DECREASED HIGH DENSITY LIPOPROTEIN CHOLESTEROL AND APOPROTEIN A-I IN PLASMA AND ULTRASTRUCTURAL PATHOLOGY IN CARDIAC MUSCLE OF YOUNG PIGS FED A DIET HIGH IN ZINC

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ABSTRACT

People who supplement their diets with extra zinc usually have potentially harmful changes in lipoprotein metabolism. Because zinc can interfere with copper metabolism and copper deficiency has produced similar changes in lipid metabolism in animals and people, the hypothesis that lipid alterations from high zinc intakes are accompanied by changes in copper metabolism was tested. Progenies of swine fed a high zinc (5000 mg/kg diet) or normal zinc (150 mg/kg diet) diet during pregnancy and lactation were weaned to practical diets of natural products containing similar amounts of zinc. Concentrations of apoprotein A-I (p<0.05) and high density lipoprotein cholesterol (p<0.004) were decreased and the age-related increase in the latter was delayed (p=0.0001) by high zinc intake. Copper in blood plasma (p=0.0008), heart (p<0.004) and liver (p<0.06) were decreased by high zinc intake which increased plasma (p=0.001) and liver zinc (p=0.0001). Quantitative electron microscopy revealed that hearts of young pigs fed high zinc had less myofibrillar volume and a higher volume ratio of mitochondria to myofibrils. Anatomical changes, lipid concentrations and copper concentrations were consonant with the induction of mild copper deficiency by high intakes of zinc. The potentially hazardous changes in lipid metabolism usually found in people who take zinc supplements probably are the result of an induction of mild copper deficiency.

KEY WORDS: Cardiopathy, Cholesterol, Copper, Copper deficiency, Dyslipidemia, Ischemic heart disease, Lipoproteins, Zinc supplements

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People who supplement their diets with extra zinc usually have potentially harmful changes in lipoprotein metabolism. This phenomenon, found first by Hooper et al. (1), has been observed in more than 76 men and women (1-4). The highest doses of zinc (300 mg/day) increased cholesterol in low density lipoproteins (LDL) and decreased cholesterol in high density lipoproteins (HDL) (2). Lesser doses of zinc (17 to 160 mg/day) lowered HDL cholesterol without a change in LDL (1,3,4).

It seems reasonable to infer that these changes in lipids are secondary to changes in copper metabolism or utilization. Copper deficiency produces potentially adverse changes in the metabolism of cholesterol, fatty acids, lipoproteins and prostaglandins (5); evidence has been obtained from experiments in several species, including *H. sapiens* (5). Zinc is a well known antagonist of copper (6-8) that can interfere with lipid metabolism.

Hypercholesterolemia in rats as a result of high zinc intakes first was observed more than 20 years ago (9). Hypercholesterolemia from copper deficiency has been "confirmed in numerous laboratories and species" (10,11) and is "generally accepted" (12).

Men fed a diet low in copper experienced an increase in LDL cholesterol and a decrease in HDL cholesterol (13); supplementation of the diet with copper reversed these changes with improvement on control values. These changes in lipid metabolism are similar to those found with zinc supplementation. Although allusion to interference with copper was made by some students of this phenomenon (e.g., 1), assessments of copper nutriture generally have not been made (1-4).

Hill et al. (14) induced copper deficiency in young pigs by feeding them a diet high in zinc after their mothers were fed high zinc during gestation. It was decided to test the hypothesis that when lipid metabolism is changed by high doses of zinc there also are changes in copper metabolism. The experiment was done by a modification of the maternal feeding method (14); cardiac anatomy, plasma lipids and trace elements in organs were evaluated.

MATERIALS AND METHODS

*Animals and diets*

Progenies of primiparous crossbred (Chester White x Landrace x Large White x Yorkshire) swine that had been fed a high zinc (5000 mg Zn/kg diet as ZnO, 11 mg Cu/kg diet) or normal zinc (150 mg Zn/kg diet, 11 mg Cu/kg diet) from mating throughout pregnancy and lactation were used. The diet is shown in Table 1; detail of diets, housing, etc. have been published (15). Three weeks after birth, piglets were weaned to similar diets containing less corn and more soybean and alfalfa meals. Oats were increased to 10% until week 10. There were eight piglets in each group; half were male. Blood samples were obtained periodically from the vena cava. Half of the pigs in each dietary group were killed by electrocution and exsanguination at age 16 weeks; plasma and organs were obtained for analysis. Remaining pigs were killed similarly at age 20 weeks.
ZINC AND THE HEART

TABLE 1

Gestation - Lactation Diet for Swine

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground</td>
<td>841</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>110</td>
</tr>
<tr>
<td>Alfalfa meal, dehyd.</td>
<td>10</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>24</td>
</tr>
<tr>
<td>Limestone</td>
<td>5</td>
</tr>
<tr>
<td>Iodized salt</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
</tr>
<tr>
<td>Trace mineral mix†</td>
<td>2</td>
</tr>
</tbody>
</table>

*Supplied the following (units/kg of complete diet): retinyl palmitate, 5,280 IU; cholecalciferol, 704 IU; dl-alpha-tocopheryl acetate, 35.2 mg; menadione sodium bisulfite, 3.52 mg; vitamin B₁₂, 26.4 μg; riboflavin, 5.28 mg; niacin, 28.16 mg; d-pantothenic acid, 21.12 mg; biotin, 8.8 μg; thiamin, 2.2 mg. The vitamin supplement was custom-mixed by International Nutritional, Omaha, NE.

†Supplied the following (mg/kg of complete diet): Fe as FeSO₄•7H₂O, 160; Mn as MnO, 20; Zn as ZnO, 100; CaCO₃ as a carrier.

Clinical chemistry

Hemoglobin and hematocrit were determined on fresh whole blood and after centrifugation at 3500 rpm for 10 minutes at 5°C. Ceruloplasmin concentration (oxidase activity) was determined colorimetrically using N,N-dimethyl-p-phenylenediamine ( assay kit from Sigma Chemical Co., St. Louis, MO 63178).

Trace elements were determined by atomic absorption spectrometry (16). Organic matter in organ samples was destroyed with nitric and sulfuric acids augmented with hydrogen peroxide (17). Lipids were determined by an automated method (Cobas Fara, Nutley, NJ 07110). Total cholesterol was determined by the method of Allain, et al. (18). Phosphotungstate precipitation aided in the determination of HDL cholesterol (19). Apoprotein A-I was measured with goat antibody (20).

Electron microscopy

A selected number of pigs (females at 16 and 20 wks of age; high Zn, n=5, normal Zn, n=4) were evaluated for cardiac histopathology to determine whether the apparent decrease in copper status due to zinc supplementation was sufficient to produce signs of cardiac pathology previously reported in copper deficiency. Hearts were rinsed and gently massaged in oxygenated Dulbecco's phosphate-buffered saline supplemented with 14 mM glucose (Gibco Laboratories Life Technologies, Grand Island, NY) three times for two minutes each at 37°C. Muscle was sampled from the left ventricle approximately 5 cm from the apex and tangential to the outer wall in order to view muscle fibers in a longitudinal plane. The excised muscle was cut into 1 mm cubes and processed into blocks for transmission electron microscopy viewing as described (21). Blocks
containing myocardium were trimmed and sectioned on a Sorvall MT-2B ultramicrotome. To check the orientation of samples, thick sections (900 nm) were first sampled using glass knives and viewed under a light microscope. Thin sections (90 nm) for TEM viewing were made with a Diatome diamond knife, collected on copper grids (75 x 300 mesh) and stained (22). Stained sections were examined with a Phillips CM-12 Transmission Electron Microscope (Eindhoven, The Netherlands). Images were exposed on film and developed as negatives followed by development of prints.

Electron micrographic prints at 7,000 X were overlaid with a plastic grid from which the volume densities ($\mu m^3/\mu m^2$) of both mitochondria and myofibrillar areas were determined by a point system as described by Wiebel (23) and Steer (22).

**Statistics**

Data on copper and zinc in plasma and lipid data were analyzed by repeated measure analysis of variance (24) with time and diet as main factors. Data on copper and zinc in organs was analyzed by two-way analysis of variance (24) and for morphometric data, one-way analysis of variance (24).

**RESULTS**

No significant effect ($p>0.05$) of the diet high in zinc was found on either hemoglobin or hematocrit; minimal mean values were 113 g/L and 0.353, respectively. Animals exposed to high zinc had lower ceruloplasmin values ($p=0.003$); the increase with age was delayed ($p=0.0001$). By week 16 the mean for the high zinc group was only about 9% lower than normal. Data are not shown because they have been published in detail (15).

Data on copper and zinc in plasma are shown in Table 2. Exposure to high zinc more than doubled plasma zinc and decreased plasma copper by approximately 20%.

**TABLE 2**

Plasma Copper and Zinc, $\mu$mol/L*

<table>
<thead>
<tr>
<th>Time, week</th>
<th>Copper</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Zn</td>
<td>High Zn</td>
<td>Normal Zn</td>
</tr>
<tr>
<td>9</td>
<td>33.2 ± 1.3</td>
<td>26.8 ± 1.9</td>
</tr>
<tr>
<td>12</td>
<td>33.7 ± 1.3</td>
<td>27.7 ± 1.5</td>
</tr>
<tr>
<td>16</td>
<td>34.5 ± 0.83</td>
<td>25.3 ± 1.0</td>
</tr>
</tbody>
</table>

*Mean ± standard error, 8 animals per group. Copper probabilities: time, >0.5; zinc, 0.0008; time x zinc, >0.1. Zinc probabilities: time <0.02; zinc, 0.001; time x zinc, >0.4. Values were calculated from traditional units by multiplying by 0.1574 and 0.1530 for copper and zinc, respectively, because balances are calibrated in grams. Approximate values in mg/dL can be obtained by dividing by 0.16.
Data on lipids are shown in Table 3. Total cholesterol, HDL cholesterol and apolipoprotein A-I were lower when animals were exposed to high zinc. The greatest decrease, approximately 18% on the average, was found in HDL cholesterol. Lipid values were dependent on the time of measurement except for apoprotein A-I. There were no interaction effects.

**TABLE 3**

Plasma Lipids*

<table>
<thead>
<tr>
<th>Time, weeks</th>
<th>Total cholesterol, μmol/L</th>
<th>HDL cholesterol, μmol/L</th>
<th>Apoprotein A-I, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Zn</td>
<td>High Zn</td>
<td>Normal Zn</td>
</tr>
<tr>
<td>11</td>
<td>2.61 ± 0.16</td>
<td>2.15 ± 0.20</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>14</td>
<td>2.87 ± 0.08</td>
<td>2.48 ± 0.17</td>
<td>1.45 ± 0.07</td>
</tr>
<tr>
<td>16</td>
<td>2.43 ± 0.11</td>
<td>2.25 ± 0.12</td>
<td>1.37 ± 0.08</td>
</tr>
</tbody>
</table>

*Mean ± standard error, 8 animals per group. Cholesterol probabilities: time, <0.05; zinc, <0.02; time x zinc, >0.5. HDL cholesterol probabilities: time, 0.0001; zinc, <0.004; time x zinc, >0.8. Apoprotein probabilities: time, >0.3; zinc, <0.05; time x zinc, >0.2. See footnote to Table 2. Approximate values in mg/dl can be obtained by dividing cholesterol values in μmol/L by 0.025.

Table 4 contains data on organ copper which decreased approximately 13 and 28% in heart and liver, respectively, under exposure to high zinc. As expected, liver zinc increased substantially, 1316 vs 261 mg/kg (p=0.0001), with high dietary zinc. There was no change in cardiac zinc.

**TABLE 4**

Organ Copper, mg/kg

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Zn</td>
<td>18 ± 0.2</td>
<td>29 ± 3.3</td>
</tr>
<tr>
<td>High Zn</td>
<td>16 ± 0.5</td>
<td>21 ± 1.7</td>
</tr>
</tbody>
</table>

Data at 16 and 20 weeks were combined because ANOVA revealed no effect of time (p>0.08). Eight animals per group.

Ultrastructural examination of myocardium from the left ventricle of pigs fed normal zinc showed normal histology. In contrast, the pigs fed the high zinc diets appeared altered in that...
mitochondria appeared abnormal in areas with their fine structure disrupted, vacuolization and disarray of the normally parallel arrays of cristae (Figure 1). The mitochondria also appeared enlarged or swollen in the zinc-supplemented myocardium. Morphometric data revealed that pigs fed high zinc diets had lower myofibrillar volume densities \((p<0.01)\) and greater mitochondria to myofibrillar volume ratios \((p=0.05)\) (Table 5). While hearts from both treatments contained glycogen granules, the pigs fed high zinc tended to have a slightly greater accumulation of the storage polysaccharide. Myofibrils in areas tended to be distorted with separated myofilaments apparent in the high zinc pig hearts.

**TABLE 5**

Cardiac Mitochondrial and Myofibrillar Volume Densities and the Ratio of Mitochondria to Myofibril Volume of Female Pigs Fed Normal and High Zinc Diets*

<table>
<thead>
<tr>
<th></th>
<th>Mitochondría ((\mu m^3/\mu m^3))</th>
<th>Myofibril ((\mu m^3/\mu m^3))</th>
<th>Mitochondría to Myofibril Volume Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Zinc</td>
<td>0.280 ± 0.023</td>
<td>0.566 ± 0.022</td>
<td>0.492 ± 0.026</td>
</tr>
<tr>
<td>High Zinc</td>
<td>0.335 ± 0.046</td>
<td>0.469 ± 0.014</td>
<td>0.721 ± 0.108</td>
</tr>
</tbody>
</table>

*Mean ± standard error, four animals in the normal zinc groups and five animals in the high zinc group. Myofibril volume density lower in the high zinc group, \(<0.01\), and mitochondria to myofibril volume ratios higher in the high zinc group, \(p=0.05\).

**DISCUSSION**

When copper and zinc interact in metabolism, the effects depend on the dose of each element. High dietary zinc can increase copper requirements and lessen copper toxicity; high dietary copper can increase zinc requirements and lessen zinc toxicity (25,26).

The present experiment was done with a practical pig diet (Table 1) containing approximately 11 mg Cu/kg diet. This amount is considerably greater than the 0.5 mg Cu/kg diet in the purified diet used by Hill et al. (14) who inferred that the copper requirement of the baby pig is about 5 mg/kg. They were unable to decrease copper in either heart or liver with high doses of zinc when dietary copper was 10 mg/kg. In contrast (Table 4), the decreased copper in heart and liver probably contributed to both ultrastructural and metabolic change.

Anemia and altered cardiac anatomy such as cardiac enlargement and ventricular aneurysm, calcification, fibrosis and rupture can be found in severe copper deficiency (16,27-31). Signs of copper deficiency in the present experiment were more subtle because deficiency was mild. Anemia was not found, but anemia is not inevitable in copper deficiency (6,32-34). Although ceruloplasmin was decreased early in the experiment and the normal increase with age was delayed, by the end of the experiment ceruloplasmin was decreased only about 9% (15) and plasma copper was decreased only about 20% (Table 2).
FIG. 1A. In control pigs the myofibrils alternate with rows of mitochondria. Mitochondria cristae demonstrate the usual parallel arrays. M, mitochondria; my, myofibrils; G, glycogen granules; I, intercalated disc. Bar = 1 μM.
FIG. 1B. Zinc-supplemented pigs demonstrate an increase volume density of mitochondria. Mitochondria appear less dense and vacuolated in areas. The cristae in many mitochondria appear fragmented and do not always demonstrate their usual parallel alignment. The myofibrils appear to have a separation of the myofilaments. Glycogen granules and myocyte separation in selected areas (*) seem more frequent in zinc supplementation. See Fig. 1A for explanation of symbols.
Hearts from pigs fed the high zinc diet resembled those reported for copper-deficient pigs, rats and steers (21,35-38). Specifically, swollen mitochondria, increase in the ratio of mitochondria to myofibril ratio, disruption in the mitochondria fine structure (i.e., cristae) and apparent increase in glycogen granules as reported in the present study have been reported for copper-deficient animals, including pigs (38).

The present study also revealed a greater increase in the mitochondria to myofibril ratio in pigs fed the high zinc diets than previously reported for copper-deficient swine. However, the greater ratio in the present study was primarily due to a decrease in myofibril volume density in pigs fed the high zinc diets. A recent study on copper-deficient swine demonstrated that the increased ratio was due to both an increase in the mitochondrial volume density and decrease in the myofibril volume density (38). The small sample size and inherent variability in morphometric analysis of electron microscopy data may explain some of these differences. Additionally, the high zinc diet may also have contributed to the pathology independent of the mild copper deficiency reported here. The accumulation of glycogen granules in heart tissue is often suggestive of an oxygen deficit. However, any such deficit must have been cellular because there was no anemia. Light microscopy of sections stained with hematoxylin and eosin revealed no effect of the high zinc diet (15).

Electron microscopy revealed abnormal cardiac anatomy with a smaller decrease in cardiac copper than found in severe copper deficiency: 38% (37) to 66% (38). No data on cardiac copper were provided by other authors (21,35,36).

The decrease in HDL cholesterol found here is similar to that experienced by men and women who supplement their diets with zinc. A smaller decrease in apoprotein A-I is consistent with the change in HDL. The former was detected only at age 11 weeks, the latter persisted from age 11 to 16 weeks.

Doses of zinc less than the 5000 mg/kg diet found effective here have modified the lipoprotein metabolism of people because the dried human diet contains less copper (mean 1.9 mg/kg diet) (39) than this pig diet (11 mg/kg diet).

The highest (2) dose of zinc to affect lipoprotein metabolism in people corresponds to somewhat more than 300 mg/kg diet per day (Klevay, unpublished data from reference 39). Lower doses of zinc, somewhat greater than 25 mg/kg diet, were found by Goodwin et al. (3) to "negate the beneficial effect of exercise on raising HDL-cholesterol". Altered lipid metabolism from impaired copper utilization may explain the significant positive correlation between the mortality rate for coronary heart disease and the ratio of zinc to copper in milk in 47 cities of the United States (40). Of course, it is unknown whether or not some people who take zinc supplements have changes in cardiac metabolism or structure.

Although some of the people whose lipid metabolism was altered by high doses of zinc may have been eating too little copper, some diets are sufficiently low in copper to disturb lipid metabolism without zinc supplementation. The recommended safe and adequate range of copper for adults is 1.5 to 3 mg/day (41). Calculations of daily copper intake from ten dietary surveys in which copper was measured by chemical analysis reveal that 35% of diets contain less than 1 mg (11). Sixteen percent of daily diets contain less than 0.65 mg. Daily diets ranging from 0.65 to 1.02 mg have been shown to contain too little copper for the more than 31 men and women in copper depletion experiments (13,42-44). Some of these volunteers responded to the low copper diet with hypercholesterolemia (42) and dyslipidemia (13).
In summary, the high dose of zinc in this experiment induced a mild copper deficiency, the principal manifestations of which were decreased HDL cholesterol and ultramicroscopic change in the cardiac muscle. The depression of HDL cholesterol by zinc is similar to that found in people who take zinc supplements. People who take excessive zinc supplements probably experience mild copper deficiency.

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