Effects of Isolated Zinc Deficiency on the Composition of Skeletal Muscle, Liver and Bone during Growth in Rats

JUNG H. Y. PARK, CARTER J. GRANDJEAN, DEAN L. ANTONSON AND JON A. VANDERHOOF

Department of Pediatrics, University of Nebraska Medical Center and the Swanson Center for Nutrition, Inc., Omaha, NE 68105

ABSTRACT We investigated the effects of zinc deficiency on body composition by using intragastric force-feeding to obviate decreased food intake and altered eating patterns. Weanling male Sprague-Dawley rats were fed a purified zinc-deficient diet: the ad libitum-fed control group (AL; eight rats) was given powdered diet and water containing 25 ppm zinc; the zinc-replete group (ZN; nine rats) was force-fed a diet blended with water containing zinc in an amount of equal caloric intake to the AL group and allowed access to water containing zinc. The zinc intake of ZN rats was approximately twice that of AL rats based on water intake. The zinc-deficient group (ZD; 13 rats) was fed similarly to the ZN group except deionized water was used for diet preparation and drinking water. After 8 d, body and muscle weight were lower in the ZD group than in the ZN group. Femur weights were similar in the two groups. Serum, liver and femur zinc concentrations were 85, 22 and 42% lower, respectively, in the ZD group than in the ZN group. Serum glucose, relative liver weight, liver glycogen and liver lipids were higher, but muscle and liver DNA were lower in the ZD group than in control groups. J. Nutr. 116: 610–617, 1986.

INDEXING KEY WORDS isolated zinc deficiency • force-feeding • skeletal muscle • liver • serum • lipids • bone • glucose • glycogen

Effects of zinc deficiency on body growth and development have been investigated extensively in the last two decades. However, most of these studies used pair-fed animals as a control, because feeding a zinc-deficient diet to rats initiates a prompt and dramatic decrease in food intake (1). Pair-fed animals not only receive restricted amounts of food but also develop a meal eating pattern, whereas zinc-deficient animals fed ad libitum develop nibbling but cyclic patterns of food intake (2, 3). Studies have shown that altering the frequency of food ingestion results in differences in nutrient metabolism and body composition (4–6).

The decrease in food intake in zinc-deficient animals can be very important for survival of the animal because limited available zinc will be used for vital functions of the body instead of being deposited in growing tissues. In addition to the low food intake, a cyclic eating pattern may slow the development of the zinc-deficiency state by providing plasma zinc for the vital functions of the body through the catabolism of tissues when food intake is low. Previously, we observed that rats force-fed a zinc-deficient diet in an amount identical to ad libitum-fed zinc-replete controls became seriously ill in 8 d (7), and the cutaneous lesions in these rats were similar to those seen in patients
with acrodermatitis enteropathica (8) or patients that received parenteral nutrition without added zinc (9). It has also been reported that anorexia is not a consistent characteristic of zinc deficiency in primates (10–12) and the metabolic effect of zinc deficiency depends, in part, on whether or not anorexia is induced. Golub et al. (12) have reported that severely anorexic pregnant monkeys lost weight but maintained a normal plasma zinc level when they were fed a zinc-deficient diet, whereas monkeys that gained 20–30 % of their body weight had severely depressed plasma zinc.

The direct effects of zinc deficiency separated from secondary effects mediated by reduced food intake have not been successfully investigated. The present study was performed to investigate the effects of isolated zinc deficiency on body composition without any decrease in food intake or change in eating pattern through the use of controlled intragastric force-feeding.

MATERIALS AND METHODS

Animals and diets. Weanling male Sprague-Dawley rats (Sasco, Omaha, NE) were housed individually in hanging stainless-steel cages under a fixed light-dark cycle (lights being on from 0800 to 2300 h). They were given a nonpurified diet (Rodent Laboratory Chow,Ralston Purina Co., St. Louis, MO) and tap water ad libitum for 2 d while they acclimated to our laboratory conditions. Initially, all animals were allowed to adapt to the intragastric tube-feeding procedure by receiving increasing amounts of liquid diet three times a day for 3 d. The diet was a purified zinc-deficient diet (Teklad, Harlan Sprague-Dawley, Inc., Madison, WI) blended with water containing 25 ppm of zinc as ZnSO₄ · 7H₂O. The liquid diet for tube-feeding was prepared by mixing 100 g of powdered diet with 100 ml of water in a Waring Blender (Waring Products Division of Dynamics Corporation of America, New Hartford, CT) for 4 min. An intragastric tube was made by connecting a 10-cm piece of Tygon microbore tubing (Westvaco, Cleveland, OH) to a 16-gauge syringe needle. Water containing 25 ppm of zinc was given ad libitum. The basal zinc-deficient diet contained less than 0.5 μg zinc/g as determined by atomic absorption spectrophotometry and consisted of the following ingredients in grams/kilogram: spray-dried egg white solids, 200; dextrose, 634; corn oil, 100; cellulose, 30; vitamin mix,¹ 10; and mineral mix,² 26. After 3 d of adaptation to the tube-feeding procedure, the rats were randomly divided into three groups. The first group (AL; eight rats) was fed ad libitum the basal zinc-deficient powdered diet and zinc-supplemented water, and daily food intakes were monitored. The second group (ZN; nine rats) was force-fed the blended diet three times per day in amounts identical to the AL group. The diet was prepared with water containing zinc, and water supplemented with zinc was given ad libitum. The third group (ZD; 13 rats) was force-fed in a manner similar to the ZN group except that deionized water was used for drinking and diet preparation. Water consumption was measured between 1200 and 1300 h. Total water consumption over an 8-d period was 94 ± 4 ml for the AL rats and 162 ± 7 ml for the ZN rats. Thus, total zinc consumption over an 8-d period for the AL and ZN rats was 2388 ± 109 and 4050 ± 177 μg, respectively. Precautions were taken to eliminate sources of zinc contamination from the environment.

Analysis. The animals were killed after 8 d of feeding the experimental diets, at which time the ZD animals showed severe cutaneous lesions (perioral and periorbital); one rat in the ZD group died. Tissue and blood samples were taken after a 12-h fast for the ZD and ZN groups and 2 h after the end of the dark cycle for the AL group. Measurements of serum glucose, zinc, triglycerides and cholesterol were made with serum from blood samples withdrawn from the inferior vena cava. The liver, femur and the psoas major muscle were removed and frozen.

¹The vitamin mix contained in milligrams/kilogram diet: biotin, 4.4; p-aminobenzoic acid, 100; ascorbic acid, 107; vitamin B₁₂, 30; calcium pantothenate, 60; choline dihydrogen citrate, 5477; folic acid, 2.0; inositol, 110; niacinamide, 50; niacin, 96; pyridoxine - HCI, 22; riboflavin, 22; thiamin - HCI, 22; retinyl palmitate, 5.0 × 10⁻³; vitamin D₃, 1000 IU; vitamin E (DL-α-tocopherol), 100 IU.

²The salt mix contained in milligrams/kilogram diet: CaCl₂, 18,747; KCl, 2,388; NaCl, 778; MgSO₄, 2,478; MnSO₄ · H₂O, 100; FeSO₄ · 7H₂O, 590; KI, 0.4; CuSO₄, 15.1. Although chromium and selenium were not added to the mineral mix, an identical study was performed to examine the possible effects of chromium and selenium on glucose metabolism. With chromium (CrK(SO₄)₂, 12H₂O, 19.3 mg/kg diet) and selenium (Na₂SeO₃, 0.22 mg/kg) added to the diet, no differences were noted in data from the follow-up study and the data presented in this paper (Park, J. H. Y. and Hart, M., unpublished data).
Serum was diluted with distilled deionized water and zinc was analyzed by atomic absorption spectrophotometry (Instrumentation Laboratory Inc., Wilmington, MA). Serum glucose was determined by the method of Bergmeyer et al. (13) by using hexokinase and glucose-6-phosphate dehydrogenase. Serum cholesterol was determined by a colorimetric method (14), and serum triglycerides were determined by an enzymatic procedure (15). A femur and part of the liver were wet ashed with concentrated nitric acid and vacuum evaporated to dryness. The resulting mixture was reconstituted with 5% nitric acid, and zinc concentration was determined by atomic absorption spectrophotometry by using a certified standard diluted with 5% nitric acid. Bovine liver standards (National Bureau of Standards, Washington, DC, Standard Reference Material 1577) were analyzed for zinc concentration to evaluate our methodological accuracy. The zinc concentration of the standard was 128 ± 1 μg/g (n = 10) as compared with the certified value of 130 ± 13 μg/g. The DNA and RNA in the liver and muscle were extracted by the method of Schmidt and Thannhauser (16) as modified by Munro and Fleck (17). The resulting RNA was quantified by reading absorbance at 260 nm. The DNA fraction was assayed according to Burton (18) as modified by Giles and Meyers (19). The glycogen content of the liver was estimated by a method utilizing glycogen precipitation on filter paper and subsequent enzymatic hydrolysis of glycogen (20). The resulting glucose was assayed according to the method of Bergmeyer et al. (13). The method of Folch et al. (21) was used to extract lipids from the liver and the lipid content was quantified by a gravimetric method. Protein was determined according to Lowry et al. (22) by using bovine serum albumin as a standard.

Statistical analyses. The data were analyzed by analysis of variance by using completely randomized design. Statistically significant differences among group means were determined by Duncan's new multiple-range test (23).

RESULTS

Changes in body weight from the beginning of experimental diets to the conclusion of the experiment are shown in figure 1. Rats force-fed the zinc-deficient diet three times per day (ZD) grew at a rate similar to rats force-fed the zinc-containing diet (ZN) until d 6 of feeding but stopped growing thereafter. Thus, at the time of killing animals in the ZD group were smaller than those in the ZN group. The mean body weight of the AL group was significantly higher than that of the ZN group at the conclusion of the experiment. Relative to ZN animals, animals in the ZD group had lower zinc content in the femur (42% reduction), although zinc deficiency did not affect the weight of the femur. Also serum zinc concentration was drastically lowered (85% reduction), but liver zinc was not lowered as much (22%). There was no difference in serum and femur zinc between the ZN and AL groups, whereas the zinc concentration of the liver was higher in the ZN group compared to the AL group (table 1).

Table 2 shows the concentration of protein, triglycerides, cholesterol and glucose in serum. Protein concentration was not changed in ZD animals compared to the ZN animals, but was lower than that from the AL group. Triglyceride concentration tended to be higher in the ZD group but was not statistically different from the ZN group. The concentration of triglycerides was significantly higher in the ZD group than in the AL group. Zinc deficiency resulted in higher serum glucose level compared to the ZN group. There was no difference in serum

![Fig. 1](https://academic.oup.com/jn/article-abstract/116/4/610/4763161) Growth curves of weanling rats fed different amounts of zinc; zinc-deficient, force-fed group (▲), zinc-replete, force-fed group (●) and ad libitum-fed control (★). Values are means ± SEM.
TABLE 1

Effects of zinc deficiency on femur weights and zinc concentrations of serum, liver and femur

<table>
<thead>
<tr>
<th>Measure</th>
<th>ZD (13)</th>
<th>ZN (9)</th>
<th>AL (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur wt, mg</td>
<td>269 ± 10^a</td>
<td>291 ± 8^b</td>
<td>311 ± 1^b</td>
</tr>
<tr>
<td>Serum zinc, µg/dl</td>
<td>23 ± 1^a</td>
<td>155 ± 4^b</td>
<td>168 ± 12^b</td>
</tr>
<tr>
<td>Liver zinc, ppm</td>
<td>29 ± 0.4^a</td>
<td>37 ± 0.7^b</td>
<td>31 ± 0.3^c</td>
</tr>
<tr>
<td>Femur zinc, ppm</td>
<td>65 ± 2^a</td>
<td>112 ± 4^b</td>
<td>134 ± 5^b</td>
</tr>
</tbody>
</table>

^a Zn deficient, force-fed; ^b Zn replete, force-fed; ^c AL, ad libitum-fed control. Number in parentheses refers to number of rats in each group. Values are means ± SEM.

Effects of zinc deficiency on the composition of the liver are shown in table 3. The mean liver weight expressed per 100 g of body weight was significantly higher in the ZD group than that of the two control groups. The concentration of DNA in the liver was lower in the ZD group than in the AL or ZN group. Zinc deficiency per se did not affect RNA and protein concentration when expressed as milligrams/gram of the liver, but the ratios of RNA/DNA and protein/DNA were higher in the ZD group than in the AL and ZN groups. Force-feeding rats the zinc-deficient diet for 8 d resulted in higher glycogen and lipid amounts in the liver than either other treatment. There was no difference in the parameters of liver composition studied in the present experiment between the force-fed and ad libitum-fed controls.

Short-term isolated zinc deficiency also had a negative impact on muscle growth (table 4). The mean muscle weight was highest in the AL group and lowest in the ZD group. If muscle weight is expressed per 100 g of body weight, the values for the ZD, ZN and AL were 4.70 ± 0.10, 5.58 ± 0.09 and 5.73 ± 0.17, respectively. DNA concentration did not vary among the three dietary groups, and thus, total DNA content of muscle paralleled muscle weight. RNA concentration expressed per gram of muscle weight was lowest in the ZD group, but RNA/DNA ratio did not vary among the three dietary groups. Surprisingly, protein concentration (milligrams/gram of muscle) and protein/DNA ratio were higher in animals force-fed the zinc-deficient diet, but total protein content in the psoas major muscle was lowest in the ZD group and highest in the AL group.

DISCUSSION

This study was designed to explore the effects of isolated zinc deficiency in the absence of a decrease in food intake. We and other investigators observed that rats force-fed a zinc-deficient diet could not survive beyond 8–10 d (7, 24). However, Faraji and Swendsen (25) observed that young adult rats tube-fed a zinc-deficient diet grew similarly to control rats for 25 d. This discrepancy between studies could be attributed to the difference in growth rate of animals. Zinc-deficient rats used by Faraji and Swendsen (25) gained 3 g/d, whereas rats in the

TABLE 2

Effects of zinc deficiency on serum protein, triglycerides, cholesterol and glucose

<table>
<thead>
<tr>
<th>Measure</th>
<th>ZD (13)</th>
<th>ZN (9)</th>
<th>AL (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g/dl</td>
<td>4.94 ± 0.09^a</td>
<td>5.18 ± 0.07^b</td>
<td>5.86 ± 0.22^b</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>387 ± 32^a</td>
<td>298 ± 10^b</td>
<td>239 ± 13^b</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>64 ± 3^a</td>
<td>93 ± 3^a</td>
<td>93 ± 3^a</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>147 ± 8^a</td>
<td>106 ± 5^b</td>
<td>179 ± 5^b</td>
</tr>
</tbody>
</table>

^a ZD, zinc deficient, force-fed; ^b ZN, zinc replete, force-fed; ^c AL, ad libitum-fed control. Number in parentheses refers to number of rats in each group. Values are means ± SEM.

Values with the same letter are not significantly different (P > 0.05) among dietary treatments.
TABLE 3

Effects of zinc deficiency on the liver composition during growth in rats

<table>
<thead>
<tr>
<th>Measure</th>
<th>Dietary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZD (13)</td>
</tr>
<tr>
<td>Liver wt, g/100 g body wt</td>
<td>4.79 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DNA concn, mg/g liver</td>
<td>1.08 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RNA concn, mg/g liver</td>
<td>10.73 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>10.1 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein concn, mg/g liver</td>
<td>176 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein/DNA</td>
<td>163 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycogen concn, mg/g liver</td>
<td>55.7 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lipid concn, mg/g liver</td>
<td>61.9 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>ZD, zinc deficient, force-fed; ZN, zinc replete, force-fed; AL, ad libitum-fed control. Number in parentheses refers to number of rats in each group. Values are means ± SEM.  <sup>b</sup>Values with the same letter are not significantly different (P > 0.05) among dietary treatments.

The present study gained 6 g/d. The concentration of serum zinc dropped to 23 μg/dl after 8 d of force-feeding a zinc-deficient diet in the present study (table 1). However, in the study of Faraji and Swendseid the value was 72 μg/dl after 32 d of feeding. In a study of pregnant rats, Masters et al. (26) observed that feeding rats a zinc-deficient diet in restricted amounts resulted in alleviation of the teratogenic effects of zinc deficiency. On the other hand force-feeding the pregnant rats for 18 d made them severely distressed. These results support the hypothesis that rapid weight gain stimulates zinc depletion due to decreased protein catabolism and increased anabolism.

The muscle mass, represented by the psoas major in the ZD animals was 20% lower than that of their respective zinc-replete controls (table 4), but the difference in total-body weight between these two groups was only 7% (fig. 1). Since muscle mass contributes 40% of total-body mass, it seems apparent that this lower body weight was due to less muscle mass. This is assuming that the psoas major is representative of the total skeletal musculature. These data indicate that the growth of skeletal muscle was compromised by zinc deficiency more than other components of the body that were measured.

Parallel changes in weight and DNA content, no change in RNA/DNA ratio, and higher protein/DNA ratio in the ZD group (table 4) suggest that the impaired muscle growth by zinc deficiency was mainly due to the decrease in DNA synthesis in muscle but not due to a decrease in synthesis of RNA or protein. In the liver from the ZD rats the DNA concentration was lower, hence the ratios of RNA/DNA and protein/DNA were higher (table 3). The detrimental effects of zinc deficiency were far more pronounced in skeletal muscle than in the liver.
zinc deficiency on DNA synthesis agree with findings of other investigators (27–29) who compared ad libitum-fed zinc-deficient rats with pair-fed controls. However, there are conflicting reports in the literature concerning the effects of dietary zinc on RNA and protein synthesis (30–34). This discrepancy is probably due to differences in experimental conditions such as age of animals, duration of experiments, the daily zinc intake of zinc-deficient or pair-fed control animals, or other factors. Mills et al. (35) pointed out that valid results cannot be obtained without eliminating feeding pattern differences between control and experimental groups, especially in isotopic studies of RNA synthesis. The results presented in tables 3 and 4 reaffirm that DNA synthesis is impaired due to zinc deficiency but not because of a decrease in food intake or a change in eating pattern.

The serum protein and cholesterol levels in the ZD group did not differ from those of the ZN group (table 2). These results are in agreement with findings of other investigators who studied animals fed a zinc-deficient diet ad libitum. Golub et al. (12) observed that total serum protein and cholesterol levels remained in the same range in marginally zinc-deficient pregnant monkeys as ad libitum–fed and food-restricted controls. Macapinlac et al. (36) also reported that total serum cholesterol and protein were comparable among zinc-deficient, restricted-fed controls, and ad libitum–fed controls. In the present study the concentration of serum triglycerides appeared to be higher in the ZD group, but was not statistically different between the ZD and ZN group (table 2). Because the appearance of some sera from the ZD rats was suggestive of hypertriglyceridemia, a subsequent experiment similar to the present experiment has now been performed (Park, J. H. Y. and Hart, M., unpublished data). Data from the recent experiment indicated that serum triglyceride levels were significantly higher in the ZD group (471 ± 46 mg/dl) than in the ZN group (279 ± 31 mg/dl).

The higher relative liver weight, glycogen and lipid concentration (table 3) and the higher level of serum glucose (table 2) and triglycerides in the ZD rats are of interest for several reasons. Hepatosplenomegaly was reported to frequently occur in zinc-deficient patients in the Middle East (37, 38). Fatty liver and glycogen infiltration of the hepatocellular cytoplasm and nucleus have been reported to occur in approximately 50% of all diabetic patients (39–41). Severe zinc deficiency would be expected to have some influence on insulin release from the pancreas and on the rate of utilization, because it has been reported that the pancreas is relatively high in zinc concentration (42) and insulin action is prolonged if insulin is provided as a zinc complex. However, reported effects of zinc deficiency on glucose tolerance and insulin levels in the pancreas and blood in animals fed zinc-deficient diets ad libitum are variable (43–45). We hypothesize that in conventionally ad libitum–fed zinc-deficient animals, the decrease in food intake and nibbling eating pattern reduce insulin depletion in the pancreas. It is also possible that the cyclic eating pattern of these animals provides opportunities to redistribute zinc through the catabolism of body tissue, so released zinc can be used for more vital functions of the body. Negative relationships have been reported between food intake and plasma zinc concentration in rats fed a zinc-deficient diet (46). In a study with pregnant female rats fed a diet deficient in both zinc and calcium, Hurley and Tao (47) showed that conditions causing bone resorption increased the availability of skeletal zinc to the fetus. Force-feeding would not only cause a rapid reduction of plasma zinc by stimulating tissue anabolism but also stimulate insulin release and depletion from the pancreas. Studies are underway pursuing the concept that production of a zinc-deficient state in rats constitutes a major pathway leading to the onset of diabetes.

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LITERATURE CITED


