Efficacy of dietary zinc supplementation on catch-up growth after protein malnutrition

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Young male rats were subjected to protein malnutrition for 21 days and then rehabilitated for 21 days with diets that varied in zinc content. The three levels of dietary zinc used during recovery were 12, 33, and 72 ppm. Compared with a baseline group, serum total protein, albumin, zinc, and tibia zinc were all significantly decreased during the period of malnutrition. During recovery the malnourished rats consumed less food but grew more than the age-matched control rats. Feed efficiency (weight gain/food intake) of the recovering rats was twice the rate of the controls. Dietary zinc in excess of 12 ppm did not significantly increase linear growth or lean tissue weights, but did increase serum total protein, albumin, zinc, and tibia zinc during recovery from protein malnutrition.

Keywords: zinc; catch-up growth; protein malnutrition

Introduction

Recovery from severe protein-energy malnutrition (PEM) is often a long process, and optimal nutritional support leading to recovery could shorten the hospital stay and reduce costs. Because PEM is a major cause of morbidity worldwide,[1] optimal dietary treatment of severe malnutrition is important. Treatment of PEM involves two phases.[2] The acute phase includes therapies designed to correct problems with dehydration, infection, and electrolyte imbalances. Once the patient is rehydrated and stabilized, the rehabilitation phase may begin. The goal of nutritional rehabilitation is to induce sufficient catch-up growth to correct deficits in physical size.

Current knowledge about the amounts of nutrients required to promote recovery of lean tissue after growth retardation is far from complete. A technical report[3] of the World Health Organization stressed that investigation of the nutrient requirements of the recovering malnourished child should be a high priority. In addition to protein and calorie needs, knowledge of the specific requirements for other nutrients is also incomplete.

The ability of children with severe PEM to recover lean body mass may be limited by nutritional factors. Aggressive refeeding of malnourished children resulted in significant fat deposition but poor recovery of lean body mass.[4,5] In contrast, other recovering children have shown impressive gains in lean tissue.[6,7] Identification of dietary factors responsible for this improved lean tissue growth remains incomplete. PEM is often concomitant with poor zinc status. Kwashiorkor, associated with inadequate or poor quality dietary protein and low plasma albumin, has been consistently related to low plasma zinc values.[8–12] Dietary zinc has been suggested as a limiting factor during catch-up growth.[13–18] The purpose of the present study was to determine if protein-malnourished rats could attain additional catch-up growth if they received zinc supplements in excess of their normal requirement.

Methods and materials

Seventy-two male Sprague-Dawley rats (SASCO, INC., Omaha, NE USA) weighing between 75–100 grams were

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Table 1 Diet composition

Ingredient	Low-protein	Control
	g/kg	g/kg
Egg-white albumin[a]	30	151
DL-methionine[a]	2	2
AIN-vitamin mix 76A[b]	10	10
Mineral mix USP 14[c]	40	40
Choline bitartrate[a]	2	2
Cellulose (Celufil)[a]	50	50
Sucrose[d]	766	645
Corn oil[e]	100	100
Sodium selenite	0.0030	0.0030
Chromium chloride	0.0102	0.0102
Cupric acetate	0.0151	0.0151
D-biotin	0.0020	0.0020
Zinc acetate[f,g]	0.0370	Varied

[a]United States Biochemical Corp., Cleveland, OH USA.
[b]United States Biochemical Corp. This vitamin mix supplied the following (g/kg): thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; niacin, 3.0; calcium pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; vitamin B12, 1.0; dry vitamin A palmitate, 0.8; dry vitamin E acetate, 10.0; vitamin D trituration, 0.25; menadione sodium bisulfite complex, 0.15.
[c]The mineral mix supplied the following (%): calcium carbonate, 6.86; calcium citrate, 30.83; calcium phosphate monobasic, 11.28; manganese carbonate, 3.52; magnesium sulfate-7-H_2O, 3.83; potassium chloride, 12.47; dipotassium phosphate, 21.88; sodium chloride, 7.71; copper sulfate-5-H_2O, 0.0777; ferric citrate, 1.52815; manganous sulfate-H_2O, 0.02008; potassium aluminum sulfate, 0.00923; potassium iodide, 0.00405; sodium fluoride, 0.05070.
[d]Imperial Sugar Co., Sugarland, TX USA.
[e]Mazola, Best Foods, CPC International, Englewood Cliffs, NJ USA.
[f]Sigma Chemical Co., St. Louis, MO USA.
[g]Analysis of the low protein diet determined the zinc content to be 12 ppm. To the control diets, zinc acetate (mg/kg) was added in amounts of 37.0, 111.1, and 222.2; analysis of the diets revealed that the total zinc contents of these diets were 12, 33, and 72 ppm.

housed in stainless-steel, wire-bottomed cages in a room with controlled temperature and a 12-hour light/dark cycle. Extreme care was taken at all times to prevent any zinc contamination during this study. Stainless-steel food cups were used. Doubly deionized water (< 0.1 ppm zinc) was available at all times from glass water bottles. During a 3-day acclimation period the rats were provided free access to a purified diet (control diet in *Table 1*) providing 12 ppm zinc. All protocols used in this study were approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center.

For the first 21 days of the study rats were given free access to either a 3% albumin low-protein (LP) diet or a 15.1% albumin control (C) diet. The diets used spray-dried egg white albumin as the protein source because it is essentially zinc free. Zinc acetate was added to the C and LP diets to adjust the zinc content to 12 ppm, which is the requirement for the rat established by the National Research Council (NRC).[19] The diet components are listed in detail in *Table 1*. A baseline group was killed on day 0 and used for tissue and blood data collection.

After the first 21 days of the study, eight rats from each of the C and LP treatments were used for analysis, while the remaining rats were divided into six groups (three for C and three for LP) with rats from all groups given free access to the control diet, which was modified to contain one of three levels of zinc (12, 36, or 72 ppm zinc). The specific zinc content of each diet was verified by analysis. A 2 × 3 factorial, randomized complete block design was used for the rehabilitation part of the study. Eight rats from each group were used for analysis after 21 days of rehabilitation.

Ketamine (40 mg/kg) and xylazine (5 mg/kg) were injected in a subcutaneous location for anesthesia. Blood was collected via cardiac puncture with an 18 gauge needle to minimize hemolysis of red blood cells. Death occurred as a result of exsanguination due to the cardiac puncture. Serum was allowed to clot for 10 minutes and then separated from the red blood cells by centrifugation for two minutes at 10,000 rpm in an Eppendorf microcentrifuge.

Serum albumin and total protein were determined by the bromcresol green[20] and biuret methods,[21] respectively. Bovine serum albumin (Sigma Chemical Co., St. Louis, MO USA) was used as the protein standard for these assays. Within 24 hours of serum collection, serum alkaline phosphatase activity was determined through a quantitative and kinetic method (Procedure No. 245, Sigma Chemical Co.).

Dietary zinc content was determined for each diet prior to the start of the study. Samples of each diet were weighed into porcelain crucibles, dried at 70° C for 24 hr, ashed at 500° C for 48 hr in a muffle furnace (Model 1500, Thermolyne, Thermolyne Corp, Dubuque, IA). After cooling, the ash was weighed and dissolved in 2 mL of 1.3 N ultrapure nitric acid. Zinc analysis of the samples was done with a Perkin-Elmer Model 703 atomic absorption spectrophotometer (Perkin-Elmer Corp, Norwalk, CT USA) with an air acetylene flame. National Bureau of Standards citrus leaves were processed in a similar manner and analyzed; the percentage recovery of zinc from the citrus leaves was > 99%.

Zinc concentration was measured in serum and tibia. A glycerol dilution technique was used to assay serum zinc.[22] Serum was diluted 1:3 with doubly deionized water, and the zinc standard prepared with 5% glycerol to produce a viscosity and aspiration rate similar to the diluted serum samples. Tibia lipids were extracted twice with diethyl ether over 48 hr. The bones were dried for weight determination and then ashed at 500° C for 24–48 hrs and wet ashed with ultrapure nitric acid prior to appropriate dilution for analysis.

One-way analysis of variance (ANOVA) was used to analyze the results after the malnutrition phase. If the ANOVA revealed a significant effect ($P < 0.05$), statistical comparison of all group means was done with the Fisher's LSD test. Two-way ANOVA was used to test for significant ($P < 0.05$) main treatment effects and interactions for the data from the rehabilitation phase. The main treatments were previous nutritional status (control of low protein) and dietary zinc (12, 33, or 72 ppm). The Number Cruncher Statistical System (NCSS, Kaysville, UT USA) was used to analyze the data.

Results

During the malnutrition phase the LP rats experienced a 10% loss of body weight, while the C rats had a 129% gain in weight (data not shown). Compared with baseline, body length increased for C rats but did not change for the LP rats. No significant loss of tissue weight occurred in LP rats for the four skeletal muscles and the two fat pads, but significant losses were found for the heart, kidney, spleen, and thymus. Spleen and thymus from the LP group showed the largest losses, 33% and 38%, respectively. Although liver weight of the LP group did not significantly change compared with the baseline group, distinct streaks of fat were observed in the livers of malnourished rats.

Table 2 Serum and tibia parameters of rats before and after receiving control or low protein diets for 21 days

	Baseline	Low protein	Control
Serum			
Protein, g/L	59.0 ± 6.1[a]	50.5 ± 6.6[b]	66.0 ± 2.4[c]
Albumin, g/L	34.8 ± 2.2[a]	26.3 ± 1.7[b]	36.5 ± 2.0[a]
Zinc, mg/L	1.67 ± 0.30[a]	0.75 ± 0.19[b]	1.68 ± 0.27[a]
Alkaline phosphatase, units/L	210 ± 65[a]	172 ± 48[a]	168 ± 30[a]
Tibia			
Tibia wt (dry), mg	149 ± 6[a]	185 ± 21[b]	330 ± 23[c]
Zinc, μg/g dry wt	167 ± 13[a]	134 ± 25[b]	188 ± 22[a]
Zinc, μg/tibia	25 ± 2[a]	25 ± 6[a]	62 ± 8[b]
Length, mm	29.9 ± 0.4[a]	30.0 ± 0.8[a]	36.1 ± 0.8[b]

Mean ± SD of eight rats per group. ANOVA was significant ($P < 0.05$) for all parameters except alkaline phosphatase. Means in a row not sharing a common superscript are statistically different.

Table 3 Body weight and tissue weights of rats fed either low protein (LP) or control (C) diets for 21 days and then rehabilitated for 21 days with diets containing supplemental zinc

Group	Dietary zinc (ppm)	Body wt (g)	Wt gain rehab period (g)	Food intake rehab period (g/d)	Wt gain / Food intake (g/g)	Body length (cm)	Tissue weights (mg) Gastrocnemius	Spleen	Thymus	Kidney	Heart
LP	12	221	132	13.6	0.35	19.6	1122	659	542	877	694
LP	33	224	133	14.5	0.33	19.5	1128	646	604	902	701
LP	72	223	136	15.0	0.32	19.7	1097	628	610	926	741
C	12	286	69	16.1	0.15	20.9	1494	635	470	1119	806
C	33	303	83	18.0	0.16	21.5	1590	679	526	1214	834
C	72	305	84	18.1	0.17	21.5	1552	677	506	1233	890
Pooled SEM		8	4	0.3	0.01	0.2	49	30	41	47	29
ANOVA											
Previous nutritional status (PNS)		S	S	S	S	S	S	NS	S	S	S
Zinc (Z)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PNS X Z		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Mean and pooled SEM of eight rats per group. Data were analyzed with a 2 × 3 ANOVA. Main treatment effects and interactions are indicated as significant (S) $P < 0.05$ or non-significant (NS).

Compared with the baseline group, serum protein, albumin, and zinc decreased 14%, 24%, and 55%, respectively, due to the LP diet *(Table 2)*. Serum alkaline phosphatase activity was not different in any of the three groups. While tibia zinc concentration decreased 20% in the LP group, total zinc content of the tibia was unchanged; however, tibia dry weight in the LP group increased in comparison with the baseline group. The LP diet prevented any increase in linear bone growth.

After 21 days of rehabilitation, the LP rats showed significant, yet incomplete, catch-up growth as they were smaller than the C rats in terms of body weight and length *(Table 3)*. During recovery the LP rats ate less food but had a larger feed efficiency (wt gain/food intake) than the C rats. With the exceptions of the spleen and thymus, all the individual tissues were significantly smaller in weight for the LP groups compared with the C groups. The spleen showed complete catch-up growth in 21 days, and the thymus accomplished a growth hypertrophy such that the LP rats had larger mean weights of the thymus compared with the C rats. None of the tissues measured in this study was able to attain additional catch-up growth after malnutrition due to dietary zinc that was supplemented in amounts exceeding the NRC requirement of 12 ppm. Likewise, no significant interaction between previous nutritional status (LP or C) and zinc supplementation was found for any of the body-weight and tissue-weight measurements.

Relative tissue sizes (tissue wt/100 g body wt) and tissue-weight gains were calculated (data not shown) for the sum of: (1) the four skeletal muscles, (2) the two fat pads, and (3) the five organs. Compared with control values, relative size of the fat pads was smaller in the LP rats, while relative organ size was larger. Skeletal muscle growth in the LP rats paralleled whole body growth and no change in relative size was found in comparison with the C rats. Tissue weight gains for the muscles and organs during recovery by the LP rats exceeded those of the C rats, and the gain in fat was similar for these two major treatments. During recovery from protein malnutrition, skeletal muscle and organs had a higher priority for growth than did adipose tissue.

Serum and tibia parameters *(Table 4)* showed some significant treatment effects due to the zinc supplementation. Serum total protein, albumin, zinc, alkaline

Table 4 Serum and tibia parameters of rats fed either low protein (LP) or control (C) diets for 21 days and then rehabilitated for 21 days with diets containing supplemental zinc

		Serum				Tibia			
Group	Dietary Zinc (ppm)	Protein (g/L)	Albumin (g/L)	Zinc (mg/L)	Alkaline phosphatase (units/L)	Tibia wt (dry) mg	Zinc (μg per tibia)	Zinc (μg/g dry wt)	Length (mm)
LP	12	65.0	35.0	1.31	140	332	32	95	37.6
LP	33	67.6	36.0	1.54	155	331	40	119	37.7
LP	72	70.0	38.0	2.10	148	333	50	151	37.5
C	12	67.9	38.5	1.68	99	420	93	221	39.8
C	33	69.0	38.4	1.85	139	447	99	221	40.1
C	72	69.8	40.4	1.81	124	453	89	197	41.0
Pooled SEM		1.4	0.8	0.09	11	10	3	7	0.3
ANOVA									
Previous Nutritional Status (PNS)		NS	S	NS	S	S	S	S	S
Zinc (Z)		S	S	S	S	NS	S	S	NS
PNS X Z		NS	NS	S	NS	NS	S	S	S

Mean and pooled SEM of eight rats per group. Data were analyzed with a 2 × 3 ANOVA. Main treatment effects and interactions are indicated as significant (S) $P < 0.05$ or non-significant (NS).

phosphatase activity, and tibia zinc concentration and content were all significantly increased by dietary zinc in excess of 12 ppm; however, linear growth and dry weight of the tibia were not increased by the zinc supplements. Previous nutritional status was again a significant factor in limiting complete recovery of the LP rats for serum albumin, tibia zinc, and tibia length. Alkaline phosphatase activity was higher for the LP rats than for the C rats. Both serum and tibia zinc showed significant interactions between previous nutritional status and zinc supplementation as LP, but not C, rats increased levels of zinc in the serum and tibia as dietary zinc increased.

Discussion

The main hypothesis tested by the present research was the efficacy of supplemental zinc to stimulate recovery from protein malnutrition. Previous studies examined the effects of supplemental dietary zinc in malnourished children.[13–18,23] Because PEM and zinc deficiency have many common clinical features (stunted growth, tissue wasting, anorexia, diarrhea, hair dyspigmentation, decreased lymphoid tissue, and increased risk of infection), the best test of zinc deficiency in the malnourished child may be a positive therapeutic response to zinc supplementation. Castillo-Duran et al.[15] provided zinc supplements to infants during recovery from marasmus and found that additional zinc decreased the percentage of anergic infants but did not significantly increase arm muscle area or plasma zinc. Walravens et al.[17] found that daily zinc supplements of about 5 mg increased the rate of weight gain in a 6-month study of failure-to-thrive infants. In a study of 16 PEM children (14 with marasmus), zinc supplementation increased the rate of weight gain, restored thymus growth and sodium pump activity, and increased plasma zinc by 57%.[13] Although muscle growth was not directly measured, the decreased energy cost of tissue deposition in response to zinc supplementation suggested that lean tissue growth had been favored over deposition of fat in the more energy-dense adipose tissue. The results of these studies suggested that supplemental zinc is needed to support additional growth during recovery from malnutrition; however, some of the studies[15,17,23] appear to have compared zinc supplements with a diet that was either low or marginal in zinc content. Also, because zinc has been shown[15,24] to improve immunocompetence, some of the improved catch-up growth in children recovering from PEM may be a secondary effect due to fewer or less severe infections after zinc supplementation. One advantage of the present animal experiment is the opportunity to study the zinc effect without infection as a confounding factor on the growth process.

Mouse pups supplemented with dietary zinc after a period of malnutrition showed that increasing dietary zinc from 5 to 10 ppm caused a significant increase in weight and protein gains; but increasing dietary zinc from 10 to 40 ppm did not significantly change gains in body weight or protein.[25] The results of this animal study appear to agree with the findings of the present study in that dietary zinc in excess of the requirement for rodents did not significantly improve weight gain or lean tissue accretion during recovery from severe malnutrition. However, the present study does show some advantage of extra dietary zinc for increasing serum total protein, albumin, zinc, and tibia zinc. Also, the present study confirmed the finding[26] that the spleen and thymus will show significant atrophy in response to a low-protein diet but will demonstrate more catch-up growth than other organs when a good quality diet is provided.

In summary, the efficacy of zinc supplementation to stimulate catch-up growth was tested against the NRC requirement (12 ppm) for the rat in the present study. Dietary zinc in excess of 12 ppm did not significantly increase linear growth or lean tissue weights during recovery from protein malnutrition; however, zinc supplementation did increase serum total protein, albumin, zinc, and tibia zinc. Thus, moderate zinc supplementa-

tion during recovery from severe protein malnutrition was needed to replenish serum and bone reservoirs of zinc but was not able to stimulate additional catch-up growth.

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