high-zinc diets had significantly lower copper status as indicated by low serum copper, serum ceruloplasmin activity, and liver copper, than rats fed normal-zinc diets. Liver superoxide dismutase or cytochrome c oxidase activities were not affected by high zinc. Observations of sections of the duodenum by electron microscopy showed that non-assembled collagen molecules of the lamina propria were more often disorganized and formed tangled masses in rats fed the high-zinc diet than in those fed normal-zinc diets. This suggests that low copper status caused by high-zinc feeding might be affecting the activity of lysyl oxidase, a copper-dependent enzyme, and thus crosslinking of the collagen molecules. However, these observations did not always correlate with low copper status. Other possible explanations include a direct competition between zinc and copper for sites on lysyl oxidase, zinc blocking of aldehyde residues on the collagen molecule, or some unrecognized process involving other enzymes or other aspects of collagen assembly. Whether such processes or affinities actually exist is still under investigation. J. Trace Elem. Exp. Med. 10:37–46, 1997. © 1997 Wiley-Liss, Inc.

Key words: zinc; copper; intestine; collagen; ultrastructure; electron microscopy; rats

INTRODUCTION

The intake of diets containing high concentrations of zinc over a long period will interfere with copper metabolism in animals and humans. The magnitude of this effect

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depends on a number of factors including the amount of zinc and/or copper in the diet and the duration of the feeding trial. Humans seem to be more sensitive than animals. Studies have shown that intakes of as little as 25 to 50 mg of zinc per day for 2 to 10 weeks in adult humans [1–4] will lower copper status, while much higher zinc intakes in adult rats, relative to body weight, are not effective [5,6].

For the past 15 years, oral zinc therapy has been used successfully to treat Wilson’s disease, an autosomal recessive disorder of copper accumulation [7]. It is believed that excess zinc blocks copper absorption at the intestinal mucosa and eventually helps lower the overall body burden of copper. Treatments with 50 mg of zinc as zinc acetate three times a day for years have had no discernable adverse effects on the patients. However, in studies with laboratory animals, we have noticed that during the removal of the intestinal mucosa from rats fed high-zinc diets, the structure of the intestinal wall seems weak. For example, the intestines from these rats tear more easily than those from rats fed normal-zinc diets. Therefore, the main objective of this study was to determine if morphological changes in the intestines relevant to tissue fragility could be detected by light and electron microscopy in rats fed high-zinc diets for an extended period.

During our previous studies in this area, we found that the type of diet seemed to alter the effects of zinc on copper status of adult rats. The use of one type of diet reduced copper status when high zinc was fed [8,9], and the use of another type did not [6]. Thus, a second objective of this study was to determine if diet type would alter the effects of high dietary zinc on copper status in young rats.

MATERIALS AND METHODS

Animals and Diets

The Animal Use Committee of the USDA, ARS, Grand Forks Human Nutrition Research Center approved this study, and it was performed according to the guidelines on the experimental use of laboratory animals issued by the National Institutes of Health [10].

Forty male Sprague-Dawley rats (Sasco, Inc., Omaha, NE), 3 weeks of age, were housed individually in hanging stainless-steel cages in a temperature/humidity controlled room with a fixed light–dark cycle of 12 h. Upon arrival at the laboratory, the rats were fed laboratory rat chow for 3 days to acclimate them to their surroundings. Then they were randomly divided into four groups of ten rats each and fed one of two purified diet preparations containing either 35 or 350 mg of zinc/kg. Both diets contained 4 mg of copper/kg of diet. The Zn:Cu molar ratios in the two diets were 8.5:1 and 85:1, respectively. Compositions of the diets are listed in Table I. The rats had free access to deionized water. Body weights were recorded weekly.

Tissue Collection and Analysis

After 7 weeks of consuming their respective diets, each rat was anesthetized with 50 mg of pentobarbital sodium/100 g body weight before collecting blood from the abdominal aorta. The blood was allowed to clot at room temperature for 16 min and then placed on ice. After centrifugation of the blood, serum was collected and analyzed immediately for ceruloplasmin amine oxidase (CPAO) activity [11], and for copper and zinc content [9]. Livers were removed and immediately frozen and stored
TABLE I. Composition of Diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Modified AIN-93G (g/kg)</th>
<th>PR-183 (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch&lt;sup&gt;a&lt;/sup&gt;</td>
<td>397.486</td>
<td>300.00</td>
</tr>
<tr>
<td>Glucose&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>313.00</td>
</tr>
<tr>
<td>Dextrinized starch&lt;sup&gt;c&lt;/sup&gt;</td>
<td>132.000</td>
<td>—</td>
</tr>
<tr>
<td>Casein&lt;sup&gt;d&lt;/sup&gt;</td>
<td>200.000</td>
<td>150.00</td>
</tr>
<tr>
<td>Sucrose&lt;sup&gt;e&lt;/sup&gt;</td>
<td>100.000</td>
<td>—</td>
</tr>
<tr>
<td>Egg white&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td>50.00</td>
</tr>
<tr>
<td>Soybean oil&lt;sup&gt;g&lt;/sup&gt;</td>
<td>70.000</td>
<td>100.00</td>
</tr>
<tr>
<td>Cellulose&lt;sup&gt;h&lt;/sup&gt;</td>
<td>50.000</td>
<td>30.00</td>
</tr>
<tr>
<td>Mineral mix&lt;sup&gt;i&lt;/sup&gt;</td>
<td>35.000&lt;sup&gt;i&lt;/sup&gt;</td>
<td>35.00&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;j&lt;/sup&gt;</td>
<td>10.000&lt;sup&gt;k&lt;/sup&gt;</td>
<td>10.00&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biotin premix&lt;sup&gt;m&lt;/sup&gt;</td>
<td>—</td>
<td>10.00</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.000</td>
<td>—</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.500</td>
<td>2.00</td>
</tr>
<tr>
<td>Tert-butylhydroquinone (TBHQ)</td>
<td>0.014</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup>Argo, CPC International, Englewood Cliffs, NJ.
<sup>b</sup>ICN Biochemicals, Cleveland, OH.
<sup>c</sup>Dyetrose, Dyets Inc., Bethlehem, PA.
<sup>d</sup>High protein, Teklad, Madison, WI.
<sup>e</sup>Crystal Sugar Co., Moorehead, MN.
<sup>f</sup>Teklad, Madison, WI.
<sup>g</sup>Crisco Oil, Procter & Gamble, Cincinnati, OH.
<sup>h</sup>Teklad, Madison, WI.
<sup>i</sup>Similar to AIN-93-MX [26] except the CuCO₃ concentration was lowered to 0.15 g/kg of mix.
<sup>j</sup>Contains in g/kg of mix: calcium phosphate (dibasic), 500; sodium chloride, 74; potassium citrate (monohydrate), 220; potassium sulfate, 52; magnesium oxide, 24; manganese 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. For the high-zinc diet, a premix of zinc carbonate and glucose was prepared so that when mixed at 1% of the diet, 325 mg of zinc/kg of diet would be obtained.
<sup>k</sup>Similar to AIN-93-VX [26].
<sup>l</sup>AIN-76 Vitamin mix, Teklad, Madison, WI.
<sup>m</sup>80 mg d-biotin/kg in powdered sucrose.
<sup>n</sup>Sulfur amino acids supplied by egg white solids.

at −80°C until analyzed for copper and zinc content [9] and for Cu, Zn-superoxide dismutase (Cu/Zn-SOD) [12] and cytochrome c oxidase (CCO) [13,14] activities. Twenty centimeters of the upper intestine, beginning at 0.5 cm from the pylorus, was excised, and the mucosal lining was scraped off with a glass slide. The scrapings were immediately frozen at −80°C, and within 1 week they were analyzed for their concentrations of metallothionein (MT) [15], zinc, and copper.

**Preparation of the Intestine for Light and Electron Microscopy**

A segment of the proximal duodenum (approximately 1 cm in length) was excised, by using scissors, just distal to the pylorus. The tissue was immediately immersed in fixative consisting of 2% glutaraldehyde (Tousimis Res. Corp., Rockville, MD) and 0.05% CaCl₂ (Sigma Chemical Co., St. Louis, MO) in 0.1 moles of sodium cacodylate/L of buffer (Electron Microscopy Science, Fort Washington, PA) at pH 7.2. After 2–3 min in the fixative, a fresh transverse cut was made with a scalpel at each
end of the tissue sample to remove contracted and potentially crushed regions. After another 0.5 min in the fixative, the piece was split longitudinally, and the luminal contents were gently removed. Subsequently, these halves were again divided along the longitudinal axis, which resulted in four to six longitudinal strips (5–7 mm long) from each original segment. These were stored overnight in the fixative at 4°C. After the samples were washed in 0.1 M cacodylate buffer with 0.05% CaCl₂ and 0.2 M sucrose at pH 7.2 (three changes, 20 min each), they were treated with 1% osmium tetroxide (Tousimis Res. Corp., Rockville, MD) in the above buffer without CaCl₂. The samples were then washed in buffer as before and dehydrated with a series of graded ethanol concentrations (30, 50, 70, 85, 95% for 10 min each, and 100%, three times for 10 min each). This was followed by two 5-min rinses in propylene oxide (PolySciences, Inc., Warrington, PA) and overnight infiltration with a 1:1 mixture of propylene oxide and EMbed-812® epoxy resin (Electron Microscopy Science, Fort Washington, PA) in tightly closed containers. The propylene oxide-resin mixture was replaced with a fresh resin and allowed to sit for 4 h at room temperature in open vials. The samples were flat-embedded in fresh resin at 60°C for approximately 18 h. Thick (1–2 μm) and thin (70–80 nm) sections were cut transversely to the longitudinal axis of the gut, by using glass and diamond knives (RMC Inc., Tucson, AZ), respectively, on an LKB Ultratome® V (LKB Produkter AB, Bromma, Sweden). Thick sections were stained for light microscopy with a mixture of 0.25% azure-IIIB (Sigma Chemical Co., St. Louis, MO) and 0.25% methylene blue in 0.25% sodium borate at 70°C for 2 min. Thin sections for electron microscopy were mounted on 200 mesh nickel grids (Ted Pella Inc., Redding, CA), stained with saturated aqueous uranyl acetate (Mallinckrodt, St. Louis, MO) for 40 min and lead citrate (Ladd Res. Industries Inc., Burlington, VT) for 3 min, according to the methods of Venable and Coggeshall [16], and viewed with a Philips EM 300 operated at 60 kV accelerating voltage (Philips Electronic Instruments Co., Schaumberg, IL).

Statistical Methods

Data for tissue minerals and enzyme activities were analyzed by analysis of variance for a 2 × 2 factorial design. Significant differences between means were accepted when \( P \leq .05 \).

RESULTS AND DISCUSSION

Copper Status

Table II shows the results of body weight gain and some blood parameters of rats after 7 weeks of consuming their respective diets. The type of diet or dietary zinc concentration had no effect on gain in body weight. However, significant effects of both diet and dietary zinc on the percent hematocrit were found. Rats fed the modified AIN-93G formula had a significantly lower (about 2%; \( P < .04 \)) hematocrit than those fed the PR-183 formula. Whether this is physiologically significant is debatable. Hematocrits of rats fed high dietary zinc were significantly lower (about 3%; \( P < .003 \)) than in rats fed normal zinc. The consumption of the high-zinc diets caused a significant reduction in both serum copper and CPAO activity. This suggests that the copper status of these rats was compromised. Serum zinc concentrations were about 12% higher (\( P < .001 \)) in the rats fed the AIN-93G formula, compared with the other
TABLE II. Effects of Diet Composition and Zinc Concentration on Weight Gain and Hematocrit, and on the Concentration of Serum Zinc and Copper, and Ceruloplasmin Activity of Rats*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Diet Zn (mg/kg)</th>
<th>Weight gain (g/d)</th>
<th>Hematocrit (%)</th>
<th>Serum zinc (μmol/L)</th>
<th>Copper (μmol/L)</th>
<th>Ceruloplasmin activity (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-93G</td>
<td>35</td>
<td>5.2 ± 0.2</td>
<td>45.4 ± 0.6</td>
<td>24.6 ± 0.6</td>
<td>14.4 ± 0.6</td>
<td>74.5 ± 2.7</td>
</tr>
<tr>
<td>AIN-93G</td>
<td>350</td>
<td>5.2 ± 0.2</td>
<td>43.5 ± 0.5</td>
<td>35.0 ± 1.7</td>
<td>6.6 ± 1.3</td>
<td>37.8 ± 5.0</td>
</tr>
<tr>
<td>PR-183</td>
<td>35</td>
<td>5.2 ± 0.2</td>
<td>46.2 ± 0.4</td>
<td>22.1 ± 0.7</td>
<td>15.0 ± 0.5</td>
<td>79.6 ± 3.3</td>
</tr>
<tr>
<td>PR-183</td>
<td>350</td>
<td>5.2 ± 0.1</td>
<td>44.8 ± 0.3</td>
<td>30.2 ± 1.7</td>
<td>8.1 ± 1.6</td>
<td>35.2 ± 9.2</td>
</tr>
</tbody>
</table>

ANOVA Table:
Source of error
| Diet (P value) | NS          | .039       | NS          | NS          | NS          | NS          |
| Zinc (P value) | NS          | .002       | <.001       | <.001       | <.001       | NS          |
| Diet × zinc (P value) | NS          | NS         | NS          | NS          | NS          | NS          |

*Values are means ± SEM for ten replicates per group.

formula. High dietary zinc also caused a significant (P < .001) elevation in serum zinc.

Although there was a significantly (P < .001) lower concentration of copper in the livers of rats fed high zinc, this was not reflected by a change in the activities of two copper-dependent enzymes, SOD and CCO (Table III). Feeding high dietary zinc did not change the zinc concentration in the liver.

Zinc in the intestinal mucosa was a reflection of the amount in the diet (Table IV). As dietary zinc increased, zinc in the mucosa increased. As expected, intestinal MT concentration was a manifestation of zinc concentration in the mucosa. However, the copper concentration in the intestinal mucosa was significantly (P < .01) lower in rats fed the high-zinc diet than in those fed normal zinc. This suggests that elevated mucosal MT is not associated with copper binding and subsequent accumulation in the mucosal cells, at least in the presence of high zinc concentrations. Zinc binds to

TABLE III. Effects of Diet Composition and Dietary Zinc Concentration on Zinc and Copper Concentrations, and Cu/Zn-SOD and CCO Activity in Liver of Rats*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Diet Zn (mg/kg)</th>
<th>Zinc (μmol/kg)</th>
<th>Copper (μmol/kg)</th>
<th>Cu/Zn-SOD activity (μkat/mg protein)</th>
<th>CCO activity (μkat/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-93G</td>
<td>35</td>
<td>386 ± 9</td>
<td>54.7 ± 0.9</td>
<td>2.51 ± 0.20</td>
<td>9.60 ± 0.66</td>
</tr>
<tr>
<td>AIN-93G</td>
<td>350</td>
<td>402 ± 18</td>
<td>43.4 ± 3.6</td>
<td>1.85 ± 0.38</td>
<td>8.96 ± 0.64</td>
</tr>
<tr>
<td>PR-183</td>
<td>35</td>
<td>395 ± 12</td>
<td>59.7 ± 2.8</td>
<td>2.37 ± 0.23</td>
<td>9.35 ± 0.83</td>
</tr>
<tr>
<td>PR-183</td>
<td>350</td>
<td>401 ± 10</td>
<td>44.7 ± 2.1</td>
<td>2.38 ± 0.42</td>
<td>8.70 ± 0.44</td>
</tr>
</tbody>
</table>

ANOVA Table:
Source of error
| Diet (P value) | NS        | NS          | NS          | NS          |
| Zinc (P value) | NS        | <.001       | NS          | NS          |
| Diet × zinc (P value) | NS        | NS          | NS          | NS          |

*Values are means ± SEM for ten replicates per group.
TABLE IV. Effects of Diet Composition and Dietary Zinc Concentration on Zinc, Metallothionein (MT), and Copper Concentrations in the Intestinal Mucosa of Rats*

<table>
<thead>
<tr>
<th>Diet</th>
<th>Diet Zn (mg/kg)</th>
<th>Intestinal mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zinc (μmol/kg)</td>
</tr>
<tr>
<td>AIN-93G</td>
<td>35</td>
<td>264 ± 10</td>
</tr>
<tr>
<td>AIN-93G</td>
<td>350</td>
<td>465 ± 37</td>
</tr>
<tr>
<td>PR-183</td>
<td>35</td>
<td>260 ± 11</td>
</tr>
<tr>
<td>PR-183</td>
<td>350</td>
<td>509 ± 38</td>
</tr>
</tbody>
</table>

ANOVA table:
Source of error
Diet (P value) NS NS NS
Zinc (P value) <.001 <.001 <.01
Diet × zinc (P value) NS NS NS

*Values are means ± SEM for 10 replicates per group.

MT in a 7:1 molar ratio. The ratio of elevated zinc to the amount of induced MT was calculated to be about 14:1. This suggests that mucosal MT was saturated with zinc and that about half of total mucosal zinc was free and/or bound to other ligands.

Structural Changes in the Intestine

Several aspects of the small intestine were examined with both light and electron microscopy in an effort to detect any morphological and ultrastructural changes resulting from dietary treatment. Overall, no changes in the gross organization of the intestine and no differences in cell–cell associations were seen among any of the treatment groups. The apical junctional complexes of the mucosal epithelium were examined with regard to tight junction (terminal bar) regions, the zonula adherens attachments, and associated desmosomes. None of the diet groups varied in these features. Changes in microvilli also were indistinguishable among the treatment groups. Because the high-zinc diets depressed blood and tissue copper concentrations, the possibility was considered that mitochondrial function and energy states might be

Fig. 1. The collagen of the lamina propria is shown in light (a,b) and transmission electron micrographs (c–g). a,b: These sections show portions of the small intestinal wall from rats fed a diet with normal zinc concentrations (a) and of those fed a high-zinc diet (b). The mucosa (m), lamina propria (lp), and smooth muscle fibers (cm) are labeled; collagen bundles (*') and fibroblasts (arrowheads) are indicated. c: This electron micrograph shows a section through collagen bundles of the lamina propria of an animal fed a normal-zinc diet. The individual collagen fibrils (arrows) are cut at various angles but appear well organized. d: Large areas of less-well-organized fibrils associated with finer fibrillar or flocculent material are common in the lamina propria of rats fed high-zinc diets. The collagen fibrils (arrows) are indicated. This micrograph is from a rat fed a high-zinc diet and demonstrating depressed copper status. e: In a higher-magnification view of the collagen of a rat from a normal-zinc diet, the newly secreted collagen molecules (arrowheads) are organized roughly parallel to each other and also in relation to the collagen fibrils (arrows). f: In animals fed a high-zinc diet the newly secreted collagen (arrowheads) often forms tangled masses with little apparent organization. The collagen fibrils (arrows) are indicated. This animal also demonstrated depressed copper status. g: A considerable degree of disorganization of collagen molecules (arrowheads) and collagen (arrows) is seen in rats fed high-zinc diets that did not experience a depressed copper status. This example is from a rat exhibiting normal copper status after having been maintained on a high-zinc diet throughout the experiment.
affected. The predicted effect would be a shift in the mitochondrial population from the orthodox configuration to a more condensed configuration, with the impediment of the electron transport system. This type of mitochondrial change was demonstrated in platelets from copper-deficient rats, and it was associated with depressed CCO activity [17]. Such an effect was not seen in these intestinal tissues, however. Although mucosal CCO was not measured, the fact that liver CCO activity was not affected (Table III) suggests that copper concentrations were not sufficiently reduced to alter this aspect of mitochondrial function and ultrastructure. While mitochondria apparently had some differences in configuration among the different cell types of the gut, there were no mitochondrial changes in any tissues relatable to dietary treatment.

As a result of observations of increased tissue fragility in the intestines of rats fed high-zinc diets, the collagen components of the intestine were examined. The lamina propria (Fig. 1a) is composed almost entirely of heavy collagen deposits and fibroblasts. This region has the most extensive and highly organized collagen bundles in the intestine. Light microscopic examination of tissue sections from the various diet groups revealed no differences in the overall organization of this region. The density and packing of collagen fibrils and the number and distribution of fibroblasts were not affected by high-zinc diets (Fig. 1a, b). Observations of collagen organization were made in the more central aspects of this layer (in the mucosa-serosa orientation), by using the electron microscope. Also, attempts were made to avoid sampling areas at the surface of fibroblasts where newly secreted collagen molecules might present an erroneous lack of organization. At the ultrastructural level, animals from all diet groups exhibited localized areas of individual, non-assembled collagen molecules between the well-organized collagen fibers of the lamina propria, with some variation in organization. In the lamina propria of rats fed the high-zinc diet, the regions of non-assembled collagen were more extensive and typically showed considerable disorganization (Fig. 1c, d). In animals fed normal-zinc diets, the collagen molecules were in loose parallel orientation with each other (Fig. 1e) while those of the animals fed high-zinc diets often formed tangled masses crossing each other at various angles (Fig. 1f).

While this degree of disorganization may seem slight, within the gross organization of the lamina propria, it is likely a reflection of the defect underlying the tissue fragility observed during the manipulation of intestinal samples from rats fed the high-zinc diets. It seems that newly secreted collagen molecules form tangled masses or diffuse unoriented associations at a point in time when they would normally be assembled into long fibrils forming parallel alignments. These associations are guided by the interaction between strongly reactive aldehyde groups near the ends of the collagen molecules. These reactive sites, responsible for the initial organization of the collagen fibril, are generated by the deamination of lysine and hydroxylysine residues of the triple-stranded collagen molecule. This reaction is dependent upon the extracellular enzyme lysyl oxidase, a copper-requiring enzyme [18–22]. This fact leads us to the simple explanation that the tissue fragility is caused by a decreased copper availability as a result of high concentration of zinc in the diet. However, not all animals fed the high-zinc diet showed a pronounced drop in copper concentrations. Some showed only moderate decreases, and some had serum and intestinal copper concentrations equivalent to those fed the normal-zinc diets.

Although copper concentrations were essentially normal in some animals fed the
high-zinc diet, defects in collagen organization were still apparent in the intestine (Fig. 1g). This suggests that elevated dietary and, consequently, tissue zinc concentrations, may be acting to perturb collagen organization other than through the depression of copper concentrations in the tissue. This effect could be through some unrecognized process involving other enzymes or other aspects of collagen assembly. Or, it could be as straightforward as a direct competition by zinc for copper sites on lysyl oxidase, as suggested by the work of Chvapil and Misiorowski [23] and Iguchi and Sano [24]. In addition, there may be a blocking of aldehyde residues on the collagen molecule by zinc, as discussed by Bornstein [25]. Whether such suggested processes or affinities actually exist is still under investigation.

This study suggests that long-term feeding of diets with high Zn:Cu ratios might begin to disrupt the intestinal mucosa. The highest dietary molar ratio in this study was 85:1. It is unlikely that this ratio would be reached in normal humans diets; however, in the treatment of Wilson disease, as much as 150 mg of zinc per day is given orally [7]. With a normal dietary copper intake of 1.5 mg/day, the Zn:Cu ratio could reach 95:1, and over a long period of treatment, some adverse effects on the intestinal mucosa might occur.

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