Effect of zinc intake on copper excretion and retention in men

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ABSTRACT To determine the influence of zinc intake on copper excretion and retention, nine men consumed diets containing 2.6 mg of copper/day and 1.8, 4.0, 6.0, 8.0, 18.5 or 20.7 mg of zinc/day for one- or two-week periods in a 63-day study. Copper and zinc in the diet and copper in plasma were determined weekly; fecal copper was determined daily and averaged within each week. The weekly mean (±SEM) plasma copper concentrations (81 ± 3.3 to 100 ± 5.8 µg/dl) remained within the normal range throughout the study. Fecal copper and apparent copper retention were influenced by the level of dietary zinc and the duration it was fed. When 18.5 mg of zinc/day was fed for two consecutive weeks following a lower zinc intake, fecal copper was elevated and apparent copper retention was reduced after a one-week lag. Thus, an intake of zinc only 3.5 mg/day above the RDA for men reduced apparent retention of copper at an intake of 2.6 mg/day.

KEY WORDS Copper, zinc, plasma copper, fecal copper, copper retention, human subjects

Introduction

Recent research (1-7) indicates that the copper content of US diets may supply less than the safe and adequate intake of 2 to 3 mg/day suggested by the Food and Nutrition Board (8). Estimation of dietary copper alone is insufficient to ascertain the adequacy of copper intake. Trace mineral interactions, as well as other dietary factors, affect mineral availability and utilization (9). High levels of dietary zinc reduce copper absorption and alter tissue distribution of copper in rats (10-15). The same antagonistic effect of high zinc supplementation on copper levels in humans in certain disease states has been reported (16, 17).

Several studies in healthy humans have produced conflicting evidence as to the effects of dietary zinc on copper utilization. Greger et al (18, 19) reported differing results from two metabolic studies in which the effect of dietary zinc on apparent copper retention was examined. No differences in fecal copper excretion or copper retention were found when 7.4 or 13.4 mg of zinc and 2.9 mg of copper/day were fed to 14 adolescent girls for 18 days (18), and all subjects were in positive copper balance. In another study in which 11 adolescent girls received 1.2 mg of dietary copper daily (19), significantly greater fecal copper excretion and reduced copper retention occurred when the girls were fed 14.7 compared to 11.5 mg of zinc/day during 10-day periods; however, all subjects remained in apparent positive copper balance. Burke et al (20) studied 11 elderly adults fed...
of copper in these adults, the mean balance for each of the four groups would become slightly negative. These investigators (22) affirmed their earlier suggestion (23) that an intake of 2 mg of copper/day may be marginally sufficient to maintain copper equilibrium in adult women.

In view of the conflicting data from these studies, the purpose of the present experiment was to examine the effect of varying the zinc intake (1.8, 4.0, 6.0, 8.0, 18.5 and 20.7 mg/day) on copper utilization in young men receiving a controlled diet containing a constant copper intake (2.6 mg/day) within the range recommended for adults (8).

Methods

Subjects

Nine healthy male students between 21 and 27 years of age served as subjects and are described in Table 1. Each gave written consent to participate prior to beginning the study. The study protocol was approved by the University of Missouri Committee to Review Research Involving Human Subjects. The subjects maintained their normal daily routines and consumed three meals daily in the metabolic unit.

Experimental design

The 63-day metabolic study was divided into nine weeks (Table 2) and was part of a larger study designed to measure zinc bioavailability (24). Zinc intake was varied by week and subject groups. To help establish similar zinc status among the 9 men prior to feeding amounts below the RDA, all subjects ingested 20.7 mg of zinc/day during week 1 and 18.5 mg of zinc/day during weeks 3, 5, 6, 8 and 9. In week 2 all subjects consumed 1.8 mg of zinc/day. During week 4 the

<table>
<thead>
<tr>
<th>Week</th>
<th>Dietary zinc intake</th>
<th>No of subjects</th>
<th>Plasma copper</th>
<th>Fecal copper</th>
<th>Copper balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.7</td>
<td>9</td>
<td>95 ± 3.3**</td>
<td>1.88 ± 0.07*</td>
<td>0.75 ± 0.07*</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>9</td>
<td>100 ± 5.8*</td>
<td>1.94 ± 0.12*</td>
<td>0.69 ± 0.12**</td>
</tr>
<tr>
<td>3</td>
<td>18.5</td>
<td>9</td>
<td>98 ± 7.1*</td>
<td>2.30 ± 0.07*</td>
<td>0.32 ± 0.07*</td>
</tr>
<tr>
<td>4</td>
<td>18.5</td>
<td>5, 4</td>
<td>99 ± 7.4*</td>
<td>1.95 ± 0.08*</td>
<td>0.67 ± 0.06*</td>
</tr>
<tr>
<td>5</td>
<td>18.5</td>
<td>9</td>
<td>88 ± 5.3**</td>
<td>1.97 ± 0.09*</td>
<td>0.66 ± 0.10*</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>9</td>
<td>86 ± 4.2**</td>
<td>2.84 ± 0.13*</td>
<td>-0.21 ± 0.13*</td>
</tr>
<tr>
<td>7</td>
<td>4.0, 6.0, 8.0</td>
<td>3, 3, 3</td>
<td>84 ± 5.3**</td>
<td>2.48 ± 0.11*</td>
<td>0.14 ± 0.11*</td>
</tr>
<tr>
<td>8</td>
<td>18.5</td>
<td>9</td>
<td>81 ± 3.3**</td>
<td>1.86 ± 0.09*</td>
<td>0.77 ± 0.09*</td>
</tr>
<tr>
<td>9</td>
<td>18.5</td>
<td>9</td>
<td>94 ± 6.0**</td>
<td>2.40 ± 0.09*</td>
<td>0.23 ± 0.09*</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± SEM.
† Copper balance = copper intake - fecal copper.
‡ Means with common letters are not significantly different at p ≤ 0.05.
subjects were divided into two subgroups; five subjects consumed 1.8 mg and four subjects consumed 8.0 mg of zinc/day. In week 7, the subjects were divided equally into three subgroups and fed 4, 6, or 8 mg of zinc/day.

Diet

The diet consisted of three meals daily. The menu remained constant each day (Table 3). The basal egg-white diet provided all essential nutrients at levels recommended by the Food and Nutrition Board (8) with the exception of zinc and protein. The basal diet provided 1.8 mg of zinc/day and 16.4 g of nitrogen/day, as determined by analysis. This basal diet was supplemented with zinc carbonate to provide the higher quantities of dietary zinc. The mean analyzed dietary copper intake was 2.63 mg/day for all metabolic periods; 2.5 mg of copper was provided as copper sulfate, and the remaining 0.13 mg was supplied by foods in the basal diet. The energy content of each subject’s diet was adjusted in an attempt to maintain weight, yet the subjects gained a mean of 2 kg during the study (Table 1). Butterballs containing no measurable amounts of protein, zinc or copper were used to adjust the energy intake. A low calorie carbonated beverage, containing no measurable amounts of protein, zinc or copper was used to adjust the energy intake. A low calorie carbonated beverage, distilled water and NaCl were allowed ad libitum.

Analyses

Blood was collected from the antecubital vein of each subject before breakfast on the last day of each experimental period using a plastic syringe and a stainless steel needle rinsed with heparin. Plasma was obtained and frozen for subsequent analysis. A portion of each plasma sample was diluted ten-fold with 10 mmol nitric acid and injected into a Perkin Elmer 306 graphite furnace atomic absorption spectrophotometer (Perkin Elmer Corporation, Norwalk, CT) for copper determination.

Throughout the study the complete 24-hour urines were collected daily into acid-cleaned polyethylene bottles containing toluene. Completeness of sample collection was verified by the relative constancy of daily creatinine excretion. Selected urine samples were acidified with concentrated nitric acid and injected into the furnace atomic absorption spectrophotometer for copper analysis. To confirm that daily urinary copper was less than 1% of the dietary intake (21, 23), urinary copper was analyzed for one subject each day during week 3 and for all subjects on day 7 of each period. The mean daily urinary copper for the one subject was 27 μg (range 12 to 47 μg), a value which is 1% of the daily copper intake and 1.2% of the mean daily fecal copper intake. The mean urinary copper for all subjects on day 7 of week 3 was 22 μg (range 17 to 30 μg), a value which is 0.84% of the daily copper intake and 0.95% of the fecal copper excretion. Since these data are similar to those reported by others (21, 23) and indicate that urinary copper constitutes about 1% or less of the copper intake of our subjects, further analyses of urinary copper were not performed.

Urine samples were separated into 7-day periods with the aid of a fecal dye marker composed of a mixture of 50 mg of FD&C Blue No 1 Certified (H Kohnstamm & Co, Inc, New York, NY) to 250 mg of mucilose flakes (Winthrop Labs, Division of Sterling Drugs, Inc, New York, NY). The marker was given in a gelatin capsule before breakfast at the beginning of each period. Fecal samples were prepared for copper analysis by an extraction-filtration technique. The fresh fecal samples collected within a day were homogenized and digested by adding approximately three volumes of 50% hydrochloric acid and heating at 120°C for one hour with constant stirring. A 2.0 ml aliquot of this mixture was transferred to a preweighed 10 ml volumetric flask, weighed, and 1 ml

Table 3

<table>
<thead>
<tr>
<th>Food items in the basal diet</th>
<th>For all subjects</th>
<th>Variable among subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angel food cake*</td>
<td>Tea†</td>
<td></td>
</tr>
<tr>
<td>Mineral-supplemented bread†</td>
<td>Decaffeinated coffee**</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>Farina§</td>
<td>Low calorie carbonated beverage††</td>
<td></td>
</tr>
<tr>
<td>Cooked egg white§</td>
<td>Butterballs‡‡</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>Micilose flakes§§</td>
<td></td>
</tr>
<tr>
<td>Cheese, American</td>
<td>NaCl</td>
<td></td>
</tr>
<tr>
<td>Tomato, canned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion, dried minced</td>
<td></td>
<td></td>
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<tr>
<td>Green pepper, fresh chopped</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multi-vitamin supplements††

- Daily amount was one cake per subject containing 90 g cake flour, 250 g sucrose, 460 g egg white, 4.8 g cream of tartar, 1.4 g noniodized salt, 6.0 g imitation vanilla, and 4.5 g corn oil.
- Daily portion per subject was 1/6 loaf. One loaf contained 7 g active dry yeast, 1 g sucrose, 520 ml distilled water, 500 g Dietetic Pay-gel Low Protein Bread Baking Mix (General Mills, Inc. 4620 West 77th St, Minneapolis, MN), 9 g non-iodized salt, 60 g dry egg white, 4 drops yellow food color, 53.4 g mineral mix. The mineral mix supplied the following amounts of minerals per day: 800 mg Ca, 800 mg P, 350 mg Mg, 10 mg Fe, 0.153 mg I, 2.5 mg Cu, 3.5 mg Mn, 2010 mg K, 0.0734 mg Cr, 0.100 mg Se.
- Contained 30 g dry Farina (Quaker Oats Co, Barrington, IL), 150 g distilled water, 10 g corn oil, 14 g butter.
- Served 107 g of frozen egg whites (Standard Brands, 625 Madison Ave, New York), at each meal, as scrambled eggs for breakfast and incorporated into Spanish or cheese omelets for the noon and evening meals.
- One-A-Day Multiple Vitamin (Miles Lab, Inc, Elkhart, IN) plus 0.15 mg vitamin K₁, 0.20 mg biotin and 7.64 mg calcium pantothenic acid.
- A maximum of two tea bags per day was allowed.
- One serving contained 0.75 g Freeze-Dried Sanka (Maxwell House Division, General Foods Corporation, White Plains, NY). A maximum of two servings was allowed per day.
- Diet 7-Lip (Seven-Up Company, St Louis, MO) was given in 16 oz bottles, as desired.
- Each butterball provided 100 Kcal, composed of 3.75 g butter, 1.5 g corn oil, 1.55 confectioners’ sugar, artificial food coloring and flavoring.
- A range of 4 to 9 g of mucilose flakes (Winthrop Labs, Division of Sterling Drugs, Inc, New York, NY) were given per day, with most subjects receiving 6 g/day.
of concentrated nitric acid added. These solutions were brought to volume with deionized distilled water, shaken and centrifuged at 1980 x g for 20 minutes. The supernates were filtered (Whatman number 40 ashless paper) into polyethylene screw cap containers. The copper content of the fecal supernates was determined using a Varian 475 flame atomic absorption spectrophotometer (Varian Company, Palo Alto, CA). The accuracy of the copper analytical procedure was checked at intervals, and an average recovery of 99% was obtained. Periodically this method was compared to the dry-ashing technique of Menden et al (25). Results using the two techniques differed by approximately 10%, with the majority of the higher values occurring in samples treated by the extraction-filtration technique. The copper contents of the daily fecal collections between the fecal dye markers were averaged to obtain a daily mean for each week and each subject.

Each week a composite of the total diet for one day for one subject was prepared and blended into a slurry using a stainless steel Waring Blender. An aliquot was prepared for analysis using a dry-ashing procedure adapted from Menden et al (25). The copper and zinc contents of the diet slurries were determined using flame atomic absorption spectrophotometry.

Statistics

Originally, a split-plot design was developed in order to test differences among all zinc intakes (26). Since there were no differences between the subgroups in week 4 and in week 7, the data were pooled within each of these weeks and the following analyses were applied. Statistical significance was determined using a two-way analysis of variance (26) in which the model contained subject and week (Table 2). Fisher's protected least significant differences (26) were used to determine significance between individual treatment means in both analyses.

Results

Plasma copper concentration

Mean plasma copper concentrations tended to decrease in the latter half of the study except for week 9 (Table 2). Plasma copper concentrations for weeks 7 and 8 were significantly lower than those for the first 4 weeks and week 9, and concentrations in weeks 2 and 4 were significantly higher than in weeks 5 through 8. Nevertheless, the weekly plasma copper concentrations for all subjects fell within the range of 64 to 152 μg/dl, values similar to the range of 64 to 164 μg/dl reported by Lahey et al (27) for 504 normal subjects. The mean (± SEM) concentrations for individual subjects throughout the nine weeks ranged from 81 ± 4.2 to 125 ± 6.3 μg/dl. Thus, varying dietary zinc intake from 1.8 to 20.7 mg/day in the present study resulted in no alteration in plasma copper concentrations outside the usual ranges reported in normal subjects.

Fecal copper excretion

During the first two weeks when the analyzed zinc intake was 20.7 and 1.8 mg/day, respectively, the mean fecal copper values were nearly the same and below the copper intake (Table 2). While the difference between the mean habitual (2.3 mg/day, range 1.2 to 3.2) and the experimental (2.63 mg/day) copper intakes was small, the lower habitual intakes of some of the subjects (1.2 mg/day in one and 1.9 to 2.1 mg/day in three others) may have reduced the group mean for fecal copper during the initial week. In week 3 when the zinc intake was 18.5 mg/day, mean fecal copper increased significantly. In weeks 4 and 5 the mean fecal copper excretions returned to quantities not significantly different from those during weeks 1 and 2. However, during week 6, which was the second week of the 18.5 mg/day intake, fecal copper rose markedly. This excretion was significantly different from all other periods and exceeded the intake, placing the subjects in negative copper balance. When the zinc intake was decreased during week 7, the mean fecal copper was significantly lower than week 6 and decreased further during week 8. During week 9, the second week of an 18.5 mg zinc intake, fecal copper increased significantly as had occurred in week 6.

These data suggest that fecal copper excretion was influenced by the amount of zinc in the diet and the length of time a particular amount of zinc was fed. Compared to the first two weeks, subjects lost significantly (p ≤ 0.05) more copper in feces: 1) when fed 18.5 mg of zinc/day for two consecutive weeks (weeks 6 and 9); 2) when fed 18.5 mg of zinc/day (week 3) following a very low zinc intake; and 3) when zinc intake was lowered to 4, 6, or 8 mg of zinc/day (week 7) following two weeks of consuming 18.5 mg of zinc/day. On two occasions (weeks 5, 6 and weeks 8, 9) a one-week lag occurred between the initial change to the diet containing 18.5 mg of zinc/day and the increase in fecal copper excretion.
Copper retention

Apparent copper balance for each week is indicated in Table 2. Copper losses in urine, sweat, skin and hair were not included in calculating the apparent balance. If it is assumed that copper lost from these sources is about 0.3 mg/day (28–33), the mean copper balance would be negative during weeks 6, 7, and 9, and at equilibrium in week 3. Thus, copper balance was not maintained throughout the 63-day study, and dietary zinc influenced the copper balance.

Discussion

This human metabolic study and two others (19, 20) suggest that dietary zinc intakes toward the high side of the physiological range may have an adverse effect on copper excretion and retention, while three other human studies do not support an adverse effect (18, 21, 22). The reason for the differences between our findings and those of others is not immediately apparent. In a recent review, Solomons (34) suggested three possibilities for discrepancies between data from elderly subjects (20) and those in adolescents (18) and women of childbearing age (21): different susceptibility to copper malabsorption at different ages; influences of the chemical forms of the two minerals (food forms versus mineral compounds) in the meals; and, adverse effects of zinc:copper mass ratios of greater than 10 in the meals.

Our subjects were young adult males, comparable in age to women used in two studies (21, 22) in which no adverse effects were reported. To our knowledge, the effect of zinc intake on copper retention has not been studied in young men previous to this study, and differences in copper requirements between men and women are unknown. The majority of the dietary copper (2.5 mg/day) in our study was supplied in a readily available form, copper sulfate. The ratio of dietary zinc to copper in our diet containing 18.5 mg of zinc/day was 7; yet fecal copper was elevated and copper retention decreased, suggesting that while the ratio may be influential, a dietary zinc:copper ratio of greater than 10 may not be the explanation.

The effect of the nitrogen intake on copper excretion and retention is unclear. Colin et al (22) reported little effect of commonly consumed nitrogen intakes (8 and 15 g/day) on these parameters. However, predictions of copper requirements using an experimentally derived regression equation suggest that as the protein intake increases (40 to 100 g/day) copper requirement decreases (35). Data on apparent copper retention and excretion when protein intake was 50 or 150 g/day (36) are consistent with this prediction, but 3.8% higher absorption of a stable isotope of copper when protein intakes were 25 to 36 versus 56 to 119 g/day may be inconsistent with this prediction (37). In our study any influence of the constant high nitrogen intake (16.4 g/day) on copper retention remains to be determined.

In two studies in young women (21, 22) the copper intake of about 2 mg/day appeared to be too low to maintain copper equilibrium when copper losses through the body surface are considered, thus possibly rendering any adverse effect of zinc intake less easily detected. In the studies with adolescent subjects copper retention was decreased at the higher zinc intakes when the copper intake was 1.2 (19) but not when it was 2.9 mg/day (18), yet the high zinc intakes were similar (14.7 and 13.4 mg/day). In the elderly subjects (20) and in young men of the present study copper intakes were 2.33 and 2.63 mg/day, respectively, amounts assumed to be adequate but not excessive (8). It may be that the apparent adverse effect of high dietary zinc on copper retention is dependent upon the amount of available copper in the diet, occurring when this level just meets or is slightly below the requirement.

A primary means by which zinc intake might influence copper excretion and retention is through absorption at the intestinal level. Van Campen and Scaife (13) first demonstrated that zinc interferes with copper absorption in the rat at a site located either in or on the duodenum. Hall et al (15) reported an increase in $^{64}$Cu bound to metallothionein in the mucosal cell of the rat but a decrease in $^{64}$Cu transferred across the mucosal cell with increasing dietary zinc. Other investigators (38–40) have suggested
that high levels of zinc induce de novo synthesis of metallothionein in the rat mucosal cell. Copper is reported to have a higher affinity for metallothionein than zinc and displaces zinc attached to the protein (40). Copper complexed with metallothionein is retained in the intestinal mucosal cell, unavailable for transfer to plasma and is ultimately lost into the lumen of the intestine as the cells exfoliate (38, 40). The approximate 5-day turnover time of the mucosal cell (41) may explain the delay between commencement of the 18.5 mg/day zinc intake and the rise in fecal copper excretion demonstrated by the second of the two-week periods in our study. The reason for the rise in fecal copper during week 3 is uncertain, but may be related to rapid induction of metallothionein by the 18.5 mg/day zinc intake following the low 1.8 mg/day intake in week 2. Rapid induction of intestinal metallothionein has been reported in short-term (4 days) zinc-depleted rats following zinc feeding (42).

In a recent study Fischer et al (43) described the distribution of copper between metallothionein and a high-molecular-weight protein fraction as influenced by dietary levels of zinc. When isolated duodenal segments from rats fed high-zinc diets were incubated in a high copper media, the majority of the copper was associated with metallothionein. Conversely, the majority of copper was bound to the high-molecular-weight protein fraction in rats fed a low-zinc diet. In all intestines incubated in the low copper media, regardless of the zinc status of the rats, the majority of copper was associated with metallothionein. Fischer et al (43) suggest that with the low-zinc diets, metallothionein was saturated with copper, and the excess copper was bound to the high-molecular-weight protein fraction. In rats fed high-zinc diets metallothionein synthesis was induced, resulting in the binding of more copper to metallothionein and less to the high-molecular-weight protein fraction. The copper bound to metallothionein was not readily available for transfer, whereas copper bound to high-molecular-weight protein was available for transfer.

If these findings in rats can be applied to the present human study, they suggest the possible induction of metallothionein synthesis in man by a zinc intake of 18.5 mg/day, the subsequent binding of copper to the metallothionein and “trapping” of this copper within the mucosal cell, and finally, the release of the copper several days later when the mucosal cell is sloughed into the intestinal lumen. Whether or not the absorptive or cellular utilization mechanisms would adapt to the 18.5 mg zinc intake over a period of time, and thus promote more favorable copper retention, is unknown. The apparent lack of effect of a zinc intake of up to 8 mg/day in this study suggests some maximum zinc intake below which copper excretion and retention is unaffected. Since all zinc intakes below 18.5 mg/day were tested for only one week, it is unknown whether any increase in fecal copper would have occurred if the length of feeding these lower intakes had been extended to two weeks.

The implications of this research require careful consideration. Dietary zinc above the requirement but within a physiological range may have an antagonistic effect on copper balance by reducing copper absorption and increasing fecal copper excretion. To complicate the problem, the estimated intake of dietary copper in US diets may be well below the suggested adequate level of 2 to 3 mg/day (1–7), creating a possible negative balance situation in man. Our study shows that feeding 18.5 mg of zinc/day, an amount only 3.5 mg above the RDA for adults (8), in diets containing 2.63 mg of copper/day resulted in elevated fecal copper excretion and reduced copper retention during two-week periods. Zinc intakes of this magnitude are quite possible in segments of the population consuming large amounts of protein and with high energy intakes, such as military personnel (44) and adolescent males (45), as well as in those individuals who consume zinc supplements. Whether the negative copper balance would persist in our subjects if they were maintained on the high zinc diet beyond two weeks is unknown. This study raises a serious question concerning the most favorably dietary quantities of zinc and copper for optimum copper absorption in human adults.

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